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The influence of plane of section on the identification of bone tissue types in amniotes with implications for paleophysiological inferences

Running title : Assessment of bone tissue types

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Research Highlights : We show that the presence of canaliculi running radially from large irregular osteocyte lacunae, and general isotropy at tissue scale, but local anisotropy at cellular scale, offer reliable characters for diagnosing woven bone in amniotes.

Abstract

The proportion of woven bone (WB) to parallel-fibered bone has been extensively used to infer bone growth rates and resting metabolic rates of extinct organisms. The aim of this study is to test in a variety of amniotes how reliably WB content can be measured using transverse sections. For this we analyzed femoral transverse mid-diaphyseal thin sections of 14 extant and extinct taxa and the corresponding longitudinal sections at a slightly metaphyseal level for comparative purposes. We used the following characters to identify WB in transverse sections because they are known to be distinct from those observed in parallel-fibered bone: an isotropic bone matrix at tissue scale; an anisotropic microlamellar arrangement in former osteoblast secretory territories at cellular scale; no alignment between osteocytes; and canaliculi running radially from large irregular osteocyte lacunae. Our null hypothesis predicts no differences between the amount of WB quantified in the transverse and the longitudinal sections of a given long bone. Qualitatively, when a stripe or a patch of WB was identified in a transverse section, the corresponding stripe or patch of WB was always found at the same location in the corresponding longitudinal section. Quantitatively, a Wilcoxon signed-rank non-parametric paired test did not detect a significant difference in the WB content of the two section planes. Thus the null hypothesis is not rejected. Considering that paleohistology is a destructive method, we recommend a workflow to efficiently establishing the proportion of WB: quantifying it in transverse sections; preparing and analyzing longitudinal sections only in cases where an ambiguity remains; reanalyzing the corresponding transverse sections.

Keywords: anisotropy, bone histology, isotropy, parallel-fibered bone, woven bone

39 Introduction

40 In vertebrate paleontological studies, bones constitute the large majority of fossilized remains.
 41 The histological analysis of skeletal elements allows to infer bone growth rates (Botha-Brink
 42 & Smith, 2011; Cubo, Le Roy, Martinez-Maza, & Montes, 2012; Padian, De Ricqlès, &
 43 Horner, 2001; Padian, Horner, & De Ricqlès, 2004), lifestyle (Houssaye, 2013; Houssaye et
 44 al., 2013; Laurin, Canoville, & Germain, 2011), biomechanical constraints (Cubo, Woodward,
 45 Wolff, & Horner, 2015) or metabolic rates (Cubo & Jalil, 2019; Fleischle, Wintrich, &
 46 Sander, 2018; Legendre, Guénard, Botha-Brink, & Cubo, 2016; Olivier, Houssaye, Jalil, &
 47 Cubo, 2017). Traditionally, bone paleohistology was based on the analysis of transverse
 48 sections (*e.g.*, all the references cited above). Paleohistologists defined two characters using
 49 this material. The first one concerns bone matrix organization and has three states: presence of
 50 woven-fibered bone matrix (WB), presence of parallel-fibered bone matrix (PFB) and
 51 presence of lamellar bone matrix (LB) (Francillon-Vieillot et al., 1990). The second one is
 52 based on the organization of bone as a tissue and has two states: presence of lamellar-zonal
 53 bone (LZB) and presence of fibro-lamellar bone (FLB) (Francillon-Vieillot et al., 1990).
 54 Prondvai, Stein, de Ricqlès, & Cubo (2014) proposed a development-based nomenclature in
 55 which LB is a particular type of PFB and FLB is a particular case of woven-parallel complex.
 56 According to Francillon-Vieillot et al. (1990), two osteohistological features can be used to
 57 identify WB and PFB: (1) collagen fiber orientation (inferred from the presence or absence of
 58 birefringence under cross-polarized light) and (2) morphology and spatial distribution of
 59 osteocyte lacunae. Under cross-polarized light, a highly organized matrix with fibers running
 60 parallel (oriented in the same direction; PFB) is anisotropic and appears bright (birefringent)
 61 when fibers are perpendicular to the axis of light propagation and it appears dark when fibers
 62 are aligned along the axis of light propagation (Bromage et al., 2003). Thus, in transverse thin
 63 sections, collagen fibers parallel to the main axis of a long bone appear dark. At low

magnification (at tissue scale), the poorly organized matrix of woven bone shows intermediate brightness values (Bromage et al., 2003), or “general isotropy” (Francillon-Vieillot et al., 1990) from all cutting directions. However, at high magnification (on cellular scale), considering that WB collagen fibers are parallel in former osteoblast secretory territories (“microlamellar arrangement”), they are anisotropic and they appear bright where they are perpendicular to the axis of light propagation (Kerschnitzki et al., 2011). The other osteohistological feature, the morphology and spatial distribution of osteocyte lacunae, reflects (i.e., is correlated to) the extracellular matrix organization (Kerschnitzki et al., 2011). In PFB the longitudinal axis of osteocyte lacunae is parallel to collagen fiber direction whereas canaliculi are perpendicular to it (Kerschnitzki et al., 2011). In contrast, in WB there is no alignment between osteocytes and canaliculi run radially from osteocyte lacunae (Kerschnitzki et al., 2011).

Stein & Prondvai (2014) drew attention to the fact (known previously but neglected before 2014) that WB, and PFB with fibers parallel to the main axis of the long bone, both appear dark in transverse thin sections. Considering that the proportion of WB to PFB has been extensively used to infer bone growth rates and resting metabolic rates of extinct organisms, mismeasure of WB content in transverse sections may have important paleophysiological consequences. The aim of this study is to test in a variety of amniotes the null hypothesis that WB content is not significantly different in the transverse and the longitudinal section planes.

Material and Methods

We analyzed femoral mid-diaphyseal transverse and slightly metaphyseal longitudinal thin sections of 14 extant and extinct taxa including five synapsids, three lepidosaurs, two testudines and four archosauromorphs. The transverse sections of most of them were prepared and used by Montes et al. (2007): *Chelodina oblonga* (Gray, 1841), *Crocodylus niloticus*

(Laurenti, 1768), *Gallus gallus* (Linnaeus, 1758), *Pelodiscus sinensis* (Wiegmann, 1835), *Varanus exanthematicus* (Bosc, 1792), *Varanus niloticus* (Linnaeus, 1766) and *Zootoca vivipara* (Von Jacquin, 1787). We prepared the corresponding longitudinal thin sections using the remaining slightly metaphyseal bone samples that were conserved embedded in epoxy resin. Vivian de Buffrénil kindly shared with us the femur of a growing *Caiman crocodilus* (Linnaeus, 1758), that was embedded in epoxy resin and cut both transversally and longitudinally. The transverse sections of four (of the five) synapsids were prepared and used by Olivier et al. (2017): *Capreolus capreolus* (Linnaeus, 1758), *Lepus europaeus* (Pallas, 1778), *Lystrosaurus* sp., and *Oudenodon bainii* Owen 1860. We used the remaining slightly metaphyseal bone samples (conserved embedded in epoxy resin) to perform the corresponding longitudinal thin sections. Transverse and longitudinal sections of the fifth synapsid of the sample (*Clepsydropus collettii* Cope 1875) and of the archosauromorph *Azendohsaurus laaroussii* Dutuit 1972 were prepared and used respectively by Laurin & Buffrénil (2016) and by Cubo & Jalil (2019). For further information on the ontogenetic stage of the specimens, see Supplementary file 1. It is expressed as % of adult body mass in extant species and inferred from bone histology in extinct ones. To check whether WB can be diagnosed in a reliable way we used a sample including different ontogenetic stages, different thermometabolic regimes (endotherms and ectotherms), and extant and extinct species of diverse amniote clades.

Transverse sections were available because they have been used in previous studies (see above). The corresponding longitudinal thin sections, $100 \pm 10\mu\text{m}$ thick, were obtained from slightly metaphyseal samples of the same specimens using the protocol described by Montes et al. (2007). Thin sections were photographed using a Nikon Eclipse E600POL microscope under linearly polarized light and cross-polarized light with lambda wave plate. Thin sections were deposited at the vertebrate hard tissues histological collection of the French National

Museum of Natural History (MNHN), where they are available upon request to the curator (accession numbers of the thin sections analyzed in this study are given in Supplementary file 1). Bone histology nomenclature follows Francillon-Vieillot et al. (1990) with addenda from Prondvai et al. (2014).

For each taxon included in this study transverse sections were analyzed first. In transverse sections, bone matrix appearing yellow/orange or blue under cross-polarized light with lambda wave plate, or bright without lambda wave plate, is composed of PFB with fibers perpendicular to the main axis of the long bone. In these transverse sections, at low magnifications (at the tissue level), bone matrix appearing red under cross-polarized light with lambda wave plate, or black without lambda wave plate, is composed of either WB or PFB with fibers parallel to the main axis of the long bone. Please note that at high magnifications (at the cellular level), woven bone is anisotropic because of the presence of microlamellar arrangement in former osteoblast secretory territories (fibers appear yellow/orange or blue where they are perpendicular to the axis of light propagation; white arrow in Figure 1). We encircled WB areas in transverse sections and measured these areas using ImageJ when we observed: (a) a bone matrix showing general isotropy at low magnification (at tissue scale); (b) an anisotropic microlamellar arrangement at high magnification (on cellular scale); (c) no alignment between osteocytes, and (d) canaliculi running radially from large irregular osteocyte lacunae. Longitudinal sections were analyzed likewise in a second time. Please note that the channels herein referred to as vascular cavities are known to not be exclusively filled with corresponding veins and arteries but accommodate a few more tissues as well such as nerves and lymphatic vessels (Starck & Chinsamy, 2002).

We tested the null hypothesis suggesting that the proportion of WB (expressed in percentage: WB area / Total matrix area) is not significantly different in transverse and in longitudinal sections. For this first we performed Shapiro-Wilk normality tests for the proportion of WB in

transverse and in longitudinal sections in R (R Development Core Team, 2008). Second, we tested the null hypothesis using a Wilcoxon signed-rank non-parametric paired test in R (R Development Core Team, 2008) because the proportions of WB in transverse and in longitudinal sections are not normally distributed.

Results

We analyzed qualitatively and quantitatively femoral transverse and longitudinal bone thin sections of the fourteen taxa of the sample in order to test whether the quantified amount of WB is the same in both section planes. First we provide detailed qualitative histological descriptions of six species of the sample: two extant endotherms (*Capreolus capreolus* and *Gallus gallus*, Figure 2), two extant ectotherms (*Crocodylus niloticus* and *Zootoca vivipara*, Figure 3) and two extinct taxa (*Lystrosaurus* sp. and *Oudenodon bainii*, Figure 4). The remaining taxa are briefly discussed later on in this section and are shown in supplementary file 2.

The femoral transverse section of *Capreolus capreolus* (Figure 2A, B) is composed of laminar bone. It contains vascular cavities circumferentially parallel to the periosteum with rare radial anastomoses (Figure 2B, black arrow). Lining these cavities there are thin layers of anisotropic LB (Figure 2B, white arrowhead) containing fibers running perpendicular to the axis of light propagation (appearing bright blue or yellow under cross-polarized light with lambda wave plate), fibers running parallel to the axis of light propagation (appearing as thin black lines), and flattened osteocyte lacunae with the main axis oriented parallel to the periosteal margin. Between two consecutive vascular cavities surrounded by LB we observe large areas appearing red under cross-polarized light with lambda wave plate (black arrowhead in Figure 2B). In these areas collagen fibers can be either woven or longitudinally organized parallel to the long axis of the bone (and so cut transversally). The small size of the

osteocyte lacunae of these areas (Figure 2B, black arrowhead) suggests that it is composed of PFB cut transversally. Finally, under cross-polarized light with lambda wave plate, we always observe bluish stripes (Figure 2B, white arrow) equidistant from two consecutive vascular cavities. The large size and irregular shape of osteocyte lacunae of these areas suggest that it is composed of WB (Figure 2B, white arrow). The bluish aspect of these stripes (Figures 1 and 2B, white arrows) may be linked to the microlamellar arrangement of collagen fibers in WB at cellular scale (Kerschnitzki et al. 2011). The analysis of the corresponding longitudinal section (Figure 2C, D) is congruent with this interpretation because the entire section but the WB area (Figure 2D, white arrow) is strongly anisotropic. Note however that the WB area (Figure 2D, white arrow) is not entirely red but rather dark purple under cross-polarized light with lambda wave plate because of the microlamellar arrangement. The LB (Figure 2D, white arrowhead) is composed of alternating anisotropic layers of fibers oriented either parallel to the main axis of the bone (appearing blue because they are perpendicular to the axis of light propagation), or perpendicular to the main axis of the bone and parallel to the axis of light propagation (appearing as black lines). The PFB is anisotropic and appears bright blue under cross-polarized light with lambda wave plate in longitudinal section (Figure 2D, black arrowhead) because collagen fibers run parallel to the main axis of the bone (and so are perpendicular to the axis of light propagation). It contains spindle shaped osteocyte lacunae (Figure 2D, black arrowhead). In summary, there is a scaffold of WB trabeculae containing large primary osteons formed by a peripheral thick layer of PFB and a inner thin layer of LB around the vascular cavities (Figure 2 A-D).

The femoral transverse section of *Gallus gallus* is characterized by a radial pattern of vascular cavities (Figure 2E, F). These ones are lined by anisotropic PFB (Figure 2F, black arrowhead). Between these layers of PFB, we can observe areas of isotropic bone matrix (Figure 2F, white arrow). The large size and irregular shape of osteocyte lacunae of these

isotropic areas suggests that it is composed of woven bone. The analysis of the corresponding longitudinal section (Figure 2G, H) is congruent with this interpretation. We can observe areas of WB (Figure 2H, white arrow) and areas of PFB (Figure 2H, black arrowhead).

The femoral transverse section of *Crocodylus niloticus* is organized into three regions (white bar in Figure 3B). It contains longitudinal vascular canals without anastomoses (Figure 3A, B). Some of them are surrounded by an anisotropic tissue (blue and yellow under cross-polarized light with lambda wave plate in Figure 3B, white arrow) indicating the presence of PFB forming primary osteons. Others are simple vascular canals (Figure 3B, black arrow) and are preferentially located in the outer half of the cortex. The inner region is formed by a layer of endosteal bone around the medullary cavity (Figure 3B, D white arrow-heads). This endosteal layer is anisotropic in the longitudinal section (Figure 3D, white arrow-head), whereas it appears blue and red in the transverse section (Figure 3B, white arrow-head), indicating that it is probably composed of spiraling PFB at low angle to the bone's long axis.

In the central region (in between the endosteal bone and the outer half of the cortex), in between primary osteons, the bone matrix is isotropic. It contains irregular large osteocyte lacunae (Figure 3B, black arrow-heads) suggesting that this matrix is composed of WB. The bone matrix of the outer half of the cortex appears blue at some locations, indicating that it is composed of PFB, and red at other locations, suggesting that it is formed by either WB or by PFB with fibers oriented parallel to the main axis of the bone. The small size of the osteocyte lacunae suggests that the second interpretation is correct. The longitudinal section was prepared from a slightly different (more metaphyseal) region and it contains only the endosteal layer and an outer region corresponding to the outer half of the cortex observed in the transverse section (bar in Figure 3D). The layer of endosteal bone is anisotropic (see above), suggesting that it is formed by PFB. The bulk of the cortex contains simple vascular canals (Figure 3D, black arrow) that appear elongated because they are parallel to the main

212 axis of the long bone (they appear rounded in the transverse section; Figure 3B, black arrow).
 213 The bone matrix of this region is anisotropic, suggesting that it is mainly composed of
 214 longitudinal PFB (Figure 3D). Note, however, that it may contain a few transversally oriented
 215 collagen fibers relative to the main axis of the long bone because bone matrix appears partly
 216 blue in the transverse section; Figure 3B).

217 The femoral transverse section of *Zootoca vivipara* (Figure 3E, F) is completely avascular. It
 218 contains an endosteal layer of PFB (Figure 3F, white arrow-head) and an outer periosteal
 219 region that is also composed of PFB according to overall birefringence under cross-polarized
 220 light with lambda wave plate. The longitudinal section also shows birefringence (Figure 3H).
 221 These observations suggest that the PFB forming the periosteal bone cortex probably contains
 222 spiraling fibers at about 45° to the bone's long axis.

223 The femoral transverse section of *Lystrosaurus* sp. is characterized by a pattern of
 224 longitudinal vascular cavities (Figure 4A, B). These ones are lined by anisotropic PFB
 225 forming primary osteons (Figure 4B, white arrowheads). These primary osteons are separated
 226 by areas of isotropic bone matrix (Figure 4B, white arrows). The large size and irregular
 227 shape of osteocyte lacunae in these isotropic areas suggests that it is composed of WB. The
 228 analysis of the longitudinal section shows that the vascular canals have an elongated aspect
 229 aligned to the main axis of the bone (Figure 4C, D). These cavities are lined by PFB forming
 230 primary osteons (Figure 4D, white arrowheads). In between these primary osteons we can see
 231 isotropic areas composed of WB (Figure 4D, white arrows).

232 Transverse and longitudinal femoral sections of *Oudenodon bainii* (Figure 4E-H) show a
 233 pattern similar to that described for *Capreolus capreolus* : there is a scaffold of trabeculae
 234 containing WB (Figure 4F, H, white arrows) and, in-between two layers, there are primary
 235 osteons (vascular cavities lined by LB; Figure 4F, H, white arrowheads). Please note that in

the transverse section of *Oudenodon* vascular cavities are not as regularly circumferentially parallel to the periosteum as in *Capreolus*.

The analysis of the six taxa described above, and of the remaining taxa (shown in supplementary file 2: *Azendohsaurus laaroussii*, *Caiman crocodilus*, *Chelodina oblonga*, *Clepsydropus collettii*, *Lepus europaeus*, *Pelodiscus sinensis*, *Varanus exanthematicus* and *Varanus niloticus*) reveals no incongruences between what is observed in the transverse sections and what is observed in the longitudinal ones. When the presence of a) general isotropy, b) a microlamellar arrangement in former osteoblast secretory territories, c) no alignment between osteocytes, and d) canaliculi running radially from large irregular osteocyte lacunae, suggested the presence of a patch or a stripe of WB in a transverse section, the analysis of the corresponding longitudinal section confirmed the presence of such stripe or patch of WB at the same relative location. Please find in supplementary file 2 pictures of all transverse and longitudinal sections analyzed in this study where WB areas, PFB areas and vascular canals were encircled.

Quantifications of proportion of WB in transverse and longitudinal sections are given in Table 1. Shapiro-Wilk normality tests showed that the proportion of WB was not normally distributed neither in transverse (p-value = 0.02398) nor in longitudinal (p-value = 0.04465) sections. A Wilcoxon signed-rank non-parametric paired test did not reject the null hypothesis suggesting that the proportion of WB (expressed in percentage: WB area / Total matrix area) is not significantly different in transverse and in longitudinal sections (p-value = 0.9056). In other words, the Wilcoxon signed-rank non-parametric paired test did not detect a significant difference in the WB content of the two section planes.

Discussion

Stein & Prondvai (2014) analyzed the bone histology of sauropod dinosaurs using transverse and longitudinal sections and concluded that the amount of woven bone has been overestimated in previous studies that used only transverse sections. They suggested that this is because, under cross-polarized light, WB and PFB with fibers parallel to the main axis of the long bone, both appear isotropic (dark) in transverse thin sections. Considering that the proportion of WB to PFB has been widely used to perform paleobiological inferences of bone growth rates and resting metabolic rates (see below), the bias put in evidence by Stein & Prondvai (2014) in sauropod dinosaurs, if proven by comparative data in other taxa, may have important paleophysiological consequences. First, we elaborate on why quantifying the proportion of WB is relevant in paleobiology by analyzing the relationships between this feature and bone growth rates and resting metabolic rates. Afterwards we test the null hypothesis that WB content is not significantly different in the transverse and the longitudinal section planes. Finally, we propose recommendations and a workflow based on our findings.

WB is formed by the process of static osteogenesis: mesenchymal cells differentiate into stationary osteoblasts oriented randomly, which differentiate into osteocytes *in situ* (Cubo, Hui, Clarac, & Quilhac, 2017; Ferretti, Palumbo, Contri, & Marotti, 2002; Marotti, 2010; Palumbo, Ferretti, & Marotti, 2004). PFB is formed by the process of dynamic osteogenesis: mesenchymal cells differentiate into osteoblasts that are all oriented in the same direction and move back as they secrete osteoid, forming a migrating lamina of osteoblasts (Cubo et al., 2017; Ferretti et al., 2002; Marotti, 2010; Palumbo et al., 2004). We will consider the relationships between WB and bone growth rate at three levels: tissue, cellular and ultrastructural (see Stein & Prondvai, 2014, for a detailed review). At the tissue level, during the initial growth stages, a number of amniotes form an initial scaffold made of WB that includes large cavities and produces a rapid volume expansion of the cortex (*e.g.* Castanet, Curry, Cubo, & Boisard, 2000, in the long bones of ratites and Margerie et al., 2004, in the

284 long bones of the king penguin). In other amniotes, the initial expansion of the cortex by
 285 appositional growth is made of PFB including, if any, smaller cavities, and producing a slow
 286 volume expansion of the cortex (*e.g.*, Castanet, 1978, in squamates). As a consequence, for
 287 the same quantity of osteoid produced, static osteogenesis and WB are associated to a higher
 288 rate of bone accretion (at the tissue level) than dynamic osteogenesis and PFB. At the cellular
 289 level, considering that in static osteogenesis stationary osteoblasts are oriented randomly, the
 290 WB matrix formed is highly porous (Stein & Prondvai, 2014). In contrast, in dynamic
 291 osteogenesis, osteoblasts are all oriented in the same direction and produce PFB, a much more
 292 compact matrix (Stein & Prondvai, 2014). Again, for the same quantity of osteoid produced,
 293 static osteogenesis will produce a higher rate of volume expansion than dynamic osteogenesis.
 294 At the ultrastructural level, the absolute growth rates at which WB and PFB are formed
 295 depend on the osteoid secretion rate of, respectively, static osteogenesis osteoblasts and
 296 dynamic osteogenesis osteoblasts (Stein & Prondvai, 2014). Considering that no
 297 ultrastructural differences have been observed between static and dynamic osteoblasts
 298 (Marotti, 2010), we expect to find similar osteoid secretion rates (Stein & Prondvai, 2014).
 299 The formation of a scaffold of WB incorporating large cavities at high rates and the
 300 perimedullary resorption of bone tissue by osteoclasts at high rates are both energy consuming
 301 (Montes et al., 2007). Thus, these mechanisms are only possible in taxa characterized by high
 302 resting metabolic rates (Fleischle et al., 2018; Legendre et al., 2016; Olivier et al., 2017).
 303 According to Padian & Horner (2002) “the types of tissue deposited in the bones of extinct
 304 animals are the most direct evidence of basal metabolic rates, because they directly reflect
 305 growth rates [...]. The sustained deposition of fast-growing bone tissues, as displayed by
 306 mammals, birds and other dinosaurs, must reflect sustained high basal metabolic rates.” This
 307 hypothesis was supported by the results obtained by Montes et al. (2007): the variation of
 308 bone growth rates significantly explains the variation of resting metabolic rates in a sample of

extant amniotes. In conclusion, precise quantifications of the proportion of WB is a key factor for performing reliable paleobiological inferences of both bone growth rates and resting metabolic rates.

Is WB content significantly different in the transverse and the longitudinal section planes?

Qualitatively, when a stripe or a patch of WB was identified in a transverse section, the corresponding stripe or patch of WB was always found at the same location in the corresponding longitudinal section (please find in supplementary file 2 pictures of all the analyzed thin sections where WB areas, PFB areas and vascular canals were encircled).

Quantitatively, the proportion of WB is not significantly different in transverse and in longitudinal sections (i.e., the Wilcoxon signed-rank non-parametric paired test did not reject the null hypothesis suggesting that the proportion of WB is not significantly different in transverse and in longitudinal sections).

Considering that traditional bone histology is a destructive method, and that performing longitudinal sections (in addition to the transverse sections) involves the destruction of supplementary volume of material, we recommend a three steps workflow to efficiently establishing the proportion of WB in the diaphysis of an appendicular bone: (a) a careful analysis of transverse sections to propose hypotheses of the presence of WB when we observe the presence of canaliculi running radially from large irregular osteocyte lacunae, and general isotropy at tissue scale, but local anisotropy at cellular scale; (b) performing and analyzing longitudinal sections only in cases where an ambiguity remains; and (c) reanalyzing the corresponding transverse sections.

Author contributions

JC conceived and designed the study, MFB wrote a first draft of the manuscript and performed quantifications and statistical tests. All three authors analyzed thin sections and wrote the final version.

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Figure legends

Figure 1. Transverse thin sections of the femur of *Capreolus capreolus* observed in cross polarized light with lambda wave plate. White arrow: woven bone showing microlamellar arrangement in former osteoblast secretory territories (fibers are anisotropic and appear blue where they are perpendicular to the axis of light propagation); Black arrow: vascular cavity. White arrowhead: lamellar bone lining vascular cavities in primary oteons; Black arrowhead: parallel-fibered bone with fibers oriented parallel to the axis of light propagation in primary osteons. Periosteum is on the top. Scale bars equal 100 μm .

442

443

444 Figure 2. Transverse (A, B, E, F) and longitudinal (C, D, G, H) thin sections of the femora of
 445 *Capreolus capreolus* (A-D) and *Gallus gallus* (E-H) in linearly polarized light (A, C, E, G)
 446 and cross polarized light with lambda wave plate (B, D, F, H). White arrows: woven bone;
 447 black arrow: radial anastomosis between circumferential canals; white arrowheads: lamellar
 448 bone; black arrowheads: parallel-fibered bone. Periosteum is on the left for A to F and top left
 449 corner for G and H. Scale bars equal 100 μ m.

450

451 Figure 3. Transverse (A, B, E, F) and longitudinal (C, D, G, H) thin sections of the femora of
 452 *Crocodylus niloticus* (A-D) and *Zootoca vivipara* (E-H) in linearly polarized light (A, C, E,
 453 G) and cross polarized light with lambda wave plate (B, D, F, H). White arrow: primary
 454 osteon; black arrows: simple vascular canals; white arrowheads: endosteal bone; black
 455 arrowheads: woven bone. Periosteum is on the right. Scale bars equal 100 μ m.

456

457 Figure 4. Transverse (A, B, E, F) and longitudinal (C, D, G, H) thin femoral sections of
 458 *Lystrosaurus* sp. (A-D) and *Oudenodon bainii* (E-H) in linearly polarized light (A, C, E, G)
 459 and cross polarized light with lambda wave plate (B, D, F, H). White arrow: primary osteon;
 460 black arrows: simple vascular canals; white arrowheads: endosteal bone; black arrowheads:
 461 woven bone. Periosteum is on the top right corner in A, B, E, F, and on the top in C, D, G, H.
 462 Scale bars equal 100 μ m.