

1 **Trabecular and cortical bone structure of the talus and distal tibia in Pan and Homo**

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ABSTRACT

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Objectives: Internal bone structure, both cortical and trabecular bone, remodels in response to loading and may provide important information regarding behaviour. The foot is well suited to analysis of internal bone structure because it experiences the initial substrate reaction forces, due to its proximity to the substrate. Moreover, as humans and apes differ in loading of the foot, this region is relevant to questions concerning arboreal locomotion and bipedality in the hominoid fossil record.

Materials and methods: We apply a whole-bone/epiphysis approach to analyse trabecular and cortical bone in the distal tibia and talus of *Pan troglodytes* and *Homo sapiens*. We quantify bone volume fraction (BV/TV), degree of anisotropy (DA), trabecular thickness (Tb.Th), bone surface to volume ratio (BS/BV), cortical thickness, and investigate the distribution of BV/TV and cortical thickness throughout the bone/epiphysis.

Results: We find that *Pan* has a greater BV/TV, a lower BS/BV and thicker cortices than *Homo* in both the talus and distal tibia. The trabecular structure of the talus is more divergent than the tibia, having thicker, less uniformly aligned trabeculae in *Pan* compared to *Homo*. Differences in dorsiflexion at the talocrural joint and in degree of mobility at the talonavicular joint are reflected in the distribution of cortical and trabecular bone.

Discussion: Overall, quantified trabecular parameters represent overall differences in bone strength between the two species, however, DA may be directly related to joint loading. Cortical and trabecular bone distributions correlate with habitual joint positions adopted by each species, and thus have potential for interpreting joint position in fossil hominoids.

1. INTRODUCTION

54
55 Aspects of the external bony morphology of the talus and distal tibia reflect kinematic differences
56 between how terrestrial bipedal humans and arboreal, quadrupedal African apes load their foot and ankle
57 during locomotion (e.g. Lewis, 1980a,b,c; Stern and Susman, 1983; Latimer et al., 1987; DeSilva, 2009;
58 Barak et al., 2013b). These morphological differences can be related to fundamental differences in foot
59 posture: the degree of dorsiflexion at the ankle, use of the foot in an inverted position, the general
60 conformation of the leg, and the presence of medial and longitudinal arches of the foot. For example,
61 compared with African apes, humans have been described as having a less mediolaterally expanded
62 anterior distal articular surface of the tibia (Latimer et al., 1987; DeSilva, 2009), an angle close to 90
63 degrees between the long axis and distal articular surface of the tibia (Latimer et al., 1987; DeSilva,
64 2009), a more symmetric talar trochlea (Latimer et al., 1987; DeSilva, 2009), a relatively stiff mid-foot
65 without a mid-tarsal break (Elftman and Manter, 1935; DeSilva, 2010), and a complex of features,
66 including the medial longitudinal arch, metatarsophalangeal joints and various soft tissues, which
67 contribute to the windlass mechanism (Griffin et al., 2015) that improves locomotor efficiency (Ker et al.,
68 1987).

69 In part due to the mosaic nature of fossil hominin morphology, but also due to reliance on fragmentary or
70 isolated postcranial elements, palaeoanthropologists often differ in their interpretations of the functional
71 significance of various morphological features. It remains unclear, based on the morphology of the ankle,
72 whether early hominins continued to engage in a significant amount of arboreal behaviour and whether
73 hominin species used kinematically similar or distinct forms of bipedalism, perhaps unlike the modern
74 human bipedal gait (e.g. Day and Wood, 1968; Lisowski et al., 1974; Lisowski et al., 1976; Oxnard and
75 Lisowski, 1980; Stern and Susman, 1983; Latimer et al., 1987; Clarke and Tobias, 1995; Harcourt-Smith
76 and Aiello, 2004; DeSilva, 2009; DeSilva and Throckmorton, 2010; Zipfel et al., 2011; Haile-Selassie et
77 al., 2012; DeSilva et al., 2013; Harcourt-Smith et al., 2015; Prang, 2015, 2016). Functional interpretation
78 of the external skeletal morphology of the foot is further complicated by the role of soft tissues in limiting

79 or enabling adoption of different foot postures (Venkataraman, 2013a,b) and by the substantial individual
80 variability in the flexibility of the modern human foot (Bates et al., 2013; DeSilva et al., 2015). As the
81 foot comprises a complex system of bones, tendons, ligaments and muscles, there are potentially many
82 different ways for it to adapt to different functions, other than by modification of external bone shape
83 (Crompton, 2015). Even modern humans are able to access numerous resources efficiently from the
84 arboreal environment (Kraft et al., 2014), without any apparent external morphological signal on the talus
85 and distal tibia (Venkataraman et al., 2013a).

86 Analysis of internal bone structure, both cortical and trabecular bone, of the talocrural and talonavicular
87 joint has potential to provide further insight into interpreting use of the foot in the past. While external
88 articular morphology indicates the joint positions a species was able to adopt, the internal bone structure
89 can provide information about how a joint was actually loaded (Ruff and Runestad, 1992; Kivell, 2016).
90 This is because both trabecular and cortical bone structure can adapt to loading during an individual's
91 lifetime (e.g. Lanyon, 1974; Robling et al., 2002; Pontzer et al., 2006; Ruff et al., 2006; Barak et al.,
92 2011; Kivell, 2016), by remodelling in response to strain (Ehrlich and Lanyon, 2002). Structural
93 adaptations can occur at the level of individual trabeculae (Schulte et al., 2013; Cresswell et al., 2015). As
94 these individual trabeculae appear able to adapt to accommodate regional strains, it is likely that regional
95 architectural parameters can provide information about how different areas of a joint are loaded. For
96 example, trabecular and cortical bone distribution close to the articular surface, radiodensity patterns, and
97 indicators of bone remodelling, correspond with predicted locations of peak loading associated with
98 specific joint positions (Patel and Carlson, 2007; Polk et al., 2008, 2010; Mazurier et al., 2010; Zeininger
99 et al., 2011; Carlson et al., 2013; Tsegai et al., 2013; Skinner et al., 2015).

100 Experimentally changing the loading regime of a joint or limb by, for example, changing the angle of the
101 joint during loading or subjecting a limb to an unnatural load, leads to predictable alterations in both
102 cortical and trabecular bone (Robling et al., 2002; Pontzer et al., 2006; Barak et al., 2011; Cresswell et al.,
103 2015). It is often difficult to relate bone structure, especially that of trabecular bone, directly to the

104 biomechanical environment, i.e. to connect specific architectural variables to joint function and loading
105 regime. Factors other than behaviour have the potential to influence, or even be the main factor
106 determining, bone form (Bertram and Swartz, 1991; Lovejoy et al., 2003; Ruff et al., 2006; Kivell, 2016).
107 There is still much that we do not fully understand about bone functional adaptation, including the genetic
108 and systemic factors that shape trabecular and cortical structure (Lieberman, 1996; Carlson et al., 2008;
109 Havill et al., 2010; Wallace et al., 2010; Paternoster et al., 2013; Wallace et al., 2013; Tsegai et al.,
110 2016a). These include the way in which bone remodels depending upon the duration, frequency, or
111 magnitude of the external load (e.g. Frost, 1987; Rubin and Lanyon, 1985; Skerry and Lanyon, 1995), or
112 how these factors might vary depending on species (e.g. Turner, 2001), anatomical region (e.g. Morgan
113 and Keaveny, 2001), age (e.g. Pearson and Lieberman, 2004) or body mass (e.g. Biewener, 1990; Doube
114 et al., 2011). Moreover, cortical and trabecular bone may respond differently to strain or even interact to
115 compensate for each other (Carlson and Judex, 2007). It is likely that these factors vary between even
116 closely related species/subspecies. For example, some of the genetic differences between modern humans
117 and Neanderthals relate to bone growth (Green et al., 2010), and changes in indirect measures of hormone
118 levels occur at different developmental stages in humans, chimpanzees and bonobos (e.g. TT3: Behringer
119 et al., 2014a; testosterone: Behringer et al., 2014b). All of these factors can confound our functional
120 interpretations of variation in bone structure. However, there is a wealth of comparative, computational
121 and in vivo research that makes clear that variation in cortical and trabecular structure reflects, at least to
122 some degree, variation in external loading (Ruff et al., 2006; Kivell, 2016).

123 The hominoid foot and ankle, specifically the talocrural and talonavicular joints, are well suited to
124 analysis of internal bone structure due to differences in foot postures adopted by modern humans and
125 extant apes, the specific structure of the joint, and the close association of the foot with the substrate.
126 Several studies have investigated the kinematics of the foot, during both quadrupedal and bipedal
127 locomotion, in humans and chimpanzees (e.g. Sockol et al., 2007; Pontzer et al., 2009; Pontzer et al.,
128 2014; O'Neill et al., 2015; Holowka et al., 2017). As modern human bipeds and chimpanzee

129 climbers/knuckle-walkers adopt divergent foot postures (DeSilva, 2009), the loading environment within
130 the foot and at the ankle is likely to differ between these groups. In *Pan troglodytes*, the ankle is loaded in
131 dorsiflexion during both vertical climbing and during quadrupedal knuckle-walking (Sockol et al., 2007;
132 DeSilva, 2009; Pontzer et al., 2009; Barak et al., 2013b; Pontzer et al., 2014), whereas the human ankle
133 adopts a more neutral posture during bipedalism (Barak et al., 2013b). The chimpanzee ankle is also
134 inverted during climbing (Lewis 1980a; Latimer et al., 1987; DeSilva, 2009). Loading at the talonavicular
135 joint is characterised by greater mobility in *Pan* compared to *Homo*, either related to dorsiflexion (i.e. the
136 midtarsal break) or to rotation (Elftman and Manter, 1935; DeSilva, 2010; Thompson et al., 2014; but see
137 Holowka et al., 2017). The high joint congruity between the distal tibia and the trochlea surface of the
138 talus (Latimer et al., 1987) indicates that the bone structure is likely to be directly related to joint use, and
139 not to other factors such as the action of muscles, as in other regions (e.g. the humeral head), where the
140 bony articulation itself does not maintain joint integrity. In the absence of muscle/tendon attachments on
141 the talus itself, and thus of tensile forces caused by muscle contractions, this region also offers an
142 opportunity to analyse the effects of locomotor forces alone on trabecular bone structure (DeSilva and
143 Devlin, 2012). Further, as the foot is in direct contact with the substrate, it directly experiences the initial
144 forces of locomotion, unlike more proximally located joints. The same is true for the hand, where clear
145 trabecular signals of the direction of loading are present (Tsegai et al., 2013; Skinner et al., 2015).

146 Previous analyses have assessed the functional significance of trabecular and cortical bone structure of the
147 ankle in humans (talus: Takechi et al., 1982; Sinha, 1985; Pal and Routal, 1998; Ebraheim et al., 1999;
148 Schiff et al., 2007; Athavale et al., 2008; Nowakowski et al., 2013; talus and distal tibia: Hvid et al.,
149 1985), and several studies have adopted a comparative approach across different taxa (talus: Su, 2011;
150 DeSilva and Devlin, 2012; Hérbert et al., 2012; Su et al., 2013; Su and Carlson, 2017; tibia: Su, 2011;
151 Barak et al., 2013b; Carlson et al., 2016). DeSilva and Devlin (2012) found interspecific differences in
152 regional patterning of trabecular structure across four quadrants of the talar body, but were unable to
153 attribute these differences to locomotor mode and a biomechanical explanation remains unclear. Analysis

154 of more localised subregions, sampling bone directly adjacent to the articular surface, has shown regional
155 patterning of degree of anisotropy (DA), elongation and primary trabecular orientation, which is distinct
156 in modern humans when compared with extant apes, with fossil hominins displaying some ape-like and
157 some human-like features (Su, 2011; Su et al., 2013; Su and Carlson, 2017). At the distal tibia, the
158 orientation of trabecular bone in humans and chimpanzees corresponds with measurements of
159 dorsiflexion at the ankle (Barak et al., 2013b). Previous studies have assessed cortical thickness and
160 radiodensity patterns of the articular surfaces of the primate talus and distal tibia (talus: Su, 2011; tibia:
161 Su, 2011; Carlson et al., 2016), and behavioural correlates have been identified from bone profiles and
162 radiodensity patterns at articular surfaces of other primate and mammalian taxa and epiphyses (Patel and
163 Carlson, 2007; Mazurier et al., 2010; Carlson et al., 2013). However, to our knowledge no previous study
164 has comparatively analysed cortical thickness maps in both the talus and distal tibia of humans and
165 chimpanzees.

166 Previous studies quantifying trabecular bone structure and/or bone strength characteristics at the ankle
167 relied on analyses of multiple volumes of interest (Su, 2011; DeSilva and Devlin, 2012; Su et al., 2013) or
168 on destructive methods (Sinha, 1985; Athavale et al., 2008). Interspecific analyses are often complicated
169 by the difficulty in identifying biologically homologous regions, and differences in VOI size and location
170 have a substantial impact on trabecular bone analysis, especially when comparing among species that
171 vary greatly in size and in morphologically complex bones (Maga et al., 2006; Kivell et al., 2011;
172 Lazenby et al., 2011). Moreover, trabecular bone close to the articular surface, which can be difficult to
173 sample using VOI-based methods that require manual discrimination between cortical and trabecular
174 bone, is more likely to be of biomechanical relevance as it experiences the initial joint reaction forces, and
175 bone closer to the articular surface differs from that in the center of the epiphysis (Singh, 1978). Analyses
176 of bone strength at the articular surface have not investigated the cortical and trabecular structure
177 independently, but have instead used methods which quantify cortical bone and some of the underlying
178 trabeculae (Patel and Carlson, 2007; Mazurier et al., 2010). In this study, we address some of these

179 challenges by using two methodologies that allow independent quantification of the trabecular and the
180 cortical structure. The trabecular bone analysis applied here enables quantification of trabecular structure
181 throughout the bone or in a pre-defined region of the epiphysis, however, statistical comparisons cannot
182 be conducted between groups. For cortical bone, we use a method that is able to compare cortical
183 thickness across the bone/epiphysis between groups, but does not allow quantification of trabecular
184 structure further than around 5mm beneath the cortex. By combining these complementary
185 methodologies, we are able to analyse patterns of both cortical and trabecular bone in the human and
186 chimpanzee talus and distal tibia. As a result, we are able to generate a fine scale, nuanced analysis
187 through the visualisation of regional patterning of both cortical and trabecular bone, which may provide
188 detailed information about joint loading.

189 In this study, we measure trabecular and cortical bone of the talus and distal tibia in *Pan troglodytes verus*
190 and *Homo sapiens*. We test the following predictions in how trabecular bone structure and distribution,
191 and cortical thickness and distribution differ between *Pan* and *Homo*. First, as both the talocrural and
192 talonavicular joint are used in a greater range of positions in *Pan*, and both joints are less mobile in
193 *Homo*, we predict a higher DA in humans in both the talus and tibia (Barak et al., 2013b; Su, 2011; Su et
194 al., 2013; Thompson et al., 2014; Su and Carlson, 2017; but see Holowka et al., 2017). Second, following
195 the findings of previous trabecular studies that sedentary modern humans have a generally low BV/TV
196 and cortical thickness (Ruff et al., 1993; Lieberman, 1996; Ruff, 2005; Chirchir et al., 2015; Ryan and
197 Shaw, 2015; Scherf et al., 2015; Chirchir et al., 2017), we predict an overall lower BV/TV and thinner
198 cortex in *Homo*. Third, we hypothesise that the regional distribution of both cortical and trabecular bone
199 will reflect differences in habitual peak loading of the talocrural and talonavicular joints. More
200 specifically, that at the talocrural joint *Pan* will show a pattern of BV/TV and cortical thickness that
201 reflects use of the foot in dorsiflexion and inversion, and at the talonavicular joint a greater degree of
202 mobility. In *Homo*, the trabecular bone distribution and cortical thickness will reflect less mobility, and a
203 more neutral ankle position.

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2. MATERIALS AND METHODS

2.1 Sample

This study analysed trabecular and cortical bone morphology of the tibia and talus of two species with divergent modes of locomotion: *Pan troglodytes verus* and *Homo sapiens*. The sample, detailed in Table 1, included fifteen wild *P. t. verus* individuals (tibiae: N = 10; tali: N = 13; of which N = 8 were paired) whose skeletal remains were collected from the Taï National Park, Cote d'Ivoire, and ten *H. sapiens* individuals (tibia: N = 8; tali: N = 9; of which N = 7 were paired) from an 18th - 19th century cemetery in Inden, Germany. Adult specimens were used, based on fusion of the epiphyses throughout the skeleton and no external signs of pathology or senescence related changes were present. The right side was chosen where both talus and tibia were available and free from damage, otherwise the left side was used.

2.2 Computed tomography

High resolution micro-computed tomography (CT) scans were collected with a BIR ACTIS 225/300 CT scanner for the tibiae and with a SkyScan1173 CT scanner for the tali, using an acceleration voltage of 130kV and 100 μ A and either a 0.5mm brass or 1mm aluminium filter, at the Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology (Leipzig, Germany). Isotropic acquisition voxel sizes were 25-36 microns for the tibia and talus of *Homo* and 19-30 microns for the tibia and talus of *Pan*. Each scan was reconstructed as a 2048 x 2048 16-bit TIFF image stack from 2500 projections with three-frame averaging. Following reconstruction, all specimens were reoriented into standardised positions using AVIZO 6.3[®] (Visualization Sciences Group, SAS) and segmented using a Ray Casting Algorithm (Scherf and Tilgner, 2009).

Prior to segmentation, all *Pan* specimens were resampled to 35 microns and all *Homo* specimens to 40 microns, due to processing constraints. The relative resolutions, a measure of how adequately the average trabecular strut is represented (i.e. mean trabecular thickness [mm] / resolution [mm]), are shown in Table 1. The average for the entire sample of 7.57 (range: 5.46 – 11.59) is consistent with previous studies of

228 trabecular bone structure (Sode et al., 2008; Kivell et al., 2011; Tsegai et al., 2013), and is appropriate for
229 microstructural analysis.

230 **2.3 Analysis of trabecular bone microstructure**

231 To quantify trabecular bone, each material in the scan (Fig. 1a), i.e. cortical bone, trabecular bone, air and
232 the internal bone cavity, were segmented automatically using an in house script in medtool v3.9 ([www.dr-
233 pahr.at](http://www.dr-pahr.at)), following Gross et al. (2014). Morphological filters were used to separate these regions, and the
234 kernel size used was adjusted for each individual according to its measured trabecular thickness, enabling
235 an accurate, subject-specific segmentation. This resulted in three data sets that were used in subsequent
236 processing steps: (1) the trabecular bone (Fig. 1b), (2) the inner region of the bone and, (3) the inner mask
237 (Fig. 1c), which contains the internal region of the bone where internal bone cavity and trabecular bone
238 are represented by different grey values and the cortex has been removed. This automated segmentation
239 was problematic in two locations in the talus, at the inferior talar neck and at the subtalar joint surfaces,
240 due to their complex morphology. Thus the results from these regions are treated with caution. The
241 proximal boundary of the distal tibia was defined as the point at which curvature of the shaft begins in
242 both medial and anterior views, which is at the proximal extent of the fibular notch, and is an equivalent
243 location across the sample.

244 From the trabecular only mask (Fig. 1b), trabecular thickness (Tb.Th), bone surface area (BS), and bone
245 volume (BV) were quantified using the BoneJ plugin (version 1.3.12; Doube et al., 2010) for ImageJ
246 v1.46r (Schneider et al., 2012). Bone surface to volume ratio (BS/BV) was subsequently calculated.

247 The inner region of the bone was used to create a 3D tetrahedral mesh with a mesh size of 1mm, using
248 CGAL 4.4 (CGAL, Computational Geometry, <http://www.cgal.org>). The inner mask (Fig. 1c) was used to
249 calculate BV/TV throughout the bone to generate 3D colour maps of bone distribution, and to calculate
250 the overall bone volume fraction (BV/TV) and degree of anisotropy (DA) using medtool v3.9. A
251 rectangular background grid, with a grid size of 2.5mm, was applied and a spherical VOI with a diameter

252 of 5mm was used to measure BV/TV at each node of the grid. A sphere size of 5mm is appropriate as
253 enough trabecular struts are sampled to adequately quantify trabecular parameters (Gross et al., 2014). To
254 create a 3D colour map of bone distribution, the BV/TV values at each node were interpolated to assign
255 each element in the 3D mesh of the trabecular region a BV/TV value (Fig. 1d). The colour maps were
256 visualized in Paraview v4.0.1 (Ahrens et al., 2005). The overall BV/TV value was calculated as the mean
257 of the values for each element in the 3D mesh, and thus is the average for the whole bone/epiphysis. The
258 mean intercept method (Whitehouse, 1974; Odgaard, 1997) was used to calculate the mean fabric tensor,
259 the arithmetic mean of all second order fabric tensors normalised using the determinants. The extracted
260 eigenvalues and eigenvectors were then used to calculate the DA ($DA = 1 - [\text{smallest eigenvalue}/\text{largest}$
261 $\text{eigenvalue}]$), whereby a DA of 1 indicates complete anisotropy and a DA of 0 complete isotropy.

262 **2.4 Analysis of cortical bone microstructure**

263 To compare cortical thickness between Pan and Homo in the talus and distal tibia, cortical bone thickness
264 maps were generated for each specimen (following Treece et al., 2010; Treece et al., 2012; Tsegai et al.,
265 2016b). This was accomplished via semi-automatic segmentation of the cortical surface, from the
266 unsegmented CT data (Fig. 1e-f) in Stradwin v5.1a (Treece, Gee, Cambridge;
267 <http://mi.eng.cam.ac.uk/~rwp/stradwin>). Following definition of the surface, around 15,000 independent
268 measurements of cortical thickness were calculated throughout the bone (Fig. 1f) and mapped onto a
269 subject specific surface (Fig. 1g). Subsequently, each surface was registered to a canonical surface using
270 wxRegSurf v13 (Fig. 1h). The canonical surface used was an average of the entire sample, each species
271 was averaged separately and then the average of the two resulting surfaces was used, to prevent the
272 difference in sample size affecting the average morphology. After registration to the canonical surface,
273 mean thickness maps were generated for each species.

274 **2.5 Statistical analysis**

275 For trabecular bone analysis, all statistical tests were performed using R v3.0.3 (R Core Team, 2016) and
276 ggplot2 was used for generating plots (Wickham, 2009). Shapiro-Wilk test for normality showed that the

277 data were not normally distributed and thus non-parametric tests were used. Mann-Whitney U tests were
278 used to test for statistical differences in trabecular bone parameters between Homo and Pan. A principal
279 component analysis was conducted to determine which parameters contributed to interspecific differences
280 in the talus and in the tibia. All variables were included in the principal component analysis: Tb.Th,
281 BV/TV, DA, BS/BV, and cortical thickness. As there are large differences in the variances of these
282 variables, prior to analysis the data was centered and scaled to unit variance. Principal components were
283 subsequently derived by singular value decomposition of the resulting data matrix. Spearman's
284 correlation test and RMA regression were used to test for correlation between trabecular parameters and
285 cortical thickness in the talus and distal tibia. To test the relationship between size and trabecular bone
286 parameters, OLS \log_{10} regressions and Pearson's correlation tests were conducted for each trabecular
287 parameter against the size of the epiphysis/bone for each taxon. The size of each bone was represented as
288 the geometric mean of several measurements, both of overall bone size and of the size of the articular
289 surfaces. For the talus, these measurements were the anteroposterior length, mediolateral width and
290 dorsoplantar height of the talus, the anteroposterior length and mediolateral width of the talar trochlea,
291 and the dorsoplantar height and mediolateral width of the talar head. For the tibia, a geometric mean was
292 derived from the maximum anteroposterior length and maximum mediolateral width of the distal tibia, the
293 anteroposterior length and mediolateral width of the distal articular surface, the anteroposterior length,
294 mediolateral width and proximodistal height of the medial malleolus. Pearson's correlation test was used
295 to compare trabecular parameters between paired tibia and tali in each taxon. Statistical parametric
296 mapping was used to identify regional cortical thickness differences between the two species (Friston et
297 al., 1995), using the SurfStat package (Worsley et al., 2009), by fitting a general linear model (GLM) to
298 the data. This model determined whether cortical thickness differences could be explained by species
299 (covariates of interest) or other factors (confounding covariates). As there is risk of systematic
300 misregistration due to shape differences, non-rigid shape coefficients were included as confounds in the
301 GLM (Gee and Treece, 2014; Gee et al., 2015). Bone size, however, was strongly correlated with species
302 and therefore not included as a confound in the GLM. Statistical parametric maps were generated using F

303 statistics and the corresponding p-values were corrected for multiple comparisons using random field
304 theory to control for the chance of false positives. Relative cortical thickness was calculated for each
305 specimen, by subtracting the individual mean value from each individual thickness measurement and
306 dividing by the standard deviation. In this way, relative patterns of cortical thickness could be analysed,
307 despite considerable interspecific differences in absolute cortical thickness. For all statistical tests, a p
308 value of <0.05 was considered significant.

309 **3 RESULTS**

310 **3.1 Trabecular and cortical architecture of the talus and tibia**

311 Means and standard deviations of measured trabecular and cortical parameters and Mann-Whitney U test
312 results are shown in Table 2, and extracted regions of trabecular bone, visualizing structural differences,
313 are shown in Figure 2. Mann-Whitney U test results (Table 2) find that the trabecular structure of Pan
314 differs from that of Homo in having a significantly greater BV/TV and lower BS/BV in both the talus and
315 the tibia. The trabecular structure is more divergent in the talus than in the tibia: with the talus of Pan
316 having significantly thicker, less uniformly-oriented trabeculae (i.e. lower DA). The cortex of Pan is
317 significantly thicker in both the talus and the tibia compared to Homo.

318 Correlations between parameters in the talus and tibia of each taxon are reported in Table 3. Significant
319 correlations between variables differ both between taxa and between skeletal regions. As such, all
320 parameters were included in the analysis, although correlations between parameters may lead to
321 overemphasis of the contribution of these variables. Table 4 shows the results of the principal component
322 (PC) analysis, and Figure 3 shows the plot of PC1 against PC2 for both the talus and tibia. Together, PC1
323 and PC2 explain 92.90% and 90.85% of the variance for the talus and tibia, respectively and in both
324 analyses, Homo and Pan are clearly separated. All four trabecular parameters and cortical thickness
325 contribute equally to PC1 in the talus, distinguishing Pan, with greater BV/TV, Tb.Th and cortical
326 thickness, but lower DA and BS/BV, from Homo. PC2 is driven by Tb.Th and BS/BV, but only separates
327 out particular individuals within each taxon. In the tibia, separation along PC1 is largely determined by

328 BV/TV, BS/BV and cortical thickness. Along PC2, most Pan individuals are distinguished from Homo in
329 having lower Tb.Th and higher DA.

330 **3.2 Allometry**

331 The results of the \log_{10} OLS regressions of each parameter against the geometric mean, a proxy for bone
332 size, are shown for Pan and Homo in Table 5 and Figures 4 and 5. There were no significant correlations
333 between any trabecular parameter and bone size. However, the relationship between size and trabecular
334 and cortical structure does differ between species and between the talus and tibia (Figs. 4-5).

335 **3.3 Correlation between the talus and tibia**

336 Paired tali and tibiae were used to compare trabecular and cortical bone parameters between the talus and
337 tibia in seven Homo and eight Pan specimens (Table 6 and Fig. 6). Within Pan, all parameters other than
338 DA are strongly correlated across the joint (i.e. $r > 0.70$), whereas in Homo, only Tb.Th and BS/BV are
339 strongly and significantly correlated.

340 **3.4 Distribution of trabecular bone in the talus and distal tibia**

341 Figure 7 shows BV/TV colour maps for the talus of one representative individual of Homo and Pan.

342 Images of the full sample are included in the Supporting Information.

343 On the dorsal surface of the talus (Fig. 7 a and f), all Pan specimens share a region of high BV/TV on the
344 lateral edge of the trochlea. In some individuals this extends posteriorly along the edge, and in others it is
345 more anteriorly confined. Some, but not all, specimens have an additional region of higher BV/TV on the
346 medial trochlea, which is not consistent in its location or antero-posterior extent (see Supporting
347 Information). In Homo, there is no consistent pattern of trabecular bone distribution on the dorsal surface
348 of the trochlea as this region is highly variable across the sample. All individuals of both Pan and Homo
349 have a region of high BV/TV on the dorsal surface of the talar neck, although this is much more
350 pronounced in Pan. In a transverse plane, where the superior portion of the talus has been removed (Fig. 7
351 b and g), there is a region of high BV/TV at the neck in Pan, although, as mentioned above, the inferior

352 region of the neck must be interpreted with a certain degree of caution due to problems segmenting
353 trabeculae from cortex. In Homo, there is no localised region of high BV/TV in the neck, but instead an
354 anteroposterior trajectory of bone running through the head and neck, which is absent in Pan. The region
355 of high BV/TV at the articular surface of the talar head (i.e. at the talonavicular joint), is more localized in
356 Homo than in Pan. This is clearly seen in anterior view (Fig. 7 c and h), where Homo has a point of high
357 BV/TV located dorsally on the head, in contrast to Pan, where there is a band running mediolaterally
358 across the head. In the coronal (Fig. 7 d and i) and sagittal (Fig. 7 e and k) planes of Homo, the centre of
359 the talar body contains a relatively higher BV/TV than in Pan. Also, in the sagittal plane (Fig. 7 e and k)
360 there is a distinct trajectory of high BV/TV running antero-posteriorly through the talar head of Homo that
361 is not found in Pan. Instead, the Pan neck has a region of high BV/TV on the dorsal surface. Comparison
362 of the individual BV/TV scales shows that Pan has a higher BV/TV than Homo in both its minimum and
363 maximum values.

364 Colour maps of the BV/TV distribution in the distal tibia of Homo and Pan are shown in Figure 8 and
365 results for the entire sample are included in the Supporting Information. On the distal articular surface of
366 the tibia (Fig. 8a and e), some specimens of Homo have a high concentration of BV/TV confined to the
367 medial side of the articular surface and in other individuals it is centrally located. This is in contrast to
368 Pan, where there are consistently three regions of higher BV/TV: anterolateral, anteromedial and
369 posterocentral. When viewed in the mid-sagittal plane of the distal tibia (Fig. 8 b and f), the anteromedial
370 and posterior concentrations of bone are visible in Pan, in contrast to the more central and continuous
371 area of high BV/TV in Homo. On the anterior edge of the distal tibia (Fig. 8 c and g), Pan has a high
372 concentration of bone extending across the edge that is absent in Homo. In the mid-coronal plane (Fig. 8 d
373 and h), Pan contains a relatively greater BV/TV in the centre of the medial malleolus, compared to Homo.
374 Unlike the talus, the range of BV/TV is more similar between the two species (Fig 7 and Fig 8, scale
375 bars).

376 **3.5 Distribution of cortical bone in the talus and distal tibia**

377 Mean relative cortical thickness maps for the talus and distal tibia of Pan and Homo, along with regions
378 of significant differences, are shown in Figures 9 and 10. In contrast to the trabecular bone maps, these
379 figures do not show the cortical thickness in just one individual, but rather the mean of all individuals by
380 taxon. As Pan has a greater cortical thickness in both the talus and the distal tibia, results are presented
381 for relative cortical thickness values, equalized by subtracting the mean value from each cortical thickness
382 value and dividing by the standard deviation for every individual in the sample.

383 Visual comparison between the relative cortical thickness maps of the talus in Homo (Fig. 9a) and Pan
384 (Fig. 9b), show that the regions of thickest cortical bone differ between the two species. On the talar head,
385 Homo has a dorsally located region of highest relative thickness, whereas in Pan the region of high
386 thickness runs mediolaterally along the dorsal half of the articular surface. At the trochlea, Pan has a
387 higher cortical thickness on the lateral edge, whereas in Homo it is the centromedial region that has the
388 highest mean thickness. Pan and Homo share thick cortical bone around the region of the talar neck,
389 however, in Pan this extends around the entire dorsal region of the neck, whereas in Homo it is confined
390 to the dorso-lateral side. In Homo the centre of the posterior subtalar articular surface has the thickest
391 cortical bone, whereas in Pan the cortical bone is thickest anterolaterally on this articular surface.

392 Differences between Pan and Homo are shown in Figure 9c, and regions where these differences reach
393 significance are shown in Figure 9d. There are several regions with significant differences located at the
394 articular surfaces of the talus. Pan has relatively thinner bone compared to Homo on the anterior surface
395 of the talar head, on the anteromedial region of the talar trochlea and on the dorsal edge of the talar head,
396 and relatively thicker bone compared to Homo in a band anterolaterally on the posterior subtalar articular
397 surface.

398 Cortical thickness maps, showing relative cortical thickness are shown for Homo and Pan in Figure 10a
399 and b, respectively. In distal view, Homo has thickest cortical bone the along the medial edge of the distal
400 articular surface and the distal end of the medial malleolus. Both taxa share regions of thicker cortical
401 bone on the distal end of the medial malleolus and the medial edge of the distal articular surface. This

402 region on the medial articular surface is relatively thicker anteriorly in Pan, whereas in Homo this feature
403 extends along the medial border of the articular surface. Pan has two additional regions of thicker cortical
404 bone on the anterolateral and posterocentral regions of the distal articular surface. Comparisons of relative
405 cortical thickness values between Homo and Pan are shown in Figure 10c and regions with significant
406 differences are shown in Figure 10d. At the distal articular surfaces, Pan has significantly thicker cortex
407 at the anteromedial corner, extending along the anteromedial edge of the medial malleolus. There is
408 significantly thicker cortical bone on the distal surface of the medial malleolus in Pan compared to Homo.

409 **4 DISCUSSION**

410 We analysed the internal bone structure of the talus and distal tibia in bipedal Homo and arboreal,
411 quadrupedal Pan. We find that trabecular and cortical bone, both the measured parameters and the
412 regional distribution of bone, differed, often significantly, between the two taxa in ways that are
413 potentially related to variation in joint position and load distribution during locomotion. In addition to
414 these differences, we find further support for previously proposed systemically weaker trabecular and
415 cortical bone in recent humans (Ruff et al., 1993; Lieberman, 1996; Ruff, 2005; Chirchir et al., 2015;
416 Ryan and Shaw, 2015; Scherf et al., 2015; Chichir et al., 2017).

417 **4.1 Identifying functional signals in internal bone structure**

418 The relationship between bone form and mechanical loading is complex. It may be influenced by
419 numerous factors that affect bone growth and structure, which are likely to differ systematically between
420 species and, as such, bone structure should be considered within the broader context of what is already
421 known about the bone architecture of each species. In both the talus and distal tibia of Homo, we find
422 support for our prediction that bone is relatively weak, having a lower BV/TV, a higher BS/BV and
423 thinner cortices, compared with the more robust Pan. BV/TV is the strongest predictor of trabecular bone
424 stiffness, or Young's modulus; it alone explains 87-89% of variance in stiffness (Stauber et al., 2006;
425 Maquer et al., 2015). Cortical bone thickness is also related to bone strength, as thin cortices are
426 associated with increased fracture risk (Augat and Schorlemmer, 2006). The difference in trabecular

427 BV/TV and cortical thickness between Pan and Homo is consistent with previous findings for the talus
428 and distal tibia (talus: Su, 2011; DeSilva and Devlin, 2012; Su and Carlson, 2017; tibia: Su, 2011; Barak
429 et al., 2013b), and with the trabecular morphology of other anatomical regions (e.g. third metacarpal:
430 Tsegai et al., 2013; calcaneus: Maga et al., 2006; Zeininger et al., 2016; first and second metatarsal:
431 Griffin et al., 2010; systemic: Chirchir et al., 2015). As the biomechanical environment of different joints
432 in the human and chimpanzee are likely to vary given their divergent modes of locomotion, this consistent
433 difference across several anatomical sites may be part of a systemic pattern (i.e. in all regions of the
434 skeleton) and not due to specific locomotor, or other, behaviour. This gracility of the modern human
435 skeleton may be associated with increased sedentism following the adoption of agriculture, as early
436 hominins and recent hunter gatherers/foragers have a more robust skeleton (Ruff et al., 1993; Lieberman,
437 1996; Ruff, 2005; Chirchir et al., 2015; Ryan and Shaw, 2015; Scherf et al., 2015). Analysis of the
438 relationship between these structural parameters and size are limited by small sample sizes.

439 There are aspects of bone structure that appear likely to reflect joint function and thus can be of use for
440 reconstructing behaviour in the fossil record. Here, we find support for our prediction that the human talus
441 has a significantly higher DA than in Pan. However, contrary to our predictions, we find no significant
442 difference for the distal tibia. During human bipedalism the mid-foot forms a relatively rigid lever during
443 push off (Morris, 1977), compared with the flexibility of the chimpanzee mid-foot (Elftman and Manter,
444 1935; Susman, 1983; Thompson et al., 2014; but see Holowka et al., 2017). There is also less mobility at
445 the ankle of Homo than in Pan (Latimer et al., 1987). The less aligned trabeculae of the Pan talus are
446 consistent with being more able to withstand forces from multiple directions associated with a wider
447 range of joint positions, whereas the more highly aligned trabecular structure of the Homo talus appears to
448 reflect more stereotypical loading (Su, 2011; DeSilva and Devlin, 2012; Su et al., 2013; Su and Carlson,
449 2017). In contrast to previous studies (Su, 2011; Barak et al., 2013b), we do not find a higher DA in the
450 distal tibia of Homo, but rather higher (although not significantly so) mean DA in Pan. However, Su
451 (2011) found that trabeculae in Homo were significantly more uniformly aligned in the talus compared

452 with the tibia, suggesting that more similar DA values in the Homo and Pan distal tibia are not
453 unexpected.

454 DA may hold a functional signal for different types of behaviour that engender more or less stereotypical
455 loads at a joint. Regional differences in DA have been useful in distinguishing between primate locomotor
456 groups, with the structure of the proximal femur being consistent with inferred differences in loading in
457 leaping and slow climbing strepsirrhines (Ryan and Ketcham, 2002a,b; MacLachy and Muller, 2002;
458 Ketcham and Ryan, 2004). The trabecular structure of the human foot is generally more highly aligned
459 than other apes (first and second metatarsal: Griffin et al., 2010; calcaneus: Maga et al., 2006; Zeininger
460 et al., 2016; but see Kuo et al., 2013; talus: Su, 2011; Su et al., 2013; Su and Carlson, 2017). It seems
461 unlikely that this would relate to differences in activity level between the taxa, and there are no consistent
462 differences in DA in the proximal femur (Ryan and Shaw, 2015) or humerus (Scherf et al., 2015) between
463 human populations with different activity levels (i.e. engaging in the same behaviours but at different
464 frequencies). Adult trabecular structure could reflect individual or interspecific differences in loading
465 during puberty, at a time when bone is more responsive to strain (e.g. Pettersson et al. 2010; for cortical
466 bone see Pearson and Lieberman, 2004). However, homologous regions of trabecular bone in adolescent
467 and adult humans have not been sampled, as many studies exploring ontogeny have investigated changes
468 in structure between non-adult groups (Ryan and Krovitz, 2006; Ryan et al., 2007; Gosman and Ketcham,
469 2009; Raichlen et al., 2015). DA in the proximal tibial metaphysis and in the ilium continue to change
470 between adolescence and adulthood (Gosman & Ketcham, 2009; Abel & Macho, 2011). Moreover,
471 chimpanzees reach adult-like locomotor behaviour by adolescence (Doran, 1992; Sarringhaus et al.,
472 2014), while humans reach this point during early childhood (e.g. Sutherland et al., 1980; Beck et al.,
473 1981; Raichlen et al., 2015). Trabecular orientation in the talus also shows plasticity later in life, as
474 degeneration of articular cartilage, i.e. changes at the joint surface that affect loading, is associated with
475 differences in trabecular orientation in humans (Schiff et al., 2007). This indicates that DA in adult
476 humans and chimpanzees is likely to reflect adult behaviour patterns, as loading from locomotion has

477 remained generally consistent during much of the later growth period. Together these results suggest that
478 the high degree of trabecular alignment throughout several elements of the human foot may be a
479 behavioural signal related to the stereotypical loading of terrestrial bipedality. We suggest that, using our
480 methodology, DA may provide functional information about loading in the talus, but not the tibia.

481 **4.2 The relationship between joint position and bone distribution**

482 We predicted that differences in the cortical and trabecular bone distribution maps would reflect variation
483 in dorsiflexion and inversion of the talocrural joint and the degree of mobility at the talonavicular joint.
484 The colour maps of cortical and trabecular bone support some, but not all, of these predictions. These
485 results are based on mean cortical thickness distribution maps and significant differences, and on BV/TV
486 distribution maps for each individual. Generation of mean morphometric maps for BV/TV was not
487 conducted due to the complexity of registering 3D meshes while ensuring homology.

488 **4.2.1 Dorsiflexion**

489 Dorsiflexion at the ankle is characteristic of both climbing and knuckle-walking in chimpanzees
490 compared to the more neutral ankle posture adopted by humans during bipedalism. We find no clear
491 signal of dorsiflexion in trabecular and cortical bone of the talar trochlea, but are able to identify
492 differences in internal bone structure of the distal tibia that we propose are related to degree of
493 dorsiflexion. In chimpanzees, during knuckle-walking the angle between the long axis of the tibia and the
494 foot is 75.2 degrees, compared with 85.6 degrees in normal human bipedalism (Barak et al., 2013b).
495 During vertical climbing the degree of dorsiflexion is much greater, with an angle between the long axis
496 of the tibia and the foot of 44.5 degrees (DeSilva, 2009). The external morphology of the talar trochlea
497 and the distal articular surface of the tibia is associated with this difference in loading of the ankle
498 (DeSilva, 2009; but see Venkataraman et al., 2013a). It might be expected that the distribution of
499 trabecular bone and cortical bone in the talar trochlea of Pan would be more anteriorly distributed,
500 reflecting this difference in joint angle. However, we find no clear signal across the study sample in either
501 the trabecular or cortical bone distribution maps. This is consistent with previous studies that did not

502 identify differences in BV/TV across quadrants of the talar body (DeSilva and Devlin, 2012), or higher
503 BV/TV and cortical thickness in the anterior talar trochlea (Su, 2011; Su and Carlson, 2017).

504 In contrast to the talus, we did find that the trabecular and cortical bone structure of the distal tibia
505 reflected the differences in joint position between Homo and Pan. Pan shows two regions of higher
506 BV/TV and thicker cortical bone, located at the anterior portion of the distal articular surface of the tibia,
507 one lateral and one medial. In addition, the anterior edge of the distal articular surface has a higher
508 BV/TV, which extends up anteriorly through the epiphysis. This is in contrast to Homo, where BV/TV
509 maps show a more central concentration of trabecular bone. In Homo, the cortex is thickest on the medial
510 edge of the articular surface, adjacent to the medial malleolus. In several (but not all) individuals in the
511 study sample (see Supporting Information), this medial region also has a high BV/TV. Although direct
512 comparison between results from different subregions is complex, some of these findings are supported
513 by the results of Su (2011). Fewer significant differences in BV/TV and cortical thickness are found
514 across the Homo tibia compared to Pan, and Pan has generally higher BV/TV anteriorly and posteriorly.
515 This is not the case for cortical thickness, where both Homo and Pan have thicker bone on the antero- and
516 postero- medial regions, and in Pan, the posterocentral region of the articular surface (Su, 2011). Perhaps
517 also relevant to the degree of flexion at the ankle, there is a region of high BV/TV and cortical thickness
518 posterocentrally on the distal articular surface in Pan, with the region of high BV/TV extending into the
519 bone. This could indicate increased loading during plantarflexion in Pan compared to Homo, however,
520 this is not supported by kinematic data. Previous findings in the distal tibia of Pan also found that the
521 posterior region has a higher BV/TV than the central region, and thicker cortical bone was found in the
522 posterocentral region (Su, 2011; Su and Carlson, 2017).

523 In the absence of detailed kinematic data on joint contact areas, in particular for Pan (for humans see Wan
524 et al., 2006; Bae et al., 2015), our understanding of the differences in the loading of the trochlea in these
525 two species is limited. Moreover, we must make assumptions about which aspects of a species'
526 locomotor, or other, behaviour contribute most to the remodelling of bone. Previous studies in humans

527 have identified areas of contact and distribution of pressure on the talus using a finite element simulation
528 of the human foot during walking (Bae et al., 2015) and on both the talar trochlear and distal articular
529 surface of the tibia under pressure using dual orthogonal fluoroscopy (Wan et al., 2006; Caputo et al.,
530 2009; Bischof et al., 2010). During human bipedalism, ground reaction forces (GRF) peak at two phases,
531 first after heelstrike and before midstance, and second at toe off (Bae et al., 2015; Alexander, 2004), with
532 contact pressure and strain increasing throughout the stride, peaking at toe off (Bae et al., 2015). After
533 heelstrike, during the first peak in GRF, there is contact between the cartilage of the talus and tibia on the
534 latero-central trochlea (Wan et al., 2006; Bae et al., 2015). During stride, the area of contact moves
535 anteriorly (Wan et al., 2006; Bae et al., 2015) and the point of highest pressure moves antero-centrally
536 until toe off, when both the contact area and point of highest pressure are located on the anterior of the
537 trochlea, just lateral to the midline (Bae et al., 2015). At the distal tibia, contact is located antero-
538 posteriorly at heel strike, moving anteriorly across the medio-lateral extent of the articular surface at mid-
539 stance, and at heel strike in the anterolateral half of the distal articular surface of the tibia (Wan et al.,
540 2006). Although some of the human sample in this study have a region of high BV/TV on the anterior
541 talus, just lateral to the midline, near the location of highest pressure (Bae et al., 2015), this is not always
542 the region of highest BV/TV, and does vary within the sample. There is also no direct correspondence
543 between regions of contact and areas with thicker cortices. There are several potential explanations for
544 why the trabecular and cortical bone structure of the talar trochlea does not, as expected, reflect
545 differences in dorsiflexion at the ankle. Firstly, experimental measures of cartilage contact and pressure
546 may not necessarily correspond to the regions experiencing the greatest forces during life. Secondly,
547 modern humans differ greatly in their gait. For example, there is inter-individual variation in the presence
548 of a mid-tarsal break, and intra-individual variation between strides (Bates et al., 2013; DeSilva et al.,
549 2015). There is also variability in foot strike patterns, with individuals making initial contact with the
550 fore-foot, midfoot or heel, that could also contribute to variability in loading of the trochlea (e.g. during
551 running: Lieberman et al., 2010; Hatala et al., 2013). Thirdly, differences in the external morphology of
552 the talus may accommodate the different distribution of forces, i.e. different shaped tali absorb loads

553 differently, thus cortical thickness and trabecular architecture do not directly reflect differences in joint
554 position.

555 Due to interest in adaptations of the human skeleton to bipedal locomotion, many biomechanical analyses
556 of Pan have focused on bipedal walking (e.g. Susman, 1983; Thorpe et al., 2004; Wang et al., 2014;
557 O'Neill et al., 2015), although several studies have investigated kinematics of knuckle-walking in
558 bonobos (e.g. Vereecke et al., 2003; D'Août et al., 2004; Schoonaert et al., 2016). Although no in vivo
559 measurements of joint movement or cartilage contact are available for Pan, there is evidence of force
560 transmission due to contact between the anterior edge of the distal tibia and the neck of the talus. This can
561 be observed when manipulating dry, associated tibia and tali, where in an extreme position of dorsiflexion
562 the ankle joint retains congruity while there is contact between the talar neck and the anterior border of
563 the tibia in African apes, but not in Homo (Latimer et al., 1987). Modern humans who regularly adopt
564 crouched positions develop squatting faces on the talus and tibia (Bouille, 2001). The BV/TV distribution
565 may reflect this and indicate high loads transmitted through this region. On the medial and lateral side of
566 the talar neck and on the anteroinferior border of the tibia, Pan has regions of high BV/TV, which are
567 absent in Homo. This may reflect habitual loading of these regions in an ankle dorsiflexed to such a
568 degree that force transmission occurs between the antero-inferior edge of the distal tibia and the talar
569 neck.

570 **4.2.2 Talonavicular mobility**

571 We find a clear signal of differences in joint mobility at the talonavicular joint in the trabecular and
572 cortical bone structure. Two features in which human bipedalism is distinct from ape quadrupedalism are,
573 firstly, weight transfer from the lateral to medial side of the foot during midstance; and secondly, in
574 having a rigid mid-foot, so that the foot acts as a lever during toe off (Elftman and Manter, 1935). The
575 medial side of the midtarsal joint (the talonavicular joint) is more mobile than the lateral side
576 (calcaneocuboid and cuboid-MT5 joints), during stance phase the talus rotates, along with the leg and
577 calcaneus, creating a close packed talonavicular joint (Elftman, 1960; Siegler et al., 1988; Scott and

578 Winter, 1991). Although investigations of mid-foot mobility in Pan have largely focused on the mid-
579 tarsal break at the lateral side (DeSilva, 2010), there is greater movement at the talonavicular joint which,
580 during passive dorsiflexion of the foot, is characterised by rotation in the coronal plane (Thompson et al.,
581 2014). Furthermore, there is greater inter-individual and intra-individual variability in mobility of the
582 human lateral midfoot than was previously assumed (Elftman and Manter, 1935; Bates et al., 2013).
583 During bipedalism, humans have greater midfoot mobility during push off, which is characterised by
584 plantarflexion and adduction, whereas chimpanzees have higher dorsiflexion at the midfoot (mid-tarsal
585 break) during the single limb support period (Holowka et al., 2017). Contrary to expectations, the human
586 midfoot was found to be overall more mobile than that of chimpanzees (Holowka et al., 2017), however,
587 precise kinematics of the talonavicular joint remain unknown.

588 There are clear differences between the study taxa in the trabecular bone distribution at the talar head,
589 where Pan has a band of high BV/TV running mediolaterally across the talar head, and in Homo there is a
590 localised point of high BV/TV. In cortical thickness, Pan has relatively thinner cortices at the talar head,
591 which is significantly thinner in the central region. Previous studies have measured both trabecular bone
592 in the medial and lateral sides of the head (DeSilva and Devlin, 2012) and trabecular bone adjacent to the
593 neck of the talus (i.e. on the anteromedial region of the talar trochlea). When comparing the medial and
594 lateral side of the head of the talus in humans to other species, DeSilva and Devlin (2012) found no
595 significant difference in DA, although the trabeculae were significantly thicker in the lateral head and
596 significantly more connected in the medial head of humans compared to other species (DeSilva and
597 Devlin, 2012). In the anteromedial trochlea, humans have a unique orientation of trabeculae compared to
598 other great apes, in having trabeculae with a primarily anteroinferior orientation, i.e. parallel to the talar
599 neck; a pattern shared with an early Pleistocene biped, KNM-ER 1464 (Su, 2011; Su et al., 2013; Su and
600 Carlson, 2017). This distinct orientation of trabeculae in bipedal species noted by Su et al. (2013) may
601 correspond to the trajectory of bone that we show here, travelling through the talar head into the trochlea.

602 The trabecular and cortical distribution of the talar head reveals a clear difference in bone structure,
603 perhaps related to differences in midfoot mobility between the study species.

604 **4.2.3 Inversion**

605 As well as dorsiflexion, inversion of the foot is characteristic of arboreal behaviour in Pan, including
606 vertical climbing (DeSilva, 2009). Species that engage in more arboreal locomotion have a less
607 symmetrical trochlea surface, where the lateral trochlea ridge is higher than the medial. This asymmetry
608 increases the difference in the radius of curvature of the medial and lateral side, thereby increasing the
609 arcuate path of the tibia over the talus (Latimer et al., 1987), a difference that has even been identified
610 between more arboreal western and more terrestrial eastern gorillas (Dunn et al., 2014). Of potential
611 interest with regard to identifying signals of inversion, is the high BV/TV on the anterolateral lip of the
612 trochlea of the talus that is consistent throughout the sample of Pan. This region also has a slightly thicker
613 cortex in Pan than in Homo, with Pan having relatively thinner cortical bone than Homo on the
614 anteromedial region of the trochlea. This is consistent with previous findings of high BV/TV, but not
615 thicker cortices, on the anterolateral two thirds of the trochlea in Pan (Su, 2011; Su and Carlson, 2017).
616 This may reflect increased shearing stresses associated with adoption of inverted foot postures, which are
617 also mitigated by having a higher lateral ridge of the talus. More detailed understanding of the kinematics
618 of climbing and knuckle-walking, along with modelling of the forces experienced by the talus, may
619 improve interpretation of this signal.

620 **5 CONCLUSION**

621 Identifying those features of internal bone structure that are directly related to joint loading is often
622 problematic. Here, we find that average architectural variables (BV/TV, BS/BV and cortical thickness)
623 that relate to overall bone strength differ between Pan and Homo. These may be part of a systemic pattern
624 unrelated to joint function, but rather due to other factors such as overall activity levels, and therefore
625 may not be relevant for reconstructing loading of individual joints. However, the degree to which

626 trabeculae are uniformly oriented (DA) in the talus does correspond to variation in joint loading due to
627 different locomotor behaviours, clearly differentiating between the more stereotypical loading regime of
628 bipedalism in *Homo* and the greater range of motion and joint loading typical of arboreal behaviours in
629 *Pan*. In contrast to these architectural variables quantified throughout the epiphysis/bone, more precise
630 information about locomotor behaviour can be obtained from patterns of trabecular and cortical bone
631 distribution. The trabecular and cortical bone distribution of the distal tibia and talus reflect differences in
632 dorsiflexion at the ankle and range of motion at the talonavicular joint in humans and chimpanzees. Thus,
633 the distribution of both trabecular and cortical bone in the talus and distal tibia holds potential for
634 interpreting loading regimes and reconstructing loaded joint positions in fossil specimens.

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642

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Table 1. Study sample

Taxon	Body mass (kg) ³	Locomotor behaviour	Tibia	Talus	Paired	Scan resolution (µm)	Relative resolution n ⁴
Homo sapiens ¹	62.1-72.1	Biped	8	9	7	40	5.72-9.06
Pan troglodytes verus ²	41.6-46.3	Arboreal/knuckle-walker	10	13	8	35	5.46-11.59

¹ Anthropological Collection of Institute of Zoology and Anthropology, University of Göttingen

² Max Planck Institute for Evolutionary Anthropology

³ Sex specific mean body mass (F-M). Body masses from Smith and Jungers (1997)

⁴ Relative resolution = mean trabecular thickness (mm)/resolution(mm)

Table 2. Mean and standard deviation of trabecular and cortical parameters in the talus and distal tibia of Homo and Pan. Results of Mann-Whitney U test between taxa are shown, with significant differences in bold.

Element	Taxon	Tb.Th (mm)	BV.TV (%)	DA	BS/BV(mm ⁻¹)	Cortical thickness (mm)
Talus	Homo	0.26 (0.03)	24.77 (2.17)	0.14 (0.07)	0.32 (0.05)	0.45 (0.06)
	Pan	0.31 (0.04)	34.65 (2.63)	0.02 (0.02)	0.19 (0.02)	0.88 (0.19)
	Significance	<0.01	<0.01	<0.01	<0.01	<0.01
Tibia	Homo	0.25 (0.04)	19.92 (2.87)	0.29 (0.10)	0.45 (0.08)	0.63 (0.07)
	Pan	0.23 (0.02)	24.17 (3.43)	0.32 (0.06)	0.31 (0.06)	1.13 (0.19)
	Significance	0.17	0.02	0.51	<0.01	<0.01

Table 3. Results of Spearman's correlation test to test relationship between trabecular parameters within Homo and Pan in the talus and distal tibia.

Element	Taxon	Parameter	Tb.Th	BV/TV	BS/BV	DA
Talus	Homo	BV/TV	0.42	-		
		BS/BV	-0.18	-0.92**	-	
		DA	-0.82**	-0.45	0.28	-
		CTh	0.57	0.50	-0.30	-0.72*
	Pan	BV/TV	0.59*	-		
		BS/BV	-0.10	-0.80**	-	
		DA	-0.98**	-0.66**	0.16	-
		CTh	0.84**	0.63*	-0.24	-0.80**
Tibia	Homo	BV/TV	0.71	-		
		BS/BV	-0.83**	-0.90**	-	
		DA	-0.74*	-0.50	0.69	-
		CTh	0.31	0.07	-0.07	-0.02
	Pan	BV/TV	0.75*	-		
		BS/BV	-0.67*	-0.95**	-	
		DA	-0.71*	-0.62	0.41	-
		CTh	0.82**	0.65*	-0.66*	-0.44

P-values indicated as $p < 0.05$ * and $p < 0.01$ **

Table 4. Results of principal component analyses showing percentage variance and loading for each principal component. The analysis was conducted separately for the talus and tibia, including Tb.Th, BV/TV, DA, BS/BV, and cortical thickness.

Element	Parameter	PC1	PC2	PC3	PC4	PC5
Talus	% variance	82.64	10.27	4.35	2.43	0.31
	(cumulative)	(82.64)	(92.90)	(97.26)	(99.69)	(100.00)
	Tb.Th	0.41	0.70	0.30	0.50	-0.08
	BV/TV	0.48	-0.28	0.09	0.08	0.82
	DA	-0.44	-0.21	0.85	0.16	0.07
	BS/BV	-0.43	0.62	-0.05	-0.40	0.51
	Cortical thickness	0.46	0.05	0.41	-0.75	-0.22
Tibia	% variance	56.45	34.41	5.41	2.88	0.86
	(cumulative)	(56.45)	(90.85)	(96.26)	(99.14)	(100.00)
	Tb.Th	0.27	-0.65	0.03	-0.71	-0.06
	BV/TV	0.57	0.13	0.42	0.06	0.69
	DA	-0.30	0.62	0.29	-0.67	0.02
	BS/BV	-0.55	-0.22	-0.36	-0.09	0.72
	Cortical thickness	0.46	0.37	-0.78	-0.20	0.05

High loadings (i.e. greater than 0.40) are shown in bold

Table 5. The relationship between bone structure and bone size in Homo and Pan. Results of OLS regression and Pearson's correlation for each trabecular parameter and cortical thickness against the geometric mean of several measurements, used as a proxy for bone size.

Taxon	Element	Parameter	Pearson's r	Slope	Lower 95% CI	Upper 95% CI	P-value	R ²
Homo	Talus	Tb.Th	-0.40	-0.51	-1.58	0.57	0.30	0.15
		BV/TV	-0.48	-0.52	-1.37	0.34	0.20	0.23
		DA	0.10	2.01	-4.28	8.29	0.48	0.08
		BS/BV	0.33	0.59	-1.23	2.41	0.47	0.08
		CTh	0.12	0.23	-1.23	1.70	0.72	0.02
	Tibia	Tb.Th	0.27	0.53	-1.47	2.53	0.54	0.07
		BV/TV	0.55	1.11	-0.57	2.80	0.16	0.30
		DA	0.09	-0.05	-6.71	6.61	0.99	0.00
		BS/BV	-0.51	-1.35	-3.56	0.87	0.19	0.27
		CTh	0.25	0.38	-1.19	1.95	0.57	0.06
Pan	Talus	Tb.Th	0.29	0.55	-0.79	1.89	0.39	0.07
		BV/TV	-0.05	-0.06	-0.88	0.76	0.87	0.00
		DA	-0.11	-2.97	-11.57	5.62	0.46	0.05
		BS/BV	0.12	0.25	-1.15	1.65	0.70	0.01
		CTh	0.19	0.60	-1.55	2.75	0.55	0.03
	Tibia	Tb.Th	0.37	0.74	-0.64	2.11	0.25	0.16
		BV/TV	0.05	0.16	-1.70	2.03	0.84	0.01
		DA	-0.35	-1.22	-3.98	1.54	0.34	0.11
		BS/BV	-0.04	-0.04	-3.36	3.27	0.98	0.00
		CTh	0.28	0.80	-1.32	2.93	0.41	0.09

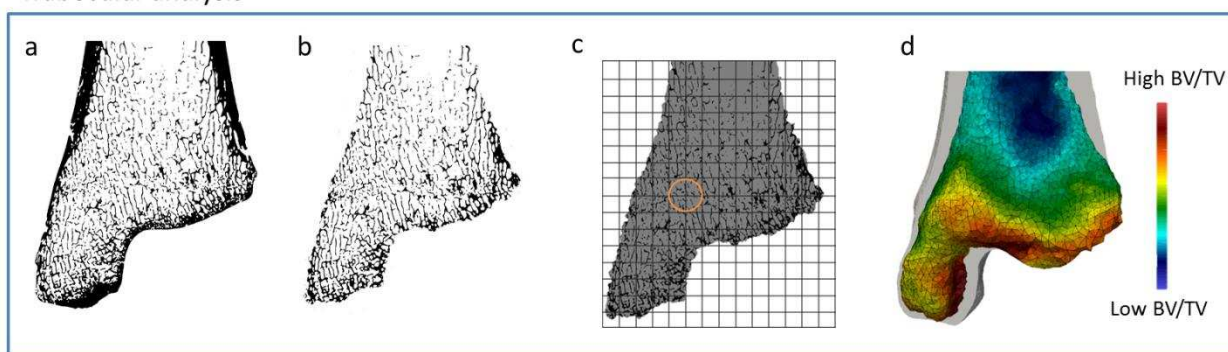
Table 6. Results of Pearson's correlation test to test relationship of each trabecular parameter and cortical thickness between the talus and distal tibia in Homo and Pan.

Taxa	Parameter	Pearson's r	P-value
Homo	Tb.Th	0.83	0.02
	BV/TV	0.72	0.07
	DA	0.55	0.20
	BS/BV	0.83	0.02
	Cortical thickness	0.43	0.33
Pan	Tb.Th	0.86	0.01
	BV/TV	0.80	0.02
	DA	0.56	0.15
	BS/BV	0.81	0.02
	Cortical thickness	0.92	<0.01

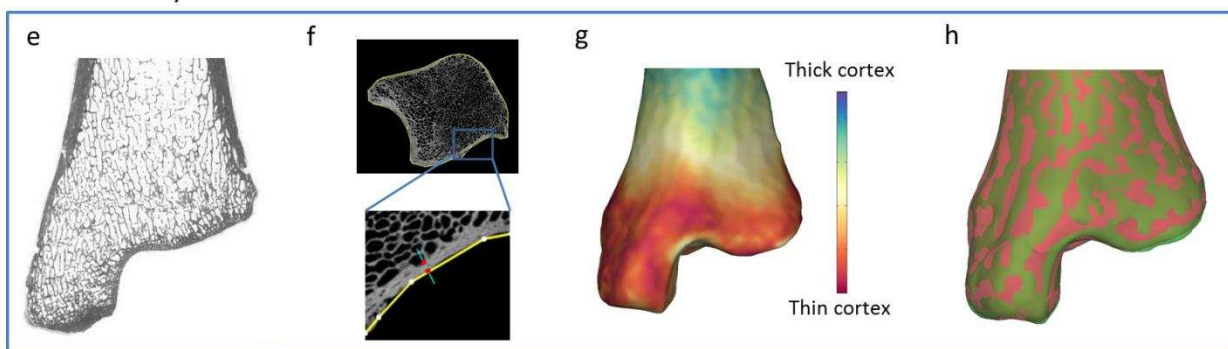
Significant correlations are shown in bold ($p < 0.05$)

1 **Fig 1.** Processing steps for trabecular and cortical bone analysis for a Pan distal tibia. a)
 2 Segmented microCT scan. b) Segmented trabecular bone. c) Inner mask, where trabecular bone
 3 and internal region of the bone are assigned different grey values, and the cortical bone has been
 4 removed. A background grid and sampling sphere are applied to calculate trabecular structure
 5 throughout the bone. d) Tetrahedral mesh with colour scalars representing trabecular bone
 6 volume fraction. e) Unsegmented voxel data. f) Process of measurement of cortical thickness.
 7 Cortical thickness values mapped to a subject-specific surface. h) Each subject-specific surface
 8 (green) is registered to a canonical surface (red) for interspecific comparisons.

Trabecular analysis



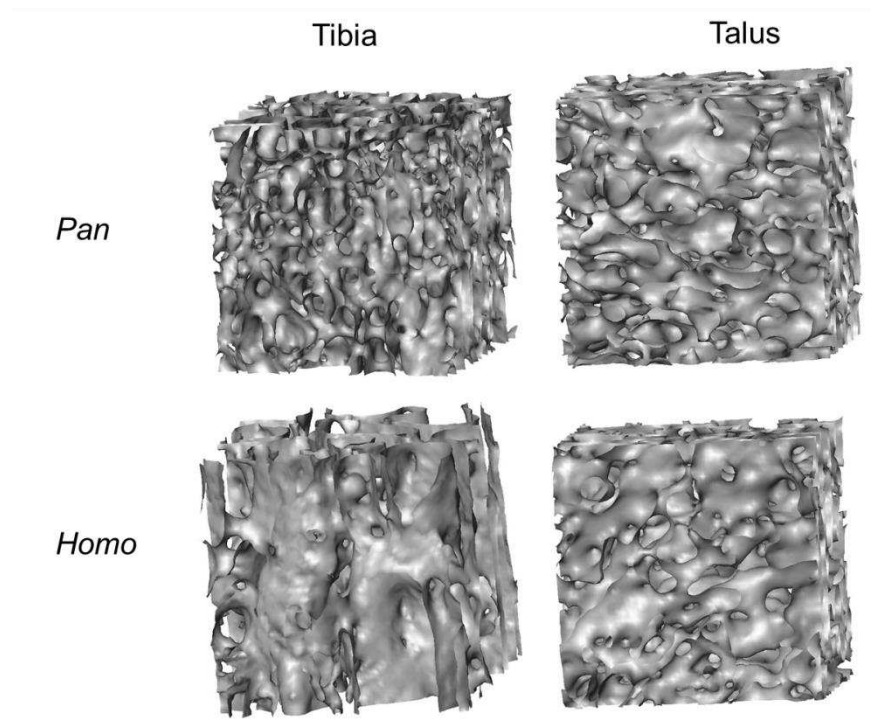
Cortical analysis



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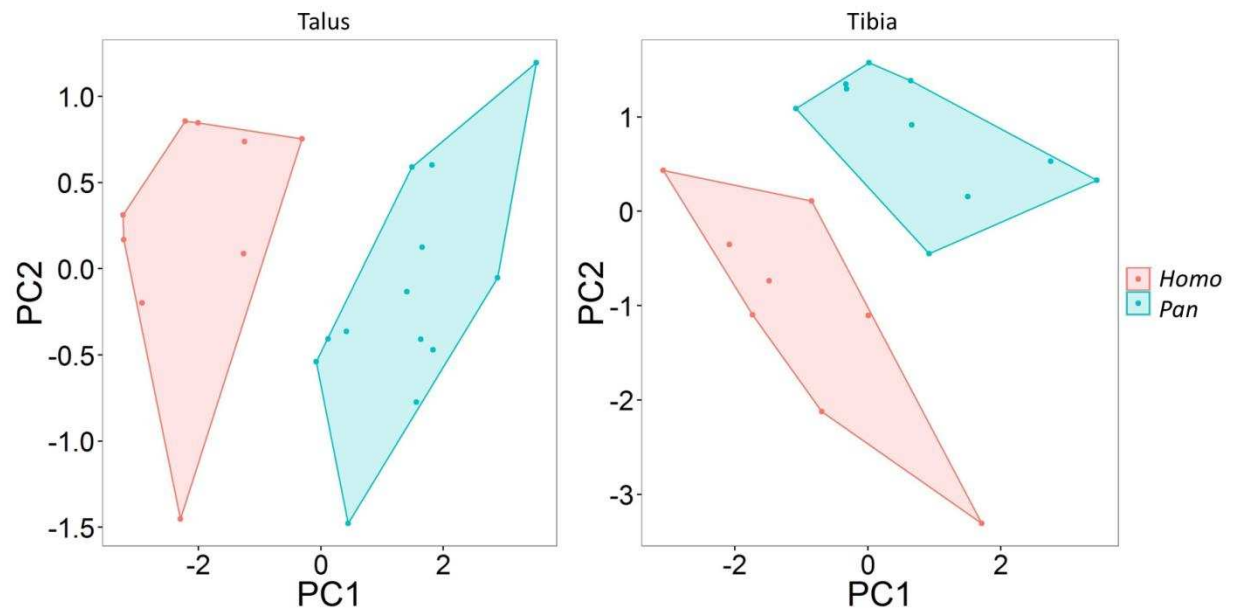
- 11 **Fig 2.** Extracted cubes of trabecular bone from approximately the same location in the talus and
12 distal tibia of Homo and Pan.



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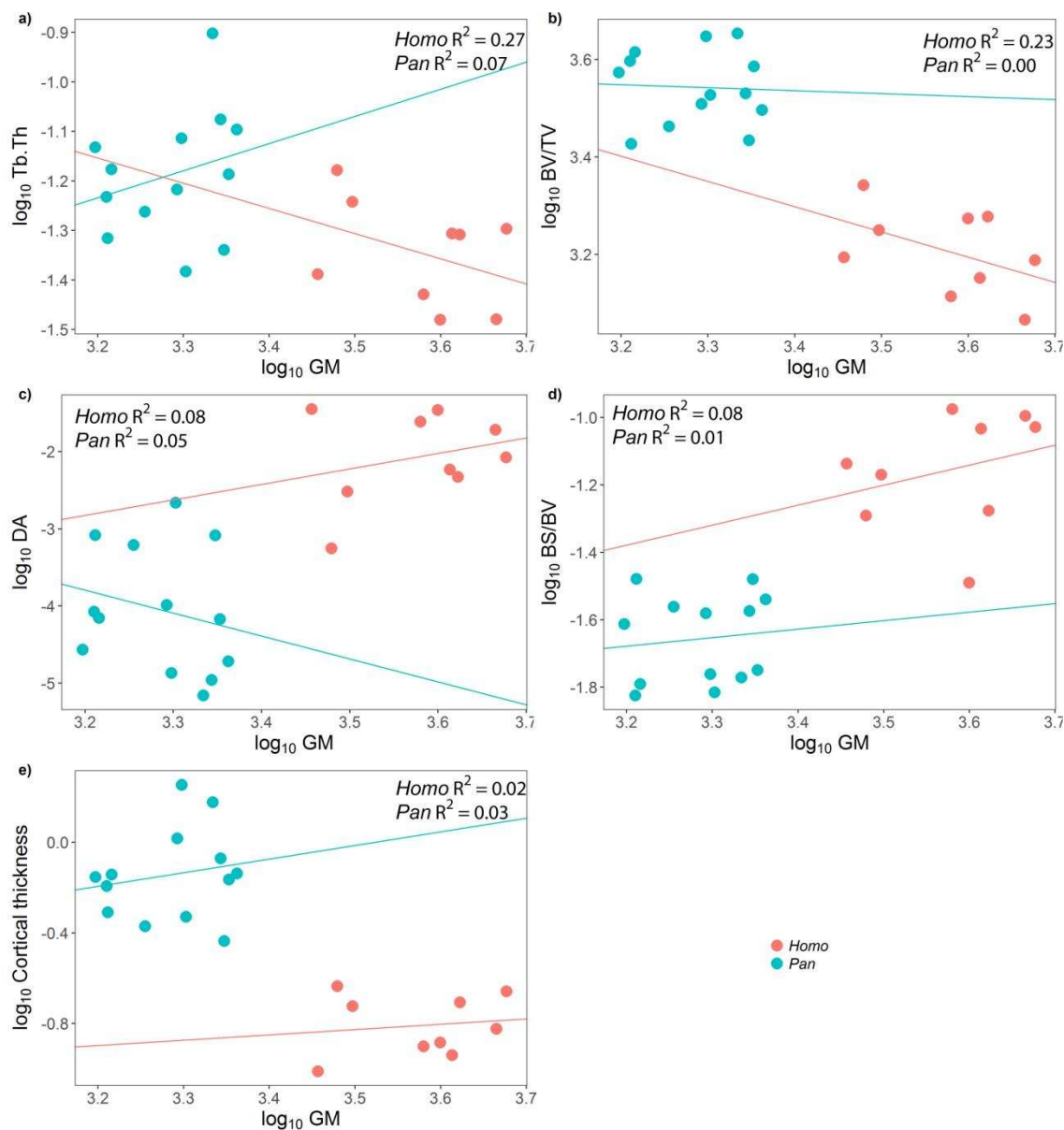
15 **Fig 3.** PC1 and PC2 for trabecular and cortical structure of the talus and distal tibia of Pan (blue)
16 and Homo (red).



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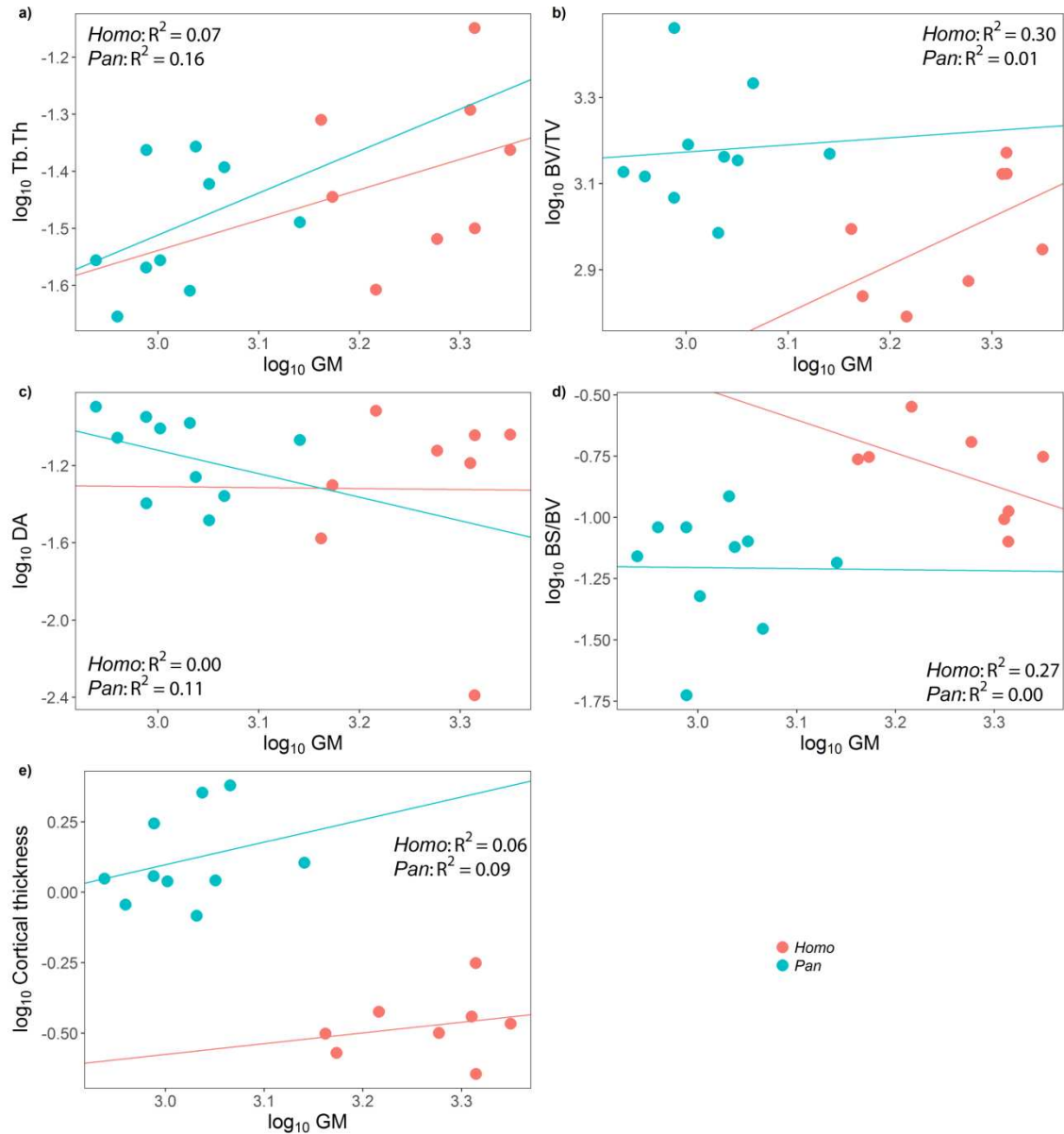
19 **Fig 4.** Relationship between talus size and trabecular and cortical parameters in Pan (blue) and
 20 Homo (red). The \log_{10} OLS regression lines are shown independently for Pan (blue) and Homo
 21 (red).



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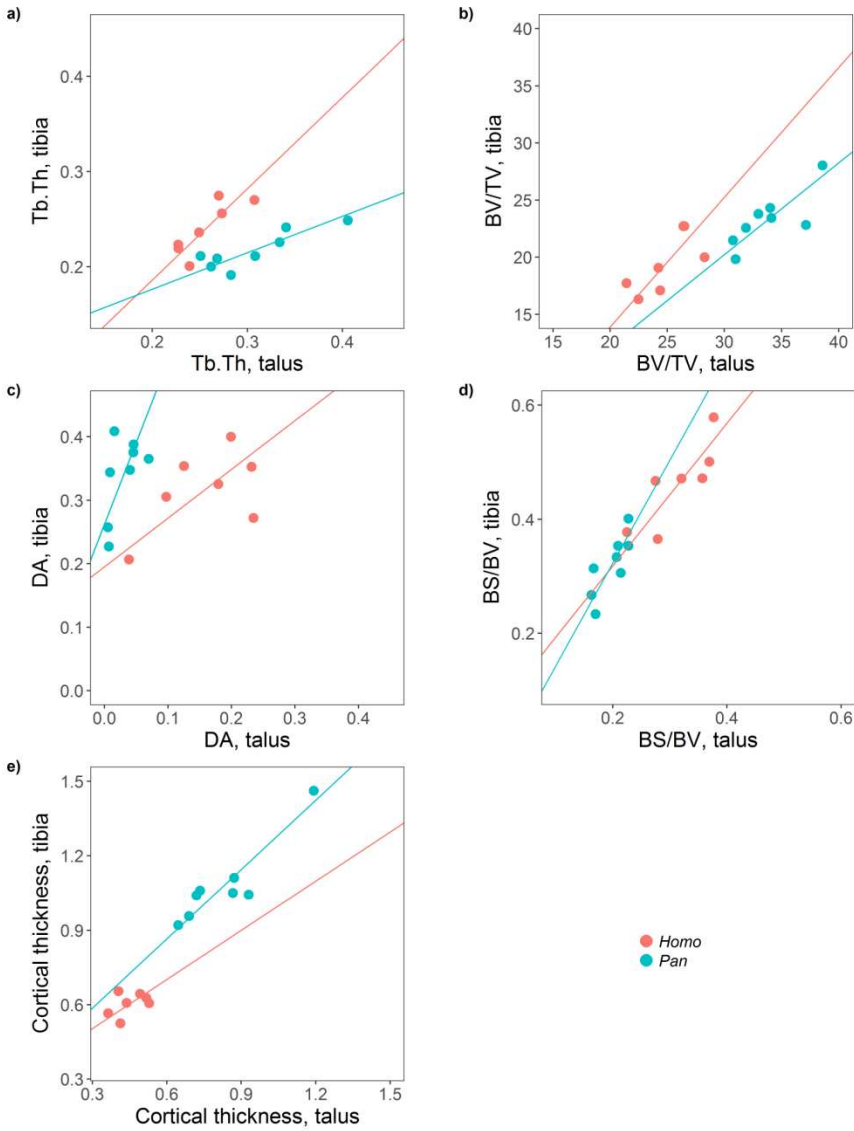
24 **Fig 5.** Relationship between tibia size and trabecular and cortical parameters in Pan (blue) and
 25 Homo (red). The \log_{10} OLS regression lines are shown independently for Pan (blue) and Homo
 26 (red).



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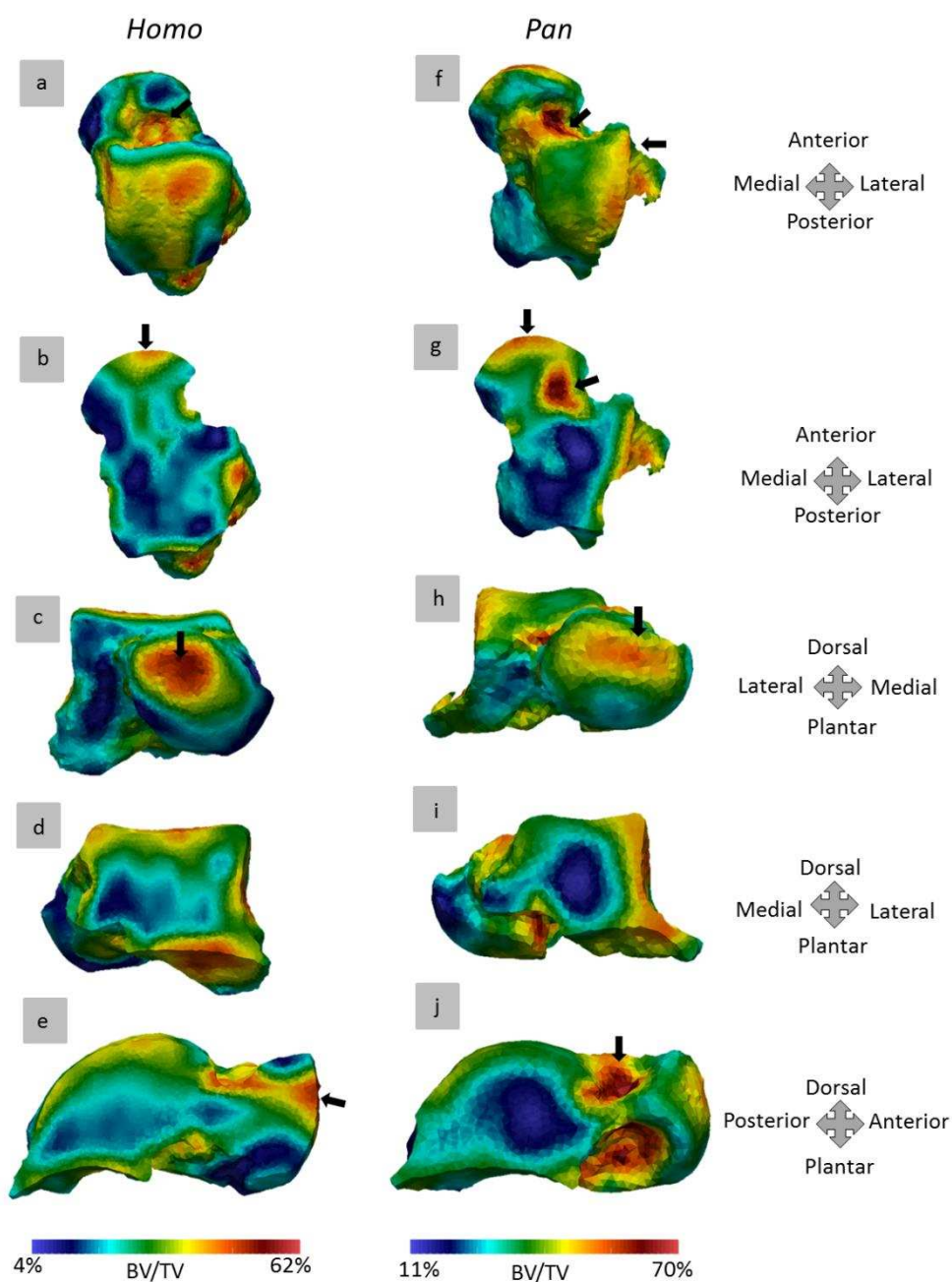
29 **Fig 6.** Comparison of trabecular and cortical structure between the talus and tibia in Pan (blue)
30 and Homo (red). The \log_{10} RMA regression lines are shown independently for Pan (blue) and
31 Homo (red).



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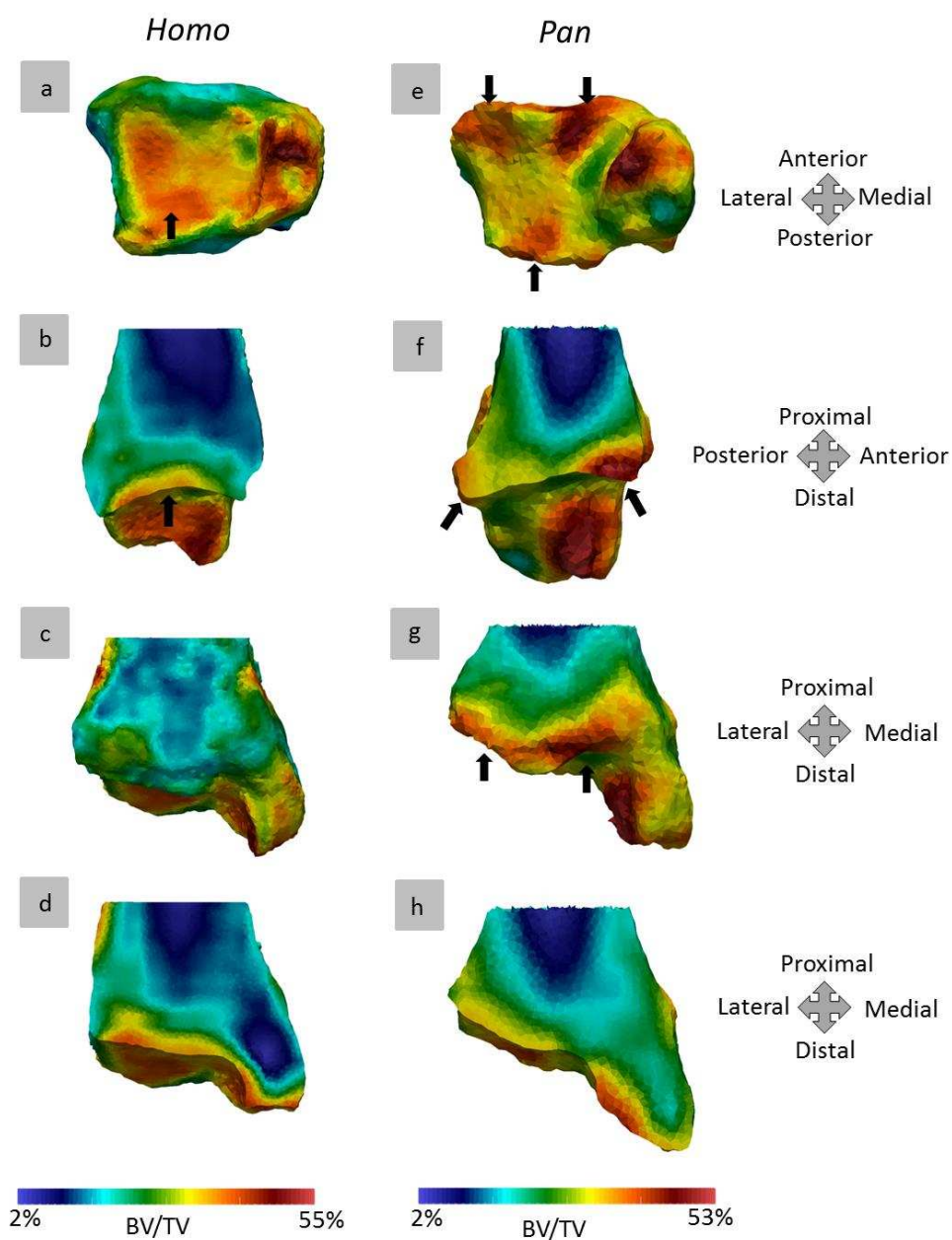
34 **Fig 7.** Morphometric maps of BV/TV in the talus in one individual of *Homo* (a-e) and *Pan* (f-j)
 35 in (from top to bottom) dorsal view, mid-transverse plane, anterior view, coronal plane (in the
 36 centre of the trochlea), and sagittal plane (in the centre of the trochlea). Each specimen is scaled
 37 to its own data range, as shown in the scale bars. Black arrows indicate regions described in the
 38 text.



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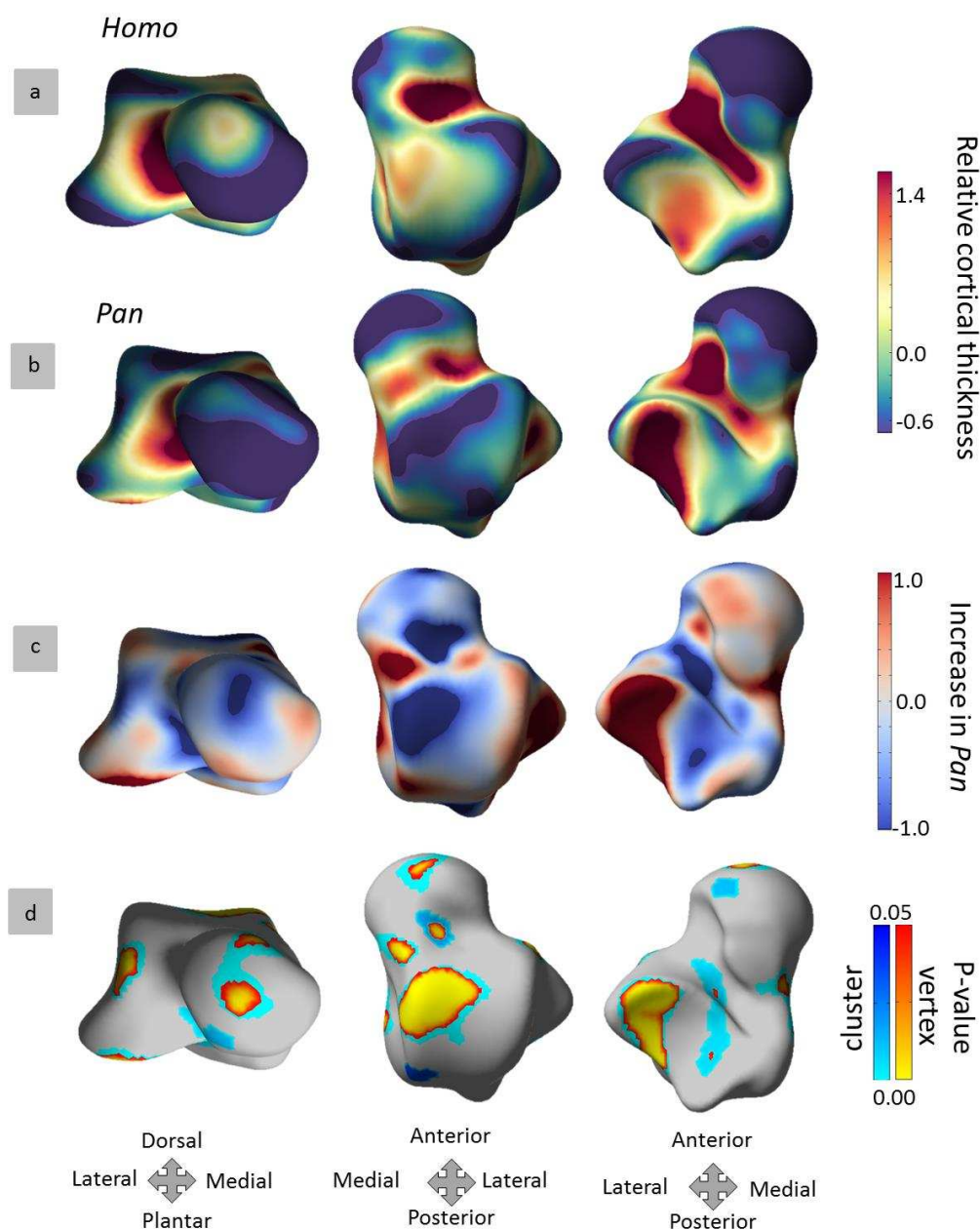
41 **Fig 8.** Morphometric maps of BV/TV in the tibia in one individual of Homo (a-e) and Pan (f-j)
 42 in (from top to bottom) distal view, mid-sagittal plane of distal tibia, anterior view and mid-
 43 coronal plane of distal tibia. Each specimen is scaled to its own data range, as shown in the scale
 44 bars. Black arrows indicate regions described in the text.



45

46

47 **Fig 9.** Morphometric maps of mean relative cortical thickness on the canonical talus in Homo (a)
 48 and Pan (b) in (from left to right) anterior, dorsal and plantar views. Red indicates thick regions
 49 and blue indicates thin regions. (c) Differences between the species are shown as the difference
 50 in Pan compared to Homo with positive values (red) indicating thicker bone and negative values
 51 (blue) indicating thinner bone. (d) Regions of significant differences between the species at
 52 vertices and clusters (red-yellow) and at clusters (blue) of the surface mesh.



54 **Fig 10.** Morphometric maps of mean relative cortical thickness on the canonical tibia in (a)
 55 Homo and (b) Pan in (from left to right) lateral, distal and anterior views. Red indicates thick
 56 regions and blue indicates thin regions. (c) Differences between the species are shown as the
 57 difference in Pan compared to Homo with positive values (red) indicating thicker bone and
 58 negative values (blue) indicating thinner bone. (d) Regions of significant differences between the
 59 species at vertices and clusters (red-yellow) and at clusters (blue) of the surface mesh.

