SHORT COMMUNICATION

Insight into the growth dynamics and systematic affinities of the Late Cretaceous *Gargantuavis* from bone microstructure

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Abstract Enigmatic avialan remains of *Gargantuavis philoinos* from the Ibero-Armorican island of the Late Creta-ceous European archipelago (Southern France) led to a debate concerning its taxonomic affinities. Here, we show that the bone microstructure of *Gargantuavis* resembles that of *Apteryx*, the extinct emeids and *Megalapteryx* from New Zealand, and indicates that like these slow-growing terrestrial birds, it took several years to attain skeletal maturity. Our findings suggest that the protracted cyclical growth in these ornithurines may have been in response to insular evolution.

Introduction

Gargantuavis philoinos is a giant flightless bird which is known from a few postcranial elements (e.g. pelvis, synsacrum, cervical vertebra, femur) from the Late Cretaceous of Southern France (Buffetaut et al. 1995; Buffetaut and Le Loeuff 1998; Buffetaut and Angst 2013). Only a few bones being known, many aspects of the anatomy of *Gargantuavis*

remain obscure. Despite suggestions that it might have been a giant pterosaur (Mayr 2009), anatomical evidence, including a broad pelvis with an anteriorly placed acetabulum and a robust femur, supports its avian status (Buffetaut and Le Loeuff 2010).

Studies of the bone microstructure of a variety of extinct vertebrates have provided information about various aspects of their biology, such as ontogenetic age, lifestyle adaptations, bone depositional rates and growth patterns (Amprino and Godina 1947; Ricqlès et al. 1991; Castanet et al. 1993; Chinsamy-Turan 2005, 2012; Erickson 2005). We examined the bone microstructure of a *Gargantuavis* femur (MDE-A08 from the Musée des Dinosaures, Espéraza, Aude, France) from the Late Campanian/Early Maastrichtian of Villespassans (Hérault, Southern France) to deduce information about its systematic position and aspects of its biology. Histological comparisons were made with non-avian dino-saurs, avialans and pterosaurs.

Materials and methods

Two bone cores (A and B; Fig. 1a; Stein and Sander 2009) from the peripheral surface of the bone to the medullary cavity were removed from the diaphysis of the approximately 23-cm long *Gargantuavis* incomplete femur (Fig. 1a). The cores were sectioned into two longitudinal (B, Fig. 1b–d) and trans-verse (A, Fig. 1e–h) sections. After embedding in Struers epoxy resin, the thin sections were prepared using an Imptech PC10 thin sectioning machine, and final polishing was done with silicon dioxide powder on a velvet cloth (Chinsamy and Raath 1992; Chinsamy-Turan 2005). The final thickness of the slides is approximately 30 μm. The thin sections were examined under a Nikon Eclipse Biological petrographic mi-croscope E200. Micrographs and measurements were per-formed using NIS Elements version 3.0.

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Results

The bone histology of the cores from the *Gargantuavis* femur is reasonably well preserved. In both cores, the bone wall is fairly thick, between 5 and 8 mm from the periosteal surface to the medullary cavity (Fig. 1b, e). A number of channels, which in life would have housed blood vessels and other connective tissue (Starck and Chinsamy 2002), are distributed over the cortical bone. They tend to have a longitudinal orientation in the transverse section (Fig. 1e–h) and a circumferential to reticular arrangement in the longitudinal section (Fig. 1b–d).

The compacta is stratified into three distinct regions, i.e. I, II and III (Fig. 1d, g). The innermost region I formed during early ontogeny and appears to be more richly 'vascularized' (Fig. 1b, e) and largely comprises a highly remodelled Haversian bone with several generations of secondary osteons (Fig. 1e, h). Many of the channels in this region are erosionally enlarged, and several show a distinct cement line that delimits the extent of erosion into the primary compacta and the subsequent centripetal deposition of lamellar bone (Fig. 1b, f, h). Nevertheless, the transverse section still shows some evidence of primary bone and lines of arrested growth (LAG; Fig. 1f). A layer of endosteal lamellar bone is visible in some areas around the medullary cavity (Fig. 1b, e, h). Region II is a transitional zone that exhibits a distinct change in bone tissue texture and a decrease in bone vascularization and bone remodelling (Fig. 1d, g; region II). The vascularization is formed of sparse large secondary osteons and few small primary osteons and simple vascular canals (Fig. 1d, g; region II). Closer to the medullary cavity, the bone has a more woven-to-parallel-fibred texture with more globular osteocyte lacunae, while in the outermost cortex, it has a more lamellar texture, and the osteocytes tend to be more flattened and regularly arranged. Sharpey's fibres are visible in regions II and III (Fig. 1g). Region III, towards the peripheral surface of the bone, has several growth marks that interrupt the deposition of the poorly vascularised parallel-fibred bone tissue (Fig. 1c–e, g). At least 10 LAGs are observed across the cortex. The earlier LAGs (closer to the medullary region) are more widely spaced than the more recently formed ones. It is possible that growth marks formed during even earlier stages of ontogeny and may have been obliterated because of extensive bone remodelling.

Discussion

The histology of *Gargantuavis* permits us to deduce various aspects of its biology and growth dynamics. The innermost woven texture and richly vascularized nature of the bone microstructure of Region I as well as the widely spaced growth marks indicate that the early growth experienced by

Fig. 1 **a** Cranial view of the *Gargantuavis* femur (MDE-A08) core-sampled mid-diaphyseally for histological analysis. *Grey circles* indicate the sampling locations of the bone cores A and B. *Red lines* indicate the planes of the cross sections. **b** Part of the longitudinal section of the bone core B (with the bone surface on *top* and the medullary cavity at the *bottom*). The cortex can be subdivided into three regions according to the organization of the bone tissue: *I* The inner cortex (about 70 % of the bone wall) is composed of a woven fibred bone matrix, with haphazard osteocyte lacunae, and is well vascularized. The vascular canals have a circumferential to reticular orientation, and some are erosionally enlarged, whilst many are completely formed secondary osteons. *II* A transitional area marked by a decrease in vascularization and a change in the texture of the tissue (from a woven texture to a more parallel-fibred to a lamellar texture) suggests a decrease in the rate of bone deposition. *III* The outermost part of the cortex with 10 LAGs visible. **c** Detail of the outermost cortex (*region III*) showing the LAGs (*arrows*) and the single-double LAG (*arrowhead*). Note that the intervals between the LAGs are irregular but tend to decrease toward the bone surface. **d** Detail of the bone core B showing the change in the texture of the bone tissue (*region II*) from the more richly vascularised woven bone to the more poorly vascularised parallel-fibred to lamellar bone tissue. **e** Transverse section of the bone core A. As in the longitudinal section (**b**), the cortex can be subdivided into three regions according to the organization of the bone tissue. **f** Detail of the inner cortex (*region I*) in (**e**). The deep cortex is highly remodelled with numerous secondary osteons and resorption cavities. In some areas, the primary bone and widely spaced growth marks are visible (*black arrows*). **g** Detail of the cross-section in (**e**) in direct (*left*) and polarized (*right*) light. The deep cortex (*region I*) is highly vascularised with numerous large secondary osteons. The *region II* marks a transition in bone organization with decrease in vascularization and remodelling. The outermost cortex (*region III*) is poorly vascularised and has several LAGs (*black arrows*). The *white arrows* point at clusters of Sharpey's fibres. **h** Detail of the innermost cortex in (**e**). The deep cortex is highly remodelled, and some areas are formed with a dense Haversian bone. A layer of lamellar endosteal bone is visible in the vicinity of the medullary cavity. *PS* periosteal surface, *ELB* endosteal lamellar bone

this animal (at least about 70 %) was rather rapid. However, the subsequent almost 30 % of the bone tissue (i.e. the more recently formed bone) is clearly interrupted by closely spaced growth marks, and the change to a more parallel-fibred texture directly indicates that the rate of bone deposition during later growth was slower (Chinsamy-Turan 2005; Erickson 2005; Ricqlès et al. 1991). The LAGs that interrupt bone deposition indicate that the animal experienced at least 10 arrests in growth, which are most likely to have been annual (e.g. Ricqlès et al. 1991; Castanet et al. 1993; Chinsamy-Turan 2005; Köhler et al. 2012) and that it continued to deposit bone, albeit at a slower rate than in early ontogeny, for at least 10 years. The closer spacing of the LAGs towards the periosteal surface (Fig. 1e) suggests that the animal was nearing somatic maturity, although we cannot be certain for how much longer it would have continued to accrue bone appositionally. Double LAGs, as indicated by the seventh LAG (Fig. 1e), are not uncommon among modern vertebrates (Castanet and Smirina 1990; Ricqlès et al. 1991; Castanet et al. 1993; Turvey et al. 2005; Erismis and Chinsamy-Turan 2010) and suggest that the animal had
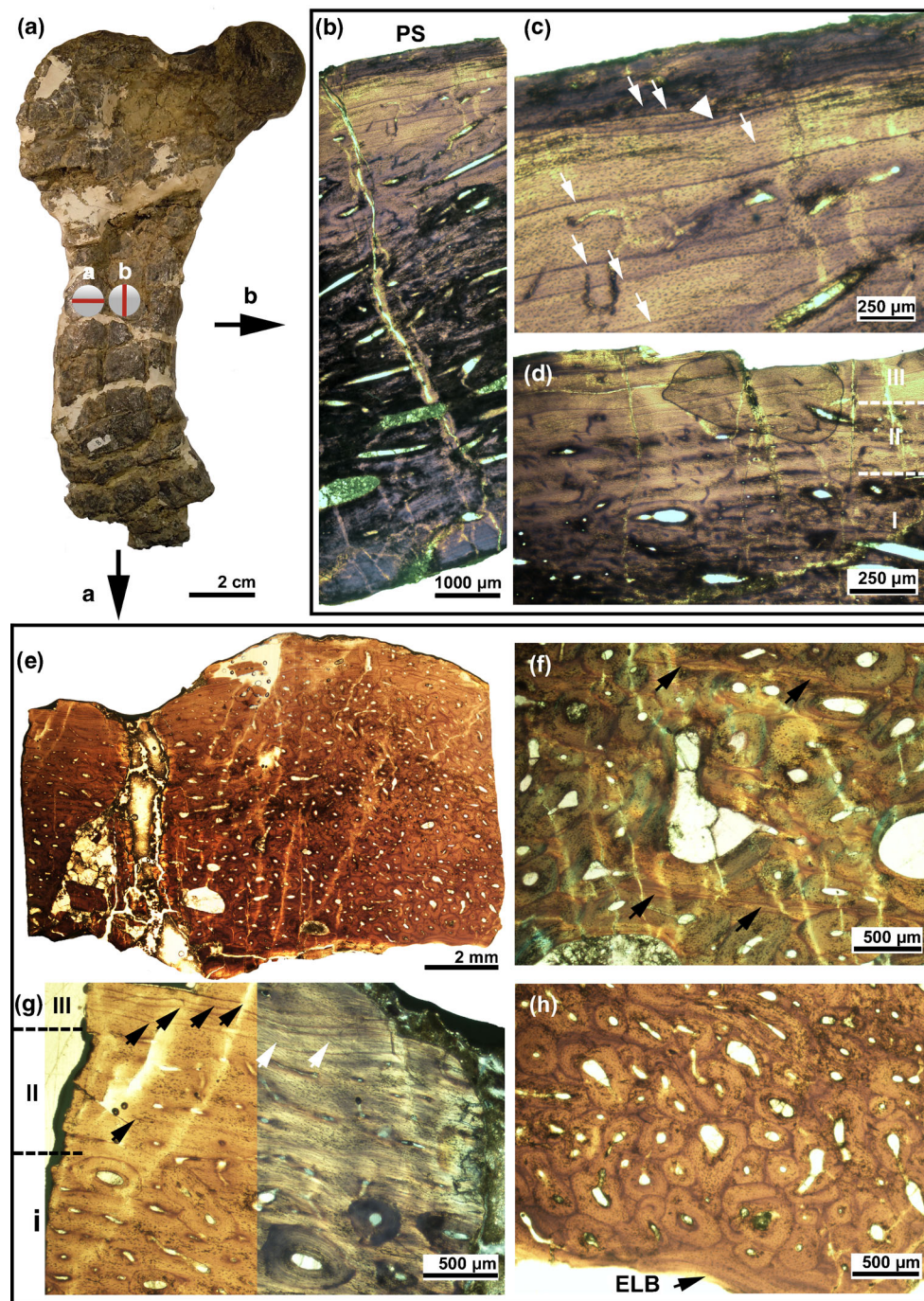
briefly resumed growth, but that for some reason, it had ceased bone deposition again.

The systematic affinities of *Gargantuavis* have been disputed, with Mayr (2009) suggesting that it might have been a large pterosaur. Recent studies have demonstrated that microanatomical and osteohistological parameters may contain a strong phylogenetic signal (Canoville and Laurin 2010; Legendre et al. 2013). Thus, considering these studies, our findings show that the bone microstructure of *Gargantuavis* is

unlike that of non-avian theropod dinosaurs (Chinsamy-Turan 1990; Chinsamy-Turan 2005; Reid 1990; Varricchio 1993), which typically have a zonal fibro-lamellar bone tissue in their femoral cortical bones (Fig. 2a). *Gargantuavis* bone also differs from that of pterosaurs (Fig. 2b), which is distinctive in having extremely thin walls (e.g. Ricqlès et al. 2000; Padian et al. 2004; Steel 2008; Chinsamy et al. 2009), in which the majority of the compacta generally comprises un-remodelled fibro-lamellar bone tissue.
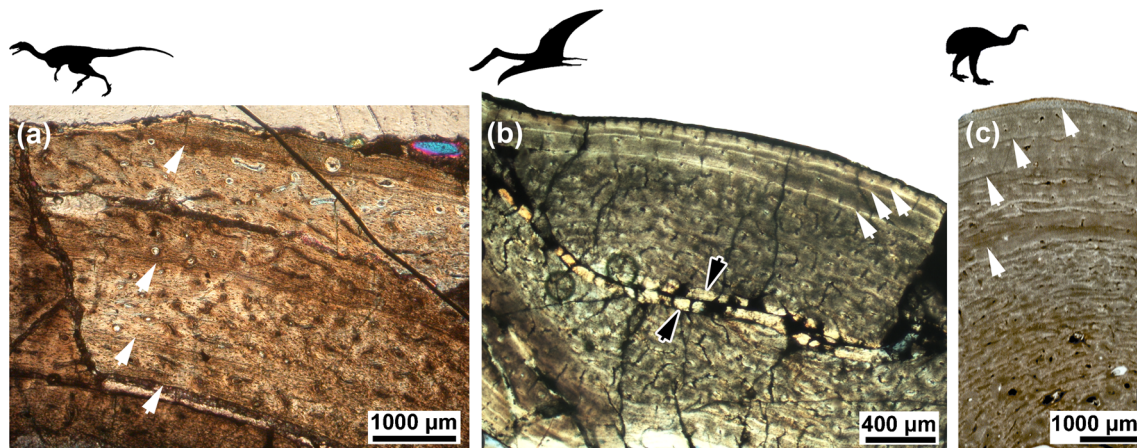
Fig. 2 Bone histology of **a** Femur of *Megapnosaurus (Syntarsus) rhodesiensis*, a small non-avian theropod showing a compacta consisting of richly vascularised zonal bone tissue. The growth marks are indicated by *white arrows*. **b** Femur of *Pterodaustro guinazui*, a pterosaur showing fibro-lamellar bone tissue interrupted by LAGs (*white arrows*). The bone wall has collapsed on the medullary cavity, and the inner circumferential layer is indicated by *black arrows*. **c** Tibiotarsus of *Megalapteryx didinus* from Takahe Valley, New Zealand. The outer cortex has a more slowly formed lamellar bone tissue with several growth marks (*white arrows*)

Interestingly, *Gargantuavis* bone microstructure deviates from that of most modern birds (including large extant ratites such as cassowary, rhea and ostriches), which typically show rapid uninterrupted growth to adult body size (Enlow and Brown 1958; Chinsamy 1995; ; Starck and Chinsamy-Turan 2002; Chinsamy-Turan 2005). It is, however, most similar to that of *Apteryx* (kiwi) and the extinct emeids (such as *Euryapteryx*, *Anomalopteryx*) and *Megalapteryx* (Turvey et al. 2005; Fig. 2c), which were large terrestrial birds from New Zealand. In a comprehensive survey of moa bone histology, Turvey et al. (2005) showed that their growth dynamics deviated from that of most other extant birds (Chinsamy-Turan 1995; 2005) in that they had an extended period of slow cyclical growth with some forms, such as the emeid *Euryapteryx geranoides*, taking at least a decade to attain skeletal maturity. In terms of histological structure, *Gargantuavis* most closely resembles *Megalapteryx* (Fig. 2c), and Emeidae. *Megalapteryx* was once considered as an emeid; however, Bunce et al. (2009) now place it outside this family. Irrespectively, the bone histology of *Gargantuavis* shows that it experienced a cyclical growth pattern similar to that of the emeids and *Megalapteryx*, and that it took at least 10 years to reach skeletal maturity.

Our findings suggest that *Gargantuavis* was a large avialan that experienced protracted growth to attain skeletal maturity. This kind of growth pattern is known to have occurred among early birds (Chinsamy-Turan 1994, 2005; Erickson et al. 2009), and although not common among modern birds, which tend to grow rapidly within a few months to adult body size, such an extended growth strategy has been observed in *Apteryx* (kiwis; Bourdon et al. 2009) and the Dinornithiformes (notably the emeids and *Megalapteryx*; Turvey et al. 2005). Curiously, both these taxa are ratites, which evolved in New Zealand, i.e. in an island environment. *Apteryx*'s extended growth is considered to have evolved in response to temperature fluctuations in the Miocene (Bourdon et al. 2009), while the atypical postnatal growth experienced by the Dinornithiformes is considered as an adaptation to the unique New Zealand ecosystem (Turvey et al. 2005). *Gargantuavis* is known only from the Ibero-Armorican island of the Late Cretaceous European archipelago (Pereda-Suberbiola 2009). This suggests a link between insular evolution and the flexible extended growth exhibited in *Gargantuavis* and the New Zealand birds (Starck and Chinsamy 2002; Köhler and Moyà-Solà 2009). However, the moas were the largest terrestrial animals in their ecosystem and apparently had a single predator, *Harpagornis moorei*, a large flying eagle (Worthy and Holdaway 2002). Unlike the moas, *Gargantuavis* was part of an ecosystem which included large terrestrial predators, namely theropods (Pereda-Suberbiola 2009). Thus, it would not have been a selective advantage for *Gargantuavis* to have a protracted growth in this environment.

In a histological study of the extinct insular bovid *Myotragus balearicus*, Köhler and Moyà-Solà (2009) found that it had a bone microstructure interrupted periodically by LAGs, which differed markedly from mainland bovids which grew in an uninterrupted manner. They deduced that this growth pattern was a consequence of periodic food shortages on the island. Similar findings were obtained by Steel (2009) who reported that *Pezophaps solitaria*, a flightless bird from Rodrigues (Mascarene Islands) exhibited LAGs in its bones in response to seasonal conditions and availability of food, while its sister taxon *Raphus cucullatus* from the bigger island Mauritius had more food resources and less seasonal variation and lacked LAGs. Another curious example of 'changed or modified' growth pattern was observed in the bone microstructure of the titanosaur *Ampelosaurus atacis* (Le Loeuff 1995), which is contemporaneous with *Gargantuavis* at

several Late Cretaceous localities in Southern France, and shows a reduction in its growth rate (as compared to other titanosaurs), which may have been related to resource limitations (Klein et al. 2012). Interestingly, sedimentological and mineralogical studies have documented episodes of semi-arid and strongly seasonal climate during the Late Cretaceous in Southern France (Cojan and Moreau 2006), notably during the Late Campanian, when both *Gargantuavis* and *Ampelosaurus* inhabited that area. Thus, the peculiar growth patterns exhibited by these animals may be a consequence of the prevailing environmental conditions in an insular setting.

Gargantuavis, the moas (especially emeids and *Megalapteryx*) and *Apteryx*, reflect a generally atypical ornithurine flexible (cyclical) life history strategy (Chinsamy-Turan 2005; Starck and Chinsamy 2002). Palaeoenvironmental studies suggest that the ecological conditions under which *Gargantuavis* and the moas acquired protracted growth were different. We propose that the punctuated growth experienced by *Gargantuavis* may have been in response to seasonality and food availability, as in various other insular tetrapods (Köhler and Moyà-Solà 2009; Steel 2009; Klein et al. 2012).

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