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1 The scaling of postcranial muscles in cats (Felidae) I: forelimb, cervical, and
2 thoracic muscles

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14

15 Abstract

16 The body masses of cats (Mammalia, Carnivora, Felidae) span a ~300-fold range from the smallest to
17 largest species. Despite this range, felid musculoskeletal anatomy remains remarkably conservative,
18 including the maintenance of a crouched limb posture at unusually large sizes. The forelimbs in felids
19 are important for body support and other aspects of locomotion, as well as climbing and prey
20 capture, with the assistance of the vertebral (and hindlimb) muscles. Here, we examine the scaling
21 of the anterior postcranial musculature across felids to assess scaling patterns between different
22 species spanning the range of felid body sizes. The muscle architecture (lengths and masses of the
23 muscle-tendon unit components) for the forelimb, cervical and thoracic muscles was quantified to
24 analyse how the muscles scale with body mass. Our results demonstrate that physiological cross-
25 sectional areas of the forelimb muscles scale positively with increasing body mass (i.e. becoming
26 relatively larger). Many significantly allometric variables pertain to shoulder support, whilst the rest
27 of the limb muscles become relatively weaker in larger felid species. However, when phylogenetic
28 relationships were corrected for, most of these significant relationships disappeared, leaving no
29 significantly allometric muscle metrics. The majority of cervical and thoracic muscle metrics are not
30 significantly allometric, despite there being many allometric skeletal elements in these regions.
31 When forelimb muscle data were considered in isolation or in combination with those of the
32 vertebral muscles in principal components analyses and MANOVAs, there was no significant
33 discrimination among species by either size or locomotory mode. Our results support the inference
34 that larger felid species have relatively weaker anterior postcranial musculature compared to smaller
35 species, due to an absence of significant positive allometry of forelimb or vertebral muscle
36 architecture. This difference in strength is consistent with behavioural changes in larger felids, such
37 as a reduction of maximal speed and other aspects of locomotor abilities.

38

39 Keywords: Felidae, muscle, scaling, body mass

40

41 Introduction

42 The carnivoran family Felidae comprises almost 40 species of extant cats, ranging in body mass from
43 a minimum body mass of around one kilogram in the rusty-spotted cat (*Prionailurus rubiginosus*) to a
44 maximum of around 300 kg in the largest tigers (*Panthera tigris*) and lions (*Panthera leo*) (Sunquist
45 and Sunquist, 2002). This spectrum of sizes expands further when fossil taxa are considered (~400-
46 500 kg estimated body masses for the largest felids; e.g. Cuff et al., 2015 and references therein).
47 This size range has led to many discussions about posture, prey capture and locomotory ability in
48 living and extinct cats (Day and Jayne, 2007; Doube et al., 2009; Meachen-Samuels and Van
49 Valkenburgh 2009a,b; Meachen-Samuels and Van Valkenburgh, 2010; Meachen et al., 2014). Of
50 particular interest is the change of limb posture, or lack thereof, across the Felidae (Day and Jayne,
51 2007; Zhang et al., 2012; Wiktorowicz et al., in review, Doube et al., 2009). Despite ranging over two
52 orders of magnitude in body mass, all extant felids appear to maintain the same crouched,
53 digitigrade posture observed in domestic cats (and presumably ancestral for all Felidae; Day and
54 Jayne, 2007). This unusual maintenance of a similar posture across such a range of body masses
55 removes one common behavioural strategy to forestall increases in supportive tissue stresses with
56 increasing body size: increasing erectness (Biewener, 1989,1990,2005). Therefore, other trade-offs,
57 such as reduced locomotor performance (e.g. range of speeds and gaits available; Alexander and

58 Jayes, 1983; Day and Jayne 2007) or bone scaling (Alexander, 1977; Biewener, 2005), should be
59 emphasized more strongly in extant (and possibly extinct) Felidae than in some other mammals.
60 Studies of long bone scaling in felids have found that the lengths of long bones in both the fore- and
61 hindlimbs scale isometrically with body mass (Anyonge, 1993; Christiansen and Harris, 2005; Doube
62 et al., 2009). However, the long bones do exhibit some degree of positive allometry in diameters and
63 cross-sectional areas, with long bones being relatively more robust in larger felids (Doube et al.,
64 2009; Lewis and Lague, 2010; Meachen-Samuels and Van Valkenburgh, 2009a,b; Meachen-Samuels
65 and Van Valkenburgh, 2010). This positive allometry has been interpreted as allowing larger felids to
66 support their greater body masses and resist the forces and moments that muscles and tendons
67 generate on and around long bones. Scapular morphology has also been shown to change with
68 increasing body size, with relative enlargement of the infra-/supraspinous fossae suggesting that the
69 attaching muscles also scale with positive allometry (Zhang et al., 2012).

70

71 As the locomotory speed of an animal increases, the length of time that the feet are in contact with
72 the substrate (stance time, Cavagna et al., 1988; Heglund and Taylor, 1988) and the proportion of
73 the stride that the limbs are in stance phase (duty factor, Keller et al., 1996; Weyand et al., 2000)
74 tend to decrease. These changes in stance time and duty factor lead to increasing limb forces with
75 increasing speed (Weyand et al., 2000; Witte et al., 2004). In mammalian quadrupeds, the forelimbs
76 tend to support around 60% of body weight (Barclay, 1953; Alexander and Jayes, 1978, 1983; Ueda
77 et al., 1981; Witte et al., 2004), so it is expected that felid forelimbs at top speeds experience
78 particularly high peak forces, and so must have sufficiently enlarged musculature to produce the
79 limb forces required. The muscles that would be most important for generating these forces are the
80 extensor (antigravity) muscles of the limbs, which should thus have large physiological cross-
81 sectional areas (PCSA) and masses (Hudson et al., 2011a).

82 In addition to their role in locomotion, the forelimbs of felids are involved in other important
83 behaviours including prey capture and tree climbing (Gonyea and Ashworth, 1975; Leyhausen,
84 1979). Most felids are well adapted to climbing; indeed, some species (e.g. *Neofelis nebulosa* and
85 *Leopardus wiedii*) show some adaptations for arboreality (Meachen-Samuels and Van Valkenburgh,
86 2009a). Some of the larger felid species (particularly the leopard, *Panthera pardus*) still climb trees
87 as adults, but the largest species climb little when they are adults, even though they are regular
88 climbers when they are younger (Schaller, 1967, 1972). All felid species also use their forelimbs to
89 capture and subdue prey before delivering a killing bite (Leyhausen, 1965). This contact becomes
90 increasingly important when the prey size is as large (or larger) than the felid. For all felids 25 kg and
91 larger, these larger prey items are the primary food sources (Carbone et al., 1999; Meachen-Samuels
92 and Van Valkenburgh, 2009a). Whilst all large felid species are capable of killing with a single bite,
93 they must initially use their forelimbs to grapple with and position the prey so they can deliver this
94 bite. Large prey items are seldom brought down by just the impact of the predator; more often, the
95 prey is pulled down by the felid, using its forelimbs, whilst the hindlimbs maintain contact with the
96 ground and the vertebral column acts as a lever between these limb pairs (Leyhausen, 1965;
97 Schaller, 1967, 1972; Gonyea, 1973; Kleiman and Eisenberg, 1973).

98

99 The limbs, however, are not isolated functional units and must work with the vertebral column,
100 which plays a critical role in supporting the torso and head, as well as linking the limbs and
101 lengthening the stride (Hildebrand, 1959; 1961; Kitchener *et al.* 2010). Recently, the nature of
102 vertebral column scaling in felids has become much better understood. Jones (2015a,b) found that
103 the length of the total thoracolumbar region, and lengths of the individual thoracic and lumbar
104 sections, present an evolutionary scaling pattern of negative allometry, such that larger felid species
105 have more robust vertebrae but a shorter posterior column length. Further, Randau *et al.* (in press)
106 found extensive positive allometric scaling within individual vertebrae, particularly for centrum
107 height, which was also observed by Jones (2015b) in the thoracic and mid-lumbar regions. Increases
108 in centrum height are directly correlated with increases in passive stiffness in the dorsoventral plane
109 (Long *et al.*, 1997; Koob and Long, 2000; Pierce *et al.*, 2011; Molnar *et al.*, 2014). Thus, these results
110 may partly explain how the felid axial skeleton copes passively with hyperextension moments,
111 although the important contribution of musculature has never been studied in a broad comparative
112 context.

113

114 Here we quantify the architecture of the forelimb and cervical-thoracic vertebral musculature across
115 a diverse sample of nine felid species spanning a large spectrum of body sizes to determine how the
116 architecture of these muscles scales with body mass and to investigate the biomechanical
117 consequences of that scaling. We anticipate that, as observed for multiple skeletal structures
118 summarized above, the locomotor musculature of felids will exhibit positive allometry of muscle
119 masses and cross-sectional areas. We also examine whether larger felids will have allometrically
120 shorter muscle fascicles and longer, heavier tendons, similar to those of prey species, such as bovids,
121 which have evolved highly cursorial limbs (Alexander, 1977; Pollock and Shadwick, 1994a,b). Our
122 study complements related research by Cuff *et al.* (submitted) on the hindlimb and lumbosacral
123 musculature of felids.

124

125 Methods

126 Specimens

127 Our study species were the black-footed cat (*Felis nigripes*: NMS.Z.2015.90; male), domestic cat
128 (*Felis catus*: Royal Veterinary College, JRH uncatalogued personal collection; female), caracal
129 (*Caracal caracal*: NMS.Z.2015.89.1; male), ocelot (*Leopardus pardalis*: NMS.Z.2015.88; male),
130 cheetah (data from Hudson *et al.*, 2009a,b) snow leopard (*Panthera uncia*: NMS.Z.2015.89.2;
131 female), jaguar (*P. onca*: NMS.Z.201467.2; female), Sumatran tiger (*P. tigris sondaica*:
132 NMS.Z.2015.91; female), and Asian lion (*P. leo persica*: NMS.Z.2015.128; female). The majority of the
133 felid specimens were obtained from various public and private zoo/park facilities around the United
134 Kingdom. The domestic cat was a pet that was euthanized after a long-term decline in health and
135 donated to the Royal Veterinary College for scientific research. No specimens were euthanized for
136 the purposes of this research. The institutional abbreviation NMS refers to the National Museums
137 Scotland, Department of Natural Sciences; source of many of our specimens as per below. All body
138 mass and dissection data are included in the Supplementary information.

139

140 **Dissection**

141 With the exception of the Asian lion, which was dissected shortly after death, all specimens were
142 freshly frozen after death and then defrosted (variably 24-48 hrs) prior to dissection. Initially, each
143 specimen had the limbs from one side removed (generally the right-hand side, but for the Asian lion
144 the left-hand side's limbs were removed) and refrozen, allowing for future dissection if the initial
145 material was incomplete or damaged. Next, the muscles from the forelimb and vertebral column
146 were dissected individually and muscle architecture was measured following standard procedures
147 (e.g. Hudson et al., 2011a).

148 For each muscle the following architectural parameters were measured: muscle belly length and
149 mass, tendon length and mass, muscle fascicle length and pennation angle (at least three for each
150 muscle, but up to 10 for some specimens, depending on muscle size and variation of fascicle
151 dimensions) (Figure 1). The belly and fascicle lengths for most muscles were measured using plastic
152 rulers or tapes (accurate to 1mm), but for some of the smallest species fascicle lengths were
153 measured using Vernier callipers (accurate to 0.1mm). Masses were measured using electronic
154 scales (accuracy between 0.001g and 0.01g). These data were used to calculate physiological cross-
155 sectional area (PCSA) for each muscle, as follows:

156 Eq. 1
$$PCSA = \frac{\text{muscle volume} \cdot \cos(\text{pennation angle})}{\text{fascicle length}}$$

157 where muscle volume is calculated using equation 2:

158 Eq.2
$$\text{Muscle volume} = \text{Muscle mass} \cdot \text{density}$$

159 In which muscle density is 1060kg m⁻³ (typical vertebrate muscle, Mendez and Keys, 1960). Any
160 muscles that were damaged or degraded in a specimen were excluded from the initial dataset,
161 although the remaining limb from the opposite side of the body was dissected to measure the
162 equivalent muscle where possible. Furthermore, where architecture data remained incomplete
163 (particularly those of the scapula and distal limb elements, which are smallest and most likely to
164 degrade during post-mortem or dissection), they were assumed to have parallel muscle fibres (i.e.
165 pennation angle of 0°), which in turn would maximize the force estimate for those muscles
166 (Supplementary table). As PCSA is calculated based on the cosine of the pennation angle, any
167 pennation angles less than 30° have a minimal effect on the PCSA, so this assumption was deemed
168 acceptable.

169

170 In total, we measured 41 forelimb muscles for all nine species, producing 246 metrics per species,
171 and 16 vertebral muscles producing 96 metrics per species. For most species, fewer than 10 metrics
172 were missing in total. The exceptions are the ocelot (which only had one usable forelimb), and the
173 cheetah, as the data taken from Hudson et al. (2011a) yielded only 50% completeness for forelimb
174 measures (no muscle length or tendon measurements were provided).

175

176 **Scaling (regression) analysis**

177 The data for muscle belly length and mass, tendon length and mass, fascicle length, and PCSA were
178 subjected to a series of analyses. As noted above, some measurements were incomplete for the

179 taxonomic sample. Where metric values were equal to zero (limited exclusively to tendon lengths
180 and masses where there were no tendons), the data were removed before scaling analyses. Metrics
181 for which there were data from less than three species were removed, but only those metrics with
182 at least six measures will be discussed (although the results from metrics with fewer measures, if
183 significant, are displayed in Tables 1-6). All data were logged, and then each logged metric was
184 regressed against \log_{10} body mass, using Standardised Reduced Major Axis (SMA) regression (“Model
185 II”; see Sokal and Rohlf, 1995) in ‘smatr’ package (Warton et al., 2013) in R 3.1.0 (R Core Team,
186 2014). Significances of the slope of the regression line and the correlation (r^2) between each metric
187 and body mass were determined using bootstrapped 95% confidence intervals (2000 replicates).
188 Isometry is defined as scaling patterns that match those expected for a given increase in body size
189 (i.e., maintaining geometric similarity), whilst allometry is an increase or decrease from that slope.
190 For these logged metrics, isometry is defined as follows: muscle masses scale against body mass with
191 slope equal to 1.00; lengths scale against body mass with a slope of 0.333 (i.e. length is proportional
192 to $\text{mass}^{1/3}$); and PCSA scales against body mass with a slope of 0.667 (i.e. area is proportional to
193 $\text{mass}^{2/3}$).

194

195 We wanted to account for the fact that closely related species tend to have characteristics more
196 similar to each other than more distantly related species; therefore, each variable was analysed for
197 phylogenetic signal using the phylosignal function in the ‘picante’ package (Kembel et al., 2010) in R,
198 which measures phylogenetic signal with the K statistic. This statistic reflects the difference between
199 the observed tip data and the expected values under a Brownian motion model for any given
200 phylogeny (Blomberg et al., 2003). A value for K close to 1.0 suggests a Brownian motion pattern,
201 while values <1.0 indicate less resemblance among related species than would be expected under
202 Brownian motion, and values >1.0 indicate more resemblance (Kembel et al., 2010). Although the
203 raw value of the K statistic assesses the fit of a Brownian motion model, the calculated p-value
204 reflects the strength of the phylogenetic signal in the data set, with a significant result ($p < 0.05$)
205 indicating that there is a significant phylogenetic signal in the data, as is the case for felid body size
206 (Cuff et al., 2015). The phylogeny used for this analysis is from Piras et al. (2013) – a combined
207 morphometric and molecular (from Johnson et al., 2006) phylogeny – and was pruned to only
208 include the taxa in this study. Only metrics for which there were significant phylogenetic signal
209 underwent correction using independent contrasts, before the contrast data were subjected to SMA,
210 as implemented in the ‘smatr’ package (Warton et al., 2013) in R software. However, as phylogenetic
211 SMA does not tolerate missing data, each metric was analysed independently, dropping any taxa
212 with missing data for that metric.

213

214 **Principal Components Analysis and MANOVAs**

215 In addition to the regression analyses, principal components (PC) analyses were carried out on the
216 unlogged muscle data. As PC analyses require complete datasets, any missing values were imputed
217 based on observed instances for each variable using R 3.1.2. The imputed data were calculated
218 iteratively using regression values for the missing data until convergence was achieved (German and
219 Hill, 2006; Ilin and Raiko, 2010). The resulting “complete” dataset was entered into PAST 2.17c
220 (Hammer et al., 2001). The “allometric vs. standard” option within the “remove size from distances”

221 tool was used to remove the effects of body size upon the metrics. This adjustment works by
222 estimating allometric coefficients with respect to a standard metric (in this case the mass), with each
223 metric being regressed against the standard metric after log-transformation (e.g. Elliot et al., 1995)
224 giving a slope b for that metric. An adjusted measurement was then computed from the original
225 value following the equation:

226 Eq. 3
$$metric_{adj} = metric_{orig} \left(\frac{\overline{mass}}{mass} \right)^b$$

227 Where $metric_{adj}$ is the new adjusted metric, $metric_{orig}$ is the original, \overline{mass} is the mean mass across
228 all species and b is the slope equation. The felid species were assigned to groups firstly by body size
229 (i.e., small cat vs. big cat, following Cuff et al., 2015, although here defined as *Panthera* vs non-
230 *Panthera* species), and in a second analysis by locomotor mode (terrestrial: *Felis nigripes*, *Acinonyx*
231 *jubatus*, *Panthera tigris*, *Panthera leo*; scansorial: *Felis silvestris*, *Caracal caracal*, *Leopardus pardalis*,
232 *Panthera uncia*, *Panthera onca*). Significant PC scores were subsequently tested for body size and
233 locomotory signal using MANOVAs with and without phylogenetic correction in the ‘geomorph’
234 package (Adams and Otárola-Castillo, 2013) in R software.

235

236 Results

237 Only modest amounts of unambiguously allometric scaling were evident in our musculoskeletal data
238 for our felid sample. For simplicity, here we focus only on these significant deviations from isometry;
239 all architectural measurement data and results from analyses of them are provided in
240 Supplementary Tables 1 and 2.

241

242 Forelimb

243 The muscle belly lengths (Figure 2) of *M. serratus ventralis cervicis*, *M. triceps lateralis*, *M.*
244 *omotransversarius*, *M. biceps brachii*, and *M. deltoideus spinosus* all displayed significant negative
245 allometry, whilst *M. abductor digitorum I* showed a significantly positive allometric slope prior to
246 phylogenetic correction (Table 2). The tendon lengths of *M. abductor digitorum I*, *M. triceps longus*,
247 *M. cleidobrachialis* and *M. infraspinatus* were all significantly positively allometric before
248 phylogenetic correction (Table 3). The *M. trapezius thoracis*, *M. latissimus dorsi*, *M. serratus*
249 *ventralis cervicis*, *M. biceps brachii* and *M. omotransversarius* fascicle lengths all scaled with
250 negative allometry, whilst the *M. flexor carpi ulnaris humeral*, *M. brachialis*, *M. pronator teres*, *M.*
251 *abductor digitorum I* and *M. flexor carpi radialis* all exhibited significant positive allometry before
252 phylogenetic correction (Table 4). Nevertheless, after phylogenetic correction, all length metrics for
253 the forelimb displayed scaling exponents that were statistically indistinguishable from isometry.

254

255 Only the *M. brachioradialis* showed positively allometric scaling of muscle belly mass both before
256 and after phylogenetic correction (Table 5). The *M. flexor carpi radialis* displayed a negatively
257 allometric tendon mass before phylogenetic correction, but no other muscles showed any scaling
258 that was statistically different from isometry (Table 6). Eleven muscles have PCSAs that scale with
259 positive allometry before phylogeny was accounted for, including the *M. brachioradialis*, which also

260 displayed significant positive allometry after phylogenetic correction whereas the other 10 muscles
261 did not (Table 7).

262

263 **Vertebral muscles**

264 The *M. splenius cervicis* muscle's belly length scaled with significant negative allometry, whilst the
265 *M. semispinalis capitis biventer*'s belly length exhibited significant positive allometry (Table 8). There
266 was no significant allometry of any muscle belly length after phylogenetic correction. The tendon
267 lengths of vertebral muscles did not show any significant allometries before or after phylogenetic
268 correction (Table 9). Only the fascicle lengths of the *M. longissimus cervicis* displayed any significant
269 deviation from isometry both before and after phylogenetic correction (positively allometric in both
270 cases) (Table 10). The *M. rectus capitis* was the only muscle with a belly mass displaying significant
271 (negative) allometric scaling before phylogenetic correction (Table 11). However, there was no
272 significant allometry observed for any muscle belly masses after phylogenetic correction. There was
273 also no significant allometry evident in tendon mass or muscle PCSA for the vertebral muscles either
274 before or after phylogenetic correction.

275

276 **Principal components analyses and phylogenetic MANOVAS**

277 A PCA of all of the metrics for the forelimb alone produced eight PC axes, encompassing 100% of the
278 total variance, with PC1 explaining 25.3% of the variation and PC2 explaining 20.9% of the variation
279 in the data set. The loadings for PC1 were dominated by positive correlations of body lengths and
280 negative correlations for tendon masses and lengths whilst PC2 was primarily body mass (positive
281 correlation), but these were not limited to any particular region. There was no significant separation
282 between size groups or locomotory modes using either a MANOVA or phylogenetic MANOVA
283 ($p \gg 0.05$ in all tests, Figure 3). When the vertebral muscles were included, the result was similar,
284 with eight significant PC axes covering all of the variance. As with the forelimb-only analysis, there
285 was no significant separation of the groupings using either size or locomotory mode (Figure 4) across
286 all axes before or after phylogenetic correction ($p \gg 0.05$ in all tests). The cheetah appeared to be an
287 outlier on many of the PC axes (Figures 3 and 4), but removal of this taxon did not significantly affect
288 any results.

289

290 **Discussion**

291 As land vertebrates evolve into larger body sizes, it becomes increasingly more physiologically and
292 mechanically demanding to maintain relatively crouched limb postures (Biewener, 1990; Fischer et
293 al., 2002; Day and Jayne, 2007; Ren et al., 2010). Despite this gravitationally induced challenge,
294 extant felids maintain roughly the same crouched posture across their range of body masses (Day
295 and Jayne, 2007). It has previously been hypothesized that the muscles associated with antigravity
296 (i.e. extensor) roles should scale with positive allometry for mass and PCSA so that they can produce
297 enough force to balance the increased moments experienced about each joint in increasingly large
298 felids (Hudson et al., 2011a). Similarly, energy savings from elastic energy storage and minimization
299 of limb inertia tends to favour the evolution of shorter muscle fascicles and longer tendons in larger,
300 extremely cursorial mammals such as bovids (Alexander, 1977; Pollock & Shadwick, 1994a,b).

301

302 Of the metrics displaying unambiguous allometry in our results, the positively allometric PCSA
303 (linked to greater maximal muscle force output) for the *M. latissimus dorsi*, *M. trapezius thoracis*
304 and *cervicis*, *M. deltoideus spinosus* and *M. rhomboideus capitis* suggest that these muscles become
305 relatively stronger with increasing body mass in felids. The negative allometry observed for the
306 fascicle lengths of the shoulder-stabilising *M. trapezius thoracis*, *M. latissimus dorsi*, and *M. serratus*
307 *ventralis cervicis* suggest that some muscles may contract slower (or with a narrower range of
308 motion), and in the case of *M. trapezius thoracis* (due to the positively allometric PCSA – linked to
309 fascicle length and pennation angles: Equation 1) more forcefully, and thus become better able to
310 support the shoulder in larger felids. As well as the muscle belly itself playing a key role in supporting
311 the increased body masses of larger felids, there may also be increased importance of the tendons
312 for some antigravity muscles of felid forelimbs, with the *M. triceps longus* and *infraspinatus* both
313 displaying positively allometric tendon lengths (i.e. longer tendons in larger taxa), with likely benefits
314 for elastic energy storage capacity (Alexander 1984; Alexander and Maloiy, 1989).

315

316 In addition to the requirements for limb muscles to support a stationary animal or an animal during
317 the more static periods of the stance phase of locomotion (i.e. antigravity-related functions) the *M.*
318 *extensor digitorum communis* (main digital extensor) also has a PCSA that scales with positive
319 allometry, with this muscle likely to have been used more in the swing phase of locomotion (Goslow
320 et al., 1973; Rasmussen et al., 1978). In addition to locomotor functions, the *M. extensor digitorum*
321 *communis* also likely plays a role in prey prehension. Thus our finding that it scales allometrically is
322 important, considering that larger felids take on larger prey, emphasizing forelimb prehension
323 (Meachen-Samuels and Van Valkenburgh, 2009a; Hudson et al., 2011a; Cuff et al., 2015). The results
324 for this muscle also qualitatively match the positively allometric scaling of PCSA for the pectoral
325 muscles, *M. abductor digitorum I* and *M. flexor carpi ulnaris* (ulnar head), muscles that are also likely
326 involved in prehension. The positively allometric (mass and PCSA) scaling for the *brachioradialis*
327 shows its importance in forearm flexion during pronation, the primary action used by felids whilst
328 gripping large prey. For both prey manipulation and climbing, the forelimb claws (unguals) of felids
329 are protracted (dorsiflexed) from their resting position in parallel with the penultimate phalanges
330 (Gonyea and Ashworth, 1975). This claw protraction requires the simultaneous co-contraction of the
331 digital flexors (particularly the deep head) and extensors (*M. extensor digitorum communis* and *M.*
332 *extensor digitorum lateralis*) (Gonyea and Ashworth, 1975). Once cats reach a body mass of 25kg
333 they regularly take prey as large, or larger, than themselves (Carbone et al., 1999; Meachen-Samuels
334 and Van Valkenburgh, 2009a,b). They drag their prey to the ground using their forelimbs and claws,
335 before a killing bite can be delivered (Leyhausen, 1965; Schaller, 1967, 1972; Gonyea 1973; Kleiman
336 and Eisenberg, 1973). Therefore it is expected that these claw-protracting muscles should scale
337 positively allometrically as felids get larger. However, cheetahs are an exception amongst felids, as
338 they have elongate claws on digits II-IV that appear to not be protractile in the same manner due to
339 their length; however, their dew claw (digit I) appears to retain the primitive function observed for
340 the dew claw in all other big cats (Russell and Bryant, 2001) in pulling prey off balance (Hudson et al.,
341 2011a). Surprisingly, the PCSA scores for the cheetah produce positive residuals (i.e. are above the
342 regression line) for most of the claw-protracting muscles, suggesting that they may continue to play

343 important functional roles beyond claw protraction and are possibly associated with ensuring grip at
344 high speeds as well as strengthening digital and metacarpophalangeal joints.

345

346 It has long been appreciated that closely-related species tend to have more similar morphologies
347 than more distantly related species (Felsenstein, 1985). Similarly to some previous studies that have
348 found significant influence of phylogeny on allometric scaling patterns across taxa (e.g. Smith and
349 Ceverud, 2002), taking phylogeny into account in our analyses dramatically changes the number of
350 significantly allometric results, with most metrics becoming statistically indistinguishable from
351 isometric scaling. Of those allometries that remain significant after phylogenetic correction, most
352 overlap with the non-phylogenetically-corrected significant results. To our knowledge, no previous
353 studies have attempted to assess whether taking phylogeny into account is an appropriate method
354 for analysing muscle scaling patterns within clades, particularly when all modern felid species
355 diverged relatively recently (within the last 10 million years: Johnson et al., 2006). To add to this
356 potential difficulty, most large felids fall within *Panthera*, and those were the large species studied
357 here. As such, the phylogenetic results presented here are probably conservative with respect to
358 which allometries are truly significant. However, further research should assess the impact of adding
359 *Puma*, the largest of the extant, non-*Panthera* felids to the dataset; or other moderately large felids.

360

361 Our results demonstrated that, despite the increasing biomechanical challenges that should be
362 imposed on larger felids by isometric scaling, most muscle metrics scale with (or at least
363 indistinguishably from) geometric similarity (i.e. isometry). We also showed that the scapular
364 muscles (specifically the M. infraspinatus mass), which had been predicted to scale with positive
365 allometry due to the broadening of the scapular fossae in felids (Zhang et al., 2012), scale
366 isometrically, or at least without unambiguously significant positive allometry. Considering that most
367 muscle PCSAs do not scale significantly differently from isometry (PCSA scales approximately to
368 $mass^{2/3}$), bigger cats must be relatively weaker than smaller cats. This inference is consistent with
369 other evidence, such as the isometry of most limb muscle moment arms and their effective
370 mechanical advantages (Wiktorowicz et al., in review; Zhang et al., 2012; but see Gálvez-López and
371 Casinos, 2012). This weakly allometric or isometric scaling of musculature might be partly
372 compensated for by the positive allometry of the limb bones in felids, which otherwise is
373 predominant in mammals larger than 300 kg (Biewener, 2005; Doube et al., 2009; although see
374 Campione and Evans, 2012).

375

376 However, the limb muscles of felids only tell part of the story, with the vertebral muscles also surely
377 playing important roles in support and locomotion, as well as predation. Most of the cervico-thoracic
378 muscles scale isometrically, particularly with respect to masses and PCSAs. Therefore, the vertebral
379 muscles also seem to get relatively weaker with increasing body mass in felids. Whilst the muscle
380 weakening of the musculature of the anterior vertebral column may be compensated for by positive
381 allometry of vertebrae and the resulting moment arms (Jones, 2015a,b; Randau et al., in press), the
382 combined result with the forelimb muscles show that there is a relative reduction in force
383 production in the musculature of the anterior half of the larger felids. But, how the biomechanics of
384 the musculoskeletal anatomy, limb posture and gait of felids interact to produce overall changes in

385 tissue stresses or safety factors across the size range of Felidae remains unclear, and would require
386 more sophisticated methods to resolve.

387

388 Generally, in fast-running quadrupeds and bipeds there tends to be a reduction in muscle mass
389 towards the distal ends of limbs, which lightens them for faster swinging and emphasizes elastic
390 energy storage in long tendons (Alexander, 1977; Payne et al., 2005; Smith et al., 2006,2007; Hudson
391 et al., 2011b). These anatomical specializations at best only delay the decline in relative locomotor
392 performance such as maximal running speed in larger species, or even emphasize efficiency and
393 endurance over maximal speed or acceleration. Such extreme specializations are not evident in
394 felids (cheetahs *Acinonyx* only representing a slight shift toward this extreme cursorial anatomy),
395 whose relatively robust distal forelimbs, digitigrade rather than unguligrade foot posture and – as
396 we have shown here -- modest muscular scaling may be linked to their maintenance of a crouched
397 limb posture and other behavioural differences (e.g. hunting and climbing) particularly compared to
398 ungulates. Whereas tigers and lions today may reach 300 kg in body mass, the largest known extinct
399 felids apparently never exceeded 400-500 kg (Peigné et al., 2005; Randau et al., 2013; Cuff et al.,
400 2015). If larger felids are relatively more poorly adapted for crouched postures than their smaller
401 relatives due to the scaling patterns we have outlined here, compensatory behavioural changes
402 would be required, including a reduction of relative or absolute maximal speeds (Garland, 1983; Day
403 and Jayne 2007) or modification of gaits, in larger extinct felids perhaps to a degree more extreme
404 even than evident in extant *Panthera*.

405

406 In mammalian quadrupeds, the forelimbs support about 60% of the total body weight in addition to
407 predominantly performing a braking function (Alexander and Jayes 1978, 1983; Witte et al., 2004),
408 whilst the hindlimbs are primarily responsible for providing a greater proportion of the propulsive
409 forces (at least at slower, steady speeds). At faster speeds this pattern changes as the forelimbs
410 become increasingly used to generate acceleratory forces (Hudson et al., 2011b). Felids seem to be
411 no exception to this pattern. Our PC analyses of forelimb muscles and of forelimb and vertebral
412 muscles combined might therefore be expected to separate body size and locomotor modes.
413 However, the body size and locomotor groupings were indistinguishable, with or without correction
414 for phylogeny. As the forelimbs in felids are used to capture and subdue prey (Leyhausen, 1965),
415 which becomes increasingly important in larger taxa (Carbone et al., 1999; Meachen-Samuels and
416 Van Valkenburgh, 2009a), our results are consistent with the inference that muscular adaptations for
417 predation behaviour supersede adaptations for supporting body weight and related locomotor
418 functions.

419

420 The data and results presented here are derived from captive animals, which died either from ill
421 health, or from euthanasia associated with a decline in health. These specimens tended to be either
422 overweight (e.g. the Asian lion) or underweight (e.g. caracal and domestic cat). Thus these animals
423 presumably had relatively smaller muscles than their wild counterparts. In a study of cheetahs, wild
424 individuals were found to have much larger limb muscles (Hudson et al., 2011a,b). Associated with
425 the animals' poor health, alterations in muscle architecture linked to a lack of physical activity are
426 likely (Blazevich et al., 2003), and muscle shortening is probable, due to rigor mortis and the freezing

427 process (Cutts, 1988). All animals, except the Asian lion, were subjected to the same post-mortem
428 procedures, and most of the muscles had angles of pennation of 30° or less, hence the cosine of the
429 pennation angle (equation 2) was close to 1. Therefore, the pennation angle in these muscles (as
430 noted in Methods, not subjected to scaling analysis here) had a very small effect on the PCSA of the
431 muscles (Calow and Alexander, 1973) and thus is a minimal concern for our study. In addition, all of
432 our measures are from a single individual from each species (or, in the case of the cheetah, lion and
433 tiger, a single subspecies), and not all of the same sex. However, there is no reason to expect that
434 these data are outliers or otherwise non-representative for their respective species, although there
435 will certainly be intraspecific variation (Hudson et al., 2011a,b). Despite these caveats, this study
436 provides the only data currently available for muscle architecture across much of the size range of
437 the Felidae. Future work and continued data collection will be able to test the stability of these
438 results with respect to the potentially complicating factors discussed above, but we do not expect
439 that our fundamental conclusions are unduly influenced by them.

440

441 Conclusions

442 The forelimb muscles of felids have 36 muscle metrics that scale with positive allometry prior to
443 phylogenetic correction. Of these metrics, the most biomechanically influential and statistically
444 consistent appear to be the positively allometric PCSAs of muscles that support the shoulders or
445 have other antigravity roles within the forelimbs, potentially indicating that these muscles may scale
446 at a rate that allows their force-producing capacity to keep pace with increasing body mass, whilst
447 the remainder of forelimb muscles are relatively weaker in larger felids. However, when phylogeny is
448 considered, most of these significant relationships disappear, and no clear pattern of muscular
449 allometry remains. Within the cervico-thoracic vertebral musculature, the majority of muscles scale
450 indistinguishably from isometry before and after phylogenetic correction, despite clear osteological
451 scaling. The latter findings support the inference that the vertebral articulations (as well as non-
452 muscular soft tissues such as intervertebral ligaments) may be playing a more active role in
453 stabilising the spine in larger felids. Finally, our PC analyses and MANOVAs demonstrated that body
454 mass and locomotor modes are indistinguishable in our dataset for felid muscle architecture,
455 suggesting that alternative functions such as prey capture may overwhelm any other signals.

456

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465

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638

639 **Tables**

640 Table 1. Specimens dissected in this study. Sex F=female, M=Male or Mix=both (unspecified).

Common name	Species	Sex	Body mass (kg)	General condition
Black-footed cat	<i>Felis nigripes</i>	F	1.1	Underweight
Domestic cat	<i>Felis catus</i>	F	2.66	Underweight
Caracal	<i>Caracal caracal</i>	M	6.6	Underweight
Ocelot	<i>Leopardus pardalis</i>	M	9.6	Overweight
Cheetah	<i>Acinonyx jubatus</i>	Mix	33.1 average	Unknown
Snow leopard	<i>Panthera uncia</i>	F	36	Ok
Jaguar	<i>P. onca</i>	F	44	Ok
Sumatran tiger	<i>P. tigris sumatrae</i>	F	86	Ok
Asian lion	<i>P. leo persica</i>	F	133	Overweight

646

647 Table 2. SMA results for log muscle belly lengths against log body mass, displaying only those that
 648 differ significantly from an isometric slope value of 0.333. Results with significant r^2 indicated in bold.
 649 No results were significant after phylogenetic correction. Upper and lower limits represent 95%
 650 confidence intervals, “slope p” represents statistical probability of the slope differing from isometry,
 651 whilst the “ r^2 p” shows the statistical significance of the correlation. All results including non-
 652 significant patterns are provided in Supplementary Information.

Muscle	slope	lower limit	upper limit	slope p	intercept	r^2	r^2 p	n
<i>Before phylogenetic correction</i>								
Serratus vent. cerv.	0.244	0.186	0.321	0.032	-1.18	0.923	0.000	8
Triceps lateralis	0.249	0.198	0.313	0.020	-1.13	0.946	0.000	8
Omotransversarius	0.250	0.201	0.312	0.021	-1.04	0.962	0.000	7
Biceps brachii	0.259	0.214	0.315	0.020	-1.19	0.962	0.000	8
Deltoideus spinous	0.267	0.245	0.292	0.001	-1.33	0.992	0.000	8
Abductor digitorum 1	0.576	0.388	0.856	0.013	-1.71	0.834	0.002	8
Coracobrachialis	1.09	0.468	2.543	0.009	-2.72	0.093	0.464	8
Brachioradialis	1.37	0.676	2.772	0.001	-2.79	0.557	0.054	7

After phylogenetic correction

None

653

654 Table 3. Significant SMA (before and after phylogenetic correction) scaling results for log tendon
 655 lengths plotted against log body, displaying only those that differ from an isometric slope value of
 656 0.333. Results with significant r^2 shown in bold. Column headings as in Table 2.

Muscle	slope	lower limit	upper limit	slope p	intercept	r^2	r^2 p	n
<i>Before phylogenetic correction</i>								
Abductor digitorum 1	0.691	0.365	1.31	0.029	-2.25	0.533	0.040	8
Triceps longus	0.727	0.420	1.26	0.014	-2.57	0.828	0.012	6
Cleidobrachialis	0.945	0.433	2.06	0.025	-3.04	0.920	0.041	4
Triceps lateralis	1.03	0.387	2.77	0.026	-3.39	0.000	0.992	7
Infraspinatus	1.18	0.751	1.84	0.000	-3.42	0.785	0.003	8

After phylogenetic correction

None

657

658 Table 4. Significant SMA (before and after phylogenetic correction) scaling results for log muscle
 659 fascicle lengths plotted against log body mass , displaying only those that differ from an isometric
 660 slope value of 0.333. Results with significant r^2 shown in bold. Column headings as in Table 2. “Flexor
 661 carpi ulnaris (h)” is the humeral head of that muscle.

Muscle	slope	lower limit	upper limit	slope p	intercept	r^2	r^2 p	n
<i>Before phylogenetic correction</i>								
Trapezius thoracis	0.168	0.112	0.254	0.004	-1.20	0.776	0.002	9
Latissimus dorsi	0.222	0.174	0.282	0.005	-0.802	0.927	0.000	9
Serratus vent. cerv.	0.234	0.165	0.332	0.049	-1.25	0.841	0.000	9
Biceps brachii	0.246	0.195	0.311	0.018	-1.76	0.931	0.000	9
Omotransversarius	0.254	0.201	0.320	0.029	-1.06	0.944	0.000	8
Flexor carpi ulnaris (h)	0.508	0.391	0.659	0.007	-2.35	0.930	0.000	8
Brachialis	0.542	0.375	0.784	0.017	-1.92	0.858	0.001	8
Pronator teres	0.601	0.339	1.07	0.045	-2.29	0.540	0.024	9
Abductor digitorum 1	0.695	0.393	1.23	0.016	-2.47	0.548	0.023	9
Flexor carpi radialis	0.706	0.467	1.07	0.002	-2.53	0.775	0.002	9
<i>After phylogenetic correction</i>								
None								

662

663 Table 5. Significant SMA (before and after phylogenetic correction) scaling results for log muscle
 664 body mass plotted against log body mass, displaying only those that differ from an isometric slope
 665 value of 1.00. Results with significant r^2 shown in bold. Column headings as in Table 2.

Muscle	slope	lower limit	upper limit	slope p	intercept	r^2	r^2 p	n
<i>Before phylogenetic correction</i>								
Brachioradialis	1.49	1.26	1.75	0.001	-4.11	0.972	0.000	8
<i>After phylogenetic correction</i>								
Brachioradialis	1.54	1.08	2.18	0.024	-0.008	0.903	0.001	8

666

667 Table 6. Significant SMA (before and after phylogenetic correction) scaling results for log tendon
 668 mass plotted against log body mass, displaying only those that differ from an isometric slope value
 669 of 1.00. Results with significant r^2 shown in bold. Column headings as in Table 2.

Muscle	slope	lower limit	upper limit	slope p	intercept	r^2	r^2 p	n
<i>Before phylogenetic correction</i>								
Flexor carpi radialis	0.660	0.450	0.967	0.037	-4.28	0.847	0.001	8
<i>After phylogenetic correction</i>								
None								

670

671 Table 7. Significant SMA (before and after phylogenetic correction) scaling results for log
 672 physiological cross-sectional area plotted against log body mass, displaying only those that differ

673 from an isometric slope value of 0.667. Results with significant r^2 shown in bold. Column headings as
 674 in Table 2. “Extensor digitorum (c)” is M. extensor digitorum complex, “Flexor carpi ulnaris (u)” is the
 675 ulnar head of that muscle.

Muscle	slope	lower limit	upper limit	slope p	intercept	r^2	r^2 p	n
<i>Before phylogenetic correction</i>								
Cleidobrachialis	0.919	0.692	1.22	0.032	-4.85	0.917	0.000	8
Latissimus dorsi	0.934	0.753	1.16	0.007	-4.60	0.941	0.000	9
Pectoralis profundus	0.942	0.714	1.24	0.021	-4.29	0.901	0.000	9
Extensor digitorum (c)	0.950	0.722	1.25	0.018	-4.91	0.904	0.000	9
Trapezius thoracis	0.953	0.722	1.26	0.018	-4.96	0.901	0.000	9
Deltoideus spinous	0.973	0.702	1.35	0.028	-4.95	0.861	0.000	9
Trapezius cervicis	0.994	0.762	1.30	0.010	-4.96	0.927	0.000	8
Pectoralis superficialis	0.999	0.704	1.42	0.029	-5.01	0.840	0.001	9
Flexor carpi ulnaris (u)	1.03	0.681	1.55	0.041	-4.67	0.776	0.002	9
Rhomboideus capitis	1.07	0.685	1.66	0.040	-5.61	0.738	0.003	9
Brachioradialis	1.44	1.040	2.00	0.001	-6.26	0.889	0.000	8
Teres minor	1.65	0.816	3.34	0.015	-6.35	0.268	0.154	9
<i>After phylogenetic correction</i>								
Brachioradialis	1.53	0.962	2.42	0.004	0.031	0.827	0.005	8
Teres minor	1.86	0.809	4.29	0.019	-0.033	0.126	0.389	9

676

677 Table 8. Significant SMA (before and after phylogenetic correction) scaling results for log muscle
 678 body lengths plotted against log body mass, displaying only those that differ from an isometric slope
 679 value of 0.333. Results with significant r^2 shown in bold. Column headings as in Table 2.

Muscle	slope	lower limit	upper limit	slope p	intercept	r^2	r^2 p	n
<i>Before phylogenetic correction</i>								
Splenius cervicis	0.234	0.174	0.316	0.029	-0.946	0.930	0.000	7
Semispinalis capitis (B)	1.36	0.683	2.71	0.002	-2.55	0.712	0.035	6
<i>After phylogenetic correction</i>								
None								

680

681 Table 9. Significant SMA (before and after phylogenetic correction) scaling results for log tendon
 682 lengths plotted against log body mass, displaying only those that differ from an isometric slope value
 683 of 0.333. Results with significant r^2 shown in bold. Column headings as in Table 2.

Muscle	slope	lower limit	upper limit	slope p	intercept	r^2	r^2 p	n
<i>Before phylogenetic correction</i>								
Serratus dorsalis thor.	-1.53	-6.00	-0.389	0.032	0.484	0.001	0.953	5
<i>After phylogenetic correction</i>								
None								

684

685 Table 10. Significant SMA (before and after phylogenetic correction) scaling results for log fascicle
 686 lengths plotted against log body mass, displaying only those that differ from an isometric slope value
 687 of 0.333. Results with significant r^2 shown in bold. Column headings as in Table 2.

Muscle	slope	lower limit	upper limit	slope p	intercept	r^2	r^2 p	n
<i>Before phylogenetic correction</i>								
Longissimus cervicis	0.734	0.457	1.18	0.006	-1.82	0.818	0.005	7
<i>After phylogenetic correction</i>								
Longissimus cervicis	0.837	0.402	1.74	0.021	-0.006	0.667	0.047	7

688

689 Table 11. Significant SMA (before and after phylogenetic correction) scaling results for log muscle
 690 body mass plotted against log body mass, displaying only those that differ from an isometric slope
 691 value of 1.00. Results with significant r^2 shown in bold. Column headings as in Table 2.

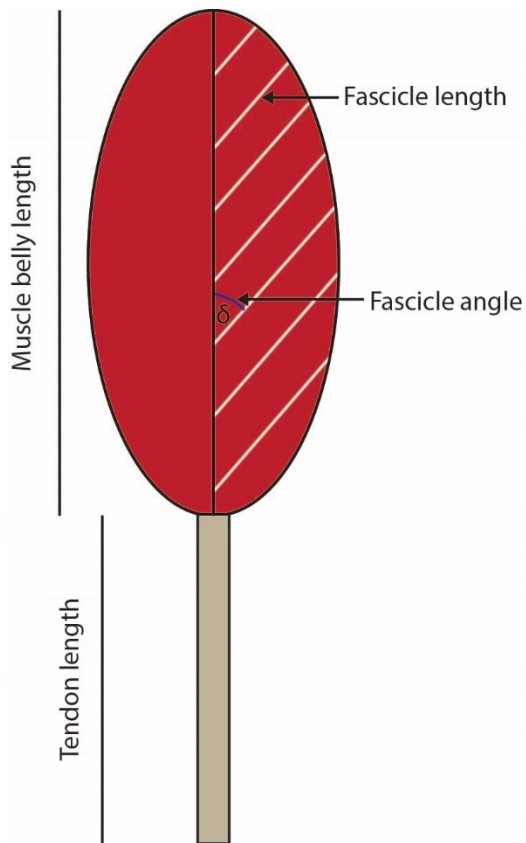
Muscle	slope	lower limit	upper limit	slope p	intercept	r^2	r^2 p	n
<i>Before phylogenetic correction</i>								
Rectus capitis	0.679	0.472	0.977	0.043	-2.58	0.959	0.004	5
<i>After phylogenetic correction</i>								
None								

692

693

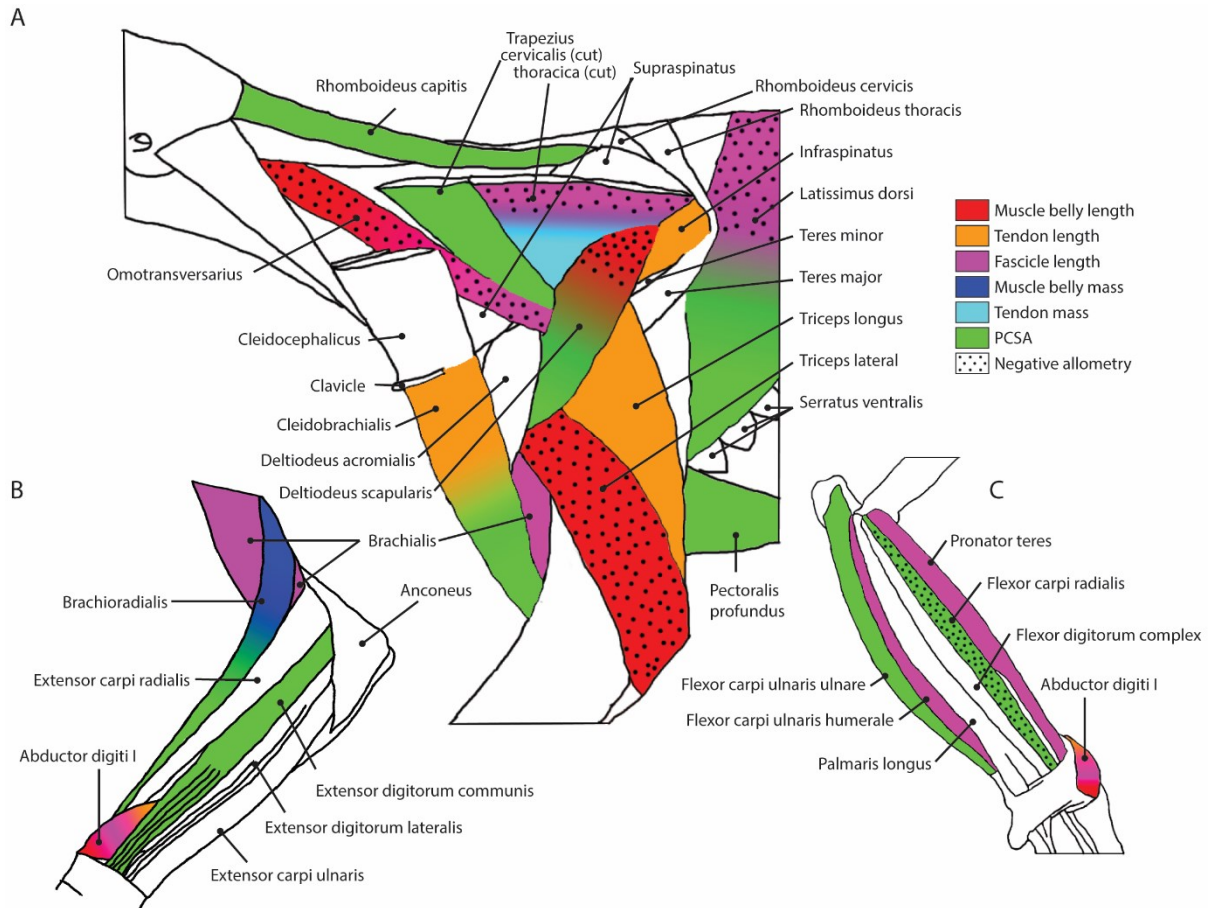
694 Figure legends

695 Figure 1. Simple diagram showing length and angle measurements of muscle architecture made
 696 during dissection.



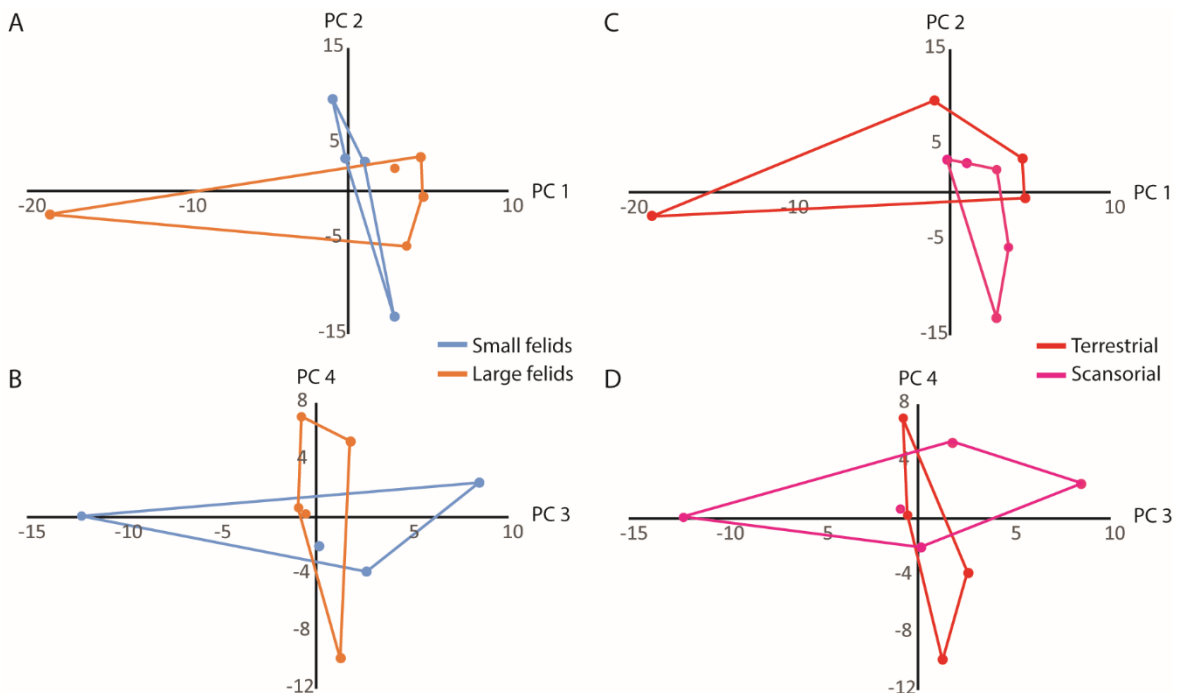
697

698 Figure 2. Muscles displaying potential allometry (prior to phylogenetic analysis) in the studied felid
 699 species are shown in colour; others as white; for a representative left forelimb. A) Lateral superficial
 700 muscles of the shoulder; B) Lateral muscles of the lower forelimb; C) Medial muscles of the lower
 701 forelimb. Colour codes for allometries: Red = muscle belly length; orange = tendon length; purple =
 702 fascicle length; navy blue = muscle belly mass; light blue = tendon mass; green = PCSA. Stippling
 703 pattern indicates negative allometry; lack of stippled colour indicates positive allometry. Muscles not
 704 shown, but displaying allometries: *M. serratus ventralis cervicis* (Table 2), *M. biceps brachii* (Tables
 705 1,3), *M. pectoralis superficialis* (Table 7). After phylogenetic correction, only the *M. brachioradialis*
 706 remains significant.



707

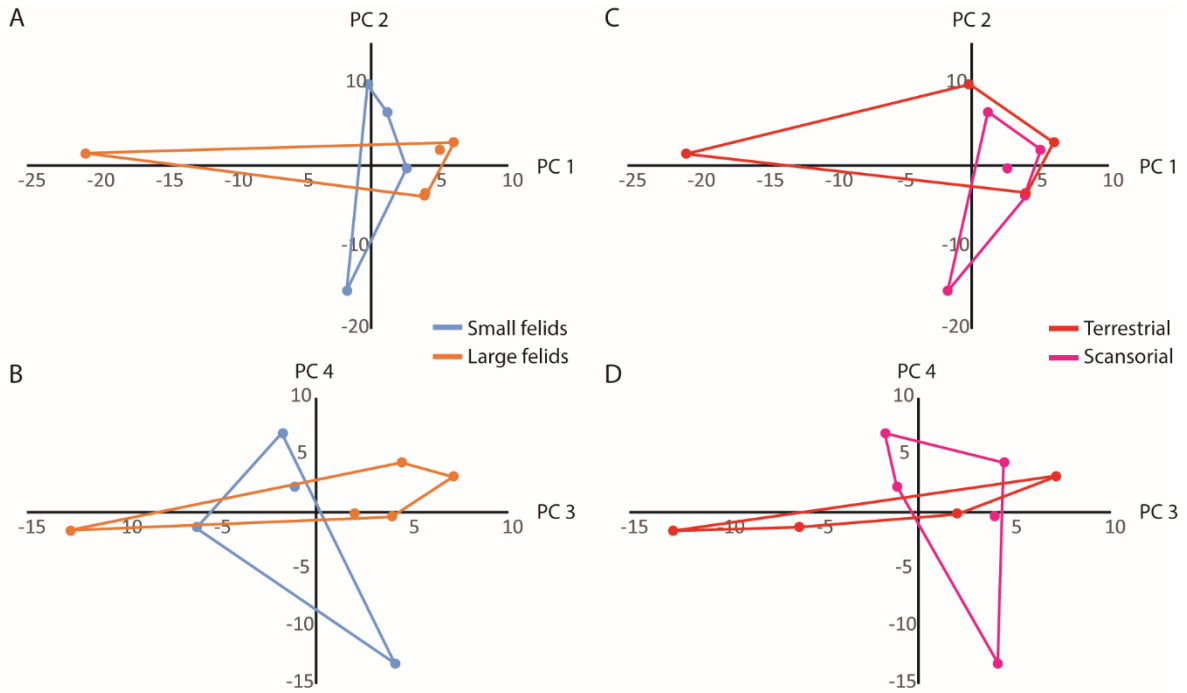
708 Figure 3 Principal component analysis of the forelimb architectural metrics, grouped by body size
 709 and locomotory mode. A) and B) body size, with blue for small felids, orange for large felids (Cuff et
 710 al., 2015); C) and D) locomotory mode, with red for terrestrial, pink for scansorial. A) and C) show
 711 PC1 (25.32% of total variance) vs PC 2 (20.86% of total variance); C) and D) show PC3 (14.08% of
 712 total variance) vs PC 4 (12.04% of total variance).



713

714

715 Figure 4. Principal component analysis of the forelimb and vertebral architectural metrics grouped
716 by body size and locomotory mode. A) and B) body size with blue for small felids, orange for large
717 felids (Cuff et al., 2015); C) and D) locomotory mode with red for terrestrial, pink for scansorial. A)
718 and C) show PC1 (25.25% of total variance) vs PC 2 (19.65% of total variance); C) and D) show PC3
719 (14.78% of total variance) vs PC 4 (12.36% of total variance).



720