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Primary bone retention in a young adult male with limb disuse: a bioarchaeological case study

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ABSTRACT

Bone mineral and mass are low in limb bones that experience prolonged lack of, or minimal, mechanical stimulation. Cases of ancient human limb paralysis offering an opportunity to examine histological markers of cortical bone modelling and remodelling are rare. To improve our understanding of the spectrum of bone tissue response to its muscular disuse environment in archaeological contexts, we tested whether bone histology in an individual afflicted with long-term loss of muscle function showed unremodelled primary bone due to minimal/absent, mechanical stimulation. We examined cortical bone histology in a 1906–1523 cal BC atrophied post-cranium of a young adult (mid-20s) male who had suffered from Klippel-Feil Syndrome Type III, experiencing minimally paraplegia and potentially complete or intermittent quadriplegia in late childhood/early adolescence. Samples taken from the humeral and femoral midshaft displayed thin cortices and extensive retention of primary bone with only localised Haversian tissue or isolated secondary osteons. The retention of widespread primary bone and thin cortices in this adult individual is evidence for stunted modelling and remodelling due to immobility during early ontogeny. Our bone histology descriptions should be of interest to palaeobiologists investigating the effects of physical inactivity on bone microstructure in fossilised and archaeological human remains.

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1. Introduction

Palaeobiologists, bioarchaeologists, and skeletal biologists can use thin section histology to study the dynamics of bone modelling (change in bone shape and size during growth), remodelling (continuous replacement of old with new bone), growth trajectories (course of bone modelling and cortical drift through ontogeny) and life histories, in specimens that range from millions of years old to modern day in age (Crowder and Stout 2011; Schlecht et al. 2012; Miszkiewicz and Mahoney 2017; Bakalova et al. 2018; Walker et al. 2020; de Buffrénil V et al. 2021; Nacarino-Meneses and Orlandi-Oliveras 2021; Garrone et al. 2021). Bone remodelling is achieved through a balanced activity of Bone Multicellular Units (BMUs) which deposit and resorb bone in the same location, whereas cortical drift occurs during modelling when a bone shaft is formed and resorbed at two opposing bone regions (Jee and Frost 1992; Crowder and Stout 2011; Maggiano et al. 2016). Cortical bone tissue matrix (re)organisation and micro-anatomical features, such as shape and size of BMU vascularity products, can be linked to various factors, including lifestyle and disease (Crowder and Stout 2011; de Buffrénil V et al. 2021).

In the experimental vein, we know that paralysis, muscle weakness, and other forms of immobilisation (e.g. physical inactivity and weightlessness) are detrimental to a young growing skeleton, leading to stunted bone length and width, and thin cancellous and cortical bone, due to a significant lack of biomechanical stimulation to bone modelling and remodelling processes (Sibonga et al. 2000; Trebacz 2001; Sievänen 2010). Congenital abnormalities such as Klippel-Feil Syndrome (KPS) present with a fusion of the cervical vertebrae which carries the risk of injury to the spinal cord (Vaidyanathan et al. 2002). From a biomechanical viewpoint, while substantial data exist for quantitative cortical and cancellous bone microarchitecture deterioration in disuse contexts, including 'disuse osteoporosis' (Donahue et al. 2006; Alexandre and Vico 2011; Ghasem-Zadeh et al. 2021), cortical bone tissue matrix examination histologically has been largely limited to post-mortem cases (Goldman et al. 2009; Schlecht et al. 2012). Outside of samples harvested clinically or post-mortem, histological analyses of ancient human skeletal remains representing rare examples of congenital disorders, and/or paralysis or immobilisation, can be a useful source of understanding the spectrum of bone tissue microscopic manifestation in extreme cases of limb disuse specimens (Kozłowski and Piontek 2000; Miszkiewicz et al. 2020), and provide methodological suggestions for fragmented archaeological and fossil specimens where limb immobilisation is suspected. However, there have been very few such cases published, with one notable example of a Medieval (12–14th century) Polish 35–50-year-old male from Gruczno who had suffered from neurogenic acute poliomyelitis that led to asymmetric lower limb atrophy (Kozłowski and Piontek 2000). Kozłowski and Piontek (2000) used histology to find evidence for abnormal osteocyte lacunae density in this individual's atrophied metatarsal. Here, we contribute further to this research area. The objective of this study was to test whether cortical bone histology of the femur and humerus in a young ancient individual afflicted with long-term loss of muscle function should reveal widespread primary bone due to minimal, if not absent, mechanical

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stimulation otherwise driving bone remodelling. To the best of our knowledge, there are no case studies that directly describe cortical bone histology of humeral or femoral samples from a KPS III-associated paraplegia/quadriplegia in the archaeological and palaeoanthropological record.

2. Materials and Methods

In collaboration with, and permissions from the Vietnamese Academy of Social Sciences, we had a unique opportunity to examine bone histology in human skeletal remains of a Mán Bạc individual (ID MB07H1M9) from 1906 to 1523 cal BC Northern Vietnam [Figure 1].

The analysis was conducted by abiding to the ethical guidelines of the American Association of Biological Anthropologists and the Australasian Society for Human Biology, with no ethics clearances required due to the antiquity of the subject. This individual was a young adult (20–29 years old) male of mixed East Asian and Australo-Papuan ancestry, diagnosed to have suffered from KPS Type III (KPS III) experiencing minimally paraplegia and potentially complete or intermittent quadriplegia in late childhood or early adolescence (Oxenham et al. 2009; Tilley and Oxenham 2011). The young adult age estimate had been obtained by Oxenham et al. (2009) using as many standard anthropological methods of age-at-death estimation as possible (recommended by Buikstra and Ubelaker 1994). Specifically, Oxenham et al. (2009) had found: >30% fusion of ecto- and endo-cranial sutures (Meindl and Lovejoy 1985), molar wear (Gilmore and Grote 2012), and complete fusion of long bone epiphyses where preserved (Buikstra and Ubelaker 1994). All these indicators had suggested this Mán Bạc individual died during their third decade.

The skeletal pathology was previously extensively described and discussed part of a differential diagnosis (Oxenham et al. 2009; Tilley and Oxenham 2011). Notably, the individual's C1-T3 vertebrae showed complete ankylosis, and the post-cranium presented with bone atrophy in addition to joint surface changes consistent with osteoarthritis (Oxenham et al. 2009; Tilley and Oxenham 2011). The maximum mid-diaphyseal diameter of this individual's humerus and femur measured 15 mm and 13.8 mm, respectively (Oxenham et al. 2009). Compared to an average calculated for a larger 'normal' Mán Bạc adult sub-sample (see Table 1 in Oxenham et al. 2009, p. 111), the MB07H1M9's data indicated significant atrophy (30.9% thinner humerus, and 49.5% thinner femur). Severe mobility restrictions including a complete loss of voluntary lower limb movement, reduced upper body movement, and neck flexion constraints were concluded (Oxenham et al. 2009; Tilley and Oxenham 2011). In this case study, we turn our attention to cortical bone histology manifestation of this individual's limbs.

Using standard light microscopy techniques (transmitted, linearly polarised, and lambda compensated polarised light), we examined bone matrix composition in undecalcified and unstained thin sections of approximate 100 µm thickness produced from small (1 × 1 cm) cortical samples extracted from the right posterior femoral midshaft and the left anterior humeral midshaft (Miszkiewicz 2016). The specific bone histology variables we studied were: primary and secondary (Haversian) bone type and its spread (widespread/isolated), type of bone vascularity (primary Haversian canals, simple radial canals, secondary osteons) (Crowder and Stout 2011), and collagen fibre orientation (dark, bright, alternating osteon 'morphotypes', Skedros et al. 2013). Given the research objective, we designed this study with qualitative analyses in mind, but it is also possible to conduct histomorphometric (quantitative) analyses to gather data about the size of BMU products (Miszkiewicz 2016). However, because our bone sampling procedures were limited given the curatorial and osteological significance of these remains, our samples did not yield enough secondary osteons (the recommended standard is 25–50 osteons per sample – Stout and Crowder 2011) to produce data that would be meaningfully reflective of bone remodelling activity across an entire bone cross-section. Therefore, we took preliminary area measurements (in μm^2) of 16 intact secondary osteons and Haversian canals (Miszkiewicz et al. 2022) seen in the humerus. We used the 'free-hand selection' tool in FIJI v. 153f51 to take three repeated area measurements an average of which is reported. The raw dataset is made available via figshare (Walker et al. 2022).

3. Results

Remarkably, the preservation of intra-cortical bone microstructure in the extracted samples was comparable to that of modern clinical biopsies, except for a band of post-mortem damage lining the endosteal and periosteal borders [Figures 2, 3]. This is a feature

Figure 1. Image showing the skeletal remains of MB07H1M09 individual *in situ*. Limb bones are disproportionally gracile compared to the skull. (Tilley and Oxenham 2011, p. 37, reprinted with permission from Elsevier under licence 5225011167999).

commonly and unavoidably found in histology sections made from archaeological bone samples (Hackett 1981). The occurrence of diagenetic alteration endosteally and periosteally is thought to reflect the early stages of post-mortem decomposition where, respectively, bacteria originating from the gut, and micro-organisms originating from the soil, start bone destruction (Hackett 1981; White and Booth 2014; Kontopoulos et al. 2016). As such, we were unable to examine the obscured bone histology in those section regions. We acknowledge that the occurrence of this diagenetic alteration limits our ability to read the entirety of each section. It is possible that useful information about bone tissue matrix organisation in this individual remains 'hidden' in those regions. However, we were able to observe that the intra-cortical bone in both the humerus and femur samples retained primary unremodelled bone punctuated with primary osteons and simple radial vascular canals [Figures 2–5].

In the humerus, in particular, the primary lamellar bone area formed an endosteal lamellar pocket (ELP) (Maggiano et al. 2011). The humerus sample displayed only localised concentration of secondary osteons, which reflected Haversian tissue (with several generations of secondary osteons), as well as instances of isolated secondary osteon units [Figures 2–4], all of which showed largely homogenous dark and sometimes alternating collagen fibre orientation. The average intact osteon area in this sample was 42,561.108 μm^2 (SD = 11,254.575), whereas the associated Haversian canals averaged 3,996.494 μm^2 (SD = 1,583.716) (see raw dataset – Walker et al. 2022). There was no evidence for widespread porosity and extensive coverage of resorption cavities resulting from BMU activity expected in cases of prolonged limb immobilisation. Only at least three isolated resorption cavities located on the anterior bone surface were seen [Figure 2]. Compared to the femur, the humeral sample showed more variability in bone microstructural composition.

In the femoral sample, layers of primary lamellar bone of avascular matrix were contained within a thin cortical wall. Some evidence for secondary osteons was seen immediately to the periosteal border [Figure 5]. Shaft cross-section, diameter, and length data for the post-cranial bones of this individual were previously reported to confirm bone atrophy (Oxenham et al. 2009). This was further reflected in the present histology samples. For example, the femoral section showed poorly developed margins. A typically raised and characteristic anatomical landmark of *linea aspera* for the insertion of gluteus muscles was essentially not expressed [Figure 5].

4. Discussion with Conclusions

Our microscopic analysis confirmed that bone growth was stunted in this individual as is evidenced by poor modelling and remodelling patterns. In addition to the previously noted (Oxenham et al. 2009; Tilley and Oxenham 2011) atrophied limbs and long bone thin cortices, this histological bone manifestation is likely due to immobility occurring early in ontogeny. During phases of modelling and remodelling in the first two life decades, long bone diaphysis re-shapes in response to biomechanical, dietary, and environmental stimuli (Robling et al. 2006). Primary and secondary bone matrix is deposited and undergoes active resorption known as 'modelling drift', whereby a bone shaft essentially 'drifts' in

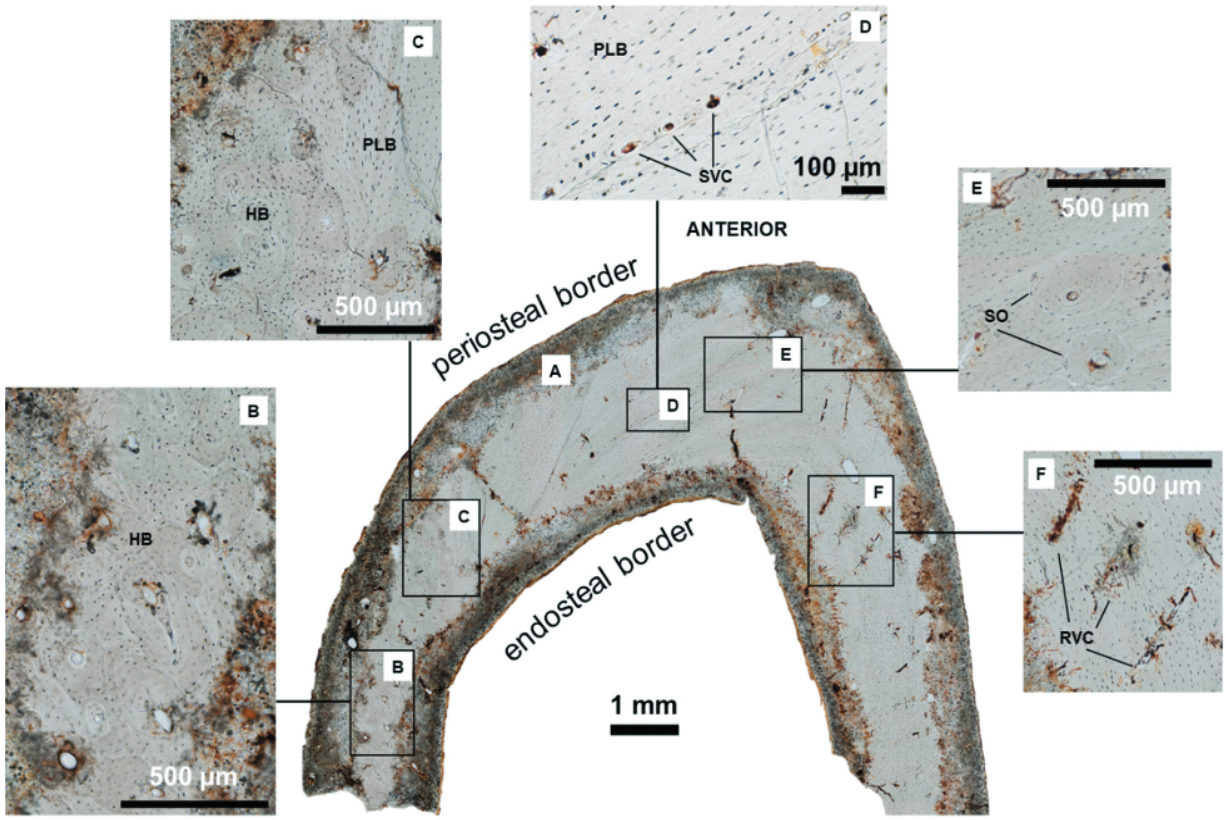

Figure 2. A: section through the anterior aspect of the left humeral midshaft in the MB07H1M9 individual. B: Haversian bone (HB) on the more lateral aspect of the section. C: remnants of HB seen transitioning into primary lamellar bone (PLB). D: PLB with simple vascular canals (SVC). E: isolated secondary osteons (SO) punctuating PLB. F: radial vascular canals (RVC) seen across PLB towards the medial aspect of the section. This image montage was taken using normal transmitted light microscopy. Because the section is unstained, the colouration is that of the current condition of the skeletal remains. The brownish discolouration following periosteal and endosteal borders is due to extrinsic post-mortem factors.

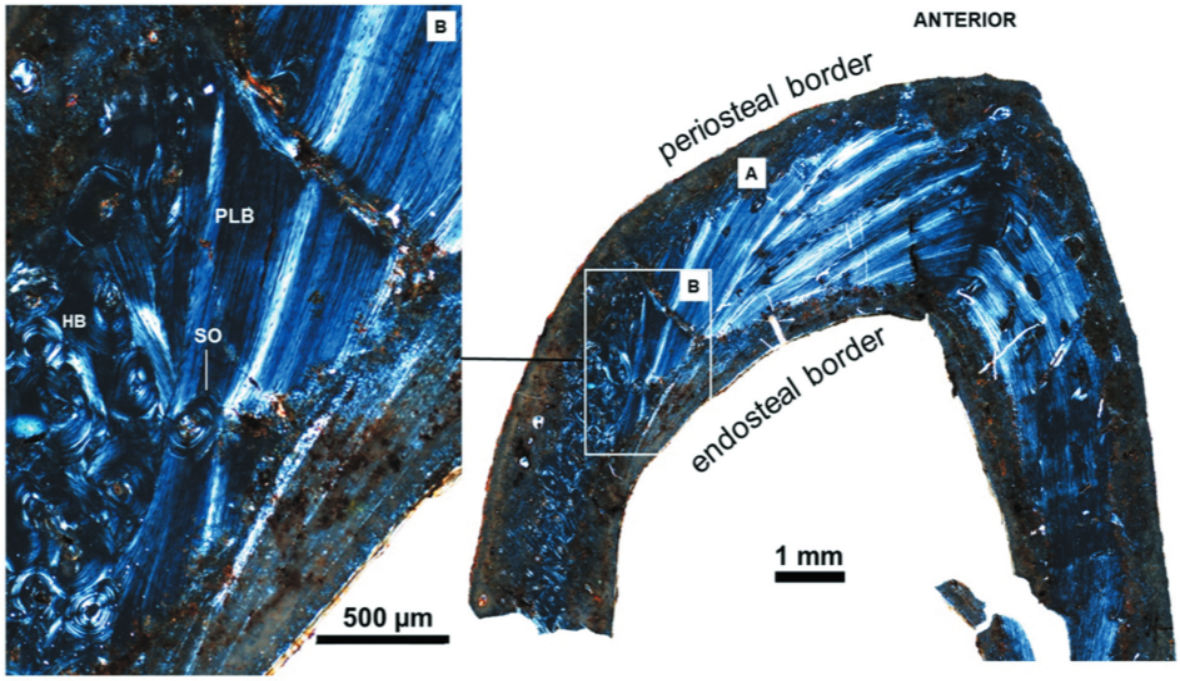

Figure 3. A: same section as shown in Figure 2 (through the anterior aspect of the left humeral midshaft in the MB07H1M9 individual) but viewed using linearly polarised light microscopy. B: remnants of Haversian bone (HB) on the more lateral aspect of the section transitioning into primary lamellar bone (PLB), and showing isolated secondary osteons (SO) beginning to remodel the PLB. Polarised light clearly identifies widespread successive primary lamellar bone layers (note the collagen birefringence). The brownish discolouration following periosteal and endosteal borders is due to extrinsic post-mortem factors.

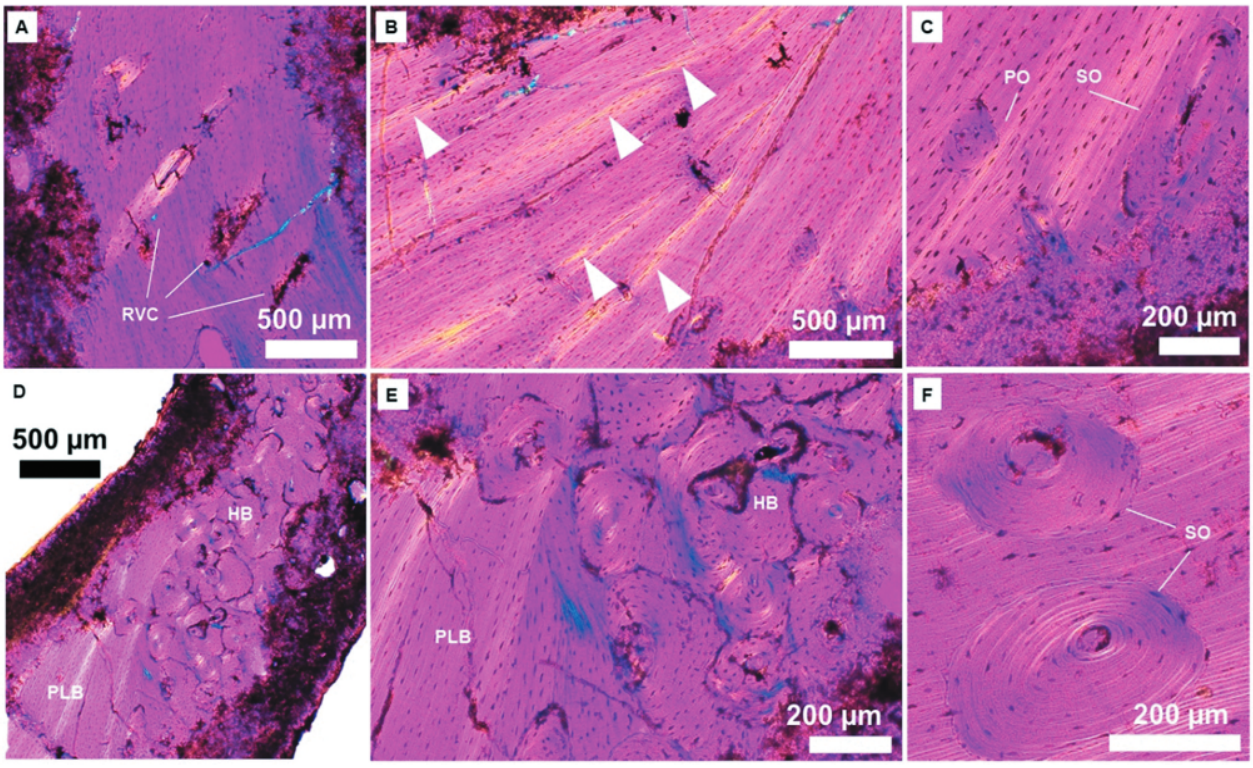

Figure 4. Series of histology regions of interest captured from across the humerus section shown in Figures 2 and 3, using lambda compensated polarised light to highlight lamellar organisation. A: radial canals in the primary lamellar bone (PLB). B: successive layers of PLB. C: isolated primary and secondary osteons punctuating PLB. D: localised Haversian bone transitioning into PLB. E: close up of the transition between HB and PLB. F: isolated secondary osteons occurring sporadically across PLB. The brownish discolouration following periosteal and endosteal borders (particularly seen in D) is due to extrinsic post-mortem factors.

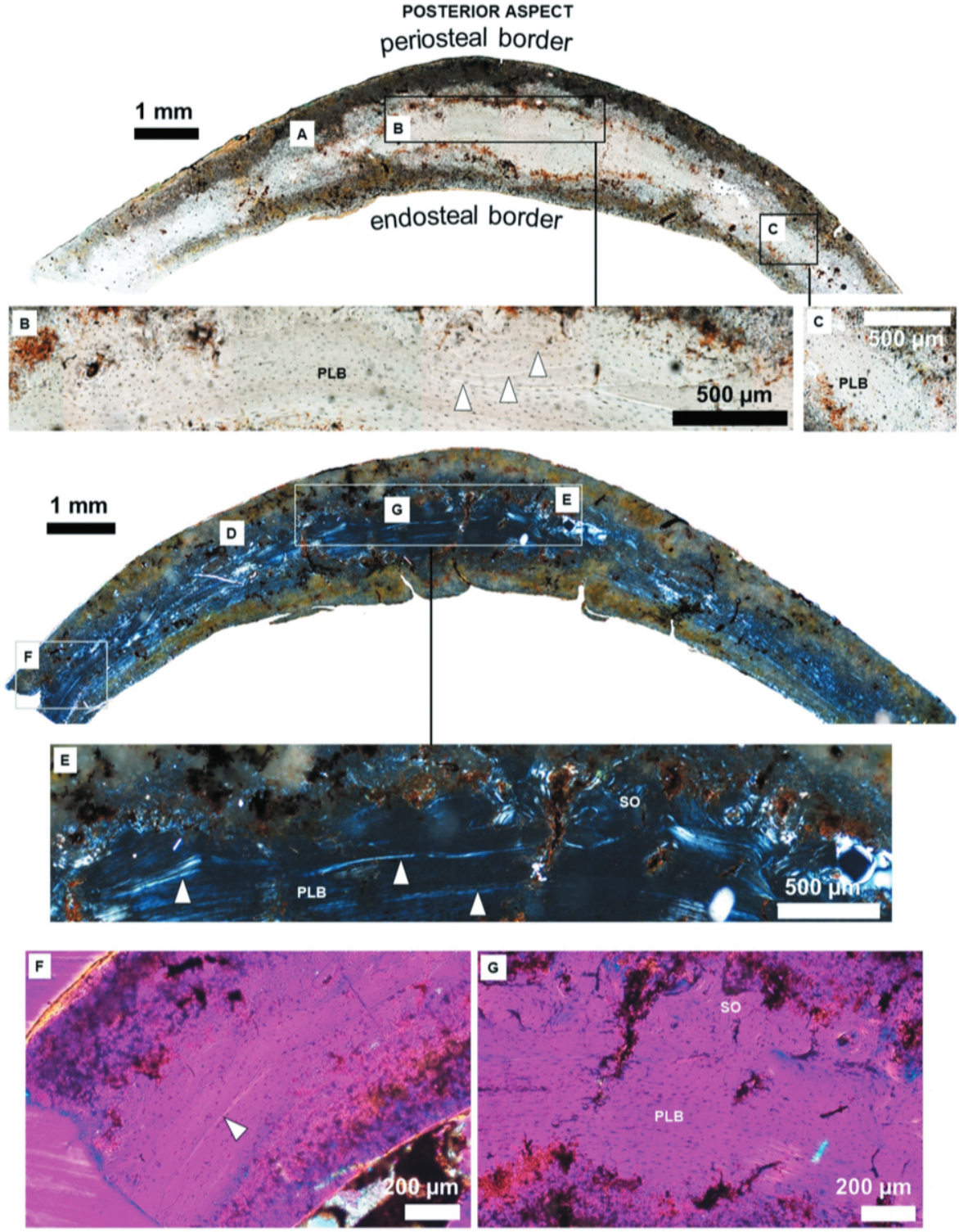

Figure 5. Montage of a series of histology images taken from a posterior femur section in the MB07H1M9 individual. A: section viewed using transmitted light. B: primary lamellar bone (PLB) layers marked with white arrow markers. C: PLB continues laterally. D: section viewed using linearly polarised light. E: PLB layers marked with white arrow markers, along with secondary osteons (SO) occurring closer to the periosteal border of the section. F: bone histology on the more medial aspect continues to retain PLB layers as viewed using lambda compensated polarised light. G: mostly avascular PLB with SO structures occurring closer to the periosteum. The brownish discolouration following periosteal and endosteal borders is due to extrinsic post-mortem factors.

a transverse plane as cortical bone is formed and resorbed at two opposing cross-section regions (Jee and Frost 1992; Maggiano et al. 2016). Endosteal lamellar pockets (ELP) are regions of new bone that are minimally impacted by remodelling as a result of these drifts because there is a time lag between primary bone deposition and subsequent remodelling events (Jee and Frost 1992; Maggiano et al. 2015). Relatively older individuals with full biomechanical capacities would no longer show ELPs due to ageing related resorption of endosteal bone and mechanically stimulated remodelling of primary bone (Maggiano et al. 2015). Because we observed an ELP

in the anterior humerus, it indicates that the cortical bone of the Mán Bạc individual had begun modelling and likely experiencing cortical drift, which did not fully remodel despite this individual's age, and likely due to the absent or minimal mechanical stimulation otherwise underlying subsequent remodelling events. However, the lack of further periosteal formation, and low remodelling of the younger lamellae, suggests limited biomechanical loading in the later stages of the early ontogeny. The large homogeneity in longitudinal collagen fibres seen in secondary osteons also reflects little variation in mechanical signals. Otherwise, we would expect a range of osteon birefringence reflecting a combination of compressive and tense forces (Skedros et al. 2013). Isolated secondary osteons within the ELP of the humerus point to some mechanical loading stimulation (Pitfield et al. 2017). However, their localised and sporadic occurrence may equally be a product of mineral homoeostasis and bone tissue maintenance. The considerable remodelling as indicated by regions of Haversian tissue, but not associated with modelling drifts, also suggests biomechanical function in early life but not active throughout all three decades of this individual's life. Otherwise, replacement of primary bone with widespread Haversian bone tissue would be expected (Pitfield et al. 2017).

Bone histology in the femoral sample was largely that of primary lamellar bone, but with very little vascularity through primary osteons and simple vascular canals. The difference in both histology and section margins between the humerus and the femur here confirm this individual had some mobility of the upper limbs, but limited or comparably no mobility of the lower limbs. The latter likely occurred early in ontogeny as only very limited secondary osteons can be seen in the sub-periosteal region of the posterior femur. The lack of *linea aspera* development, compared to better-defined muscular attachments on the humerus (e.g. the deltoid tuberosity, see Oxenham et al. 2009), further support this interpretation. A typical human femoral midshaft modelling drift subdivided by ontogenetic stages occurs: posteriorly and medially in toddlers, antero-laterally in late childhood, and laterally in adults (Goldman et al. 2009; Maggiano et al. 2015). As such, the poor femoral bone growth in the MB07H1M9 individual would have stunted at a toddler or early childhood stage.

These histological observations related to interrupted limb mobility as a result of KPS III spinal cord injury are consistent with clinical cases where limb function is intermittent (Gupta et al. 2007). Remarkably, the individual we examined survived for about three decades despite 'carrying' primary bone and an atrophied post-cranium. The support and care system this individual would have received is covered elsewhere (Tilley and Oxenham 2011), but we highlight the possibility of primary cortical bone retention in cases of KPS III paralysis, and muscle disuse generally. While we did not observe evidence for disuse osteoporosis, the retention of primary bone in an adult skeleton is testament to the significant biomechanical needs in the growing human skeleton. Without a significant mechanical input during the first two decades of growth, the long bones of this individual's post-cranium stayed 'pencil-like' (Robling et al. 2006) with very limited secondary bone remodelling. The retention of primary bone in this adult skeleton agrees with our prediction based upon the gross anatomical gracile expression of this individual's post-cranium. As such, our case study provides future methodological avenues for palaeobiological investigations into bone remodelling and modelling from atrophied skeletal remains where immobilisation and/or paralysis are suspected. Where curatorial permissions for destructive analyses are granted, small cortical bone samples could be extracted and examined histologically for evidence of primary and secondary bone tissue composition and remodelling characteristics. This type of microscopic information could help resolve questions surrounding the timing of atrophy development in evolutionarily significant cases such as the Shanidar 1 Neanderthal's (*Homo neanderthalensis*) post-cranial pathologies (Lietava 1988). Checking for bone tissue type microstructurally could clarify whether these abnormalities occurred during growth or adulthood (Trinkaus and Zimmerman 1982).

We acknowledge this short project has a series of limitations as it centres on a specimen for whom biomedical markers and medical records, along with life history information, are not possible to obtain. It would have been useful to also have a rib sample to concurrently evaluate histology in a bone with minimal biomechanical load, but we respected the curatorial significance of these remains by sampling only a limited number of elements. However, future research of similar study design to ours, where larger sample size is available, might want to include the rib in analyses. The presented bone histology descriptions should be of interest to palaeobiologists, bioarchaeologists, and skeletal biologists who research the spectrum of cortical bone microarchitecture manifestation in limb disuse contexts.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availablity statement

Qualitative descriptions of histology are shown in the manuscript alongside histology images. Raw measurements of secondary osteons are available from figshare via Walker et al. (2022) *MB07H1M9 humerus osteon measurements* https://doi.10.6084/m9.figshare.18131063.

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