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1 **Sesamoid bones also show functional adaptation in their microanatomy -**  
2 **the example of the patella in Perissodactyla**

3  
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8  
9 RH: Patella microanatomical adaptation in Perissodactyla

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24 **Abstract**

25 The patella is the largest sesamoid bone of the skeleton. It is strongly involved in the knee,  
26 improving output force and velocity of the knee extensors, and thus plays a major role in  
27 locomotion and limb stability. However, the relationships between its structure and functional  
28 constraints, that would enable a better understanding of limb bone functional adaptations, are  
29 poorly known. This contribution proposes a comparative analysis, both qualitative and  
30 quantitative, of the microanatomy of the whole patella in perissodactyls, which show a wide  
31 range of morphologies, masses and locomotor abilities, in order to investigate how the  
32 microanatomy of the patella adapts to evolutionary constraints. The inner structure of the  
33 patella consists of a spongiosa surrounded by a compact cortex. Contrary to our expectations,  
34 there is no increase in compactness with bone size, and thus body size and weight, but only an  
35 increase in the tightness of the spongiosa. No particular thickening of the cortex associated  
36 with muscle insertions is noticed but a strong thickening is observed anteriorly at about mid-  
37 length, where the strong intermediate patellar ligament inserts. The trabeculae are mainly  
38 oriented perpendicularly to the posterior articular surface, which highlights that the main  
39 stress is antero-posteriorly directed, maintaining the patella against the femoral trochlea.  
40 Conversely, anteriorly, trabeculae are rather circumferentially oriented, following the  
41 insertion of the patellar ligament and, possibly also, of the quadriceps tendon. A strong  
42 variation is observed among perissodactyl families but also intraspecifically, which is in  
43 accordance with previous studies suggesting a higher variability in sesamoid bones. Clear  
44 trends are nevertheless observed between the three families. Equids have a much thinner  
45 cortex than ceratomorphs. Rhinos and equids, both characterized by a development of the  
46 medial border, show an increase in trabecular density laterally suggesting stronger stresses  
47 laterally. The inner structure in tapirs is more homogeneous despite the absence of medial  
48 development of the medial border with no “compensation” of the inner structure, which  
49 suggests different stresses on their knees associated with a different morphology of their  
50 patellofemoral joint.

51

52 Key words: Bone microanatomy; Functional adaptation; Patella; Perissodactyla

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54

## 55 **Introduction**

56

57 The skeleton is influenced by diverse constraints including phylogenetic heritage, functional,  
58 developmental, and structural constraints (Seilacher, 1970; Gould, 2002; Barr, 2018; Jones et  
59 al., 2018). Bones show a strong form-function relationship in their outer shape but also in  
60 their inner structure (Carter et al., 1991; Currey, 2003; Kley et al. 2007; Yang et al., 2017;  
61 Dunn, 2018). More plastic than bone morphology, bone micronanatomy bears a strong  
62 functional signal and is generally only slightly influenced by phylogeny (Ferry et al., 2011;  
63 Dumont et al., 2013; Kivell, 2016; Vlachopoulos et al., 2017; Montoya-Sanhueza and  
64 Chinsamy, 2018). Beyond the evolutionarily inherited traits, bone microanatomy also adapts  
65 to functional constraints during the lifetime of organisms (Warden et al., 2007; Sievänen,  
66 2010; Reina et al., 2017). At both time scales, bone inner structure is influenced by size and  
67 weight (Houssaye & Tafforeau, 2012; Dumont et al., 2013; Houssaye et al., 2016a; Iwaniec &  
68 Turner, 2016; Saers et al., 2020).

69 Many studies have been conducted in order to link bone microanatomical features to animal  
70 functional requirements in an evolutionary perspective. Various bones have been investigated,  
71 such as skull bones (Zioupos et al., 1997; Amson et al., 2018), vertebrae (Dumont et al., 2013;  
72 Houssaye et al., 2014), ribs (Buffrénil et al., 2010 ; Canoville et al., 2016), pelvic bones  
73 (Volpato et al., 2008 ; Díaz Berenguer et al., 2018), limb long bones (Quemeneur et al., 2013;  
74 Montañez-Rivera et al., 2018), and autopod bones (Thomason, 1985 ; Dunmore et al., 2019).  
75 However, very few data exist about the inner structure of sesamoid bones.

76 In the context of understanding limb bone functional adaptations, one sesamoid bone of  
77 particular interest is the patella. This bone, which is the largest of the sesamoid bones, has  
78 long been considered to ossify into the main extensor tendon of the quadriceps femoris.  
79 However, it may instead form initially alongside the femur from which it is subsequently  
80 detached by the formation of a synovial joint. The patella then becomes embedded in the  
81 quadriceps tendon (Eyal et al. 2015; 2019) separating it into two parts: the quadriceps tendon  
82 and the patellar ligament(s) (also referred to as patellar tendon; Samuels et al., 2017; Dan et  
83 al. 2018); and also articulates with the femur. It is thus part of the patellofemoral joint, one of  
84 two articulations (with the tibiofemoral joint) composing the knee. The patella ensures the  
85 continuity between the quadriceps tendon and the patellar ligament. It is assumed to protect  
86 the tendons and articular cartilage from excessive compression and to increase the lever arm  
87 of the quadriceps extensor mechanism, thereby improving its efficiency (Aglietti &

88 Menchetti, 1995; Mottershead, 1988; Sarin & Carter, 2000). This enables the improvement of  
89 both output force and output velocity of the knee extensors (Allen et al., 2017). However, the  
90 link between this bone structure and the functional constraints experienced at the knee joint  
91 remain only partially known. Some studies have investigated the possible mechanical  
92 advantage of this bone (Ellis et al., 1980; Alexander & Dimery, 1985; Lovejoy, 2007; Allen et  
93 al., 2017; Pina et al., 2020). The inner structure has been investigated in humans (Raux et al.,  
94 1975; Toumi et al., 2006). But investigations of the form-function relationships through a  
95 comparative analysis including various animals are scarce (Valois, 1917; Raymond &  
96 Prothero, 2010; Pina et al., 2020).

97 Within mammals, the patella displays a wide range of sizes but is described as rather  
98 conservative in shape (Samuels et al., 2017). The structure is hemispherical, with a convex  
99 outer surface covered by fibrocartilage and tendon fibers (Clark & Stechschulte, 1998;  
100 Benjamin et al. 2006), and a concave inner surface articulating with the femoral groove. As  
101 for the inner structure, it is essentially spongy, surrounded by a compact cortex (Raux et  
102 al., 1975). Data on the variability in the structure of this bone and its possible drivers are thus  
103 scarce.

104 The present contribution proposes to analyze the inner structure of this bone in various  
105 mammals in order to investigate how its microanatomy adapts to evolutionary constraints.  
106 One group of interest for this question is the perissodactyls. The odd-toed ungulates nowadays  
107 include the equids, tapirs and rhinos, and thus animals with a wide range of morphologies,  
108 masses (from a hundred kilos in lightly built horses to over two tonnes in massive rhinos),  
109 habitats (from mountainous forests to savannahs), and locomotor abilities (with distinct  
110 running performances between the three families). High body weight is considered to be  
111 associated with increased bone compactness (i.e. volume of osseous tissue over the total bone  
112 volume; Currey & Alexander, 1985; Houssaye et al., 2016b;c). The patella transmits to the  
113 femur, through the patellofemoral joint, forces of the quadriceps muscle that can reach up to  
114 20-25 times body weight (Aglietti & Menchetti, 1995; Samuels et al., 2007) so that we can  
115 first assume patellae of heavy animals to be more compact than those of lighter ones. During  
116 this process, the patella is also under high tensile stress, being pulled both proximally and  
117 distally. Since trabeculae tend to orient themselves in the direction of principal stress (Turner,  
118 1998; Pontzer et al., 2006; Barak et al., 2011), we could also expect the main trabecular  
119 orientation to be proximo-distal, at least away from the compression of the patella against the  
120 femur. Moreover, the distal part of the femur varies between the perissodactyl families and  
121 suggests various functional requirements; equids and rhinos are notably characterized by a

122 strongly asymmetrical femoral trochlea supposed to increase the locking mechanism of the  
123 knee (Hermanson & MacFadden, 1996; Schuurman et al. 2003; Janis et al., 2012). Because of  
124 the patellofemoral joint, variations of the functional constraints on the patellae are also  
125 expected and should be reflected in the organization of the trabecular network since bone  
126 inner structure adapts to the functional constraints it undergoes (Wolff, 1986; Huiskes et al.,  
127 2000). We thus additionally assume the trabecular organization to vary between these taxa  
128 according to morphological changes of the patellofemoral joint. Moreover, while the link  
129 between muscle activity and attachment site morphology is dubious (Zumwalt, 2005; Rabey  
130 et al. 2015), some studies have highlighted that cortical bone appears to be thicker at muscle  
131 attachment sites, so that, finally, we expect differences in muscle topography to be reflected in  
132 variations in cortical bone thickness (Niinimäki et al 2013; Djukic et al., 2015; Harbers et al.  
133 2020). The link between muscles and bone structure is complex, as it relies on various  
134 parameters in addition to muscle activity and attachment site, including the type of entheses  
135 and the angle of muscle attachment to the bone surface (Djukic et al., 2015). In this study we  
136 plan to document variations in cortical bone thickness along the bones to highlight possible  
137 relationships with muscle/tendon attachment sites.

138 To examine the microanatomical adaptive features in this sesamoid bone, we thus investigate  
139 both cortical and trabecular bone. This analysis takes the whole bones into consideration, in  
140 three dimensions, both qualitatively and quantitatively, and in a comparative perspective. This  
141 allows us to describe in detail the inner structure of this sesamoid bone in perissodactyls, and  
142 its variation within perissodactyls, to characterize the relationships between the inner structure  
143 and the functional constraints undergone by this bone, and notably its association with mass,  
144 support, and locomotion in the evolutionary history of perissodactyls.

145

## 146 **Material and Methods**

147

### 148 **Material**

149 The sample consists of 29 patellae belonging to the five modern rhino species (15), three of  
150 the four modern species of tapirs (7) and four of the seven modern species of equids (7),  
151 which enables us to encompass most of the diversity of modern Perissodactyla (Table 1; Fig.  
152 1). The bones come from the collections of the Museum National d'Histoire Naturelle  
153 (MNHN, Paris, France), of the Natural History Museum (NHM, London, UK), and of the  
154 Naturhistorisches Museum (NHM, Basel, Switzerland). The number of bones by species

155 relies on accessibility and on the state of preservation since patellae are often not preserved in  
156 the collections (maybe thrown away with the tendon) or drilled during mounting.

157

158

## 159 **Methods**

160

### 161 *Data acquisition*

162 Bones were scanned using high-resolution computed tomography at the: 1) MRI platform,  
163 hosted at the ISEM, University of Montpellier (UMR 5554; EasyTom 40-150, RX Solutions);  
164 2) micro-CT laboratory of the Natural History Museum, London, UK (Nikon HMX 225 ST  
165 system), and 3) AST-RX platform at the Museum National d'Histoire Naturelle, Paris (UMS  
166 2700; GE phoenix|X-ray v|tome|xS 240), with reconstructions performed using X-Act (RX  
167 Solutions), the CT-agent software (Nikon Metrology, Leuven, Belgium), and DATOX/RES  
168 software (phoenix datos|x). Voxel size naturally varies depending on specimen size (Table 1).  
169 Bone tissues were entirely segmented to analyze the microstructure of the complete bones.  
170 The compact cortex was then separated from the spongiosa by manual segmentation (allowing  
171 the distinction between bone and soft tissue even when the grey levels are very close) coupled  
172 with the 'smooth labels' function (avoiding artificial increments between successive  
173 reconstructed slices and thus offering a more natural contour) in Avizo 9.4 (VSG, Burlington,  
174 MA, USA) in order to be relatively objective and reproducible so that they could be analyzed  
175 separately. Then, in order to quantify the thickness of the layer of compact cortex, we isolated  
176 the outer surface of the bone and an inner surface (corresponding to the inner limit of the  
177 compact cortex or outer limit of the trabecular area) for each bone, using 'fill', and adding  
178 'remove islands' to remove the intracortical cavities. The cortical thickness was calculated  
179 using 'distance' between these two surfaces. This also enabled us to obtain bone cartographies  
180 in order to visualize the relative variation in cortical thickness along each bone. The different  
181 volumes obtained (whole bone tissue, compact bone tissue, trabecular bone tissue, spongiosa,  
182 whole bone) were then converted into image stacks in order to measure several parameters  
183 (see below).

184

185 In addition, for qualitative comparisons three virtual sections were obtained for each patella,  
186 following Barone (2010) for orientation (the apex being the distalmost point and the relief  
187 vertical in posterior view, the relief being rather rectilinear [with the most posterior parts  
188 aligned] and the most medial part of the bone becoming not observable in lateral view) and

189 terminology (Fig. 2): 1) one coronal section (CS; in the medio-lateral plane) at the level of the  
190 posteriormost extremity of the lateral angle, and 2) two sagittal sections (in the antero-  
191 posterior) plane, at the level of the deepest part of the medial articular facet (SS1) and of the  
192 apex (distal extremity on the posterior surface), along the relief (SS2; Fig. 2). Since no  
193 reference sections for the patella already exist in the literature, these sectional planes were  
194 chosen because they enable us to illustrate large portions of the bone to analyze the  
195 organization of the trabecular network (e.g. main orientation, density of the trabeculae) and  
196 could easily be created with good reproducibility for all specimens.

197 Image segmentation and visualization were performed from the reconstructed image data  
198 using Avizo 9.4, and virtual sections with VGSTUDIO MAX, versions 2.2 (Volume Graphics  
199 Inc.).

#### 200 *Data analysis*

201 Various quantitative parameters were used to describe the inner structure (Table S1), based on  
202 values directly obtained in Avizo, following Houssaye et al. (2018): 1) Whole bone volume  
203 (WBV), based on the segmentation of the osseous tissues and filling of the inner cavities; 2)  
204 the bone compactness (C), which was calculated based on WBV and on the bone tissue  
205 volume (BTV):  $C = BTV * 100 / WBV$ ; 3) the relative fraction of trabecular bone tissue  
206 (%Trab):  $\%Trab = \text{trabecular bone tissue volume} * 100 / BTC$ ; 4) the trabecular compactness  
207 (TC):  $TC = \text{trabecular bone tissue volume} * 100 / \text{trabecular volume}$  (= volume of the spongiosa,  
208 i.e., trabecular bone and intertrabecular spaces). In addition, two parameters were obtained  
209 using ‘distance’, based on MaxT: maximal cortical thickness and MeanT: mean cortical  
210 thickness: 5) RMaxT and 6) RMeanT (relative maximal and mean thicknesses), obtained by  
211 dividing MaxT and MeanT by a mean radius, calculated from WBV approximating the bone  
212 to a sphere.

213 We selected the following parameters: bone compactness (C), relative fraction of trabecular  
214 bone tissue (%Trab), trabecular compactness (TC), relative maximal (RMaxT) and mean  
215 (RMeanT) thicknesses. All these parameters are dimensionless ratios. We kept the whole  
216 bone volume (WBV) as a size estimate, in order to test the influence of size on all parameters  
217 by performing linear regressions of each parameter to WBV (lm function [stats]). ANOVAs  
218 and ANCOVAs (when the influence of size on the parameter was significant) were performed  
219 to test for differences between the three families.

220 Despite the small sample size and intraspecific variation, we tested the phylogenetic signal for  
221 each of these parameters in order to provide a rough estimate of the strength (weak, average



222 or strong) of the relationship between the parameters and phylogeny. We followed Steiner and  
223 Ryder (2011) for the phylogeny, which includes all analyzed species, setting all branch  
224 lengths to one and without testing for alternative phylogenetic hypotheses related to the  
225 unresolved position of *Dicerorhinus* (Willersley et al., 2009; Gaudry, 2017), given the gross  
226 nature of this test. For that we averaged the values obtained for each species when several  
227 specimens from the same species were available. Then we calculated the K-statistic following  
228 Blomberg et al. (2003) for each parameter and performed randomization tests. The K-statistic  
229 compares the observed phylogenetic signal in a trait with the signal under a Brownian motion  
230 model of trait evolution. A K-value  $> 1$  implies more similarity between relatives than  
231 expected under Brownian motion;  $K < 1$  highlights convergences. In order to analyze  
232 quantitatively the distribution of the different specimens in the microanatomical morphospace,  
233 but also for the purpose of evaluating how the different microanatomical parameters drive the  
234 variation observed in our sample, we conducted normalized PCAs (David & Jacobs, 2014) on  
235 the raw data (Table S1). All statistical analyses were performed using statistical software R (R  
236 Core Team 2014 [FactoMineR, ggplot2, ape]).

237

## 238 **Results**

### 239 **Qualitative description**

240 The bones essentially consist of a spongiosa, which is surrounded by a compact cortex.

241

#### 242 *Coronal sections*

243 In all rhino specimens, the spongiosa is dense and tightly packed with some large vascular  
244 canals piercing the cortex and not directed according to the main orientations of the trabecular  
245 network (Fig. 3A-E). The abundance of these large canals varies depending on specimens,  
246 being higher in *Rhinoceros sondaicus* MNHN-ZM-AC-A7970 and NHMUK ZD  
247 1871.12.29.7 and in *Ceratotherium simum* MNHN-ZM-MO-2005-297, and lower in  
248 *Dicerorhinus sumatrensis* NHMUK ZE 1948.12.20.1 and NHMUK ZD 1894.9.24.1 and in *R.*  
249 *sondaicus* NHMUK ZD 1921.5.15.1; it thus appears not to be linked with taxon or size. The  
250 cortex is thin but it thickens around the borders of the medial and lateral angles and of the  
251 apex (Fig. 3A-E). The trabeculae are rather homogeneously organized with no preferential  
252 orientation. A large part of the spongiosa near the lateral angle is compacted in specimens of  
253 *Diceros bicornis* (MNHN-ZM-AC-1944-278 and NHM 1876.9.26.6), *R. unicornis* (NHMUK

254 ZD 1884.1.22.1.2 and NHMUK ZD 1972.822), *R. sondaicus* (NHMUK ZD 1861.3.11.1), and  
255 *Ds. sumatrensis* (NHMUK ZD 1894.9.24.1).

256 In tapirs, the spongiosa is very homogeneous and the cortex is thin but thickens along the  
257 lateral border (Fig. 3F-H). There seems to be a slight change in the orientation of the  
258 trabeculae between the upper and lower parts of the bone in the two specimens of *Tapirus*  
259 *terrestris* (Fig. 3H).

260 In equids, the cortex is proportionally much thinner (Fig. 3I-L). There is a circumferential  
261 orientation of the trabeculae at the bone periphery from the bottom of the medial angle to that  
262 of the lateral one. A thin compact layer is observed in periphery medially to the apex in *Equus*  
263 *asinus* MNHN-ZM-2005-717, *Equus caballus* MNHN-ZM-AC-A541, and *Equus caballus*  
264 *przewalskii* MNHN-ZM-AC-1975-124 (Fig. 3I-J); this feature is thus not linked with taxon or  
265 size. Moreover, there is a tendency to show a change in the orientation of the trabeculae  
266 between the upper and lower parts of the bone, with a more proximo-distal orientation in the  
267 lower part; this feature is notably visible in *Equus asinus* MNHN-ZM-2005-717, *Equus*  
268 *caballus przewalskii* MNHN-ZM-AC-1975-124, and *Equus zebra* MNHN-ZM-AC-1948-54  
269 (Fig. 3I,J,L).

#### 270 271 *Sagittal sections*

272 *SSI*. In rhinos, a thick layer of compact cortex surrounds the anterior part of the bone, with a  
273 maximal thickness in the lower half of the bone (Fig. 4A-B). The thickness of compact bone  
274 on the posterior border varies between taxa, being minimal in *Ceratotherium simum* MNHN-  
275 ZM-MO-2005-297, *Dicerorhinus sumatrensis* NHMUK ZE 1948.12.20.1, and *Rhinoceros*  
276 *sondaicus* MNHN-ZM-AC-A7970, and maximal in *Ds. sumatrensis* NHMUK ZD  
277 1894.9.24.1 (Fig. 4A-B). Trabeculae are antero-posteriorly oriented on the proximal half of  
278 the bone whereas they are more randomly oriented in the distal half; this pattern is not  
279 distinguished in *Rhinoceros unicornis* MNHN ZM-AC-1967-101, NHMUK ZD 1972.822 and  
280 NHMUK ZE 1961.5.10.1. There is a circumferential orientation of the trabeculae along the  
281 anterior cortex essentially in the distal half of the bone.

282 In tapirs, the cortex is also thickened on the anterior border (Fig. 4C-D). The trabecular  
283 network is rather loose in *Tapirus terrestris* and *T. pinchaque* with a clearly dominant antero-  
284 posterior (more precisely, at about 90° to the posterior border) orientation of the trabeculae,  
285 except along the anterior border where they are circumferentially oriented, especially in the  
286 proximal half of the bone (Fig. 4D). The trabeculae are less antero-posteriorly oriented in *T.*  
287 *indicus* (Fig. 4C).

288 In equids, the cortex is rather thin with only a clear thickening along the anterior border  
289 around its mid-length (Fig. 4E-F). The orientation of the trabeculae follows that of most tapirs  
290 with a wider layer of circumferentially arranged trabeculae distalo-anteriorly.

291

292 *SS2*. In rhinos there is a clear thickening of the cortex along the anterior border, extending, to  
293 a much lower extent, along the anterior parts of the proximal and distal borders (Fig. 5A-C).

294 The spongiosa is rather dense and tightly built, i.e., with numerous trabeculae and small  
295 intertrabecular spaces. Tightness strongly increases around the core of the posterior border in  
296 *Ceratotherium simum* (Fig. 5A) and two specimens of *Rhinoceros sondaicus* (MNHN-ZM-  
297 AC-A7970 and NHMUK ZD 1871.12.29.7), whereas it is much less obvious in the other  
298 specimens. In the most proximal part of the bone, the trabeculae are antero-posteriorly  
299 oriented but rather randomly oriented elsewhere, except along the anterior border where they  
300 show a circumferential organization in most specimens. However, in *Dicerorhinus*  
301 *sumatrensis* the antero-posterior orientation appears dominant proximo-distally and is not  
302 restricted to the most proximal part of the bone (Fig. 5C).

303 In *Tapirus indicus* the organization is rather similar as in *Ds. sumatrensis*, with some  
304 thickening of the bone around the core of the posterior border, but with a slightly less tight  
305 spongiosa. Tightness is much lower in the two other tapirs and the thickening around the  
306 posterior border is discrete (Fig. 5E).

307 The pattern in equids is very similar to that of the specimens of *Ds. sumatrensis* (Fig. 5F).

308

### 309 *Bone cartographies*

310 Bone cartographies enabled us to visualize the relative variation in cortical thickness along the  
311 entire bone and to make comparisons between the various bones. In all taxa analyzed, cortical  
312 thickness is maximal on the anteriormost part of the bone (Fig. 6). The thickened area is  
313 rather wide, with a peak that is not clearly distinct and that is rather central and slightly  
314 proximally located.

315 In *Ceratotherium*, *Dicerorhinus*, *Rhinoceros sondaicus* and *R. unicornis*, there is also a more  
316 localized thickening in posterior view around the core of the lateral facet, whereas a more  
317 laterally localized thickening is observed in *Diceros*, with both occurring in *Rhinoceros*  
318 *unicornis* (Fig. 6A-F).

319 In tapirs there is a slight posterior thickening on the core of the relief in one specimen of  
320 *Tapirus indicus* (MNHN-ZM-AC-1931-528; Fig. 6G) but not on the others. In both specimens  
321 of *T. terrestris*, a thickening is observed at mid-length of the lateral border (Fig. 6J).

322 In equids, there is a thickening posteriorly in the proximal half of the relief in *Equus*  
323 *hemionus*, in one specimen of *Equus asinus* (MNHN-ZM-AC-1893-634), and in *Equus zebra*  
324 (Fig. 6K,M).

325

## 326 **Quantitative analyses**

327 Five quantitative parameters were analyzed: bone compactness (C), relative fraction of  
328 trabecular bone tissue (%Trab), trabecular compactness (TC), relative maximal (RMaxT) and  
329 mean (RMeanT) thicknesses.

330 A significant, at  $p=0.05$ , phylogenetic signal is obtained for %Trab, RMaxT and RMeanT,  
331 with K values around 1 for thickness indices and clearly higher for %Trab. Size (estimated  
332 based on WBV) also shows a significant phylogenetic signal (Table 2). No parameter shows a  
333 significant correlation with size (Table 2). Compactness significantly differs between the  
334 three families (Mean values of  $65.4\pm 7.3$ ,  $56.6\pm 10.4$ , and  $56.6\pm 6.3$  in rhinos, tapirs, and  
335 equids, respectively; ANOVA.  $p=0.02$ ) but the range of values is rather wide in the three  
336 families, especially for rhinos and tapirs (Fig. 7). Within rhinos, *Diceros* and *R. unicornis*  
337 show the highest values, except *R. unicornis* NHMUK ZD 1884.1.22.1.2 that groups with the  
338 three other species. *Dicerorhinus* shows the lowest values. Within tapirs, there is a large  
339 intraspecific variation. For %Trab, there is a clear distinction (with almost no overlap)  
340 between equids and ceratomorphs (i.e. tapirs and rhinos; Fig. 1) even when size is taken into  
341 consideration (Mean values of  $55.5\pm 5.8$ ,  $53.3\pm 6.0$ , and  $71.1\pm 4.8$  in rhinos, tapirs, and equids,  
342 respectively; ANCOVA;  $p<0.001$ ). Equids thus clearly show a much thinner cortex than  
343 ceratomorphs (Fig. 7). Within rhinos and tapirs, intraspecific variation is high. TC does not  
344 significantly differ between the three families (Mean values of  $51.5\pm 7.9$ ,  $41.6\pm 12.3$ , and  
345  $48.2\pm 6.2$  in rhinos, tapirs, and equids, respectively; ANOVA;  $p=0.27$ ). Within rhinos, it is  
346 higher in *Diceros* and *R. unicornis*, except again for *R. unicornis* NHMUK ZD 1884.1.22.1.2  
347 (Fig. 7). Again, intraspecific variation is high. RMaxT and RMeanT are significantly lower in  
348 equids even when size is taken into consideration (Mean values of  $32.3\pm 3.5$ ,  $27.3\pm 6.5$ , and  
349  $19.6\pm 3.3$  for RMaxT and  $8.0\pm 1.8$ ,  $6.1\pm 2.0$ , and  $3.5\pm 1.2$  for RMeanT in rhinos, tapirs, and  
350 equids, respectively; ANCOVA;  $p<0.001$  for the two parameters). There is intraspecific  
351 variation but a tendency for *Diceros* to show the highest values within rhinos and  
352 *Ceratotherium* the lowest ones, and *T. indicus* the highest values within tapirs and *T.*  
353 *pinchaque* the lowest ones for RMaxT but not for RMeanT (Fig. 7).

354

355 The first two axes of the PCA, which represent 95.2% of the variance (66.9 and 28.3%,  
356 respectively; Table S2), enable us to distinguish equids from rhinos and tapirs but not rhinos  
357 from tapirs (Fig. 8; neither does PC3). The first axis distinguishes equids, on the negative  
358 side, from rhinos, on the positive one, whereas tapirs are more widely distributed along PC1.  
359 This axis is essentially driven by RMeanT, RMaxT, C and, to a lesser extent, TC (Fig. 8).  
360 Rhinos thus show more compact bones with a relatively thicker compact cortex than equids.  
361 PC2 distinguishes tapirs, on the negative side, from equids, on the positive one, with a slight  
362 overlap, while rhinos have a distribution overlapping with the distribution of tapirs and  
363 equids. PC2 is essentially driven by %Trab, TC and, to a lesser extent, C (Fig. 8). Equids have  
364 a significantly wider spongiosa rather more compact than tapirs. Neither PC1 nor PC2 is  
365 correlated with size (Table 2).

366

## 367 **Discussion**

### 368 **Common patterns among Perissodactyla**

369 In all perissodactyls sampled the inner structure of the patella consists of a spongiosa  
370 surrounded by a compact cortex. The maximal cortical thickness is observed anteriorly at  
371 about mid-length, at the insertion of the intermediate patellar ligament (Fig. 6, 9A), which is  
372 very strong in ungulates (Budras et al. 2003; Barone, 2010; Abumandour et al. 2020).  
373 Ligament and muscle insertions on the patella are rather similar between horses and rhinos  
374 and thus supposedly across all perissodactyls (Barone, 2010; Etienne et al., 2021). The  
375 intermediate patellar ligament is surrounded by the lateral and medial patellar ligaments,  
376 which also insert on the anterior side (Fig. 9A), but no clear specific thickening associated  
377 with the insertion of these two ligaments is observed in the sampled taxa. The lateral ligament  
378 probably inserts where thickness is relatively high around the peak, but no thickening is  
379 observed medially. These ligaments link the anterior part of the patella to the tibial tuberosity  
380 and thus play a major role in locomotion and limb stability (Barone, 2010). Some specimens  
381 show a thickening of the cortex between the relief and the lateral angle in posterior view. This  
382 could be linked to the insertion of the lateral patellofemoral ligament, which (with the medial  
383 ligament) help to maintain the patella in front of the trochlea, though it inserts on the  
384 parapatellar fibrocartilage (Barone, 2010) and is thus less likely to be directly associated with  
385 a cortical thickening on the patella. There is no thickening proximally and more generally no  
386 thickening that appears associated with the insertions of the quadriceps femoris muscle (Figs  
387 6, 9B).

388 As for the spongiosa, there is a clearly dominant antero-posterior orientation of the trabeculae,  
389 which is approximately perpendicular to the posterior articular surface. The main trabecular  
390 orientation is assumed to reflect the direction of maximal stress (Wolff, 1986; Huiskes et al.,  
391 2000). Accordingly, the main stress would be antero-posteriorly directed. This might be  
392 associated with maintaining the patella against the femoral trochlea, which would be  
393 consistent with previous studies showing the strong shape adaptation between the patellar  
394 articular surface (ridge and facets) and the femoral trochlea allowing a more stable patellar  
395 alignment (Fujikawa et al. 1983; Wang et al. 2016). This would also be consistent with the  
396 main functional role of the patella being to increase the moment arm. This compression  
397 results from the tension exerted proximally and distally by the quadriceps tendon and the  
398 patellar ligament (Ellis et al., 1980; Aglietti & Menchetti, 1995) and the primary orientation  
399 would reflect the resultant force of this tension. However, considering the diversity in the  
400 orientation of the trabeculae in long bones, in both the epiphyses and metaphyses, and in short  
401 bones (e.g., Thomason, 1985; Jang & Kim, 2009; Kivell et al. 2018; Bird et al. 2021; AH.  
402 Pers. Obs.), if an anteroposterior orientation of the trabeculae in the core of the bone is  
403 coherent, it nevertheless appears surprising not to see a progressive shift from the proximal  
404 and distal surfaces to the core of the bone, from one direction of tension (and thus a rather  
405 proximo-distal main orientation) to the resultant (antero-posterior) force. The resultant force  
406 thus seems to act all along the bone rather homogeneously, with only limited local tension,  
407 except along the anterior side. A similar general orientation of the trabeculae is also observed  
408 in humans (Raux et al., 1975; Toumi et al., 2006).

409 The dominant antero-posterior orientation in perissodactyls is stronger in the proximal than in  
410 the lower half of the bone, suggesting either stronger compressive stresses in the proximal  
411 half of the patella, or stronger tension in the lower half. And, indeed, equids and, to a lesser  
412 extent tapirs, show a second slight proximo-distal orientation of the trabeculae (observed on  
413 the CS) in the lower half, that would rather follow proximo-distal tension. In all specimens,  
414 trabeculae near the anterior cortex tend to align rather proximo-distally but along the anterior  
415 surface, probably reflecting the traction of the patellar ligament, and possibly also of the  
416 quadriceps tendon.

417 For all specimens, there is a slightly more intense thickening of the cortex and increase in the  
418 compactness of the spongiosa posteriorly in SS2 than in SS1. It is probably associated with a  
419 stronger compressive stress at the apex than in the medial articular facet, which is also in  
420 accordance with some cortical thickening on the apex in most specimens.

421 Contrary to our hypothesis, there is no significant correlation of bone - including trabecular -  
422 compactness with size and thus with body mass. Only an increase in the tightness of the  
423 spongiosa is observable, i.e. trabeculae appear more numerous and thinner, with reduced  
424 intertrabecular spaces, which is in accordance with multiple microanatomical studies (e.g.  
425 Dumont et al., 2013; Plasse et al., 2019).

426

### 427 **Trends among Perissodactyla**

428 The quantitative analyses highlight a strong variation within the perissodactyl families. There  
429 is also high intraspecific variation, notably observed among tapirs in our sample, since rhino  
430 specimens from the same species tend to group together in most cases and since only one  
431 equid species is represented by two specimens although these are two subspecies with strong  
432 differences in size and gracility. Intraspecific variation would need a larger sample to be  
433 analyzed in more detail. Information relative to captive versus wild origin, sex and even age  
434 (between subadult and adult specimens) are incomplete (Table 1) but no strong relationship of  
435 intraspecific variation with these three factors that might have been visible in such a limited  
436 sample is observed. This important intrafamily and intraspecific variation is consistent with  
437 previous studies highlighting a stronger variability of sesamoid bones as compared to other  
438 bones, in part because they are embedded in soft tissues that are also variable (Raymond &  
439 Prothero, 2012; Clark & Stechschulte, 1998; Samuels et al., 2007). Despite this variation,  
440 some clear differences are observed between the three perissodactyl families.

441 Equids have a clearly thinner cortex than ceratomorphs and this is notably characterized,  
442 beyond a generally lower thickness, by a much lower thickening in anterior view. This  
443 notable feature might be associated with a higher gracility and a relatively high position of the  
444 knee in equids (with proportionally much longer zeugopod and, especially, autopod bones)  
445 engendering different biomechanical constraints on the patella. Comparable analyses in other  
446 groups, such as early rhinocerotoids, notoungulates, artiodactyls and proboscideans would be  
447 required in order to functionally understand this adaptive trend.

448 In equids (also very slightly in some rhinos but not in tapirs) there is a circumferential  
449 orientation of the trabeculae between the apex and the medial border (observed in CS). This  
450 might be associated with the medial extension of the medial angle, with a medial  
451 displacement of the medial patellar ligament.

452 Among perissodactyls, both rhinos and equids show a strongly asymmetrical femoral trochlea,  
453 with a much larger medial trochlear ridge (Hermanson & MacFadden, 1996; Janis et al.,  
454 2012). This feature convergently evolved in these two lineages and in others, such as

455 artiodactyls and notoungulates (Hermanson & MacFadden, 1996; Shockey, 2001; Janis et al.  
456 2012). It is supposed to increase the locking mechanism of the knee and thus to constitute a  
457 passive stay-apparatus (Hermanson & MacFadden, 1996; Shockey, 2001; Janis et al. 2012).  
458 This would be of particular interest in animals with long periods of standing, like *Equus*  
459 *caballus* that stands for more than 20 hours a day (Boyd et al. 1988), but also in heavy taxa,  
460 so that this locking mechanism has been inferred to be also associated with body weight  
461 support (Hermanson & MacFadden, 1996). Shockey (2001) insisted on the associated increase  
462 in the tension of the medial patellar ligament that would also restrict the medio-lateral  
463 movements of the tibia and thus increase limb stability during locomotion (Janis et al. 2012).  
464 As a consequence, knee asymmetry was tentatively associated with a galloping locomotor gait  
465 and high body mass. This would explain its evolution within perissodactyls, with all modern  
466 perissodactyls being able to gallop (Hildebrand, 1977; Leach & Dagg, 1983; Alexander &  
467 Pond, 1992; MacLaren & Nauwelaerts, 2016). However, if the transverse gallop is most  
468 common in equids and rhinos, tapirs essentially resort to rotary gallop (Economou et al.,  
469 2021). This distinction in the type of gallop is common between small and large ungulates and  
470 might explain the joint association of locomotion and weight with knee asymmetry (larger  
471 galloping ungulates showing more asymmetric knees; Janis et al., 2012). Despite the  
472 development of the medial border, it is laterally and not medially that trabecular bone is  
473 denser in rhinos and equids (whereas it is more homogeneous in tapirs), suggesting stronger  
474 stress laterally (since bone compactness generally increases with stress intensity; Fyhrie &  
475 Kimura, 1999; Keaveny et al. 2001; Perilli et al. 2008). This feature is in accordance with  
476 more forces being transmitted laterally because of the laterally deviated position of the knee.  
477 Trabecular tightness appears looser, i.e., less numerous trabeculae and relatively thicker  
478 trabeculae and intertrabecular spaces, in tapirs but compactness does not differ from that of  
479 rhinos and equids. Considering that tapirs do not show a development of the medial angle, we  
480 could have expected a “compensation” of the inner structure, with an increased compactness  
481 medially associated with higher stresses, but this is not the case, which suggests different  
482 repartition of stresses on their knees associated with a different patellofemoral joint.

483

## 484 **Conclusion**

485 The microanatomy of the entire patella in perissodactyls consists of a spongiosa surrounded  
486 by a compact cortex. As against our predictions, there is no increase in compactness with size.  
487 The muscle insertions are not associated with local thickening of the cortex; however, a strong



488 thickening occurs at the insertion of the intermediate patellar ligament. The main orientation  
489 of the trabeculae, and therefore of the main stress, is anteroposterior, whereas anteriorly the  
490 trabeculae are more circumferentially oriented, following the insertion of the patellar ligament  
491 and, possibly, the quadriceps tendon. Variations occur within and between the three modern  
492 families. Ceratomorphs have a relatively thicker cortex than equids. The presence of a medial  
493 border in rhinos and equids is associated with increased trabecular density laterally,  
494 suggesting greater lateral stress. In contrast, the internal structure is more homogeneous in  
495 tapirs. These differences suggest that the diverse morphologies of the patellofemoral joints in  
496 the three families are associated with distinct stresses on their knees, raising the interest of  
497 further biomechanical investigations.

498

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513

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516

517

## 518 **References**

519

520 Abumandour, M.M.A., Bassuoni, N.F., El-Gendy, S., Karkoura, A. & El-Bakary, R. (2020).  
521 Cross-anatomical, radiographic and computed tomographic study of the stifle joint of donkeys  
522 (*Equus africanus asinus*). *Anatomia, Histologia, Embryologia*, 49, 402–416.

523 Aglietti, P. & Menchetti, P.P.M. (1995) Biomechanics of the patellofemoral joint, in: The  
524 Patella. Springer, pp. 25–48.

525 Alexander, R. & Pond, C. (1992) Locomotion and bone strength of the white rhinoceros,  
526 *Ceratotherium simum*. *Journal of Zoology*, 227, 63–69.

527 Alexander, R.M. & Dimery, N.J. (1985) The significance of sesamoids and retro-articular  
528 processes for the mechanics of joints. *Journal of Zoology*, 205, 357–371.  
529 <https://doi.org/10.1111/j.1469-7998.1985.tb05622.x>

530 Allen, V., Kambic, R., Gatesy, S. & Hutchinson, J. (2017) Gearing effects of the patella (knee  
531 extensor muscle sesamoid) of the helmeted guineafowl during terrestrial locomotion. *Journal*  
532 *of Zoology*, 303, 178–187.

533 Amson, E., Billet, G. & de Muizon, C. (2018) Evolutionary adaptation to aquatic lifestyle in  
534 extinct sloths can lead to systemic alteration of bone structure. *Proceedings of the Royal*  
535 *Society B: Biological Sciences*, 285, 20180270.

536 Barone, R., (2000). Anatomie comparée des mammifères domestiques. Tome 2 : Arthologie et  
537 myologie. Paris: Vigot Frères.

538 Barone R. (2010). Anatomie comparée des mammifères domestiques. Tome 1 : Ostéologie.  
539 Paris: Vigot Frères.

540 Barr, W.A. (2018) Ecomorphology. *Methods in paleoecology*, 339–349.

541 Benjamin, M., Toumi, H., Ralphs, J., Bydder, G., Best, T. & Milz, S. (2006). Where tendons  
542 and ligaments meet bone: attachment sites (‘entheses’) in relation to exercise and/or  
543 mechanical load. *Journal of anatomy*, 208, 471–490.

544 Bird, E.E., Kivell, T.L. & Skinner, M.M. (2021). Cortical and trabecular bone structure of the  
545 hominoid capitate. *Journal of Anatomy*.

546 Blomberg, S.P., Garland, T. & Ives, A.R. (2003) Testing for phylogenetic signal in  
547 comparative data: behavioral traits are more labile. *Evolution*, 57, 717–745.

548 Bongianini, M. (1988) Simon and Schuster’s Guide to Horses and Ponies of the World. Simon  
549 and Schuster.

550 Boyd, L.E., Carbonaro, D.A. & Houpt, K.A. (1988) The 24-hour time budget of Przewalski  
551 horses. *Applied Animal Behaviour Science*, 21, 5–17.

552 Budras, K.D., Sack, W.O. & Rock, S. (2003). *Anatomy of the horse: an illustrated text*.  
553 Schlütersche.

554 Buffrénil, V de., Canoville, A., D’Anastasio, R. & Domning, D.P. (2010) Evolution of  
555 Sirenian pachyosteosclerosis, a model-case for the study of bone structure in aquatic  
556 tetrapods. *Journal of Mammalian Evolution*, 17, 101–120. [https://doi.org/10.1007/s10914-](https://doi.org/10.1007/s10914-010-9130-1)  
557 010-9130-1

558 Canoville, A., Buffrénil, V. & Laurin, M. (2016) Microanatomical diversity of amniote ribs:  
559 an exploratory quantitative study. *Biological Journal of the Linnean Society*, 118, 706–733.

560 Carter, D.R., Wong, M. & Orr & T.E. (1991) Musculoskeletal ontogeny, phylogeny, and  
561 functional adaptation. *Journal of Biomechanics*, 24, 3–16.

562 Clark, J. & Stechschulte, D.J. (1998) The interface between bone and tendon at an insertion  
563 site: a study of the quadriceps tendon insertion. *Journal of Anatomy*, 192, 605–616.  
564 <https://doi.org/10.1046/j.1469-7580.1998.19240605.x>

565 Currey, J.D. (2003) The many adaptations of bone. *Journal of Biomechanics*, 36, 1487–1495.  
566 [https://doi.org/10.1016/S0021-9290\(03\)00124-6](https://doi.org/10.1016/S0021-9290(03)00124-6)

567 Dan, M., Parr, W., Broe, D., Cross, M. & Walsh, W.R. (2018). Biomechanics of the knee  
568 extensor mechanism and its relationship to patella tendinopathy: A review. *Journal of*  
569 *Orthopaedic Research*, 36, 3105–3112.

570 David, C.C. & Jacobs, D.J. (2014) Principal Component Analysis: A Method for Determining  
571 the Essential Dynamics of Proteins, in: *Protein Dynamics, Methods in Molecular Biology*.  
572 Humana Press, Totowa, NJ, pp. 193–226. [https://doi.org/10.1007/978-1-62703-658-0\\_11](https://doi.org/10.1007/978-1-62703-658-0_11)

573 Díaz-Berenguer, E., Badiola, A., Moreno-Azanza, M. & Canudo, J.I. (2018) First adequately-  
574 known quadrupedal sirenian from Eurasia (Eocene, Bay of Biscay, Huesca, northeastern  
575 Spain). *Scientific Reports*, 8, 1–13. <https://doi.org/10.1038/s41598-018-23355-w>

576 Doube, M., Kłosowski, M.M., Arganda-Carreras, I., Cordelières, F.P., Dougherty, R.P.,  
577 Jackson, J.S., Schmid, B., Hutchinson, J.R. & Shefelbine, S.J. (2010) BoneJ: Free and  
578 extensible bone image analysis in ImageJ. *Bone*, 47, 1076–1079.

579 Dumont, M., Laurin, M., Jacques, F., Pelle, E., Dabin, W. & de Buffrenil, V. (2013) Inner  
580 architecture of vertebral centra in terrestrial and aquatic mammals: a two-dimensional  
581 comparative study. *Journal of Morphology*, 274, 570–84. <https://doi.org/10.1002/jmor.20122>

582 Dunmore, C.J., Bardo, A., Skinner, M.M. & Kivell, T.L. (2020) Trabecular variation in the  
583 first metacarpal and manipulation in hominids. *American Journal of Physical Anthropology*,  
584 171, 219-241. <https://doi.org/10.1002/ajpa.23974>

585 Dunn, R.H. (2018) Functional morphology of the postcranial skeleton, in: *Methods in*  
586 *Paleoecology*. Springer, pp. 23–36.

587 Economou, G., McGrath, M., Wajsberg, J. & Granatosky, M.C. (2021) Perissodactyla  
588 Locomotion, in: Vonk, J., Shackelford, T. (Eds.), *Encyclopedia of Animal Cognition and*  
589 *Behavior*. Springer International Publishing, Cham, pp. 1–8. [https://doi.org/10.1007/978-3-](https://doi.org/10.1007/978-3-319-47829-6_887-1)  
590 [319-47829-6\\_887-1](https://doi.org/10.1007/978-3-319-47829-6_887-1)

591 Ellis, M.I., Seedhom, B.B., Wright, V. & Dowson, D. (1980) An evaluation of the ratio  
592 between the tensions along the quadriceps tendon and the patellar ligament. *Engineering in*  
593 *Medicine*, 9, 189–194. [https://doi.org/10.1243/EMED\\_JOUR\\_1980\\_009\\_049\\_02](https://doi.org/10.1243/EMED_JOUR_1980_009_049_02)

594 Etienne, C., Houssaye, A. & Hutchinson, J.R. (2021). Limb myology and muscle architecture  
595 of the Indian rhinoceros *Rhinoceros unicornis* and the white rhinoceros *Ceratotherium simum*  
596 (Mammalia: Rhinocerotidae). *PeerJ* 9: e11314.

597 Eyal, S., Blitz, E., Shwartz, Y., Akiyama, H., Schweitzer, R. & Zelzer, E. (2015). On the  
598 development of the patella. *Development*, 142, 1831–1839.

599 Eyal, S., Rubin, S., Krief, S., Levin, L. & Zelzer, E. (2019). Common cellular origin and  
600 diverging developmental programs for different sesamoid bones. *Development*, 146,  
601 [dev167452](https://doi.org/10.1242/dev.167452).

602 Ferry, B., Duclos, M., Burt, L., Therre, P., Le Gall, F., Jaffré, C. & Courteix, D. (2011) Bone  
603 geometry and strength adaptations to physical constraints inherent in different sports:  
604 comparison between elite female soccer players and swimmers. *Journal of bone and mineral*  
605 *metabolism*, 29, 342–351.

606 Fujikawa, K., Seedhom, B.B. & Wright, V. (1983). Biomechanics of the patello-femoral joint.  
607 Part I: A study of the contact and the congruity of the patello-femoral compartment and  
608 movement of the patella. *Engineering in Medicine*, 12, 3–11.

609 Fyhrie, D.P. & Kimura, J.H. (1999). Cancellous bone biomechanics. *Journal of*  
610 *Biomechanics*, 32, 1139–1148.

611 Gould, S.J. (2002) *The structure of evolutionary theory*. Harvard University Press.

612 Hermanson, J.W. & Macfadden, B.J. (1996) Evolutionary and functional morphology of the  
613 knee in fossil and extant horses (Equidae). *Journal of Vertebrate Paleontology*, 16, 349–357.

614 Hildebrand, M. (1977) Analysis of asymmetrical gaits. *Journal of Mammalogy*, 58, 131–156.

615 Houssaye, A. & Tafforeau, P. (2012). What vertebral microanatomy reveals about the ecology  
616 of juvenile mosasaurs (Reptilia, Squamata). *Journal of Vertebrate Paleontology*, 32, 1042–  
617 1048.

618 Houssaye, A., Fernandez, V. & Billet, G. (2016a) Hyperspecialization in some South  
619 American endemic ungulates revealed by long bone microstructure. *Journal of Mammalian*  
620 *Evolution*, 23, 221–235.

621 Houssaye, A., Sander, P.M. & Klein, N. (2016b) Adaptive patterns in aquatic amniote bone  
622 microanatomy—More complex than previously thought. *Integrative and Comparative*  
623 *Biology*, 56, 1349–1369.

624 Houssaye, A., Tafforeau, P. & Herrel, A. (2014) Amniote vertebral microanatomy—what are  
625 the major trends? *Biological Journal of the Linnean Society*, 112, 735–746.

626 Houssaye, A., Waskow, K., Hayashi, S., Cornette, R., Lee, A.H. & Hutchinson, J.R. (2016c)  
627 Biomechanical evolution of solid bones in large animals: a microanatomical investigation.  
628 *Biological Journal of the Linnean Society*, 117, 350–371.

629 Houssaye, A., Taverne, M. & Cornette, R. (2018) 3D quantitative comparative analysis of  
630 long bone diaphysis variations in microanatomy and cross-sectional geometry. *Journal of*  
631 *anatomy*, 232, 836–849.

632 Huiskes, R., Ruimerman, R., Van Lenthe, G.H. & Janssen, J.D. (2000) Effects of mechanical  
633 forces on maintenance and adaptation of form in trabecular bone. *Nature*, 405, 704–706.

634 Iwaniec, U.T. & Turner, R.T. (2016) Influence of body weight on bone mass, architecture and  
635 turnover. *Journal of Endocrinology*, 230, R115–R130.

636 Janis, C.M., Shoshitaishvili, B., Kambic, R. & Figueirido, B. (2012) On their knees: distal  
637 femur asymmetry in ungulates and its relationship to body size and locomotion. *Journal of*  
638 *Vertebrate Paleontology*, 32, 433–445.

639 Jang, I.G. & Kim, I.Y. (2009). Computational simulation of trabecular adaptation progress in  
640 human proximal femur during growth. *Jouranl of Biomechanics*, 42, 573–580.

641 Jones, K.E., Benitez, L., Angielczyk, K.D. & Pierce, S.E. (2018) Adaptation and constraint in  
642 the evolution of the mammalian backbone. *BMC Evolutionary Biology*, 18, 1–13.  
643 <https://doi.org/10.1186/s12862-018-1282-2>

644 Keaveny, T.M., Morgan, E.F., Niebur, G.L. & Yeh, O.C. (2001). Biomechanics of trabecular  
645 bone. *Annual review of biomedical engineering*, 3, 307–333.

646 Kivell, T.L. (2016) A review of trabecular bone functional adaptation: what have we learned  
647 from trabecular analyses in extant hominoids and what can we apply to fossils? *Journal of*  
648 *Anatomy*, 228, 569–594.

649 Kivell, T.L., Davenport, R., Hublin, J.J., Thackeray, J.F. & Skinner, M.M. (2018). Trabecular  
650 architecture and joint loading of the proximal humerus in extant hominoids, Ateles, and  
651 Australopithecus africanus. *American Journal of Physical Anthropology* 167: 348–365.

652 Kley, N., Kearney, M. & Hall, B. (2007) Adaptations for digging and burrowing. Fins into  
653 limbs: evolution, development, and transformation, 284–309.

654 Leach, D.H. & Dagg, A.I. (1983) A review of research on equine locomotion and  
655 biomechanics. *Equine Veterinary Journal*, 15, 93–102. [https://doi.org/10.1111/j.2042-](https://doi.org/10.1111/j.2042-3306.1983.tb01726.x)  
656 [3306.1983.tb01726.x](https://doi.org/10.1111/j.2042-3306.1983.tb01726.x)

657 Lovejoy, C.O. (2007) The natural history of human gait and posture: Part 3. The knee. *Gait*  
658 *and posture* 25, 325–341.

659 MacLaren, J.A. & Nauwelaerts, S. (2016) A three-dimensional morphometric analysis of  
660 upper forelimb morphology in the enigmatic tapir (Perissodactyla: *Tapirus*) hints at subtle  
661 variations in locomotor ecology. *Journal of Morphology*, 277, 1469–1485.

662 Montañez-Rivera, I., Nyakatura, J.A. & Amson, E. (2018) Bone cortical compactness in ‘tree  
663 sloths’ reflects convergent evolution. *Journal of Anatomy*, 233, 580–591.

664 Montoya-Sanhueza, G. & Chinsamy, A. (2018) Cortical bone adaptation and mineral  
665 mobilization in the subterranean mammal *Bathyergus suillus* (Rodentia: Bathyergidae):  
666 effects of age and sex. *PeerJ*, 6, e4944.

667 Mottershead, S. (1988) Sesamoid bones and cartilages: an enquiry into their function. *Clinical*  
668 *Anatomy*, 1, 59–62.

669 Perilli, E., Baleani, M., Öhman, C., Fognani, R., Baruffaldi, F. & Viceconti, M. (2008).  
670 Dependence of mechanical compressive strength on local variations in microarchitecture in  
671 cancellous bone of proximal human femur. *Journal of Biomechanics*, 41, 438–446.

672 Pina, M., DeMiguel, D., Puigvert, F., Marcé-Nogué, J. & Moyà-Solà, S. (2020) Knee function  
673 through finite element analysis and the role of Miocene hominoids in our understanding of the  
674 origin of antipronograde behaviours: the *Pierolapithecus catalaunicus* patella as a case study.  
675 *Palaeontology*, 63, 459–475.

676 Plasse, M., Amson, E., Bardin, J., Grimal, Q. & Germain, D. (2019) Trabecular architecture in  
677 the humeral metaphyses of non-avian reptiles (Crocodylia, Squamata and Testudines):  
678 Lifestyle, allometry and phylogeny. *Journal of Morphology*, 280, 982–998.  
679 <https://doi.org/10.1002/jmor.20996>

680 Quemeneur, S., Buffrénil, V. & Laurin, M. (2013) Microanatomy of the amniote femur and  
681 inference of lifestyle in limbed vertebrates. *Biological Journal of the Linnean Society*, 109,  
682 644–655.

683 Raux, P., Townsend, P.R., Miegel, R., Rose, R.M. & Radin, E.L. (1975) Trabecular  
684 architecture of the human patella. *Journal of Biomechanics*, 8, 1–7.

685 Raymond, K.R. & Prothero, D.R. (2010) Comparative variability of intermembranous and  
686 endochondral bones in Pleistocene mammals. *Palaeontologia Electronica*, 13, 4A.

687 Reina, N., Cavaignac, E., Trousdale, W.H., Laffosse, J. & Braga, J. (2017). Laterality and grip  
688 strength influence hand bone micro-architecture in modern humans, an HR p QCT study.  
689 *Journal of Anatomy*, 230, 796–804.

690 Saers, J.P., Ryan, T.M. & Stock, J.T. (2020) Baby steps towards linking calcaneal trabecular  
691 bone ontogeny and the development of bipedal human gait. *Journal of Anatomy*, 236, 474–  
692 492. <https://doi.org/10.1016/j.jhevol.2016.05.012>

693 Saers, J.P.P., Cazorla-Bak, Y., Shaw, C.N., Stock, J.T. & Ryan, T.M. (2016) Trabecular bone  
694 structural variation throughout the human lower limb. *Journal of Human Evolution*, 97, 97–  
695 108.

696 Samuels, M.E., Regnault, S. & Hutchinson, J.R. (2017) Evolution of the patellar sesamoid  
697 bone in mammals. *PeerJ*, 5, e3103.

698 Sarin, V.K. & Carter, D.R. (2000) Mechanobiology and joint conformity regulate  
699 endochondral ossification of sesamoids. *Journal of Orthopaedic Research*, 18, 706–712.

700 Seilacher, A. (1970) Arbeitskonzept zur Konstruktions-Morphologie. *Lethaia*, 3, 393–396.

701 Shockey, B.J. (2001) Specialized knee joints in some extinct, endemic, South American  
702 herbivores. *Acta Palaeontologica Polonica*, 46, 277–288.

703 Sievänen, H. (2010). Immobilization and bone structure in humans. *Archives of biochemistry  
704 and biophysics*, 503, 146–152.

705 Steiner, C.C. & Ryder, O.A. (2011) Molecular phylogeny and evolution of the Perissodactyla.  
706 *Zoological Journal of the Linnean Society*, 163, 1289–1303.

707 Thomason, J.J. (1985) Estimation of locomotory forces and stresses in the limb bones of  
708 Recent and extinct equids. *Paleobiology*, 11, 209–220.

709 Toumi, H., Higashiyama, I., Suzuki, D., Kumai, T., Bydder, G., McGonagle, D., Emery, P.,  
710 Fairclough, J. & Benjamin, M. (2006) Regional variations in human patellar trabecular  
711 architecture and the structure of the proximal patellar tendon enthesis. *Journal of Anatomy*,  
712 208, 47–57.

713 Valois, H. (1917) La valeur morphologique de la rotule chez les mammifères. *Bulletins et  
714 Mémoires de la Société d'Anthropologie de Paris*, 8, 1–34.

715 Vlachopoulos, D., Barker, A.R., Ubago-Guisado, E., Fatouros, I.G., Knapp, K.M., Williams,  
716 C.A. & Gracia-Marco, L. (2017) Longitudinal adaptations of bone mass, geometry, and  
717 metabolism in adolescent male athletes: The PRO-BONE Study. *Journal of Bone and Mineral  
718 Research*, 32, 2269–2277.

719 Volpato, V., Viola, T.B., Nakatsukasa, M., Bondioli, L. & Macchiarelli, R. (2008) Textural  
720 characteristics of the iliac-femoral trabecular pattern in a bipedally trained Japanese macaque.  
721 *Primates*, 49, 16–25. <https://doi.org/10.1007/s10329-007-0053-2>

722 Wang, X.M., Liu, H.X., Niu, J.H., Duan, G.M. & Wang, F. (2016). Relationship between the  
723 patellar ridge and the femoral trochlea in the patellar tracking. *Orthopaedic Surgery*, 8, 468–  
724 474.

725 Warden, S.J., Fuchs, R.K., Castillo, A.B., Nelson, I.R. & Turner, C.H. (2007). Exercise when  
726 young provides lifelong benefits to bone structure and strength. *Journal of Bone and Mineral*  
727 *Research*, 22, 251–259.

728 Wilson, D.E. & Mittermeier, R.A. (2011) Handbook of the Mammals of the World. Vol. 2.  
729 Lynx Edicions, Barcelona.

730 Wolff, J. (1986) Concept of the law of bone remodelling, in: The Law of Bone Remodelling.  
731 Springer, pp. 1–1.

732 Yang, H., Embry, R.E. & Main, R.P. (2017) Effects of loading duration and short rest  
733 insertion on cancellous and cortical bone adaptation in the mouse tibia. *PloS one*, 12.

734 Zioupos, P., Currey, J.D., Casinos, A. & Buffrénil, V.de (1997) Mechanical properties of the  
735 rostrum of the whale *Mesoplodon densirostris*, a remarkably dense bony tissue. *Journal of*  
736 *Zoology*, 241, 725–737. <https://doi.org/10.1111/j.1469-7998.1997.tb05744.x>

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740 **Tables**

741

742 Table 1. List of the material analyzed in this study. Mass from Bongiani (1988), Dinerstein  
 743 (2011), Medici (2011), Rubenstein (2011), Wilson and Mittermeier (2011), and Yilmaz et al.  
 744 (2012). Institutional abbreviations: NMB, Naturhistorisches Museum Basel, Switzerland;  
 745 MNHN, Museum National d’Histoire Naturelle, Paris, France; NHMUK, Natural History  
 746 Museum, London, UK. So: sample origin; C: captive; W: wild. S: sex; F: female; M: male. A:  
 747 age; Ad; adult; SA: subadult. ?: unknown. Resol: resolution. ML: microtomography location;  
 748 L: London, M: Montpellier, P: Paris.

749

Family	Taxon	Mass (kg)	Collection number	So	S	A	Resol (µm)	ML
Rhinocerotidae	<i>Ceratotherium simum</i>	2300	MNHN-ZM-MO-2005-297	C	M	Ad	61.8	M
			NHMUK ZD 2018.143	?	?	Ad	64.4	L
	<i>Diceros bicornis</i>	800-1300	MNHN-ZM-AC-1944-278	C	M	Ad	64.2	M
			MNHN-ZM-AC-1936-644	?	F	SA	56.6	M
			NHM 1876.9.26.6	?	?	?	74.8	L
	<i>Dicerorhinus sumatrensis</i>	600-950	NHMUK ZD 1894.9.24.1	W	?	Ad	57.5	L
			NHMUK ZE 1948.12.20.1	?	?	Ad	56.8	L
	<i>Rhinoceros sondaicus</i>	1200-1500	MNHN-ZM-AC-A7970	?	?	Ad	66.8	M
			NHMUK ZD 1871.12.29.7	W	M	Ad	64.4	M
			NHMUK ZD 1921.5.15.1	W	F	SA	64.4	M
			NHMUK ZD 1861.3.11.1	W	?	SA	51.6	M
	<i>Rhinoceros unicornis</i>	2000	MNHN ZM-AC-1967-101	C	F	Ad	68.8	M
			NHMUK ZE 1961.5.10.1	W	M	Ad	82.2	L
			NHMUK ZD 1972.822	?	?	Ad	74.8	L
		NHMUK ZD 1884.1.22.1.2	W	F	Ad	74.8	L	
Tapiri dae	<i>Tapirus indicus</i>	280-400	MNHN-ZM-AC-1931-528	C	M	Ad	44.9	M
			NMB 8125	C	F	Ad	52.2	P

			MNHN ZM-AC-1935-460	C	M	Ad	52.2	P
	<i>Tapirus pinchaque</i>	150-200	MNHN-ZM-AC-1982-34	C	M	Ad	44.9	M
			MNHN indet.	?	?	?	52.2	P
	<i>Tapirus terrestris</i>	180-300	MNHN-ZM-MO-1990-20	C	M	Ad	44.3	M
			MNHN-ZM-AC-1937-1	C	?	Ad	52.2	P
Equidae	<i>Equus caballus</i>	380-600	MNHN-ZM-AC-1891-107	C	M	Ad	65.7	M
			MNHN-ZM-AC-A541	C	F	?	55.2	M
	<i>Equus caballus przewalskii</i>	200-300	MNHN-ZM-AC-1975-124	C	F	?	56.5	M
	<i>Equus asinus</i>	90-460	MNHN-ZM-2005-717	?	?	Ad	65.8	M
			MNHN-ZM-AC-1893-634	C	M	?	50.9	M
	<i>Equus hemionus</i>	200	MNHN-ZM-AC-1880-1103	C	M	Ad	44.3	M
	<i>Equus zebra</i>	240-380	MNHN-ZM-AC-1948-54	C	F	Ad	56.9	M

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751

752 Table 2. Values obtained for the tests of a phylogenetic signal (data averaged by species) and  
753 allometric effect (all specimens separately) on the parameters analyzed. In bold when  
754 significant at 5%.

755

	C	%Trab	TC	RMaxT	RMeanT	WBV	PC1	PC2
Phyl	K=0.59 p=0.36	<b>K=1.54</b> <b>p&lt;0.01</b>	K=0.60 p=0.28	<b>K=1.05</b> <b>p=0.01</b>	<b>K=1.00</b> <b>p=0.02</b>	<b>K=0.84</b> <b>p=0.04</b>	-	-
Size	r=0.31 p=0.11	r=0.16 p=0.42	r=0.36 p=0.06	r=0.04 p=0.85	r=0.11 p=0.59	-	r=0.18 p=0.38	r=0.34 p=0.08

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762 **Figure legends**

763

764 Figure 1. Phylogenetic tree including the taxa sampled; from Steiner and Ryder (2011).

765

766 Figure 2. Patellae of *Ceratotherium simum* NHMUK ZD 2018.143 (A,B), *Equus caballus*  
767 MNHN-ZM-AC-A541 (C,D) and *Tapirus indicus* MNHN-ZM-AC-1931-528 (E,F)  
768 illustrating the planes of the virtual sections.

769

770 Figure 3. Virtual coronal sections (CS) of the patellae of A, *Ceratotherium simum* MNHN-  
771 ZM-MO-2005-297; B, *Diceros bicornis* MNHN-ZM-AC-1944-278; C, *Dicerorhinus*  
772 *sumatrensis* NHMUK ZD 1894.9.24.1; D, *Rhinoceros sondaicus* NHMUK ZD 1871.12.29.7;  
773 E, *Rhinoceros unicornis* NHMUK ZD 1972.822; F, *Tapirus indicus* MNHN ZM-AC-1935-  
774 460; G, *Tapirus pinchaque* MNHN indet.; H, *Tapirus terrestris* MNHN-ZM-AC-1937-1; I,  
775 *Equus caballus przewalskii* MNHN-ZM-AC-1975-124; J, *Equus asinus* MNHN-ZM-2005-  
776 717; K, *Equus hemionus* MNHN-ZM-AC-1880-1103; L, *Equus zebra* MNHN-ZM-AC-1948-  
777 54. Scale bars equal 1 cm. Arrows point to large vascular canals.

778

779 Figure 4. Virtual sagittal sections (SS1) of A, *Ceratotherium simum* MNHN-ZM-MO-2005-  
780 297; B, *Dicerorhinus sumatrensis* NHMUK ZD 1894.9.24.1; C, *Tapirus indicus* NMB 8125;  
781 D, *Tapirus terrestris* MNHN-ZM-MO-1990-20; E, *Equus caballus* MNHN-ZM-AC-1891-  
782 107; F, *Equus hemionus* MNHN-ZM-AC-1880-1103. Scale bars equal 1 cm.

783

784 Figure 5. Virtual sagittal sections (SS2) of A, *Ceratotherium simum* NHMUK ZD 2018.143;  
785 B, *Rhinoceros unicornis* NHMUK ZD 1972.822; C, *Dicerorhinus sumatrensis* NHMUK ZD  
786 1894.9.24.1; D, *Tapirus indicus* MNHN ZM-AC-1935-460; E, *Tapirus pinchaque* MNHN  
787 indet.; F, *Equus caballus* MNHN-ZM-AC-A541. Scale bars equal 1 cm.

788

789 Figure 6. Cartographies illustrating the relative differences in thicknesses among each bone  
790 individually (the maximal value differs between bones). Color varies from 0 (blue) to  
791 specimen's maximal thickness (red) (see Table S1). A-B, *Dicerorhinus sumatrensis* NHMUK  
792 ZE 1948.12.20.1; C-D, *Rhinoceros sondaicus* NHMUK ZD 1921.5.15.1; E, *Rhinoceros*  
793 *unicornis* NHMUK ZE 1961.5.10.1; F, *Rhinoceros unicornis* NHMUK ZD 1972.822; G-H,

794 *Tapirus indicus* MNHN-ZM-AC-1931-528 ; I, *Tapirus pinchaque* MNHN-ZM-AC-1982-34;  
795 J, *Tapirus terrestris* MNHN-ZM-MO-1990-20 ; K-L, *Equus hemionus* MNHN-ZM-AC-1880-  
796 1103; M-N, *Equus zebra* MNHN-ZM-AC-1948-54; O-P, *Equus caballus przewalskii* MNHN-  
797 ZM-AC-1975-124; in posterior (A, C, E, G, J, K, M, O) and anterior (B, D, F, H, I, L, N, P)  
798 views. Scale bars equal 1 cm.

799

800 Figure 7. Violin plots illustrating the variation of the various parameters among and between  
801 the three families. R: Rhinocerotidae; T: Tapiridae; E: Equidae. C: bone compactness; %Trab:  
802 relative fraction of trabecular bone tissue; TC: trabecular compactness; RMaxT: relative  
803 maximal cortical thickness; RMeanT: relative mean cortical thickness; WBV: whole bone  
804 volume.

805

806 Figure 8. Distribution of the specimens in the morphospace along the two first axes of the  
807 PCA with, on the top right, the contribution of the different parameters according to the  
808 principal component (PC) axes PC1 and PC2. Color code for the species and complete names  
809 of the parameters, as in Fig. 7.

810

811 Figure.9. Drawings illustrating the knee-joint of a horse in anterior view, notably the patellar  
812 ligament insertions (A; modified from Barone, 2000), and muscle insertions on a horse patella  
813 (B; modified from Barone, 2010). QF: *Quadriceps femoris* insertions.