Paleobiological implications of the bone histology of the extinct Australian marsupial *Nimbadon lavarackorum*

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Abstract.—Despite the recognition that bone histology provides much information about the life history and biology of extinct animals, osteohistology of extinct marsupials is sorely lacking. We studied the bone histology of the ca. 15-million-year-old *Nimbadon lavarackorum* from Australia to obtain insight into its biology. The histology of thin sections of five femora and five tibiae of juveniles, subadult, and adult *Nimbadon lavarackorum* was studied. Growth marks in the bones suggest that *N. lavarackorum* took at least 7–8 years (and likely longer) to reach skeletal maturity. The predominant bone tissue during early ontogeny is parallel-fibered bone, whereas an even slower rate of bone formation is indicated by the presence of lamellar bone tissue in the periosteal parts of the compacta in older individuals. Deposition of bone was interrupted periodically by lines of arrested growth or annuli. This cyclical growth strategy indicates that growth in *N. lavarackorum* was affected by the prevailing environmental conditions and available resources, as well as seasonal physiological factors such as decreasing body temperatures and metabolic rates.

Introduction

During life, bone is a living tissue that records various aspects of the life history and biology of an animal. It is therefore fortuitous that despite millions of years of fossilization, the integrity of the microscopic structure of bones of fossil vertebrates remains intact (Chinsamy-Turan, 2005), thereby allowing paleobiological deductions. Although the bone histology of placental mammals has been well studied (e.g., Enlow and Brown, 1958; Klevezal and Kleinenberg, 1969; Kolb et al., 2015; Montoya-Sanhueza et al., 2021a, 2021b), the osteohistology of marsupials is sorely lacking (Chinsamy and Warburton, 2021), despite work on them having begun in the nineteenth century (Quekett, 1849) and early twentieth century (Foote, 1916; Amprino, 1947), and continuing sporadically thereafter (e.g., Enlow and Brown, 1958; Singh et al., 1974; Werning, 2013; Walker et al., 2020). Growth marks in marsupials were first reported in a large extinct diprotodontid (unknown species) (Leahy, 1991), and thereafter in a kangaroo (Chinsamy-Turan, 2005; Hurum and Chinsamy-Turan, 2012). The most comprehensive study of marsupial osteohistology (in terms of number of extinct and extant taxa) was done by Werning (2013) (although *Nimbadon* was not analyzed). More recently, Chinsamy and Warburton (2021) undertook the only osteohistology study documenting histological changes through ontogeny of the Western Grey Kangaroo, *Macropus fuliginosus* Desmarest, 1817. Bone disease in wombats, *Vombatus ursinus* Shaw, 1800, has been examined by

(Slon et al., 2014). Walker et al. (2020) studied the forelimbs of extinct and extant wombats to assess their biomechanical and physiological adaptations to their fossorial lifestyle. In a study looking at limb bone scaling in macropods and quadrupedal artiodactyles, Doube et al. (2018) speculated that the hopping behavior of macropods might have enhanced repair of fatigue damage by increasing bone remodeling. However, unexpectedly, they found that a femur of *Macropus gianteus* Shaw, 1790, lacked any bone remodeling. In a study of skeletal lesions caused by fluorosis in six species of marsupials, Death et al. (2017) found that the pathologies evident in their bones concurred with those of other fluoride-related skeletal changes described for other mammals.

Our research focuses on juvenile and adult *Nimbadon lavarackorum* Hand et al., 1993 (Zygomaturinae, Diprotodontidae, Marsupialia), that lived during the Middle Miocene (ca. 15 Ma; Woodhead et al., 2016) of Australia. Diprotodontids are a taxonomically (18 genera, 30 species) and ecologically diverse group of extinct, large-bodied (70–2000 kg) marsupials commonly found in late Oligocene to Pleistocene deposits of Australia and New Guinea. At ~70 kg, *N. lavarackorum* is regarded as one of the smallest diprotodontids, but one of the largest mammals to have occupied the forest canopies of Australia (Black et al., 2012a). Anatomically, its strong forelimbs, highly mobile shoulder and elbow joints, semi-opposable digit 1 on the manus and pes, and large recurved claws make it similar in some respects to modern koalas (*Phascolarctos cinereus* Goldfuss, 1817) and highly adapted for an arboreal lifestyle. The proportionately short hindlimbs (relative to forelimbs) of *N. lavarackorum* may also indicate some suspensory

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behavior (Black, 2012a). Its closest living relatives are wombats and koalas.

We hypothesize that the postcranial bone histology of *Nimbadon lavarackorum* will show growth marks in the form of alternating periods of fast and slow growth, and that lines of arrested growth (LAGs) will be associated with the periods of slow growth. Since we are examining both juvenile and adult bones of the species, we predict that the number of growth marks in the bones of *N. lavarackorum* will increase through ontogeny, and we expect to see a slowing down in both appositional periosteal growth and in endochondral growth (i.e., growth in length).

Materials and methods

Ten skeletal elements were studied: two juvenile femora and two juvenile tibiae (identified on the basis of dimensions and comparison with more complete specimens, as well as degree of epiphyseal fusion), three larger (probably adult) femora, and three larger tibiae (Fig. 1, Table 1). All of these were recovered from the Middle Miocene (14.82 ± 0.27 Ma and 14.64 ± 0.46 Ma; Woodhead et al., 2016) AL90 site, Riversleigh World Heritage Area (Boodjamulla) in Waanyi area of northwestern Queensland (project numbers, DE130100467, DP170101420). All specimens were prepared using dilute acetic acid to process limestone speleothems (flowstone) that had accumulated in a cave deposit (Arena et al., 2014). Current understanding is that the paleohabitat surrounding the AL90 cave during the Middle Miocene was species-rich, lowland rainforest (Archer et al., 1994; Travouillon et al., 2009, 2012; Black et al., 2012a, b; Arena et al., 2014; Myers et al., 2017) that also contained isolated, ephemeral, internally drained lakes.

Prior to embedding in resin for thin sectioning, all the bones were pre-treated by leaving them in 95% alcohol for 8 hours. Thereafter, the alcohol was discharged and fresh alcohol was poured over the specimens. This was done three times and was followed by two overnight baths in acetone. After the acetone treatment, the bones were left to dry completely. The pre-treatment procedure removes any remaining organic material (such as collagen) in the bones and prevents the specimen "lifting" off the slide during thin-section preparation (Chinsamy et al., 2020).

Following pre-treatment, the bones were sampled for thin sectioning (Fig. 1). Transverse sections along the diaphysis (including the midshaft) were made to obtain information about the organization of the bone along the shaft. Longitudinal thin sections were made of bones that preserved articular ends to obtain information about endochondral growth (Fig. 1). Once segments of the bones were removed, thin sections were prepared following the methodology outlined in Chinsamy and Raath (1992). Resulting thin sections were examined under a Zeiss microscope/Nikon E200 microscope, and photomicrographs were taken using a Zeiss Axio camera/Nikon camera.

Histology terminology used generally follows the traditional bone terminology of Francillon-Vieillot et al. (1990) and Chinsamy-Turan (2005, 2012). It should be noted that the orientation of the canals in the bone generally are used as a proxy to describe the extent and orientation of vascular canals present in the bone. However, each vascular canal can house a variable number of blood vessels, nerves, and other connective tissues (Chinsamy-Turan, 2005). It is also worth noting that the shape of the canals do not reflect the orientation of the actual blood vessels therein (Starck and Chinsamy, 2002; Chinsamy-Turan, 2005).

Growth marks (GMs) are indicated by the stratification of the compacta into alternating bands of rapidly formed bone tissue (zones), and more slowly formed bone tissue (annuli) that are often associated with lines of arrested growth (LAGs), which indicate periodic cessation of growth (Francillon-Vieillot et al., 1990; Chinsamy-Turan, 2005, 2012).

Repositories and institutional abbreviations.—AR, paleontological collections in the School of Biological, Earth and Environmental Sciences, University of New South Wales; QM F, paleontological collection of the Queensland Museum. All thin sections will be housed with the original collections from which the material originated.

Results

Detailed descriptions of the cross-sectional histology and, where possible, the longitudinal osteohistology of *N. lavarackorum* specimens follows.

AR21803a juvenile partial tibia (associated with partial femur, AR21803b).—The compacta is mostly damaged by diagenesis, but details of the histology are still evident. Several GMs interrupt the primary bone compacta (Fig. 2.1).

AR21803b juvenile partial femur (associated with partial tibia, AR21803a).—The bone tissue is affected by diagenesis (probably manganese infiltration), but despite this, it is evident that the compacta is well vascularized with many mainly radially organized vascular canals. The bone appears to have a more lamellar texture, and at least three growth marks are evident in the compacta (Fig. 2.2), although these are not consistently visible around the bone. The endosteal margin is highly resorptive, and several large erosion cavities are visible in the perimedullary region (Fig. 2.2). A narrow strip of endosteal lamellar bone tissue is present in some parts of the bone wall. It is notable that the bone tissue changes quite dramatically from one area of the thin section to the next. Figure 2.3 shows a radial tract of bone tissue that is composed of more compacted coarse cancellous bone (cccb) as compared to the primary lamellar bone on the one side and a more woven textured primary bone on the other side.

AR21802a juvenile tibia (associated with partial femur, AR21802b).—A partial thin section of the tibial bone wall is preserved. The compacta is well vascularized. Here, the histology is quite variable across the thin section. Several closely spaced LAGs are visible in the peripheral region of one part of the bone wall (Fig. 3.1), but if you follow the LAGs around the compacta, they then become wider apart. Also, in the latter region, the bone wall is very uneven. The outer third of the bone wall consists of primary bone tissue, but there are different types of bone tissue internal to this, of
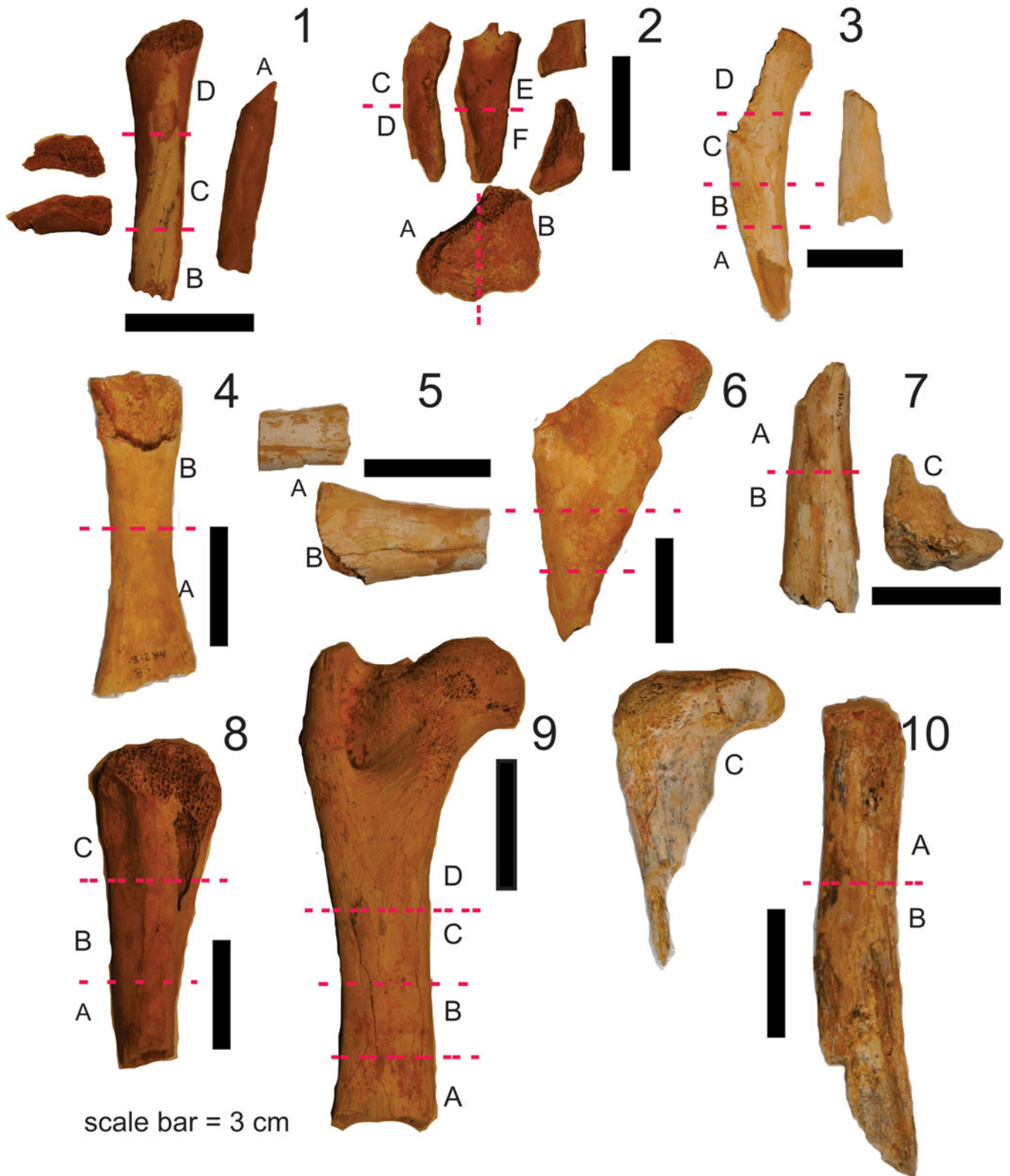
Figure 1. *Nimbodon lavarackorum* material used in this study. (**1**) AR21802a, partial juvenile tibia; (**2**) AR21802b, partial juvenile femur; (**3**) AR21803a, partial juvenile tibia; (**4**) AR21803b, partial femur; (**5**) AR21804, femur fragments; (**6**) AR21805, proximal femur; (**7**) QM F50691 partial tibia; (**8**) QM F41185, partial tibia; (**9**) QM F50842a, partial femur; (**10**) QM F50692, partial femur. Dashed red lines indicate where the bones were sectioned into different blocks. labeled A, B, C, etc. Abbreviations: AR, paleontological collections in the School of Biological, Earth and Environmental Sciences, University of New South Wales; QM F, paleontological collection of the Queensland Museum; a, b indicate associated material.

Table 1. Specimens studied and thin sections that were prepared from each element. Note that specimens AR21802a and AR21802b were associated and are likely from the same juvenile (juv) individual. The same applies to specimens AR21803a and AR21803b. frag = fragmentary; prox = proximal, dist = distal; TS = transverse section, LS = longitudinal section.

Specimen No.	Skeletal element	Comments	Thin sections
AR21802a	tibia	juv, partial	7 TS (AI, II, III, BI, BII, BIII, CI, CII) shaft. 3 LS (DI, DII, DIII)
AR21802b	femur	juv, partial	7 TS (AI, AII, AII, AIV; BI, BII, BIII) shaft; 4 TS dist artic end (CI, CII; F, D)
AR21803a	tibia	juv, frag	7TS (A, B shaft); 3LS (A, BI, BII)
AR21803b	femur	juv, partial	6 TS shaft
AR21804	femur	partial	2TS (A, B)
AR21805	femur	prox	2TS (A, B) shaft. 2 LS (D3, D4))
QM F41185	tibia	partial	6 TS shaft, 3 LS artic end
QM F50691	tibia	partial	7TS (A, B, BII shaft), 3 LS (CI, CII, CIII)
QM F50692	tibia	partial	2TS (A, B); 1LS(D)
QM F50842a	femur	partial	4 TS (A, AII, B, BII shaft), 2LS prox

which cccb appears to be the predominant type (Fig. 3.1–3.3). Some enlarged erosion cavities and several secondary osteons are visible in the perimedullary area (Fig. 3.1). The endosteal margin is resorptive in places, but in other parts, there is a narrow band of lamellar bone tissue that lines the medullary cavity (Fig. 3.1). The longitudinal section of the bone is quite
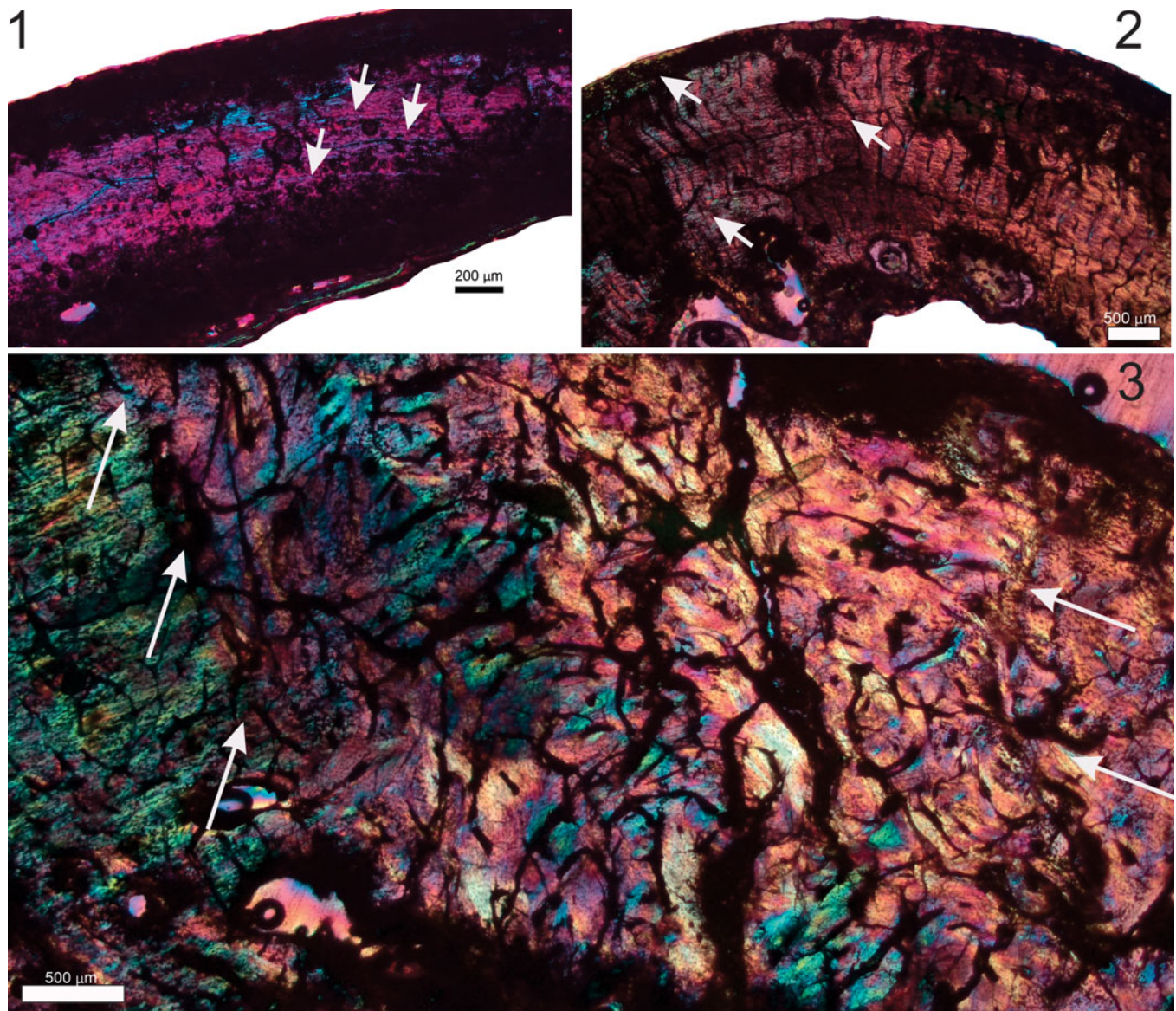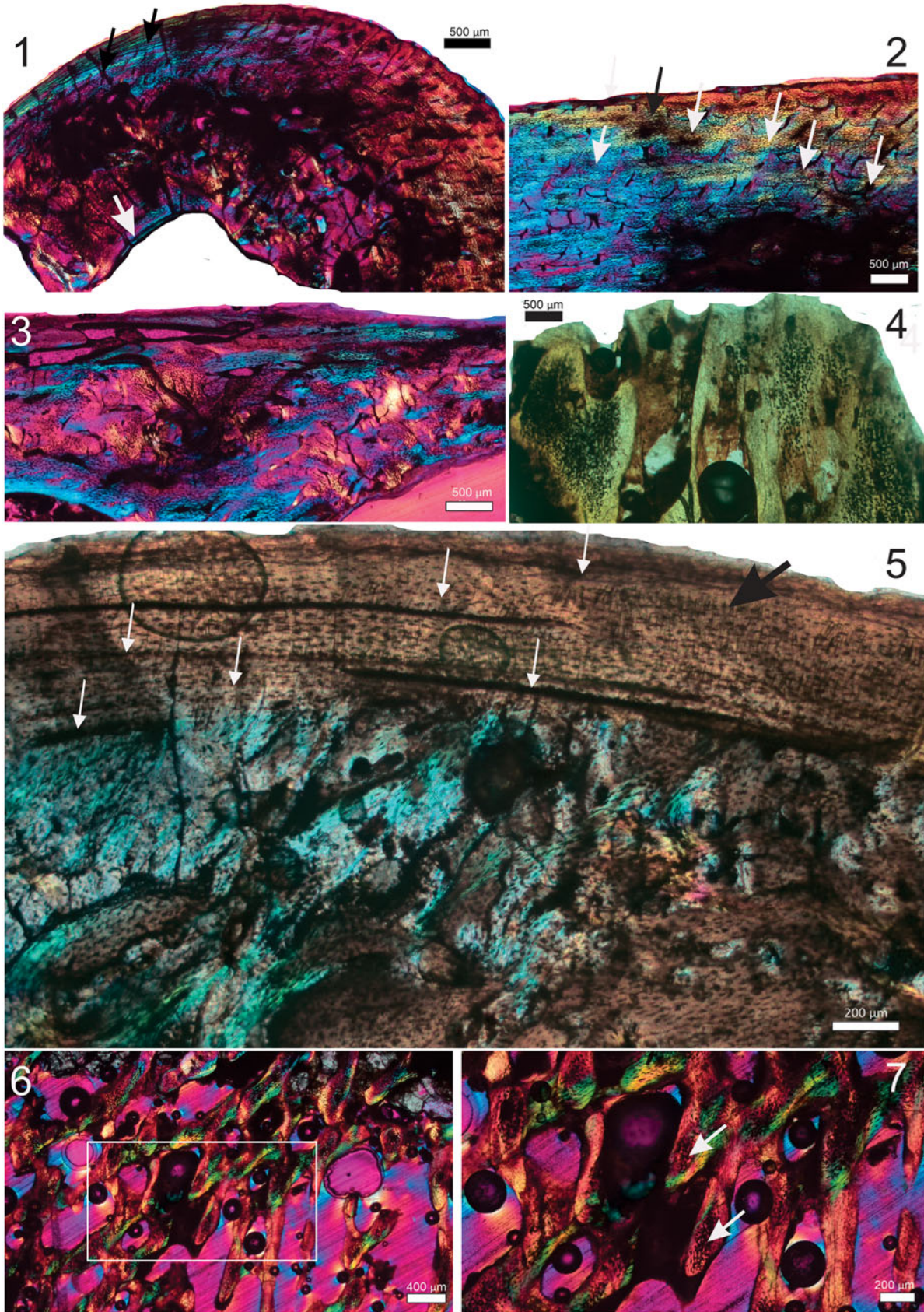

Figure 2. Specimen AR21803. (**1**) AR21803a, thin section BII; section of partial tibia; arrows indicate growth marks in the compacta; (**2**) AR21803b, thin section AI; section of partial femur showing a low-magnification overview of the compacta; arrows inindicate growth marks in the compacta (note the resorptive endosteal margin of the bone wall); (**3**) AR21803b, thin section AI showing radial tract of compacted coarse cancellous bone (indicated by the white arrows). Images taken under polarized light with a one-quarter-λ compensator.

Figure 3. Specimen AR21802. (**1–4**) AR21802a, partial tibia; (**1**) AR21802a, thin section AII; black arrows indicate growth marks in the compacta, white arrow indicates a narrow band of inner circumferential lamellar bone tissue lining the medullary cavity (note that the endosteal margin of the bone wall is resorptive); (**2**) AR21802a, thin section DI; arrows indicate growth marks formed by narrow annuli; the vascular canals tend to be radially organized in this part of the bone wall; (**3**) AR21802a, thin section CI; a different part of the bone wall showing the predominance of compacted coarse cancellous bone tissue; (**4**) AR21802b, partial femur, thin section D; longitudinal section showing some calcified cartilage. (**5–7**) AR21802b, partial femur, thin section AIV; (**5**) arrows indicate several LAGs that interrupt deposition of the poorly vascularized parallel-fibered bone tissue; several short Sharpey's fibers are visible towards the periosteal region of the bone wall (black arrow); a region of reconstructed secondary bone tissue (blueish color) is present below the narrow strip of primary bone; (**6, 7**) overview of trabecular bone tissue; (**7**) higher magnification of framed region in (**6**); arrows indicate islands of calcified cartilage in the trabeculae. Images taken under polarized light with a one-quarter-λ compensator.

badly affected by diagenesis, but a patchy distribution of calcified cartilage is visible (Fig. 3.4).

AR21802b juvenile femur (associated with partial tibia, AR21802a).—This thin section also shows some diagenetic alteration, but the histology of the outer part of the cortex is visible (Fig. 3.5). There appears to be a distinct band of parallel-fibered bone tissue in the peripheral region, which is interrupted by deposition of several LAGs (Fig. 3.5). Some of the LAGs are very closely associated and could be double LAGs, but this is not certain (Fig. 3.5). The osteocyte lacunae in this region have abundant canaliculi that extend from them. Below this band of tissue, there is a wider region consisting of cccb. The longitudinal sections show cancellous bone tissue in which there are patches of calcified cartilage located in the trabeculae (Fig. 3.6, 3.7).

AR21804 femur fragment(s).—Bone is diagenetically altered. There are large amounts of a dark inclusion (probably manganese) that penetrates the bone wall. The medullary cavity seems to be infilled by a calcite-like material. The organization of the tissue that makes up the bone wall is not visible; however, it is evident that there are several vascular spaces and erosion cavities in the compacta. Thin sections of this specimen are not illustrated.

AR21805 proximal femur.—Unfortunately, this section was not a proper transverse section (i.e., not taken perpendicular to the long axis of the bone), therefore the histology is not comparable to other sections. The osteocytes have been sectioned in different orientations and reveal a narrow outer compact bone wall with GMs and inner more cancellous tissue. The longitudinal section shows several rows of calcified cartilage, but this does not appear to have been fast-growing tissue because the aligned calcified cartilage rows are not numerous (Chinsamy and Warburton, 2021). Several Sharpey's fibers are visible in the preserved articular region of the bone (Fig. 4.1).

QM F41185 partial tibia.—Overall, the compacta is well vascularized with a large amount of secondary osteons. However, bands of lamellar deposits of bone (annuli) are visible between the secondary osteons. At least seven annuli (often with LAGs) are observed (Fig. 4.2). At least three closely spaced growth marks are visible in the outermost region. Different parts of the cross section show different types of bone tissue. In some parts of the compacta there is a dense distribution of secondary osteons that leads to dense Haversian bone (Fig. 4.3). In the region of the trochanter there appears to be a secondary resorption of some of the secondary

osteons, which leads to a more porous textured bone tissue (Fig. 4.4), and in other areas there is a large amount of remodeling in the perimedullary region that leads to a substantially narrow bone wall with several radially organized vascular canals (Fig. 4.5). No calcified cartilage is visible in the longitudinal section of the tibia (Fig. 4.6).

QM F50692 partial tibia.—A thick, well-compacted outer region of the bone wall is present (Fig. 5.1), while the inner perimedullary region appears to be more cancellous (Fig. 5.2). The histology appears variable across the thin section, with different parts of the bone wall having different types of bone tissue. The outer fifth of the compacta consists predominantly of poorly vascularized primary periosteal parallel-fibered to lamellar bone tissue that is interrupted by at least 3–4 LAGs that are associated with narrow annuli. Internal to this outer tissue, the compacta is extensively remodeled, with a large number of secondary osteons present, many of which overlap (Fig. 5.1, 5.2). At higher magnification, several Sharpey's fibers (arrows) are visible near the peripheral margin of the bone wall (Fig. 5.2). In parts of the cross section, secondary reconstruction reaches up to the periosteal margin (Fig. 5.2).

QM F50691 partial tibia.—The bone wall is highly altered by diagenesis, although there is patchy preservation of the original bone tissue (Fig. 6.1). In some areas, at least three growth marks are evident; secondary osteons are also visible (Fig. 6.2). The altered bone has the characteristics of damage caused by bacteria (e.g., Nacarino-Meneses et al., 2021), and there is a large amount of black infilling substance in the bone tissue.

Curiously, bits of plant material are preserved within the medullary cavity of this partial tibia (Fig. 6.3, 6.4). The cross section of plant material shows a central section surrounded by a few smaller cross sections. At higher magnification, the epidermis appears to be quite thick-walled, below which is a thick layer of rounded sclerenchyma, which could suggest a culm of a grass or restio. However, because grasses did not become part of the Australian flora until the Pliocene, this may represent a more recent inclusion or some other plant from the Miocene.

QM F50842a partial femur.—Although this bone also shows evidence of diagenesis, the histological structure can still be deciphered (Fig. 7.1). Overall, the thin sections from this femur demonstrate how variable the histology is across the transverse section of the bone wall, as well as in different parts of the bone shaft. In a single cross section, it is evident that some parts of the bone wall are much thicker than others, whereas other areas are heavily remodeled and result in a narrow bone wall. The outer third of the bone wall appears to
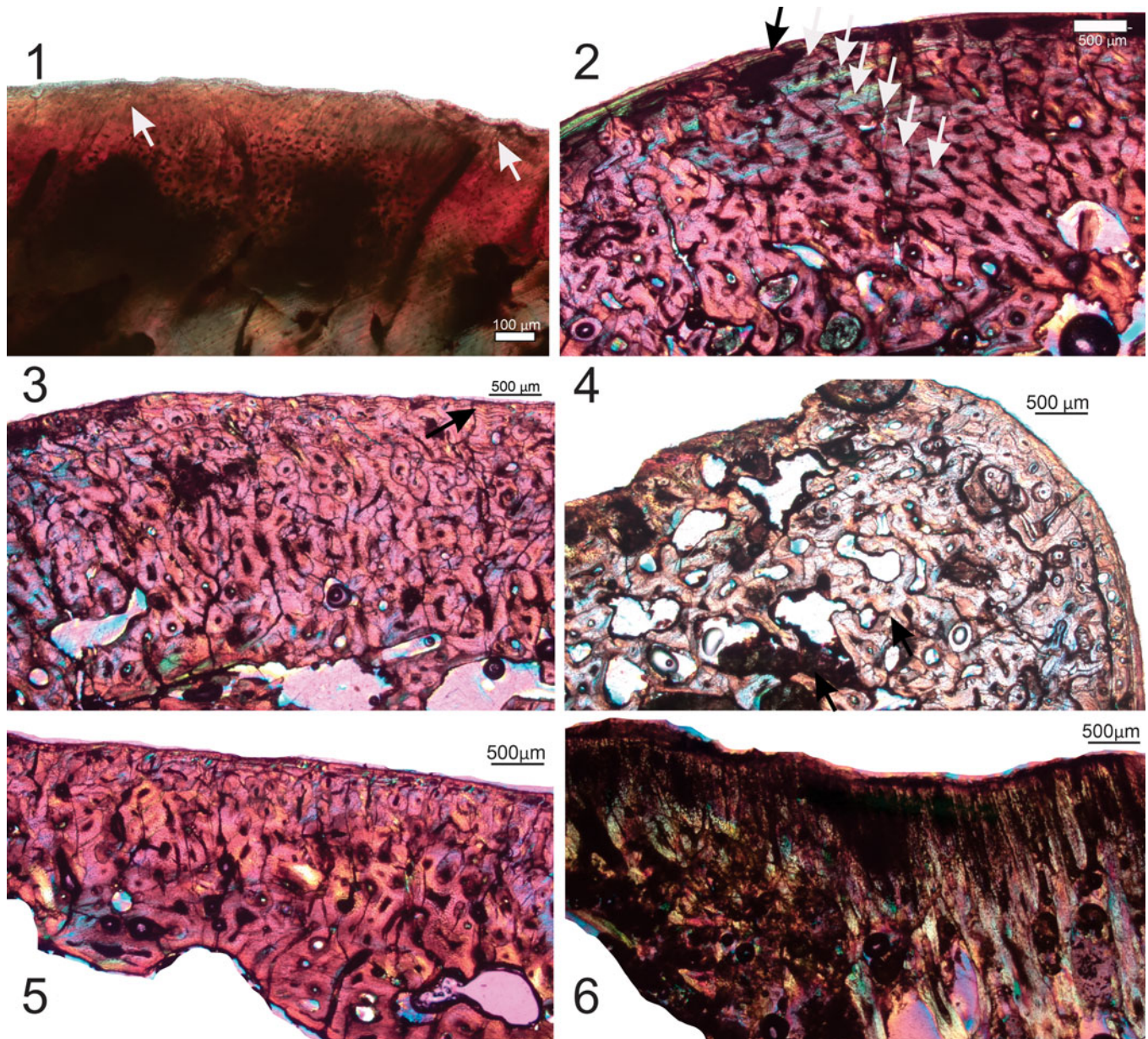
Figure 4. (**1**) Specimen AR21805 CII, proximal femur, arrows indicate Sharpey's fibers. (**2**) QM F41185, thin section AIII partial tibia, black and white arrows indicate growth marks (note the large number of erosion cavities present in the inner parts of the compacta. (**3**) Different part of the tibial thin section AIII from QM F41185 showing the highly remodeled cortex; the arrow indicates a group of closely spaced growth marks. (**4**) QM F41185, thin section AIII tibia; several erosion cavities are visible in the region of the trochanter, giving the bone a porous texture. (**5**) QM F41185, thin section BIII tibia; substantial remodeling in the perimedullary region leads to a narrow bone wall (note the uneven, resorptive endosteal bone margin). (**6**) Thin section CIII; no calcified cartilage is visible in the longitudinal section of the tibia, QM F41185. Images taken under polarized light with a one-quarter-λ compensator.

be composed of a more parallel-fibered to lamellar bone tissue with a mix of short radial and longitudinal vascular canals (Fig. 7.1). This tissue is interrupted by at least five GMs, several of which appear to be groups of double/multiple LAGs (Fig. 7.1). Towards the periosteal margin, there are many closely spaced LAGs (Fig. 7.1). Note that several of the vascular canals appear to cross the LAGs. Internal to this region there is a lot more secondary reconstruction, and several overlapping generations of secondary osteons are visible (Fig. 7.1, 7.2). Large erosion cavities are present nearer to the medullary cavity (Fig. 7.1). The longitudinal section of the bone shows that some endochondral ossification is still

underway, since a narrow band of calcified cartilage is evident along the "articular end" of the femur (Fig. 7.3).

Discussion

Despite substantial alteration by diagenetic factors, the histology of the femora and tibia of *Nimbadon lavarackorum* could be deciphered and has provided substantial information about the overall growth and biology of this diprotodontid. Juvenile individuals have a bone compacta that is composed of a mix of different types of tissues. For example, a large amount of primary bone tissue is present, although in parts of the compacta
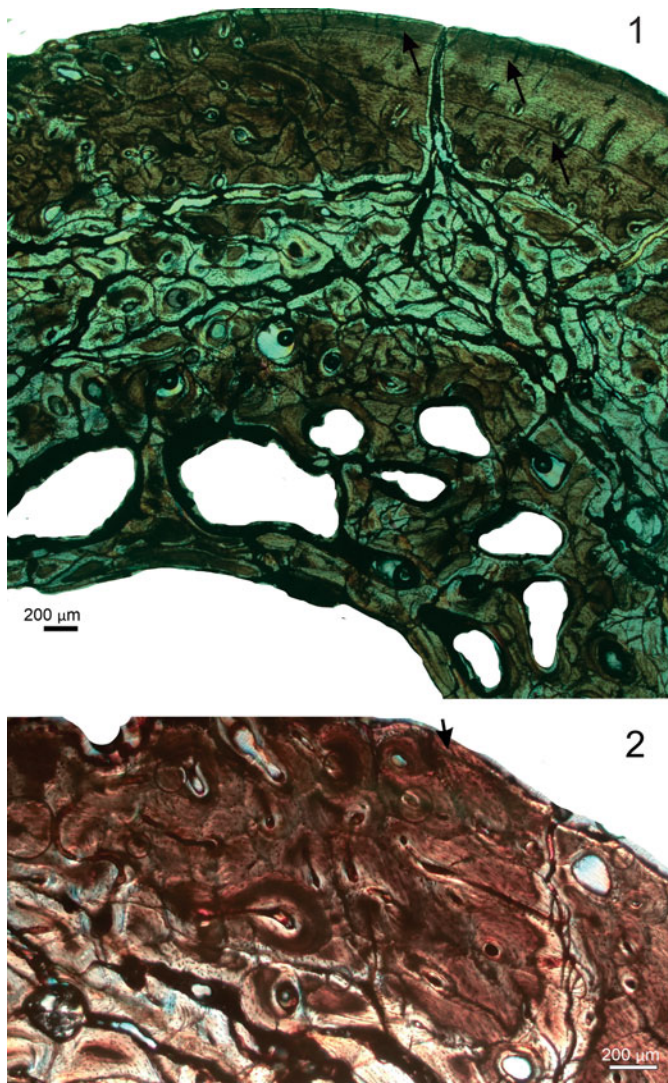
Figure 5. Specimen QM F50692, partial tibia. (**1**) Low-magnification view of thin section BI showing thick bone wall; at least three LAGs (black arrows) are visible in outer part of the compacta. Several erosion cavities are visible in the perimedullary part of the bone wall, which give this region a cancellous texture; (**2**) thin section AIV; clumps of Sharpey's fibers (arrow) are visible towards the peripheral region of the bone wall; secondary remodeling extends right up to the periosteal surface in this part of the bone wall. Images taken under polarized light with a one-quarter-λ compensator.

secondary reconstruction has begun but is not extensive enough to replace the original bone tissue entirely. The endosteal margin is clearly erosive in several of the juvenile specimens, and large erosion cavities are restricted to the endosteal margins. Some parts of the compacta are affected by local functional constraints. For example, attachment sites of muscles have a different histology from the rest of the compacta (Chinsamy and Warburton, 2021).

It is well recognized that the rate at which bone forms results in different types of bone tissues (e.g., fibro-lamellar bone tissue is deposited at a faster rate as compared to parallel-fibered or lamellar bone tissue [Amprino, 1947; de Margerie et al., 2002; Starck and Chinsamy, 2002]). Thus, the presence of large amounts of parallel-fibered bone as the principal primary bone tissue in most of the bones studied, including the

juveniles, suggests that growth in *Nimbadon* occurred at a moderate rate as compared to other juvenile terrestrial mammals (including juvenile kangaroos, which tend to have large amounts of fibro-lamellar bone tissue [Chinsamy and Warburton, 2021]). However, it must be noted that although the bones studied here are juvenile, they may not be very young individuals because substantial bone remodeling and reconstruction have occurred in the bones. Thus, it is quite likely that the earliest stages of growth may have had faster deposition of bone tissue (but this is not visible in the compacta).

Based on partial femur size and morphology, specimen QM F50842a (Fig. 7) could be sub-adult, nearing adult body size. Indeed, although it is a partial specimen, its femur midshaft circumference measurement (62.5 mm) is smaller than those of adult femora (with fused epiphyses evident), which range from 65.5–68.6 mm, and there appears to be a few layers of calcified cartilage in the epiphyseal region suggesting that growth in length was still ongoing at the time of death. However, it is noteworthy that the most recently formed periosteal bone tissue in QM F50842a, as well as in the partial tibia QM F50692, is more lamellar textured, which suggests an even slower rate of bone formation than observed in the smaller (younger) long bones studied.

All the bones display growth marks (GMs) (e.g., some having at least 3–4 GMs in the peripheral region, and the partial tibia QM F41185 has at least seven GMs visible in the outer cortex; Fig. 4.2), which directly indicates that the moderate growth rates of *N. lavarackorum* were periodically arrested (Chinsamy-Turan, 2005). As noted for extant kangaroos (Chinsamy and Warburton, 2021), double/multiple LAGs are visible in some individuals, indicating multiple pauses and resumption of growth in the "unfavorable season" (Castanet et al., 1993). The close spacing of growth marks in the outer cortex of larger bones (e.g., QM F50692, Fig. 5.1; QM F50842a, Fig. 7.1) and the lamellar texture of the bone tissue indicate slow incremental growth and suggest impending skeletal maturity (although the partial femur of QM F50842a still shows some calcified cartilage [Fig. 7.3], indicating that growth in length was still occurring). Thus, as reported for the Western Grey Kangaroo (Chinsamy and Warburton, 2021), we found that appositional growth occurred at a different rate than endochondral growth.

Among modern vertebrates, it is well recognized that the presence of growth marks (single or multiple) indicate that the growth of the animal responded to the prevailing environmental conditions, including food availability and nutritional content, as well as to endogenous physiological factors (e.g., Köhler et al., 2012; Angst et al., 2017). Growth marks recorded in the bones of the Western Grey Kangaroo, *Macropus fuliginosus* (Chinsamy and Warburton, 2021), have been suggested to correspond to periodic decreases and stoppages in growth dynamics that occurred in response to heterothermy that the animals experienced during the harsh summers (Maloney et al., 2011). Similar cyclical growth patterns have been recorded in the Desert Antelope *Addax nasomaculatus* de Blainville, 1816 (Marín-Moratalla et al., 2013). Thus, the presence of lines of arrested growth indicates that the environment in which *N. lavarackorum* lived caused some physiological or nutritional stress periodically (Köhler et al., 2012). Based on paleoecological, biostratigraphic, and biochronological data, the
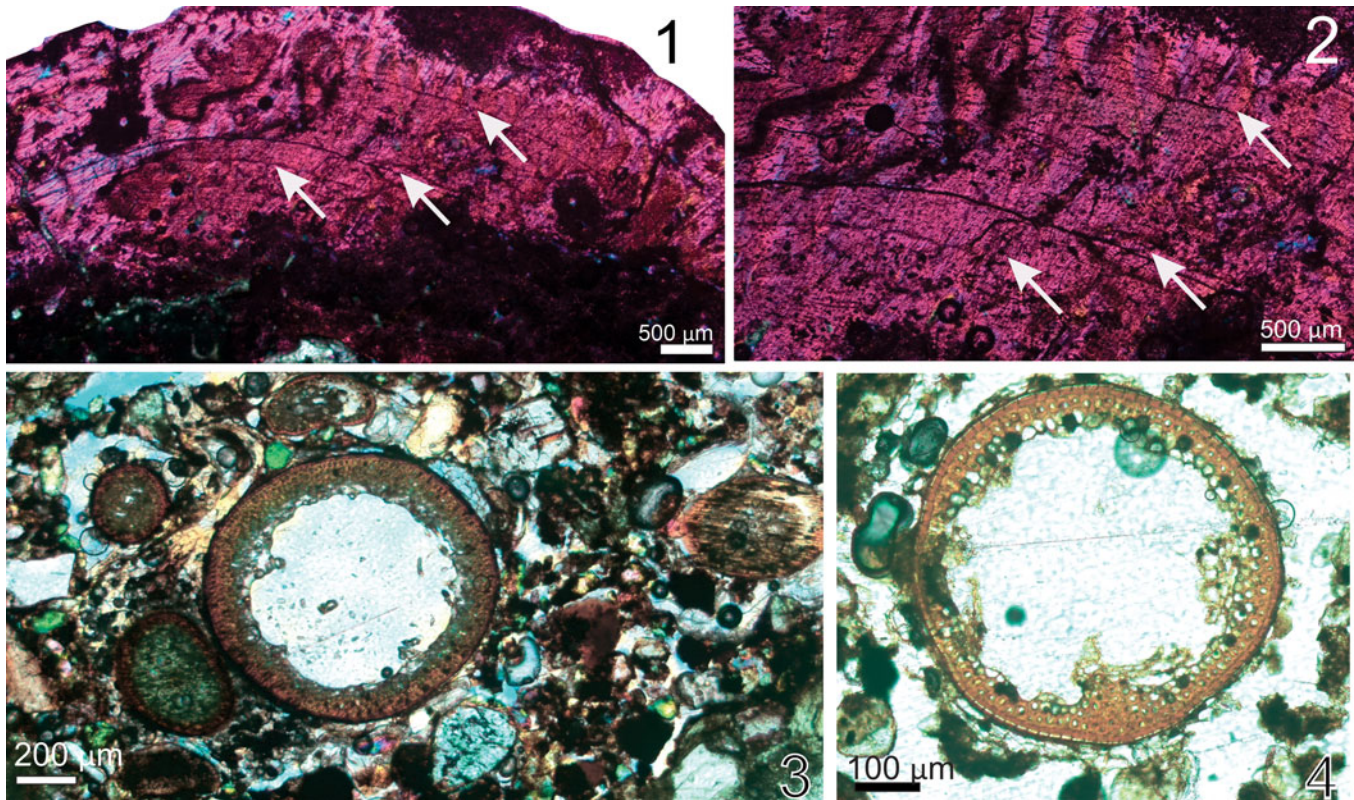
Figure 6. QM F50691, partial tibia. The section is damaged by diagenesis. (**1**) Thin section AIII; at least three growth marks (white arrows) are visible in the compacta; (**2**) higher magnification of (**1**) showing the LAGs (white arrows) and a few secondary osteons; (**3, 4**) thin section BI showing plant material preserved inside the medullary cavity of the bone. Images (**1, 2**) taken under polarized light with a one-quarter-λ compensator; images (**3, 4**) taken under ordinary light.

paleohabitat of *N. lavarackorum* is interpreted to have been rainforest (Foote, 1916; Travouillon et al., 2009; Black et al., 2012a; Zhang and Moise, 2016; Myers et al., 2017). Although the environmental conditions in the Middle Miocene of Australia involved less intense seasonality than today (personal communication to MA by Michael Tyler, 1995, based on fossil frog communities), it is quite likely that there may have been seasonal differences in rainfall because of monsoonal influences in the north of the continent, which might have seasonally affected the type and availability of food. The bilophodont molar morphology of *N. lavarackorum* suggests it was a browser, however, recent dental microwear analyses are inconsistent with obligate folivory and indicate the inclusion of a high proportion of fruit in *Nimbadon*'s diet (Larisa De Santis, personal communication, 2022). Fruit production in the Middle Miocene rainforests of Riversleigh may have been correlated with rainfall and if so, the periodic availability of this highly nutritive food source could have contributed to the periodic increased growth rates, while the seasonal scarcity of fruit may have caused growth cessation and the formation of a LAG. Given that there is increasing evidence that several homeothermic endotherms show cyclical growth, which often is combined with seasonal decreases in body temperature and metabolic rate (e.g., Köhler et al., 2012; Marín-Moratalla et al., 2013), it is likely that the growth dynamics of *N. lavarackorum* also may have been influenced by similar intrinsic physiological factors in conjunction with resource availability. Furthermore, although we cannot be certain, we postulate that the cyclical growth observed in this large extinct

marsupial may have been linked to heterothermy, as documented for the modern Western Grey Kangaroo, *Macropus fuliginosus* (Maloney et al., 2011).

It should also be noted that xenarthrans (e.g., sloths, anteaters, armadillos) all show flexible (cyclical) growth rates (Straehl et al., 2013). Interestingly, Black et al. (2012a) found that, in terms of limb proportions, *N. lavarackorum* was unique among marsupials in having significantly reduced hindlimbs relative to their forelimbs, which is a feature associated in extant prosimians with suspensory climbing behaviors and/or increased pedal friction on vertical surfaces. Exploring the idea that bone compactness and structural geometry is related to lifestyle, two lineages of modern tree sloths (*Choloepus* and *Bradypus*) that are known to have slow arboreal lifestyles were examined (Girondot and Laurin, 2003). They were found to have lower cortical compactness as compared to other similar-sized taxa (Nyakatura, 2012; Montañez-Rivera et al., 2018) and other xenarthrans, and they also show high rates of bone remodeling that appear to be maintained at high levels throughout ontogeny (Montañez-Rivera et al., 2018). Following these results, slow arboreal animals (e.g., tree sloths, lorisid, koala, palaeopropithecids, and *Megaladapis*), which all share suspensory vertical climbing and low metabolic rates, were studied to verify if they would all show low bone cortical compactness (CC) linked to their slow arboreal lifestyle (Alfieri et al., 2021). However, the results showed that extinct tree sloths, koalas, and lorisids all have high CC (Alfieri et al., 2021), following the general mammalian condition (e.g., Kolb et al., 2015).
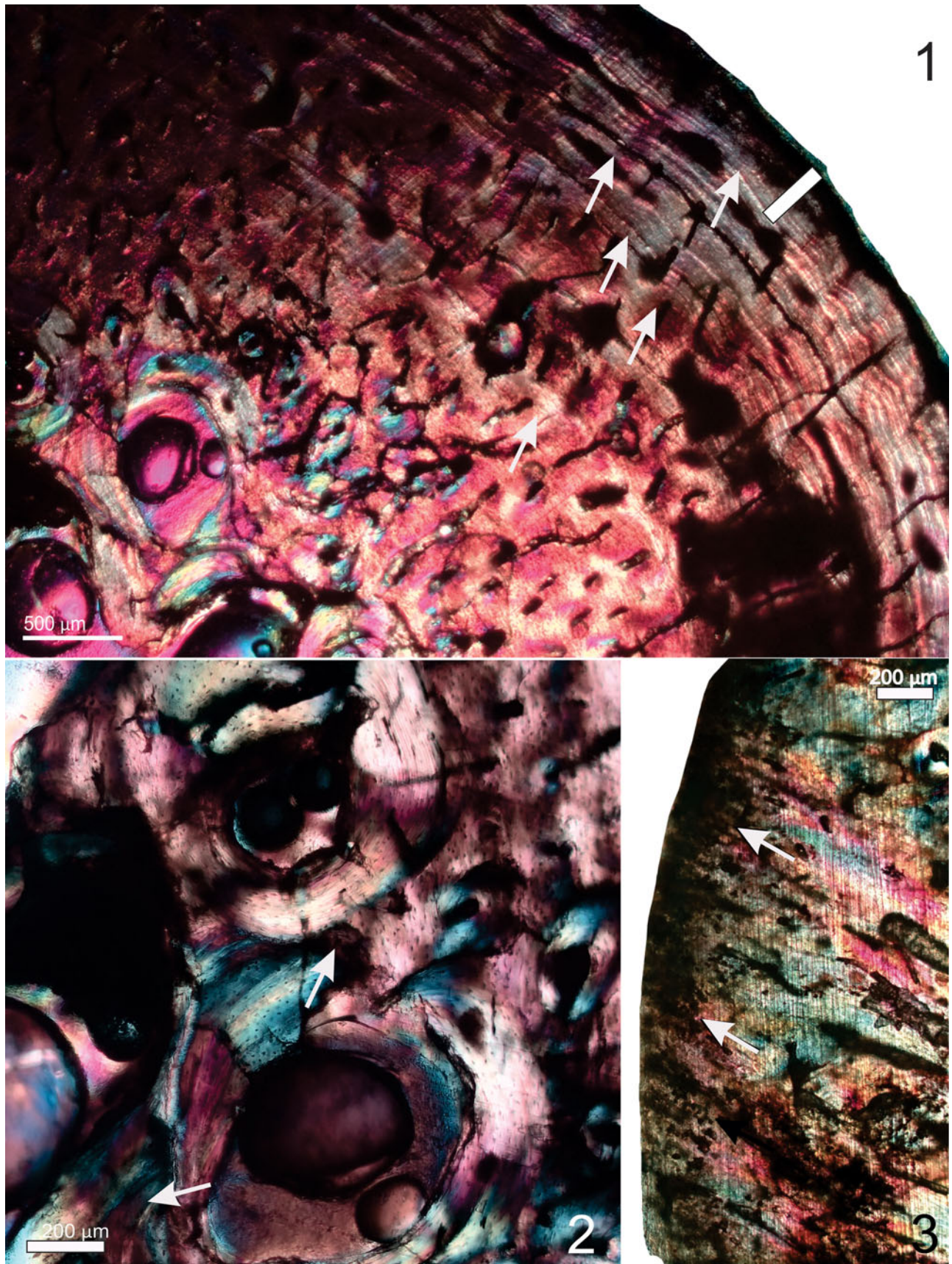
Figure 7. QM F50842a, partial femur. (**1**) Thin section AII; the outer third of the bone wall appears to be composed of more-lamellar bone tissue with a mix of short radial and longitudinally organized vascular canals; several growth marks interrupt the bone tissue; note that several of the vascular canals appear to cross the LAGS (arrows). In the mid-cortical region, a large amount of secondary reconstruction occurs. Many closely spaced LAGs occur in the peripheral region (white bar). (**2**) Thin section AII showing several large erosion cavities (white arrows) that occur nearer to the medullary cavity; overlapping generations of secondary osteons are also visible in this region. (**3**) Thin section DIV; the longitudinal section of the femur shows a narrow region of calcified cartilage (white arrows) at the "articular end" of the bone. Images taken under polarized light with a one-quarter-λ compensator.

Our *N. lavarackorum* sections were rarely complete, therefore we were unable to deduce overall CC, but based on preservation of the bone wall, it is evident that the specimens do not display low CC, and the bone wall appears to be comparable to other terrestrial vertebrates (e.g., Kolb et al., 2015), although clearly not as compact as fossorial animals (Montoya-Sanhueza and Chinsamy, 2017; Legendre and Botha-Brink, 2018; Montoya-Sanhueza et al., 2021b). Note that the significant amounts of cccb observed in the compacta of *N. lavarackorum* stems from the metaphyseal location of the sections, rather than from an adaptation for fossoriality, as observed in, for example, *Ornithorhynchus* (Chinsamy and Hurum, 2006), *Bathyergus* (Montoya-Sanhueza and Chinsamy, 2017), *Dasypus* (Heck, 2019), and *Leitheia* (Kolb et al., 2015). Black et al. (2012a) noted that the hindlimbs of *Nimbadon* are short and robust and far less specialized for climbing than the forelimbs. Furthermore, because of the lack of material, we were unable to verify if, like koalas, *Nimbadon* would have had higher humeral CC as compared to femora.

Interestingly, secondary osteons were not found in the cortex of koalas or in most of the marsupial bones studied (Werning, 2013), yet in our *N. lavarackorum* specimens we observed a lot of secondary reconstruction throughout ontogeny. Notably though, the secondary remodeling did not reach dense Haversian proportions, but in some instances, especially in the larger individuals, overlapping generations of secondary osteons occurred. This seems to be similar to the observations made in other vombatomorphian taxa such as *Vombatus ursinus* Shaw, 1800 and *Diprotodon optatum* Owen, 1838 (Werning, 2013), yet, as previously noted by Werning (2013), it is difficult to determine whether this is a result of large body size, phylogeny, or both. Although they tend not to be extensively developed, secondary osteons have been reported in kangaroos (Chinsamy and Hurum, 2012; Chinsamy and Warburton, 2021), but Doube et al. (2018) did not observe any secondary remodeling in a *Macropus giganteus* femur.

Conclusions

Our histological findings support our hypothesis that *Nimbadon lavarackorum* experienced cyclical growth rates, and that the number of growth rings increased through ontogeny. In addition, we found that, as expected, slower rates of bone deposition were apparent in the bone compacta as the individuals reached adult body size. The presence of seven closely spaced growth marks in the partial tibia, QM F41185, suggests that they needed at least 7–8 years to reach skeletal maturity. Indeed, given the extensive remodeling observed in the bones, it is likely that earlier growth marks may have been removed, suggesting that they may have needed even more time to reach skeletal maturity. The cyclical growth dynamics evident in the skeletal elements indicate that their growth was responsive to prevailing environmental conditions (including resources) in conjunction with intrinsic physiological factors. Bone tissue that formed during the favorable growing season appears to have been deposited at a much slower rate than in modern kangaroos. This observation suggests that *N. lavarackorum* grew at much slower rates, which indicates that it probably had a protracted life span to reach its large body size. Unlike koalas, *N. lavarackorum* has a large amount of secondary reconstruction in their bones, which suggests that they had higher demands for calcium mobilization. Because secondary remodeling is also related to biomechanical stresses during locomotion (e.g., McFarlin et al., 2008), these findings suggest that, unlike koalas, *N. lavarackorum* may have more actively traversed the rainforest canopy in search of fruit.

Declaration of competing interests

The authors declare none.

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