Slow life history and physiological plasticity: survival strategies of a large mammal in a resource-poor environment.

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Abstract:

Because of their physiological and life history characteristics, mammals exploit adaptive zones unavailable to ectothermic reptiles. Yet, they perform best in energy-rich environments because their high and constant growth rates and their sustained levels of resting metabolism require continuous resource supply. In resource-limited ecosystems such as islands, therefore, reptiles frequently displace mammals because their slow and flexible growth rates and low metabolic rates permit them to operate effectively with low energy flow. An apparent contradiction of this general principle is the long- term persistence of certain fossil large mammals on energy-poor Mediterranean islands.

The purpose of the present study is to uncover the developmental and physiological strategies that allowed fossil large mammals to cope with the low levels of resource supply that characterize insular ecosystems. Long-bone histology of *Myotragus*, a Plio-Pleistocene bovid from the Balearic Islands, reveals lamellar-zonal tissue throughout cortex, a trait exclusive to ectothermic reptiles. The bone microstructure indicates that *Myotragus* grew unlike any other mammal but similar to crocodiles at slow and flexible rates, ceased growth periodically, and attained somatic maturity extremely late by about 12 years. This developmental pattern denotes that *Myotragus*, much like extant reptiles, synchronized its metabolic requirements with fluctuating resource levels. Our results suggest that developmental and physiological plasticity was crucial to the survival of this and, perhaps, other large mammals on resource-limited Mediterranean Islands, yet it eventually led to their extinction through a major predator, *Homo sapiens*.

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Introduction:

Energy availability is a key factor in the evolution of physiological and life history strategies of organisms. Therefore, much interest has recently been shown in the ecophysiological adaptations of vertebrates endemic to ecosystems with low energy flux (1). Ectotherms, though frequently thought of as primitive (2), are actually specialists in coping with low levels of available energy (3, 4). Ectotherm vertebrates have slow and flexible growth rates and a notable physiological plasticity, which allows a close matching of their energy requirements to prevailing resource conditions (3, 5, 6). Endotherms, instead, typically have high and steady growth rates, a constant thermometabolic regime, and they depend on high and continuous food intake to maintain their elevated metabolism (7). Therefore in environments such as islands, where resource bases are narrow and resource availability is unpredictable (1), reptiles frequently replace mammals (8, 9).

Certain mammals, however, were dominant faunal elements on Mediterranean islands, where they persisted for long time periods, some of them over millions of years (10). This is particularly perplexing in the case of insular dwarf mammals such as elephants, deer and hippos, which should be expected to have even higher resource requirements than small mammals because of the scaling of metabolic rate with body mass. Unsurprisingly, therefore, hypotheses aimed to explain the evolution of dwarfism and gigantism on islands (the Island Rule, Van Valen 1973) (11) traditionally evoked resource availability as the driving force behind these, often dramatic, changes in body size (8, 9). More recently, however, several studies drew attention to the tight correlation between body size and life history traits, suggesting that not body size itself but fitness-related life history traits were the chief goal of selection on islands (12, 13). Thus, it has been argued that dwarfing is a corollary of selection for an increase in production rate in low-mortality environments (12-15) through an *increase in growth rate* (14) and a *decrease in age at maturity* (14, 15). This contrasts with a model that predicts shifts in adult body size in function of the magnitude of adaptive changes in growth rate and age at maturity in response to resource availability and extrinsic mortality (16). For environments such as islands, where resources are scarce and extrinsic mortality is low,

this model predicts a decrease in adult body size through a *decrease in growth rate*, associated to an *increase in age at maturity* (16). Data that might provide empirical support for any of these essentially theoretical approaches, however, are scarce and come from observations on small extant vertebrates only (see 16 for a more comprehensive review), because almost all large insular mammals went extinct following human settlement (1). The only way to reconstruct the physiological and life history strategies of dwarfed insular mammals, hence, is the study of their fossil remains. *Myotragus*, a dwarf bovid from the Plio-Pleistocene of Majorca (Balearic Islands, Spain), is particularly suitable for this purpose because it evolved under known selective pressures (chronically low resource levels and lack of predators) (10, 17) in a completely isolated ecosystem, conditions that closely resemble experiments on natural populations but at a time scale that only the fossil record can provide.

Physiological and life history strategies of fossil vertebrates are recorded in their hard tissues. Long-bone tissues of slow and flexibly growing ectotherms and fast and constantly growing endotherms differ substantially. Ectotherms are characterized by lamellar-zonal bone throughout cortex. This bone is formed in a periodic manner whereby the deposition of lamellar (parallel-fibered) bone (18, 19) cyclically comes to a halt. These seasonal pauses in bone formation are recorded in the bone tissue as growth rings or lines of arrested growth (LAGs) (20-22). Endotherms are characterized by uninterrupted (azonal) fast growing fibrolamellar tissue throughout cortex and a thin outer cortical layer (OCL) of slow growing lamellar bone deposited after attainment of somatic / sexual maturity (21, 23, 24). LAGs, if present, appear near periosteum in the OCL (21, 23). An "intermediate" pattern, the fibrolamellar-zonal complex (25) composed of alternating zones of fibrolamellar tissue and LAGs, can be observed in extinct tetrapods only (dinosaurs and non-mammalian therapsids) (24, 25). Fossil evidence indicates that fast and uninterrupted growth has been acquired independently by birds and mammals (21). The capability to stop growth periodically is therefore considered to be a plesiomorphic trait reflecting an intermediate physiological condition (20) that has been lost in modern vertebrates (20, 24), or simply as a phylogenetic legacy (26).

Results:

Ontogenetic stages of bone tissue

Our descriptions of bone tissues of *Myotragus* are based on the typological classification established by Ricqlès (18, see also de Margerie et al. (19)). Thin-sections from an ontogenetic series of 57 long bones of *Myotragus* reveal that the primary bone tissue consists of zonal bone throughout (Fig. 1A, C, E, F; 2B-E), comparable to that of crocodiles (compare Fig. 1B, D). LAGs appear as simple (Fig. 1A, E, F), double or even triple rest-lines (Fig. 2B-E). They are spaced fairly homogeneously throughout cortex (Fig. 2C). In older individuals, LAGs are closer spaced the more they approach the periosteal surface (Fig. 1A), indicating that growth rate decreased with age. At an early ontogenetic stage (Fig. 1F; 2B), fibrolamellar-zonal (23) tissue or lamellar bone with primary osteons (LPO) prevails, alternating with annuli of lamellar non-vascular bone (LNV) with flattened osteocytes and LAGs (Fig. 2B, D). Vascularization is moderate with an essentially circumferential orientation of the channels. Early remodeling becomes manifest at the inner medullary surface (erosion of innermost primary tissue and deposition of inner circumferential layers, first Haversian systems) (Fig. 1F; 2B). The primary bone pattern of this early ontogenetic stage indicates a moderately rapid rate of bone deposition interrupted by low rates of bone deposition and growth arrest. It sharply contrasts with the early ontogenetic stage of other bovids (here *Gazella borbonica*, Fig. 1I), which is characterized by azonal fibro-lamellar complex (FLC) throughout cortex deposited during uninterrupted fast growth. At a later juvenile stage (Fig. 2C, E), alternating LNV and LPO bone becomes predominant and vascularization decreases. Equidistant LAGs embedded in LNV tissue with flattened osteocytes (annuli) denote that growth slowed down and ceased periodically. Haversian systems become increasingly abundant throughout the inner half of the cortex. Older individuals (Fig. 1E) show very slow growing lamellar-zonal bone in which non-vascular annuli (LNV) and / or LAGs alternate with poorly vascularized zones (LNV/LPO) throughout cortex. This pattern of bone microstructure is frequent among wild alligators (27) (compare Fig. 1B, D), but contrasts with the presence of fast growing fibrolamellar bone (FLC) throughout cortex in other artiodactyls (here adult *G. borbonica* Fig. 1H, and adult *Cervus* indet. Fig. 1G). None of the *Myotragus* specimens available for sectioning shows a distinct OCL that might indicate an rather abrupt onset of somatic maturity and / or sexual maturity as in

other mammals. Instead, some of the specimens simply show an increasingly closer spacing of LAGs towards the outer cortex, a trait that characterizes crocodiles but not mammals (compare Fig. 1A with Eocene crocodile Fig. 1B). Table 1 summarizes the main histological traits of *Myotragus* in comparison with the bone microstructure of crocodiles and large mammals.

Fig. 1 goes here

Table 1 goes here

Skeletochronology

Skeletochronology is consistent with the slow and variable-rate growth pattern deduced from the long-bone tissue. The earliest ontogenetic stage available for sectioning is a very tiny and immature humerus without epiphyses (IPS-26158-1, length approximately 4 cm; Fig. 1F). The tissue consists largely of FCL with longitudinal and circular osteons, though at the middle of the bone wall the tissue is more compact and of LPO type. Two clearly distinguishable generations of endosteal bone are deposited along the medullary cavity. At periosteum, one LAG is observable followed by a thin annulus and, most peripherally, by FLC tissue, indicating that the individual resumed growth after the unfavorable season but died shortly after at the age of somewhat more than one year. A small proximal femur without epiphysis (IPS-26444e, Fig. 2Ab, B, D) shows FLC bone around the medullary cavity followed by LPO and LNV bone. Erosion is observable along the medullary cavity and some Haversian systems are scattered over the inner bone wall. At the middle of the bone wall there is a double LAG embedded in lamellar (LNV) annuli with flattened osteocytes, followed by alternating LPO/LNV bone. This tissue indicates that the individual recovered a faster growth rate after a period of slow growth and growth arrest. Age at death, hence, was at approximately two years. A slightly larger immature femur (IPS-26324, Fig. 2Ac) that still lacks epiphyses, trochanter major and trochanter minor, shows little vascularized primary tissue of LPO type throughout. It presents two LAGs, large erosion cavities on the inner cortical (medullary) surface, endosteal bone and more extensive Haversian remodeling, providing a minimum age of

almost three years. We found a similar tissue pattern with two LAGs in a similar-sized humerus without epiphyses (IPS 26430). A minimum of six LAGs has been observed in a tibia of only two thirds the size of a fully-grown tibia in which the proximal epiphysis is not completely fused (IPS 44923-c), providing a minimum age of 7 years. Seven LAGs and, hence, a minimum age of close to 8 years, correspond to a juvenile femur of nearly adult size that still lacks both proximal and distal epiphyses and that shows an initial fusion of the trochanter major (IPS 26321; Fig. 2d, C, E). The primary bone largely consists of LNV tissue type; LAGs are mostly double or triple. Haversian systems invaded the inner cortical wall, erosion and formation of endosteal bone along the medullary cavity is advanced. The presence of a minimum of 11 LAGs in fully-grown individuals with epiphyses completely or almost completely fused (Fig. 1A, IPS 44929) denotes that *Myotragus* grew for *at least* 12 years before it attained skeletal / sexual maturity, more than six-fold the time of bovids of similar body mass (28), and even longer than large males of highly dimorphic *Bison*, which stop somatic growth at 7 years (29).

Fig. 2 goes here

Discussion

The peculiar bone histology of *Myotragus* provides direct evidence of the developmental and growth strategy, and indirect evidence regarding the physiology of this insular dwarf mammal. The presence of lamellar-zonal bone throughout cortex indicates that *Myotragus* grew at slow and variable rates and ceased growth cyclically, which was associated with an important delay in the attainment of skeletal (sexual) maturity. Consistent with life history theory (30), the extended juvenile development of *Myotragus* was associated with an extended life span as indicates the elevated number of very old individuals in the fossil assemblages (10). Our empirical finding, hence, does not support the prediction that life history traits of insular dwarfs accelerate to increase reproductive investment (12-15), but instead it lends support to the model that predicts a shift in life history traits towards the slow end of the slow-fast continuum with a delay in age at maturity and an extended life span (16).

True zonal bone with growth marks deposited seasonally throughout ontogeny is a general ectotherm characteristic (20). In ectotherms, bone matrix consists of slow growing lamellar bone (LNV, LSV, LPO). However, cyclically interrupted fast growing fibrolamellar bone (fibrolamellar-zonal complex (25)) has been found in many dinosaur taxa, in basal birds (20, 31) and in non-mammalian therapsids (24), leading to an ongoing controversy over whether zonal bone indicates an intermediate physiological condition along the transition between poikilothermic ectothermy and homeothermic endothermy (20) or whether it merely represents the ghost of past physiologies (26). Inferences about the physiologies of these extinct vertebrate groups, however, remain conjectural because they don't have living equivalents. Our finding of true lamellar-zonal bone in a fossil representative of phylogenetically modern mammals, hence, may shed some light on the physiological correlates of zonal bone.

Ungulates, like other endotherms, are characterized by azonal fast growing bone tissue and a thin outer cortical layer that may contain several growth lines. Sporadically, a single, isolated LAG has been observed within the fast growing fibrolamellar bone of cervids (23, 26) (see also Fig.1G). The occasional presence of LAGs in these large endothermic (non-hibernating and non-estivating) mammals led some (26) to conclude that such growth lines reflect phylogenetic legacy rather than a physiological response to environmental cycles or stresses. Nevertheless, evidence is accumulating that certain ungulates significantly reduce endogenous heat production to cope with energetically challenging situations (food shortage, harsh climatic conditions) (32). Thus, seasonal fluctuations in metabolic rate and in body temperature (heterothermy) have been described for ungulates with a winter nadir in northern species (32, 33, 34) and with a summer nadir in desert species (35, 36). Taking into account these recent advances in ungulate physiology, the zonal bone of *Myotragus* quite likely reflects seasonal fluctuations in metabolic rate and / or body temperature over an extended juvenile period in response to fluctuating resource conditions on the island.

Insular ecosystems are intrinsically resource-limited (1, 8, 9, 16, 37) because their limited landmass can only support a limited number of primary producers, which in turn affects the energy flow at higher trophic levels. Therefore, energy-poor islands are depauperate in competitors and predators (8, 9). Under these conditions, the pivotal achievements of

endothermy – (i) sustained aerobic capacities (7), (ii) an enhanced behavioral repertoir (7), (iii) high growth rates (38), and (iv) high reproductive rates (38) - are not only dispensable but the elevated metabolic rate to fuel these activities, is incompatible with the low insular resource bases. Therefore, insular endotherms should be expected to reduce these expensive traits. Indeed, among extant birds and mammals, small-island endemics have lower basal metabolic rates than their continental counterparts (8, 9, 39), while heterothermic small mammals such as dormice were dominant faunal elements in the fossil record of Mediterranean islands (10).

Majorca was such a resource-poor island, which is evidenced by nutrition-related malformations and other symptoms of starvation and undernourishment in fossil endemic populations (10), as well as by the low species diversity and the absence of predators (10). In agreement with the reasoning given above, *Myotragus* not only decreased aerobic capacities (low-gear locomotion) (40) and behavioral traits (reduction of brain and sense organs) (41) but it flexibly synchronized growth rates and metabolic needs to the prevailing resource conditions as do ectothermic reptiles. Our present study, hence, provides the first evidence that in energy-poor environments where reptiles usually replace mammals, selection for energy saving may be so imperious that mammals may revert to some ectotherm-like state that includes both physiological and developmental plasticity. Completely unexpected, this reversal is possible even in large mammals of phylogenetically modern groups such as bovids.

The reptile-like physiological and life history traits found in *Myotragus* were certainly crucial to the survival on a small island for the amazing period of 5,2 million years, more than twice the average persistence of continental species (42). Therefore, we expect similar physiological and life history traits to be present in other large insular mammals such as dwarf elephants, hippos and deer. However, precisely because of these traits (very tiny and immature neonates, low growth rate, decreased aerobic capacities, and reduced behavioral traits) *Myotragus* did not survive the arrival of a major predator, *Homo sapiens*, some 3000 years ago.

Materials and Methods:

Materials:

Fossil material: *Myotragus*: Plio-Pleistocene of Majorca, Spain (Cova de Llenaire, Es Bufador, Son Apats, Cova de Moleta, Avenc de Nècora); crocodile (genus and species indet.): Eocene (Lutetian), La Boixedat (Huesca, Spain); *Gazella borbonica*: Middle Pliocene, Layna (Spain); cervid (genus and species indet.): Upper Pliocene, Vilarroya (Spain). Specimens labeled #IPS are housed at the Institut Català de Paleontologia (ICP), Universitat Autònoma de Barcelona, Bellaterra, Spain. Specimens labeled #mbcn are housed at the Museu Balear de Ciències Naturals, Sóller, Majorca (Spain).

In order to avoid irreversible damaging of valuable material, we preferred fragmented specimens for sectioning (except for IPS-44923-c, IPS-26158-1, IPS-26324, and IPS-26321). Slices were made at mid-shaft following standard procedures (21, 23), and examined under transmitted light and under polarized and circularly polarized light with 1λ filter. Micrographs Figures 1C-I and 2B, D, E were taken on slices previously moistened with a drop of alcohol (98%) on their uncovered surface (a common procedure in petrography and crystallography). This emphasizes the original tissue structure where this is affected by microbial attack or diagenetic processes, without damaging the fossil. Micrographs were taken with Polarization Microscope Leica DM 2500 P.

Age assessment:

There is a general agreement that growth marks represent annual cycles (21, 22, 25). We estimated the age of individuals by counting cortical growth rings in histological sections of each specimen. Estimation of the number of lost or masked growth marks was not possible because removal of inner cortical bone starts at early ontogenetic stages. Therefore, estimated ages are minimum ages.

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Figure Legends:

Figure 1: Micrographs of long bone tissues.

A *Myotragus balearicus* (IPS 44929; entire section through cortical wall), adult distal tibia with completely fused epiphysis, 11 lines of arrested growth (LAGs), some Haversian systems; **B** crocodile (IPS 4913, Eocene, Spain; entire section through cortical

wall), adult femur. Observe the similarities with *Myotragus* (A) in the spacing of growth lines. **C** *Myotragus balearicus* (IPS 44923c), subadult tibia, annuli (bars) interrupting FLC and LPO bone; **D** crocodile gen. et sp. indet. (IPS 4930-h, Eocene, Spain), proximal femur with alternating lamellar annuli and fibrolamellar zones; **E** *Myotragus balearicus* (IPS -e), complete tibia with alternating LAGs, lamellar annuli and fibrolamellar zones (elongated vascular channels, red). Note the resemblances with crocodile (D); **F** *Myotragus balearicus* (IPS 26158-1), very tiny humerus of approx. 4 cm length, FLC bone, one LAG (yellow arrow), two generations of endosteal bone (white arrows); **G** Cervid gen. et sp. indet. (IPS 3811-f, Pleistocene, Spain), adult distal tibia with completely fused epiphysis. Densely vascularized FLC tissue with alternating formation of radial, concentric and irregular oriented channels, one isolated LAG (arrow). Compare with the almost non-vascular zonal bone of *Myotragus* (E); **H** *Gazella borbonica* (IPS 26760-c, Pliocene, Spain), adult proximal femur with uninterrupted FLC bone. Compare with the zonal bone of adult *Myotragus* (E); **I** *Gazella borbonica* (IPS 26780, Pliocene, Spain) juvenile distal femur without epiphysis. Loosely formed azonal tissue of FLC type with rounded osteocytes. Compare with the more compact and organized bone with flattened osteocytes and advanced remodeling of juvenile *Myotragus* (F). Periosteal surface in all micrographs bottom left (left in A, B); **A, B** transmitted light, **C-I** polarized light with 1λ filter, **F** polarized light. **A, B** composed of various micrographs. Scale bars: **A, D-I** 200μ, **B** 1000μ, **C** 100μ.
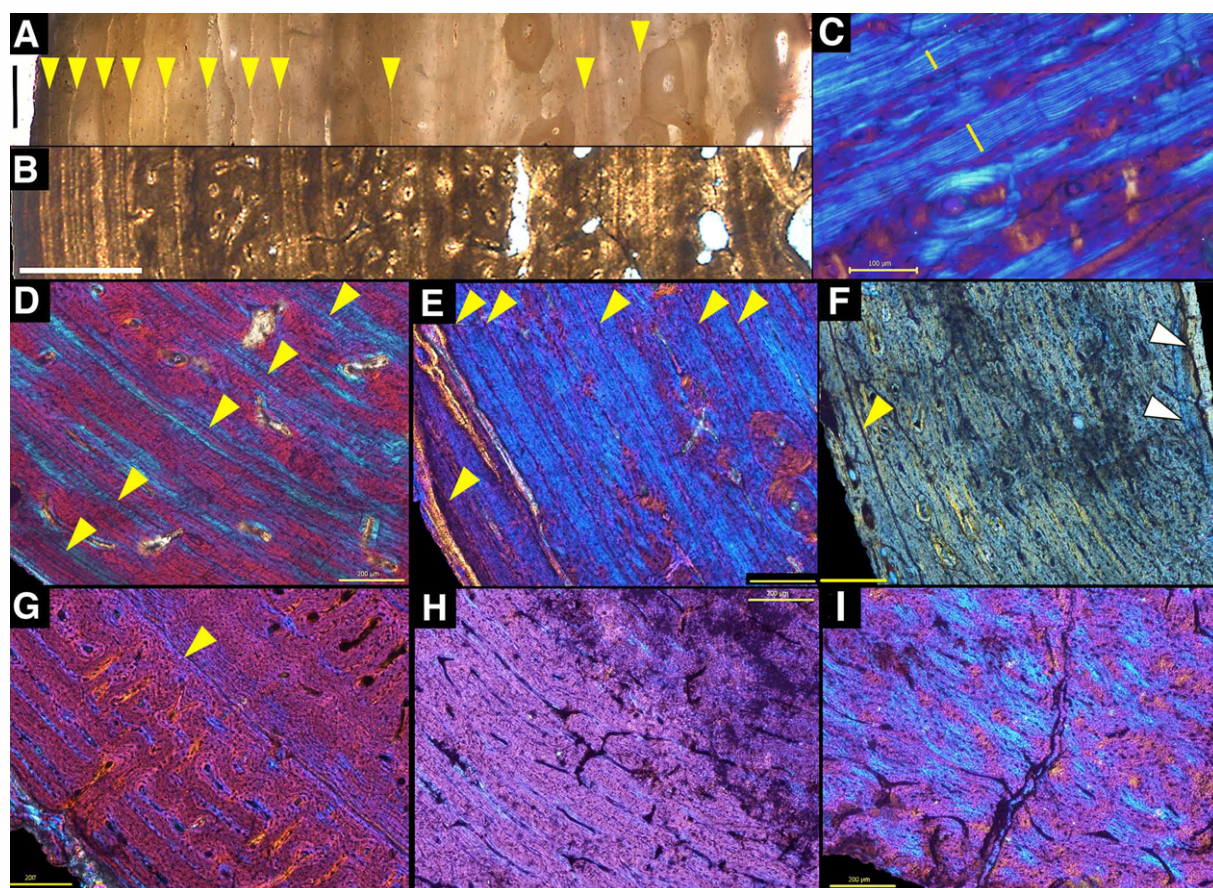
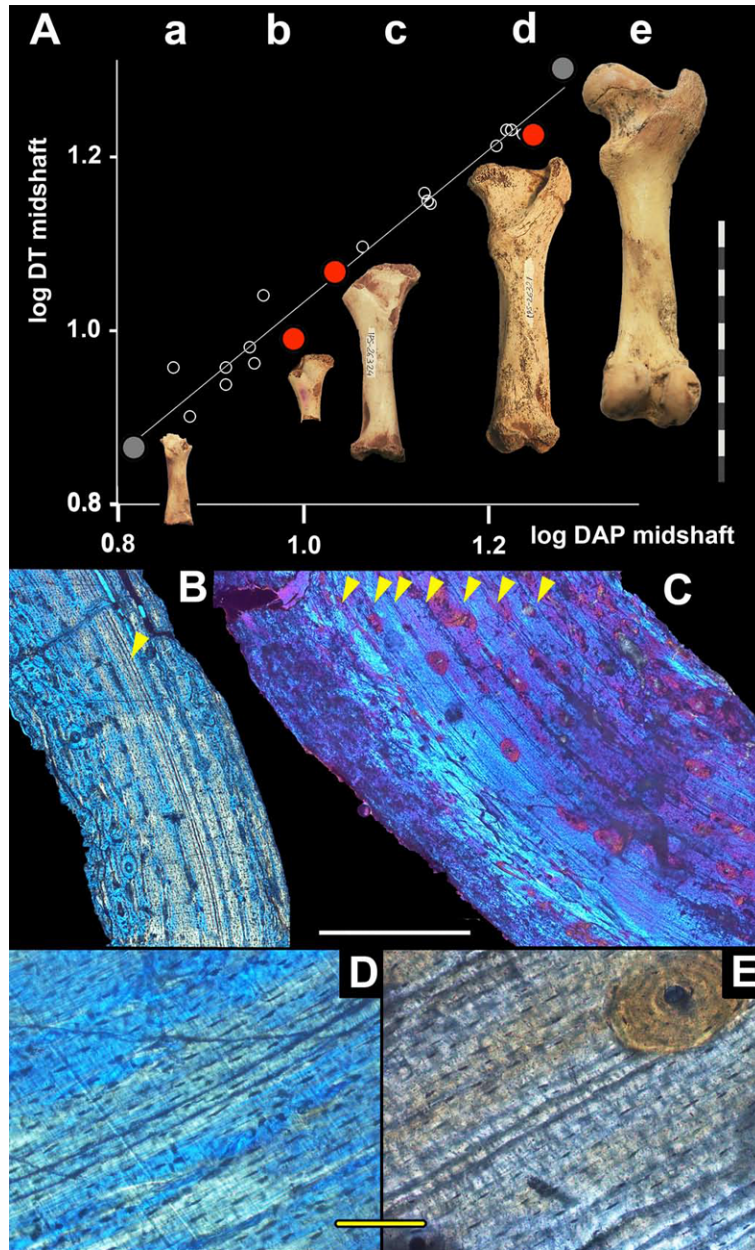**Figure 2: Growth series for three aged femora and micrographs of their sections**. **A** Growth series of femora from the smallest juvenile (a, grey dot; mbcn7160) to a large adult individual (e, grey dot; mbcn7260) (logarithmic regression of anterior-posterior diameter (DAP) against transversal diameter (DT) at midshaft). Sectioned specimens (red dots) provided an age of two (b; IPS 26444e), three (c; IPS 26324), and eight (d; IPS 26321) years. Notice the surprisingly small size at two and three years. Scale bar 10 cm. **B** Section through cortical wall of IPS 26444e (femur Ab) with a double LAG (arrow head) in the central cortical wall, embedded in LNV annuli. Bone tissue is of FLC type close to the medullary cavity, and of LPO type before and after the annuli. **C** Section through cortical wall of IPS 26321 (femur Ad) with seven LAGs (arrow heads). Their

regular distances and their presence only on the central cortex suggest that there might have been more LAGs that have been deleted by microbial attack (dark clouds) and remodeling; Haversian systems (red) scattered throughout inner cortex, erosion and endosteal bone along medullary cavity. **D** Higher magnification of IPS 26444-e-1 (femur Ab) showing the double LAG embedded in FNV tissue with flattened osteocytes. **E** Higher magnification of IPS 26321 (femur Ad) showing multiple LAGs embedded in FNV tissue with flattened osteocytes. Scale bar for **B, C** 500μ, for **D, E** 100μ. **C** composed of multiple micrographs.

Table 1: Main histological traits of crocodiles, large mammals and *Myotragus*. Bone microstructure of *Myotragus* is essentially similar to that of crocodiles in tissue pattern, periodicity of bone formation, transition to slower bone formation at skeletal / sexual maturity, and in degree and pattern of vascularization. It resembles, however, other large mammals in pattern and rate of remodeling. LNV: lamellar non-vascular bone; LSV: lamellar bone with simple vascular canals; LPO: lamellar bone with primary osteons; FLC: fibrolamellar complex (18, 19). Trait description for crocodiles and large mammals modified after (21, 23, 27).

A a b c d e

	crocodiles	*Myotragus*	large mammals
inner and central cortex *primary bone*	zonal, LNV, LSV and FLC	zonal, LNV, LPO and FLC	azonal, FLC through LPO
annuli	present	present	absent
resting lines (LAGs)	cyclically throughout cortex	cyclically throughout cortex	rare, near periosteum
outer cortical pattern in adults	increasingly closer spacing of LAGs	increasingly closer spacing of LAGs	outer cortical layer (OCL)
vascularization	sparce to avascular	sparce to avascular	densely vascularized
orientation of vascular channels	mostly longitudinal	longitudinal and concentric	irregular, variable, increasingly organized with age
remodeling	little, in females extensive during egg-shell formation	extensive	extensive
erosion and endosteal bone	rare	from an early age onwards	at subadult age
Haversian systems	rare	frequent	extensive