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# Unravelling the postural diversity of mammals: Contribution of humeral cross-sections to palaeobiological inferences

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1                   **Unravelling the postural diversity of mammals:**  
2                   **Contribution of humeral cross-sections to palaeobiological**  
3                   **inferences**

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5  
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14  
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16                   Palaeobiology

17 **Abstract**

18 Mammals have an evolutionary history spanning hundreds of millions of years. Today,  
19 mammals represent one of the most diverse groups of tetrapod vertebrates. In particular, they  
20 present a great postural diversity. The humerus adopts different positions: small mammals have  
21 a “crouched” posture with a quasi-horizontal humerus, while in the largest species, the humerus  
22 is more vertical. Some monotremes have more transversely oriented humeri similar to those of  
23 reptiles. The forelimb of moles is also modified in relation to their burrowing lifestyle. This  
24 postural diversity is accompanied by an important microanatomical disparity. Indeed, the bones  
25 of the appendicular skeleton support the weight of the body and are subjected to various forces  
26 that partly shape their external and internal morphology. We show here how geometric and  
27 microanatomical parameters measured in cross-section such as the polar section modulus or the  
28 position of the medullo-cortical transition can be related to posture. Using statistical methods  
29 that take phylogeny into account, we develop a postural model from a sample of humerus cross-  
30 sections belonging to 41 species of extant mammals. Our model can be used by palaeontologists  
31 to infer the posture of extinct synapsids. As an example, we infer the posture of two emblematic  
32 taxa: *Dimetrodon natalis* and *Peratherium cuvieri*. The results of the analysis indicate a  
33 sprawling posture for *Dimetrodon* and a crouched posture for *Peratherium*. This work  
34 contributes to unravel the complex interaction between phylogeny, humeral microanatomy and  
35 geometry, body mass, lifestyle and posture in mammals.

## 36 **Introduction**

37 Mammals are a highly successful group of tetrapod vertebrates with a long evolutionary history.  
38 Their earliest stem members, i.e. the first synapsids (the term “stem mammal” is used  
39 throughout this study to refer to any taxon that is more closely related to Mammalia than to  
40 Reptilia but that does not belong to the mammalian crown group), originated in the  
41 Carboniferous, about 330 million years ago, with the emergence of the first amniotes (Didier  
42 and Laurin 2020), and have undergone several episodes of diversification ever since. The Late  
43 Carboniferous and Early Permian is dominated by eupelycosaurs. These are followed by  
44 therapsids by the middle Permian; they comprised medium-sized herbivorous and carnivorous  
45 taxa that became extinct by the end of the Triassic, except for cynodonts, which gave rise to the  
46 Mammaliaformes during the Triassic (Kemp 2005; Brocklehurst et al. 2013). It is generally  
47 thought that most Mesozoic mammals were small, nocturnal creatures with more or less  
48 burrowing habits and a generalised insectivory (Jenkins and Parrington 1976; Kielan-  
49 Jaworowska et al. 2004; Gerkema et al. 2013; but see Hu et al. 2005; Gill et al. 2014; Meng  
50 2014; Debuyschere 2015). The K-Pg extinction event was the starting point for the Cenozoic  
51 radiation of mammals which led them to colonise the ecological niches left empty by non-avian  
52 dinosaurs (Rose 2006; but see Wilson et al. 2012). Today, mammals are extremely diverse, both  
53 taxonomically and ecologically. With over 5,000 currently recognised extant species (Upham  
54 et al. 2019), they are found all over the world, at all levels of the trophic network, on land, in  
55 the seas and in the air (Vaughan et al. 2015). They range in size from the bumblebee bat, which  
56 weighs only a few grams, to the blue whale (the largest animal on the planet), which weighs  
57 more than 100 tons, the weight of a few dozen elephants. Mammals are also particularly diverse  
58 in their posture: small mammals like rodents have a “crouched” posture, with a quasi-horizontal  
59 humerus (Jenkins 1971), while in the cursorial and graviportal taxa, such as artiodactyls and  
60 proboscideans, the humerus is more vertical (Gregory 1912). Some monotremes have more  
61 transversely oriented humeri (Pridmore 1985). The forelimb of moles is also modified in  
62 relation to their burrowing lifestyle (Lin et al. 2019).

63         The shape of biological structures is determined by at least three types of constraints  
64 that can be positioned at the three vertices of an abstract triangle in the framework of  
65 constructional morphology (Seilacher 1970). These are phylogenetic (heredity), structural  
66 (development), and adaptive (function) constraints. The bones of the appendicular skeleton are  
67 subject to these different constraints, especially functional constraints relative to posture.  
68 Indeed, limb bones support the weight of the body and are affected by various forces that partly

69 shape their external and internal morphology. While many studies have already identified the  
70 link between lifestyle (from aquatic to terrestrial) and bone microanatomy, including using  
71 multivariate quantitative methods that take phylogeny into account (Germain and Laurin 2005;  
72 Krilloff et al. 2008; Canoville and Laurin 2009, 2010; Laurin et al. 2011; Quemeneur et al. 2013;  
73 Amson et al. 2014; Ibrahim et al. 2014; Nakajima et al. 2014; Cooper et al. 2016; Houssaye et  
74 al. 2016a; Klein et al. 2016; Houssaye and Botton-Divet 2018; Kilbourne and Hutchinson 2019;  
75 Canoville et al. 2021; Fabbri et al. 2022), fewer have attempted to link the geometric and  
76 microanatomical properties of limb bones to posture (Houssaye et al. 2016b; Bishop et al.  
77 2018a, 2018b, 2018c; Plasse et al. 2019; Main et al. 2021; Wagstaffe et al. 2022).

78 Although it is accepted that mammals, like reptiles such as dinosaurs and  
79 pseudosuchians (Hutchinson 2006), experienced a postural transition from approximately  
80 transversely-oriented to more parasagittally-oriented limbs, the timing of this transition has  
81 been widely debated without reaching a consensus. Some authors (Jenkins 1973; Pridmore  
82 1985; Sereno 2006) have argued that early mammals had already acquired a parasagittal limb  
83 posture and gait by the Late Triassic/Early Jurassic, while others (Gambaryan and Kielan-  
84 Jaworowska 1997; Kielan-Jaworowska and Hurum 2006) favoured the hypothesis of a later  
85 acquisition in early therians. Even today, posture in mammals and in older stem taxa such as  
86 *Dimetrodon*, raises many questions that triggered numerous studies that enrich our knowledge  
87 of the evolution of locomotion in mammals (Abbott 2019; Regnault et al. 2020; Jones et al.  
88 2021; Brocklehurst et al. 2022).

89 Our study is a logical extension of these works. Using generalised least squares, we  
90 investigate the relationship between humeral posture and geometric and microanatomical data  
91 collected from humeral bone cross-sections belonging to 41 extant mammalian species, while  
92 taking phylogeny into account. Body mass and lifestyle were also included in our models as  
93 both are known to be related to posture and/or bone microanatomy, e.g. large taxa tend to have  
94 greater bone compactness and more upright limbs (Biewener 1989b; Houssaye et al. 2016b);  
95 fossorial talpids have greater extension of the medullo-cortical transition compared to terrestrial  
96 talpids (Meier et al. 2013). We use the collected data to generate a phylogenetically informed  
97 postural inference model capable of generating postural predictions in extinct taxa. We apply  
98 the model to two taxa: *Dimetrodon natalis* (a small *Dimetrodon* species) and *Peratherium*  
99 *cuvieri* (“Cuvier’s Sarigue”). While *Dimetrodon* is a stem mammal, *Peratherium* is a putative  
100 marsupialiform. *Dimetrodon* and *Peratherium* lived in the Early Permian and Late Eocene,  
101 respectively, well before and well after the Mesozoic postural transition in mammals. The

102 posture of *Dimetrodon* has been extensively studied since its discovery in the second half of the  
103 19th century. The recent interpretation indicates a more or less sprawling posture. In  
104 comparison, *Peratherium* has been the subject of less work, but its younger geological age and  
105 general anatomy are compatible with a more crouched posture. These two taxa are therefore  
106 perfect candidates to test our method.

## 107 **Materials and Methods**

### 108 BIOLOGICAL SAMPLE

109 To train our statistical model, we compiled a set of geometric and microanatomical data  
110 collected on humeral mid-diaphyseal cross-sections from a large number of mammalian taxa  
111 with a known posture. The dataset included 43 individuals from 41 extant species (Table 1;  
112 Online Resource 1). We built our dataset to be as taxonomically exhaustive as possible and to  
113 maximise coverage of the postural diversity of Mammalia. We used our postural models to infer  
114 the posture of two extinct taxa of interest: the Early Permian stem mammal *Dimetrodon natalis*  
115 (IPBSH-4) and the Late Eocene herpetotheriid *Peratherium cuvieri* (MNHN-F-GY679b); see  
116 Online Resource 1.

### 117 POSTURAL DEFINITIONS

118 Most non-flying mammals are obligate quadrupeds, that is, they move exclusively on four limbs  
119 (Vaughan et al. 2015). A few taxa, especially among rodents, marsupials and primates, are  
120 known to be facultatively bipedal or quadrupedal, meaning that they alternate between  
121 bipedalism and quadrupedalism (D'Août et al. 2004; Russo and Kirk 2017). Some pangolins  
122 (*Smutsia temminckii*) are also able to move on their hind limbs for some distance (Pietersen et  
123 al. 2020). Obligate bipedalism is restricted to humans (Niemitz 2010).

124 Yet, mammals show great diversity in limb posture, especially regarding the humerus  
125 (Fig. 1). During normal walking, the specialised “erect” (or upright) forelimb posture of  
126 mammals exhibits fairly low humeral abduction (less than 10 degrees to the parasagittal plane;  
127 Jenkins 1971). The humerus is oblique with the elbow always functioning below the shoulder  
128 joint between approximately 30 and 75 degrees from the horizontal. Erect limbs are found in  
129 “hoofed” mammals such as artiodactyls and proboscideans (Gregory 1912), in carnivorans  
130 (Blob 2000) and in cursorial and graviportal mammals in general (Gregory 1912; Jenkins 1971;  
131 but see Stein and Casinos 1997; Carrano 1999). Taxa with more generalised locomotor  
132 adaptations, such as rodents, have a so-called “crouched” posture (Jenkins 1971). The humerus

133 **Table 1** List of the mammalian taxa included in this study. Taxa are presented in alphabetical  
 134 order. Body mass is rounded to the nearest gram. \*Data collected on  
 135 <https://www.morphosource.org>. Abbreviations: Aq, semi-aquatic; Ar, arboreal; C, crouched; E,  
 136 erect; Fo, fossorial; M, modified; S, sprawling; Te, terrestrial. Institutional abbreviations:  
 137 IPBSH/STIPB, Steinmann-Institut, Universität Bonn, Germany; MNHN, Muséum national  
 138 d'Histoire naturelle, Paris, France; NHMUK, Natural History Museum, London, United  
 139 Kingdom; UFGK, Ur- und Frühgeschichte Köln, Cologne, Germany; UMZC, Cambridge  
 140 University Museum of Zoology, Cambridge, United Kingdom

Taxon		Collection number	Humeral posture	Lifestyle	Body mass (g)	
<b>Afrosoricida</b>	Chrysochloridae	<i>Chrysochloris asiatica</i>	MNHN-ZM-MO-1991-626	C	Fo	37
<b>Artiodactyla</b>	Bovidae	<i>Cephalophus silvicultor</i>	NHMUK ZD 1961.8.9.80-1	E	Te	62,007
		<i>Rupicapra rupicapra</i>	STIPB M1639	E	Te	35,383
		<i>Syncerus caffer</i>	NHMUK ZD 1874.11.2.4	E	Te	646,333
	Cervidae	<i>Alces americanus</i>	UMZC H.17,691	E	Te	368,500
		<i>Cervus elaphus</i>	MNHN unnumbered specimen	E	Te	160,167
		<i>Rangifer tarandus</i>	STIPB M47	E	Te	101,250
	Suidae	<i>Sus scrofa</i>	MNHN unnumbered specimen	E	Te	135,000
		<i>Sus scrofa</i>	STIPB M56	E	Te	135,000
<b>Carnivora</b>	Canidae	<i>Vulpes vulpes</i>	STIPB M12	E	Te	4,580
	Felidae	<i>Felis silvestris</i>	UFGK unnumbered specimen	E	Te	5,037
		<i>Panthera leo</i>	MNHN-ZM-AC-1912-398	E	Te	149,062
	Mustelidae	<i>Martes martes</i>	STIPB unnumbered specimen	C	Ar	1,300
		<i>Mustela putorius</i>	STIPB unnumbered specimen	C	Te	809
	Ursidae	<i>Ursus americanus</i>	MNHN-ZM-MO-1902-1415	E	Te	132,405
<b>Cingulata</b>	Dasypodidae	<i>Dasypus novemcinctus</i>	MNHN-ZM-MO-2001-1317	C	Te	3,949
<b>Diprotodontia</b>	Macropodidae	<i>Macropus giganteus</i>	MNHN-ZM-AC-A10098	C	Te	41,455
		<i>Thylogale stigmatica</i>	umzc:vertebrates:a12.44/1*	C	Te	4,306
	Potoroidae	<i>Aepyprymnus rufescens</i>	msu:mr:mr.4680*	C	Fo	2,820
	Vombatidae	<i>Vombatus ursinus</i>	MNHN-ZM-AC-A3289	C	Fo	25,750
<b>Eulipotyphla</b>	Erinaceidae	<i>Erinaceus europaeus</i>	STIPB unnumbered specimen	C	Te	778

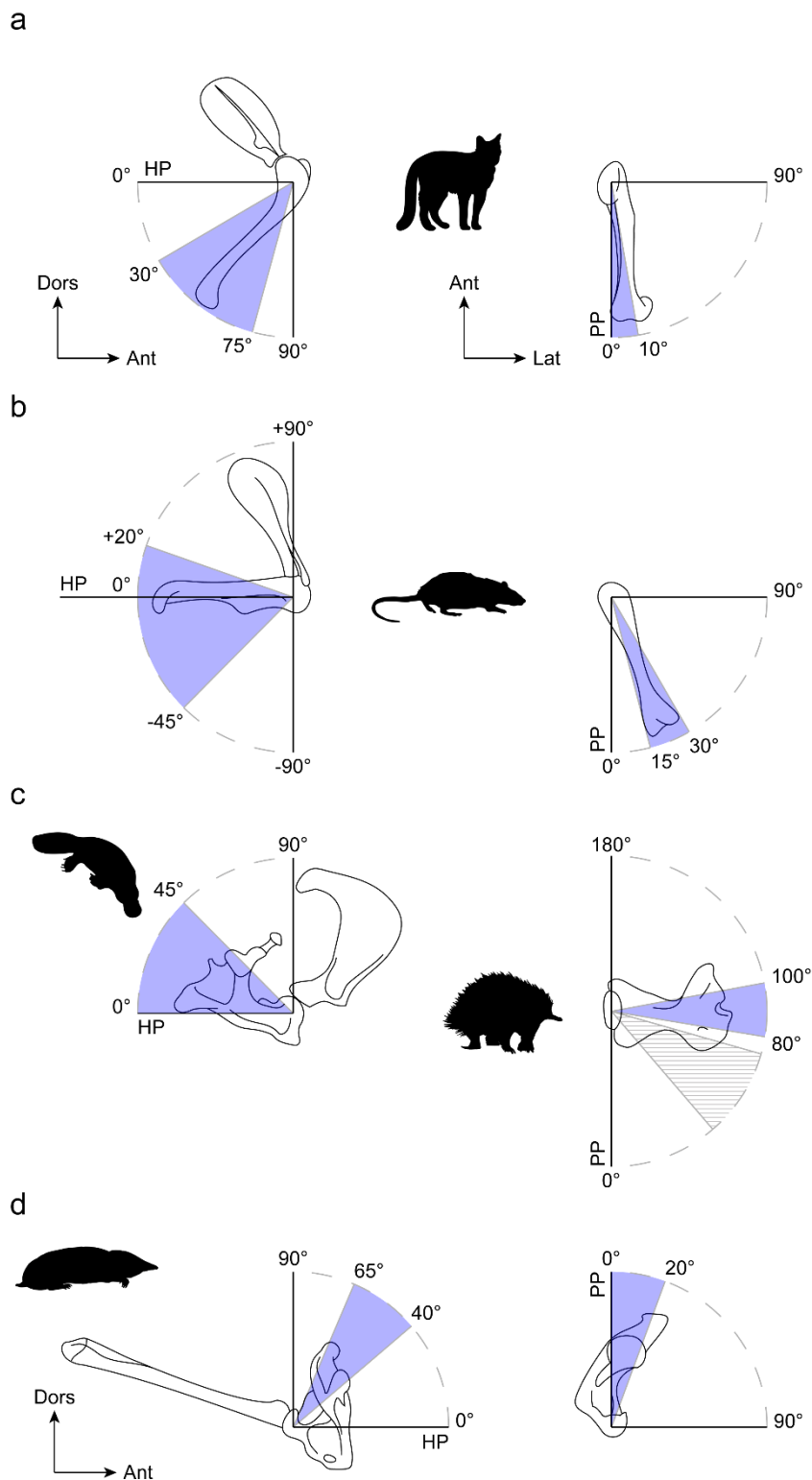
	Solenodontidae	<i>Solenodon paradoxus</i>	MNHN-ZM-MO-1980-237	C	Fo	900
	Talpidae	<i>Euroscaptor micrura</i>	MNHN-ZM-MO-1959-1795	M	Fo	60
		<i>Talpa europaea</i>	MNHN-ZM-MO-1953-829	M	Fo	110
		<i>Talpa europaea</i>	STIPB unnumbered specimen	M	Fo	110
<b>Monotremata</b>	Ornithorhynchidae	<i>Ornithorhynchus anatinus</i>	MNHN-ZM-AC-1906-484	S	Aq	1,225
	Tachyglossidae	<i>Tachyglossus aculeatus</i>	MNHN-ZM-AC-1884-1125	S	Fo	3,170
<b>Pholidota</b>	Manidae	<i>Smutsia temminckii</i>	MNHN-ZM-AC-1897-134	C	Te	9,587
<b>Primates</b>	Cercopithecidae	<i>Chlorocebus aethiops</i>	MNHN-ZM-AC-1909-262	E	Ar	5,104
		<i>Macaca radiata</i>	MNHN-ZM-AC-1845-271	E	Ar	5,132
	Hominidae	<i>Pan paniscus</i>	amnh:mammals:m-202870*	E	Ar	35,120
	Lemuridae	<i>Lemur catta</i>	MNHN-ZM-AC-1910-101	C	Ar	2,555
<b>Rodentia</b>	Dipodidae	<i>Allactaga elater</i>	UF:mammal:30045*	C	Fo	59
		<i>Dipodomys ordii</i>	MNHN-ZM-MO-1958-294	C	Fo	50
		<i>Zapus princeps</i>	uwbm:mammal specimens:74482*	C	Te	28
		<i>Zapus trinotatus</i>	uwbm:mammal specimens:OG-7813*	C	Te	27
	Hystriidae	<i>Hystrix cristata</i>	MNHN-ZM-AC-1922-386	C	Fo	19,167
	Muridae	<i>Gerbillus campestris</i>	MNHN-ZM-MO-1990-10	C	Fo	28
		<i>Meriones libycus</i>	MNHN-ZM-MO-1981-619	C	Fo	91
	Pedetidae	<i>Pedetes capensis</i>	MNHN-ZM-AC-1883-1640	C	Fo	2,775
	Sciuridae	<i>Marmota marmota</i>	STIPB unnumbered specimen	C	Fo	3,500
<b>Scandentia</b>	Tupaiaidae	<i>Tupaia belangeri</i>	STIPB unnumbered specimen	C	Ar	200
<b>Tubulidentata</b>	Orycteropodidae	<i>Orycteropus afer</i>	MNHN-ZM-AC-1919-19	C	Fo	56,175

141

142 is more abducted than in erect mammals (between 15 and 30 degrees relative to the parasagittal  
143 plane; Jenkins 1971); and the humerus is also more horizontal, with the elbow oscillating from  
144 about 20 degrees above the shoulder joint to 45 degrees below it. Both erect and crouched taxa  
145 belong to the same morpho-functional continuum, with graviportal and cursorial taxa  
146 representing the two extreme morphologies (Carrano 1999).

147 Besides this general pattern, some taxa have atypical humeral postures. The monotremes  
148 *Tachyglossus* and *Ornithorhynchus* have more “sprawling” humeri, akin to what exists in extant





149 **Fig. 1** Humeral excursion at the shoulder joint in lateral (left) and dorsal (right) views in several  
 150 mammals with different postures (Jenkins 1971; Pridmore 1985; Lin et al. 2019). The scapula  
 151 and humerus are shown in lateral view, but the scapula is not shown in dorsal view for better  
 152 visibility of the humerus. a. erect humerus in *Felis domestica*; b. crouched humerus in *Rattus*  
 153 *norvegicus*; c. sprawling humerus in *Ornithorhynchus* (left and hatched area on right) and

154 *Tachyglossus* (right); d. modified humerus in *Scalopus aquaticus*. Abbreviations: HP,  
155 horizontal plane; PP, parasagittal plane. Silhouettes come from phylopic.org

156 ectothermic reptiles (Bakker 1971; but see Pridmore 1985; Gambaryan and Kuznetsov 2013).  
157 In *Tachyglossus*, the humerus is completely horizontal. The elbow oscillates laterally between  
158 80 and 100 degrees from the parasagittal plane (Jenkins 1971; Pridmore 1985). In  
159 *Ornithorhynchus*, the humerus is abducted between 40 and 75 degrees from the parasagittal  
160 plane, with the elbow operating above the shoulder joint up to 45 degrees from the horizontal  
161 (Pridmore 1985). The forelimb of moles (Talpidae) is also highly modified in relation to their  
162 burrowing behaviour. The humerus is slightly abducted (less than 20 degrees from the  
163 parasagittal plane). The elbow oscillates cranially relative to the shoulder joint, rising 40 to 65  
164 degrees from the horizontal plane (Lin et al. 2019).

#### 165 DATA ACQUISITION

166 We measured various geometric and microanatomical parameters that have been previously  
167 associated in the literature with locomotion and posture, and more generally with lifestyle in  
168 amniotes (Canoville and Laurin 2009, 2010; Amson et al. 2014; Houssaye et al. 2016b;  
169 Houssaye and Botton-Divet 2018; Scheidt et al. 2019). This was done on cross-sections of  
170 mammalian humeral shafts obtained mainly from CT data retrieved from the literature and from  
171 morphosource.org. We scanned some of the specimens on the AST-RX platform of the Muséum  
172 national d'histoire naturelle and on the MRI platform of the Université de Montpellier. We  
173 extracted a cross-section from the CT data where the perimeter of the shaft was the smallest  
174 because this is an area where mechanical stresses generally are important (Beck et al. 1996;  
175 Tommasini et al. 2005; Campione and Evans 2012), resulting in more or less mid-diaphyseal  
176 cross-sections. We also incorporated into our data mid-diaphyseal traditional histological  
177 sections (unpublished data from Quemeneur et al. 2013). Mixing sections with slightly different  
178 reference planes in comparative studies is not considered a problem as long as the species of  
179 interest does not show excessive longitudinal microanatomical variation (Amson and Kolb  
180 2016; Houssaye et al. 2018). The scans were processed in ImageJ (Abràmoff et al. 2004) and  
181 MorphoDig (Lebrun 2018). Each bone was oriented so that the section plane was as  
182 perpendicular as possible to the long axis of the diaphysis. Data for all left humeri were  
183 symmetrised so that the sample consisted of right side bones only. We binarised the cross-  
184 sections before taking our geometric and microanatomical measurements in ImageJ with the  
185 BoneJ plugin (Doube et al. 2010) and in R (R Core Team 2013) with the BoneProfileR package

186 (Girondot and Laurin 2003; Gônet et al. 2022). A sample of the mammalian cross-sections used  
187 in this study are presented in Fig. 2.

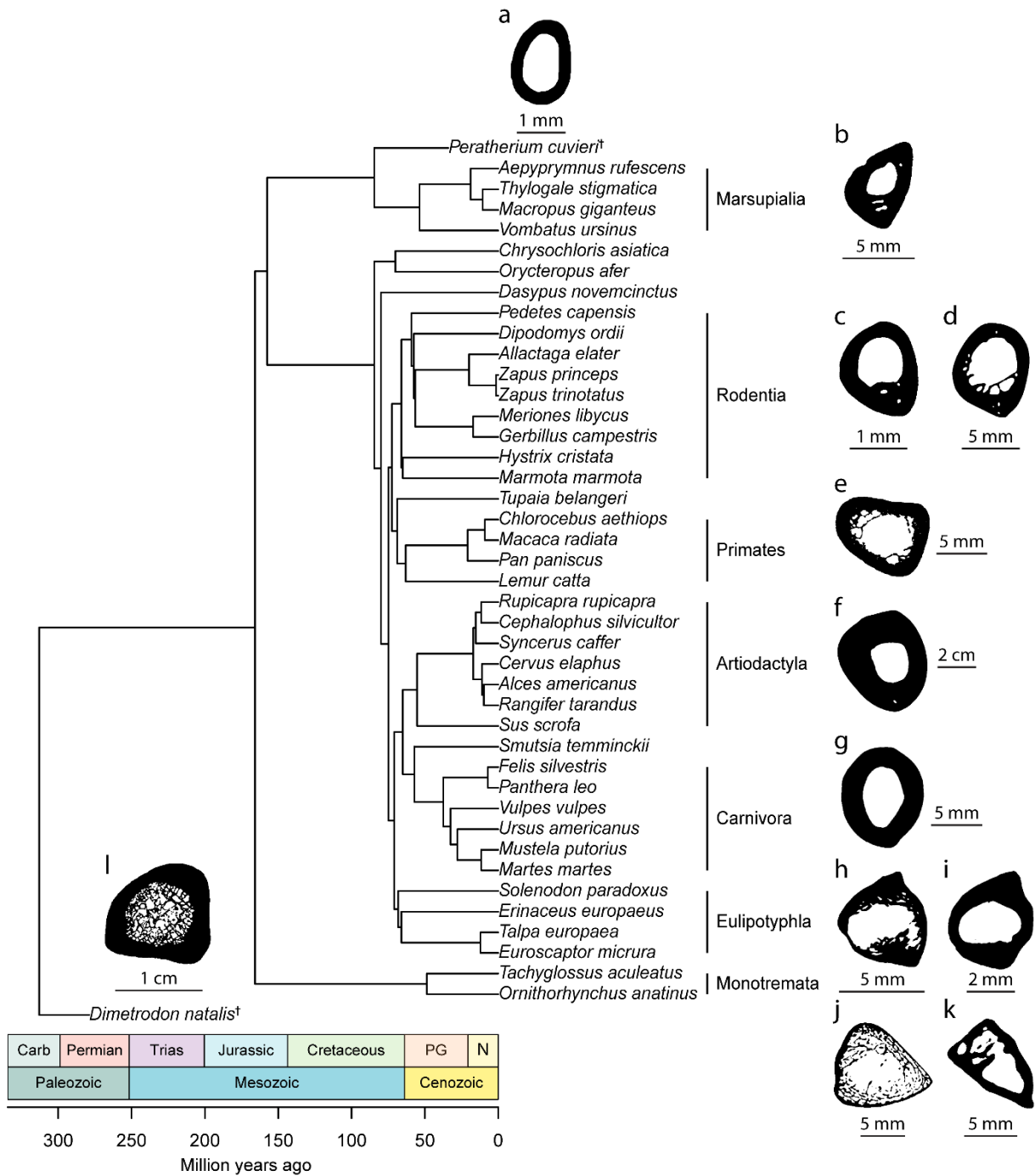
188 We measured six geometric parameters with BoneJ (Fig. 3):  $P_{\min}$ , the minimum  
189 perimeter of the shaft; BCSA, the area occupied by the bone on the section; TCSA, the total  
190 area of the section; Ecc, the eccentricity of the section corresponding to the ratio of the area  
191 moments of inertia (I) around the major and minor axes ( $I_{\max}/I_{\min}$ ); SR, the slenderness ratio (a  
192 high SR indicates a slender bone, while a low SR indicates a more robust bone; see Eq. 1);  $Z_{\text{pol}}$ ,  
193 the polar section modulus reflecting the resistance of the shaft to torsion (the higher  $Z_{\text{pol}}$ , the  
194 more resistant the bone will be to torsion). Although  $Z_{\text{pol}}$  can be used with subcircular cross-  
195 sections, which is the case for most taxa, it is ideally used with circular cross-sections.

196 
$$\text{Slenderness ratio} = \frac{\text{Bone length}}{\sqrt{\frac{I_{\min}}{\text{TCSA}}}} \quad (1)$$

197 We used BoneProfileR to measure seven microanatomical parameters (Fig. 3). We set  
198 BoneProfileR to determine the position of the centre of unmineralisation, i.e. the centre of the  
199 unmineralised spaces in the bone section, and segment the cross-section into 100 concentric  
200 circles. Bone compactness (measured by the number of bone pixels relative to the total number  
201 of pixels) was measured in each circle from the centre of the medulla to the edge of the cross-  
202 section. We extracted several parameters from the resulting compactness profiles: P, the  
203 distance of the medullo-cortical transition from the centre of the cross-section (a high P  
204 generally reflects low bone compactness); S, the inverse of the asymptote of the slope at point  
205 P (a high S corresponds to a gradual transition between the medulla and the cortex, as in the  
206 case of cancellous bone, while a low S reflects an abrupt transition). BoneProfileR also  
207 computes an observed global compactness value,  $C_{\text{obs}}$ . In addition, we performed a radial  
208 analysis to extract the radial component of the parameters P and S: the cross-section is  
209 segmented into 60 slices of 6 degrees and a compactness profile is drawn for each slice. The  
210 radial component of P (RP) and S (RS) is the average of the P and S of all slices. The standard  
211 deviation associated with RP and RS is RPSD and RSSD, respectively. When a species in our  
212 sample was represented by more than one individual, we calculated the mean value for each  
213 microanatomical parameter.

#### 214 BUILDING REFERENCE PHYLOGENIES

215 We constructed a set of 100 reference trees of mammals to include phylogenetic uncertainty in  
216 our statistical analyses (Fig. 2). The trees were manipulated in R using the packages phytools



217 **Fig. 2** Tree 1 of our set of 100 time-calibrated composite phylogenies displaying the  
 218 evolutionary relationships among the extant and extinct species included in this study, with  
 219 some of the humeral cross-sections analysed. a. *Peratherium cuvieri*† (Late Eocene) MNHN-  
 220 F-GY679b (unknown); b. *Aepyprymnus rufescens* MorphoSource: msu:mr:mr.4680  
 221 (crouched); c. *Meriones libycus* MNHN-ZM-MO-1981-619 (crouched); d. *Marmota marmota*  
 222 STIPB unnumbered specimen (crouched); e. *Macaca radiata* MNHN-ZM-AC-1845-271  
 223 (crouched); f. *Syncerus caffer* NHMUK ZD 1874.11.2.4 (erect); g. *Felis silvestris* UFGK  
 224 unnumbered specimen (erect); h. *Talpa europaea* MNHN-ZM-MO-1953-829 (modified); i.  
 225 *Euroscaptor micrura* MNHN-ZM-MO-1959-1795 (modified); j. *Tachyglossus aculeatus*

226 MNHN-ZM-AC-1884-1125 (sprawling); k. *Ornithorhynchus anatinus* MNHN-ZM-AC-1906-  
227 484 (sprawling); l. *Dimetrodon natalis*† (Early Permian) IPBSH-4 (unknown). The cross-  
228 sections are anatomically oriented (anterior to the top and lateral to the right) except for  
229 *Dimetrodon*. Trees were compiled in R using the work of Selva (2017), Upham et al. (2019),  
230 and Didier and Laurin (2020)

231 (Revell 2012) and TreePar (Stadler 2011). We extracted 100 trees with only the species of  
232 interest from a distribution of 10 000 Bayesian supertrees of mammals calibrated in time (node-  
233 dating, 5911 species) from the publication of Upham et al. (2019) and available on [vertlife.org](http://vertlife.org).  
234 The statistical analyses in this study required reference trees that included the taxa for which  
235 we wanted to infer posture. Therefore, *Dimetrodon natalis* (Sphenacodontidae) was branched  
236 at 313 Ma based on Didier and Laurin (2020). We followed Selva (2017) in considering  
237 *Peratherium cuvieri* an herpetotheriid and set the age of divergence between Herpetotheriidae  
238 and Marsupialia at approximately 85 Ma. The trees in Newick tree format are provided in  
239 Online Resource 2

#### 240 BODY MASS ESTIMATES AND LIFESTYLE

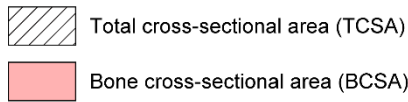
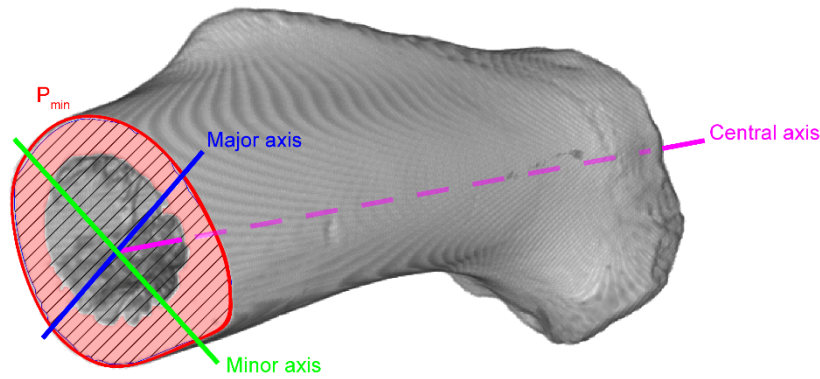
241 Body mass affects posture and bone microanatomy. Indeed, among mammals, the largest taxa  
242 tend to have more erect/upright limbs and greater bone compactness (Biewener 1989b, 2005;  
243 Hutchinson 2021). We therefore collected body mass estimates from the literature for each  
244 taxon in our sample (Table 1; Online Resource 1). We relied entirely on the database of  
245 Myhrvold et al. (2015), which compiles median body mass for a large number of extant  
246 amniotes (we rounded values to the nearest gram).

247 Lifestyle is also known to be related to bone microanatomy, e.g., fossorial talpids have  
248 greater extension of the medullo-cortical transition compared to terrestrial talpids (Meier et al.  
249 2013). Thus, we defined four lifestyle categories based on limb use (semi-aquatic, terrestrial,  
250 fossorial, and arboreal) to explore the potential relationship between lifestyle and posture (Table  
251 1; Online Resource 1).

#### 252 STATISTICAL TREATMENT IN A PHYLOGENETIC FRAMEWORK

253 *Phylogenetic signal*—We used the `phylosig` function from the R package `phytools` (Revell 2012)  
254 to estimate the phylogenetic signal in each geometric and microanatomical parameters. The  
255 `phylosig` function computes the K-statistic of Blomberg et al. (2003). A K-statistic greater than  
256 1 indicates that closely related species in the tree show more similarity between them than what

## Geometric parameters

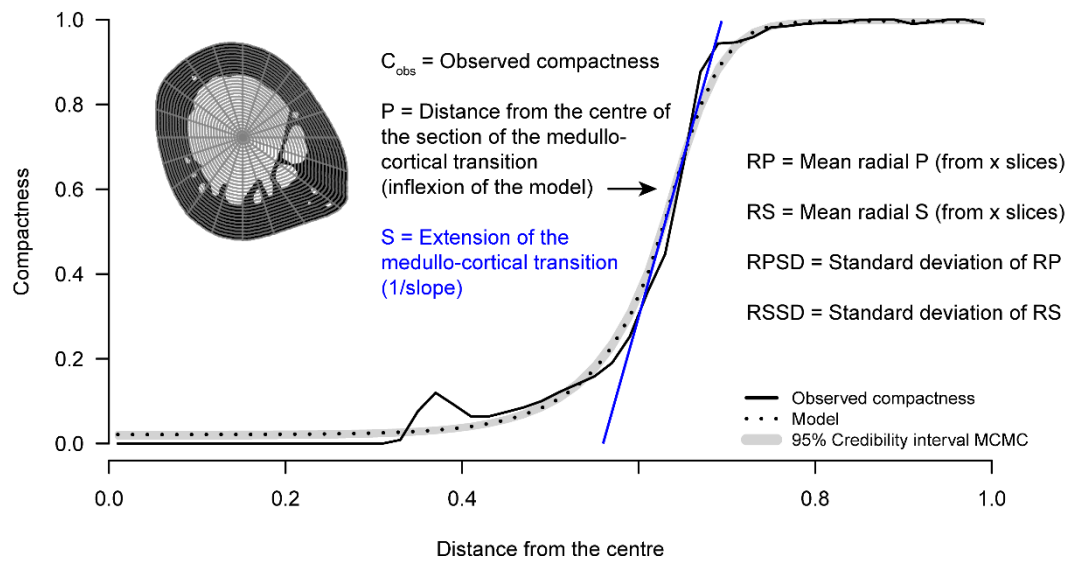


$$\text{Eccentricity (Ecc)} = \frac{\text{Area moment of inertia around the major axis } (I_{\max})}{\text{Area moment of inertia around the minor axis } (I_{\min})}$$

$$\text{Polar section modulus } (Z_{\text{pol}}) = \frac{\text{Polar moment of inertia (around the central axis)}}{\text{Radius of the cross-section}}$$

$$\text{Slenderness ratio (SR)} = \frac{\text{Bone length}}{\sqrt{\frac{I_{\min}}{\text{TCSA}}}}$$

## Compactness parameters



257 **Fig. 3** All the geometric and compactness parameters measured in this study, illustrated here  
 258 with a humerus of *Marmota marmota* (STIPB unnumbered specimen). The section on the  
 259 compactness profile is divided into 30 concentric circles and 20 slices for better readability, but  
 260 more were used in the analyses (100 and 60, respectively)

261 would be expected with a Brownian model of evolution, suggesting the existence of a  
 262 substantial phylogenetic signal in the data. Conversely, a K-statistic lower than 1 implies that

263 closely related species are more different than expected highlighting evolutionary convergence  
264 or higher variance between clades rather than within them.

265 We used the delta-statistic (Borges et al. 2019), which was designed for categorical  
266 traits, to estimate the phylogenetic signal in posture. The delta-statistic depends on the  
267 uncertainty associated with the inference of ancestral states: low uncertainty implies low  
268 entropy (Shannon 1948) and a high delta-statistic. The higher the delta, the stronger the  
269 phylogenetic signal.

270 A *P*-value is obtained by randomisation, i.e. a redistribution of the measured traits  
271 among terminal branches. *K* and delta are computed 1000 and 100 times, respectively, with a  
272 random distribution, then the pool of values obtained is compared to the *K* and delta with the  
273 actual distribution. This was done for each tree in our phylogenetic tree sample.

274 *Generalised least squares*—We used the *gls* function from the R package *nlme* (Pinheiro et al.  
275 2021) to model the relationship between each geometric and microanatomical parameter and  
276 postural groups while accounting for phylogeny, body mass and lifestyle. The *gls* function fits  
277 a linear model using least squares to optimise coefficients and allows a covariance structure to  
278 be set between observations. Here, the expected covariance of a given trait (or a relationship  
279 between traits) between two taxa corresponds to its evolution under a Brownian motion during  
280 the time from the root to their last common ancestor.

281 *Phylogenetic flexible discriminant analysis*—We used phylogenetic flexible discriminant  
282 analysis (PFDA) to explain posture in our sample of extant mammals and to predict humeral  
283 posture in *Dimetrodon* and *Peratherium* based on geometric and microanatomical  
284 measurements taken from humeral cross-sections while accounting for phylogeny. PFDA is a  
285 classification model based on a combination of linear regressions. It is derived from flexible  
286 discriminant analysis (FDA; Hastie et al. 1994) and corresponds to its phylogenetically  
287 informed version (Motani and Schmitz 2011). In practice, PFDA corresponds to a *gls* where  
288 categories are split in dummy variables and treated as continuous variables while phylogeny is  
289 incorporated as a phylogenetic covariance matrix whose terms are multiplied by lambda to  
290 make phylogenetic inertia variable through model optimisation (Pagel 1999). Lambda is  
291 assigned a value between 0 and 1 that minimises the model error, that is, the share of variance  
292 explained by phylogeny: 0 indicating that phylogeny does not explain the distribution of the  
293 trait on the tree; 1 indicating that phylogeny explains as much variance in the trait as is expected  
294 under a Brownian model of evolution.

295 Overfitting occurs when a model becomes overly complex by including too many  
296 parameters (Everitt and Skrondal 2010). An overfitted model will perform well in explaining  
297 initial data (training) but will perform poorly with new data or predictions (testing). The key to  
298 preventing overfitting lies in optimising the choice of parameters to include in the model in  
299 order to minimise test error. We chose the percentage of correct classification (PCC) obtained  
300 through leave-one-out cross-validation (CV; Stone 1974) as our selection criterion. The higher  
301 the PCC, the better the model performed under test conditions. Prior to performing CV  
302 procedures, we generated a dissimilarity matrix from the correlation coefficients of the  
303 geometric and microanatomical parameters before performing a hierarchical cluster analysis to  
304 identify and eliminate highly correlated variables (correlation coefficient  $> 0.95$ ) in order to  
305 avoid subsequent complications related to the existence of singular variance-covariance  
306 matrices.  $Pe_{min}$ , BCSA, TCSA and  $Z_{pol}$  were all inter-correlated. We decided to keep only the  
307 parameter  $Pe_{min}$  which, in a paleobiological inference context, is the easiest to measure and least  
308 likely to be impacted by taphonomy. The parameters P and RP were also correlated. We kept  
309 the former and removed the latter from our data set. CV was performed with all possible  
310 combinations of the remaining parameters ( $Pe_{min}$ , Ecc, SR,  $C_{obs}$ , P, S, RS, RPSD and RSSD)  
311 and for each of the 100 phylogenetic trees for a total of more than 50,000 CV. In the end, only  
312 three parameters out of the original 13 were retained for our humerus inference model ( $Pe_{min}$ ,  
313 SR and P). The R script and associated R environment allowing to replicate the postural  
314 inferences presented in the results of this study and allowing new inferences to be produced in  
315 other extinct synapsids from our dataset are available in Online Resources 3 and 4, respectively.

316 We then designed linear models in R to examine the association of the coordinates of  
317 the sampled taxa on the first and second axis of the PFDA model with body mass and lifestyle.  
318 When lifestyle was significant, we performed pairwise post-hoc tests with false discovery rate  
319 (FDR) correction using the `emmeans_test` function in the R package `rstatix` (Alboukadel 2021).

## 320 **Results**

### 321 PHYLOGENETIC SIGNAL IN THE DATA

322 All geometric parameters except for cross-sectional eccentricity (Ecc) were significantly  
323 associated with phylogeny (Table 2). For  $Pe_{min}$ , BCSA, TCSA and  $Z_{pol}$ , the K-statistic was  
324 below 1, indicating that intra-clade variation is greater than inter-clade variation and suggesting  
325 patterns of evolutionary convergence. However, K was close to 1 for the slenderness ratio (SR),  
326 implying that the distribution of this trait on the phylogeny is consistent with what would be



327 expected under a Brownian model of evolution and therefore reflects a phylogenetic signal. S  
 328 and RPSD were the only microanatomical parameters to be significantly associated with  
 329 phylogeny (Table 2). K was lower than 1 in each case, highlighting convergences. Posture also  
 330 contained a substantial phylogenetic signal ( $P$ -value < 0.01), with the delta-statistic ranging  
 331 from 3.194 to 21.471 (mean = 11.982; see Table 2).

332 **Table 2** Phylogenetic signal in the data. Values reported in the table are means obtain from 100  
 333 phylogenetic trees. The  $P$ -values for K (Blomberg et al. 2003) and delta (Borges et al. 2019)  
 334 are obtained from 1000 and 100 randomisations, respectively. Minimum and maximum values  
 335 obtained from our distribution of 100 phylogenetic trees are given in parentheses. All geometric  
 336 and microanatomical parameters, except ratios, were log-transformed in R

Parameter	K-statistic	Delta-statistic	$P$ -value
$Pe_{min}$	0.58 (0.506–0.706)		0.001** (0.001–0.003)
BCSA	0.616 (0.536–0.745)		0.001** (0.001–0.003)
TCSA	0.593 (0.517–0.72)		0.001** (0.001–0.002)
Ecc	0.185 (0.1–0.251)		0.403 (0.194–0.63)
$Z_{pol}$	0.611 (0.531–0.742)		0.001** (0.001–0.002)
SR	0.915 (0.796–1.069)		0.001** (0.001–0.001)
P	0.237 (0.15–0.298)		0.151 (0.051–0.334)
S	0.392 (0.339–0.472)		0.007** (0.002–0.017)
$C_{obs}$	0.169 (0.078–0.254)		0.52 (0.177–0.781)
RP	0.225 (0.144–0.279)		0.186 (0.092–0.359)
RS	0.272 (0.237–0.332)		0.059 (0.03–0.105)
RPSD	0.697 (0.586–0.813)		0.001** (0.001–0.003)
RSSD	0.234 (0.2–0.274)		0.159 (0.081–0.278)
Posture		11.982 (3.194–21.471)	< 0.001*** (< 0.001–0.01)

337

### 338 GEOMETRIC AND MICROANATOMICAL COMPARISON OF POSTURAL GROUPS

339 The microanatomical parameter S and the geometric parameters  $Pe_{min}$ , BCSA, TCSA,  $Z_{pol}$  and  
 340 SR are all significantly associated with posture (Table 3). They were also always significantly  
 341 related to body mass and never to lifestyle except for SR and S, which are significantly  
 342 associated with both body mass and lifestyle. RPSD was significantly associated only with

343 **Table 3** Effect of posture, body mass and functional ecology/lifestyle on the humeral geometric  
 344 and microanatomical parameters. Values reported are means obtained from 100 phylogenetic  
 345 trees. Minimum and maximum values obtained from our distribution of 100 phylogenetic trees  
 346 are given in parentheses. Body mass and all geometric and microanatomical parameters, except  
 347 ratios, were log-transformed in R. Abbreviations: BM, body mass; LS, lifestyle; POS, posture

GLS model formula	Independent variables	Chi-square values	P-values
<b>Pe<sub>min</sub> ~ BM + LS + POS</b>	POS	14.653 (10.196–18.37)	0.003** (< 0.001–0.017)
	BM	536.358 (366.969–653.646)	< 0.001***
	LS	3.311 (2.21–4.439)	0.35 (0.218–0.53)
<b>BCSA ~ BM + LS + POS</b>	POS	13.884 (12.008–15.852)	0.003** (0.001–0.007)
	BM	551.749 (509.506–597.076)	< 0.001***
	LS	4.869 (4.208–5.937)	0.184 (0.115–0.24)
<b>TCSA ~ BM + LS + POS</b>	POS	18.178 (13.67–22.052)	< 0.001*** (< 0.001–0.003)
	BM	645.451 (478.171–755.691)	< 0.001***
	LS	4.688 (3.44–6.266)	0.2 (0.099–0.329)
<b>Ecc ~ BM + LS + POS</b>	POS	0.55 (0.274–0.987)	0.907 (0.804–0.965)
	BM	0.02 (< 0.001–0.161)	0.912 (0.689–0.999)
	LS	3.645 (2.009–5.04)	0.311 (0.169–0.571)
<b>Z<sub>pol</sub> ~ BM + LS + POS</b>	POS	20.982 (18.529–23.534)	< 0.001***
	BM	794.205 (735.737–861.524)	< 0.001***
	LS	7.175 (5.93–8.665)	0.069 (0.034–0.115)
<b>SR ~ BM + LS + POS</b>	POS	15.664 (13.844–17.459)	0.001** (0.001–0.003)
	BM	13.869 (11.131–16.525)	< 0.001*** (< 0.001–0.001)
	LS	8.718 (7.529–10.025)	0.034* (0.018–0.057)
<b>P ~ BM + LS + POS</b>	POS	1.071 (0.614–1.524)	0.784 (0.677–0.893)
	BM	0.088 (0.003–0.248)	0.778 (0.619–0.956)
	LS	1.318 (0.848–1.788)	0.725 (0.617–0.838)
<b>S ~ BM + LS + POS</b>	POS	12.697 (11.016–14.076)	0.006** (0.003–0.012)
	BM	7.031 (6.033–8.012)	0.008** (0.005–0.014)
	LS	11.017 (9.13–13.989)	0.013* (0.003–0.028)
<b>C<sub>obs</sub> ~ BM + LS + POS</b>	POS	1.288 (0.51–2.173)	0.733 (0.537–0.917)

	BM	0.063 (0.002–0.183)	0.812 (0.668–0.961)
	LS	2.529 (1.128–4.161)	0.478 (0.245–0.77)
<b>RP ~ BM + LS + POS</b>	POS	1.404 (0.826–1.963)	0.705 (0.58–0.843)
	BM	0.025 (< 0.001–0.134)	0.895 (0.714–0.999)
	LS	2.234 (1.449–2.94)	0.527 (0.401–0.694)
<b>RS ~ BM + LS + POS</b>	POS	4.919 (4.157–6.171)	0.18 (0.104–0.245)
	BM	9.705 (8.243–11.851)	0.002** (0.001–0.004)
	LS	6.89 (5.721–8.036)	0.077 (0.045–0.126)
<b>RPSD ~ BM + LS + POS</b>	POS	5.334 (4.365–6.047)	0.151 (0.109–0.225)
	BM	1.048 (0.373–2.005)	0.317 (0.157–0.541)
	LS	9.671 (8.443–11.623)	0.022* (0.009–0.038)
<b>RSSD ~ BM + LS + POS</b>	POS	2.482 (2.011–3.219)	0.48 (0.359–0.57)
	BM	5.234 (4.379–6.227)	0.023* (0.013–0.036)
	LS	3.955 (3.268–4.565)	0.268 (0.207–0.352)

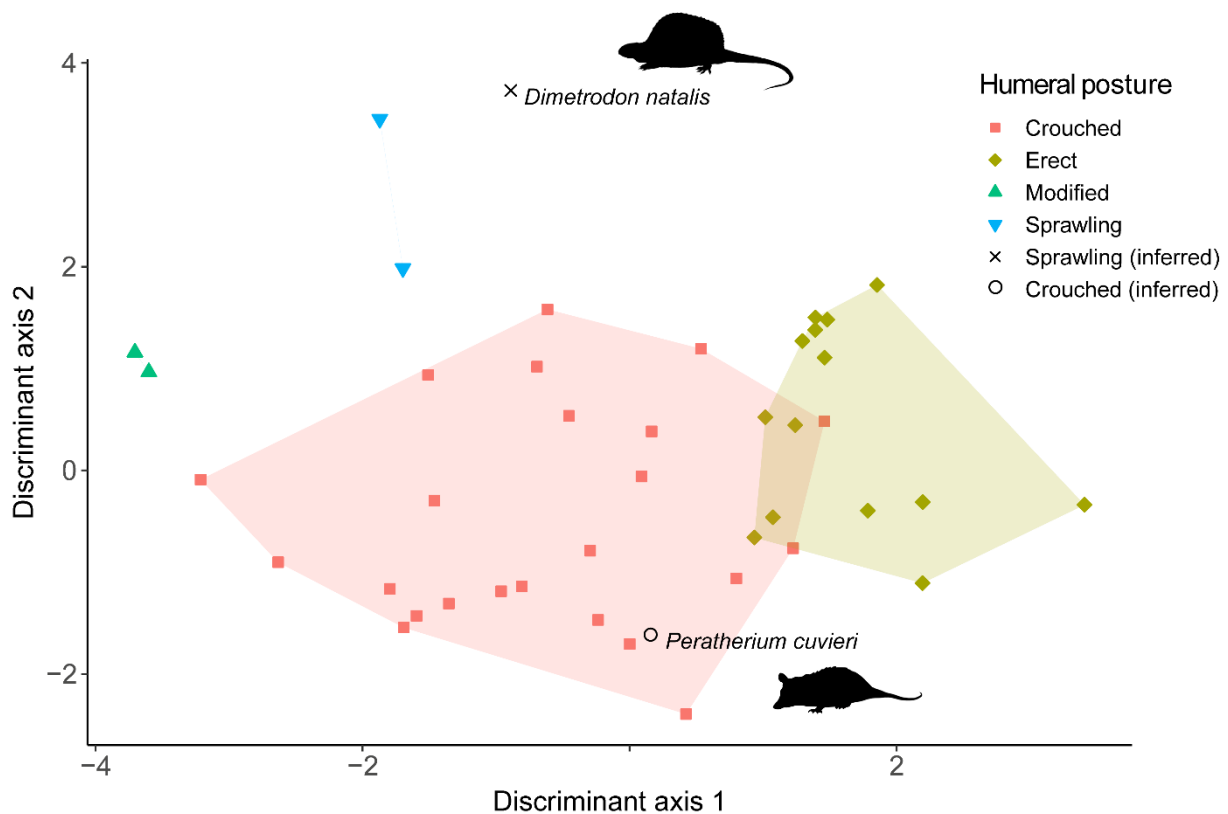
348

349 lifestyle, RS and RSSD only with body mass.

### 350 PHYLOGENETIC DISCRIMINATION OF POSTURAL GROUPS

351 The PFDA model was very successful in discriminating between postural groups (Fig. 4).  
352 Indeed, the mean training PCC reached 88% (88-90%). Most of the time, crouched taxa were  
353 correctly classified at 83% (19 out of 23 taxa). The rest of the time (5% of the phylogenetic  
354 trees), they reached 87% (20 out of 23 taxa). With all tree hypotheses, the erect taxa were  
355 correctly classified at 93% (13 out of 14 taxa) while the two monotremes (sprawling) and the  
356 two talpids (modified) both achieved 100% of correct classifications. *Dimetrodon* and  
357 *Peratherium* were always inferred as sprawling and crouched, respectively. Lambda ranged  
358 from 0.04 to 0.17 (mean = 0.099), indicating a present but low influence of the phylogeny.

359 Body mass was significantly associated with the taxon coordinates on the first and  
360 second PFDA axes (Table 4). Lifestyle was significantly associated with the taxon coordinates  
361 on the first PFDA axis but not on the second, although the *P*-values were close to the  
362 significance level. The results of the post-hoc tests with the first PFDA axis revealed that  
363 arboreal taxa were significantly different from semi-aquatic, terrestrial and fossorial taxa, and  
364 that fossorial taxa were significantly different from terrestrial taxa (Table 5).



365 **Fig. 4** Phylogenetic discriminant space generated from PFDA (Motani and Schmitz 2011) on  
 366 postural groups. Based on posterior probabilities, *Dimetrodon* and *Peratherium* are inferred as  
 367 “sprawling” and “crouched”, respectively. Silhouettes come from phylopic.org

## 368 Discussion

### 369 CROSS-SECTIONAL CHARACTERISATION OF POSTURAL GROUPS

370  $Pe_{min}$ , BCSA, TCSA were all significantly associated with posture (Table 3). This is not  
 371 surprising since  $Pe_{min}$  is related to body mass (Campione and Evans 2012), and it is well known  
 372 that body mass and limb posture in mammals are related (Biewener 1989b, 2005; Houssaye et  
 373 al. 2016b). Indeed, large mammals tend to have more erect (columnar) limbs that help reduce  
 374 tissue stress (Gregory 1912; Biewener 1989a, Gatesy and Biewener 1991; Hutchinson 2021).  
 375 This is because bone resists compression better than tension or torsion (Currey 2013).  
 376 Logically,  $Pe_{min}$  increases with increasing bone size and so do both BCSA and TCSA. The  
 377 significant association between  $Z_{pol}$  and posture is interesting, as it probably reveals a difference  
 378 in torsional strength in the humerus between postures. Indeed, previous studies, including in-  
 379 vivo measurements, have shown that crouched taxa, just like non-avian reptiles with a  
 380 sprawling posture, exhibit increased torsional stress compared to erect taxa, which are primarily  
 381 loaded in bending (Biewener 1990; Blob and Biewener 1999, 2001; Butcher et al. 2008, 2011).  
 382 SR and S are the only parameters to be significantly associated with both posture and lifestyle.

383 **Table 4** Effect of body mass and lifestyle on the taxon coordinates on the first and second axes  
 384 of the PFDA model. Values reported are means obtained from 100 phylogenetic trees.  
 385 Minimum and maximum values obtained from our distribution of 100 phylogenetic trees are  
 386 indicated in parentheses. Abbreviations: BM, body mass; LS, lifestyle

Linear model formula	Independent variable	F-value	P-value
Coordinates of the taxa on the first PFDA axis ~ BM + LS	BM	53.88 (46.071–57.117)	< 0.001***
	LS	11.807 (11.244–12.138)	< 0.001***
Coordinates of the taxa on the second PFDA axis ~ BM + LS	BM	14.499 (13.927–15.613)	< 0.001*** (< 0.001–0.001)
	LS	2.7 (2.526–2.767)	0.06 (0.056–0.073)

387

388 **Table 5** Differences in taxon coordinates on the first PFDA axis between lifestyle categories as  
 389 shown by pairwise comparison. Values reported are means obtained from 100 phylogenetic  
 390 trees. Minimum and maximum values obtained from our distribution of 100 phylogenetic trees  
 391 are indicated in parentheses. Abbreviations: Aq, semi-aquatic; Ar, arboreal; Fo, fossorial; Te,  
 392 terrestrial

Pairwise comparison	Adjusted P-value
Aq vs. Ar	0.006** (0.005–0.007)
Aq vs. Fo	0.547 (0.533–0.563)
Aq vs. Te	0.161 (0.145–0.171)
Ar vs. Fo	< 0.001***
Ar vs. Te	0.001** (< 0.001–0.001)
Fo vs. Te	0.028* (0.023–0.036)

393

394 Monotremes and talpids have some of the most robust humeri. These taxa are also the most  
 395 fossorial species in our sample. This is because burrowing habits generally go hand in hand  
 396 with robust, stocky forelimbs for digging in hard substrates (Shimer 1903). Similarly, the  
 397 primates in our sample have the slenderest humeri. They are also the species with the most  
 398 arboreal habits. Indeed, arboreal species generally have slender, elongated forelimbs that allow  
 399 them to move more efficiently in trees by increasing reach and reducing energy expenditure  
 400 during vertical climbing, as longer arms allow them to lean back more, thereby increasing  
 401 friction between the foot and the substrate (Preuschoft et al. 1996; Isler 2005). The talpids show

402 the highest S values in the sample. Two reasons can explain these high S values: (1) The  
403 presence of cancellous bone considerably extending the transition between the medulla and the  
404 cortex. The corresponding compactness profile is flattened, resulting in a low slope of the  
405 asymptote at point P and thus a high S value ( $S = 1/\text{slope}$ ). (2) Heterogeneity of cortical  
406 thickness. Variations in cortical thickness depending on the position within the cross-section  
407 mimic an extensive transition between the medulla and the cortex on the overall compactness  
408 profile. Talpids clearly show a thickening of the cortex antero-posteriorly (Fig. 2). An increase  
409 in mechanical stress in these regions, due to the attachment of strong muscles involved in the  
410 adduction/abduction cycle of the humerus (Rose et al. 2013), could explain these variations in  
411 cortical thickness.

#### 412 PHYLOGENETIC DISCRIMINANT MODEL AND PALAEOBIOLOGICAL INFERENCES

413 Lambda was always greater than 0, indicative of a phylogenetic involvement in the PFDA,  
414 which attempts to maximise the relationships between humeral posture and the  
415 microanatomical parameters. This result is far from being surprising. Indeed, we saw that  
416 humeral posture was significantly associated with phylogeny (Table 2). Nevertheless, this  
417 confirms our choice to use a classification method accounting for species relatedness. With a  
418 mean correct classification rate exceeding 85%, the PFDA model was very successful in  
419 discriminating the postural groups. Even monotremes and talpids, represented by only four  
420 individual taxa (*Ornithorhynchus* and *Tachyglossus*, and *Euroscaptor* and *Talpa*, respectively),  
421 are always correctly classified.

422 The Late Eocene herpetotheriid *Peratherium cuvieri* was inferred to be “crouched” with  
423 all trees in our phylogenetic tree sample. Studies on herpetotheriid locomotion are very sparse.  
424 Kurz (2005) designated *Amphiperatherium* and another undetermined herpetotheriid as  
425 “cursorial” based on lumbar vertebral morphology and tail length. Horovitz et al. (2008)  
426 described *Herpetotherium* as “agile” based on femoral morphology. The literature is more  
427 abundant regarding their extant close relatives, the Didelphidae. The didelphids are commonly  
428 used as models to study the evolution of therian locomotion (Jenkins 1971; Jenkins and Weijs  
429 1979; Argot 2001; Butcher et al. 2011). Didelphids, like most small mammals, have a crouched  
430 posture (Jenkins 1971). Thus, a crouched posture in *Peratherium cuvieri* is deemed very  
431 plausible.

432 The posture of permo-carboniferous synapsids (the earliest stem mammals) has been  
433 extensively studied, in comparison to that of herpetotheriids. Indeed, it is widely accepted,

434 based on anatomical, biomechanical, and ichnological evidence, that the earliest stem mammals  
435 had sprawling limbs (Jenkins 1973; Hunt and Lucas 1998; Blob 2001; Benton 2015; Hopson  
436 2015; Wright 2018; Cavanaugh 2021). Therefore, it is not surprising that *Dimetrodon natalis*  
437 was inferred to be a sprawler by the PFDA model, although the postural has not yet been clearly  
438 established. Sometimes described as “lizard-like” (Bakker 1971; Desmond 1975), the sprawling  
439 posture of monotremes may in fact be close to the ancestral condition of synapsids, yet distinct  
440 from the sprawling posture of squamates and urodeles (Gambaryan and Kuznetsov 2013;  
441 Regnault et al. 2020), or it may be derived from early mammals with parasagittal limbs  
442 (Pridmore 1985). Similarly in reptiles, Crocodylia, with their “semi-erect” limbs, were  
443 commonly considered “primitive” posturally (Bakker 1971; Charig 1972), when in fact they  
444 are descended from more erect forms (Parrish 1987; Gatesy 1991; Reilly and Elias 1998).

445         Body mass seems to have a confounding effect on our PFDA model (Table 4). This is  
446 not surprising since the parameter  $P_{e_{min}}$  was used in the model. We have already mentioned  
447 that body mass and posture in mammals are strongly intertwined. But we do not see this as a  
448 problem, on the contrary. Indeed, our goal is to build a model that can effectively discriminate  
449 between humeral postures in mammals based on easily measurable parameters, including in  
450 fossils, so that inferences can be produced for extinct taxa. If body mass is a powerful parameter  
451 to achieve this goal, we should use it by including, or rather not excluding, parameters  
452 associated with it, such as the perimeter of the cross-section. However, body mass, although  
453 useful, is not sufficient to distinguish between postures. Indeed, some species have equivalent  
454 body mass but different posture; e.g. *Marmota* (crouched) and *Tachyglossus* (sprawling). It  
455 should also be mentioned here that some ungulates with erect limbs weigh less than 10 kg, e.g.  
456 dik-diks (genus *Madoqua*). The case of small ungulates, although beyond the scope of this  
457 study, is worthy of further investigation. Therefore, we believe that femoral geometric and  
458 microanatomical parameters contain a functional signal that a multivariate quantitative  
459 approach such as PFDA can effectively exploit. Lifestyle was significantly associated with the  
460 first axis of the model and was close to the significance level for the second axis. This is most  
461 likely due to the presence of SR in the model. Indeed, we saw that the slenderness ratio was  
462 significantly associated with both body mass, posture, and lifestyle.

463         Post-hoc tests revealed that virtually all lifestyle categories were significantly different  
464 on the first axis with the exception of semi-aquatic taxa (Table 5). However, the only semi-  
465 aquatic taxon in our analysis was *Ornithorhynchus*. Therefore, this is most likely due to the  
466 small sample size, which results in a lack of statistical power. However, semi-aquatic taxa, such

467 as otters, deserve a separate study, as they tend to show pachyostosis and/or osteosclerosis (an  
468 increase in periosteal bone deposits and widespread spongiosa, respectively), which affect  
469 buoyancy (Houssaye et al. 2016a).

470 At first glance, it is surprising that  $S$  was not retained in the PFDA model since it seems  
471 to be significantly associated with posture unlike  $P$  (Table 3). However, the result of the cross-  
472 validation with the parameters  $Pe_{min}$ ,  $SR$  and  $S$  gives only 73% of correct classification (15%  
473 less compared to the original model). Ultimately, joint use of  $Pe_{min}$ ,  $SR$  and  $P$  seems to be the  
474 best parameter configuration to discriminate mammalian posture with our sample.

## 475 **Conclusion**

476 Using generalised least squares, we showed that all parameters that were significantly  
477 associated with posture, i.e. minimum humeral shaft perimeter ( $Pe_{min}$ ), bone cross-sectional  
478 area (BCSA), total cross-sectional area (TCSA), polar section modulus ( $Z_{pol}$ ), slenderness ratio  
479 (SR) and the reciprocal of the slope of the asymptote at point  $P$  on the compactness profile ( $S$ ),  
480 were also significantly associated with body mass. This was expected as body mass is known  
481 to have an impact on posture in mammals, with smaller species having a crouched posture and  
482 larger species having more erect limbs to minimise body weight-induced stresses. The  
483 association between  $Z_{pol}$  and posture was also expected since  $Z_{pol}$  corresponds to the resistance  
484 of the shaft to torsion, and previous studies have shown that in mammals (and other taxa)  
485 crouched limbs are subject to higher torsional stresses than erect limbs, which are primarily  
486 loaded in flexion. We showed that  $SR$  and  $S$  were also related to lifestyle, with burrowing taxa  
487 having more robust humeri and arboreal taxa having slender humeri, and moles exhibiting  
488 heterogeneity in cortical thickness most likely related to the attachment of strong muscles on  
489 the anterior and posterior surfaces of the humerus involved in the limb adduction/abduction  
490 cycle.

491 A number of parameters were significantly associated with phylogeny ( $Pe_{min}$ , BSCA,  
492 TCSA,  $Z_{pol}$ ,  $S$  and RPSD), as well as posture itself. The lambda values from the PFDA model  
493 indicated an influence of the phylogeny in the data, justifying the use of a phylogenetically  
494 informed classification method. Elimination of overly correlated parameters followed by cross-  
495 validation procedures ultimately yielded a PFDA model with three variables ( $Pe_{min}$ ,  $SR$  and  $P$ )  
496 that successfully discriminated postural groups (88% average correct classification into four  
497 categories based on 100 mammalian phylogenetic trees). Despite the small sample size, the  
498 model was able to correctly classify moles (modified humeral posture) and monotremes



499 (sprawling humeral posture). Application of the model to extinct taxa yielded plausible results.  
500 *Peratherium cuvieri* and *Dimetrodon natalis* are inferred to have had a crouched and sprawling  
501 humeral posture, respectively. The PFDA model appeared to be significantly influenced by  
502 body mass and lifestyle, but nevertheless allows quantitative postural discrimination that size  
503 or lifestyle parameters alone would not achieve, while producing plausible inferences in extinct  
504 taxa.

505 Our study highlights the complex interplay between body mass, lifestyle, posture and  
506 the geometry and microanatomy of the humerus in mammals. Our model can be used by  
507 palaeontologists to infer the humeral posture of other extinct species based on humeral cross-  
508 sections alone. Extending our method to other appendicular skeletal elements could refine the  
509 inferences produced for extinct taxa, particularly those relevant to the context of shifts in limb  
510 posture (more sprawling to more erect/parasagittal limbs) in early mammals, which tend to  
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532 *Data availability*—The data that support the findings of this study are included in this published  
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534 *Conflict of interest*—The authors declare that they have no conflicts of interest.

## 535 **References**

536 Abbott CP (2019) The *Dimetrodon* dilemma: reassessing posture in sphenacodontians and  
537 related non-mammalian synapsids. Undergraduate honors dissertation, College of  
538 William and Mary

539 Abràmoff MD, Magalhães PJ, Ram SJ (2004) Image processing with ImageJ. *Biophotonics Int*  
540 11:36–42

541 Alboukadel K (2021) rstatix: pipe-friendly framework for basic statistical tests. R package  
542 version 0.7.0

543 Amson E, Kolb C (2016) Scaling effect on the mid-diaphysis properties of long bones—the  
544 case of the Cervidae (deer). *Sci Nat* 103:1–10. [https://doi.org/10.1007/s00114-016-](https://doi.org/10.1007/s00114-016-1379-7)  
545 1379-7

546 Amson E, de Muizon C, Laurin M, Argot C, de Buffrénil V (2014) Gradual adaptation of bone  
547 structure to aquatic lifestyle in extinct sloths from Peru. *Proc R Soc Lond B Biol Sci*  
548 281:20140192. <https://doi.org/10.1098/rspb.2014.0192>

549 Argot C (2001) Functional-adaptive anatomy of the forelimb in the Didelphidae, and the  
550 paleobiology of the Paleocene marsupials *Mayulestes ferox* and *Pucadelphys andinus*. *J*  
551 *Morphol* 247:51–79. [https://doi.org/10.1002/1097-4687\(200101\)247:1<51::AID-](https://doi.org/10.1002/1097-4687(200101)247:1<51::AID-JMOR1003>3.0.CO;2-%23)  
552 *JMOR1003*>3.0.CO;2-%23

553 Bakker RT (1971) Dinosaur physiology and the origin of mammals. *Evolution* 25:636–658.  
554 <https://doi.org/10.1111/j.1558-5646.1971.tb01922.x>

555 Beck TJ, Ruff CB, Mourtada FA, Shaffer RA, Maxwell-Williams K, Kao GL, Sartoris DJ,  
556 Brodine S (1996) Dual-energy X-ray absorptiometry derived structural geometry for

557 stress fracture prediction in male U.S. marine corps recruits. *J Bone Miner Res* 11:645–  
558 653. <https://doi.org/10.1002/jbmr.5650110512>

559 Benton MJ (2015) *Vertebrate Palaeontology*. Wiley, Hoboken

560 Biewener AA (1989a) Mammalian terrestrial locomotion and size. *Bioscience* 39:776–783.  
561 <https://doi.org/10.2307/1311183>

562 Biewener AA (1989b) Scaling body support in mammals: limb posture and muscle mechanics.  
563 *Science* 245:45–48. <https://doi.org/10.1126/science.2740914>

564 Biewener AA (1990) Biomechanics of mammalian terrestrial locomotion. *Science* 250:1097–  
565 1103. <https://doi.org/10.1126/science.2251499>

566 Biewener AA (2005) Biomechanical consequences of scaling. *J Exp Biol* 208:1665–1676.  
567 <https://doi.org/10.1242/jeb.01520>

568 Bishop PJ, Hocknull SA, Clemente CJ, Hutchinson JR, Barrett RS, Lloyd DG (2018a)  
569 Cancellous bone and theropod dinosaur locomotion. Part II—a new approach to  
570 inferring posture and locomotor biomechanics in extinct tetrapod vertebrates. *PeerJ*  
571 6:e5779. <https://doi.org/10.7717/peerj.5779>

572 Bishop PJ, Hocknull SA, Clemente CJ, Hutchinson JR, Farke AA, Barrett RS, Lloyd DG  
573 (2018b) Cancellous bone and theropod dinosaur locomotion. Part III—inferring posture  
574 and locomotor biomechanics in extinct theropods, and its evolution on the line to birds.  
575 *PeerJ* 6:e5777. <https://doi.org/10.7717/peerj.5777>

576 Bishop PJ, Hocknull SA, Clemente CJ, Hutchinson JR, Farke AA, Beck BR, Barrett RS, Lloyd  
577 DG (2018c) Cancellous bone and theropod dinosaur locomotion. Part I—an  
578 examination of cancellous bone architecture in the hindlimb bones of theropods. *PeerJ*  
579 6:e5778. <https://doi.org/10.7717/peerj.5778>

580 Blob RW (2000) Interspecific scaling of the hindlimb skeleton in lizards, crocodylians, felids  
581 and canids: does limb bone shape correlate with limb posture? *J Zool* 250:507–531.  
582 <https://doi.org/10.1111/j.1469-7998.2000.tb00793.x>

583 Blob RW (2001) Evolution of hindlimb posture in nonmammalian therapsids: biomechanical  
584 tests of paleontological hypotheses. *Paleobiology* 27:14–38.  
585 [https://doi.org/10.1666/0094-8373\(2001\)0272.0.CO;2](https://doi.org/10.1666/0094-8373(2001)0272.0.CO;2)

- 586 Blob RW, Biewener AA (1999) In vivo locomotor strain in the hindlimb bones of *Alligator*  
587 *mississippiensis* and *Iguana iguana*: implications for the evolution of limb bone safety  
588 factor and non-sprawling limb posture. *J Exp Biol* 202:1023–1046.  
589 <https://doi.org/10.1242/jeb.202.9.1023>
- 590 Blob RW, Biewener AA (2001) Mechanics of limb bone loading during terrestrial locomotion  
591 in the green iguana (*Iguana iguana*) and American alligator (*Alligator mississippiensis*).  
592 *J Exp Biol* 204:1099–1122. <https://doi.org/10.1242/jeb.204.6.1099>
- 593 Blomberg SP, Garland T, Ives AR (2003) Testing for phylogenetic signal in comparative data:  
594 behavioral traits are more labile. *Evolution* 57:717–745. [https://doi.org/10.1111/j.0014-](https://doi.org/10.1111/j.0014-3820.2003.tb00285.x)  
595 [3820.2003.tb00285.x](https://doi.org/10.1111/j.0014-3820.2003.tb00285.x)
- 596 Borges R, Machado JP, Gomes C, Rocha AP, Antunes A (2019) Measuring phylogenetic signal  
597 between categorical traits and phylogenies. *Bioinformatics* 35:1862–1869.  
598 <https://doi.org/10.1093/bioinformatics/bty800>
- 599 Brocklehurst N, Kammerer CF, Fröbisch J (2013) The early evolution of synapsids, and the  
600 influence of sampling on their fossil record. *Paleobiology* 39:470–490.  
601 <https://doi.org/10.1666/12049>
- 602 Brocklehurst RJ, Fahn-Lai P, Regnault S, Pierce SE (2022) Musculoskeletal modeling of  
603 sprawling and parasagittal forelimbs provides insight into synapsid postural transition.  
604 *iScience* 25:103578. <https://doi.org/10.1016/j.isci.2021.103578>
- 605 Butcher MT, Espinoza NR, Cirilo SR, Blob RW (2008) In vivo strains in the femur of river  
606 cooter turtles (*Pseudemys concinna*) during terrestrial locomotion: tests of force-  
607 platform models of loading mechanics. *J Exp Biol* 211:2397–2407.  
608 <https://doi.org/10.1242/jeb.018986>
- 609 Butcher MT, White BJ, Hudzik NB, Gosnell WC, Parrish JH, Blob RW (2011) In vivo strains  
610 in the femur of the Virginia opossum (*Didelphis virginiana*) during terrestrial  
611 locomotion: testing hypotheses of evolutionary shifts in mammalian bone loading and  
612 design. *J Exp Biol* 214:2631–2640. <https://doi.org/10.1242/jeb.049544>
- 613 Campione NE, Evans DC (2012) A universal scaling relationship between body mass and  
614 proximal limb bone dimensions in quadrupedal terrestrial tetrapods. *BMC Biol* 10:1–  
615 22. <https://doi.org/10.1186/1741-7007-10-60>

- 616 Canoville A, Laurin M (2009) Microanatomical diversity of the humerus and lifestyle in  
617 lissamphibians. *Acta Zool* 90:110–122. [https://doi.org/10.1111/j.1463-](https://doi.org/10.1111/j.1463-6395.2008.00328.x)  
618 [6395.2008.00328.x](https://doi.org/10.1111/j.1463-6395.2008.00328.x)
- 619 Canoville A, Laurin M (2010) Evolution of humeral microanatomy and lifestyle in amniotes,  
620 and some comments on palaeobiological inferences. *Biol J Linn Soc Lond* 100:384–  
621 406. <https://doi.org/10.1111/j.1095-8312.2010.01431.x>
- 622 Canoville A, de Buffrénil V, Laurin M (2021) Bone microanatomy and lifestyle in tetrapods.  
623 In: de Buffrénil V, de Ricqlès AJ, Zylberberg L, Padian K, Laurin M, Quilhac A (eds)  
624 *Vertebrate Skeletal Histology and Paleohistology*. CRC Press, Boca Raton, pp 724–743
- 625 Carrano MT (1999) What, if anything, is a cursor? Categories versus continua for determining  
626 locomotor habit in mammals and dinosaurs. *J Zool* 247:29–42.  
627 <https://doi.org/10.1111/j.1469-7998.1999.tb00190.x>
- 628 Cavanaugh T (2021) Reconstructing body size and center of mass in synapsids. Dissertation,  
629 Harvard University
- 630 Charig AJ (1972) The evolution of the archosaur pelvis and hindlimb: an explanation in  
631 functional terms. In: Joysey KA, Kemp TS (eds) *Studies in Vertebrate Evolution*. Oliver  
632 and Boyd, Edinburgh, pp 121–155
- 633 Cooper LN, Clementz MT, Usip S, Bajpai S, Hussain ST, Hieronymus TL (2016) Aquatic  
634 habits of cetacean ancestors: integrating bone microanatomy and stable isotopes. *Integr*  
635 *Comp Biol* 56:1370–1384. <https://doi.org/10.1093/icb/icw119>
- 636 Currey JD (2013) *Bones: Structure and Mechanics*. Princeton University Press, Princeton.  
637 <https://doi.org/10.1515/9781400849505>
- 638 D’Août K, Vereecke EE, Schoonaert K, De Clercq D, Van Elsacker L, Aerts P (2004)  
639 Locomotion in bonobos (*Pan paniscus*): differences and similarities between bipedal  
640 and quadrupedal terrestrial walking, and a comparison with other locomotor modes. *J*  
641 *Anat* 204:353–361. <https://doi.org/10.1111/j.0021-8782.2004.00292.x>
- 642 Debuysschere M (2015) Origine et première diversification des mammaliaformes : apport des  
643 faunes du Trias supérieur de Lorraine, France. Dissertation, Muséum national d’Histoire  
644 naturelle, Paris Desmond AJ (1975) *The Hot-Blooded Dinosaurs*. Blond and Briggs,  
645 London

646 Didier G, Laurin M (2020) Exact distribution of divergence times from fossil ages and tree  
647 topologies. *Syst Biol* 69:1068–1087. <https://doi.org/10.1093/sysbio/syaa021>

648 Doube M, Klosowski MM, Arganda-Carreras I, Cordelières FP, Dougherty RP, Jackson JS,  
649 Schmid B, Hutchinson JR, Shefelbine SJ (2010) BoneJ: free and extensible bone image  
650 analysis in ImageJ. *Bone* 47:1076–1079. <https://doi.org/10.1016/j.bone.2010.08.023>

651 Everitt BS, Skrondal A (2010) *The Cambridge Dictionary of Statistics*. Cambridge University  
652 Press, Cambridge

653 Fabbri M, Navalón G, Benson RBJ, Pol D, O’Connor J, Bhullar BAS, Erickson GM, Norell  
654 MA, Orkney A, Lamanna MC, Zouhri S, Becker J, Emke A, Dal Sasso C, Bindellini G,  
655 Maganuco S, Auditore M, Ibrahim N (2022) Subaqueous foraging among carnivorous  
656 dinosaurs. *Nature* 603:1–6. <https://doi.org/10.1038/s41586-022-04528-0>

657 Gambaryan PP, Kielan-Jaworowska Z (1997) Sprawling versus parasagittal stance in  
658 multituberculate mammals. *Acta Palaeontol Pol* 42:13–44

659 Gambaryan PP, Kuznetsov AN (2013) An evolutionary perspective on the walking gait of the  
660 long-beaked echidna. *J Zool* 290:58–67. <https://doi.org/10.1111/jzo.12014>

661 Gatesy SM (1991) Hind limb movements of the American alligator (*Alligator mississippiensis*)  
662 and postural grades. *J Zool* 224:577–588. <https://doi.org/10.1111/j.1469-7998.1991.tb03786.x>

664 Gatesy SM, Biewener AA (1991) Bipedal locomotion: effects of speed, size and limb posture  
665 in birds and humans. *J Zool* 224:127–147. <https://doi.org/10.1111/j.1469-7998.1991.tb04794.x>

667 Gerkema MP, Davies WI, Foster RG, Menaker M, Hut RA (2013) The nocturnal bottleneck  
668 and the evolution of activity patterns in mammals. *Proc R Soc Lond B Biol Sci*  
669 280:20130508. <https://doi.org/10.1098/rspb.2013.0508>

670 Germain D, Laurin M (2005) Microanatomy of the radius and lifestyle in amniotes (Vertebrata,  
671 Tetrapoda). *Zool Scr* 34:335–350. <https://doi.org/10.1111/j.1463-6409.2005.00198.x>

672 Gill PG, Purnell MA, Crumpton N, Brown KR, Gostling NJ, Stampanoni M, Rayfield EJ (2014)  
673 Dietary specializations and diversity in feeding ecology of the earliest stem mammals.  
674 *Nature* 512:303–305. <https://doi.org/10.1038/nature13622>

- 675 Girondot M, Laurin M (2003) Bone Profiler: a tool to quantify, model, and statistically compare  
676 bone-section compactness profiles. *J Vertebr Paleontol* 23:458–461
- 677 Gônet J, Laurin M, Girondot M (2022) BoneProfileR: the next step to quantify, model and  
678 statistically compare bone section compactness profiles. *Palaeontol Electron* 25:a12  
679 <https://doi.org/10.26879/1194>
- 680 Gregory WK (1912) Notes on the principles of quadrupedal locomotion and on the mechanism  
681 of the limbs in hoofed animals. *Ann N Y Acad Sci* 22:267–294.  
682 <https://doi.org/10.1111/j.1749-6632.1912.tb55164.x>
- 683 Hastie T, Tibshirani R, Buja A (1994) Flexible discriminant analysis by optimal scoring. *J Am*  
684 *Stat Assoc* 89:1255–1270. <https://doi.org/10.1080/01621459.1994.10476866>
- 685 Hopson JA (2015) Fossils, trackways, and transitions in locomotion: a case study of  
686 *Dimetrodon*. In: Dial KP, Shubin N, Brainerd EL (eds) *Great Transformations in*  
687 *Vertebrate Evolution*. University of Chicago Press, Chicago, pp 125–141
- 688 Horovitz I, Ladevèze S, Argot C, Macrini TE, Martin T, Hooker JJ, Kurz C, Muizon C de,  
689 Sánchez-Villagra MR (2008) The anatomy of *Herpetotherium cf. fugax* Cope, 1873, a  
690 metatherian from the Oligocene of North America. *Palaeontographica Abt A* 284:109–  
691 141. <https://doi.org/10.1127/pala/284/2008/109>
- 692 Houssaye A, Botton-Divet L (2018) From land to water: evolutionary changes in long bone  
693 microanatomy of otters (Mammalia: Mustelidae). *Biol J Linn Soc Lond* 125:240–249.  
694 <https://doi.org/10.1093/biolinnean/bly118>
- 695 Houssaye A, Sander PM, Klein N (2016a) Adaptive patterns in aquatic amniote bone  
696 microanatomy—more complex than previously thought. *Integr Comp Biol* 56:1349–  
697 1369. <https://doi.org/10.1093/icb/icw120>
- 698 Houssaye A, Waskow K, Hayashi S, Cornette R, Lee AH, Hutchinson JR (2016b)  
699 Biomechanical evolution of solid bones in large animals: a microanatomical  
700 investigation. *Biol J Linn Soc Lond* 117:350–371. <https://doi.org/10.1111/bij.12660>
- 701 Houssaye A, Taverne M, Cornette R (2018) 3D quantitative comparative analysis of long bone  
702 diaphysis variations in microanatomy and cross-sectional geometry. *J Anat* 232:836–  
703 849. <https://doi.org/10.1111/joa.12783>

704 Hu Y, Meng J, Wang Y, Li C (2005) Large Mesozoic mammals fed on young dinosaurs. *Nature*  
705 433:149–152. <https://doi.org/10.1038/nature03102>

706 Hunt AP, Lucas SG (1998) Vertebrate tracks and the myth of the belly-dragging, tail-dragging  
707 tetrapods of the Late Paleozoic. *N M Mus Nat Hist Bull* 12:67–69

708 Hutchinson JR (2006) The evolution of locomotion in archosaurs. *C R Palevol* 5:519–530.  
709 <https://doi.org/10.1016/j.crpv.2005.09.002>

710 Hutchinson JR (2021) The evolutionary biomechanics of locomotor function in giant land  
711 animals. *J Exp Biol* 224:jeb217463. <https://doi.org/10.1242/jeb.217463>

712 Ibrahim N, Sereno PC, Dal Sasso C, Maganuco S, Fabbri M, Martill DM, Zouhri S, Myhrvold  
713 NP, Iurino DA (2014) Semiaquatic adaptations in a giant predatory dinosaur. *Science*  
714 345:1613–1616. <https://doi.org/10.1126/science.1258750>

715 Isler K (2005) 3D-kinematics of vertical climbing in hominoids. *Am J Phys Anthropol*  
716 126:6681. <https://doi.org/10.1002/ajpa.10419>

717 Jenkins FA (1971) Limb posture and locomotion in the Virginia opossum (*Didelphis*  
718 *marsupialis*) and in other non-cursorial mammals. *J Zool* 165:303–315.  
719 <https://doi.org/10.1111/j.1469-7998.1971.tb02189.x>

720 Jenkins FA (1973) The functional anatomy and evolution of the mammalian humero-ulnar  
721 articulation. *Am J Anat* 137:281–297. <https://doi.org/10.1002/aja.1001370304>

722 Jenkins FA, Parrington FR (1976) The postcranial skeletons of the Triassic mammals  
723 *Eozostrodon*, *Megazostrodon* and *Erythrotherium*. *Philos Trans R Soc Lond B Biol Sci*  
724 273:387–431. <https://doi.org/10.1098/rstb.1976.0022>

725 Jenkins FA, Weijs WA (1979) The functional anatomy of the shoulder in the Virginia opossum  
726 (*Didelphis virginiana*). *J Zool* 188:379–410. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-7998.1979.tb03423.x)  
727 [7998.1979.tb03423.x](https://doi.org/10.1111/j.1469-7998.1979.tb03423.x)

728 Jones KE, Dickson BV, Angielczyk KD, Pierce SE (2021) Adaptive landscapes challenge the  
729 “lateral-to-sagittal” paradigm for mammalian vertebral evolution. *Curr Biol*  
730 31:18831892. <https://doi.org/10.1016/j.cub.2021.02.009>

731 Kemp TS (2005) *The Origin and Evolution of Mammals*. Oxford University Press, Oxford.  
732 <https://doi.org/10.1093/oso/9780198507604.001.0001>



- 733 Kielan-Jaworowska Z, Hurum JH (2006) Limb posture in early mammals: sprawling or  
734 parasagittal. *Acta Palaeontol Pol* 51:393–406
- 735 Kielan-Jaworowska Z, Cifelli RL, Luo Z-X (2004) *Mammals from the Age of Dinosaurs:  
736 Origins, Evolution, and Structure*. Columbia University Press, New York
- 737 Kilbourne B, Hutchinson JR (2019) Morphological diversification of biomechanical traits:  
738 mustelid locomotor specializations and the macroevolution of long bone cross-sectional  
739 morphology. *BMC Evol Biol* 19:37. <https://doi.org/10.1186/s12862-019-1349-8>
- 740 Klein N, Sander PM, Krahl A, Scheyer TM, Houssaye A (2016) Diverse aquatic adaptations in  
741 *Nothosaurus* spp. (Sauropterygia)—inferences from humeral histology and  
742 microanatomy. *PLoS One* 11:e0158448. <https://doi.org/10.1371/journal.pone.0158448>
- 743 Krilloff A, Germain D, Canoville A, Vincent P, Sache M, Laurin M (2008) Evolution of bone  
744 microanatomy of the tetrapod tibia and its use in palaeobiological inference. *J Evol Biol*  
745 21:807–826. <https://doi.org/10.1111/j.1420-9101.2008.01512.x>
- 746 Kurz C (2005) Ecomorphology of opossum-like marsupials from the Tertiary of Europe and a  
747 comparison with selected taxa. *Kaupia* 14:21–26
- 748 Laurin M, Canoville A, Germain D (2011) Bone microanatomy and lifestyle: a descriptive  
749 approach. *C R Palevol* 10:381–402. <https://doi.org/10.1016/j.crpv.2011.02.003>
- 750 Lebrun R (2018) MorphoDig, an open-source 3D freeware dedicated to biology. *IPC5 Abstract  
751 Book*:399
- 752 Lin Y-F, Konow N, Dumont ER (2019) How moles walk; it's all thumbs. *Biol Lett*  
753 15:20190503. <https://doi.org/10.1098/rsbl.2019.0503>
- 754 Main RP, Simons EL, Lee AH (2021) Interpreting mechanical function in extant and fossil long  
755 bones. In: de Buffrénil V, de Ricqlès AJ, Zylberberg L, Padian K, Laurin M, Quilhac A  
756 (eds) *Vertebrate Skeletal Histology and Paleohistology*. CRC Press, Boca Raton, pp  
757 688–723
- 758 Meier PS, Bickelmann C, Scheyer TM, Koyabu D, Sánchez-Villagra MR (2013) Evolution of  
759 bone compactness in extant and extinct moles (Talpidae): exploring humeral  
760 microstructure in small fossorial mammals. *BMC Evol Biol* 13:110.  
761 <https://doi.org/10.1186/1471-2148-13-55>

- 762 Meng J (2014) Mesozoic mammals of China: implications for phylogeny and early evolution  
763 of mammals. *Natl Sci Rev* 1:521542. <https://doi.org/10.1093/nsr/nwu070>
- 764 Motani R, Schmitz L (2011) Phylogenetic versus functional signals in the evolution of form–  
765 function relationships in terrestrial vision. *Evolution* 65:2245–2257.  
766 <https://doi.org/10.1111/j.1558-5646.2011.01271.x>
- 767 Myhrvold NP, Baldrige E, Chan B, Sivam D, Freeman DL, Ernest SKM (2015) An amniote  
768 life-history database to perform comparative analyses with birds, mammals, and  
769 reptiles. *Ecology* 96:3109–3109. <https://doi.org/10.1890/15-0846R.1>
- 770 Nakajima Y, Hirayama R, Endo H (2014) Turtle humeral microanatomy and its relationship to  
771 lifestyle. *Biol J Linn Soc Lond* 112:719–734. <https://doi.org/10.1111/bij.12336>
- 772 Niemitz C (2010) The evolution of the upright posture and gait—a review and a new synthesis.  
773 *Naturwissenschaften* 97:241–263. <https://doi.org/10.1007/s00114-009-0637-3>
- 774 Pagel M (1999) Inferring the historical patterns of biological evolution.  
775 *Nature* 401:877–884. <https://doi.org/10.1038/44766>
- 776 Parrish JM (1987) The origin of crocodylian locomotion. *Paleobiology* 13:396–414.  
777 <https://doi.org/10.1017/S0094837300009003>
- 778 Pietersen DW, Jansen R, Swart J, Panaino W, Kotze A, Rankin P, Nebe B (2020) Temminck’s  
779 pangolin *Smutsia temminckii* (Smuts, 1832). In: Challender DWS, Nash HC, Waterman  
780 C (eds) *Pangolins: Science, Society and Conservation*. Elsevier, Amsterdam, pp 175–  
781 193. <https://doi.org/10.1016/B978-0-12-815507-3.00011-3>
- 782 Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2021) nlme: linear and nonlinear  
783 mixed effects. R package version 3.1–153
- 784 Plasse M, Amson E, Bardin J, Grimal Q, Germain D (2019) Trabecular architecture in the  
785 humeral metaphyses of non-avian reptiles (Crocodylia, Squamata and Testudines):  
786 lifestyle, allometry and phylogeny. *J Morphol* 280:982–998.  
787 <https://doi.org/10.1002/jmor.20996>
- 788 Preuschoft H, Witte H, Christian A, Fischer M (1996) Size influences on primate locomotion  
789 and body shape, with special emphasis on the locomotion of ‘small mammals’. *Folia*  
790 *Primatol* 66:93112. <https://doi.org/10.1159/000157188>

791 Pridmore PA (1985) Terrestrial locomotion in monotremes (Mammalia: Monotremata). *J Zool*  
792 205:53–73. <https://doi.org/10.1111/j.1469-7998.1985.tb05613.x>

793 Quemeneur S, de Buffrénil V, Laurin M (2013) Microanatomy of the amniote femur and  
794 inference of lifestyle in limbed vertebrates. *Biol J Linn Soc Lond* 109:644–655.  
795 <https://doi.org/10.1111/bij.12066>

796 R Core Team (2013) R: a language and environment for statistical computing. R Foundation  
797 for Statistical Computing, Vienna

798 Regnault S, Fahn-Lai P, Norris RM, Pierce SE (2020) Shoulder muscle architecture in the  
799 echidna (Monotremata: *Tachyglossus aculeatus*) indicates conserved functional  
800 properties. *J Mamm Evol* 27:591–603. <https://doi.org/10.1007/s10914-020-09498-6>

801 Reilly SM, Elias JA (1998) Locomotion in *Alligator mississippiensis*: kinematic effects of  
802 speed and posture and their relevance to the sprawling-to-erect paradigm. *J Exp Biol*  
803 201:2559–2574. <https://doi.org/10.1242/jeb.201.18.2559>

804 Revell LJ (2012) phytools: an R package for phylogenetic comparative biology (and other  
805 things). *Methods Ecol Evol* 3:217–223. [https://doi.org/10.1111/j.2041-](https://doi.org/10.1111/j.2041-210X.2011.00169.x)  
806 [210X.2011.00169.x](https://doi.org/10.1111/j.2041-210X.2011.00169.x)

807 Rose KD (2006) *The Beginning of the Age of Mammals*. Johns Hopkins University Press,  
808 Baltimore

809 Rose JA, Sandefur M, Huskey S, Demler JL, Butcher MT (2013) Muscle architecture and out-  
810 force potential of the thoracic limb in the eastern mole (*Scalopus aquaticus*). *J Morphol*  
811 274:1277–1287. <https://doi.org/10.1002/jmor.20178>

812 Russo GA, Kirk EC (2017) Another look at the foramen magnum in bipedal mammals. *J Hum*  
813 *Evol* 105:24–40. <https://doi.org/10.1016/j.jhevol.2017.01.018>

814 Scheidt A, Wölfer J, Nyakatura JA (2019) The evolution of femoral cross-sectional properties  
815 in sciuriform rodents: influence of body mass and locomotor ecology. *J Morphol*  
816 280:11561169. <https://doi.org/10.1002/jmor.21007>

817 Seilacher A (1970) Arbeitskonzept zur Konstruktions-Morphologie. *Lethaia* 3:393–396.  
818 <https://doi.org/10.1111/j.1502-3931.1970.tb00830.x>

- 819 Selva C (2017) Morphologie et fonction du système vestibulaire de l'oreille interne des  
820 mammifères souterrains. Dissertation, Muséum national d'Histoire naturelle, Paris
- 821 Sereno PC (2006) Shoulder girdle and forelimb in multituberculates: evolution of parasagittal  
822 forelimb posture in mammals. In: Carrano MT, Gaudin TJ, Blob RW, Wible JR (eds)  
823 Amniote Paleobiology: Perspectives on the Evolution of Mammals, Birds, and Reptiles.  
824 University of Chicago Press, Chicago, pp 315–366
- 825 Shannon CE (1948) A mathematical theory of communication. *Bell Sys Tech J* 27:379–423.  
826 <https://doi.org/10.1002/j.1538-7305.1948.tb01338.x>
- 827 Shimer HW (1903) Adaptations to aquatic, arboreal, fossorial and cursorial habits in mammals.  
828 III. Fossorial adaptations. *Am Nat* 37:819–825. <https://doi.org/10.1086/278368>
- 829 Stadler T (2011) Mammalian phylogeny reveals recent diversification rate shifts. *Proc Natl*  
830 *Acad Sci U S A* 108:6187–6192. <https://doi.org/10.1073/pnas.1016876108>
- 831 Stein BR, Casinos A (1997) What is a cursorial mammal? *J Zool* 242:185–192.  
832 <https://doi.org/10.1111/j.1469-7998.1997.tb02939.x>
- 833 Stone M (1974) Cross-validatory choice and assessment of statistical predictions. *J R Stat Soc*  
834 *Series B Stat Methodol* 36:111133. [https://doi.org/10.1111/j.2517-](https://doi.org/10.1111/j.2517-6161.1974.tb00994.x)  
835 [6161.1974.tb00994.x](https://doi.org/10.1111/j.2517-6161.1974.tb00994.x)
- 836 Tommasini SM, Nasser P, Schaffler MB, Jepsen KJ (2005) Relationship between bone  
837 morphology and bone quality in male tibias: implications for stress fracture risk. *J Bone*  
838 *Miner Res* 20:1372–1380. <https://doi.org/10.1359/JBMR.050326>
- 839 Upham NS, Esselstyn JA, Jetz W (2019) Inferring the mammal tree: species-level sets of  
840 phylogenies for questions in ecology, evolution, and conservation. *PLoS Biol*  
841 17:e3000494. <https://doi.org/10.1371/journal.pbio.3000494>
- 842 Vaughan TA, Ryan JM, Czaplewski NJ (2015) *Mammalogy*. Jones and Bartlett Learning,  
843 Burlington
- 844 Wagstaffe AY, O'Driscoll AM, Kunz CJ, Rayfield EJ, Janis CM (2022) Divergent locomotor  
845 evolution in “giant” kangaroos: evidence from foot bone bending resistances and  
846 microanatomy. *J Morphol* 283:313–332. <https://doi.org/10.1002/jmor.21445>

- 847 Wilson GP, Evans AR, Corfe IJ, Smits PD, Fortelius M, Jernvall J (2012) Adaptive radiation  
848 of multituberculate mammals before the extinction of dinosaurs. *Nature* 483:457–460.  
849 <https://doi.org/10.1038/nature10880>
- 850 Wright M (2018) Functional morphology of the hindlimb during the transition from sprawling  
851 to parasagittal gaits in synapsid evolution. Dissertation, Harvard University and  
852 University of Groningen