Virtual osteohistology of paravian dinosaurs: from 2D to 3D perspective

Starting in 2016, a Japan-Slovak research platform in integrative paleobiology was established at SPring-8. This platform aims to utilize synchrotron radiation to detect information about the life of extinct organisms accessible in fossilized remains. Several pilot projects were carried out to test imaging technology at SPring-8 on different kinds of fossil objects such as individual bones of recently extinct giant birds of New Zealand and Madagascar (2016A1038) and of non-avialan and avialan dinosaurs from the Mesozoic of China (2018B1543), as well as objects more challenging for scanning, including high-density dinosaur eggs containing embryos (2017A1714, 2017B1755) and flattened fossils associated with soft tissues (2019B1381). Here, we demonstrate our first results referring to both the imaging quality and research achievements in studying dinosaur evolution.

Two major investigation approaches, invasive (or destructive) and non-invasive (non-destructive), have been used to access the inner structure of fossilized objects: skulls, individual bones, teeth, eggs, eggshells, mineralized soft tissues, and amber inclusions. The destructive approach was used by earlier paleontologists to make hidden anatomy available for study, for example, to assess the shapes of the inner cranial cavities, canals, and sinuses by sawing fossilized skulls. From a historical perspective, these studies can be seen as revolutionary in revealing unknown biological features that significantly influenced our understanding of less accessible morphology. Despite the invasive nature, those studies had inductive effects on the modernization of our views concerning the capacity of fossils to preserve much more than anatomic information. Thus, a semi-destructive approach is still broadly applied for making physical thin sections to investigate developmental events recorded in hard tissues.

Modern experimental approaches are expected to reduce invasive intervention to a minimum. They are required to possess enhanced functionalities for the accurate visualization of qualitative traits and quantitative characters in three dimensions on a micro- or nanoscale. It is mainly the quantitative aspect of the ancient tissue microstructure that is currently seen as a promising, but mostly underexplored, source of evolutionary information. This source provides the correlation interface for the reconstruction of biological phenomena such as ontogenic chronology [1], embryonic development [2], growth dynamics, metabolic rates, thermoregulation, physiological crises, sexual maturity, reproduction, and which may further facilitate progress in new disciplines: paleoproteomics and paleogenomics. X-ray imaging techniques turned out to be well-adapted and prospective for multiscale purposes of the paleobiological investigation. We claim that at SPring-8 **BL20B2** and **BL28B2**, combined with other imaging beamlines, provide an extraordinary experimental environment that makes SPring-8 one of the leading facilities suited for exploring new cellular and molecular frontiers of ancient life. As far as virtual osteology is concerned, we succeeded in imaging the 3D microstructure of 120 million-year-old bones with an exceptional histological resolution that is comparable to 2D physical thin sections (Fig. 1(a)). This histological quality resulted from the well-sized samples (preserved in volcanic rocks) and modulation of scanning parameters (reasonable X-ray energy, exposure time, and effective propagation distance) for reaching the voxel size between 1 and 2 microns. The

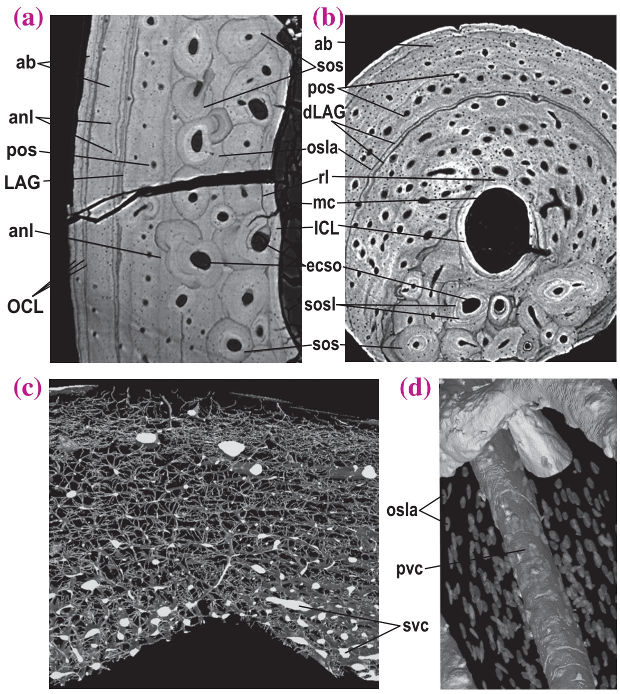

Fig. 1. (**a,b**) Virtual paleo-osteohistology of dinosaur bones (Paraves) approaching histological quality. (**c,d**) 3D-rendered vasculature and osteocyte lacunae of the extinct giant bird (Dinornithiformes). Abbreviations: ab, avascular bone; anl, annulus; dLAG, double ICL; ecso, expanded cavity with sos; inner circumferential layer; LAG, line of arrested growth; OCL, outer circumferential layer; osla, osteocyte lacuna; pos, primary osteon; rl, resorption line; sos, secondary osteon.

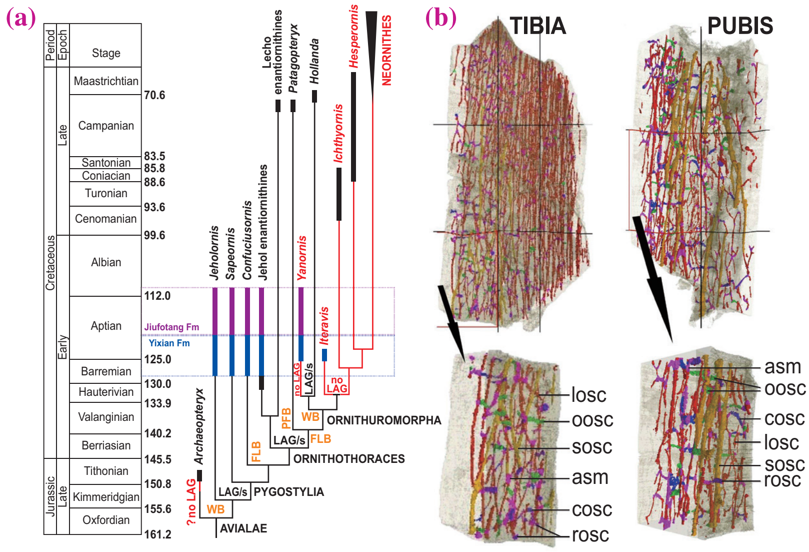

Fig. 2. (**a**) Simplified cladogram depicting the ornithuromorph lineages, in which rapid uninterrupted growth (red lines) evolved. (**b**) 3D-rendered vasculature models of the limb and pelvic bones of *Yanornis martini*. Abbreviations: asm; anastomosis; cosc, circumferential osc (in blue); FLB, fibrolamellar bone; LAG, line of arrested growth; losc, longitudinal osc (in red); oosc, oblique osc (in green); osc, osteonal canal; rosc, PFB, parallel-fibered bone; radial osc (in purple); sosc, secondary osc (in orange); WB, woven bone.

phase contrast modality enabled the reconstruction of complex vascularization in fossil and subfossil bones (Fig. 1(b)) with a high precision that is critical for further quantitative investigation.

Osteohistology of the long bones of extant amniotes usually indicates higher vascularization. Higher vascularization is typically associated with high metabolic activity and non-interrupted growth (Fig. 2(a)) in birds and mammals. In general, there is a direct correlation between metabolic rates and body temperature. We used bone vasculature variables as a proxy to determine growth rates and thermophysiology of the Mesozoic avialan dinosaur *Yanornis martini* (one of the oldest birds with non-interrupted growth) using 2D synchrotron osteohistological sections [3].

We found that *Yanornis* exhibits a dual pattern in growth rates between the pelvic bone (pubis) and the inferior limb bone (tibia) (Fig. 2(b)). Having plotted the extant ectothermic and endothermic proxies, based on vascular ratios, the estimated thermic values for *Yanornis* are aligned with those of marsupial and saurian taxa rather than with most of the endothermic taxa (birds and placentals), indicating a rather mesothermic thermophysiology. We further test this interpretation using 3D synchrotron-based data of *Yanornis*, the non-avialan dinosaur *Liaoningvenator* (Fig. 3), and extant amniotes (e.g., *Opisthocomus*, *Arvicola*) using enhanced analytical tools able to fragment the neurovascular complex by diameter and angulation of osteonal canals.

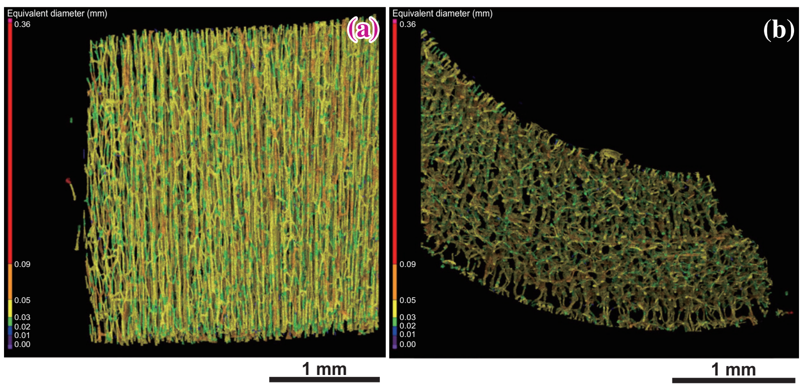

Fig. 3. Fragmentation of the bone neurovascular network by diameter in a paravian dinosaur. (**a**) Radial view. (**b**) Longitudinal view.

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References

[1] C. Shen *et al.*: Historical Biol. **31** (2019) 253.
[2] M. Kundrát *et al.*: Current Biol. **30** (2020) 1.
[3] *Q. Monfroy, M. Kundrát, K. Uesugi and M. Hoshino: Historical Biol.* ***34*** *(2022) 1039.*