

The five digits of the giraffe metatarsal

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Evolution has shaped the limbs of hoofed animals in specific ways. In artiodactyls, it is the common assumption that the metatarsal is composed of the fusion of digits III and IV, whereas the other three digits have been lost or are highly reduced. However, evidence from the fossil record and internal morphology of the metatarsal challenges these assumptions. Furthermore, only a few taxonomic groups have been analysed. In giraffes, we discovered that all five digits are present in the adult metatarsal and are highly fused and modified rather than lost. We examined high-resolution micro-computed tomography scans of the metatarsals of two mid and late Miocene giraffid fossils and the extant giraffe and okapi. In all the Giraffidae analysed, we found a combination of four morphologies: (1) four articular facets; (2) four or, in most cases, five separate medullary cavities internally; (3) a clear, small digit I; and (4) in the two fossil taxa of unknown genus, the presence of external elongated grooves where the fusions of digits II and V have taken place. *Giraffa* and *Okapia*, the extant Giraffidae, show a difference from all the extinct taxa in having more flattened digits tightly packed together, suggesting convergent highly fused digits despite divergent ecologies and locomotion. These discoveries provide evidence that enhances our understanding of how bones fuse and call into question current hypotheses of digit loss.

ADDITIONAL KEYWORDS: digit loss – fossil – giraffe – Giraffidae – limb evolution – metatarsal – micro-CT scan – Miocene – pentadactyly – ungulate.

INTRODUCTION

In mammals, two types of embryonic structures are encountered throughout ontogeny. The first structures are those found only in the embryos and are lost in the adult. For example, the major part of Meckel's cartilage, which is observed as ossified in basal mammal-like fossils (Ji *et al.*, 2009), mostly deteriorates during embryonic morphogenesis of the extant mammalian middle ear (Rowe, 1996; Sánchez-Villagra *et al.*, 2002). The second embryonic structures, comprising the majority, are still present in the adult but altered from their original form. From the example above, most of Meckel's cartilage deteriorates to separate the ear from the jaw, but the caudal part of the mammalian Meckel's cartilage ossifies into the malleus and incus, forming the two additional mammalian ossiles (Anthwal, *et al.*, 2013, 2017). Comparative anatomy books are full of examples of the fates of embryonic structures (e.g.

Carroll, 2005; Held, 2009; Shubin, 2009), and thorough functional, palaeontological and embryonic work is necessary to understand these fates.

The ungulate limb has posed a curious case. Although it was originally hypothesized that five digits are present embryonically and are lost or reduced throughout development, recently it has been shown that, for example, in the anterior limb of the horse, the adult retains all five digits but that they are altered in many ways (Solounias *et al.*, 2018). In other words, although the horse appears to be monodactyl, it is still pentadactyl because it retains all digits throughout ontogeny, fusing and modifying them rather than losing them (Solounias *et al.*, 2018). This study has recently been corroborated by an early horse embryonic study, which showed that the horse indeed begins its development with five digits (Kavanagh, *et al.*, 2020).

Artiodactyla are a clade of hoofed mammals colloquially referred to as 'even-toed ungulates', but are more appropriately characterized as being paraxonic, or where the centre of mass falls between digits III

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and IV (Groves & Grubb, 2011). Ruminant artiodactyls (Ruminantia), in particular, are ungulates with cranial appendages (i.e. horns, antlers or ossicones), and extant families include Cervidae, Bovidae, Antilocapridae and Giraffidae. The metatarsals of the ruminant foot are usually a single structure rather than separate bones (Rios *et al.*, 2016). The metatarsal is composed of the cannon bone, which is formed by the fusion of digits III and IV (Janis & Scott, 1987). In the adult, there are two cavities inside the metatarsal, and the fusion line is observable on the surface. The distal keels of the fused metatarsals and the two independent toes confirm this: one metatarsal and two toes inferiorly (Rios *et al.*, 2016).

Developmentally, artiodactyl digit reduction has been attributed to both increased embryonic cell death around the digits (camel) and developmental repatterning (pig) (Cooper *et al.*, 2014), suggesting that digit I was predisposed to loss and that paraxony might have evolved twice (Cooper *et al.*, 2014). However, the fossil record challenges current hypotheses that the ruminant metapod is simply the fusion of digits III and IV, with the other digits being lost. A previous study of a tragulid-like ancestor shows more digits present (Janis & Scott, 1987) and implies that digits II and V fit in the medial and lateral grooves separating the four articular facets for digits II and V; digits were separate or lost, but the grooves remained. However, this study failed to consider the four proximal articular facets as four actual digits. Furthermore, as we explain in this study, these facets alone might not always represent the four metatarsals. Further evidence from fossils in other ruminant families, combined with analyses of internal anatomy, are necessary to understand the evolutionary shift from five digits to two, testing whether digits have been completely lost or whether the digits are maintained in the adult but have been modified significantly through fusion of digits.

In this study, we examine whether three of the five digits are lost or are retained but modified in the adult proximal metatarsal of Giraffidae, an understudied clade of artiodactyls. Giraffidae were abundant in the middle and late Miocene of Eurasia and Africa, reaching ~53 species (Hamilton, 1978; Solounias, 2007; Rios *et al.*, 2016). During the Pliocene and Pleistocene,

the numbers of Giraffidae declined starkly, and today only the giraffe (*Giraffa* Brisson, 1772, with four very closely related species; Fennessy *et al.*, 2016) and the okapi [*Okapia johnstoni* (Lankester, 1901)] survive in Africa. Fifteen years ago, when N.S. was studying a Miocene fossil giraffid metatarsal (†YGSP 21930) from the Siwaliks of Pakistan, it was noticed that the five digits were clearly observable from external features of the facets and grooves and, in particular, that a small peg could potentially be digit I, inspiring deeper investigation of the pentadactyl hypothesis.

MATERIAL AND METHODS

INSTITUTIONAL ABBREVIATIONS

AMNH, American Museum of Natural History, New York, NY, USA; MCZ, Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA; NS, Nikos Solounias osteology collection; YGSP, The Yale/Geological Survey of Pakistan collection.

SPECIMENS

All specimens are listed in Table 1. †YGSP 21930 was collected by N.S. and is a new genus and species, yet to be named, from the Chinji Formation of Pakistan. It is a proximal left metatarsal fragment from locality Y0454 and dated to ~11.664 Mya. †YGSP 28199 is also of a new and different genus and species, yet to be named, from the Chinji Formation of Pakistan. It is from locality Y0754 and of age 11.520 Mya. These two fossils are housed at the Peabody Museum at Harvard, on loan from the Geological Survey of Pakistan. *Okapia johnstoni* (AMNH 51196) and two specimens of *Giraffa camelopardalis* (NS 289; AMNH 83458) were also analysed (Table 1). The external features of the extant outgroup taxa, including *Hyemoschus aquaticus* Ogilby, 1841; Supporting Information, Fig. S1 (Tragulidae; MCZ 220395 and AMNH 53640), and the extinct giraffid †*Samotherium boissieri* Forsyth Major, 1888; Supporting Information, Fig. S2 were also observed. Images are available in the Supporting Information (Appendix S1).

Table 1. List of specimens and scanning parameters

Species	Specimen identity	Voltage (kV)	Current (µA)	Voxel size(mm)
<i>Okapia johnstoni</i>	AMNH 51196	109	62	0.03186721
<i>Giraffa camelopardalis</i>	NS 289	150	61	0.04503476
<i>Giraffa camelopardalis</i> *	AMNH 83458	88	78	0.05023628
Fossil	†YGSP 21930	111	80	0.03444403
Fossil	†YGSP 28199	115	89	0.0384953

*No copper filter was used.

MICRO-COMPUTED TOMOGRAPHY SCANNING

All specimens were mounted in foam and scanned using the high-resolution Nikon H225 ST micro-computed tomography (μ CT) scanner at Yale University. Scans of each specimen were obtained using a 0.1 mm copper filter, which facilitates stronger X-ray penetration without oversaturating the image. Scan parameters varied for each specimen, but they were set to ensure X-ray penetration and to maximize the distribution of greyscale values (Table 1).

IMAGE PROCESSING AND SEGMENTATION

Raw output from the scans was reconstructed using in-house Nikon software and imported into VGSTUDIO MAX v.3.3 (2014; Volume Graphics GmbH, Heidelberg, Germany). In the fossil scans, medullary cavities were clearly distinct from the rest of the bone as darker structures with clear boundaries. The cavities were carefully traced and segmented by hand in each image slice ($N = 3143$) of the μ CT-scan image stack. Boundaries of fused regions in digits III and IV were determined by cracks in the external bone, although they were clearly separated in the proximal end. Each medullary cavity represented a distinct digit, and the segmented regions of interest were extracted and viewed in three dimensions to determine their internal orientation. In the extant taxa, using the external ridges as a guide, hypothesized boundaries of the five digits, based on where the trabecular bones separated from one another, were traced.

RESULTS

EXTERNAL SURFACE FEATURES

In all specimens, the hypothesized five digits are clearly visible in the proximal aspect (Fig. 1; Supporting Information, Appendix S1). There are four articular facets. The articular kidney-shaped facet for digit III is positioned more dorsally than the facet for the similarly shaped digit IV, and a deep furrow

separates the two, relating to the internal structure and the fusion of digits III and IV. These features are typical for all ruminants. Ventrally, however, there are two more articular facets. These are interpreted to be the proximal ends of digit II medially and digit V laterally. A small tubercle is also observable at the medial side of the metatarsal. This is interpreted to be digit I. In the lateral aspect of the fragment, a long groove extends distally, which is a continuation of the separation between the facets of digits IV and V. In the medial aspect of the fragment, a long groove extends distally, which is a continuation of the small tubercle. In the ventral aspect, a deep trough separates the ventral extent of the proposed digit V laterally and digit II medially. The trough terminates proximally in a rounded shape, and there is a deep, slit-like groove at the articular surface separating digits V and II. On either side of this groove, the articular heads are polished as if part of the synovial cavity extending ventrally (Fig. 1). In *Giraffa* and *Okapia*, two ridges extend ventrally along the shaft of the giraffid metatarsal (Supporting Information, Figs S4C, S5C), suggesting that these ventral ridges are components of digits II and V. Although they are not as well defined in *Okapia* and in *Giraffa*, they are common and clearly observable in extinct Giraffidae (Supporting Information, Figs S2B, S3C).

In the fossil specimens, Figure 1B shows the external structure of †YGSP 28199. The features are generally the same for †YGSP 21930 (Fig. 1C). However, in †YGSP 21930 the articular facet of digit II forms a sharper ventrally extending edge, and the facet of digit V is a smaller elongated oval (Fig. 1C; Supporting Information, Fig. S3C). In the medial aspect, the groove separating digit I from digit II is well defined, and digit I is clearly a separate structure (Supporting Information, Fig. S3B). Digit I does not extend distally, and it terminates in a rounded knob (Fig. 1C; Supporting Information, Fig. S3B). The anatomy of the metatarsal of †*Samotherium* (Supporting Information, Fig. S2) is similar to what is described for the other fossil giraffids.

In the closely related outgroup, *Hyemoschus aquaticus* AMNH 53640 (Supporting Information, Fig. S1),

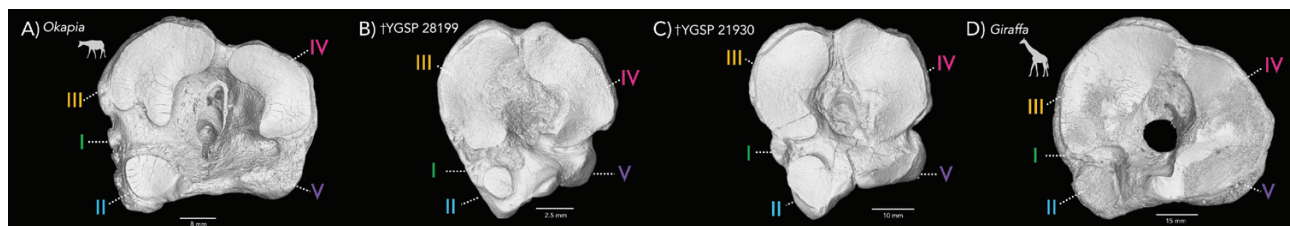


Figure 1. Proximal ends of metatarsals from the four species of interest, marked with the hypothesized digits signalled by the distal grooves and gulleys. A, D, left metapods. B, C, right metapods of fossil specimens, mirrored to facilitate comparison.

metatarsals III and IV are only fused proximally, and at the articular end there are two facets for metatarsals III and IV. The two bony shafts form a long contact with each other. Metatarsal II forms its own distinct facet; it is thin and slender and nearly as long as metatarsals III and IV, much longer than what is observed in giraffids. Metatarsal V is more robust than what is observed in giraffids, also forming its own facet.

INTERNAL FEATURES

The internal structures reconstructed from the μ CT scans of †YGSP 28199 and †YGSP 21930 are displayed in Figure 2. In both specimens, there are five distinct medullary cavities corresponding to each digit, respectively. The size of each cavity is proportional to the external expression of each digit. The cavity for digit I is ventral rather than the inner aspect of the external ridge of digit I.

In the extant taxa, almost the entire internal portion of the proximal metapods is composed of trabecular bone (Fig. 3). Although the distinct digit cavities are

not as visible as the fossil internal structures, the pattern of the trabecular bone shifts where it meets a new digit. Figure 3 demonstrates the hypothesized outlines of the digit fusions from qualitative analysis of the digit patterns. Digit I is ‘squeezed’ between digits II and III and extends medially through the internal part of the bone.

DISCUSSION

Early hypotheses about ungulate digit reduction assumed that embryological evidence of apoptosis surrounding the digits suggested that the digit never developed (Cooper *et al.*, 2014). Recently, however, evidence from horse fossils demonstrated the presence of all five digits in the adult throughout horse evolution (Solounias *et al.*, 2018), whereas it was previously thought that digits I and V never developed and that digits II and IV were highly reduced. Embryologically, five digits appear to condense during morphogenesis and persist into late developmental stages (Kavanagh *et al.*, 2020). Although horses are mesaxonic, we

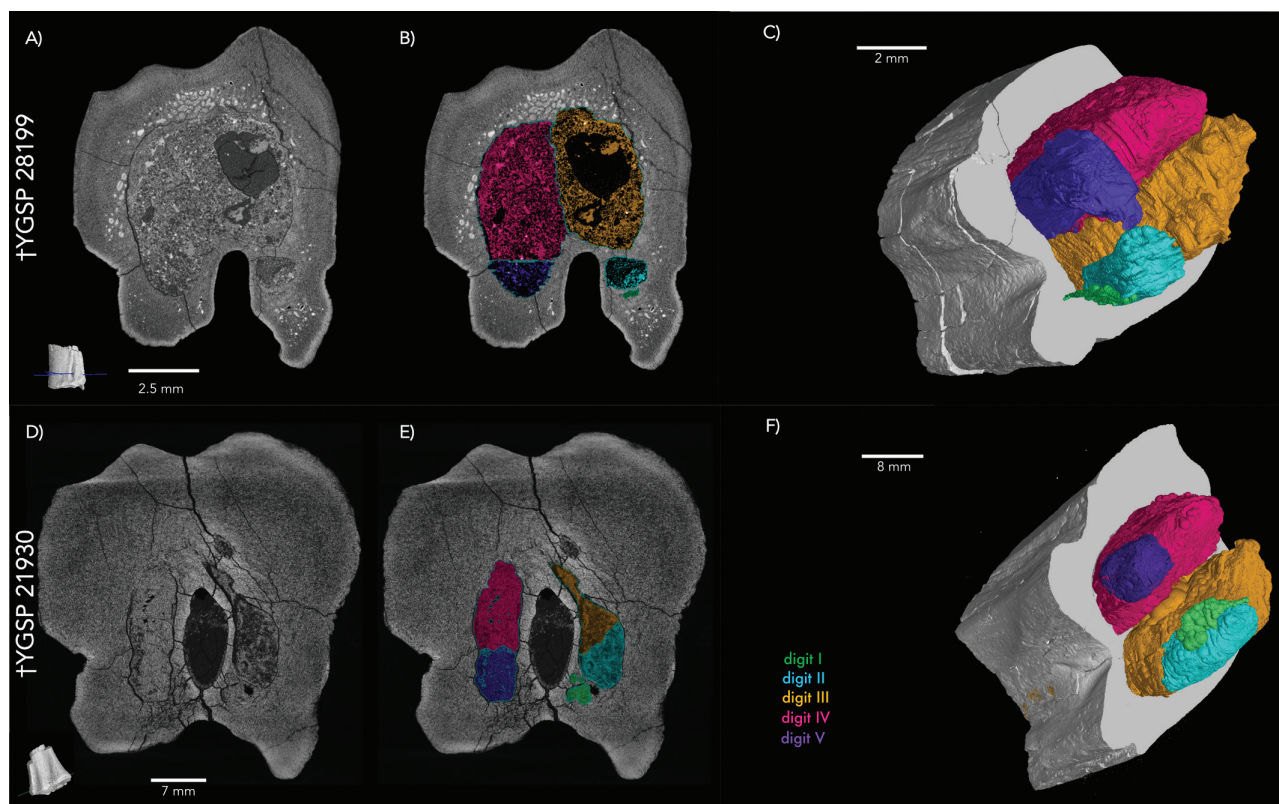


Figure 2. Transverse sections of fossil specimens †YGSP 28199 (A–C) and †YGSP 21930 (D–F). Insets in A and D show where in the fossil the section is taken. A, D, hyper-contrasted sections without segmenting. B, E, the separated medullary cavities that are hypothesized to come from each of the five digits are outlined. C, F, reconstructed segmentations of each medullary cavity in three dimensions, with the fossil clipped away to demonstrate the internal structure.

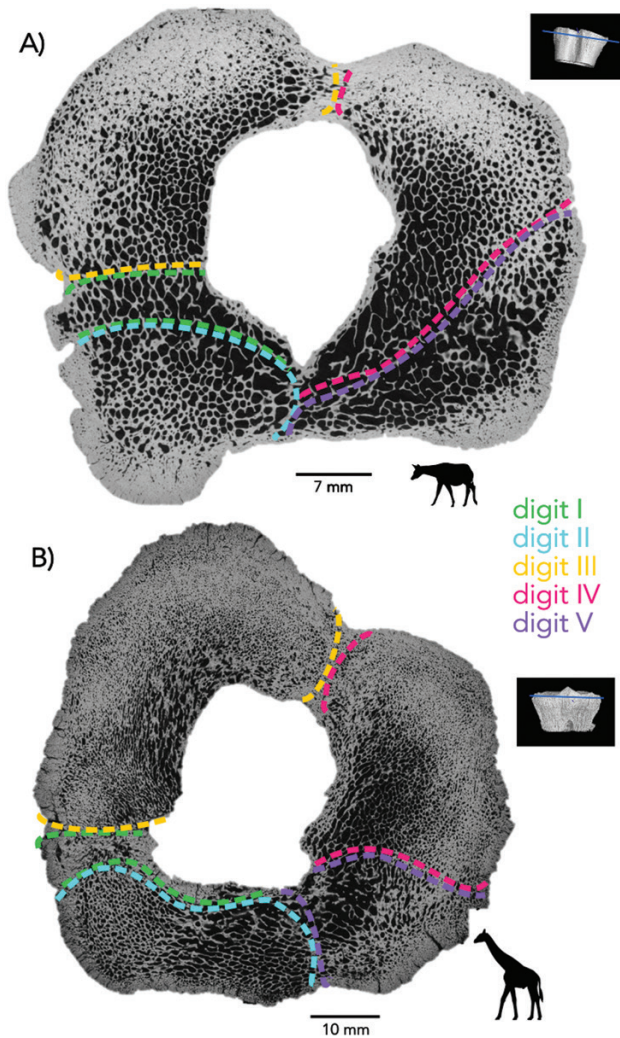


Figure 3. Transverse section of extant *Okapia* (A) and *Giraffa* (NS 289) (B). Coloured lines indicate hypothesized boundaries of the five digits, informed from qualitative analysis of the patterns of the trabecular bone. Blue lines along the bone are the transverse slice indicator position.

demonstrate that retention of pentadactyly is more widespread in ungulates than previously thought. Giraffids also demonstrate evidence of pentadactyly in the adult (Fig. 1), and although the phylogenetic positions of the fossils are not yet established, the taxa in this study support the hypothesis that the digits have fused and reduced through time (Fig. 2), becoming more and more tightly woven in extant taxa (Fig. 3). Understanding the evolutionary processes that led to the fusion of five digits rather than digit loss will help to untangle the complicated patterns of parallel digit 'loss' observed in ungulates.

We first show that the external features of the giraffid metapod support pentadactyly, suggesting that ridges and grooves reflect the internal morphology (Supporting Information, Figs S2–S5). The traditional understanding is that ruminant cannon bones are composed of two fused digits, namely III and IV, detailed in the distal gully morphology (Janis & Scott, 1987). The four articular facets may be plastic to their topography, and it is crucial to interpret what the external morphology might really demonstrate. For example, in the distantly related extant ruminant, *Hyemoschus*, digits II and V are separate (unfused), and the four articular facets are probably formed solely on digits III and IV (Windle & Parsons, 1903). Thus, digits III and IV appear to have four articular facets. It is possible that more material of digits I, II and V is present in the cannon bone, but the internal structures need to be investigated to explore these possibilities.

In Giraffidae, however, we argue that the four articular facets are the four digits. As the digits continue to fuse, the grooves complement where the digits merge. They also contain distinct internal cavities, which corroborates our assumption (Fig. 2). In the extinct taxa (Supporting Information, Figs S2, S3), the lateral grooves also indicate the proposed anatomy. Thus, it is the combination of articular facets, internal distinct cavities and the lateral ridges that provides the evidence. Rios *et al.* (2016) explored the diversity of Giraffidae metapodials, and they were useful for the determination of the various species. In their study, they named the four proximal distinct structures of the proximal end as epicondyles: medial epicondyle ventral and dorsal head and lateral epicondyle ventral and dorsal head. In this study, we propose these heads be named as the four digits.

The best explanation for the observed proximal morphology of the metatarsals in Giraffidae is that all five metatarsals are present. Furthermore, no study until ours has suggested that the smaller external ridge at the medial-proximal surface might also be digit I. The internal and external features of hypothesized digit I also support the pentadactyly hypothesis. The dorsal facets are digits III and IV, which is already widely accepted. We also propose that the ventral facets of Giraffidae are best interpreted as metatarsals II and V (Fig. 1). Material of digit I is visible on the surface as a peg, but more material of digit I is embedded in the canon bone (Fig. 1; Supporting Information, Figs S4, S5). The modification and fusion of digits rather than digit reduction might be attributable to the contact of the proximal metatarsal with the joint. The complexity of joints might select for the maintenance of digit contact; for example, the ancient contact of digits II and V. If these digits were truly lost, the joint would have to re-establish contact with digits III and IV. Such is the

case with the mammalian inner ear bones, in which the malleus and incus are highly modified bones of the jaw joints, but much of the articulation and contact of these bones is retained (Anthwal *et al.*, 2013).

Evidence from internal structures of both extinct and extant taxa corroborate the external patterns on the metapod surface. The five distinct medullary cavities in the fossils (Fig. 2) show that the five hypothesized digits display less fusion than the extant taxa, emphasizing the importance of studying fossil taxa. Surprisingly, the internal structures of both the okapi and the giraffe show convergent complete fusion of the trabecular bone of the five digits. Through observation of the trabecular bone pattern, we suggest that digits III and IV fuse in the lateral region, whereas digit V mostly ‘articulates’ with digit IV and digit I curls inferiorly is incorporated between digits II and III. We infer that much of the material from digit I might be on the internal surface (Fig. 3), and this might be why it has remained unobserved in past studies. It is surprising that digit I appears between digits II and III rather than between digits II and V. We hypothesize that as digits II and V reposition centrally, because digit I often has independent mechanisms of development (Woltering & Duboule, 2010), it does not get dragged ventrally but becomes tucked in between digits II and III (Supporting Information, Fig. S6). The tight articulation of the digit trabecular bone probably strengthens the digit fusion. Future studies need be done biomechanically to understand the effects on load-bearing of digit fusion compared with digit modification after digit reduction/loss (e.g. McHorse, *et al.*, 2017). Our findings also open up new hypotheses to investigate morphogenesis of digit fusion developmentally.

In summary, we have discovered that giraffids maintain pentadactyly not only through late-stage development, but also throughout adulthood. We argue that the giraffid metatarsal is not formed from the fusion of two digits with the loss of the remaining three, but from the fusion of all five digits. Future work needs to be done to gain a better understanding of the ubiquity of this pattern in ungulates, which might challenge our understanding of limb development and evolution further.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. The five digits of the giraffe metatarsal.

Figure S1. *Hyemoschus aquaticus*. A, MCZ 220395, proximal view. B, AMNH 53640, ventral view.

Figure S2. †*Samotherium boissieri* from the Miocene of Samos, Greece Musée cantonal de Géologie de Lausanne (S382).

Figure S3. †YGSP 21930 from the Chinji Formation, Siwaliks of Pakistan.

Figure S4. *Giraffa camelopardalis*, AMNH 83458. A, proximal. B, medial.

Figure S5. *Okapia johnstoni*, AMNH 51196. A, proximal. B, medial.

Figure S6. Hypothesized ontogenetic pattern of adult metatarsal morphogenesis in giraffids from embryonic patterning (bottom) to adult (top).