Preliminary assessment of bone histology in the extinct elephant bird Aepyornis (Aves, Palaeognathae) from Madagascar

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General Palaeontology, Systematics and Evolution (Palaeohistology)

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Examen préliminaire de l'histologie osseuse de l'oiseau-éléphant éteint Aepyornis *(Aves, Palaeognathae) de Madagascar*

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ABSTRACT

Aepyornis, a giant subfossil ratite from Madagascar, shows a well-preserved bone histology. Hindlimb bones exhibit an extensive histodiversity; the cortex is initially made of fibrolamellar, well-vascularized primary bone that modulates locally into plexiform or laminar patterns. Lines of arrested growth are generally weakly expressed. Haversian reconstruction can be complete. Perimedullar endosteal deposition is variable but can be extensive. The complex causality (phylogenetic, systematic, ontogenetic and functional... factors) involved in the production of the observed data is discussed.

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RÉSUMÉ

Aepyornis, ratite géant subfossile de Madagascar, montre une histologie osseuse bien préservée. Les os longs des pattes présentent une forte diversité histologique ; l'os primaire des corticales est initialement du type général fibrolamellaire, fortement vascularisé selon des patrons plexiformes ou laminaires. Les lignes d'arrêt de croissance sont généralement peu exprimées. Le remaniement haversien peut être complet. Le dépôt endostéal périmédullaire est variable, mais peut être très important. La causalité complexe (facteurs phylogénétiques, systématiques, ontogénétiques, fonctionnels...) pouvant rendre compte des structures observées est abordée.

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1. Introduction

The Ratitae, generally recognized as a clade of flightless palaeognathous birds with a mostly Gondwanian distribution, have been the subject of intensive inquiries regarding their phylogenetic status in connection with issues in biogeography (e.g. Bourdon et al., 2009a; Buffetaut and Angst, 2014; Cracraft, 2001). Numerous phylogenetic hypotheses of ratite birds have been recently published and the current situation is not consensual, with several alternative hypotheses at hand (Baker and Pereira, 2009; Bourdon et al., 2009a; Haddrath and Baker, 2012; Harshman et al., 2008; Johnston, 2011; Livezey and Zusi, 2007; Mitchell et al., 2014; Phillips et al., 2010; Smith et al., 2013; Worthy and Scofield, 2012), some studies even rejecting ratite monophyly (Harshman et al., 2008; Johnston, 2011; Mitchell et al., 2014; Phillips et al., 2010; Smith et al., 2013).

Nevertheless, this group has been recently used as a test case to check the possible phylogenetic relevance of a new set of phenotypic characters available in both extant and extinct taxa, namely histological characteristics of the skeleton (Legendre et al., 2014). Indeed, a current discussion is whether bone histology carries a 'phylogenetic signal', i.e. whether the phylogenetic relationships between species of a given sample have a significant influence on the variation of histological characters measured for these species. Moreover, in order to be reliable, this 'signal' must not be blurred by other signals, notably autapomorphic adaptations to specific functions, a situation prone to create deceptive homoplastic character-states (de Ricqlès et al., 2008).

A recent comparative study on ratites using up-to-date statistical assessments has detected a significant phylogenetic signal in their bone histology (Legendre et al., 2014). This study included most available extant and extinct ratite taxa. Most of them, such as the ostrich, moa and kiwi, have already been described histologically (Amprino and Godina, 1947; Bourdon et al., 2009b; Chinsamy, 1995; Enlow and Brown, 1957; Turvey et al., 2005) but some species included in the new material have never been described histologically. Among these are sections from the famous subfossil 'elephant bird' *Aepyornis* from Madagascar. The purpose of the present study is to provide a preliminary qualitative description of *Aepyornis* bone histology, as a complement to the quantitative data computed in Legendre et al. (2014).

2. *Aepyornis*, a brief history

Knowledge of the occurrence of a large terrestrial bird in the Island of Madagascar is probably as old as written history in this geographic area. References to the mythological 'Rokh' in Arabic fairy tales might be related to early encounters of Arabic sailors in the island of Madagascar and its peculiar fauna (Buffetaut, 2013; Decary, 1937). As for many large flightless island birds, interactions with human populations proved lethal for *Aepyornis* in a relatively short amount of time, in combination with climate change (Goodman and Jungers, 2014). This is probably due, in part, to some specialized life history traits that appeared during the very long time-spans when those island ecosystems evolved without any human interference. We will come back (see discussion) on how the present study can be relevant to this issue. The fossil record of *Aepyornis* is restricted to Pleistocene and Holocene deposits on Madagascar (Monnier, 1913; Wiman, 1935). Skeletal remains have been typically found in peat deposits interpreted as remnants of small ponds that served as watering holes (Last, 1894; Monnier, 1913; Wiman, 1935). The last report of elephant birds in Madagascar was provided in the mid-seventeenth century by Admiral Étienne de Flacourt (1658), who had been sent to Madagascar by the French government to restore order among the French settlers (Buffetaut, 2013). This suggests that *Aepyornis* may have survived until the seventeenth century in some remote areas of Madagascar (Buffetaut, 2013). However, this does not provide a definite date for its extinction, which is estimated as about 750 years BP based on radiocarbon dates of eggshell remains and information from archaeological excavations (Goodman and Jungers, 2014).

Most of the early scientific knowledge on *Aepyornis* has been gathered during the nineteenth century thanks to the field discoveries of French settlers and European explorers in Madagascar, who recovered important collections of subfossil bones and eggs. By the mid-nineteenth century, enough material had reached Paris that an anatomical description of *Aepyornis maximus* could be published by Geoffroy Saint-Hilaire (1851). This original description was followed by numerous publications that provided additional information on its anatomy and named several additional *Aepyornis* species, as well as a second aepyornithid genus, *Mullerornis* (e.g. Andrews, 1894, 1896, 1897, 1904; Burckhardt, 1893; Lamberton, 1930, 1934; Lowe, 1930; Milne Edwards and Grandidier, 1894; Monnier, 1913; Wiman, 1935, 1937a, 1937b; Wiman and Edinger, 1941). Aepyornithids have a strong and conical bill adapted for foraging underground tubercles or breaking hard fruits (Goodman and Jungers, 2014; Lamberton, 1930, 1934; Monnier, 1913).

Variability in size, shape and proportions among specimens prompted the creation of several species, although the taxonomic diversity seems to be distinctly lower than among New Zealand Moa (Worthy and Holdaway, 2002). The taxonomy of the elephant birds is extremely confused, however, and there has been much debate as to the actual number of species involved (Goodman and Jungers, 2014; Hume and Walters, 2012). The present consensus is that seven species once occurred on Madagascar (Davis, 2002; Goodman and Jungers, 2014; Lamberton, 1934). *Aepyornis maximus*, the largest known aepyornithid, is the most massive bird ever discovered, and could reach a height of 3 m and a weight close to 400 kg (Amadon, 1947; Monnier,1913). This gigantic bird deposited the largest known eggs, which exceeded 7.0 L in volume (Hawkins and Goodman, 2003). The embryonic skeleton contained in one of these eggs was recently described thanks to the advent of high-resolution X-ray computed tomography (Balanoff and Rowe, 2007). The genus *Aepyornis* comprises three additional, smaller species, namely *Aepyornis medius* Milne Edwards and Grandidier, 1866, *Aepyornis hildebrandti* Burckhardt, 1893 and *Aepyornis gracilis* Monnier, 1913 (Davis, 2002). The four species have

massive leg bones compared to those of ostriches (hence the nickname 'elephant bird') and short pedal phalanges. The genus *Mullerornis* Milne Edwards and Grandidier, 1894 currently includes three species (Davis, 2002), namely *Mullerornis agilis* (height 1.49 m), *Mullerornis betsilei* and *Mullerornis rudis*. *Mullerornis* species are smaller than *Aepyornis* species, with more slender leg bones and elongate, pointed pedal phalanges (Milne Edwards and Grandidier, 1894; Lamberton, 1934). The distinction between species within each genus has been mainly based on size. The significance of this systematic treatment is open to questioning (Hume and Walters, 2012). The example of New Zealand Moa, the taxonomic diversity of which has been reduced by recent DNA studies (e.g., Bunce et al., 2009), suggests that this traditional conception of aepyornithid systematics may be rather remote from biological reality. Differences once ascribed to systematic diversity may also be the expression of other biological variables, including ontogenetic trajectories, skeletal maturity, sexual dimorphism and cycles, epigenetic adaptations to local ecological conditions favouring local ecomorphs, etc. The present paper at least allows raising such issues (see discussion), and further systematic, histological as well as genetic studies will be needed to resolve this conundrum.

3. Material and methods

Four long bones from *Aepyornis maximus* and one from *A. medius* were used for this study. The same material was also used in quantitative studies (Legendre et al., 2014) and a short descriptive summary was published elsewhere (de Ricqlès et al., 2013). All the material comes from the Quaternary of Madagascar, and is housed in the paleontological collections of the MNHN (Museum national d'histoire naturelle, Paris, France). The specimens are as follows: *A. maximus*: MAD 378 (MNHN 1908-5, unknown locality), left femur; MAD 364 (MNHN 1931-6, Ankazoabo locality), femur; MAD 8814 (MNHN 1908-5, unknown locality), right tibiotarsus; MAD 8813 (MNHN 1910-12, Belo locality), tarsometatarsus. *A. medius*: MAD 8826 (MNHN 1906-16, unknown locality), left tibiotarsus.

Mid-bone shafts were processed according to current thin sections techniques (e.g., Lamm, 2013) including embedding in resin, sectioning with a thin diamond powder circular saw and further grinding and polishing. The large size of the cross section prevented mounting on standard size glass slides, therefore the specimen was subdivided in several parts, each mounted on a different slide.

Location and orientation of the thin sections relative to the anatomical features and orientations of the entire bones were accurately reported in order to describe potential histological differences between two orthogonal transects in a given section.

Histological descriptions follow the vocabulary of bone tissues classification as defined in Francillon-Vieillot et al. (1990). We are aware that this vocabulary has been recently criticized on various grounds (Prondvai et al., 2014; Stein and Prondvai, 2014) but for our purposes here the traditional vocabulary has been retained.

4. Histological descriptions

4.1. A. maximus femur MAD 378

This large bone has a diameter of 8 cm at the shaft. The bone cortex is rather thin (maximal thickness: 1.5 cm, locally far less). Extensive perimedullar erosion bays spread into the cortex, which explains its local thinness. There is some amount of medullar bone trabeculae. On a thick section observed with the naked eye, 5 to 6 "cycles" of growth may be deciphered in the cortex (Fig. 1A). Examination of the whole cortex at low magnification (Fig. 2) reveals that it is entirely built by primary periosteal bone tissue. The "cycles" of growth are far less conspicuous at this magnification (actually only one is obvious). The primary bone fits well with the general concept of fibrolamellar bone complex. It is here a very densely vascularized tissue with longitudinal, circular and radial vascular canals. It can be mostly described as plexiform because numerous radial canals occur. However the tissue locally modulates into laminar or longitudinal, depending on the lack of radial, and of radial and circular canals, respectively. As usual, the vascular canals form the lumen of primary osteons, themselves embedded into the "fibrous" component of the complex. The lumen of vascular canals varies locally in diameter, depending on the amount of centripetal lamellar deposition of the primary osteons. Around the marrow cavity and some erosion bays, a thin deposit of endosteal tissue occurs.

Along the caudal transect, the tissue follows the above description, with typical plexiform tissue close to the periphery (Fig. 1B) in the middle of the cortex, where the "fibrous" and "lamellar" components of the complex can be precisely observed (Fig. 1C) and close to the marrow cavity with a thin endosteal deposition (Fig. 1D). Along the medial transect, the primary tissue is generally similar but is locally modified by an extensive amount of Sharpey's fibres that pervade most of the cortex (Fig. 1E). All Sharpey's fibres spread from the periosteal (fibrous) component of the complex, not from the osteonal component. Along the rostral transect, the general structure is the same but there are no Sharpey's fibres and the vascular canals are predominantly circular, giving to the tissue a distinct laminar pattern (Fig. 1F). No Haversian reconstruction is observed, even in the deepest perimedullar regions of the cortex.

4.2. A. maximus femur MAD 364

The bone has a diameter of 8–9 cm at the shaft. The cortex is relatively thin, with a maximal thickness of 1 cm. There is no active perimedullar erosion, hence the contrast between the cortex and the free marrow cavity is obvious. Only some thin endosteal bone trabeculae protrude from place to place in the marrow cavity (Fig. 3A). Histologically, the cortex has been entirely reworked by Haversian reconstruction into superimposed generations of secondary osteons (Fig. 3B, C).

4.3. A. maximus tarsometatarsus MAD 8813

The bone shaft cross section is oval and irregular, about 3 × 6 cm. The cortex is relatively thick, from 1 to 2 cm. There
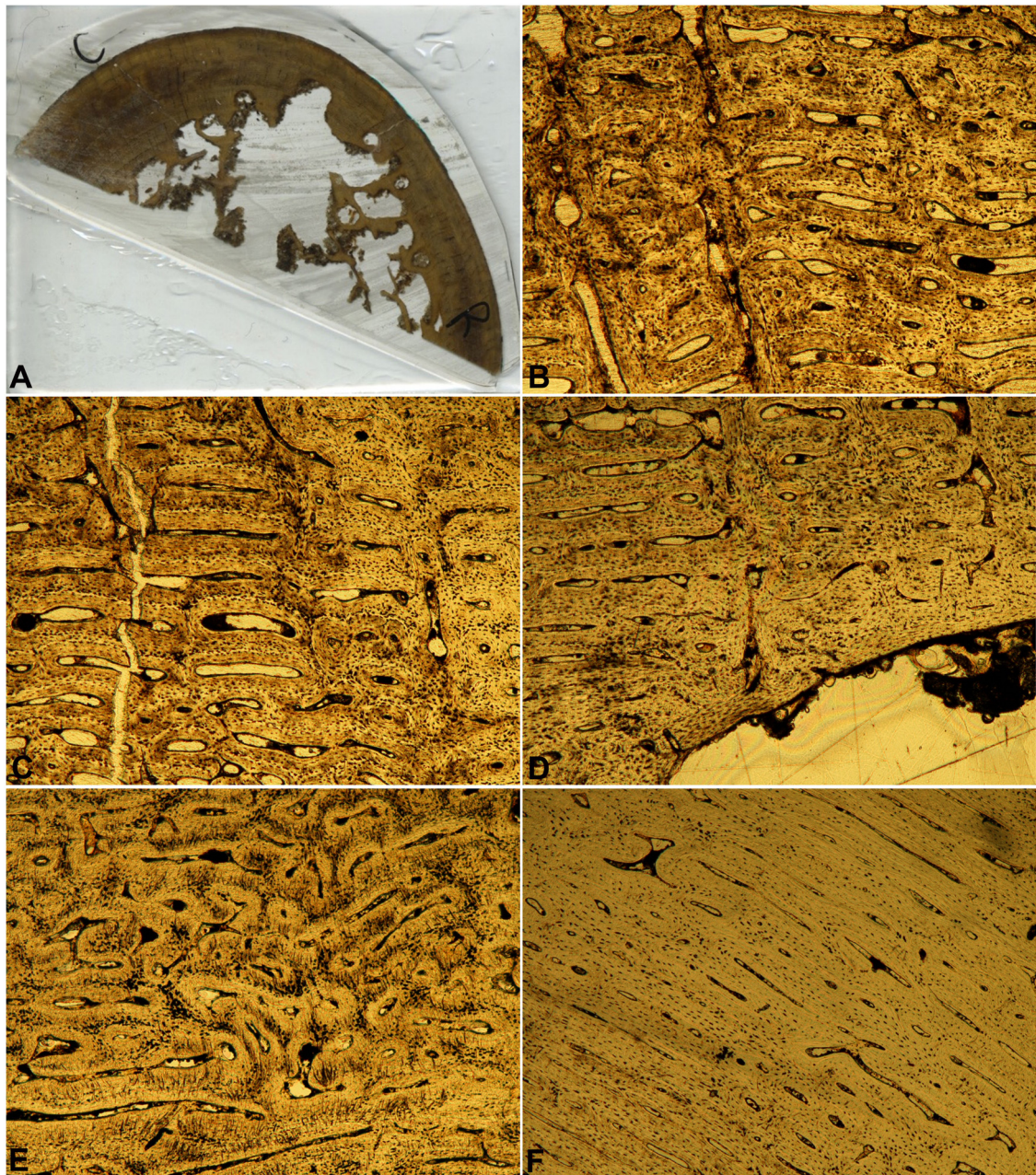
Fig. 1. (Colour online.) Whole cross-section (A) and details of the microstructure (B–F, see Histological description) in the femur MAD 378 of *Aepyornis maximus* in ordinary light, low magnification. Scale bar: 2.5 cm (A) or 1 mm (B–F).
Fig. 1. (Couleur en ligne.) Section transversale totale (A) et détails de la microstructure (B–F, voir Histological description) du fémur MAD 378 d'*Aepyornis maximus* en lumière ordinaire, à faible grossissement. Barre d'échelle : 2,5 cm (A) ou 1 mm (B–F).

is a strong contrast between the compact deep cortex and the well-delineated marrow cavity. The marrow cavity contains a high amount of well-developed trabeculae (Fig. 3D), some lining round or oval marrow spaces, others having the shape of crane-like "X struts" with a likely biomechanical significance. As in femur MAD 364, the cortex has been entirely reworked by Haversian reconstruction into

superimposed generations of secondary osteons (dense Haversian bone) (Fig. 3E, F).

4.4. A. maximus tibiotarsus MAD 8814

The bone has a diameter of 6 to 7 cm at the shaft. The cortex is relatively thick, with a maximal thickness of 2 cm.

Fig. 2. (Colour online.) Caudal transect of the cross-section in the femur MAD 378 of *Aepyornis maximus* in ordinary light, low magnification. Scale bar equals 5 mm.

Fig. 2. (Couleur en ligne.) Transect caudal de la coupe transversale du fémur MAD 378 d'*Aepyornis maximus* en lumière ordinaire, à faible grossissement. Barre d'échelle : 5 mm.

Some perimedullar erosion bay locally invades the deep cortex, while relatively thick extrusions, cone- or dome-shaped, of the deep cortex protrude in the marrow cavity. The external cortex is entirely built by a compact primary tissue with a circular organization. Close observation shows that this tissue is actually the laminar modulation of the fibrolamellar complex. However, the primary osteons of the tissue have experienced a full maturation, such that extensive lamellar bone build-ups of osteonal origin took place while the lumen of the vascular canals became reduced. Scattered Haversian substitution has taken place, and fully "mature" secondary osteons (e.g. with a much reduced Haversian canal) are set even close to the external surface of the cortex (Fig. 4A). The mid- and deep cortices have a structure basically similar to that of the outer cortex, however the amount of secondary osteons is higher (Fig. 4B), and one (perhaps two) lines of arrested growth (LAGs) have interrupted the centrifugal deposition of the laminar tissue (Fig. 4C).

The inner cortex is quite peculiar, the bone is organized as a compacta but has an endosteal origin and is formed of very finely lamellar tissue, crossed by a few simple primary vascular canals (not primary osteons). Locally the bone forms compact, undulating thick "waves" of lamellae. There is much evidence of erosion-reconstruction cycles throughout (Fig. 4D), especially near the marrow limit, where highly remodelled bone trabeculae locally protrude inwards (Fig. 4E). Other evidence of erosion-reconstruction is given by enormous, "endosteal secondary osteons" with an incomplete redeposition (Fig. 4F). The observation of all three complete orthogonal transects in the cortex does not bring evidence of obvious qualitative histological differences between them.

4.5. *A. medius tibiotarsus MAD 8826*

The bone diameter is about 5 cm at the shaft. The cortex is relatively thick, up to 2 cm. The marrow cavity is well delineated from the deep cortex but is peculiar as there is apparently no "free" marrow cavity, most of its space being invaded by numerous thin bony trabeculae forming an extensive spongiosa; only a small, kidney-shaped space remains free of bony trabeculae.

The histology of this bone is closely similar to that of MAD 8814. The primary cortex is built of the laminar tissue pattern of the fibrolamellar complex, namely with extensive circular and longitudinal vascular canals. Here, the woven (periosteal) component of the complex is very much reduced and most of the bone tissue is thus formed by the lamellar (osteonal) component of the complex (Fig. 5A-B). Because most of the bony material, as observed on cross section, is lamellar with a general circular orientation (primary osteons around circular canals) the tissue superficially looks like a simple centripetal deposition of "lamellar-zonal" tissue laid down by the periosteum. This similarity is enforced by the very complete deposition of the osteonal material around many almost obliterated circular canals. This well matured primary tissue is extensively pervaded by Haversian substitution, from the deep cortex to the free bone surface (Fig. 5C-D). Many open cavities represent the early phase of the substitution, by local osteoclastic erosion of the primary cortex. More mature secondary osteons are observed diffusely throughout the cortex, but generally do not form extensive superposed generations of osteons.

The innermost cortex, again, shows similarity with the one in MAD8814 and is formed by endosteal finely lamellar tissue. In some places this endosteal deposit is regular and rather thin, in other regions it shows an extensive development. On the medullar side it turns into highly remodelled, complex endosteal trabeculae (Fig. 5E-F); on the external side, i.e. towards the deepest (oldest) periosteal cortex, there are a high number of large erosion bays with highly remodelled walls formed by endosteal lamellae. There are complete and extensive transitions, through large "endosteal osteons" from those large erosion bays to more typical intracortical secondary osteons (Fig. 5E-F). There are no obvious differences among the three orthogonal transects observed, apart from the different developments of the complex endosteal region.

5. Discussion

Description of the available data in *Aepyornis* has shown a good amount of microanatomical and histological diversity. The purpose of this discussion is to try to make sense of this histodiversity in a biological-evolutionary framework.
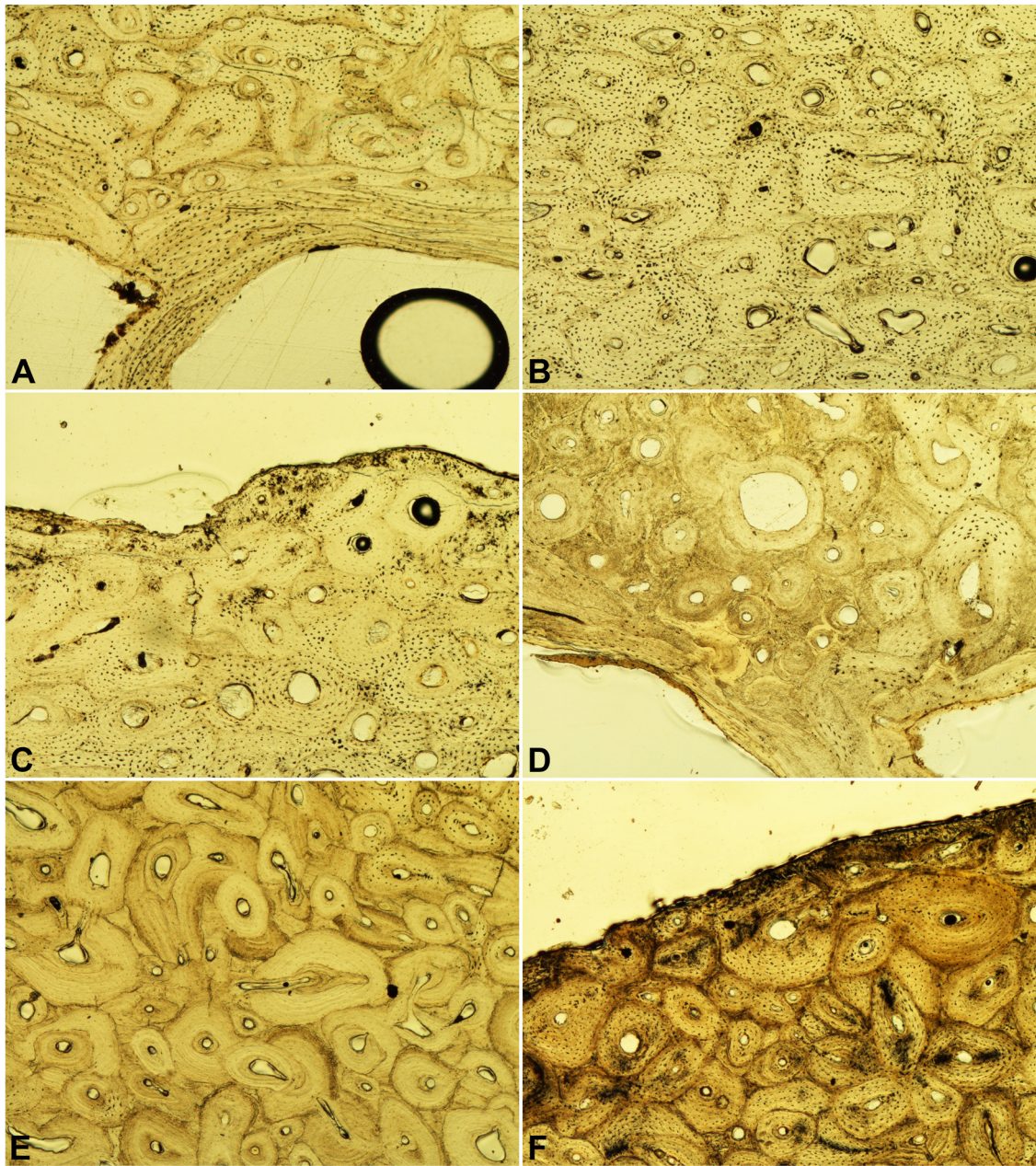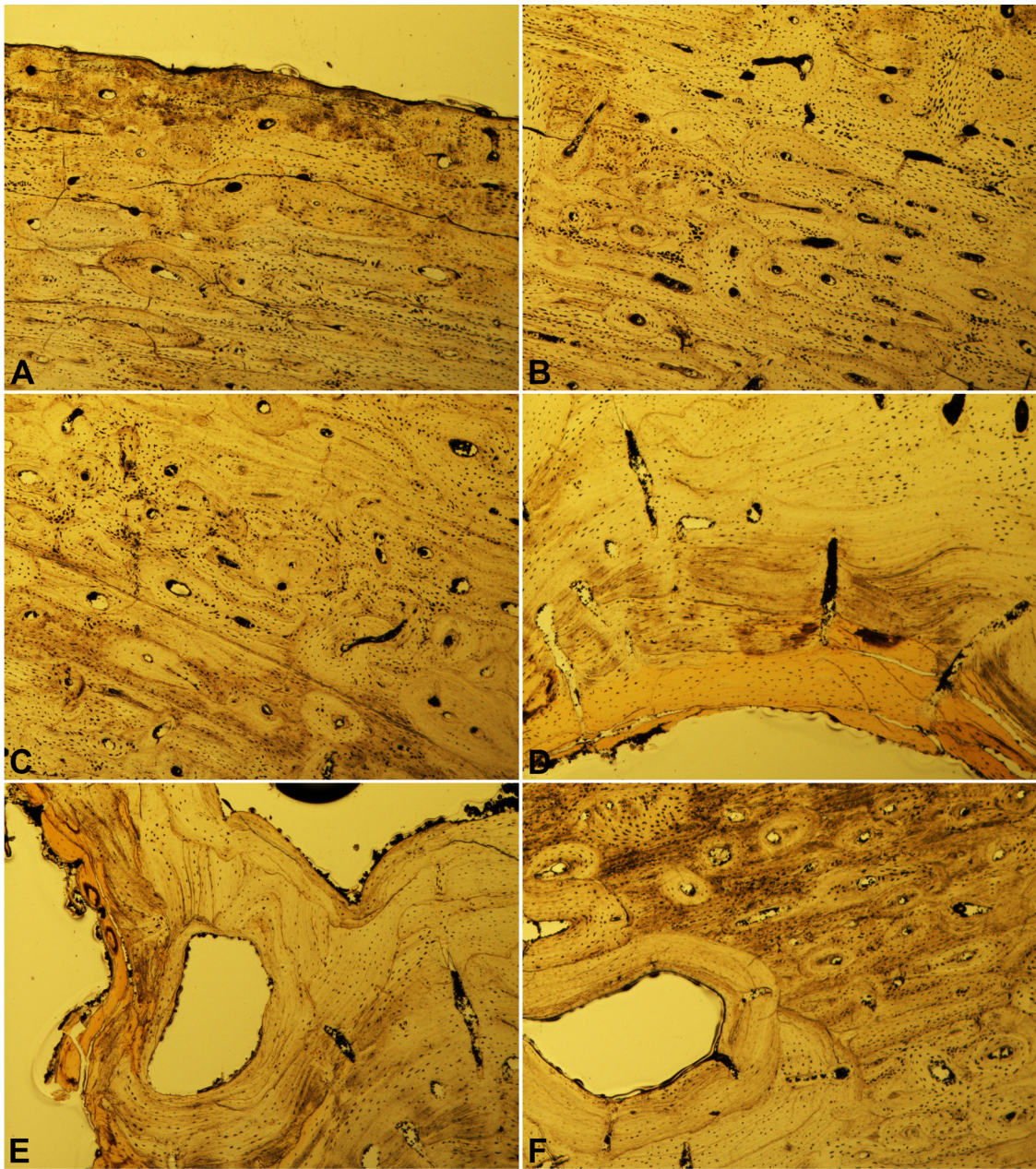
Fig. 3. (Colour online.) Cross-sections of hindlimb bones of *Aepyornis maximus* in ordinary light. A–C, Femur MAD 364 showing dense Haversian bone. A. Inner cortex and marrow cavity. B. Middle cortex. C. Outer cortex. D–F, tarsometatarsus MAD 8813 showing dense Haversian bone. D. Deep cortex and marrow cavity. E. Middle cortex. F. Outer cortex. Scale bar: 1 mm.

Fig. 3. (Couleur en ligne.) Coupes transversales d'os de la patte postérieure d'*Aepyornis maximus* en lumière ordinaire. A–C, Fémur MAD 364 montrant un os Haversien dense. A. Corticale interne et cavité médullaire. B. Mi-épaisseur de la corticale. C. Corticale externe. D–F, tarsometatarsus MAD 8813 montrant un os Haversien dense. D. Corticale interne et cavité médullaire. E. Mi-épaisseur de la corticale. F. Corticale externe. Barre d'échelle : 1 mm.

5.1. Phylogenetic signal

The putative presence of a phylogenetic signal in bone histological data has been a matter of discussion in recent years. Cubo et al. (2005) concluded that phylogenetic signal was highly significant at the microstructural level for some histological traits, but not for all of them. de Ricqlès et al. (2008) argued that 'the histological level of organization by itself may reflect at best a weak signal'. Legendre et al. (2013) performed supplementary analyses in a sample including fossils and concluded that most osteohistological features show a significant phylogenetic

Fig. 4. (Colour online.) Cross-sections of the tibiotarsus of *Aepyornis maximus* MAD 8814 in ordinary light. A. Outer cortex. B. Middle cortex. C. Middle cortex showing one line of arrested growth. D–F. Inner cortex. Scale bar: 1 mm.
**Fig. 4.** (Couleur en ligne.) Coupes transversales de tibiotarse d'*Aepyornis maximus* MAD 8814 en lumière ordinaire. A. Corticale externe. B. Mi-épaisseur de la corticale. C. Mi-épaisseur de la corticale avec ligne d'arrêt de croissance. D–F. Corticale interne. Barre d'échelle : 1 mm.

signal, a conclusion confirmed by supplementary analyses performed on an exhaustive sample of extant ratites (Legendre et al., 2014).

The observations show that most primary bone tissues laid down in the shaft cortex of at least the femur and tibiotarsus of *Aepyornis* are of the fibrolamellar general type. Tissues modulate locally, being perhaps more plexiform in the femur, more laminar in the tibiotarsus. These tissues can receive moderate to extensive Haversian replacement, Haversian bone sometimes entirely obliterating the primary tissues previously laid down during earlier life. Similar characteristics are often observed among non-avian dinosaurs of "moderate" body sizes, actually among archosaurs generally. Does that mean that one is dealing here with a "dinosaurian" (even archosaurian) histological "signature", in other words a strong "phylogenetic signal" carried by bone histology? This may well be the case but alternative explanations should not be neglected. It seems
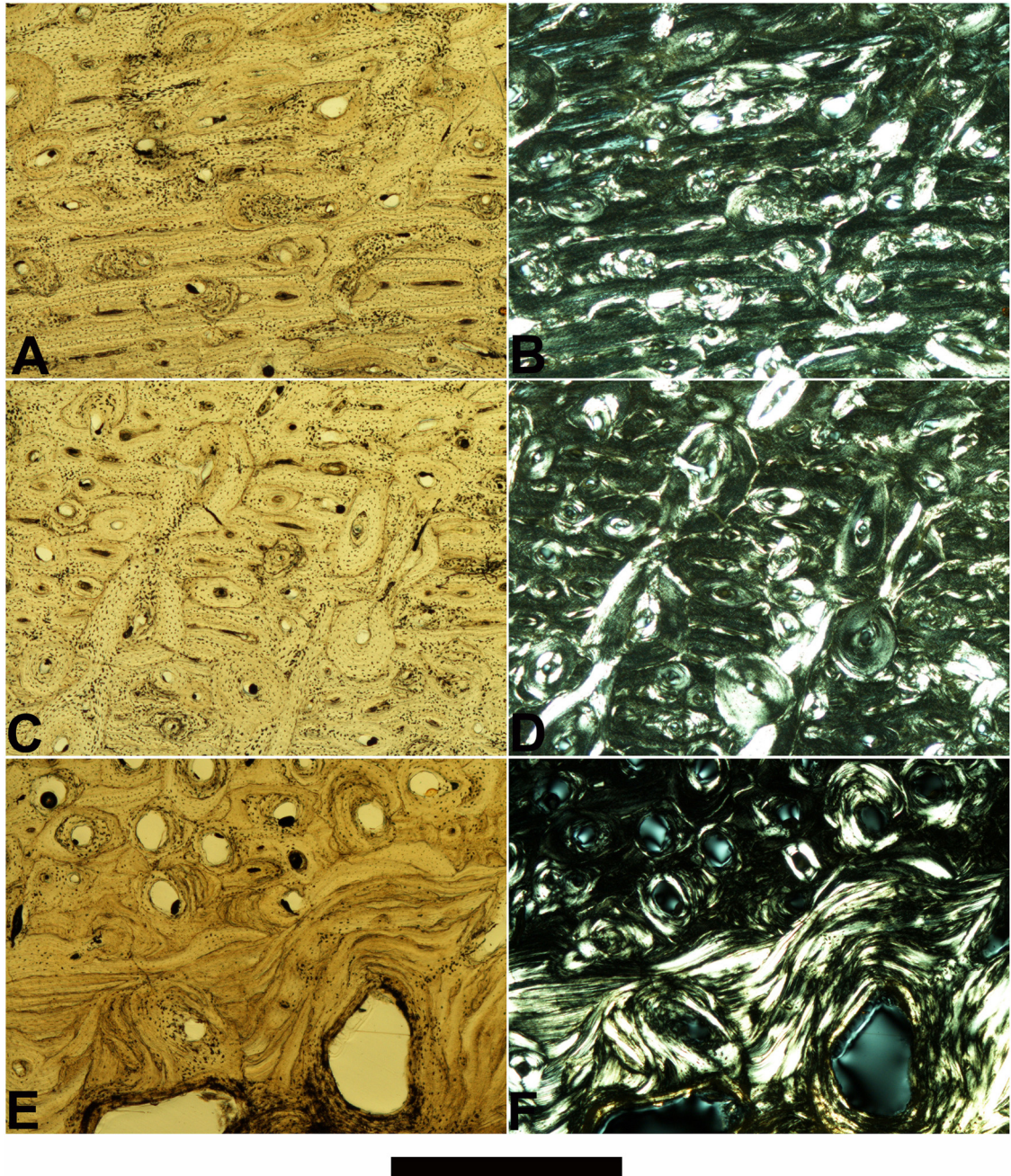
Fig. 5. (Colour online.) Details of the microstructure (see Histological description) of the cross-section in the tibiotarsus MAD 8826 of *Aepyornis medius* in ordinary (A, C, and E) and polarized (B, D, and F) light, low magnification. Scale bar: 1 mm.
Fig. 5. (Couleur en ligne.) Détails de la microstructure (voir Histological description) de la coupe transversale du tibiotarse MAD 8826 d'*Aepyornis medius* en lumière ordinaire (A, C et E) et polarisée (B, D et F), à faible grossissement. Barre d'échelle : 1 mm.

unlikely that all the ancestral taxa of *Aepyornis*, down to the theropodian ancestor of birds, have always and continuously expressed those character-states in their bone tissues phenotypes (de Ricqlès, 2000). Because immediate bird ancestor and early birds were small (Turner et al., 2007), it is unlikely that their bone histological phenotypes were similar to those of most dinosaurs and large ratites. If so, the occurrence of such tissues among ratites would be a

secondary development. It may be that the genetic capacity to produce fibrolamellar tissues and dense Haversian bone – given certain ontogenetic requisites – has always been available among archosaurs as a plesiomorphic trait (de Ricqlès, 1993) and retained even without actual phenotypic expression. If so, rather than a simple homoplastic development, the histological situation among large ratites would express "deep homology". Nevertheless, the

control of actual phenotypic tissue characters in a given taxon would be, first and foremost adaptive-ontogenetic autapomorphies, linked with species-specific life history traits (de Ricqlès et al., 2008).

In the context of the hypothesis suggesting that the presence (or the genetic basis) of a fibrolamellar complex may be the primitive condition among amniotes, we can hypothesize that each expression of a fibrolamellar complex in less inclusive clades is homologous among themselves. However, secondary homology tests (according to which only a character state that occurs once on a tree is taken to be homologous; de Pinna, 1991) refute this last hypothesis (for instance, the last common ancestor of sauropsids and that of diapsids does not show a fibrolamellar complex, because neither testudines nor lepidosaurs show this character state, whereas the ancestral condition for archosaurs involves the presence of fibrolamellar complex). Secondary homology tests through parsimony show that each expression of a fibrolamellar complex in less inclusive clades (e.g. ratite birds, if this group is indeed a clade, see introduction) constitutes an independent synapomorphy. This last view is congruent with the facts that:

- each historical event is by definition unique;
- even if the genetic basis underlying the formation of the fibrolamellar complex is "roughly" the same in synapsids and in archosaurs, the epigenetic context of each new expression is clade-specific and thus, unique (a good example illustrating this pattern can be found in Dillman et al., 2010).

This view is also congruent with Dollo's (1893) law of irreversibility, according to which a structure or organ that has been lost or discarded through the process of evolution (e.g. absence of fibrolamellar complex at the nodes Sauropsida and Diapsida) will not reappear in the exact same form in that line of organisms (e.g. presence of a fibrolamellar complex at the node Archosauria). In conclusion, the fibrolamellar complex observed in synapsids and in archosaurs probably share the same genetic basis ("deep homology"), but each expression of this character state in less inclusive clades may constitute independent synapomorphies at the level of these clades.

5.2. Growth cycles

In the case of *Aepyornis maximus*, the large body size may not have been reached as fast as in long-legged ratites (i.e. ostrich, rheas, emu and cassowaries), in spite of the high growth rates suggested by the occurrence of plexiform bone tissue. In ornithurine birds including most modern birds, *Hesperornis* and *Ichthyornis*, skeletal development is achieved in less than a year, so that bone growth marks are either absent or restricted to the outer cortical layer of bone cortices (Chinsamy, 2005). The evidence for several LAGs in adult-sized femur – if confirmed by further studies – suggests that *Aepyornis maximus* took several years to reach skeletal maturity. Interestingly, proctracted osteogenesis interrupted by several LAGs is found in New Zealand ratites, including the subfossil moa

(Dinornithiformes; Turvey et al., 2005) and the extant kiwi (*Apteryx*; Bourdon et al., 2009b). It has been suggested that prolonged cyclical growth in New Zealand ratites evolved in response to insular environment with low predation pressure (Bourdon et al., 2009b; Turvey et al., 2005). Protracted cyclical growth also occurs in the putative bird *Gargantuavis*, which is known only from the Ibero-Armorican island of the Late Cretaceous European archipelago (Chinsamy et al., 2014). Chinsamy et al. (2014) proposed that the growth pattern of *Gargantuavis* may be linked to the prevailing environmental conditions in an insular setting. About 88 million years ago, Madagascar became isolated from other Gondwanian landmasses, which led to the development of a unique ecosystem with a high level of endemic species (Goodman and Jungers, 2014). This evidence suggests that *Aepyornis* probably evolved cyclical growth in response to the insular environment of Madagascar. The ontogeny of *Aepyornis* undoubtedly followed a K-selected evolutionary pathway, as suggested by the enormous size of the eggs. As for the moa (Turvey et al., 2005), it is clear that such strategies would have proved lethal as soon as these bird populations were confronted to human predation, although the extinction of elephant birds seems to have a complex causality (Goodman and Jungers, 2014). The total number of LAGs in *Aepyornis* cannot be ascertained in this preliminary study, and a precise assessment of its ontogeny will have to await more extensive investigations. On the issue of "growth lines", it may be useful to pinpoint again that the optimal conditions to observe these may be quite different from the optimal conditions to observe fine histology or cells sizes/shapes.

5.3. Ontogenetic trajectories

Comparison of the two femora of roughly the same size (MAD 378 and 364) but with very distinct histologies shows that at least a part of the ontogenic trajectories are faithfully recorded by bone tissue. While MAD 378 strongly suggests a subadult condition with growth still ongoing, MAD 364 histology would record a fully adult or perhaps even an old individual.

5.4. Bone-specific size/shape modelling

Because there is no control in our material of different bones pertaining to the same individual or not, it is difficult to assess the significance of bone-specific differences as observed. Obviously there are microanatomical and histological differences between femora, tibiotarsi and tarsometatarsus. What do they mean? Are they caused by species-specific, organ-specific, ontogenetic or biomechanical or sexual differences? Or express all of them simultaneously?

Along a proximodistal axis in the limb bones, there seems to exist a "gradient" in which the histological "maturation" of cortical bone tissues differ. The femur would show a more "juvenile" histology than the tibiotarsus, and the tibiotarsus more "juvenile" than the other, more distal (and often smaller) bones. This has been fairly well documented in ostriches (Amprino and Godina, 1947) and

dinosaurs (Horner et al., 1999) and is not restricted to cursorial limbs. The data available for *Aepyornis*, although weak, seem to concur: the tarsometatarsus is entirely substituted into secondary bone, an adult-sized femur is still mostly formed by primary tissues, and the tibiotarsi fall in between. This situation may be explained by the bone-specific growth dynamics of different bones within an individual skeleton. For example if a small metatarsal takes the same amount of time to reach its adult size than the much larger tibiotarsus and femur in the same individual (growth isochrony), it makes sense than different tissue types adapted to different growth rates will be laid down in the different bones. Poorly vascularized "lamellar-zonal" tissues would prevail in the smallest bones, and well vascularized "fibrolamellar" ones in the largest. In turn, the difference in initial vascularization of the primary tissues laid down may control the rate and amount of their secondary reconstruction, hence relatively more Haversian bone in the smaller, most distal bones and a more "mature" histology.

5.5. Biomechanics

There is a distinct difference in the cortico-medullary ratios among the different bones studied. While the tarsometatarsus (especially) and tibiotarsus have a relatively thick cortex, the femur has a relatively thin one. Also, the amount of cancellous trabeculae in the marrow cavity seems to be very different from bone to bone, minimal in the femur, maximal in the tibiotarsus (MAD 8826). Assuming that our sample brings significant data, such differences may express in part the differences in biomechanical loading experienced among the leg bones. In cursorial birds, the femur, during locomotion, may assume an almost horizontal orientation with little actual motions while the more distal limb bones may experience intensive loading changes during each locomotory cycle (Gatesy, 1990). This could be expressed in the cortico-medullary ratios, development of Haversian substitution in the cortex, and amount of the perimedullary spongiosa. In the tarsometatarsus, the bone section departs strongly from a circular shape: this may be linked to the obvious "Warren struts" or X-shaped trabeculae observed in the marrow cavity.

5.6. Sexual cycles

In the tibiotarsus of both species, one has noted the peculiar development of endosteal deposition at the periphery of the marrow cavity. The endosteal bone tissue takes several aspects, from a dense compacta with undulating lamellae to highly remodelled trabeculae forming a spongiosa. The bone is apparently prone to intensive erosion/reconstruction phases, with production of very large "endosteal osteons", etc. This situation may suggest that this endosteal deposit is linked to the reproductive physiology of the bird, especially to the storage/releasing cycles of calcium involved in yolk and especially eggshell production. Similar situations have been well documented among extant birds (e.g. Meister, 1951; Schweitzer et al., 2005; Taylor and Moore, 1953), and have been suggested in

several dinosaurs as well (Chinsamy, 2005; Chinsamy et al., 2013; Hubner, 2012; Schweitzer et al., 2005).

5.7. Species-specific changes

A clue may be the comparison of the tibiotarsi in *A. maximus* (MAD 8814) and *A. medius* (MAD 8826). As stated above, the taxonomy of elephant birds is controversial, and some or all aepyornithid species may represent just one variable or sexually dimorphic species within each genus (Hume and Walters, 2012). Pronounced sexual size dimorphism occurs in other large ratites that evolved in an insular environment, namely the New Zealand Moa (Bunce et al., 2003). Assuming one is really dealing with two different species, with specific body-size differences, the histology of MAD 8826 would indeed suggest a lower growth rate than in MAD 8814, and also a more mature "older" bone, as suggested by the more generalized Haversian replacement. Alternatively, the data could simply express interindividual differences within a population. Be it as it may, the tibiotarsi histology is more congruent with each other than with any other bones of the sample, suggesting again a strong organ-specific (autapomorphic) signal, even if the samples pertain to different (but closely related) species.

6. Concluding remarks

As stated in the introduction, this preliminary work has helped to set the situation of the interpretation and significance of bone histodiversity, here for *Aepyornis*, a spectacular subfossil ratite. Some of the possible causal factors "explaining" this diversity have been sorted out and discussed specifically and separately, to try to analyse qualitatively their input. Nevertheless we believe they all interact together into an intrinsically complex causality. As far as *Aepyornis* is concerned, it is clear that much more material should be studied before its ontogeny and life history traits can be further deciphered thanks to its bone histology.

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