The scaling of postcranial muscles in cats (Felidae) II: hindlimb and lumbosacral muscles

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Abstract

In quadrupeds the musculature of the hindlimbs is expected to be responsible for generating most of the propulsive locomotory forces, as well as contributing to body support by generating vertical forces. In supporting the body, postural changes from crouched to upright limbs are often associated with an increase of body mass in terrestrial tetrapods. However, felids do not change their crouched limb posture despite undergoing a three-hundred-fold size increase between the smallest and largest extant species. Here, we test how changes in the muscle architecture (masses and lengths of components of the muscle-tendon units) of the hindlimbs and lumbosacral region are related to body mass, to assess if there are muscular compensations for the maintenance of a crouched limb posture at larger body sizes. We use regression and principal component analyses to detect allometries in muscle architecture, with and without phylogenetic correction. Of the muscle lengths that scale allometrically, all scale with negative allometry (i.e. relative shortening with increasing body mass), whereas all tendon lengths scale isometrically. Only two muscles' belly masses and two tendons' masses scale with positive allometry (i.e. relatively more massive with increasing body mass). Of the muscles that scale allometrically for physiological cross-sectional area, all scale positively (i.e. relatively greater area with increasing body mass). These muscles are mostly linked to control of hip and thigh movements. When the architecture data are phylogenetically corrected, there are few significant results, and only the strongest signals remain. None of the vertebral muscles scaled significantly differently from isometry. Principal component analysis and MANOVAs showed that neither body size nor locomotor mode separate the felid species in morphospace. Our results support the inference that, despite some positively allometric trends in muscle areas related to thigh movement, larger cats have relatively weaker hindlimb and lumbosacral muscles in general. This decrease in power may be reflected in relative decreases in running speeds and is consistent with prevailing evidence that behavioural changes may be the primary mode of compensation for a consistently crouched limb posture in larger cats.

Keywords: biomechanics, anatomy, mammal, effective mechanical advantage, locomotion, morphometrics

Introduction

In terrestrial tetrapods, where there are evolutionary increases in body masses there tends to be changes in limb posture from crouched to upright to avoid potential increases in stresses within the supportive tissues, whose relative strengths tend not to vary (Biewener 1989, 1990, 2005). Extant felids are unusual in that they maintain the same crouched posture from the smallest species to the largest (Day and Jayne, 2007) throughout their ~1 - 300kg range of body masses (Cuff et al., 2015). In addition, felids mostly capture prey using ambushes and short, high-speed pursuits. Larger felids (above cheetah, *Acinonyx jubatus*, size) seem to suffer from reduced locomotor performance relative to their smaller relatives (e.g. range of speeds: Garland, 1983; Day and Jayne, 2007), which may be emphasised more strongly in Felidae than in some other mammals due to their conserved limb postures. Previous work on the scaling of the limb bones in felids shows that long bone lengths in both the hind- and forelimbs scale isometrically with body mass (Christiansen and Harris, 2005; Anyonge, 1993; Doube et al., 2009). However, diameters and cross-sectional areas of those bones scale with positive allometry, meaning long bones become relatively more robust (and stiffer and stronger as a consequence) in larger felid species (Doube et al., 2009; Lewis and Lague, 2010; Meachen-Samuels and Van Valkenburgh, 2009; Meachen-Samuels and Van Valkenburgh, 2010). Similar patterns have been found for vertebral dimensions in felids, indicating that some degree of skeletal allometry may help to support loads on the spine that might otherwise incur greater stresses as body mass increases. However, the lumbar region tends to show relatively weaker allometry than is observed in the cervicothoracic regions (Jones, 2015; Randau et al., in press).

Muscles generate greater moments around joints partly by increasing moment arms (i.e. by lengthening the distance of muscle action from the joint), increasing the mechanical advantage of the muscle; e.g. as potentially present for the M. gastrocnemius on felid calcanei (Gálvez-López and Casinos, 2012). Whilst if larger animals might not forestall increases in tissue stresses if they do not straighten their limbs to increase their limbs' effective mechanical advantage (EMA) (Biewener 1989, 1990, 2005), maintaining a crouched posture at larger body sizes may otherwise increase the ability to generate horizontal (as opposed to vertical) forces, needed in accelerations and manoeuvring. As the hindlimbs generally are the main propulsive drivers in the locomotion of felids, their muscles must be able to provide forces and power that are capable of generating the required forward movement and acceleration. Across mammalian quadrupeds, this force requirement tends to be largely achieved through an increase of the volume of hip extensor musculature (Alexander et al., 1981; Usherwood and Wilson, 2005; Williams et al., 2008, 2009). The same or similar extensor (e.g. antigravity) muscles must also be able to support the animal's body weight. The impulse (force-time integral) required for this support is equivalent to the product of the animal's body weight and stride time (Alexander and Jayes, 1978). At faster speeds the foot is in contact with the ground for a shorter period of time (shorter stance time) and a smaller proportion of the stride (decreasing duty factor). Therefore, peak limb force must increase (Witte et al., 2004) and the muscles must be able to generate larger amounts of forces and joint moments to sustain this limb force.

In addition, during the swing phase the hindlimbs must be protracted quickly enough to reposition them in time for the next stance phase. This capacity for limb protraction is limited by the limbs' inertia (Lee et al., 2004), the internal muscle architecture (including maximal contraction velocity of the muscle fibres), and the moment arms of the muscles (Hudson et al., 2011a,b). In fast-running tetrapods there tends to be a reduction in muscle mass towards the distal ends of limbs, in which the distal muscles transmit their forces down long tendons (Alexander et al., 1981; Alexander and Jayes, 1983; Payne et al., 2005; Smith et al., 2006, 2007; Hudson et al., 2011a,b). This tapering of the limbs reduces their inertial properties and therefore reduces the amount of power that would otherwise be required from the muscles to swing the limb (Hudson et al., 2011b). Additional energy savings are achieved by using long tendons to store elastic strain energy, contributing to the bouncing dynamics of locomotion and enabling the muscles to remain closer to optimal isometric activity during steady-state locomotion (Alexander, 1984; Alexander and Maloiy, 1989). In addition to the limbs, the vertebral musculature is important for locomotion in quadrupeds, whether being used in active dynamic flexion and extension of the spine, or for stabilisation of the spine in larger taxa (Boszczyk et al., 2001).

Here we measure the architecture of the musculature of the hindlimb and lumbosacral vertebrae in a range of felid species, spanning almost their full spectrum of body sizes, to quantify patterns of musculoskeletal scaling and interpret their biomechanical consequences. This work follows that of Cuff et al. (in review) on scaling of the forelimb, cervical and thoracic musculature across extant felids. We hypothesise that, as in the forelimbs (Cuff et al., in review), many of the muscles involved in limb and body support scale with positive allometry such that the muscles are more adept at supporting the increasing body masses. We further hypothesise that muscle fascicles scale with negative allometry (i.e. shortening), while tendons scale with positive allometry (i.e. lengthening), as is common in other cursorial tetrapods (Alexander, 1977; Pollock and Shadwick, 1994a,b). We finally predict that, as with the cervico-thoracic vertebral muscles (Cuff et al., in review), the lumbosacral musculature scales indistinguishably from isometry.

Methods

Muscle data collection

The methodological protocol used here is identical to that described in detail in Cuff et al. (in review). In brief, the species studied in this study were the black-footed cat (*Felis nigripes*: NMS.Z.2015.90; male), domestic cat (*Felis catus*: Royal Veterinary College, JRH uncatalogued personal collection; female), caracal (*Caracal caracal*: NMS.Z.2015.89.1; male), ocelot (*Leopardus pardalis*: NMS.Z.2015.88; male), cheetah (*Acinonyx jubatus*: data from Hudson et al., 2009a,b) snow leopard (*Panthera uncia*: NMS.Z.2015.89.2; female), jaguar (*P. onca*: NMS.Z.2014.67.2; female), Sumatran tiger (*P. tigris sondaica*: NMS.Z.2015.91; female), and Asian lion (*P. leo persica*: NMS.Z.2015.128; female) (Table 1). No specimens were euthanized for the purposes of this research. The institutional abbreviation NMS refers to the National Museums Scotland, Department of Natural Sciences. All body mass and dissection data are included in the Supplementary information.

Dissection

All specimens were frozen shortly after death and then defrosted (variably 24-48 hrs) prior to dissection except the Asian lion, which was dissected one day post-mortem without any freezing or thawing. Initially, each specimen had the limbs from one side removed and refrozen, allowing for future dissection if the initial material was incomplete or damaged. The muscles from the hindlimb and vertebral column were dissected individually and muscle architecture was measured following standard procedures (e.g. Alexander et al., 1981; Hudson et al., 2011a). For each muscle the following architectural parameters were measured: muscle belly length and mass, tendon length and mass, muscle fascicle length and pennation angle (at least three for each muscle, but up to 10 for some specimens, depending on muscle size and variation of fascicle dimensions). These data were used to calculate physiological cross-sectional area (PCSA) for each muscle using equation 1:

Eq. 1 $$Muscle\ volume = Muscle\ mass \cdot density,$$

Where density is 1060kg m^{-3} (typical vertebrate muscle, Mendez and Keys, 1960), and then with equation 2:

Eq.2 $$PCSA = \frac{muscle\ volume \cdot \cos(pennation\ angle)}{fascicle\ length}$$

In total 38 hindlimb muscles were measured for all nine species, producing up to 228 metrics per species, and three vertebral muscles, producing up to 18 metrics per species. For most species, fewer than 12 metrics were missing in total. The exception is the cheetah, as the data taken from Hudson et al. (2011a) yielded only 50% completeness for hindlimb measures (only muscle mass, fascicle length and PCSA were usable; no tendon measurements were provided).

Scaling (regression) analysis

The data for muscle belly length and mass, tendon length and mass, fascicle length, and PCSA were subjected to a series of scaling analyses. Where tendon lengths and masses could not be measured (because there were no tendons), those data were removed before scaling analyses. Metrics for which there were data from less than three species were removed, but only metrics with at least six measures will be discussed (although the results from metrics with fewer measures, if significant, are displayed in Tables 1-6). The data were $\log_{10}$-transformed, and then each logged metric was regressed against $\log_{10}$ body mass, using Standardised Reduced Major Axis (SMA) regression in the 'smatr' package (Warton et al., 2013) in R 3.1.0 (R Core Team, 2014) software. Significances of the regression line relative to isometry and the correlation (r^2) between each metric and body mass were determined using bootstrapped 95% confidence intervals (2000 replicates). Isometry is defined as scaling patterns that match the slope expected for a given increase in body size (i.e., maintaining geometric similarity), whilst allometry represents increases or decreases from that slope. For the logged metrics, isometry is defined as follows: muscle or tendon masses scale against body mass with slope equal to 1.00; muscle or tendon lengths scale against body mass with a slope of 0.333 (i.e. length is proportional to $mass^{1/3}$); and muscle PCSA scales against body mass with a slope of 0.667 (i.e. area is proportional to $mass^{2/3}$).

As closely related species tend to have characteristics more similar to each other, and as in felids large body masses are only found in a few clades (Cuff et al., 2015) we tested variables for phylogenetic signal. Each variable was analysed using the phylosignal function in the 'picante' package (Kembel et al., 2010) in R, which measures phylogenetic signal using the K statistic. The phylogeny used for this analysis was from Piras et al. (2013), which was pruned to include only the taxa in this study. Metrics which were found to have significant phylogenetic signal underwent correction using independent contrasts in R, before the contrast data were subjected to SMA, as implemented in the 'smatr' package (Warton et al., 2013) in R software. However, as phylogenetic SMA does not tolerate missing data, each metric was analysed independently, dropping any taxa with missing data for that metric.

Principal Components Analysis and MANOVAs

Principal component (PC) analyses were also carried out on the unlogged muscle data. As PC analyses require complete datasets, any missing values were imputed based on observed instances for each variable, using R 3.1.2 software. The imputed data were calculated iteratively until convergence was achieved (German and Hill, 2006; Ilin and Raiko, 2010). The resulting “complete” dataset was entered into PAST 2.17c (Hammer et al., 2001) software. The “allometric vs. standard” option within the “remove size from distances” tool was used to remove the effects of body size upon the metrics. The felid species were assigned to groups firstly by body size (i.e., small cat vs. big cat species, following Cuff et al., 2015, although here defined as *Panthera* vs non-*Panthera* species), and in a second analysis by locomotor mode (following Meachen-Samuels and Van Valkenburgh, 2009; terrestrial: *Felis nigripes*, *Acinonyx jubatus*, *Panthera tigris*, *Panthera leo*; scansorial: *Felis silvestris*, *Caracal caracal*, *Leopardus pardalis*, *Panthera uncia*, *Panthera onca*). Significant PC scores were then tested for body size and locomotory signal using MANOVAs with and without phylogenetic correction in the ‘geomorph’ package (Adams and Otarola-Castillo, 2013) in R software.

Results

Limb muscles

Prior to phylogenetic correction the belly lengths for M. piriformis, M. peroneus brevis, M. soleus, M. gastrocnemius medialis and M. semitendinosus all displayed significant negative allometry (i.e. relative shortening as body mass increases) (Table 2, Figure 1). After phylogenetic correction, only the M. soleus remained significantly negatively allometric (Table 2, Figure 2). None of the tendon lengths exhibited significant allometry before or after phylogenetic correction (Table 3). Prior to phylogenetic correction, the fascicle lengths for M. extensor digitorum lateralis and M. vastus intermedius showed significant allometry: the M. lateral digital extensor fascicles scaled with negative allometry (again, relative shortening), and M. vastus intermedius scaled with positive allometry (Table 4). After phylogenetic correction no fascicle lengths scaled significantly differently from isometry (slope of 0.333) (Table 4).

For the muscle belly masses two muscles initially showed significant allometry; the M. vastus intermedius scaled with negative allometry (i.e. relatively less massive with increasing body mass) and the M. gluteus medius scaled with positive allometry (Table 5, Figure 1). After phylogenetic correction, only the M. gluteus medius retained significantly positive allometry (Table 5, Figure 2). The tendon masses for the M. psoas major and M. extensor digitorum longus both showed significant positive allometry prior to phylogenetic correction, but no tendon masses scaled significantly differently from isometry after phylogenetic correction (Table 6). Before phylogenetic correction seven muscles’ PCSAs scaled with positive allometry (Table 7, Figure 1) (i.e. relatively greater area with increasing body mass); the M. gluteus medius, M. gemelli, M. biceps femoris, M. tensor fascia latae, M. caudofemoralis, M. tibialis caudalis, and the M. tibialis cranialis. After phylogenetic correction only the PCSA of the M. tibialis cranialis remained significantly positively allometric with body mass (Table 7, Figure 2).

Vertebral muscles

None of the vertebral muscle metrics showed significant difference from isometry either before or after phylogenetic correction (Table S2).

Principal components analyses and phylogenetic MANOVAS

PCA of all of the metrics for the hindlimb muscles alone produced eight significant PC axes according to the Joliffe cutoff, which is automatically generated in PAST. PC1 represented 28.5% of the total variance, PC2 was 15.4%, with PC3-8 representing between 12.8% and 4.5% (Figure 3). There was no significant separation between body size or locomotory groups using either a MANOVA or phylogenetic MANOVA of all PCs ($p \gg 0.05$ in all analyses). Adding data from lumbosacral vertebral muscles did not improve the ability to distinguish among either body size or locomotor groupings ($p \gg 0.05$).

Discussion

In quadrupeds, the hindlimbs are usually the main propulsive drivers (Alexander,1977; Alexander et al., 1981; Hudson et al., 2011a) and as such play more roles than just limb maintaining support against gravity. The muscles responsible for such roles are primarily the hip extensors (Alexander,1977; Alexander et al., 1981; Usherwood and Wilson, 2005; Williams et al., 2008, 2009; Hudson et al. 2011a). Therefore it should be expected that these muscles will scale with at least isometry, or possibly positive allometry, for the muscle body measurements and PCSA (a metric which is linked to force production). Our results showed that most thigh muscle metrics actually scaled isometrically, or at least with allometry that is indistinguishable from isometry, in our dataset. In the thigh only the M. gluteus medius, M. tensor fascia latae, M. caudofemoralis, M biceps femoris have PCSAs that scale positively allometrically, with the M. biceps femoris (weakly positively allometric), and the M. gluteus medius being responsible for thigh extension (the rest are used in adduction or rotation). Because the muscles' cross-sectional areas scaled isometrically proportional to mass$^{2/3}$, most muscles of the thigh appear to be relatively weaker in larger species of felids.

In quadrupeds able to move rapidly, as taxa become larger, there tends to be a reduction in muscle mass towards the distal ends of limbs, in which the distal muscles transmit their forces down long tendons (Alexander and Jayes, 1983; Payne et al., 2005; Smith et al., 2006, 2007). Cheetahs have been noted to exhibit some similar degree of limb tapering (Hudson et al., 2011a,b). This reduction of distal limb muscle mass does not appear to be the case in felids in general, with all distal muscles' masses scaling isometrically, and only the tendon mass of M. extensor digitorum longus scaled with positive allometry. In felids, this would result in an increase in inertial properties and therefore require more work and power from the muscles to swing the hindlimbs (Hudson et al., 2011b), and with no apparent increase in elastic energy storage by the tendons (Alexander, 1984; Alexander and Maloiy, 1989), thereby reducing the overall efficiency of the hindlimbs in larger taxa. This may be because most felids have to retain limbs that are powerful enough for climbing and capturing prey, as well as being "light" enough for fast locomotion. Perhaps owing to its fast pursuit of prey, the cheetah is the only felid that shows marked limb tapering and as a consequence of its less powerful limbs, tends to feed on relatively smaller prey. Interestingly, a few muscle belly lengths actually scale with negative allometry (Table 4), but this length is not compensated for in any way with positively allometric tendons or muscle fascicles that display unambiguous negative allometry. Previous work indicates that the bone lengths of felid limbs scale isometrically (Anyonge, 1993; Christiansen and Harris, 2005; Doube et al., 2009), but if there is a shortening of some muscle bellies, and no corresponding increase in tendon lengths, there may potentially be some subtle positional changes of these muscles between the taxa or an increase in musculotendinous compliance (Roberts, 2002). Alternatively, with the small sample size, there may just be some outliers within our data, but this would require more specimens to test.

The lack of general allometric increase in muscle PCSAs suggests that felid limbs become relatively weaker at larger body sizes, especially with no reduction in distal limb muscle mass and no increase in tendon masses or lengths across most of the limb, and no change in limb posture (Day and Jayne, 2007; Zhang et al., 2012; Wiktorowicz et al., in review, Doube et al., 2009) as alternative compensatory mechanisms. As terrestrial mammals get larger, maintaining a crouched posture becomes increasingly energetically expensive due to the muscles of the limbs having to balance the moments incurred by the body weight, and the resulting vertical ground reaction forces. The advantage of remaining crouched is that it maximizes the horizontal component of the ground reaction forces' moment arms, potentially allowing for increased locomotor performance in a horizontal direction (Biewener, 1989,1990,2005). However, as felid limb posture does not seem to change with body mass and the muscle force-capacities (linked to PCSA) appear to decrease, it might be predicted that larger felids become relatively slower and incur greater metabolic costs during similar behaviours due to lower mechanical efficiency. Indeed, Day and Jayne (2007) found that the velocity of locomotion within felids (during walking) is broadly similar across all species, consistent with the theory of dynamic similarity (Alexander and Jayes, 1983). Furthermore, Garland (1983) found that larger cats (beyond an optimal body mass of ~40kg) move more slowly than smaller ones. However, felids may partially compensate for the near-isometric muscle scaling by the seemingly increased mechanical advantage of the felid calcaneus (Gálvez-López and Casinos, 2012). Although evidence for allometry of that mechanical advantage is not strong, if present it may help counter the isometric scaling of the gastrocnemius, which is the largest (in terms of PCSA and thus force potential) antigravity muscle in the hindlimb, although further work is required on both the muscles and bones.

Muscle fascicle lengths are linked to contractile speed and range of motion, with longer fascicles able to contract faster and over a longer range of motion than smaller ones (Alexander,1977; Alexander et al., 1981). Typically for most Carnivora the fascicle lengths scale indistinguishably from isometry across the hindlimb (Alexander et al., 1981). Our results broadly fit this pattern of near-isometric scaling, with one exception. In our dataset, inverse allometry (where the slope is actually negative rather than only less than the isometric slope) was detected for the M. digitorum extensor lateralis. Thus, bigger cats have shorter fascicle lengths (in an absolute and relative sense) than in smaller cats for the M. digitorum extensor lateralis, which becomes increasingly multipennate in form, resulting in a slower digital extension or more limited range of motion in larger cat species. What role this may play in their ecology and locomotion is uncertain, however.

The limb muscles, nonetheless, do not work in isolation; the vertebral muscles also play important roles in support and locomotion. All of vertebral muscles' metrics from the lumbosacral region scale isometrically in felids; therefore the vertebral muscles also seem to get relatively weaker with increasing body mass. Whilst this relative weakening of the musculature of the vertebral muscles may be compensated for by positive allometry of vertebrae and the resulting moment arms in other vertebral regions (Jones, 2015; Randau et al., in press). The combined results for the vertebral muscles (here and Cuff et al., in review) show that there is a relative reduction in force production capacity in the spinal musculature of larger felids. This lack of clear allometry of the intervertebral musculature may have consequences for the maximum extension of the spine (a vital component in maximising stride length and, therefore, maximum speed: Hildebrand, 1959), although positive allometry in the lever arms may compensate (Jones, 2015; Jones and Pierce, 2016). However, how the complex interactions of musculoskeletal anatomy, limb posture, range of spinal motion and gait relate to tissue stresses or safety factors across the body size range of Felidae remains unclear and deserves further study. We also accept there are limitations to the current study as all the individuals were captive, of varying degrees of health, and all of our measurements were from a single individual from each species (or, in the case of the lion and tiger, a single subspecies), and not all of the same sex (with the largest species all represented by females), but we have no reason to expect this would change our overall conclusions. For a more in-depth discussion of these limitations see Cuff et al., (in review).

In the forelimbs of felids, only those metrics with the strongest allometric signals remained significantly different from isometry after phylogenetic correction (Cuff et al., in review), and indeed broadly similar results were obtained for the hindlimbs of felids, with only two metrics out of 228 displaying allometry after correction. With so many muscles scaling indistinguishably from isometry (or scaling only weakly allometrically), there is no separation of the taxa using PCAs or MANOVAs when assessing body mass groupings (Cuff et al., 2015) or locomotor mode either before or after phylogenetic correction. This will remain an issue in muscle scaling studies at least until larger sample sizes are studied, particularly in felids, with many of the largest felids being closely related members of the genus *Panthera* (the exceptions being the cheetah and puma, which convergently evolved larger body sizes: Cuff et al., 2015). This close relationship of large-bodied felids (i.e. *Panthera*) means that any potentially allometric patterns are more difficult to tease apart from the null hypothesis of similarity due to common ancestry, and thus more difficult to distinguish modest allometry from true isometry in the musculoskeletal system of Felidae. However, the dataset provided here is an important step forward in understanding how felid locomotor muscles scale with body mass, and future efforts can test our findings by building on this dataset.

Conclusions

Unlike the predominantly supportive, deceleratory and prehensile roles of the forelimb muscles, the musculature of the hindlimb is responsible for generating most of the acceleratory forces during typical (e.g. steady-state) locomotion in felids. However, the majority of propulsive (and other) hindlimb muscles appear to scale isometrically across Felidae, with only the strongest allometries remaining significant after phylogenetic correction. As a consequence, larger felids have relatively weaker hindlimb muscles than those of their smaller relatives, consistent with the reduction in relative and even absolute locomotor speeds as observed in other studies (Garland, 1983; Day and Jayne, 2007). The vertebral muscles emphasize these results further, with all of the metrics scaling indistinguishably from isometry. Furthermore, multivariate analysis (PCA) of muscle metrics were unable to distinguish between locomotor modes and body mass difference, which may be due in part to the phylogenetic proximity of most large- and small-bodied felids (Cuff et al., 2015).

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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	*Felis nigripes*	F	1.1	Underweight
Domestic cat	*Felis catus*	F	2.66	Underweight
Caracal	*Caracal caracal*	M	6.6	Underweight
Ocelot	*Leopardus pardalis*	M	9.6	Overweight
Cheetah	*Acinonyx jubatus*	Mix	33.1 average	Unknown
Snow leopard	*Panthera uncia*	F	36	OK
Jaguar	*P. onca*	F	44	OK
Sumatran tiger	*P. tigris sumatrae*	F	86	OK
Asian lion	*P. leo persica*	F	133	Overweight

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

Muscle	**slope**	**lower limit**	**upper limit**	**pval**	**intercept**	**r^2 p**	**pval**	**n**
Before correction								
Piriformis	**0.167**	**0.101**	**0.276**	**0.013**	**-1.43**	**0.722**	**0.008**	**8**
Peroneus brevis	**0.192**	**0.112**	**0.330**	**0.047**	**-1.14**	**0.677**	**0.012**	**8**
Soleus	**0.212**	**0.147**	**0.304**	**0.021**	**-1.06**	**0.863**	**0.001**	**8**
Gastrocnemius medialis	**0.262**	**0.216**	**0.317**	**0.022**	**-1.14**	**0.963**	**0.000**	**8**
Semitendinosus	**0.279**	**0.242**	**0.322**	**0.023**	**-0.980**	**0.980**	**0.000**	**8**
After correction								
None								

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

Muscle	**slope**	**lower limit**	**upper limit**	**pval**	**intercept**	**r^2 p**	**pval**	**n**
Before correction								
Superficial dig. flex.	0.887	0.369	2.134	0.031	-2.48	0.007	0.846	8
After correction								
None								

Table 4. Significant RMA (before and after phylogenetic correction) scaling results for log muscle fascicle lengths plotted against log body mass , displaying only those that differ from an isometric slope value of 0.333. Results with significant r^2 are shown in bold. Column headings as in Table 2.

Muscle	**slope**	**lower limit**	**upper limit**	**pval**	**intercept**	**r^2 p**	**pval**	**n**
Before correction								

Lateral dig. ext.	**-0.185**	**-0.300**	**-0.114**	**0.022**	**-1.26**	**0.684**	**0.006**	**9**
Vastus intermedius	**0.617**	**0.374**	**1.018**	**0.021**	**-2.15**	**0.659**	**0.008**	**9**
Peroneus brevis	0.716	0.349	1.469	0.038	-2.60	0.234	0.187	9
Psoas major	0.936	0.417	2.101	0.019	-2.11	0.580	0.078	6
Adductor magnus	1.20	0.567	2.523	0.002	-2.02	0.162	0.282	9
After correction								
None								

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

Muscle	**slope**	**lower limit**	**upper limit**	**pval**	**intercept**	**r^2 p**	**pval**	**n**
Before correction								
Vastus intermedius	**0.796**	**0.650**	**0.976**	**0.033**	**-2.619**	**0.947**	**0.000**	**9**
Gluteus medius	**1.22**	**1.12**	**1.33**	**0.001**	**-2.800**	**0.991**	**0.000**	**9**
After correction								
Gluteus medius	**1.25**	**1.08**	**1.45**	**0.010**	**0.010**	**0.978**	**0.000**	9

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

Muscle	**slope**	**lower limit**	**upper limit**	**pval**	**intercept**	**r^2 p**	**pval**	**n**
Before correction								
Long dig. ext.	**1.57**	**1.06**	**2.31**	**0.029**	**-4.610**	**0.841**	**0.001**	**9**
Superficial dig. flex.	**1.71**	**1.15**	**2.54**	**0.014**	**-4.47**	**0.836**	**0.001**	
Psoas major	**1.72**	**1.08**	**2.76**	**0.042**	**-5.129**	**0.999**	**0.024**	**7**
After correction								
None								

Table 7. Significant RMA (before and after phylogenetic correction) scaling results for log physiological cross-sectional area plotted against log body mass, displaying only those that differ from an isometric slope value of 0.667. Results with significant r^2 are shown in bold. Column headings as in Table 2.

Muscle	**slope**	**lower limit**	**upper limit**	**pval**	**intercept**	**r^2 p**	**pval**	**n**
Before correction								
Biceps femoris	**0.862**	**0.680**	**1.09**	**0.037**	**-4.18**	**0.929**	**0.000**	**9**
Caudal tibial	**0.977**	**0.790**	**1.21**	**0.003**	**-4.90**	**0.943**	**0.000**	**9**
Gluteus medius	**1.00**	**0.769**	**1.31**	**0.008**	**-4.39**	**0.910**	**0.000**	**9**
Tensor fascia latae	**1.05**	**0.725**	**1.52**	**0.022**	**-4.75**	**0.821**	**0.001**	**9**
Gemelli	**1.10**	**0.739**	**1.64**	**0.021**	**-5.05**	**0.832**	**0.002**	**8**

Tibialis cranialis	**1.12**	**0.847**	**1.49**	**0.003**	**-5.08**	**0.897**	**0.000**	**9**
Caudofemoralis	**1.17**	**0.781**	**1.74**	**0.012**	**-5.40**	**0.788**	**0.001**	**9**
After correction								
Tibialis cranialis	**1.14**	**0.698**	**1.85**	**0.036**	**0.017**	**0.743**	**0.006**	**9**
Caudofemoralis	1.32	0.680	2.56	0.045	-0.036	0.491	0.053	9

Figures

Figure 1. Muscles displaying potential allometry (prior to phylogenetic analysis) in the studied felid species are shown in colour; others as white; for a representative right hindlimb. A) Lateral superficial muscles of hip and knee; B) Lateral, deeper muscles of the hindlimb; C) Medial muscles of the thigh and shank; D) Lateral muscles of the lower leg; E) Medial muscles of the lower leg. Red = muscle belly length; orange = tendon length; navy blue = muscle mass; light blue = tendon mass; green = PCSA. Stippling pattern is for negative allometry. Muscles not shown: M. psoas majorum (Table 6); M. vastus intermedius (Table 4,5); M. lateral digital extensor (Table 4), , M. superficial digital flexor (Table 6); M. peroneus brevis (Table 2).

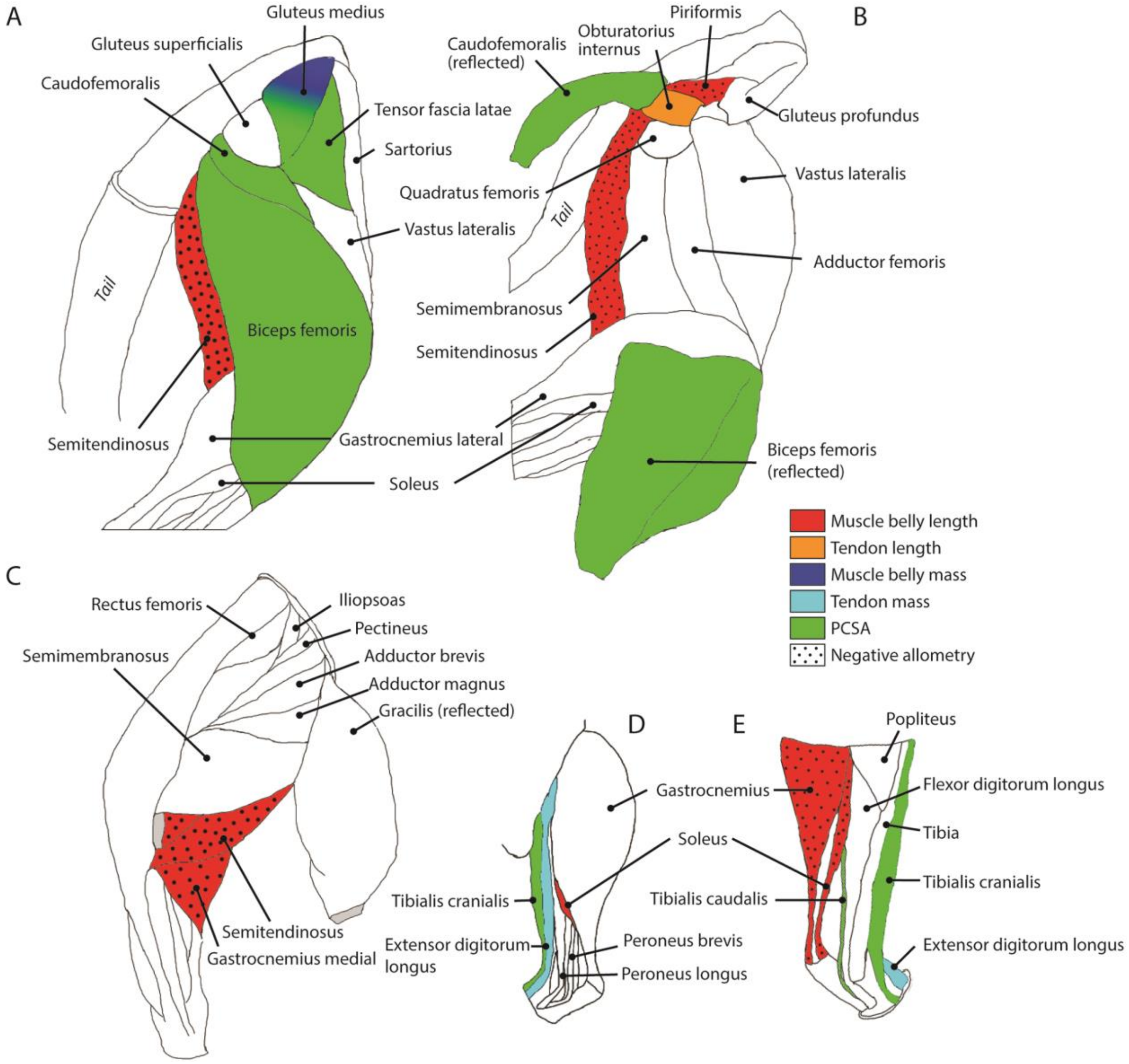


Figure 2. Muscles displaying potential allometry (after phylogenetic analysis) in the studied felid species are shown in colour; others as white; for a representative right hindlimb. A) Lateral superficial muscles of hip and knee; B) Lateral, deeper muscles of the hindlimb; C) Medial muscles of the thigh and shank; D) Lateral muscles of the lower leg; E) Medial muscles of the lower leg. Navy blue = muscle mass; green = PCSA. Stippling pattern is for negative allometry.

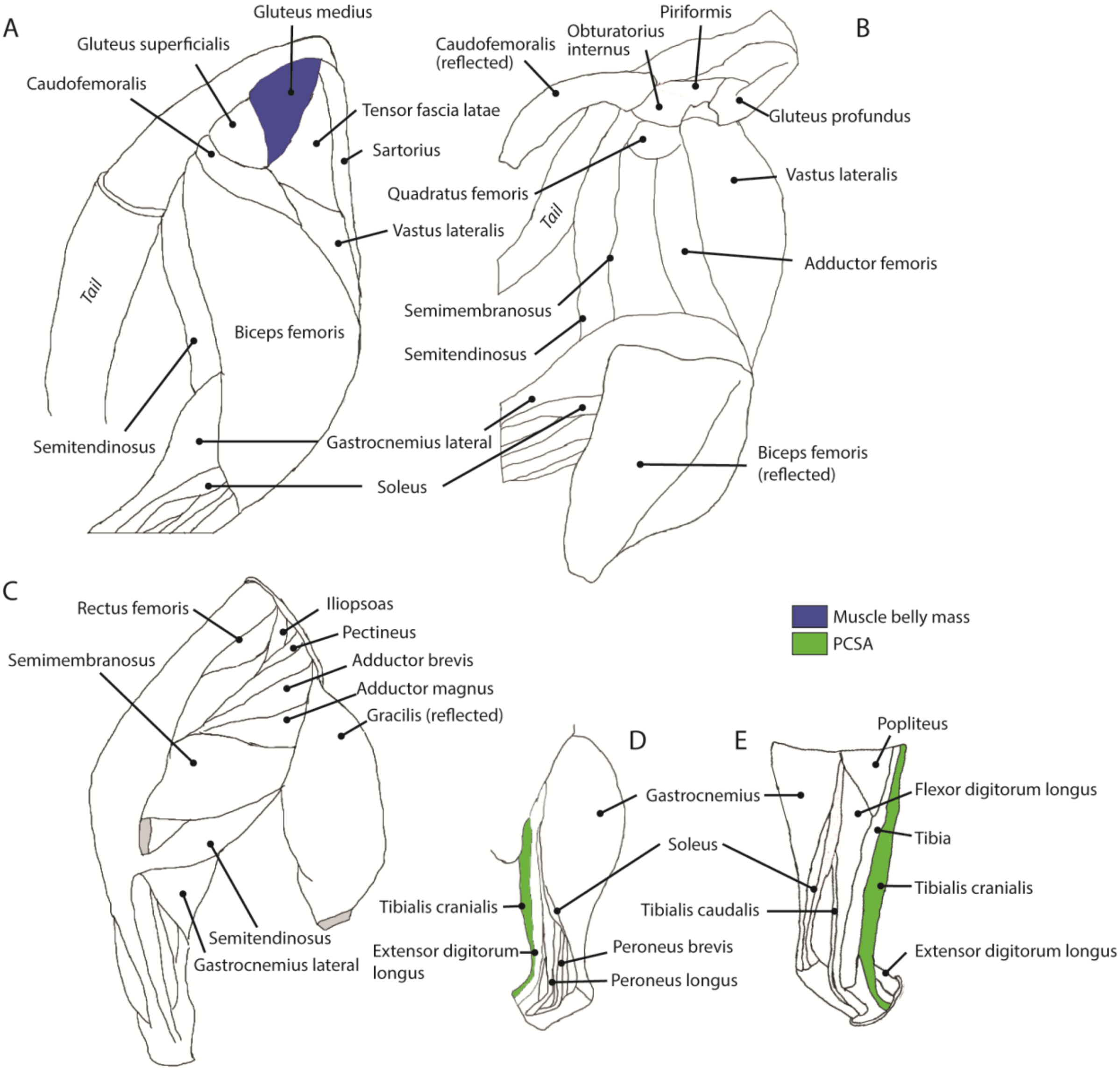
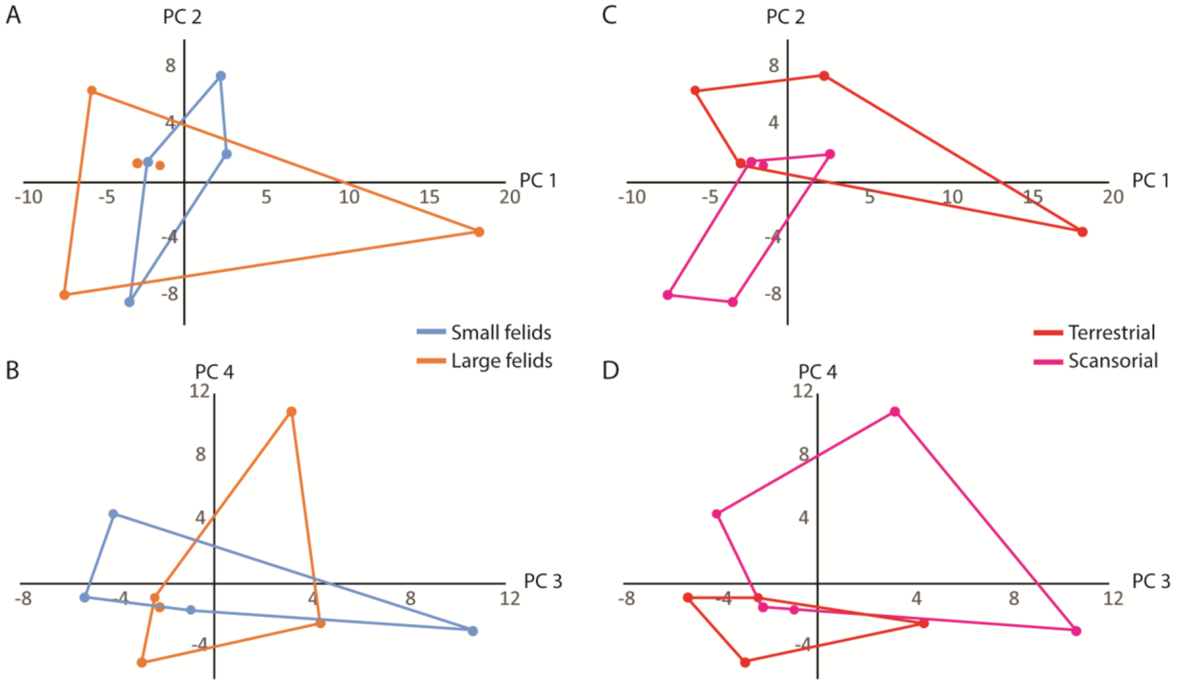


Figure 3. Principal component analysis of hind limb muscle architecture metrics. A) and B) show body size groups, with blue for small felids and orange for large felids (groupings follow Cuff et al., 2015); C) and D) show locomotory mode groups with red for terrestrial and pink for scansorial. A) and C) show PC1 (28.48% of total variance) vs PC 2 (15.39% of total variance); C) and D) show PC3 (12.83% of total variance) vs PC 4 (11.24% of total variance).


A
PC 2
PC 1
-10
-5
5
10
15
20
8
4
-4
-8
Small felids
Large felids
B
PC 4
PC 3
12
8
4
-4
-8
-4
4
8
12
C
PC 2
PC 1
Terrestrial
Scansorial
D
PC 4
PC 3