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1 **Systemic patterns of trabecular bone across the human and chimpanzee skeleton**

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3 Short title: Human and chimpanzee systemic trabecular patterns

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Abstract

Aspects of trabecular bone architecture are thought to reflect regional loading of the skeleton, and thus differ between primate taxa with different locomotor and postural modes. However, there are several systemic factors that affect bone structure that could contribute to, or be the primary factor determining, interspecific differences in bone structure. These systemic factors include differences in genetic regulation, sensitivity to loading, hormone levels, diet, and/or activity levels. Improved understanding of inter/intraspecific variability, and variability across the skeleton of an individual, is required to properly interpret potential functional signals present within trabecular structure. Using a whole-region method of analysis, we investigated trabecular structure throughout the skeleton of humans and chimpanzees. Trabecular bone volume fraction (BV/TV), degree of anisotropy (DA) and trabecular thickness (Tb.Th) were quantified from high resolution micro-computed tomographic scans of the humeral and femoral head, third metacarpal and third metatarsal head, distal tibia, talus and first thoracic vertebra. We find that BV/TV is, in most anatomical sites, significantly higher in chimpanzees than in humans, suggesting a systemic difference in trabecular structure unrelated to local loading regime. Differences in BV/TV between the forelimb and hindlimb do not clearly reflect differences in locomotor loading in the study taxa. There are no clear systemic differences between the taxa in DA and, as such, this parameter may reflect function and relate to differences in joint loading. This systemic approach reveals both the pattern of variability across the skeleton and between taxa, and helps identify those features of trabecular structure that may relate to joint function.

Keywords: Cancellous bone, Functional morphology, *Homo sapiens*, *Pan troglodytes*, Locomotion, Sedentism, Hominids

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Introduction

The behaviour of extinct species can be reconstructed from plastic features of bony morphology that reflect an individual's behaviour during life (Ruff et al., 2006). Experimental studies have demonstrated the ability of bone to adapt to external loading (e.g. Lanyon, 1974; Robling et al., 2002; Mori et al., 2003; Pontzer et al., 2006; Barak et al., 2011; Wallace et al., 2013), a process often referred to as Wolff's Law (Wolff, 1986; Martin et al., 1998), or more generally as bone functional adaptation (Cowin, 2001; Ruff et al., 2006). Trabecular bone has potential for reconstructing the behaviour of fossil taxa (Kivell, 2016), as it remodels rapidly during life in response to strain (Ehrlich and Lanyon, 2002), in comparison to the slower rate of remodelling of cortical bone (Eriksen, 1986, 2010). Thus, the structure of trabecular bone could provide information about the mechanical loading history of a joint, in terms of both the load magnitude and direction. Studies among primates, including fossil specimens, have attempted to identify behavioural signals in trabecular structure with varying degrees of success (e.g. Fajardo and Müller, 2001; Ryan and Ketcham, 2002b; Griffin et al., 2010; Ryan and Shaw, 2012; Tsegai et al., 2013; Skinner et al., 2015; Stephens et al., 2016; Zeininger et al., 2016). The ultimate goal and framework within which these studies have been conducted is to first identify trabecular differences in living species that are related to behaviour, for example locomotor or manipulatory behaviours. Once this relationship between structure and behaviour has been established, similarities between the trabecular structure of fossil specimens and living taxa could be used to infer specific behaviours, or joint loading regimes, in fossil species.

However, the relationship between trabecular structure and behaviour in extant species is often unclear. For example, many trabecular bone analyses have focused on the primate proximal humerus (e.g. Fajardo and Müller, 2001; Fajardo et al., 2007; Ryan and Walker, 2010; Shaw and

68 Ryan, 2012; Scherf et al., 2013; Scherf et al., 2015) and, for historical reasons (Skedros and
69 Baucom, 2007), the proximal femur (e.g. Fajardo and Müller, 2001; MacLatchy and Müller,
70 2002; Ryan and Ketcham, 2002a, b, 2005; Scherf, 2008; Ryan and Walker, 2010; Saporin et al.,
71 2011; Ryan and Shaw, 2012; Shaw and Ryan, 2012). However, few of these studies have found
72 clear differences in the trabecular structure of these joints that can be directly related to
73 locomotor mode and predicted joint function. Where structural differences in trabecular
74 architecture have been identified across locomotor groups, there is often no clear biomechanical
75 explanation, and trabecular architecture is not always consistent with predictions based on
76 biomechanical models. For example, studies of strepsirrhines have found that trabeculae within
77 the femoral head was more uniformly oriented in vertical clinging and leaping species compared
78 with slow climbing and/or quadrupedal taxa (MacLatchy and Müller, 2002; Ryan and Ketcham,
79 2002b, 2005). However, finite element analysis of the femoral head was unable to identify
80 differences in bone strain at a range of load orientations in vertical clinging and leaping *Galago*
81 compared to slow quadrupedal/climbing *Loris* (Ryan and van Rietbergen, 2005). This implies
82 that different trabecular structures may be able to mitigate stress in similar ways, and that joint
83 loading at the femoral (and potentially humeral) heads may actually be more similar than
84 predicted across divergent locomotor modes (Ryan and van Rietbergen, 2005; Fajardo et al.,
85 2007).

86 Since the first three-dimensional analysis of trabecular structure in primates (Fajardo and Müller,
87 2001), trabecular architecture has been described across a range of species and anatomical sites.
88 This body of work has revealed particular interspecific patterns in the variation of trabecular
89 structure, which suggests that any given species may have a similar trabecular structure across
90 several elements of their skeleton. As a notable example, recent humans have been shown to

91 have low trabecular bone volume throughout the postcranial skeleton, including highly-loaded
92 lower limb bones, such as the femur (e.g. Maga et al., 2006; Griffin et al., 2010; Tsegai et al.,
93 2013; Chirchir et al., 2015; Ryan and Shaw, 2015; Saers et al., 2016; Stephens et al., 2016;
94 Chirchir et al., 2017). In contrast, chimpanzees tend to have high bone volume across different
95 skeletal elements in comparison to other hominoids (e.g. Maga et al., 2006; Griffin et al., 2010;
96 Tsegai et al., 2013). Although few trabecular studies include bonobos, their metacarpals and
97 metatarsals have the highest bone volume amongst the great apes (Griffin et al., 2010; Tsegai et
98 al., 2013), which is not readily explained by variation in body size, locomotor mode, or activity
99 level (Susman et al., 1980; Doran, 1992, 1993a). Although bone volume fraction is the trabecular
100 parameter most strongly correlated with bone stiffness (Stauber et al., 2006; Maquer et al.,
101 2015), it does not seem to correspond directly to predictions of joint loading based on locomotor
102 mode.

103 There are several genetic and environmental factors, other than specific locomotor behaviours,
104 that could have a systemic effect on bone remodelling and trabecular structure (Bertram and
105 Swartz, 1991; Ruff et al., 2006; Kivell, 2016). Aspects of loading that are not evidently related to
106 specific positional or locomotor behaviours include loading magnitude due to body mass (Doube
107 et al., 2011; Fajardo et al., 2013; Ryan and Shaw, 2013), differences in loading frequency
108 associated with overall activity levels (Lieberman, 1996), and other factors that may affect the
109 frequency, magnitude or orientation(s) of load and thus potentially impact remodelling of both
110 cortical and trabecular bone (Rubin and Lanyon, 1985; Frost, 1987; Skerry and Lanyon, 1995;
111 Wallace et al., 2013). Genetic factors that might contribute to species-specific trabecular
112 structure include hormonal differences or differences in bone regulation, even between closely
113 related species (Lovejoy et al., 2003; Behringer et al., 2014a; Behringer et al., 2014b), between

114 males and females (Riggs and Melton, 1995; Reginster and Burlet, 2006; Eckstein et al., 2007)
115 or at different life stages (Riggs and Melton, 1995; Tanck et al., 2001; Reginster and Burlet,
116 2006; Ryan and Krovitz, 2006; Gosman and Ketcham, 2009). These genetic differences may also
117 manifest as phylogenetic differences in bone structure, unrelated to locomotor mode (Fajardo et
118 al., 2013; Ryan and Shaw, 2013). Other aspects of the environment, such as diet and the
119 intestinal microbiome, could also have a systemic effect on bone structure (Prentice, 1997; Shea
120 et al., 2002; Cashman, 2007; Cao et al., 2009; Charles et al., 2015; McCabe et al., 2015). As the
121 rate of remodelling of bone is higher during growth, behaviours during development may be
122 more important for explaining trabecular morphology than those during adulthood (Bertram and
123 Swartz, 1991; Pettersson et al., 2010). This is of particular relevance for African apes, as the
124 percentage of knuckle-walking and suspension change significantly during development (Doran,
125 1992, 1997; Sarringhaus et al., 2014; Sarringhaus et al., 2016), although long bone cross-
126 sectional geometry in African apes continues to change into adulthood and reflect locomotor
127 behaviour at different life stages (Ruff et al., 2013; Sarringhaus et al., 2016; but see Demes et al.,
128 1998; Demes et al., 2001; Lieberman et al., 2004; Carlson, 2005). Trabecular morphology may
129 differ due to anatomical location (Morgan and Keaveny, 2001; Eckstein et al., 2007; Wallace et
130 al., 2015); for example, distal limb elements may be adapted to have a lower bone mass (bone
131 mineral density measured using pQCT and multiplied by joint size) and BV/TV than more
132 proximal limb elements (Chirchir, 2015; Saers et al., 2016).

133 The absence of detailed locomotor, positional and biomechanical data on particular primate
134 species may also contribute to limited identification of clear functional signals in trabecular
135 bone. For example, accurate information on locomotor frequencies is rare, in part because
136 several primate taxa are challenging to study in the wild due to lack of habituated populations,

137 rarity of the species itself, and/or high density forest cover (Crompton et al., 2010). Many
138 species, especially hominoids, engage in multiple positional and locomotor behaviours (Hunt,
139 1991; Thorpe and Crompton, 2006; Myatt et al., 2011), beyond often over-simplified locomotor
140 categories. Furthermore, due to the difficulty –both ethically and practically– of studying the
141 biomechanics of locomotion in humans and especially non-human primates, there is little
142 accurate biomechanical data concerning loading orientations and joint reaction forces to inform
143 trabecular studies. Morphological differences related to locomotion have been investigated in
144 primate taxa through finite element analysis (e.g. Ryan and van Rietbergen, 2005; Richmond,
145 2007; Nguyen et al., 2014). Although finite element analyses enable more informed predictions,
146 they are often limited by a necessity to artificially reduce the complexity of the trabecular
147 structure (due to computational limitations) and a lack of validation (Richmond et al., 2005;
148 Ryan and van Rietbergen, 2005; Strait et al., 2005; Nguyen et al., 2014). Thus it is difficult to
149 determine which behaviour, or combinations of behaviours, are reflected in trabecular bone
150 structure.

151 To fully understand the functional significance of the trabecular bone structure of fossil
152 hominins, we need to further explore variation in trabecular bone across the skeleton of living
153 species. Previous studies have largely focused on one anatomical site (e.g. DeSilva and Devlin,
154 2012; Tsegai et al., 2013; Stephens et al., 2016) or region (Lazenby et al., 2011a; Schilling et al.,
155 2014; Tsegai et al., 2017), or have been limited to comparisons between the humerus and femur
156 (Fajardo and Müller, 2001; Ryan and Walker, 2010; Ryan and Shaw, 2012; Shaw and Ryan,
157 2012), and thus lack the context of how trabecular structure in any particular element or region
158 might reflect, at least in part, a broader systemic pattern. Several recent studies have addressed
159 the question of why previous comparative studies of trabecular bone have found notably gracile

160 bone in modern humans. Chirchir et al. (2015) conducted an analysis of trabecular structure
161 across several skeletal elements in a sample of modern humans, fossil hominins and other extant
162 primates, showing that gracile trabecular structure in humans is a relatively recent (i.e. Holocene)
163 phenomenon. Ryan and Shaw (2015) further demonstrated, through a 3D volume of interest
164 analysis of trabecular structure in the proximal femur of modern humans varying in subsistence
165 strategies (foragers vs. agriculturalists), that gracile bone structure of recent humans is likely
166 linked to a reduction in overall activity level with the adoption of agriculture. This gracilisation
167 of the skeleton of agriculturalists is apparent across the lower limb, in the proximal and distal
168 epiphyses of the femur and tibia, although all populations share a proximo-distal reduction in
169 bone volume and increase in anisotropy (Saers et al., 2016). A similar pattern of gracilisation in
170 recent humans, compared to a Neolithic population, is also present in the proximal humerus
171 (Scherf et al., 2015). Chirchir et al. (2017) quantified trabecular bone fraction from pQCT data in
172 the forelimb and hindlimb of five groups of modern humans, with a range of lifestyles, from
173 foraging to industrial sedentary populations. This revealed a reduction in hindlimb robusticity
174 with increased sedentism, and more variable changes in forelimb robusticity. Variability in
175 trabecular architecture across the skeleton of recent humans has been documented, largely in the
176 clinical literature. There is high intra-individual variability in trabecular structure, with low
177 correlation between anatomical sites in several measures of trabecular architecture, quantified
178 using 2D and 3D stereological methods (Amling et al., 1996; Parkinson and Fazzalari, 2003),
179 pQCT (Groll et al., 1999; Chirchir, 2016), and microCT (Hildebrand et al., 1999; Ulrich et al.,
180 1999; Eckstein et al., 2007). However, as yet, no study has conducted a comprehensive
181 trabecular analysis, including parameters other than trabecular bone volume, across several
182 skeletal elements in humans in comparative context with other primates. Thus, it remains

183 unknown how potential systemic patterns in trabecular bone might vary intraspecifically and
184 interspecifically.

185 In this study we address this issue through quantification of trabecular bone volume fraction
186 (BV/TV), degree of anisotropy (DA) and trabecular thickness (Tb.Th) in several anatomical sites
187 within associated skeletons of recent humans and chimpanzees. Based on previous findings
188 described above, we test three predictions: first, we predict that chimpanzees will have a higher
189 BV/TV throughout the skeleton compared to humans (Maga et al., 2006; Griffin et al., 2010;
190 Tsegai et al., 2013; Chirchir et al., 2015). Second, as humans and chimpanzees adopt locomotor
191 behaviours that involve differential loading of the forelimb and hindlimb, we predict that BV/TV
192 will be relatively similar across both limbs in chimpanzees, while BV/TV will be low across the
193 forelimb compared to the hind limb in humans. Previous studies have demonstrated that humeral
194 and femoral head trabecular structure does not reflect this difference in locomotor loading
195 (Fajardo and Müller, 2001; Ryan and Walker, 2010; Shaw and Ryan, 2012), thus in this study we
196 aim to test whether this pattern is consistent in other elements of the fore- and hindlimb. Third,
197 as trabecular fabric has previously been associated with load direction and variability, we expect
198 DA to differ between taxa in ways that reflect loading differences (Ryan and Ketcham, 2002b;
199 Barak et al., 2013b; Su et al., 2013). Although Tb.Th is strongly correlated with body size
200 (Doube et al., 2011; Barak et al., 2013a; Fajardo et al., 2013; Ryan and Shaw, 2013), it is also
201 highly correlated with BV/TV (Barak et al., 2013a), and as such could parallel the systemic
202 pattern of BV/TV. However, since the taxa in this study sample have a similar body mass, we
203 predict that there will be no differences in trabecular thickness between these taxa, as has been
204 found in general in previous studies (Cotter et al., 2009; Scherf et al., 2013; Ryan and Shaw,
205 2015; Zeininger et al., 2016; but see Barak et al., 2013b; Su and Carlson, 2017).

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Methods

Sample

Trabecular bone structure was analysed in the skeletons of *Pan troglodytes* (N = 7) and recent *Homo sapiens* (N = 7) individuals. Full details of the study sample are shown in Table 1. All chimpanzee specimens belong to a single subspecies, *P. t. verus*, and were wild-collected skeletons from the Taï National Park, Republic of Côte d'Ivoire. The human sample was collected from two skeletal collections: one from a 19th century cemetery in Inden, Germany and the other from 13-15th century medieval cemeteries in Canterbury, UK. All specimens were free from external signs of pathology. Trabecular architecture was quantified in two anatomical locations in the forelimb (humeral head and third metacarpal head [MC3]), four anatomical sites in the hindlimb (femoral head, distal tibia, talus, and third metatarsal head [MT3]) and one site in the axial skeleton (first thoracic vertebra [T1]) (Fig. 1). These anatomical sites were chosen to include elements from both limbs, and an element from the axial skeleton that is less affected by differential loading of the fore- and hindlimb. We aimed to sample all bones of the forelimb and hindlimb from the same side, but when elements were not adequately preserved, all elements from either the forelimb or hindlimb were taken from the contralateral side where possible. For example, if the right femur was absent, then the femur, tibia, talus and MT3 were taken from the left side where possible.

Micro-CT scanning

All specimens were CT scanned using either a SkyScan 1173 or a BIR ACTIS 225/300 scanner housed at the Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology (Leipzig, Germany). All scans were reconstructed as 16-bit tiff stacks with isotropic voxel sizes of 21-38 μm . All specimens were reoriented into standardised anatomical positions and were downsampled, due to computational constraints, using Avizo 6.3. Specimens

230 were analysed at a range of resolutions (25-45 μ m), with adequate representation of trabeculae as
231 demonstrated by the relative resolution (4.25-9.83), which indicates how many pixels represent
232 the average trabecular strut (Sode et al., 2008). Following this, all specimens were segmented
233 using the Ray Casting Algorithm of Scherf and Tilgner (2009).

234 **Trabecular bone quantification**

235 Analysis of trabecular bone structure was conducted using an in-house script in medtool v3.9
236 (www.dr-pahr.at), following Gross et al. (2014). Morphological filters were used to
237 automatically segment the cortical and trabecular bone, resulting in definition of three materials:
238 (1) cortical bone, (2) trabecular bone and (3) air inside the bone (Fig. 2A). In this way, the
239 trabecular bone throughout an entire region (or the whole bone, in the case of the talus) could be
240 analysed. Tb.Th was calculated using the BoneJ plugin (v1.3.12; Doube et al., 2010) for ImageJ
241 (v1.46r; Schneider et al., 2012) from the segmented trabecular only region (Fig. 2B). To quantify
242 the other trabecular parameters in medtool (following protocols outlined in Gross et al., 2014), a
243 2.5mm background grid was applied to each specimen, and a 5mm spherical volume of interest
244 was used to measure BV/TV at each node of the background grid. A 3D tetrahedral mesh was
245 created of the inner region of the bone (Fig. 2C), to which each node was assigned a BV/TV
246 value (Fig. 2D) interpolated from the background grid. A mesh size of 1mm was used for the
247 larger specimens (humeral head, femoral head, distal tibia, and talus) and a mesh size of 0.5mm
248 for the smaller specimens (MC3, MT3, and T1). As the background grid size was constant for
249 the sample, the results are independent of mesh size. The overall BV/TV was calculated as the
250 mean of all elements in the 3D region of interest (ROI; see below). The mean intercept length
251 method was used to calculate the local fabric tensor for each tetrahedron and these were
252 normalised by the determinants (Luisier et al., 2014). Similar to BV/TV, an arithmetic mean of

253 all of the second order fabric tensors was computed within the ROI. The DA was calculated as
254 the $DA = 1 - [\text{smallest eigenvalue}/\text{largest eigenvalue}]$, such that a DA of 1 represents “complete”
255 anisotropy (i.e. all trabeculae are aligned, and there are no crossing trabeculae) and a DA of 0
256 reflects complete isotropy (i.e. there is no preferential alignment of trabeculae). Often the DA is
257 bound between a DA of 1 representing isotropy and a $DA > 1$ representing increasing anisotropy,
258 however here we use an alternative, “normalised” DA.

259 In both humans and chimpanzees trabecular bone of the long bone epiphyses extends beyond the
260 epiphysis and into the shaft. As such, the ROIs for long bones were defined in order to sample as
261 much of the trabecular bone-filled region as possible, which could potentially contribute to
262 systemic differences in trabecular structure. For each skeletal element the ROI was defined as
263 follows (Fig. 1). For the proximal humerus, this was defined as the point where curvature of the
264 humeral head begins to expand from the shaft both medially and laterally (Fig. 1A). In the
265 proximal femur, the femoral head was extracted with the inferior margin being at the most
266 inferior point of the femoral head and the medial margin at the most medial point of the femoral
267 head (Fig. 1B). In the proximal femur, it was only possible to sample the femoral head, and small
268 region of the femoral neck, due to computational constraints in processing large data sets. The
269 ROI in the distal tibia was defined distally where curvature of the shaft begins in both medial and
270 anterior views, which is at the proximal extent of the fibular notch (Fig. 1C). In the MC3 and
271 MT3, the distal end (head) was defined as the point at which the shaft curves laterally in
272 palmar/plantar view (Fig. 1D & E). In the T1, only the trabeculae in the vertebral body were
273 quantified (Fig. 1F). For the talus the trabecular bone in the entire element was quantified.
274 Identification of homologous regions is complex due to the potential effect of differences in
275 location and size of the region being analysed. For example, sometimes dramatic differences in

276 quantification of trabecular bone structure have been found with variation in position or size of
277 small volumes of interest within a bone or epiphysis (Fajardo and Müller, 2001; Kivell et al.,
278 2011; Lazenby et al., 2011b). Here, our 3D ROI includes a much larger region of trabecular
279 structure (e.g. the entire epiphysis), but quantified values may also be affected by how the ROI is
280 defined between taxa. Therefore, a test of intra-observer error was conducted for the humerus and
281 tibia of one human and one chimpanzee, with the ROI defined five times on five consecutive
282 days. The percentage difference in BV/TV compared to the original quantified value, ranged
283 from -0.97% to 0.22% for the humerus and from -2.29% to 0.73% for the tibia.

284 **Statistical analysis**

285 Statistical analysis was conducted using R v3.3.2 (R Core Team, 2016) and ggplot2 (Wickham,
286 2009) for plot generation. Due to small sample sizes non-parametric tests were used. Taxonomic
287 differences in trabecular structure at each anatomical site were tested for using Mann-Whitney U
288 tests between taxa. To identify systemic patterns within species, Friedman tests were used to
289 identify whether there were overall significant differences between the ranks of anatomical sites
290 in humans and in chimpanzees. Following the results of the Friedman tests, Wilcoxon exact tests
291 with p-values corrected with a post-hoc Bonferroni adjustment, were used to identify significant
292 pairwise differences between anatomical sites within humans and within chimpanzees.

293 Differences in the systemic pattern between taxa were identified by comparing the results of
294 within-species Wilcoxon exact tests. To identify correlations between trabecular parameters in
295 different regions within humans and within chimpanzees. Spearman's correlation test was used
296 with p-values corrected with a post-hoc Bonferroni adjustment. For all statistical tests a p-value <
297 0.05 was considered significant.

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Results

Taxonomic differences

Means and standard deviations of trabecular parameters in each anatomical region and results of Mann-Whitney U tests for significant differences between species are shown in Table 2. Figure 3 shows box-and-whisker plots of the results for each taxon. There were no significant differences in Tb.Th between chimpanzees and humans in any anatomical region. Chimpanzees had significantly higher BV/TV than humans in the humeral, femoral, and MT3 heads as well as the talus. Chimpanzees also had significantly more anisotropic trabeculae in the humeral head and T1, and less anisotropic trabeculae in the talus and MT3.

Taxonomic differences in the patterning of BV/TV are further illustrated in Figure 4, where the BV/TV values are shown for each individual. In one human individual BV/TV values were much higher in every anatomical region, and this is the only individual that overlapped with chimpanzees in humeral, metatarsal, femoral, and talar BV/TV. Excluding this specimen from the statistical comparisons presented above led to significantly lower BV/TV in the human MC3 ($p = 0.03$), while the BV/TV values in the thoracic vertebra and tibia approached significance ($p = 0.05$).

Intraspecific and interspecific systemic patterns

Comparisons of trabecular structure within individuals are presented in Table 3, as the mean rank of each element for each trabecular parameter. This demonstrates the systemic pattern of trabecular bone structure within each taxon, with elements having a higher mean rank indicating generally higher values in that anatomical region across individuals. Across both chimpanzees and humans, all hindlimb elements, except for the MT3, had a higher mean rank for Tb.Th than

322 the forelimb and axial elements, and the humerus had a higher mean rank for Tb.Th than the
323 metacarpal. In chimpanzees, the order of mean ranks of the different anatomical sites for BV/TV
324 was similar to that of Tb.Th. The only difference was a switch between the humerus and the T1.
325 In humans, the ranks of anatomical sites for BV/TV followed the pattern for Tb.Th less closely.
326 Notably, the humerus was the lowest ranking element for BV/TV in humans. The mean ranks of
327 DA differed between the taxa. Within the hindlimb of chimpanzees, the DA had the highest
328 mean rank in the tibia, MT3 and femur, with the talus having the most isotropic trabeculae. The
329 pattern in humans differed from that of chimpanzees in that the MT3 had a higher DA rank
330 compared to the other hindlimb anatomical sites. In the forelimb, the MC3 had a higher mean
331 rank for DA than the humerus in both taxa.

332 Results of Friedman tests (Table 3) indicated the presence of significant differences between
333 ranks of anatomical sites in all three trabecular parameters in both humans and chimpanzees.
334 Post-hoc Wilcoxon test comparisons with a Bonferroni adjustment are shown in Table 4. For
335 Tb.Th (Table 4), significant differences were largely due to thicker trabecular bone in the femur,
336 tibia and talus compared to other elements in both taxa. The humerus had significantly thicker
337 trabeculae than the MT3 in humans, and both the MC3 and MT3 in chimpanzees. Significant
338 differences in BV/TV between elements were largely due to low BV/TV in the human humerus
339 and to high BV/TV in the chimpanzee femur and talus (Table 4). Significant differences in DA
340 were largely due to high DA in the tibia and low DA in the talus in chimpanzees. In humans,
341 most significant differences were due to the high DA of the MT3.

342 **Trabecular correlations between anatomical sites**

343 Spearman's correlation tests, to identify whether trabecular parameters were correlated between
344 anatomical sites within each taxon, revealed only two significant correlations. In chimpanzees,

345 there was a significant correlation in Tb.Th between the humerus and femur ($r = 0.96$, $p = 0.01$)
346 and between the talus and MT3 ($r = 1.00$, $p < 0.01$). There were no significant correlations
347 between anatomical sites in humans.

348 **Discussion**

349 This study provides the first comprehensive 3D analysis of potential systemic patterns in
350 trabecular architecture across the skeleton of humans and chimpanzees using a whole
351 bone/region approach. We find both similarities and differences in regional patterning of
352 trabecular structure across individuals and between taxa. Due to substantial variation in the
353 morphology of the bones/epiphyses included in this study, direct comparison of trabecular bone
354 architecture between anatomical sites is complex, as it may be influenced by factors such as
355 articular surface area or the proximo-distal location of the element (Chirchir, 2015; Saers et al.,
356 2016; for cortical bone see Lieberman et al., 2003). However, by identifying both shared and
357 distinct systemic patterns of trabecular structure, relative (rather than absolute) comparisons can
358 be made across anatomical sites and between taxa. In this comparative context, we find that the
359 systemic pattern of BV/TV, Tb.Th and DA differs between chimpanzees and humans. However,
360 this pattern is not always consistent across the skeleton, or clearly related to joint function based
361 on predicted loading during locomotion.

362 **Taxonomic differences in BV/TV**

363 Recent modern humans have been found to have a lower BV/TV than non-human primates in
364 various anatomical sites (e.g. Maga et al., 2006; Griffin et al., 2010; Shaw and Ryan, 2012;
365 Scherf et al., 2013; Tsegai et al., 2013; Chichir et al., 2015; Ryan and Shaw, 2015), thus we
366 predicted that chimpanzees would have higher BV/TV in all anatomical regions sampled in our
367 study. We find general support for this hypothesis, with chimpanzees having significantly higher

368 BV/TV than humans in the humeral, femoral and MT3 heads and the talus, and higher mean BV/
369 TV values, but not significantly so, in the distal tibia, MC3 and T1. Thus, using a whole-bone/
370 region approach across the skeletons of the same individuals, our results provide further support
371 of a general pattern of higher BV/TV in chimpanzees compared with humans documented in
372 previous studies.

373 Recent trabecular analyses have demonstrated the potential influence of activity levels on
374 trabecular architecture in modern humans, including BV/TV quantified from micro-CT scans or
375 converted from pQCT measures of volumetric mineral density (Chirchir et al., 2015; Ryan and
376 Shaw, 2015; Scherf et al., 2015; Saers et al., 2016; Chirchir et al., 2017). Recent modern humans
377 have lower BV/TV, calculated from pQCT scans, in both the upper and lower limb compared to
378 early modern humans and other fossil hominins, including *H. neanderthalensis* and members of
379 *Australopithecus* (Chirchir et al., 2015). The trabecular architecture in the centre of the proximal
380 humerus of recent modern humans is weaker (e.g. lower BV/TV and Tb.Th) than in Neolithic
381 modern humans (5,700-4,900BP) (Scherf et al., 2015). The timing of this reduction in BV/TV
382 may be related to changes in overall activity level, with recent mobile foragers having stronger
383 bone (higher BV/TV, higher Tb.Th, lower bone surface to volume ratio) in the proximal and
384 distal femur and tibia compared to recent sedentary agriculturalists (Ryan and Shaw, 2015; Saers
385 et al., 2016) and differences in trabecular BV/TV, quantified using pQCT, in particular of the
386 lower limb, can be related to subsistence strategy in recent populations (Chirchir et al., 2017).

387 In the sample included in this study, one human individual has higher BV/TV in every region of
388 the skeleton, which overlaps with chimpanzees in all anatomical locations. Unfortunately, no
389 historical information is available regarding the activity level or occupation of this individual.
390 However, it provides further support for a systemic pattern of trabecular BV/TV that could be

391 related to systemic factors, such as higher activity levels promoting bone remodelling throughout
392 the skeleton (Lieberman, 1996). Across canids, felids and cercopithecines, species with longer
393 travel distances have a higher relative trabecular bone mass, quantified from pQCT, than species
394 with shorter travel distances, indicating the potential influence of overall activity on trabecular
395 structure in a range of taxa (Chirchir et al., 2016a).

396 An explanation is not readily available for the high BV/TV in chimpanzees, in comparison to
397 both active populations of humans and other primate taxa. In the femoral head, chimpanzees
398 have higher BV/TV than closely related *Gorilla* and modern humans, having the highest BV/TV
399 amongst 32 primate taxa (Ryan and Shaw, 2013), and when compared to humans with different
400 subsistence strategies (Ryan and Shaw, 2015). In the humeral head, chimpanzees have higher
401 BV/TV than Neolithic modern humans, recent modern populations and *Pongo* (Scherf et al.,
402 2013; Scherf et al., 2015). Thus, activity levels alone may not explain the systemic difference in
403 BV/TV between humans, chimpanzees, and other primate taxa. This is of particular importance
404 for functional inferences drawn from trabecular structure in fossil hominins, where some
405 anatomical regions or isolated specimens are also characterised by high trabecular BV/TV,
406 similar to or higher than that of chimpanzees (Barak et al., 2013b; Chirchir et al., 2015; Skinner
407 et al., 2015).

408 **Functional signals in systemic patterns of BV/TV**

409 We predicted that the patterns of trabecular BV/TV in the forelimb and hindlimb of chimpanzees
410 and humans would reflect differential loading during locomotion, such that quadrupedal
411 chimpanzees would have more similar BV/TV values in the forelimb and hindlimb, whereas
412 bipedal humans would have higher BV/TV in the hindlimb elements. It is important to make
413 comparisons between elements at a similar anatomical location due to the proximo-distal

414 decrease in trabecular bone mass (bone mineral density measured using pQCT and multiplied by
415 joint size) and BV/TV in hominoids and populations of humans with different subsistence
416 strategies (Chirchir, 2015; Saers et al., 2016). Thus, here we discuss differences between the
417 humeral and femoral head and between the MC3 and MT3 head.

418 We find that both chimpanzees and humans have significantly higher BV/TV in the femoral head
419 compared with the humeral head. This is consistent with previous comparisons of trabecular bone
420 in the humerus and femur in a range of anthropoid species, where all individuals (Fajardo and
421 Müller, 2001; Ryan and Walker, 2010), or the majority of individuals (Shaw and Ryan, 2012),
422 were found to have higher BV/TV in the femoral head compared to the humeral head. Mean
423 trabecular BV/TV, derived from micro-CT and pQCT, is higher in the femoral head compared to
424 the humeral head in extant chimpanzees, modern humans, early modern humans, and *H.*
425 *neanderthalensis* (but not in *Australopithecus africanus*) (Chirchir et al., 2015; Chirchir, 2016),
426 but this difference is not significant in modern humans (Chirchir, 2016). Previous analyses of
427 proximal femoral trabecular properties in humans, although not incorporating the humeral head,
428 or the same anatomical sites as the present study, have also found relatively high trabecular BV/
429 TV in the femoral neck (Amling et al., 1996; Eckstein et al., 2007 [in men but not women]) and
430 femoral head (Hildebrand et al., 1999; Ulrich et al., 1999; Parkinson and Fazzalari, 2003)
431 compared to other anatomical sites analysed (but see Chirchir, 2016).

432 However, the skeletal pattern is more complex when the BV/TV of other anatomical sites is
433 considered. We find that, compared to other anatomical regions, chimpanzees have very high
434 femoral BV/TV, having the highest mean rank of all anatomical sites, whereas in humans femoral
435 BV/TV ranks lower than the talus. In contrast, humeral BV/TV in humans has the lowest mean
436 rank, whereas in chimpanzees it ranks above the MT3 and MC3. Thus, chimpanzees have

437 relatively high femoral BV/TV and humans have very low humeral BV/TV, compared to other
438 anatomical sites. This finding supports our prediction that trabecular BV/TV would reflect
439 reduced loading of the human forelimb, but the pattern in chimpanzees does not support our
440 prediction of similar loading between the two limbs. This could be due to the ‘hindlimb driven’
441 quadrupedal locomotion of chimpanzees, and other primate taxa, whereby the hindlimb
442 experiences greater vertical reaction forces than the forelimb, and propulsion is driven by the
443 hindlimb (Kimura et al., 1979; Demes et al., 1994). Thus, high BV/TV in femoral head of
444 chimpanzees and other primate taxa may reflect this difference in function of the hindlimb
445 during quadrupedal locomotion.

446 Comparisons between the MC3 and MT3 also do not support the hypothesis of higher BV/TV in
447 the hindlimb of humans and more similar BV/TV between the forelimb and hindlimb of
448 chimpanzees. On average, both humans and chimpanzees have higher BV/TV in the MC3
449 compared to the MT3, and, in contrast to our predictions, this pattern is more pronounced in
450 humans. In all human specimens in the study sample, and in 57% of the chimpanzees, the MC3
451 has higher BV/TV than the MT3, with this difference being significant in humans. This is
452 consistent with previous findings, where on average bone density in humans is higher in the
453 metacarpal head while in chimpanzees it is higher in the metatarsal head (Chirchir et al., 2015).
454 Thus, comparisons of BV/TV (derived both from micro-CT and pQCT scans) between the MT3
455 and MC3 does not reflect higher loading of the human hindlimb and more equal loading of the
456 forelimb and hindlimb in chimpanzees. These patterns identified between the femoral and
457 humeral heads, the MC3 and MT3, and throughout the skeleton may reflect the complex
458 relationship between mechanical load, activity level, and anatomical site (Judex et al., 2004;
459 Wallace et al., 2012; Wallace et al., 2013; Wallace et al., 2015).

460 **Taxonomic differences and systemic patterning of DA and Tb.Th**

461 Trabecular structure across the skeleton of humans and chimpanzees supports our prediction that
462 there would be no consistent taxonomic differences in DA. We found no consistent pattern in
463 DA values across the seven anatomical regions within each species. Humans had significantly
464 more anisotropic trabeculae in the talus and MT3, and significantly more isotropic trabeculae in
465 the humeral head and T1 compared to chimpanzees. This variability between taxa and
466 anatomical sites may indicate that DA is primarily reflecting differences in joint loading (see
467 below).

468 Tb.Th has previously been found to scale with body size in a range of primate taxa and
469 anatomical sites (Doube et al., 2011; Barak et al., 2013a; Fajardo et al., 2013; Ryan & Shaw,
470 2013), but also to correlate with BV/TV (Barak et al., 2013a). Here, in support of our prediction,
471 we found no significant differences in absolute Tb.Th between humans and chimpanzees.
472 Considering the smaller body size of chimpanzees, this indicates that they have relatively thick
473 trabeculae compared to humans, however due to the small difference in body size this is unlikely
474 to lead to significant differences. We did, however, find that the systemic pattern of Tb.Th
475 followed a similar pattern in both taxa, being generally higher in the hindlimb (femoral head,
476 talus and distal tibia) and lower in the forelimb (humerus and MC3) in both taxa. This is
477 supported by previous comparisons of Tb.Th between the humerus and femur, which found
478 thicker femoral trabeculae in most taxa/individuals (Ryan and Walker, 2010; Shaw and Ryan,
479 2012; Ryan and Shaw, 2013). However, the MT3 had thin trabecular bone compared to the rest
480 of the hindlimb in both humans and chimpanzees, despite different loading regimes between
481 these two taxa. Differences in BV/TV, but not Tb.Th, indicate potential differences in trabecular
482 number (Tb.N) between these taxa. Previous studies have found differences in Tb.N between

483 humans and chimpanzees (e.g. distal tibia: Su, 2011; Barak et al., 2013b; vertebra: Cotter et al.,
484 2009; femoral head: Ryan and Shaw, 2012; Shaw and Ryan, 2012; humeral head: Ryan and
485 Shaw, 2012; Shaw and Ryan, 2012; Scherf et al., 2013) with chimpanzees having more
486 numerous trabeculae, although this is not the case for the talus (Su, 2011; DeSilva and Devlin,
487 2012) or calcaneus (Kuo et al., 2013; Zeininger et al., 2016).

488 **Functional signals in systemic patterns of DA**

489 The degree of anisotropy of trabeculae, and other related measures such as primary trabecular
490 orientation and elongation index, are often able to distinguish between locomotor mode,
491 especially when comparisons are made between different regions of an epiphysis (e.g.
492 MacLatchy and Müller, 2002; Ryan and Ketcham, 2002b; Maga et al., 2006; Griffin et al., 2010;
493 Hebert et al., 2012; Barak et al., 2013b; Su et al., 2013; Zeininger et al., 2016; Su and Carlson,
494 2017). However, not all trabecular analyses have identified differences in DA or orientation-
495 based variables between locomotor groups (e.g. Fajardo et al., 2007; Kuo et al., 2013). In
496 general, DA is thought to reflect the range of joint positions in which a joint experiences high
497 loads, with more uniformly aligned trabeculae being associated with more stereotypical load
498 orientations, and more isotropic trabeculae with a greater range of adopted joint positions
499 (Fajardo and Müller, 2001; Ryan and Ketcham, 2002b). There is evidence of a systemic pattern
500 in a proximo-distal increase in DA in the human femur and tibia (Saers et al., 2016), which is
501 also found in the present study between the proximal femur and distal tibia. However, this could
502 be a structural adaptation to the proximo-distal reduction in BV/TV, or could be related to other
503 factors, such as differences in gross morphology, and thus loading stereotypy, between the femur
504 and tibia (Saers et al., 2016).

505 We predicted that DA in the hindlimb and humeral head of chimpanzees would reflect
506 differences in loading between the study taxa. In general, humans experience more stereotypical
507 loading of the hindlimb than chimpanzees, whose locomotor repertoire includes knuckle-walking
508 quadrupedalism and several arboreal behaviours (e.g. climbing, clambering and suspension) that
509 require a greater range of joint positions (Hunt, 1991; Doran, 1992, 1993b, 1997; Sarringhaus et
510 al., 2014). We find some support for this prediction. The hindlimb of humans has significantly
511 higher DA in the MT3 head and talus compared to chimpanzees, perhaps reflecting the more
512 stereotypical loading during bipedalism, especially in the foot. Moreover, DA is significantly
513 higher in the MT3 than the MC3 of humans, but not in chimpanzees. However, this is not the
514 case for the distal tibia, where chimpanzees have higher DA (contrary to Barak et al., 2013b). In
515 the chimpanzee forelimb, we find significantly higher DA in the humeral head (contrary to
516 Scherf et al., 2013), and higher mean DA in the metacarpal head (supporting the findings of
517 Tsegai et al., 2013; Chirchir et al., 2016b) compared to humans. In the T1 we find significantly
518 higher DA in chimpanzees compared to humans. A previous analysis of DA in eighth thoracic
519 vertebra found no significant difference in DA between chimpanzees and humans, but did
520 identify a negative correlation between BV/TV and DA in humans, which was absent in non-
521 human apes (Cotter et al., 2009), indicating a complex interplay between these trabecular
522 parameters in the spinal column.

523 Although DA appears to correspond with the type of loading in some anatomical sites, other
524 anatomical areas do not (e.g. the humeral head and distal tibia), nor do they always support the
525 findings of previous studies. This may be related to the whole-region method applied in this
526 study, where trabecular bone from a larger region is quantified, in comparison to previous studies
527 where DA was measured in smaller sub-regions (e.g. volume of interest). Whether trabecular

528 alignment in a small subregion, or in an entire region, is a better indicator of overall loading is
529 unclear. Another potential explanation, is that our predictions of joint loading are often
530 oversimplified, and the impact of different behaviours on bone structure is unknown. For
531 example, a lower DA might have been expected for the chimpanzee humeral head, based on their
532 adoption of a range of arboreal behaviours and thus varied load orientations. However, knuckle-
533 walking is the most frequent locomotor behaviour used by adult chimpanzees (Doran, 1992;
534 Sarringhaus et al., 2014), and as such, may contribute more to trabecular anisotropy than less
535 frequent arboreal locomotor bouts.

536 **Trabecular structure and articular morphology**

537 Comparisons of trabecular bone structure between anatomical regions, or indeed of the same
538 anatomical region between different taxa, are potentially influenced by differences in the gross
539 morphology of the articular region, and by articular function. Primate taxa differ in relative
540 articular surface area and absolute articular size, due to differences in both the magnitude of load
541 and the range of joint excursion, which can be related to locomotor mode (Ruff, 1988; Godfrey
542 et al., 1991; Ruff and Runestad, 1992; Godfrey et al., 1995; Ruff, 2002). Moreover, the
543 relationship between articular surface area and joint mobility may differ between joint types; for
544 example in a ball-and-socket joint, an increase in surface area may have more of an impact on
545 joint mobility than in a hinge joint (Ruff, 2002). Although our discussion has focused largely on
546 the comparative context, i.e. differences in the systemic pattern between humans and
547 chimpanzees, it is important to recognise the potential impact of these aspects of external joint
548 morphology on the findings of this study. It is beyond the scope of the present study to explore
549 this further, however, it is an important and relatively unexplored area of trabecular research (but
550 see Rafferty and Ruff, 1994). Future research into systemic patterns of trabecular structure

551 should further investigate the relationship between trabecular morphology and external articular
552 morphology, both within and between taxa.

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Conclusion

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Here we demonstrate that an understanding of the way in which trabecular bone varies across the skeleton can have important implications for inferring joint load, function, and ultimately behaviour, from trabecular structure. Chimpanzees and humans have systemically different trabecular BV/TV throughout their skeleton, such that humans (except for one individual within our sample) had lower BV/TV in all anatomical regions compared with chimpanzees. However, differences in BV/TV between the humeral and femoral head and the MC3 and MT3 do not directly reflect predicted differences in loading of the fore- and hindlimb in each taxon. Rather, overall BV/TV may be driven by other factors, such as overall activity level (Ryan and Shaw, 2015). Mean Tb.Th values across the skeleton do not differ significantly between chimpanzees and humans, and trabeculae are generally thicker in the hindlimb compared with the forelimb in both taxa. These systemic patterns must be considered when inferring the magnitude of joint load in any one skeletal area (e.g. high BV/TV may not necessarily reflect solely higher load/activity levels). This is particularly true, but also especially challenging, when inferring function in fossil taxa when only isolated elements are preserved, and thus potential systemic patterns are unknown. In contrast to BV/TV, the degree to which trabeculae are preferentially oriented (DA) did not differ consistently across the skeleton within chimpanzees or humans. Although the pattern of DA across different skeletal elements did not always fit our predictions, the pattern suggests that trabecular alignment may more directly reflect differences in the magnitude and direction of joint loading, and thus behaviour, than BV/TV (and Tb.Th).

Author contributions

574 Concept/design: ZJT, MMS, JJH, TLK; Acquisition of data: ZJT; Data analysis/interpretation: ZJT,
575 MMS, DHP, TLK; Drafting and revision of the manuscript: ZJT, MMS, DHP, JJH, TLK; Approval of the
576 article: ZJT, MMS, DHP, JJH, TLK

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Tables

864 Table 1. Study sample

Taxon	Collection ¹	Specimen ID	Sex	Elements
<i>H. sapiens</i>	UG	INDEN_91	M	R Hum, R MC3 R Fem, R Tib, R Tal, L MT3 T1
<i>H. sapiens</i>	UG	INDEN_113	M?	R Hum, L MC3 R Fem, L Tib, L Tal, L MT3 T1
<i>H. sapiens</i>	UG	INDEN_118	F	R Hum, R MC3 R Fem, L Tib, L Tal, L MT3 T1
<i>H. sapiens</i>	UG	INDEN_311	M	R Hum, R MC3 R Fem, L Tib, L Tal, R MT3 T1
<i>H. sapiens</i>	UK	NGA_88_SK_766	U	L Hum, L MC3 R Fem, R Tib, R Tal, R MT3 T1
<i>H. sapiens</i>	UK	NGA_88_SK_825	U	R Hum, R MC3 L Fem, L Tib, R Tal, R MT3 T1
<i>H. sapiens</i>	UK	NGA_88_SK_880	U	R Hum, R MC3 L Fem, L Tib, L Tal, L MT3 T1
<i>P. troglodytes verus</i>	MPIEVA	MPITC_11781	M	L Hum, L MC3 R Fem, R Tib, R Tal, R MT3 T1
<i>P. troglodytes verus</i>	MPIEVA	MPITC_11778	F	L Hum, L MC3 R Fem, R Tib, R Tal, R MT3 T1
<i>P. troglodytes verus</i>	MPIEVA	MPITC_14996	F	L Hum, L MC3 R Fem, R Tib, R Tal, R MT3 T1
<i>P. troglodytes verus</i>	MPIEVA	MPITC_15001	F	L Hum, L MC3 R Fem, R Tib, R Tal, R MT3 T1
<i>P. troglodytes verus</i>	MPIEVA	MPITC_15002	F	L Hum, L MC3 R Fem, R Tib, R Tal, R MT3 T1
<i>P. troglodytes verus</i>	MPIEVA	MPITC_15012	M	R Hum, L MC3 R Fem, R Tib, R Tal, L MT3 T1
<i>P. troglodytes verus</i>	MPIEVA	MPITC_15013	F	L Hum, L MC3 R Fem, R Tib, L Tal, R MT3 T1

865 ¹ MPIEVA – Max Planck Institute for Evolutionary Anthropology, UK – University of Kent, UG – University of
 866 Göttingen

867 ² M – Male, F – Female, U – Unknown, ? – indicates uncertainty concerning sex. Data taken from collection records.

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869 Table 2. Trabecular structure in each taxon across anatomical sites. Mean values with standard
 870 deviation in parentheses, and p-values resulting from Mann-Whitney U tests between taxa.
 871 Significant differences are shown in bold.

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Element	Taxon	Tb.Th (mm)	BV/TV (%)	DA
Humerus	<i>Homo</i>	0.21 (0.02)	12.72 (4.07)	0.11 (0.04)
	<i>Pan</i>	0.22 (0.02)	25.32 (3.82)	0.17 (0.02)
	p-value	0.90	<0.01	<0.01
MC3	<i>Homo</i>	0.19 (0.02)	21.25 (3.16)	0.20 (0.08)
	<i>Pan</i>	0.18 (0.01)	22.75 (1.58)	0.23 (0.04)
	p-value	0.32	0.16	0.46
T1	<i>Homo</i>	0.22 (0.04)	21.29 (5.91)	0.12 (0.05)
	<i>Pan</i>	0.20 (0.02)	26.08 (3.78)	0.18 (0.05)
	p-value	0.38	0.16	0.03
Femur	<i>Homo</i>	0.26 (0.03)	22.72 (5.45)	0.16 (0.05)
	<i>Pan</i>	0.33 (0.07)	38.58 (6.85)	0.08 (0.09)
	p-value	0.07	<0.01	0.13
Tibia	<i>Homo</i>	0.26 (0.02)	21.66 (3.11)	0.29 (0.06)
	<i>Pan</i>	0.24 (0.03)	25.98 (4.31)	0.34 (0.05)
	p-value	0.16	0.10	0.05
Talus	<i>Homo</i>	0.27 (0.03)	26.26 (3.43)	0.11 (0.06)
	<i>Pan</i>	0.31 (0.04)	35.94 (3.87)	0.02 (0.03)
	p-value	0.07	<0.01	<0.01
MT3	<i>Homo</i>	0.17 (0.02)	17.54 (3.47)	0.31 (0.03)
	<i>Pan</i>	0.18 (0.03)	22.89 (3.93)	0.22 (0.03)
	p-value	0.90	0.01	<0.01

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875 Table 3. Comparisons of trabecular structure between anatomical sites within each taxon. Mean
 876 rank of each trabecular variable within individuals from lowest (1) to highest (7) in *Homo* and
 877 *Pan*. Results of Friedman tests indicate the presence of significant differences between
 878 anatomical sites in *Homo* and in *Pan*. Significant differences are shown in bold.

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Taxon	Element	Rank		
		Tb.Th	BV/TV	DA
<i>Homo</i>	Humerus	3.43	1.00	2.29
	MC3	2.29	4.29	4.57
	T1	3.57	4.43	2.43
	Femur	5.71	5.14	3.71
	Tibia	6.00	4.29	6.14
	Talus	6.00	6.57	2.29
	MT3	1.00	2.29	6.57
	p-value	<0.01	<0.01	<0.01
<i>Pan</i>	Humerus	3.57	3.57	3.29
	MC3	2.14	2.00	5.29
	T1	3.00	3.71	3.86
	Femur	6.57	6.85	2.57
	Tibia	4.86	4.00	6.86
	Talus	6.43	6.14	1.00
	MT3	1.43	1.71	5.14
	p-value	<0.01	<0.01	<0.01

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882 Table 4. Comparison between anatomical regions within each taxon. P-values from pairwise
 883 Wilcoxon tests **with a post-hoc Bonferroni correction** between all anatomical sites in *Homo*
 884 (shaded) and *Pan* (unshaded). Significant differences are shown in bold.

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	Humerus	MC3	T1	Femur	Tibia	Talus	MT3
Tb.Th	Humerus	0.146	1.000	0.086	0.049	0.024	0.012
	MC3	0.024	1.000	0.012	0.012	0.012	1.000
	T1	1.000	0.795	1.000	0.233	0.367	0.795
	Femur	0.049	0.012	0.024	1.000	1.000	0.012
	Tibia	1.000	0.012	0.795	0.551	1.000	0.012
	Talus	0.012	0.012	0.012	1.000	0.147	0.012
	MT3	0.367	1.000	1.000	0.024	0.086	0.012
BV/TV	Humerus	0.086	0.147	0.049	0.086	0.012	0.551
	MC3	1.000	1.000	1.000	1.000	0.367	0.367
	T1	1.000	0.795	1.000	1.000	1.000	1.000
	Femur	0.086	0.012	0.049	1.000	1.000	1.000
	Tibia	1.000	1.000	1.000	0.086	0.795	0.367
	Talus	0.012	0.012	0.024	1.000	0.024	0.086
	MT3	1.000	1.000	1.000	0.024	1.000	0.012
DA	Humerus	0.551	1.000	1.000	0.012	1.000	0.012
	MC3	0.049	0.551	1.000	1.000	0.551	0.024
	T1	1.000	1.000	1.000	0.012	1.000	0.012
	Femur	1.000	0.147	1.000	0.233	1.000	0.012
	Tibia	0.012	0.086	0.024	0.012	0.012	1.000
	Talus	0.012	0.012	0.012	0.795	0.012	0.012
	MT3	0.086	1.000	1.000	0.551	0.049	0.012

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Figure legends

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Figure 1. Region of interest defined for each element. Grey boxes represent the definition of each region in specimens of *Pan* for (A) humeral head, (B) femoral head, (C) distal tibia, (D) third metacarpal head, (E) third metatarsal head, and (F) first thoracic vertebral body (shown in a mid-sagittal section, as transverse process obscures a clear view of the vertebral body). For the talus, not shown here, trabecular structure was quantified throughout the entire bone.

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Figure 2. Quantification of trabecular bone. (A) Segmented voxel data where cortex, trabecular bone and air inside the bone are assigned different grey values. (B) Trabecular only region which was imported into BoneJ to measure Tb.Th. (C) 3D tetrahedral mesh of cortex and inner region of bone. (D) Each element in the tetrahedral mesh of the inner region was assigned a BV/TV value, as visualised here where regions of low BV/TV are in blue and high BV/TV in red.

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Figure 3. Variation in trabecular bone structure across the skeleton of *Homo* and *Pan*. Boxplots showing (A) Tb.Th, (B) BV/TV and (C) DA in the humeral head (Hum), third metacarpal head (MC3), femoral head (Fem), distal tibia (Tib), talus (Tal), third metatarsal head (MT3), and first thoracic vertebra (T1) in *Homo* (red) and *Pan* (blue). Significant differences are indicated by brackets with * for $p < 0.05$ and ** for $p < 0.01$.

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Figure 4. Systemic differences in BV/TV across the skeleton of *Homo* (red) and *Pan* (blue). BV/TV in each individual of *Homo* (red) and *Pan* (blue) in the humeral head (Hum), third metacarpal head (MC3), femoral head (Fem), distal tibia (Tib), talus (Tal), third metatarsal head (MT3), and first thoracic vertebra (T1)

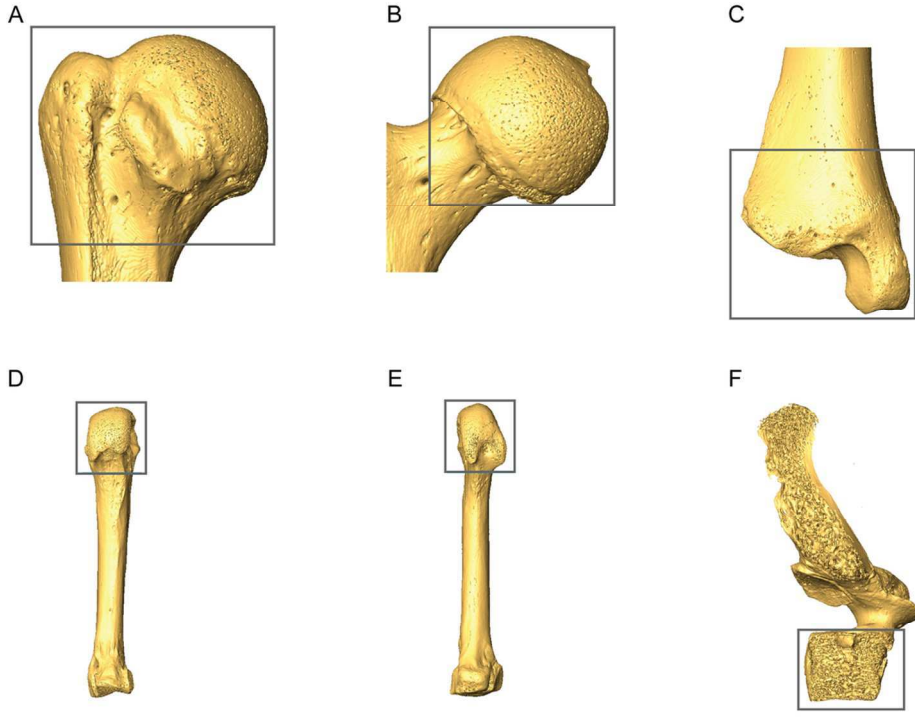


Figure 1

110x80mm (300 x 300 DPI)

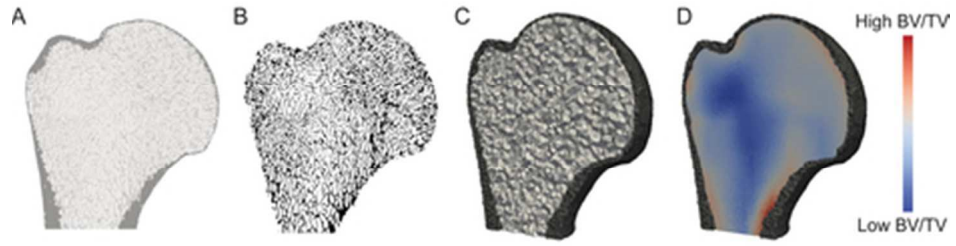


Figure 2

39x10mm (300 x 300 DPI)

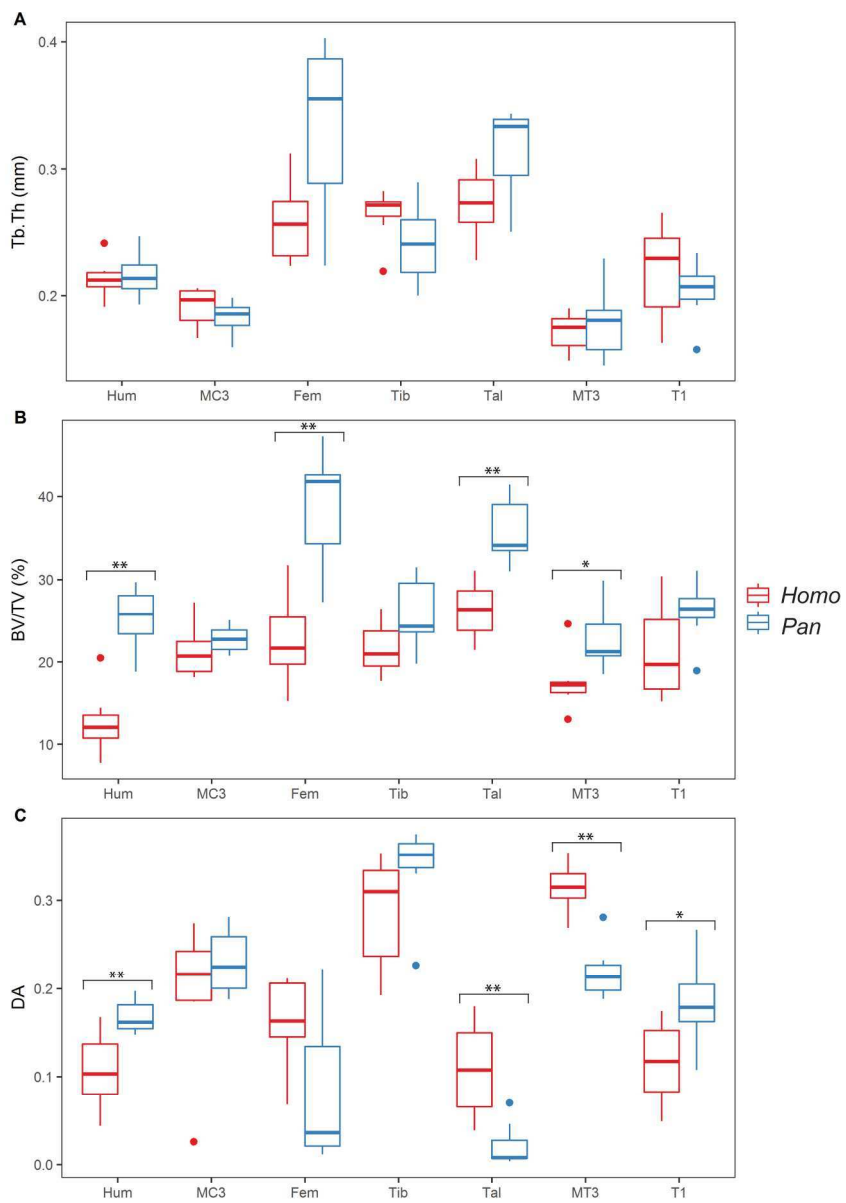


Figure 3

208x295mm (300 x 300 DPI)

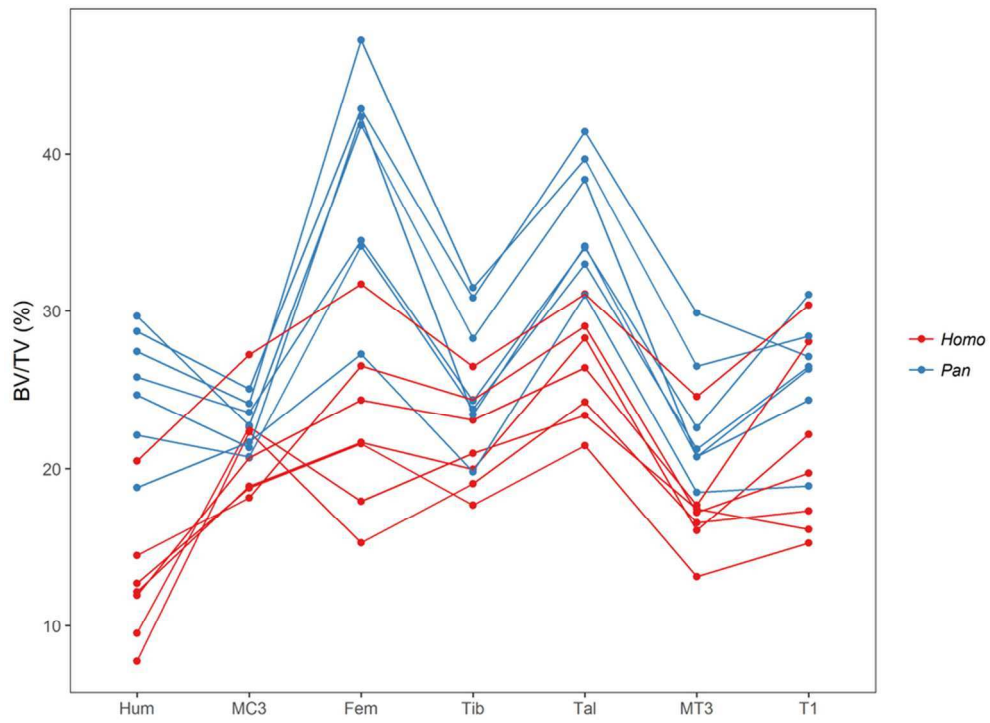


Figure 4

87x63mm (300 x 300 DPI)