

How does bone microanatomy and musculature covary? An investigation in the forelimb of two species of martens (Martes foina , Martes martes)

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40 Abstract

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The long bones and associated musculature play a prominent role in the support and 42 43 movement of the body and are expected to reflect the associated mechanical demands. But in addition to the functional response to adaptive changes, the 44 45 conjoined effects of phylogenetic, structural and developmental constraints also shape the animal's body. In order to minimize the effect of the aforementioned 46 47 constraints and to reveal the biomechanical adaptations in the musculoskeletal 48 system to locomotor mode, we here study the forelimb of two closely-related 49 martens: the arboreal pine marten (Martes martes) and the more terrestrial stone 50 marten (Martes foina), focusing on their forelimb muscle anatomy and long bone 51 microanatomy; and, especially, on their covariation. To do so, we quantified muscle 52 data and bone microanatomical parameters and created 3D and 2D-maps of the 53 cortical thickness distribution for the three long bones of the forelimb. We then analysed the covariation of muscle and bone data, both qualitatively and 54 55 quantitatively. Our results reveal that species-specific muscular adaptations are not clearly reflected in the microanatomy of the bones. Yet, we observe a global 56 57 thickening of the bone cortex in the radius and ulna of the more arboreal pine marten, 58 as well a stronger flexor muscle inserting on its elbow. We attribute these differences 59 to variation in their locomotor modes.

60 Analyses of our 2D maps revealed a shift of cortical thickness distribution pattern linked to ontogeny, rather than species-specific patterns. We found that although 61 intraspecific variation is not negligible, species distinction was possible when taking 62 muscular and bone microanatomical data into consideration. Results of our 63 64 covariation analyses suggest that the muscle-bone correlation is linked to ontogeny rather than to muscular strength at zones of insertion. Indeed, if we find a correlation 65 66 between cortical thickness distribution and the strength of some muscles in the 67 humerus, that is not the case for the others and in the radius and ulna. Cortical 68 thickness distribution appears rather linked to bone contact zones and ligament insertions in the radius and ulna, and to some extent in the humerus. We conclude 69 70 that inference on muscle from bone microanatomy is possible only for certain 71 muscles in the humerus.

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73 Key-words: Martens; Bone Microanatomy; Muscles; 3D Geometric Morphometrics;

74 Functional Morphology

75 INTRODUCTION

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The vertebrate skeleton ensures various functions, among which movement, by 77 78 being the passive structure on which the force-producing muscles are attached. Like all biological structures, limb anatomy results from the conjoined effects of 79 80 phylogenetic, structural and functional constraints (e.g., Gould, 2002; Cubo, 2004). 81 Since long bones play a prominent role in the support and movement of the body, 82 their external morphology is expected to reflect the biomechanical demands they face (Iwaniuk et al., 1999, 2000; Schmidt and Fischer, 2009; Fabre et al., 2013a, 2015; 83 Janis and Figueirido, 2014). But their inner structure (bone microanatomy) also bears 84 a strong functional signal (Ruff & Hayes, 1983; Turner, 1998; Ruimerman et al., 85 86 2005; Habib & Ruff, 2008; Nikander et al., 2010; Houssaye et al., 2018), and can 87 thus reflect habitat (Laurin et al., 2011; Quemeneur et al., 2013; Nakajima et al., 88 2014), locomotor mode (Ryan & Ketcham, 2002, 2005; Carlson, 2005; Marchi, 2005; 89 Carlson et al., 2006, 2008; Shaw and Stock, 2009, 2010; Bishop et al, 2018), body-90 weight support requirements (Davies and Stock, 2014; Houssaye et al., 2016), and 91 behavior (Warden et al., 2007; Wilks et al., 2009). While bone microanatomical features are inherited from evolution, bone microanatomy also adapts to functional 92 93 constraints during the lifetime of organisms (Reina et al., 2017; Sievänen, 2010; 94 Warden et al., 2007). Wolff's law (1986) states that bones adapt, if they have time, to 95 mechanical stresses and gravity (Ruff et al., 2006). Bones thus tend to be stiffer and 96 stronger when subjected to high stresses, with an increase in cortical thickness and trabeculae orientated in the direction of the maximal strain (Wolff, 1986; Ruimerman 97 98 et al., 2004; Volpato et al., 2008; Barak et al., 2013).

99 During an individual's lifetime, cortical bone can thicken in response to mechanical 100 forces applied either through the direct insertion of the solicited muscles on the bone, 101 or through charge-transfer by the trabeculae from the articulating surfaces (Hoyte & 102 Enlow, 1966; Henrikson *et al.*, 1974). In the case of muscle insertion, apophyses (i.e., 103 bony tuberosities) may or may not form on the contact areas between the bone and 104 muscle (Niinimäki *et al*, 2013; Sanchez *et al.*, 2013).

105 The close association between muscles and bones is broadly intuitive because 106 muscle forces and skeletal (cranial and post-cranial) structure are linked through the 107 process of bone modelling (i.e., bone formation during growth) and remodelling (i.e., 108 bone resorption and deposition; Frost, 2001; Tatara et al., 2014). Thus, we could 109 expect muscular strain to be reflected in the bone inner anatomy. However, the 110 covariation between muscle and bone microanatomy has been scarcely studied. 111 although it would enable to better understand bone functional adaptation. Muscles and bones are often studied separately; and most of the studies that do combine 112 113 muscle and bone data have focused on the skull (e.g., Fabre et al., 2014; Fabre et 114 al., 2018; Toro-Ibacache et al., 2016; Sella-Tunis et al., 2018, Brassard et al., 2020). 115 Only a few studies have investigated the relationship between postcranial bones and 116 muscles, and even fewer have done so quantitatively. Moreover, those few studies 117 have focused exclusively on the external anatomy of the bones: Warburton et al. (2013) and Böhmer et al. (2018) both investigated the bone/muscle relationship in 118 119 the forelimb of terrestrial mammals, finding significant sexual dimorphism (in the form 120 of a positive allometry in the musculature of male kangaroos) and species-specific 121 differences (stronger muscles in *M. martes* than in *M. foina*), respectively. In both 122 cases, the differences were explored in muscular anatomy but not in muscle/bone 123 covariation. Martin et al. (2019) found a significant correlation between muscle 124 strength (approximated by muscle mass and cross-sectional area) and bone external 125 shape in digging marsupials.

126 Many bone microanatomical studies rely on two-dimensional transverse slices of the 127 diaphysis. However, cortical thickness and trabecular distribution are not uniform 128 along the shaft, so that a single section provides limited information and thus can 129 hardly be informative regarding muscular attachments. More recent studies use 130 three-dimensional (3D) visualisations of the whole bone instead, which allow investigating the relationship between muscle insertion and cortical bone: e.g., 131 132 Harbers et al. (2020) found that captivity induced an increase in cortical bone volume 133 and muscle force in the humerus of suids. We here analyse the covariation of muscle and bone microanatomy in two phylogenetically closely-related mustelids: the 134 135 arboreal pine marten (Martes martes), and the more terrestrial stone marten (Martes 136 foina). Although mustelids are an extremely diverse family, varying in size, 137 geographic range, and predation behaviour, the two studied species occur 138 sympatrically and are very similar in overall appearance. They distinctly differ in habitat preference (forest versus urban environments) and locomotor mode (arboreal
versus more terrestrial; Overskaug *et al.*, 1994; Goszczynski *et al.*, 2007;
Wereszczuk & Zalewski, 2015). Differences in their shoulder and forelimb muscles
have been highlighted and interpreted to reflect the greater climbing ability of the pine
marten (Böhmer *et al.*, 2018). This raises the question whether this difference can be
observed in the shape of the bone, as well as at the bone microanatomical level.

145 The aim of this study is to investigate whether cortical thickness covaries with the strength of the muscles at zones of insertion by local thickening. In order to test this 146 147 hypothesis, we first investigate how the differences in the locomotor mode of the two 148 sympatric species are reflected 1) in the muscles attaching on the bone diaphysis 149 and epiphyses and 2) in the microanatomy and, especially, cortical bone distribution; 150 then we analyse 3) if there is a covariation between the distribution of cortical bone 151 thickness and the insertion areas of the muscles in accordance with their relative 152 strength; and 4) the relative strength of this covariation at the intraspecific and 153 interspecific levels.

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155 MATERIAL AND METHODS

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157 Sample

159 The study focused on *M. foina* (Erxleben, 1777), a medium-sized terrestrial mustelid 160 occurring in rural areas of Europe and Asia, with comparative specimens of its sister-161 taxon *M. martes* (Linnaeus, 1758). Ten specimens of *M. foina* and three specimens 162 of *M. martes* were analysed (Table 1). Carcasses were received from the Faculty of 163 Veterinary Medicine of Ludwig-Maximilians-Universität in Munich (Germany), the 164 INRAP Centre de Recherches Archéologiques de l'Oise in Compiègne (France), and 165 the taxidermy laboratory of the Muséum National d'Histoire Naturelle in Paris 166 (France).

Species discrimination was based on external attributes and dental characteristics: the throat patch in *M. martes* is yellowish, whereas it is white in *M. foina*; the crown morphology of the third maxillary premolar in occlusal view is concave in *M. martes* and convex in *M. foina* (Libois, 1991; Llorente Rodríguez *et al.*, 2011). Specimens did not display any pathology. All three *M. martes* specimens and six *M. foina* specimens were adult animals. Four *M. foina* specimens were juveniles (see Table 1). Age status was determined by the degree of fusion of the epiphyses of the long bones
after dissection (completely fused in adults and unfused in juveniles; Nickel *et al.*,
2003).

The present analyses on the *M. foina* specimens enabled us to study the link between bone and muscles at the intraspecific level, with specimens of various ontogenetic stages and body sizes. The comparison with *M. martes* allowed us to estimate species-specific characteristics as well as the relationship between intraand interspecific variation.

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182 Muscle data

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In total, we studied the left and right forelimbs of 13 specimens. Nine specimens (three *M. martes* and six *M. foina*) were previously dissected and muscle data already reported in Böhmer *et al.* (2018). The remaining specimens (four juvenile *M. foina* specimens) were dissected for the present study. A number of 37 extrinsic and intrinsic muscles attach on at least one of the three long bones of the forelimb (humerus, radius, ulna; Table 2). Each muscle was identified and systematically dissected. The dissection protocol follows that described by Böhmer *et al.* (2018).

191 The following architectural features were quantified by a single examiner (C.Bö) in 192 order to avoid operator bias. First, the blotted dry muscles were weighed on a digital 193 precision balance (Mettler; ±0.1 mg) and the muscle mass data were collected. 194 Muscle belly length (the mean of the maximum and minimum lengths) was measured 195 directly on the muscle using a standard ruler. Next, the fibre length (the mean of 15 196 randomly selected fibres) of each excised muscle was recorded. To do so, muscle 197 fibres were separated by digesting the muscles in a 30% aqueous nitric acid solution 198 for about 24 hours, after which they were transferred to a 50% aqueous glycerin 199 solution (see Antón, 1999; Herrel et al., 2008). For each muscle, individual fibres 200 were teased apart and scaled digital photographs were taken. The length of the 15 201 randomly selected fibres was measured using the software ImageJ v.1.48 (Schneider 202 et al., 2012) and then mean fibre length was calculated. The documented parameters 203 (muscle mass and fibre length) allowed us to determine the following variables: a) 204 Muscle volume (V) was calculated by dividing muscle mass (m) by a standard density 205 (p) for mammalian muscles of 1.06 g/cm3 (Mendez & Keys, 1960): V [cm³] = m [g] / ρ 206 [g/cm³] (1); b) Anatomical cross-sectional area (ACSA), which is a function of muscle

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volume and fibre length (I_f) (Powell *et al.*, 1984; Sacks & Roy, 1982), was calculated using the following equation: ACSA [cm²] = (V [cm³] / (I_f [cm]) (2). Eventually, the ACSA of each muscle from the left and right forelimbs was used to calculate c) the mean ACSA of each muscle for each specimen.

211 Contrary to the physiological cross-sectional area (PSCA; e.g., Kupczik et al., 2015; 212 Rosin & Nyakatura, 2017; Böhmer et al., 2018), the ACSA does not take into account 213 the pennation angle of muscle fibres. In muscles with high pennation angles, ACSA 214 might be less accurate in predicting the force-producing capability per muscle volume 215 (Lieber & Friden, 2001). However, muscle output is related to the cosine of pennation 216 and, thus, neglecting small angles causes only a small percentage of error in force 217 estimates (Scott & Winter, 1991). Typically, the pennation angles appear to be rather 218 small in carnivoran forelimb muscles (Williams et al., 2008; Moore et al., 2013; 219 Böhmer et al., 2018). Furthermore, the two species of martens studied here (M. 220 martes and M. foina) share similar pennation angles for the same muscles (Böhmer 221 et al., 2018). Additionally, the surface pennation angle of a muscle may vary 222 significantly from its deep pennation angle (Sopher et al., 2017) and, consequently, 223 only micro-dissection or micro-computed tomography analyses may allow accurate 224 analysis of the pennation of all fascicles that make up the muscle (e.g., Kupczik et al, 225 2015; Rosin & Nyakatura, 2017).

Although muscles are versatile organs that contribute to more than one functional role, the consideration of each muscle's main function facilitates interpretation. We assigned muscles to functional groups based on their topology and on the manipulation of dissected specimens (Table 2). Anatomical terminology primarily follows Böhmer *et al.* (2020).

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233 Quantitative muscle analyses

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235 Quantitative analyses were performed on two data sets: (1) A first data set 236 comprising all *M. foina* specimens (intraspecific sample), and (2) a second data set 237 comprising all adult specimens (interspecific sample).

To facilitate later interpretation, we calculated the mean ACSA for each long bone of each specimen by summing up the ACSA of all muscles attaching on the respective bone's diaphysis (since the subsequent covariation analyses were performed on diaphyseal microanatomical parameters) and dividing the obtained value by the number of muscles, which allows the muscles to be grouped into two categories: 'strong' muscles are muscles with an ACSA higher than the mean ACSA; 'weak' muscles are muscles with an ACSA smaller than the mean ACSA. We hypothesize that strong muscles apply more stress on the bone and thus may potentially induce bone thickening on their attachment sites. These strength categories were used for qualitative analyses only; quantitative analyses were conducted on the ACSA values of each muscle inserted on the bones using MANOVAs.

For size-correction, the obtained ACSA data were logarithmically (log 10) transformed and then regressed against log 10-transformed total ACSA (i.e., the sum of the ACSA of all muscles) for each specimen (Supplementary Table 1). All subsequent statistical analyses were performed on 1) log-transformed ACSA values (not size-corrected) and 2) the resulting residuals of the regression (size corrected data), in order to differentiate between variations due to size and variations due to other factors.

256 Principal component analyses (PCA) were used to reduce the multidimensionality of 257 the collected data and to visualise the distribution of the specimens in the 258 morphospace delimited by the PCs. We established two data sets for each of the 259 three bones of interest: (1) the first data set includes all muscles that attach on the 260 bone under study; (2) the second data set includes only muscles that attach on the 261 bone's diaphysis, as geometric morphometric analyses (GMMs) were performed on 262 the diaphysis only (see below). Linear regressions of muscle ACSA values on muscle length were used to check for the presence of an allometric relationship in adult 263 264 specimens (evolutionary allometry).

PCAs were performed and visualised using the '*FactoMineR*' package in R (Lê *et al.*,
2008). Analyses of variance (MANOVA) were used to test for a difference of muscle
ACSA between the two species of martens. All analyses were performed in R (R
Core Team. 2020, version 4.0.2) using RStudio (RStudio Team. 2020, version
1.3.959-1).

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271 X-ray microtomography on bones

After dissection, the remaining skeleton was cleaned and the forelimb bones were collected from each specimen. The right humerus, radius and ulna of all *M. martes* and *M. foina* specimens were scanned using X-ray microtomography (Easy Tom 40276 150, RX Solutions) at the MRI-ISEM (Montpellier Ressources Imagerie – Institut des 277 Sciences de l'Evolution de Montpellier, UMR 5554, University of Montpellier), with 278 reconstructions performed using X-Act (RX Solutions). Voxel size varies between 279 specimens depending on their size, from 238 µm to 413 µm. Bone tissues were 280 segmented on the complete bones. The trabecular area, consisting of the medullary 281 cavity and the trabeculae, was then separated from compact cortical bone manually 282 following Houssaye et al. (2018) in order to calculate some microanatomical 283 parameters (see below) and to generate bone cartographies in order to visualise the 284 cortical thickness distribution. Image segmentation and visualisation were performed 285 from the reconstructed image data using Avizo 9.4 (VSG, Burlington, MA, USA).

286 Bones were aligned along their longitudinal axis following Ruff (2002). Bone maximal 287 length (MaxL) was obtained virtually by using the Landmark software (UC Davis, 288 USA). The distance between proximal and distal extremities for each bone was 289 measured in cranial view. In the humerus, this equates to the distance between the 290 most proximal extremity of the humeral head and the most distal part of the medial 291 epicondyle. In the radius it is the distance between the head of the radius and the 292 extremity of the styloid process; and in the ulna it is the distance between the most 293 proximal part of the olecranon process and the extremity of the styloid process. The 294 difference in adult bone length between the two species was tested by performing a 295 t-test.

296 Diaphyses needed to be isolated for subsequent quantitative analyses. Epiphyses 297 were removed by choosing a homologous landmark on each bone based on Botton-298 Divet (2017), which enabled us to define an orthogonal cutting plane on each 299 extremity of the bone (humerus: disto-caudal tip of the humeral head, most proximal 300 point of the caudal side of the supracondylar foramen; radius: most proximal point of 301 the ulnar notch, maximum curvature of the depression on the cranial side of the radial 302 tuberosity; ulna: proximal point of the m. *brachialis* insertion groove, most proximal 303 point of the distal ulno-radial articulation).

The conversion of the segmented scans into a binary image stack enabled measurements of microanatomical parameters (Table 3) using the BoneJ plugin (Double *et al.*, 2010) of ImageJ (Wayne Rasband National Institutes of Health, USA): 1) 3D compactness of the complete bone (*C*), i.e., the volume occupied by bone (cortex and spongious bone) divided by the whole volume; 2) Relative mean thickness of the cortical layer along the diaphysis (*RmeanT*), calculated as the 310 absolute value of mean cortical thickness (AmeanT) divided by the radius of the bone's diaphysis if assimilated as a tube (R); and 3) Relative maximum thickness of 311 312 the cortical layer along the diaphysis (*RmaxT*), calculated as the absolute value of 313 maximum cortical thickness (AmaxT) divided by R. Finally, we used the 314 'MaterialStatistics' module in Avizo to calculate the trabecular ratio of the complete bone (%Trab), i.e., the surface occupied by the trabecular bone over the total surface 315 316 of osseous tissue (in 3D). Since the ossification was incomplete in juvenile specimens, some parameters (*C*, %*Trab*) could not be calculated and as such were 317 318 excluded from analyses on the *M. foina* dataset.

319 All these parameters being ratios, no size-correction was required.

PCAs on the microanatomical parameters (performed using the '*FactoMineR*' package in R) were used to visualise the distribution of the specimens in the morphospace delimited by the PCs. We performed linear regressions on the first two PCs using bone *MaxL* as a size estimate in order to check for a size effect within the PCAs.

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326 Bone thickness mapping and geometric morphometrics

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328 1. 3D mapping

329 3D-mapping of the bone cortical thickness is an approach which provides both a 330 graphical output and a set of numerical parameters, allowing for a holistic functional 331 interpretation of the bone structure. Several methods have so far been used to 332 measure and create 3D maps (see below).

333 In order to obtain 3D maps of the bone cortical thickness, cortical and trabecular 334 bone need to be separated first. Some studies have focused on dissociating cortical 335 bone from trabecular bone by automatic segmentation and analysing them 336 separately (Lublinsky et al., 2007; Gross et al., 2014), using a thickness calculation 337 algorithm (Hildebrand & Rüegsegger, 1997) in order to obtain 3D maps of the cortical 338 thickness, while another (Tsegai et al., 2016) used an automatic threshold-based 339 segmentation of the bone using grey-scale variation of the slices to obtain an outer 340 surface and an inner surface, delimiting the cortex. These methods allow to map the entire bone and as such are also applicable to short and irregular bones. 341

Recently, the use of 2D maps of bone cortical thickness generated by unrolling 3D maps has allowed the quantitative comparison of cortical thickness maps using

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GMMs. These studies assimilated the diaphyses of long bones to cylinders (obtaining 345 3D cortical thickness maps of the diaphyses) before unrolling them to compare their 346 topographies (Bondioli *et al.*, 2010; Puymerail *et al.*, 2012), thus obtaining two-347 dimensional (2D) cortical thickness maps. However, this method is only applicable to 348 long bone diaphyses, since the complex shape of epiphyses does not allow 349 comparable unrolling. Similarly, it is not applicable to irregularly shaped bones.

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351 Here, we use a combination of these methods, obtaining both 3D maps of the entire 352 bones, allowing us to study cortical thickness variation in the whole bones including 353 the epiphyses, and 2D maps of the diaphyses, to be analysed quantitatively in order 354 to compare the cortical thickness distribution using GMMs. We first isolated an outer 355 surface (corresponding to the outer surface of the bone) and an inner surface 356 (corresponding to the inner limit of the compact cortex) for each bone. This required 357 to remove the cavities located in the compact cortex and the trabeculae in the 358 medullary space. The removal was performed on Avizo. The 'PointWrap' function 359 was added to manual segmentation in order to increase smoothness and avoid 360 artificial discrepancies between sections. We then generated 3D bone cartographies 361 using the 'SurfaceDistance' module in Avizo, i.e., calculating the thickness of cortical 362 bone by measuring the distance between the outer and the inner surfaces of the 363 cortex, and generating 3D cortical thickness maps of the entire bones using absolute 364 values. These cartographies enabled the visualisation of variations in cortical 365 thickness among each bone, to make comparisons (in absolute values) between the 366 bones, with the objective to relate these results with the zones of insertion of muscles 367 and ligaments on the bones.

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2. 2D mapping

We used the 'morphomap' package in R (Profico *et al.*, 2020) in order to quantitatively analyse bone cartographies by comparing their planar representations, using GMMs, once converted to 2D maps. 3D bone cartographies are required to create the 2D maps; they were obtained using the '*morphomap3Dmap*' and '*morphomapThickness*' functions. The rendering of the 3D maps made in Avizo (see above) enabled us a better visualisation of the cortical thickness distribution in the epiphyses, so that these 'morphomap' cartographies were only used for 2D mapping 377 (that cannot be obtained based on the Avizo cartographies). The conversion of 3D 378 cortical maps to 2D maps requires the 3D maps to be assimilated to tubes and thus 379 the removal of the epiphyses. The epiphyses were removed by specifying in the 380 *'morphomapCore'* function the percentage of the bone's length where the mapping 381 was to begin and to end. We chose to place this limit at 20% and 80% for the 382 humerus and radius (following Profico et al., 2020) because it was consistent with the 383 epiphyses' proportions, and 20% and 75% for the ulna, since the ulnar proximal 384 epiphysis is proportionally longer. In order to obtain a planar representation of the topographic thickness variation, the 3D cortical thickness maps were virtually 385 unzipped along a vertical line and unrolled into a plane using the 'morphomap2Dmap' 386 387 function (Fig. 1). During this operation, all maps were standardized to the same size, 388 and the thickness value associated with each pixel (visually represented by its colour) 389 was standardized between 0 and 1. All subsequent comparisons were thus made on relative (not absolute) values. Since the maps fully overlap and contain the same 390 391 number of pixels, it is possible to perform GMM analyses by considering each pixel 392 as a semi-landmark and exporting them in a dataframe using the 'morphomapDF' 393 function. GMM procedures were performed using the procSym function of the 'Morpho' package of R (Schlager, 2017), as well as the procD.Im and 394 395 morphol.disparity functions of the 'geomorph' package (Adams & Otarola-Castillo, 396 2013).

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398 Quantitative bone analyses

400 Analyses were performed on the two datasets described above, comprising all M. 401 foina specimens and all adult specimens, respectively. We performed Procrustes 402 ANOVAs on the 2D superimposed maps in order to check for differences in cortical 403 thickness distribution between the two species of martens, as well as between the 404 juvenile and adult *M. foina* specimens. Similarly, as for the quantitative muscle data, 405 a PCA was performed to visualise the distribution of the specimens in the 406 morphospace delimited by the PCs. Since all maps were standardized, this PCA was 407 performed on the entirety of the sample, thus on adult and juvenile specimens and 408 from the two species. We tested the effect of size within the PCAs using linear 409 regression on the first two PCs with *MaxL* as a size estimate.

We used t-tests to test for difference in mean thickness (*AmeanT*, *RmeanT*) and maximal thickness (*AmaxT*, *RmaxT*) in the *M. foina* and the adult datasets and for difference in compactness (C), and trabecular ratio (%*Trab*) in the adult dataset.

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414 Covariation between muscle strength and inner bone anatomy

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We investigated the correspondence between areas of high cortical thickness and the muscles' insertion areas, i.e., the places where the mechanical forces of the muscles apply on the bone.

We first visually compared, on the entire bones, the areas of high cortical thickness (the highest values indicated in red on the 3D cortical bone maps) to the muscles' origin and insertion areas.

Then, using the 2D unrolled cortical maps, we performed two-block partial least squares (2-block PLS) analyses using the '*two.b.pls*' function in the R package 'geomorph', to quantify the degree of covariation between muscle ACSA and superimposed bone thickness maps (following Harbers *et al.*, 2020). PLS were performed on all muscles attached to the diaphysis regardless of their strength category or their functional group, as well as on the different functional groups of muscles (Table 2, Supplementary Table 1).

429 A 2-block PLS was also performed to quantify the degree of covariation between430 muscle ACSA and the microanatomical parameters for each bone.

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432 RESULTS

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- 434 *Muscle analyses*
- 435

436 Principal Components Analyses

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The following results are for the size-corrected muscle data; analyses on uncorrected
muscle ACSA values yielded similar results (see Table 3 for details).

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Results of the PCA on all muscles attaching to the humerus show that the two first PCs, which represent 37.3% and 23.1% of the variance respectively, enable the differentiation between adult specimens of the two taxa (Fig. 2A); the overlap on the first axis is due to a single specimen, Mf7. All muscles contribute to the separation (along PC1) similarly, though with differing intensities. The muscle that contributes the most to this separation is the *M. flexor digitorum profundus* (FDP), which is a major flexor of the wrist. There is no effect of size on the first two axes of the PCA (PC1: p=0.42; PC2: p=0.95). When taking only muscles inserting on the diaphysis into account, there is an almost complete overlap of the two taxa along the first axis (Fig. 2B).

PCAs on the ACSA of the muscles inserting on the radius and ulna show that the two
taxa broadly overlap, whether we consider all muscles or only those that attach on
the diaphysis (Supplementary data Fig. 1, 2)

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Results of the PCAs on the *M. foina* dataset show that when all muscles attaching to the humerus are taken into account, adult and juvenile specimens are slightly differentiated along the first two axes (PC1=35.1%; PC2=18.5%), mainly under the influence of the FDP muscle. When taking only muscles inserting on the diaphysis into account, there is an almost complete overlap of adult and juvenile specimens.

Similarly as in the adults only dataset, we observe an almost complete overlap of adult and juvenile specimens of *M. foina* in the results of the PCAs on muscles inserted on the radius and ulna, whether we considered all muscles or only those that attach on the diaphysis (Supplementary data Fig. 3).

464

465 <u>MANOVAs</u>

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467 The linear regressions (ACSA~muscle length) detected no significant allometry within 468 the entire muscle dataset (adult and juveniles) (p=0.57, $r^2=0.54$).

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470 MANOVAs on the adult only dataset indicated that when all muscles (n=26) attaching 471 to the humerus are taken into account, there is a significant difference between the 472 two species (p=0.04, r²=0.25). No discrimination between the two species is 473 observed when focusing only on muscles that attach on the humeral diaphysis (n=15; 474 MANOVA: p=0.91, r²=0.05). In order to investigate if that difference was also found 475 when considering juvenile specimens, we used MANOVAs on the entire sample 476 (adult and juveniles); we found no significant difference between the two species, 477 whether considering all muscles attaching on the humerus (p=0.27, $r^2=0.1$) or on the 478 humeral diaphysis only (p=0.96, $r^2=0.03$) (Supplementary data Fig. 4).

479 MANOVAs on ACSA of all muscles show no significant difference between the two

480 species neither for the radius (p=0.58, r^2 =0.10) nor for the ulna (p=0.45, r^2 =0.10);

481 MANOVAs on ACSA of muscles inserting on the diaphysis yielded similar results

482 (radius: p=0.42, r²=0.12; ulna: p=0.47, r²=0.10).

483 MANOVAs on uncorrected muscle data yielded similar results for all cases listed 484 above (see Table 3).

485

486 MANOVA on the *M. foina* sample detected significant differences in muscle ACSA 487 between adult and juvenile *M. foina* specimens when using uncorrected muscle data, 488 in all three bones. These differences were not found when using size-corrected 489 muscle ACSA. Results were similar whether we considered all muscles or only 490 muscles attached to the diaphyses (see Table 3).

491

492 Microanatomical analyses

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494 1. Microanatomical parameters

PCAs on the microanatomical parameters show a distinction between adult specimens of the two taxa along the first axis for the three bones (Fig. 3, Supplementary Fig. 5). PC1 represents over 65% of the variance in each case, and the four variables have a similar contribution along that axis. Although there is always a small overlap, the two species tend to be discriminated along the first axis, with *RmeanT* and *RmaxT* having a predominant contribution.

501 In *M. foina* specimens, juvenile had a significantly lower absolute mean cortical 502 thickness between than the adults, in all three bones (*AmeanT*: humerus: p=0.016; 503 radius: p=0.016; ulna: p=0.03). This difference was not detected when using relative 504 values (*RmeanT*: humerus: p=0.94; radius: p=0.48; ulna: p=0.45).

There was no significant difference in compactness nor in trabecular ratio between adults of the two species of martens (Table 3). Although the two taxa did not differ in mean and maximum cortical thickness for the humerus, difference was significant for the radius (*RmeanT*: p=0.004; *RmaxT*: p=0.008) and ulna (*RmeanT*: p=0.003; *RmaxT*: p=0.007), specimens of *M. foina* having a smaller *RmeanT* and *RmaxT* than

- 510 those of *M. martes.*
- 511
- 512
- 513 2. 2D maps

514 PCAs on the 2D cortical thickness maps show a difference in distribution between the 515 two taxa along the first axis for each bone, PC1 representing more than 80% of the 516 variance for the humerus and radius (Fig. 4, Supplementary Fig. 6). In each PCA, the 517 specimens are distributed in the same order with a small overlap along the first axis: 518 first the *M. martes* specimens, followed by the adult then juvenile *M. foina* specimens, 519 with the exception of the Mm10 specimen in the PCA on ulna maps. Linear 520 regression detected no effect of size in the first PC of the humerus PCA (p=0.61, 521 $r^{2}=0.03$), but a significant effect of size in its second PC (p=0.02, $r^{2}=0.35$), this axis representing 3.33% of the variation. There was no effect of size along the first axes of 522 523 the PCAs on the radius and ulna (radius: PC1: p=0.09, PC2: p=0.59; ulna: PC1: 524 p=0.09, PC2: p=0.80).

525 Procrustes ANOVAs on the 2D maps detected no significant difference between the 526 two taxa, but there was a significant difference between adult and juvenile *M. foina* 527 specimens in the humerus (p=0.002, r^2 =0.61) and the radius (p=0.02, r^2 =0.44) but not 528 the ulna (p=0.014, r^2 =0.64).

529

530 Covariation between muscle anatomy and inner bone structure

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- 532 1. Qualitative comparisons based on 3D maps

533 Humerus

Among the muscles that attach on the humeral diaphysis (n=15), seven muscles are generally considered 'strong' (i.e., ACSA larger than the mean ACSA of all humeral muscles). This includes all four pectoral muscles (PAB+PMJ and PMN+XH) (except PMN+XH for Mf7) and two triceps muscles (TBM and TBLA). In some specimens (Mm2, Mf3, Mf5, Mf6, Mf7, Mf8 and Mf10), one of the two deltoid muscles (DA) is considered 'strong' as well.

540 Some of the strong muscles are attached to areas of high cortical thickness: the zone 541 of insertion of the TBM muscle (Fig. 5) always appears thicker than the rest of the 542 bone, either in its distal part only (Mf1, Mf2, Mf3, Mf7, Mf9 and Mm2) or in its entirety 543 (Mf5, Mm1 and Mm3). The insertions of the superficial pectoral muscles (PAB+PMJ) 544 are areas of high cortical thickness in most specimens (Mf1, Mf2, Mf3, Mf7, Mm2 and 545 Mm3), but not all (Mf5, Mf9, Mm1). Similarly, the TBLA muscle is attached to areas of 546 high cortical thickness in some specimens (Mf2, Mf3 and Mf5) but not in others. One 547 of the strong muscles (PMN) is attached to areas of low cortical thickness. The DA is 548 a particular case: it is strong in some specimens (Mf2, Mf9 and Mm3), in which the 549 crest on which it inserts is thicker than the rest of the bone, and weak in others (Mf1, 550 Mf3, Mf5, Mf7, Mm1, and Mm2), where it almost always inserts on thin cortical bone 551 (except for Mf5).

552 Some areas of high cortical thickness are insertion areas of weak muscles: The BCH 553 muscle inserts on almost half of the diaphysis (Fig. 5A, B, C). In all specimens, at 554 least part of its insertion appears thick, whether it be more proximal (Mf5, Mm2) or 555 distal (Mf2, Mf3, Mf7, Mm1, Mm3). The ANC muscle is inserted on the caudal side of 556 the lateral crest, which is always very thick as compared to the rest of the bone (Fig. 557 5C, G). The CB muscle is also sometimes inserted on thick cortical bone since it is 558 attached on the medial side of the cranial crest (Fig. 5A), which is sometimes thicker 559 than the rest of the bone (Mf1, Mf3 and Mf7). Additionally, there are two zones that 560 are almost always thick but on which no muscle is inserted: the first one (except for 561 Mf5) is the distal part of the medial side, which is the junction between the diaphysis 562 and the medial epicondyle. This area bears several ligament insertions that 563 contribute to the stability of the elbow joint capsule. The second one (in all 564 specimens, although it is slightly less visible on Mf5) is the cranial extension of the 565 TBM insertion, visible on the medial side.

There is almost no variation of the cortical thickness distribution in the proximal 566 567 epiphysis: all adult specimens display the same pattern of thickening on both tuberosities (lesser and greater); although the proximal epiphysis bears several 568 569 muscle attachments, these cortical thickenings do not correspond to any of those 570 areas of attachment in particular. The proximal epiphysis is missing in two of the 571 juvenile specimens (Mf8 and Mf10), but the pattern is similar in the other two. The 572 cortex of the lesser tuberosity of the Mf4 specimen appears thicker than in the 573 greater tuberosity.

There is more variation in the cortical thickness distribution in the distal epiphysis: all specimens display a very thick medial supracondylar ridge, on which both the PT and TBA muscles originate (Fig. 5C, D), and a caudal part of the olecranon thicker than its cranial part. Both epicondyles are generally thicker than the rest of the epiphysis 578 but there is no clear relationship with muscle insertions apart from that of the FCUH 579 muscle on the medial side and sometimes EDL (Mf2, Mf10) and/or ECU (Mf1, Mf2, 580 Mf10) on the lateral side. The most distal part of the epiphysis appears sometimes 581 thicker than the rest, and corresponds to the PL+FDP origin (Mf3, Mf4, Mf7, Mf9, 582 Mf10, Mm3).

583

584 Despite their absolute cortical thickness being distinctly thinner than in adults, 585 juvenile specimens show the same pattern of cortical thickness distribution. The 586 specimen with the thinnest cortex (Mf4) does not yet have sufficient variation in 587 cortical thickness distribution along the shaft to clearly determine if there are cortical 588 thickenings corresponding to muscle insertions: the lateral crest and the diaphysis 589 are thicker than the epiphyses, but the posterior crest is still very thin. The other three 590 juveniles show the same global pattern of cortical thickness distribution as adults, 591 only thinner and less defined. The Mf10 specimen exhibits a thickening of the cortex 592 in the very proximal part of the diaphysis, which corresponds to the TBM insertion.

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594 We found no obvious differences in cortical thickness distribution between *M. martes* 595 and *M. foina*.

596 This comparative analysis does not show a true correlation between muscle strength 597 and cortical thickness distribution in the humerus.

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599 <u>Radius</u>

600 Among the muscles that attach on the radial diaphysis (n= 6), only two are 601 considered 'strong': the pronator teres muscle (PT) (except for Mf1, Mf2, Mf4 and 602 Mf10) and the FDP muscle. Other muscles are 'strong' in two or three specimens 603 only (the EP muscle in Mf3, Mf5, Mf7; the FCR muscle in Mm3 and Mf5; and the BCR 604 muscle in Mf1, Mf2 and Mf3), and as such are considered weak. The PT muscle 605 inserts onto the proximo-medial part of the radius diaphysis, which is an area of 606 increased cortical thickness in all adult specimens (Fig. 6A, D), although it seems to 607 be slightly thinner in *M. martes* than in *M. foina*. The FDP muscle inserts into the 608 lateral part of the diaphysis and partly on the proximal part of the distal crest (Fig. 609 6D). Most of the time it is also attached to areas of increased cortical thickness, 610 which corresponds to the zone of contact with the ulna and the interosseous 611 membrane connecting the radius to the ulna. The four remaining muscles (EP, FCR,

612 PQ and SUP), as well as the BCR muscle that inserts into the styloid process of the radius, are considered 'weak'. The EP, PQ and BCR muscles insert into areas of 613 614 'standard' cortical thickness, while the FCR muscle is often attached to areas of increased cortical thickness. However, it is attached between the PT and FDP 615 616 muscles (Fig. 6D) so it is impossible to ascertain to which muscle insertion the 617 cortical thickening is linked. Apart from the "cranial crest" leading to the radial styloid 618 process that has a very thick cortex in all specimens (Fig. 6F, G), there is no particular area of high cortical thickness that does not correspond to any muscle (with 619 620 the exception of the proximal epiphysis of Mf10).

There are no muscle attachments on the proximal epiphysis of the radius, and one
attachment (BCR muscle) on the distal one, on the styloid process of the radius (Fig.
623 6A, D).

624 There is little variation in the proximal epiphysis. Almost all specimens display a slight 625 thickening of the cortex along the articular circumference (Mf1, Mf2, Mf3, Mf6, Mf7, 626 Mf8, Mm1, Mm2, Mm3) and/or the articular fovea, which is in contact with the medial epicondyle of the humerus (Mf1, Mf3, Mf5, Mf7, Mf8, Mm3), and around the radial 627 628 tuberosity (except for Mf10), which bears a part of the PT muscle's insertion. Two 629 specimens display a homogenous distribution (very thin) of the cortical thickness in 630 the radial head: a juvenile (Mf4) and an adult (Mf9). Two of the juveniles (Mf6 and Mf8) display the same pattern as that of the adult, while Mf4 is too thin to see any 631 632 cortical thickness variation. The last juvenile specimen, Mf10, is the only one to 633 deviate from the general pattern, with an extremely thick lateral articular fovea and 634 circumference.

The cortical distribution in the distal epiphysis of the radius is similar in all *M. foina* specimens, with a slightly thicker cortex in the carpal articular surface (CAS) and the styloid process. The three specimens of *M. martes* have a thinner cortex in the CAS than in the rest of the bone, and a thicker cortex in their styloid processes.

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Juveniles exhibit a similar distribution pattern as adults. Mf4 is too thin to clearly distinguish cortical thickness variation but the cortical bone is thicker in the diaphysis than in the epiphyses, and the zone of contact with the ulna is very thin, surrounded by a thicker cortex, as found in adults. The distal epiphyses are missing in both Mf8 and Mf10. The lateral crest is not fully ossified in Mf6, and Mf10 displays a very thick cortex on the lateral part of the proximal epiphysis.

647 We found no obvious differences in cortical thickness distribution between *M. martes*648 and *M. foina*.

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650 <u>Ulna</u>

Among the muscles that attach on the ulnar diaphysis (n=7), two are strong muscles: the FDP muscle and the *M. biceps brachii* (BB except in Mf4 and Mf9). Another muscle (FCUU, the *M. flexor carpi ulnaris*) is strong in some specimens (Mm1, Mm2, Mf1, Mf2, Mf3). The four remaining muscles (EI, EP, BCH and PQ) are considered 'weak'.

656 Areas of high cortical thickness correspond to the contact zones between the radius 657 and the ulna, the interosseous membrane connecting them on the cranial side (Fig. 658 7E), and to the lateral crest on the caudal side on which the FDP, FCUU and EP 659 muscles are inserted (Fig. 7G). This pattern is similar in all adult specimens 660 regardless of the FCUU muscle's status. All the strong muscles are thus inserted on 661 areas of high cortical thickness. Similarly, juveniles (with the exception of Mf4 whose 662 cortex is too thin to discern patterns) exhibit the same pattern, though they are 663 naturally overall thinner. The distal crest corresponds to an area of increased cortical 664 thickness in seven individuals (Mf1, Mf2, Mf3, Mf5, Mf9, Mm2, Mm3).

There are numerous muscle insertions on the ulnar head, and none on its distal epiphysis. The same pattern is found in almost all specimens: the cortex of the proximal epiphysis is thicker on the caudo-medial side of the olecranon, which corresponds to the TBLO muscle insertion (Fig. 7C, G), as well as on the trochlear notch and the medial coronoid process (Fig. 7H).

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There are two exceptions, both juveniles: Mf4, which shows a very thin cortex and a thickening of the TBLO insertion zone but not of the trochlear notch and coronoid process, and Mf10, which displays a completely different distribution of the cortical thickness. The olecranon of Mf10 is thicker on the cranio-medial side, which corresponds to the TBLA insertion zone, and the area around the TBLO insertion zone is thicker than the insertion zone itself. The medial coronoid process is extremely thick.

The cortical thickness distribution of the distal epiphysis is similar in almost all specimens: both medial and lateral sides are very thin, with a thicker area above the styloid process. There are three exceptions (Mf4, Mf6 and Mm1), and two specimens
with missing epiphyses (Mf8 and Mf10). There is no discernible cortical thickness
variation in Mf4 and Mm1, and in Mf6 the styloid process appears to be thicker than
the area above it.

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685 We found no obvious difference in cortical thickness distribution between *M. martes* 686 and *M. foina*.

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2. Quantitative analyses of the covariation

691 2b-PLS on the superimposed maps and muscle data uncorrected for size detected 692 no covariation between cortical thickness distribution and muscle ACSA (see Table 693 4). However, 2b-PLS on the superimposed maps and size-corrected muscle data 694 indicate a correlation between the two parameters in all three bones. The results are 695 similar whether we consider all muscles or functional groups separately, with the 696 exception of the PQ muscle, for which no correlation is detected when using 697 corrected muscle data (Supplementary Table 3). An increase in muscle ACSA value 698 is correlated to an increased contrast in thickness along the diaphyses (Fig. 8A, B, 699 C). The two extreme deformations of the cortical thickness maps appear to 700 correspond to juvenile et adult patterns respectively, the adult pattern having the 701 greater contrast in cortical thickness. And indeed, these correlations are found only 702 when taking juvenile specimens into account, but not when considering adult 703 specimens only (Table 4).

In the humerus and radius, all strong muscles have a comparable impact on the covariation. In the ulna the effect of the FCUU muscle is more important than that of the other two muscles.

In the humerus an increase in muscle ACSA values is generally associated with a shift in higher cortical thickness toward the distal part of the caudal side, which corresponds to the insertion zone of three muscles (ANC, distal part of the TBM and BCH). In the radius, we observe almost an inversion of the cortical thickness distribution when the muscle ACSA values increase: high muscle ACSA values are associated with a thick cortex in the areas of contact with the ulna while low muscle ACSA values are associated with a thicker cortex surrounding these areas, with a very thin radial tuberosity. In the ulna all muscle ACSA values are associated with similar patterns of cortical thickness distribution, but we observe a sharp increase in contrast between the different zones when muscle ACSA values increase, forming the cranial and caudal thickenings of the proximal part of the shaft.

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719 DISCUSSION

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1. Muscular variation at the inter- and intra-specific levels

722 Taverne et al. (2018) showed that the forelimb musculature evolved in a convergent 723 manner in carnivoran arboreal species, resulting in more developed wrist 724 flexors/rotators and elbow flexors. But if these authors studied the musculature of the 725 forelimb as a whole, here we focused on the muscles attaching to the humerus, 726 radius and ulna separately. Although there is a slight trend towards stronger wrist 727 flexors in the more arboreal *M. martes*, the difference in guantitative muscular 728 anatomy between *M. martes* and *M. foina* in our study is not significant. The only 729 exception is found in the distal humeral epiphysis, on which a powerful flexor is 730 attached (Fig. 2A); this muscle allows the flexion of the wrist, its greater strength thus 731 appears to be advantageous for arboreal locomotion. The main muscle contributing to this difference is the M. flexor digitorum profundus (FDP), a powerful elbow 732 733 extensor and wrist flexor inserting on the medial epicondyle of the humeral distal 734 epiphysis, that is also involved in pronation/supination movements. Studies have 735 linked better radio-ulnar rotation capacities to a more arboreal lifestyle in mustelids 736 (Fabre et al., 2013b; 2015). The functional role of the FDP muscle suggests that this 737 muscular difference between the two species is linked to the more arboreal lifestyle 738 of the pine marten. This difference was less significant when adding the four *M. foina* 739 juveniles (Suppl. Fig.1). The humeral muscle strength of the *M. foina* juveniles 740 appears to be more similar to that of the adult *M. martes* than to that of the adult *M.* 741 foina. We lack data on the hindlimb, but it may be possible that a relatively strong 742 forelimb in the young stone martens may compensate for their overall physiological 743 immaturity. During ontogeny, relative muscle strength in the forelimb appears to 744 decrease. In primates, a decrease in relative manual grasping force from juvenile to 745 adult mouse lemurs has been shown and linked to a shift in recruitment of both the 746 fore- and hindlimbs (in juveniles) to a hindlimb-dominated recruitment (in adults)

747 (Boulinguez-Ambroise *et al.*, 2020).

These results highlight the integrative nature of the animal's body plan. Indeed, if analysing one body part, such as a single long bone, is not sufficient to identify specific variations linked to differences in locomotor mode, because the forelimb works as a functional unit, these variations become noticeable by combining analyses on the three long bones of the forelimb, and their possible causes can be investigated.

There is a significant difference between the two taxa when focusing on all the muscles attaching on the humerus, but not when considering only muscles attaching on the humeral diaphysis. This suggests that the functional signal is stronger in muscles that insert or originate near the shoulder and elbow joint.

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2. Bone microanatomy and cortical thickness distribution

The external limb bone morphology of *M. martes* and *M. foina* is very similar (Fabre *et al.*, 2013a, 2013b) despite their different locomotor modes. In light of almost unnoticeable external morphological differences, this makes them an ideal case study to investigate whether microanatomical changes do occur and whether they reflect different mechanical stress distribution.

765 Although the muscular differences between the two taxa lie predominantly in the 766 elbow joint, no accompanying variation of cortical thickness distribution in the 767 humeral distal epiphysis is observed between the two species. However, despite the 768 absence of interspecific muscular differences in the zeugopod, we found a 769 significantly thicker cortex (relative mean and maximal thicknesses) in the radius and 770 ulna of *M. martes* and with no effect of size. Although they do not differ significantly, 771 both the compactness and the trabecular ratio are higher in *M. foina* than in *M.* 772 martes, suggesting a slight trend toward more compact bone in semi-arboreal 773 species.

Although *M. martes* and *M. foina* share similar feeding habits, *M. foina*'s diet includes more plant food and insects than that of *M. martes*; it is especially the case when the two species are sympatric since they avoid competing with one another by assuming different ecological niches (Posłuszny *et al.*, 2007; Granata *et al.*, 2021). Additionally, pine martens are able to run along branches and jump from tree to tree in pursuit of prey, while stone marten tend to hunt on the ground, occasionally climbing trees to 780 reach on bird nests but without pursuing prey in the treetops (Heptner & Sludskii, 781 2002; Sidorovitch et al., 2005; Grabham et al., 2018). The differences we observed 782 between the forelimbs of the two species could thus be related to their distinct 783 locomotor modes (running along branches vs. running on the ground, jumping 784 between trees vs. occasionally climbing one), but also by their different hunting 785 behaviors (more grasping of prey for the pine marten, although Fabre et al. (2013b) 786 put both species in the same grasping category, namely "poorly developed grasping ability"). This suggests that the radius and ulna respond differently to an arboreal 787 788 lifestyle and hunting habits than the humerus. Since there was no difference in maximal length nor absolute mean/maximal thickness, the difference in relative 789 790 thickness might result in a slimmer medullar cavity in *M. martes*, without external 791 shape modification.

The covariation of the radius and ulna is not surprising in itself since they are closely linked in their functional roles. The different response of the humerus is interesting, since several studies found that the shape of the humerus covaried more with the radius than with the ulna in response to changes in body-mass and muscle anatomy (Fabre *et al.* 2013a; Martin *et al.*, 2019). Here, we found instead similar microanatomical variations in the radius and ulna, but not with the humerus.

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In the three bones, we found no interspecific difference in the 2D diaphyseal maps. Both species exhibit the same distribution in cortical thickness, although it appears visually thicker (absolute values) in the arboreal pine marten. These results support the quantitative microanatomical analyses suggesting that the higher cortical thickness observed in the radius and ulna of *M. martes* was associated with a reduced medullar cavity.

805 If there was no difference between the two species, the 2D patterns of diaphyseal 806 cortical topography were however significantly different between adult and juvenile 807 specimens of *M. foina*. After each map's minimum and maximum values were 808 standardized, we were able to compare the relative cortical thickness distribution of 809 the whole sample without being hindered by lower absolute values in juvenile 810 specimens. The extreme cortical topographies provided by the covariation analysis of 811 the 2D diaphyseal cortical topography correspond to the shift from a juvenile to an 812 adult pattern, with more contrast in relative thickness along the shaft: in the humerus, 813 the thickest parts of the diaphysis shift from the proximal to the distal part of the shaft,

814 which bears the insertions of two elbow extensors, the *M. anconeus* (ANC) and the 815 *M. triceps brachii* (TBM) muscles. In the radius, we observe a sharp increase of 816 relative cortical thickness in the zones of contact with the ulna where the cortex was 817 previously very thin, while in the ulna these areas are already thicker than the rest of 818 the shaft in juveniles, and their absolute thickness increases during growth without 819 change in the relative thickness general pattern. This indicates that changes in 820 cortical thickness distribution during growth are more pronounced in the humerus and 821 radius than in the ulna. This shift is most likely related to muscular insertions that are 822 not yet highly solicited in young individuals; a wider sample with more juveniles 823 representing the various ontogenetic stages might allow for a better understanding of 824 the link between muscle and bone microanatomy during the growth of the individuals.

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3. Covariation between muscles and bone microanatomy

The third objective of this study was to assess whether the ACSA of the muscles (as a proxy of muscle strength) is reflected in the bone microstructure considering that bones adapt their outer and inner structures to mechanical stresses.

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831 Our 3D maps show mixed results concerning the relationship between cortical 832 thickness and muscular attachments: we observed a clear link for some muscles, but 833 not for all. It is coherent with the fact that muscle attachments can sometimes cover 834 wide areas (e.g., the BCH muscle on the humerus). In those cases, the mechanical 835 strain can be distributed unevenly along the bone surface, or be too spread out to 836 have an impact on the bone cortical thickness. This result supports the observations 837 of Cuff et al. (2020), who explored the relationship between bony attachment areas 838 and both muscle mass and muscle insertions. Cuff et al. (2020) concluded that 839 although "muscle scars" were rarely correlated with muscle mass, and as such could 840 not be used for inferences in fossil taxa, those who were correlated were highly so. 841 Further studies may be able to determinate for which muscles inferences are 842 possible, and to which extent.

We observed a covariation between muscle ACSA and cortical thickness distribution (2D maps) in all three bones, but only when juveniles were included, and only on data uncorrected for size. As our small sampling could be responsible for the nonsignificance when testing for a muscle/bone covariation, the tests were repeated a number of times with a smaller sample (n=5) of random specimens, and a covariation
was observed only when juveniles were present. This suggests that the covariation is
linked to changes during ontogeny; that is coherent with the limited variations in
cortical thickness observed on 3D maps among adult specimens.

851 However, if the cortical thickness distribution does not seem to reflect muscle 852 strength, it appears to reflect the attachment of ligaments. It is visible in the radius 853 and ulna where the thickest areas correspond to the contact zones between the 854 bones and to the membrane and ligament linking them, rather than to specific muscle 855 insertions. These observations, while surprising, are consistent with recent results, 856 like those of Harbers et al. (2020), who investigated the impact of captivity and 857 domestication on limb bone cortical morphology in suids. In their study Harbers et al. 858 found no correlation between muscle ACSA and cortical thickness distribution in the 859 humerus of adult wild boars, but found that the cortical thickness distribution was correlated to the age and body mass of the specimens. These results are also 860 861 consistent with those of Houssaye et al. (2021), who studied the microanatomy of the 862 patella in perissodactyls and found a strong thickening of the cortex where the strong 863 patellar ligament inserts, but observed no particular thickenings associated with 864 muscle insertions. Similarly, on the calcaneum of suids, the ligaments insertion zones appear to be the main factors affecting the cortical thickness distribution (Cottereau, 865 866 pers. com. 2021). All of these observations are congruent with the hypothesis of 867 Zumwalt (2005), stating that muscular load does not affect the bony attachment 868 areas unless they are pathological.

869 Muscle strength was not reflected at the microanatomical level. However, previous 870 studies analysing 3D histology have found clearer correlations between bone 871 histology and musculo-tendinous insertions: Sanchez et al. (2013) were able to 872 determine the position of entheses in fossil vertebrates as well as the approximate 873 orientation of the attached muscle. In addition, Cury et al. (2016) and Zhao et al. 874 (2017) observed histological changes in tendon insertion zones and ligament 875 insertion zones respectively, indicating that cortical bone holds significant information 876 regarding muscular anatomy. Thus, if the histological level is impacted by muscle 877 insertion, the microanatomical organization appears rather poorly affected and a less 878 efficient level of investigation to infer muscle structure based on skeletal elements.

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4. Intraspecific vs. interspecific variation

We observed more separation between adult and juvenile specimens of *M. foina* than between adults of the two species, for all microanatomical parameters. This was due to a greater mean cortical thickness in the forelimb of the pine martens; in the *M. foina* sample, the thickness appeared to increase proportionally during growth.

Using muscle data, we also observed a greater distinction at the interspecific level than at the intraspecific level in the humerus. As stated above, this variation was almost entirely driven by the strength of one muscle, far greater in the arboreal pine marten than in the more terrestrial stone marten. The lack of differences between adult and juvenile specimens when using size-corrected data suggests that the intraspecific variation is mainly driven by the increase of muscle force during growth.

891 Despite these results, the cortical maps revealed greater differences within the M. 892 foina sample than compared to the *M. martes* sample. This can largely be attributed 893 to the presence of juveniles within the former, in which the zones of high cortical 894 thickness observed in adults are not yet fully developed. When taking only adult M. 895 foina into consideration, the range of variation is similar to the one observed in the M. 896 martes specimens. This is congruent with the fact that the only muscular difference 897 was found in the humeral epiphysis, which is not visible on the 2D cortical maps. The 898 greater cortical thickness detected in the pine marten's stylopod was not reflected in 899 the maps either, since all values were standardized and showed relative variation in 900 the cortical thickness pattern only.

901 Using muscular and microanatomical data, we found overall more differences 902 between the two species than within the *M. foina* sample, indicating that we can 903 distinguish between these two species, despite their strong similarity, using muscular 904 and microanatomical data. However, the ontogenic variation is not negligible: muscle 905 strength in juvenile specimens of *M. foina* is similar to that of the adult *M. martes*, 906 thus masking the species-specific muscular distinction in the humerus when taking all 907 specimens in consideration.

The relatively limited number of specimens in this study, as well as the lack of juvenile specimens of *M. martes*, prevent us to conclude with certainty that the intraspecific variation, because of ontogenetic variation, is greater than the interspecific one. This would notably require a future study using a wider sample of both juvenile and adult specimens in order to better characterize the covariation of muscle and bone during ontogeny.

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- 914 CONCLUSION
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916 The present study of the forelimb muscular anatomy and bone inner structure in two 917 sympatric species of *Martes* revealed a functional signal in the muscle anatomy: we observed a stronger flexor muscle in the elbow of *M. martes* than in the elbow of *M.* 918 919 foina. While this difference was not directly reflected in the bones' microanatomy, we 920 also observed a global thickening of the cortical bone in the radius and ulna, but not 921 in the humerus, of *M. martes*, and conclude that the stylopod and zeugopod respond 922 in a different manner to a more arboreal lifestyle. Our 2D maps revealed a clear 923 difference between adult and juvenile specimens of *M. foina*, but not between the 924 adults of the two species, and thus a shift of cortical thickness distribution pattern 925 during ontogeny, rather than species-specific patterns. However, using both muscle 926 and bone microanatomical data, we were able to distinguish the two taxa, indicating 927 that although it is not negligible, the intraspecific variation does not hinder species 928 distinction, even between two very close species. Finally, our results show that 929 cortical thickness varies only to some extent with muscular strength at zones of 930 muscle attachment. While the correlation is clear for some muscles, it is not for 931 others. It rather appears that cortical thickness responds prevalently to strains 932 applied by contact between bones and ligament insertions. We thus conclude that 933 inference of muscle information from cortical thickness distribution is possible but 934 only for certain muscles in the humerus.

935

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AUTHOR'S CONTRIBUTIONS

A.H., C.Ba. and C.Bö designed the study.. AH. did the bone data acquisition, C.Bö. did the muscular data acquisition. C.Ba. and M.A. conducted the segmentation. C.Ba and C.Bö. conducted the analyses. C.Ba. and C.Bö. prepared the figures and drafted the manuscript. All authors contributed to the final manuscript, read it and approved it.

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1272 1273

1274 Table 1: Sample studied (A: adult, J: juvenile; MaxL: maximum length of the bone;

Specimen	Species	Age category	Humerus MaxL (cm)	Radius MaxL (cm)	Ulna MaxL (cm)
Mm1	Martes martes	A	6.77	5.13	6.31
Mm2	Martes martes	A	7.45	6.01	7.18
Mm3	Martes martes	A	7.04	5.32	6.49
Mf1	Martes foina	A	5.78	4.48	5.57

1275 NA: not available because one or both epiphyses were missing).

Mf2	Martes foina	A	6.44	4.79	5.81
Mf3	Martes foina	A	6.68	5.34	6.57
Mf4	Martes foina	J	NA	NA	NA
Mf5	Martes foina	A	6.51	5.31	6.57
Mf6	Martes foina	J	NA	NA	NA
Mf7	Martes foina	A	6.78	5.09	6.21
Mf8	Martes foina	J	NA	NA	NA
Mf9	Martes foina	A	7.03	5.38	6.72
Mf10	Martes foina	J	NA	NA	NA

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Table 2: Muscles of the forelimb in martens (based on Martes martes). Add: adductor, abd: abductor, fl: flexor, ext: extensor, pro: pronator, sup: supinator. In

grey, muscles inserted on the epiphyses.

Muscle	Acronym	Origin	Insertion	Main function	Functional
					group
M. supraspinatus	SSP	Supraspinous fossa and scapular spine	Greater tubercle of humerus	Shoulder joint extensor and humeral protractor	

	lion				
vi. intraspinatus		Lateral on greater tubercle of humerus (Infraspinatus muscle facet)	Shoulder joint flexor and lateral humeral rotator		
M. subscapularis	SUB	Subscapular fossa	Lesser tubercle of humerus	Scapular adductor	
M. teres minor	TMN	Caudal border of scapula (near glenoid fossa)	Lateral on greater tubercle of humerus (distal to <i>M.</i> <i>infraspinatus</i>)	Shoulder joint flexor and lateral humeral rotator	
<i>M. triceps brachii caput accessorium</i>	ТВА	Distal caudomedial aspect of humeral diaphysis (along medial epicondylar crest)	Medial aspect of olecranon	Elbow joint extensor	
M. extensor carpi ulnaris	ECU	Lateral epicondylar crest of humerus (distal to origin of <i>M.</i> <i>extensor digitorum</i> <i>lateralis</i>)	Base of metacarpal V	Elbow joint flexor and wrist joint extensor	
M. extensor digitorum EDL Lateral epicondylar lateralis (distal to origin of M. extensor digitorum communis		Tendons into distal phalanges of digits IV-V	Elbow joint flexor, wrist joint extensor and digital extensor		
M. extensor digitorum communis	M. extensor digitorum communis EDC (distal to origin of M.extensor carpi radialis)		Tendons into distal phalanges of digits II-V	Elbow joint flexor, wrist joint extensor and digital extensor	
M. extensor carpi radialis brevis	M. extensor carpi radialis brevis ECRB Proximal lateral epicondylar crest of humerus (together with or distal to origin of M. extensor carpi radialis longur)		Base of metacarpal	Elbow joint flexor and wrist joint extensor	
M. palmaris longus	M. palmaris longus PL Medial epicondyle of humerus		Tendons into distal phalanges of digits II-V or palmar aponeurosis	Wrist joint flexor and digital flexor	
M. flexor carpi ulnaris, caput humerale	FCUH	Medial epicondyle of humerus	Sesamoid proximal to metacarpal V (Pisiform)	Wrist joint flexor	
M. flexor carpi radialis	FCR	Medial epicondyle of humerus	Base of metacarpal II and III	Wrist joint flexor	FI
M. flexor digitorum profundus (4 heads)	FDP	Medial epicondyle of humerus and medial aspect of olecranon	Tendons into distal phalanges of digits II-V	Wrist joint flexor and digital flexor	FI
M. pectoantebrachialis	<i>M. pectoantebrachialis</i> PAB Ventrolateral surface of cranial portion of sternum (Manubrium)		Cranial proximal aspect of humeral diaphysis (superficial to insertion of <i>M.</i> <i>pectoralis minor</i>)	Humeral adductor	Add
M. pectoralis major	PMJ	Ventral surface of cranial portion of sternum (Manubrium) and body of sternum	Craniomedial middle of humeral diaphysis (superficial to insertion of <i>M.</i> <i>pectoralis minor</i>)	Humeral adductor and protractor	Add
M. pectoralis minor PMN Ventral surface of body of sternum		Cranial proximal aspect of humeral diaphysis (deep to insertion of <i>M.</i> <i>pectoantebrachialis</i> and <i>M. pectoralis</i> <i>major</i>)	Humeral adductor and retractor	Add	

M. xiphihumeralis	ХН	Ventrolateral surface of caudal portion of sternum (Xiphoid	Craniomedial middle of humeral diaphysis	Humeral adductor and retractor	Add
M. clavobrachialis	СВ	process) Clavicle (if present) and raphe with <i>M.</i> <i>clavotrapezius</i> and <i>M.</i> <i>cleidomastoideus</i>	Cranial distal aspect of humeral diaphysis	Humeral protractor	FI
M. teres major	ТМЈ	Caudal border of scapula (Teres major muscle facet)	Craniomedial on humeral diaphysis (Teres major tuberosity; near pectoral crest)	Shoulder joint flexor and humeral retractor	FI
<i>M. triceps brachii caput mediale, intermediate and long portion</i>	ТВМ	Mediocaudal humeral diaphysis	Medial aspect of olecranon	Elbow joint extensor	Ext
M. articularis humeri	АН	Coracoid process of scapula	Medial proximal aspect of humeral diapyhsis	Shoulder joint stabilizer and humeral adductor	Add
M. triceps brachii caput laterale	TBLA	Proximal aspect of deltoid crest of humerus	Caudolateral aspect of olecranon (lateral to <i>M. triceps brachii</i> <i>caput longum</i>)	Elbow joint extensor	Ext
M. anconeus	ANC	Distal caudal aspect of humeral diaphysis (along lateral epicondylar crest)	Lateral aspect of olecranon (deep to <i>M. triceps brachii</i> <i>caput laterale</i>)	Elbow joint extensor and forearm pronator	Ext
M. acromiodeltoideus	DA	Acromion	Deltoid crest of humerus (superficial to <i>M.</i> spinodeltoideus)	Shoulder joint flexor and humeral abductor	Abd
M. spinodeltoideus	DS	Scapular spine (Superficial to <i>M.</i> <i>infraspinatus</i>)	Deltoid crest of humerus	Shoulder joint flexor and humeral abductor	Abd
M. extensor carpi radialis longus	ECRL	Proximal lateral epicondylar crest of humerus (distal to origin of <i>M.</i> <i>brachioradialis</i> - if present)	Base of metacarpal II	Elbow joint flexor and wrist joint extensor	FI
M. brachioradialis	BCR	Proximal lateral epicondylar crest of humerus (proximal to origin of <i>M. extensor</i> <i>carpi radialis</i>)	Distal medial aspect of radius	Elbow joint flexor and forearm supinator	FI
M. brachialis	BCH	Proximal caudolateral humeral diaphysis	Tendon into bicipital tuberosity of radius or coronoid process of ulna (adjacent to insertion of <i>M.</i> <i>biceps brachii</i>)	Elbow joint flexor and forearm supinator	FI
M. extensor pollicis	EP	Lateral ulnar and radial diaphysis (and interosseus membrane between radius and ulna)	Base of metacarpal I (and sesamoid proximal to metacarpal I)	Wrist joint extensor and extensor of digit I	Ext
M. pronator teres	PT	Medial epicondyle of humerus (dorsal to origin of <i>M. flexor</i> <i>carpi radialis</i>)	Medial aspect of radial diaphysis	Forearm pronator	Pro
M. pronator quadratus	PQ	Distal medioventral surface of ulna	Distal medioventral surface of radius	Forearm pronator	Pro
M. supinator	SUP	Lateral epicondyle of humerus	Dorsomedial aspect of radial diaphysis	Forearm supinator	Sup

	1				
M. epitrochlearis	EPI	Lateroventral on <i>M.</i> teres major and <i>M</i> .	Caudal aspect of olecranon tuber	Humeral retractor and	
		latissimus dorsi		elbow joint	
		(Caudal border of scapula)		extensor	
M. triceps brachii caput	TBLO	Caudal border of	Caudal aspect of	Shoulder joint	
longum		scapula (near glenoid	olecranon tuber	flexor and elbow	
		fossa; medial to <i>M.</i>	(deep to <i>M.</i>	joint extensor	
M. extensor indicis	EI	diaphysis	phalanges of digits I and II	Extensor of digits I and II	Ext
M. flexor carpi ulnaris, caput ulnare	FCUU	Medial aspect of olecranon	Sesamoid proximal to metacarpal V (Pisiform)	Wrist joint flexor	FI
M. biceps brachii	BB	Tendon from the supraglenoid tubercle of scapula (and from coracoid process of scapula if second head present)	Tendon into bicipital tuberosity of radius or coronoid process of ulna	Shoulder joint extensor and elbow joint flexor	FI

Table 3: Results from the statistical analyses. %Trab: trabecular ratio; C: compactness; AmeanT: absolute mean thickness; RmeanT: relative mean thickness; *RmaxT*: relative maximum thickness; *AmaxT*: absolute maximum thickness; ACSA: anatomical cross-sectional area. In grey: not available because of missing epiphyses in juvenile specimens.

Bone microanatomy t-test	Muscles (diaphysis only) MANOVA	Muscles (all) MANOVA	Cortical mapping Procruste s ANOVA
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		%Tra b	С	Amean T	Rmean T	Amax T	RmaxT	log(ACS A)	residual s	log(ACS A)	residual s	2D maps
	Humeru s	p=0.6 0	p=0.3 0	p=0.08	p=0.46	p=0.0 9	p=0.40	p=0.85 r ² =0.05	p=0.91 r ² =0.05	p=0.03 r²=0.25	p=0.04 r ² =0.25	p=0.09 r ² =0.28
Interspecifi c variation (n=9)	Radius	p=0.6 2	p=0.2 5	p=0.13	p=0.00 4	p=0.5 7	p=0.00 8	p=0.41 r²=0.1	p=0.42 r ² =0.12	p=0.56 r²=0.1	p=0.58 r ² =0.10	p=0.29 r ² =0.13
(- /	Ulna	p=0.2 0	p=0.1 9	p=0.17	p=0.00 3	p=0.3 8	p=0.00 7	p=0.80 r ² =0.07	p=0.47 r ² =0.10	p=0.49 r²=0.1	p=0.45 r ² =0.10	p=0.23 r²=0.16
Intraspecifi c variation (n=10)	Humeru s			p=0.01 6	p=0.94	p=0.4 8	p=0.72	p=0.004 r²=0.44	p=0.99 r ² =0.05	p=0.003 r²=0.47	p=0.15 r ² =0.15	p=0.002 r²=0.61
	Radius			p=0.01 6	p=0.48	p=0.3 3	p=0.36	p=0.001 r²=0.59	p=0.81 r ² =0.06	p=0.001 r²=0.57	p=0.96 r ² =0.04	p=0.02 r²=0.44
	Ulna			p=0.03	p=0.45	p=0.1 9	p=0.36	p=0.002 r²=0.52	p=0.71 r ² =0.07	p=0.001 r²=0.45	p=0.93 r ² =0.05	p=0.08 r²=0.26

1377 1378

Table 4: Results from the covariation analyses (Two-block partial least squares analyses) using a) the log(ACSA) values and b) residuals from the linear regression. ACSA: anatomical cross-sectional area; MP: Microanatomical parameters.

a)

All specimens (n=13)	<i>M. foina</i> (adults+juveniles) (n=10)	Adult s (<i>M. marte</i> (r	pecimens s + <i>M. foina</i>) n=9)	Juvenile specimens (n=4)	<i>M. martes</i> (n=3)
ACSA/2D maps	ACSA/2D maps	ACSA/MP	ACSA/2D maps	ACSA/2D maps	ACSA/2D maps

Humerus	p=0.003	p=0.02	p=0.4	p=0.32	p=0.02	p=0.91
	r-PLS=0.89	r-PLS=0.85	r-PLS=0.53	r-PLS=0.68	r-PLS=0.99	r-PLS=0.94
Radius	p=0.001	p=0.02	p=0.63	p=0.76	p=0.14	p=0.08
	r-PLS=0.83	r-PLS=0.76	r-PLS=0.30	r-PLS=0.54	r-PLS=0.86	r-PLS=0.99
Ulna	p=0.003	p=0.04	p=0.29	p=0.42	p=0.02	p=0.59
	r-PLS=0.85	r-PLS=0.83	r-PLS=0.48	r-PLS=0.65	r-PLS=0.96	r-PLS=0.91

b)

	All specimens (n=13)	<i>M. foina</i> (adults+juveniles) (n=10)	Adult specimens (<i>M. martes</i> + <i>M. foina</i>) (n=9)		Juvenile specimens (n=4)	<i>M. martes</i> (n=3)
	ACSA/2D maps	ACSA/2D maps	ACSA/MP	ACSA/2D maps	ACSA/2D maps	ACSA/2D maps
Humerus	p=0.99	p=0.86	p=0.26	p=0.80	p=0.80	p=0.91
	r-PLS=0.33	r-PLS=0.61	r-PLS=0.78	r-PLS=0.62	r-PLS=0.93	r-PLS=0.86
Radius	p=0.90	p=0.82	p=0.90	p=0.50	p=0.37	p=0.26
	r-PLS=0.44	r-PLS=0.56	r-PLS=0.45	r-PLS=0.69	r-PLS=0.97	r-PLS=0.99
Ulna	p=0.72	p=0.71	p=0.74	p=0.57	p=0.37	p=0.42
	r-PLS=0.48	r-PLS=0.54	r-PLS=0.49	r-PLS=0.65	r-PLS=0.83	r-PLS=0.95

1404 FIGURE LEGENDS

Figure 1: 2D cortical mapping of the ulna of a specimen of *Martes martes* (Mm2) with the corresponding 3D cortical maps in posterior, medial, anterior and lateral views (P: posterior, M: medial, A: anterior, L: lateral). Cortical thickness is represented by a gradient ranging from cold (low cortical thickness) to warm (high cortical thickness)colours. Refer to material and methods section for explanation on selected limits.

1412

Figure 2: Results of the Principal Components Analysis of size-corrected anatomical cross-sectional area (ACSA) considering A) all muscles attached on the humerus and B) muscles attached on the humeral diaphysis. The plots display the variation along the first two axes, along with the muscle contributions (arrows). For muscle abbreviations, see Table 2. Variables contributing the least are not shown in order to increase visibility of the graph. Mm = *Martes martes*, Mf = *Martes foina* (adult specimens only).

1420

Figure 3: Results of the Principal Components Analysis displaying the variation along the two first axes using microanatomical parameters of the ulna, along with their contributions (arrows). *%Trab*: trabecular ratio, *C*: Compactness, *RmeanT*: relative mean cortical thickness, *RmaxT*: relative maximal cortical thickness. Mm = *Martes martes*, Mf = *Martes foina* (adult specimens only).

1426

Figure 4: Results of the Principal Components Analysis displaying the variation along the two first axes using 2D cortical mappings of the humerus. Adults are visualised in the solid circles and juveniles in the open circles. Mm = *Martes martes*, Mf = *Martes foina*.

1431

Figure 5: Representations of the origin (red) and insertion (blue) of muscles on the humerus, with the corresponding cortical mapping of the humerus of *Martes martes* (Mm2) in cranial, lateral, caudal and medial view. Cortical thickness is represented by a gradient ranging from cold (low cortical thickness) to warm (high cortical thickness) colours on the 3D mapping. For muscle abbreviations see Table 2.

1437

Figure 6: Representations of the origin (red) and insertion (blue) of muscles on the radius (transparent ulna), with the corresponding cortical mapping of the radius of *Martes martes* (Mm2) in cranial, lateral, caudal and medial view. Cortical thickness is represented by a gradient ranging from cold (low cortical thickness) to warm (high cortical thickness) colours on the 3D mapping. For muscle abbreviations see Table 2. Figure 7: Representations of the origin (red) and insertion (blue) of muscles on the ulna (transparent radius), with the corresponding cortical mapping of the ulna of *Martes martes* (Mm2) in cranial, lateral, caudal and medial view. Cortical thickness is represented by a gradient ranging from cold (low cortical thickness) to warm (high cortical thickness) colours on the 3D mapping. For muscle abbreviations see Table 2.

Figure 8: Results of the Two-block partial Least Squares (2b-PLS) regressions between the 2D cortical maps of the A) humerus, B) radius, C) ulna, and anatomical cross-sectional area (ACSA) blocks. Adults are visualised by solid circles and juveniles by open circles. The black line represents the PLS regression line. Singular vectors for muscle ACSA blocks are shown using barplots, extreme cortical thickness patterns are shown using 2D mappings. Mm = *Martes martes*, Mf = *Martes foina*.

1456

1457 Supplementary Material:

1458

Supplementary Figure 1: Results of the Principal Components Analysis of sizecorrected anatomical cross-sectional area (ACSA) considering A) all muscles attached on the radius and B) muscles attached on the radial diaphysis. The plots display the variation along the first two axes, along with the muscle contributions (arrows). For muscle abbreviations, see Table 2. Variables contributing the least are not shown in order to increase visibility of the graph. Mm = *Martes martes*, Mf = *Martes foina* (adults only).

1466

Supplementary Figure 2: Results of the Principal Components Analysis of sizecorrected anatomical cross-sectional area (ACSA) considering A) all muscles attached on the ulna and B) muscles attached on the ulnar diaphysis. The plots display the variation along the first two axes, along with the muscle contributions (arrows). For muscle abbreviations, see Table 2. Variables contributing the least are not shown in order to increase visibility of the graph. Mm = *Martes martes*, Mf = *Martes foina* (adults only).

1474

1475 Supplementary Figure 3: Results of the Principal Components Analysis of size-1476 corrected anatomical cross-sectional area (ACSA) considering all muscles attached 1477 to the A) humerus, B) radius, C) ulna and muscles attached on the D) humeral diaphysis, E) radial diaphysis, F) ulnar diaphysis. The plots display the variation along the first two axes, along with the muscle contributions (arrows). For muscle abbreviations, see Table 2. Variables contributing the least are not shown in order to increase visibility of the graph. Mf = *Martes foina*. Adults are visualised by red circles and juveniles by blue circles.

1483

1484 Supplementary Figure 4: Results of the Principal Components Analysis of sizecorrected anatomical cross-sectional area (ACSA) considering A) all muscles 1485 1486 attached on the humerus and B) muscles attached on the humeral diaphysis 1487 (juveniles and adults). The plots display the variation along the first two axes from, 1488 along with the muscle contributions (arrows). Adults are visualised by solid circles 1489 and juveniles by open circles. For muscle abbreviations, see Table 2. Variables 1490 contributing the least are not shown in order to increase visibility of the graph. Mm = 1491 *Martes martes*, Mf = *Martes foina*.

1492

Supplementary Figure 5: Results of the Principal Components Analysis of the microanatomical parameters of the A) humerus and B) radius. The plots display the variation along the first two axes, along with the parameters' contributions (arrows). *%Trab*: trabecular ratio, *C*: Compactness, *RmeanT*: relative mean cortical thickness, *RmaxT*: relative maximal cortical thickness. Mm = *Martes martes*, Mf = *Martes foina* (adults only).

1499

Supplementary Figure 6: Results of the Principal Components Analysis of the 2D cortical mappings of the A) radius and B) ulna. Adults are visualised by solid circles and juveniles by open circles. Mm = *Martes martes*, Mf = *Martes foina*.

1503

Supplementary Figure 7: 2D maps of the A) humerus, B) radius, C) ulna of the entire sample. Cortical thickness is represented by a gradient ranging from blue (low cortical thickness) to red (high cortical thickness). Mm = *Martes martes*, Mf = *Martes foina*.

1508

Supplementary Figure 8: Mid-diaphyseal frontal virtual sections of the A) humerus, B)
radius, C) ulna of the entire sample ordered by descending humerus length. Mm = *Martes martes*, Mf = *Martes foina*.