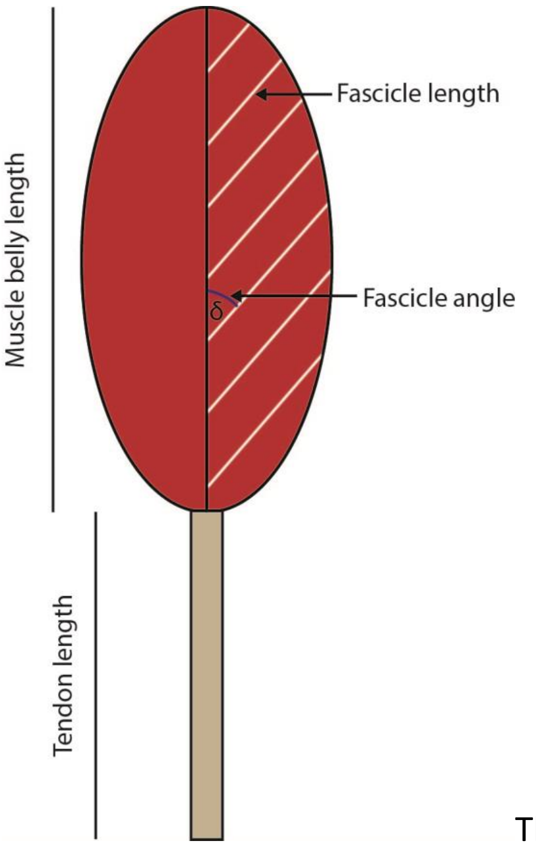

The scaling of postcranial muscles in cats (Felidae) I: forelimb, cervical, and thoracic muscles

Andrew R. Cuff[1,2], Emily L. Sparkes[2], Marcela Randau[1], Stephanie E. Pierce[3,2], Andrew C. Kitchener[4], Anjali Goswami[1*], John R. Hutchinson[2,1*]

1. GEE, University College London, Darwin Building, Gower Street, London, WC1E 6BT, United Kingdom

2. Structure and Motion Lab, Department of Comparative Biomedical Sciences, The Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts, AL9 7TA, United Kingdom

3. Museum of Comparative Zoology and Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA

4. National Museums Scotland, Chambers Street, Edinburgh, EH1 1JF, United Kingdom and Institute of Geography, University of Edinburgh, Drummond Street, Edinburgh, EH8 9XP, United Kingdom

*joint senior authors

Abstract

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

Keywords: Felidae, muscle, scaling, body mass

Introduction

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

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

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

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

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

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

Methods

Specimens

Our study species 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 (data from Hudson et al., 2009a,b) snow leopard (*Panthera uncia*: NMS.Z.2015.89.2; female), jaguar (*P. onca*: NMS.Z.201467.2; female), Sumatran tiger (*P. tigris sondaica*: NMS.Z.2015.91; female), and Asian lion (*P. leo persica*: NMS.Z.2015.128; female). The majority of the felid specimens were obtained from various public and private zoo/park facilities around the United Kingdom. The domestic cat was a pet that was euthanized after a long-term decline in health and donated to the Royal Veterinary College for scientific research. No specimens were euthanized for the purposes of this research. The institutional abbreviation NMS refers to the National Museums Scotland, Department of Natural Sciences; source of many of our specimens as per below. All body mass and dissection data are included in the Supplementary information.

Dissection

With the exception of the Asian lion, which was dissected shortly after death, all specimens were freshly frozen after death and then defrosted (variably 24-48 hrs) prior to dissection. Initially, each specimen had the limbs from one side removed (generally the right-hand side, but for the Asian lion the left-hand side's limbs were removed) and refrozen, allowing for future dissection if the initial material was incomplete or damaged. Next, the muscles from the forelimb and vertebral column were dissected individually and muscle architecture was measured following standard procedures (e.g. 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) (Figure 1). The belly and fascicle lengths for most muscles were measured using plastic rulers or tapes (accurate to 1mm), but for some of the smallest species fascicle lengths were measured using Vernier callipers (accurate to 0.1mm). Masses were measured using electronic scales (accuracy between 0.001g and 0.01g). These data were used to calculate physiological cross-sectional area (PCSA) for each muscle, as follows:

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

where muscle volume is calculated using equation 2:

Eq.2
$$Muscle\ volume = Muscle\ mass\ \cdot\ density$$

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

In total, we measured 41 forelimb muscles for all nine species, producing 246 metrics per species, and 16 vertebral muscles producing 96 metrics per species. For most species, fewer than 10 metrics were missing in total. The exceptions are the ocelot (which only had one usable forelimb), and the cheetah, as the data taken from Hudson et al. (2011a) yielded only 50% completeness for forelimb measures (no muscle length or 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 analyses. As noted above, some measurements were incomplete for the taxonomic sample. Where metric values were equal to zero (limited exclusively to tendon lengths and masses where there were no tendons), the data were removed before scaling analyses. Metrics for which there were data from less than three species were removed, but only those 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). All data were logged, and then each logged metric was regressed against $\log_{10}$ body mass, using Standardised Reduced Major Axis (SMA) regression ("Model II"; see Sokal and Rohlf, 1995) in 'smatr' package (Warton et al., 2013) in R 3.1.0 (R Core Team, 2014). Significances of the slope of the regression line 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 those expected for a given increase in body size (i.e., maintaining geometric similarity), whilst allometry is an increase or decrease from that slope. For these logged metrics, isometry is defined as follows: muscle masses scale against body mass with slope equal to 1.00; lengths scale against body mass with a slope of 0.333 (i.e. length is proportional to $mass^{1/3}$); and PCSA scales against body mass with a slope of 0.667 (i.e. area is proportional to $mass^{2/3}$).

We wanted to account for the fact that closely related species tend to be have characteristics more similar to each other than more distantly related species; therefore, each variable was analysed for phylogenetic signal using the phylosignal function in the 'picante' package (Kembel et al., 2010) in R, which measures phylogenetic signal with the K statistic. This statistic reflects the difference between the observed tip data and the expected values under a Brownian motion model for any given phylogeny (Blomberg et al., 2003). A value for K close to 1.0 suggests a Brownian motion pattern, while values <1.0 indicate less resemblance among related species than would be expected under Brownian motion, and values >1.0 indicate more resemblance (Kembel et al., 2010). Although the raw value of the K statistic assesses the fit of a Brownian motion model, the calculated p-value reflects the strength of the phylogenetic signal in the data set, with a significant result ($p<0.05$) indicating that there is a significant phylogenetic signal in the data, as is the case for felid body size (Cuff et al., 2015). The phylogeny used for this analysis is from Piras et al. (2013) – a combined morphometric and molecular (from Johnson et al., 2006) phylogeny – and was pruned to only include the taxa in this study. Only metrics for which there were significant phylogenetic signal underwent correction using independent contrasts, 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

In addition to the regression analyses, principal components (PC) analyses were 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. The imputed data were calculated iteratively using regression values for the missing data 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). The "allometric vs. standard" option within the "remove size from distances" tool was used to remove the effects of body size upon the metrics. This adjustment works by estimating allometric coefficients with respect to a standard metric (in this case the mass), with each metric being regressed against the standard metric after log-transformation (e.g. Elliot et al., 1995) giving a slope *b* for that metric. An adjusted measurement was then computed from the original value following the equation:

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

Where $\mathrm{metric}_{\mathrm{adj}}$ is the new adjusted metric, $\mathrm{metric}_{\mathrm{orig}}$ is the original, $\overline{\mathrm{mass}}$ is the mean mass across all species and ^{b} is the slope equation. The felid species were assigned to groups firstly by body size (i.e., small cat vs. big cat, following Cuff et al., 2015, although here defined as *Panthera* vs non-*Panthera* species), and in a second analysis by locomotor mode (terrestrial: *Felis nigripes*, *Acinonyx jubatus*, *Panthera tigris*, *Panthera leo*; scansorial: *Felis silvestris*, *Caracal caracal*, *Leopardus pardalis*, *Panthera uncia*, *Panthera onca*). Significant PC scores were subsequently 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

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

Forelimb

The muscle belly lengths (Figure 2) of M. serratus ventralis cervicis, M. triceps lateralis, M. omotransversarius, M. biceps brachii, and M. deltoideus spinous all displayed significant negative allometry, whilst M. abductor digitorum I showed a significantly positive allometric slope prior to phylogenetic correction (Table 2). The tendon lengths of M. abductor digitorum I, M. triceps longus, M. cleidobrachialis and M. infraspinatus were all significantly positively allometric before phylogenetic correction (Table 3). The M. trapezieus thoracis, M. latissimus dorsi, M. serratus ventralis cervicis, M. biceps brachii and M. omotransversarius fascicle lengths all scaled with negative allometry, whilst the M. flexor carpi $\mathrm{ulnaris}_{\mathrm{humeral}}$, M. brachialis, M. pronator teres, M. abductor digitorum I and M. flexor carpi radialis all exhibited significant positive allometry before phylogenetic correction (Table 4). Nevertheless, after phylogenetic correction, all length metrics for the forelimb displayed scaling exponents that were statistically indistinguishable from isometry.

Only the M. brachioradialis showed positively allometric scaling of muscle belly mass both before and after phylogenetic correction (Table 5). The M. flexor carpi radialis displayed a negatively allometric tendon mass before phylogenetic correction, but no other muscles showed any scaling that was statistically different from isometry (Table 6). Eleven muscles have PCSAs that scale with positive allometry before phylogeny was accounted for, including the M. brachioradialis, which also displayed significant positive allometry after phylogenetic correction whereas the other 10 muscles did not (Table 7).

Vertebral muscles

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

Principal components analyses and phylogenetic MANOVAS

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

Discussion

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

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

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

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

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

However, the limb muscles of felids only tell part of the story, with the vertebral muscles also surely playing important roles in support and locomotion, as well as predation. Most of the cervico-thoracic muscles scale isometrically, particularly with respect to masses and PCSAs. Therefore, the vertebral muscles also seem to get relatively weaker with increasing body mass in felids. Whilst the muscle weakening of the musculature of the anterior vertebral column may be compensated for by positive allometry of vertebrae and the resulting moment arms (Jones, 2015a,b; Randau et al., in press), the combined result with the forelimb muscles show that there is a relative reduction in force production in the musculature of the anterior half of the larger felids. But, how the biomechanics of the musculoskeletal anatomy, limb posture and gait of felids interact to produce overall changes in tissue stresses or safety factors across the size range of Felidae remains unclear, and would require more sophisticated methods to resolve.

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

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

The data and results presented here are derived from captive animals, which died either from ill health, or from euthanasia associated with a decline in health. These specimens tended to be either overweight (e.g. the Asian lion) or underweight (e.g. caracal and domestic cat). Thus these animals presumably had relatively smaller muscles than their wild counterparts. In a study of cheetahs, wild individuals were found to have much larger limb muscles (Hudson et al., 2011a,b). Associated with the animals' poor health, alterations in muscle architecture linked to a lack of physical activity are likely (Blazevich et al., 2003), and muscle shortening is probable, due to rigor mortis and the freezing process (Cutts, 1988). All animals, except the Asian lion, were subjected to the same post-mortem procedures, and most of the muscles had angles of pennation of 30° or less, hence the cosine of the pennation angle (equation 2) was close to 1. Therefore, the pennation angle in these muscles (as noted in Methods, not subjected to scaling analysis here) had a very small effect on the PCSA of the muscles (Calow and Alexander, 1973) and thus is a minimal concern for our study. In addition, all of our measures are from a single individual from each species (or, in the case of the cheetah, lion and tiger, a single subspecies), and not all of the same sex. However, there is no reason to expect that these data are outliers or otherwise non-representative for their respective species, although there will certainly be intraspecific variation (Hudson et al., 2011a,b). Despite these caveats, this study provides the only data currently available for muscle architecture across much of the size range of the Felidae. Future work and continued data collection will be able to test the stability of these results with respect to the potentially complicating factors discussed above, but we do not expect that our fundamental conclusions are unduly influenced by them.

Conclusions

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

Acknowledgements

This work was funded by Leverhulme Trust grant RPG 2013-124 to AG and JRH. ACK thanks the Aspinall Foundation (Port Lympne Wild Animal Park), the Zoological Society of East Anglia (Banham Zoo), the Cat Survival Trust, Thrigby Hall Wildlife Gardens, Cromer Zoo and the Zoological Society of London (London Zoo) for donation of specimens used in this study. ACK is grateful to the Negaunee Foundation for its support of the Curatorial Preparator at National Museums Scotland. We thank Julie Meachen and two anonymous reviewers for comments that substantially improved this manuscript.

References

Adams DC, Otarola-Castillo E (2013) geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution* **4**, 393-399.

Alexander RMcN (1977) Allometry of the limbs of antelopes (Bovidae). *J. Zool. Lond.* **183**, 125-146.

Alexander RMcN, Jayes AS (1978) Vertical movements in walking and running. *J Zool Lond* **185**, 27–40.

Alexander RMcN, Jayes AS (1983) A dynamic similarity hypothesis for the gaits of quadrupedal mammals. *Journal of Zoology* **201**, 135-152.

Alexander RMN, Maloiy GMO (1989) Locomotion of African mammals. *Sym Zool S* **61**, 163-180.

Anyonge W (1993) Body mass in large extant and extinct carnivore. *J Zoo.* **231**, 339-384.

Barclay OR (1953). Some aspects of the mechanics of mammalian locomotion. *J Exp Biol* **30**, 116-120.

Biewener AA (1989) Scaling body support in mammals: limb posture and muscle mechanics. *Science **245**, 45-48.*

Biewener AA (1990) Biomechanics of mammalian terrestrial locomotion. *Science **250**, 1097-1103.*

Biewener AA (2005) Biomechanical consequences of scaling. *J Exp Biol **208**, 1665-1676.*

Blazevich AJ, Gill ND, Bronks R, Newton RU (2003). Training-specific muscle architecture adaptation after 5-wk training in athletes. *Med Sci Sports Exerc* **35**, 2013-2022.

Blomberg SP, Garland TJ, Ives AR (2003) Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution* **57**, 717-745.

Calow LJ, Alexander RMN (1973) A mechanical analysis of a hind leg of a frog (*Rana temporaria*). *J Zoo.* **171**, 293-321.

Campione NE, Evans DC (2012) A universal scaling relationship between body mass and proximal limb bone dimensions in quadrupedal terrestrial tetrapods. *BMC Biology* **10**, 60.

Carbone C, Mace GM, Roberts SC, Macdonald DW (1999) Energetic constraints on the diet of terrestrial carnivores. *Nature* **402**, 286-288.

Cavagna GA, Franzettti P, Heglund NC, Willems P (1988) The determinants of the step frequency in running, trotting and hopping in man and other vertebrates. *Journal of Physiology* **399**, 81-92.

Christiansen P, Harris JM (2005) The body size of *Smilodon* (Mammalia:Felidae). *J Morph* **266**, 369-384.

Cuff AR, Randau M, Head J, Hutchinson JR, Pierce SE, Goswami A (2015) Big cat, small cat: Reconstructing body size evolution in living and extinct Felidae. *Journal of Evolutionary Biology* **28**, 1516-1525.

Cuff AR, Sparkes E, Randau M et al. (submitted). The scaling of hindlimb and lumbosacral muscles across cats (Felidae)

Cutts A (1988). Shrinkage of muscle fibres during the fixation of cadaveric tissue. *J Anat.* **160**, 75–78

Day LM, Jayne BC (2007) Interspecific scaling of the morphology and posture of the limbs during the locomotion of cats (Felidae). *J Exp Biol* **210**, 642-654.

Doube M, Wiktorowicz-Conroy A, Christiansen P, Hutchinson JR, Shefelbine S (2009) Three-dimensional geometric analysis of felid limb bone allometry. *PLoS One* **4**, e4742.

Elliott NG, Haskard K, Koslow JA (1995) Morphometric analysis of orange roughy (*Hoplostethus atlanticus*) off the continental slope of southern Australia. *Journal of Fish Biology* **46**, 202-220.

Felsenstein J (1985) Phylogenies and the comparative method. *The American Naturalist* **125**, 1-15.

Fischer MS, Schilling N, Schmidt M, Haarhaus D, Witte H (2002). Basic limb kinematics of small therian mammals. *The journal of experimental biology* **205**, 1315-1338.

Gálvez-Lopéz E, Casinos A (2012) Scaling and mechanics of the felid calcaneus: geometric similarity without differential allometric scaling. *J Anat* **220**, 555-563.

Garland TG Jr. (1983) The relation between maximal running speed and body mass in terrestrial mammals. *J Zool Lond* **199**, 157-170.

German A, Hill J (2006) *Data analysis using regression and multilevel/hierarchical models (Analytical methods for social research)*. Cambridge University Press: New York.

Gonyea WJ (1973) Some functional aspects of the postcranial anatomy of the Felidae (Carnivora). Thesis, University of Chicago, Illinois.

Gonyea W, Ashworth R (1975) The form and function of retractile claws in the Felidae and other representative carnivorans. *J Morphol.* **145**, 229-238.

Goslow CE Jr., Reinking RM, Stuart DG (1973) The cat step cycle: Hind limb joint angles and muscle lengths during unrestrained locomotion. *J. Morph* **141**, 1-41.

Hammer Ø, Harper DAT, Ryan PD (2001) Past: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4**: 9.

Heglund NC, Taylor CR (1988) Speed, stride frequency and energy cost per stride: how do they change with body size and gait? *Journal of Experimental Biology* **138**, 301-318.

Hildebrand M (1959) Motions of the running cheetah and horse. *Journal of Mammalogy* **40,** 481-495.

Hildebrand M (1961) Further studies on the locomotion of the cheetah. *Journal of Mammalogy* **42,** 84-91.

Hudson PE, Corr SA, Payne-Davis RC, Clancy SN, Lane E, Wilson AM (2011a). Functional anatomy of the cheetah (*Acinonynx jubatus*) hindimb. *J. Anat.* **218**, 363-374

Hudson PE, Corr SA, Payne-Davis RC, Clancy SN, Lane E, Wilson AM (2011b). Functional anatomy of the cheetah (*Acinonynx jubatus*) forelimb. *J. Anat.* **218**, 375-385

Ilin A, Raiko T (2010) Practical approaches to principal component analysis in the presence of missing values. *Journal of Machine Learning Research* **11**, 1957-2000.

Johnson WE, Eizirik E, Pecon-Slatter J, et al. (2006) The late Miocene radiation of modern Felidae: a genetic assessment. *Science* **311**, 73-77.

Jones KE (2015a) Evolutionary allometry of lumbar shape in Felidae and Bovidae. *Biol. J. Linn. Soc. Lond*. **116**, 721–740.

Jones KE (2015b). Evolutionary allometry of the thoracolumbar centra in felids and bovids. *J. Morphol.* **276,** 818–831.

Keller TS, Weisberger AM, Ray JL, Hasan SS, Shiavi RG, Spengler DM (1996) Relationship between vertical ground reaction force and speed during walking, slow jogging, and running. *Clinical Biomechanics* **11**, 253-259.

Kembel SW, Cowan PD, Helmus MR et al. (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26**, 1463–1464.

Kitchener AC, Van Valkenburgh B, Yamaguchi N, (2010) Felid form and function. *In:* Macdonald, D.W. and Loveridge, A.J. (eds.). *Biology and conservation of wild felids,* pp. 83-106. Oxford: Oxford University Press.

Kleiman DG, Eisenberg JF (1973) Comparisons of canid and felid social systems from an evolutionary perspective. *Anim Behav.* **21**, 637-659.

Koob TJ, Long JH (2000). The vertebrate body axis: Evolution and mechanical function. *Am Zool* **40**, 1-18.

Lewis ME, Lague MR (2010) Interpreting sabretooth cat (Carnivora; Felidae; Machariodontinae) postcranial morphology in light of scaling patterns in felids. *Carnivoran Evolution: New Views on Phylogeny, Form and Function*.Cambridge: Cambridge University Press. pp 411–465.

Leyhausen P (1965) Über die Funktion der Relativen Stimmungshierarchie (Dargestellt am Beispiel der phylogenetischen und ontogenetischen Entwicklung des Beutefangs von Raubtieren. *Z. Tierpsychol* **22**, 246-272.

Leyhausen P (1979) Cat behavior: the predatory and social behavior of domestic and wild cats. New York: Garland STPM Press.

Long JH, Pabst DA, Shepherd WR, McLellan WA (1997 Locomotor design of dolphin vertebral columns: bending mechanisms and morphology of *Delphinus delphis*. *J Exp Biol* **200**, 65-81.

Meachen JA, O'Keefe FR, Sadleir RW (2014) Evolution in the sabretooth cat *Smilodon fatalis* in response to Pleistocene climate change. *J Exp Biol* **27**, 714-723.

Meachen-Samuels J, Van Valkenburgh B (2009a) Forelimb indicators of prey-size preference in the Felidae. *J Morphol* **270**, 729-744.

Meachen-Samuels J, Van Valkenburgh B (2009b) Craniodental indicators of prey size preference in the Felidae. *Biol J Linn Soc* **96**, 784-799.

Meachen-Samuels JA, Van Valkenburgh B (2010) Radiographs reveal exceptional forelimb strength in the sabretooth cat, *Smilodon fatalis*. *PLoS One* **5**, e11412.

Mendez J, Keys A (1960) Density and composition of mammalian muscles. *Metabolism* **9**, 184-188.

Molnar J, Pierce SE, Hutchinson JR (2014) An experimental and morphometric test of the relationship between vertebral morphology and joint stiffness in Nile crocodiles (*Crocodylus niloticus*). *J Exp Biol* **217**, 757-768. doi: 10.1242/jeb.089904

Payne RC, Hutchinson JR, Robilliard JJ, et al., (2005) Functional specialisation of pelvic limb anatomy in horses (*Equus caballus). J Anat* **206**, 557–574.

Peigné S, de Bonis L, Likius A, Mackaye HT, Vignaud P, Brunet M (2005) A new machairodontine (Carnivora, Felidae) from the Late Miocene hominid locality of TM 266, Toros-Menalla, Chad. *Comtes Rendus Palevol* **4**, 243-253.

Pierce SE, Clack JA, Hutchinson JR (2011) Comparative axial morphology in pinnipeds and its correlation with aquatic locomotory behaviour. *J Anat* **219,** 502–514.

Piras P, Maiorino L, Teresi L, et al. (2013) Bite of the cats: relationships between functional integration and mechanical performance as revealed by mandible geometry. *Systematic Biology* **62**, 878-900.

Pollock CM, Shadwick RE (1994a) Allometry of muscle, tendon, and elastic energy storage capacity in mammals. *Am J Physiol Regulatory Integrative Comp Physiol* **266**, 1022-1031.

Pollock CM, Shadwick RE (1994b) Relationship between body mass and biomechanical properties of limb tendons in adult mammals. *Am J Physiol Regulatory Integrative Comp Physiol* **266**, 1016-1021.

R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Randau M, Goswami A, Hutchinson JR, Cuff AR, Pierce SE (in press) Cryptic complexity in felid vertebral evolution: shape differentiation and allometry of the axial skeleton. *Zool J Linnean Soc*

Randau M, Carbone C, Turvey ST (2013) Canine Evolution in Sabretoothed Carnivores: Natural Selection or Sexual Selection? *PLoS ONE* **8**(8), e72868. doi: 10.1371/journal.pone.0072868

Rasmussen S, Chan AK, Goslow CE Jr (1978) The cat step cycle: Electromyographic patterns for hindlimb muscles during posture and unrestrained locomotion. *J. Morph* **155**, 253-269.

Ren L, Miller C, Lair R, Hutchinson JR (2010) Integration of biomechanical compliance, leverage, and power in elephant limbs. *Proceedings of the National Academy of Sciences USA* **107**, 7078-7082.

Revell LJ (2012) phytools: An R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol*. **3**, 217-223.

Russell AP, Bryant HN (2001) Claw retraction and protraction in the Carnivora: the cheetah (*Acinonyx jubatus*) as an atypical felid. *J. Zool. Lond.* **254**, 67-76.

Shaller GB (1967) *The deer and the tiger: a study of wildlife in India*. Chicago: University Chicago Press.

Shaller GB (1972) *The Serengeti lion: a study of predator-prey relations*. Chicago: University Chicago Press.

Smith RJ, Cheverud JM (2002) Scaling of sexual size dimorphism in body mass: a phylogenetic analysis of Rensch's rule in primates. *International Journal of Primatology* **23**, 1095-1135.

Smith NC, Wilson AM, Jespers KJ, et al., (2006) Muscle architecture and functional anatomy of the pelvic limb of the ostrich (*Struthio camelus*). *J Anat* **209**, 765–779.

Smith NC, Wilson AM, Jespers KJ, et al., (2007) Muscle moment arms of pelvic limb muscles of the ostrich (*Struthio camelus*). *J Anat* **211**, 311-324.

Sokal RR, Rohlf FJ (1995) Biometry: the principles and practice of statistics in biological research, 3rd ed. Freeman: New York. 887 pp.

Sunquist M, Sunquist F (2002) *Wild cats of the World*. Chicago: University of Chicago Press.

Ueda Y, Niki Y, Yoshida K, Masumitsu H (1981) Force plate study of equine biomechanics—floor reaction force of normal walking and trotting horses. *Bull.Equine Res. Inst*. **18**, 28–41.

Warton DI, Duursma RA, Falster DS, Taskinen S (2012) smatr 3 - an R package for estimation and inference about allometric lines. *Methods in Ecology and Evolution* **3**(2), 257-259.

Weyand PG, Sternlight DB, Bellizzi MJ, Wright S (2000) Faster top running speeds are achieved with greater ground forces not more rapid leg movements. *Journal of Applied Physiology* **89**, 1991-1999.

Witte TH, Knill K, Wilson AM (2004) Determination of peak vertical ground reaction force from duty factor in the horse (Equus caballus). *Journal of Experimental Biology* **207**, 3639-3648.

Zhang KY, Wiktorowicz-Conroy A, Hutchinson JR, Doube M, Klosowski M (2012) 3D Morphometric and Posture Study of Felid Scapulae Using Statistical Shape Modelling. *PLoS ONE* **7**(4), e34619.

Tables

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. SMA 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 indicated in bold. No results were significant after phylogenetic correction. Upper and lower limits represent 95% confidence intervals, "slope p" represents statistical probability of the slope differing from isometry, whilst the "r^2 p" shows the statistical significance of the correlation. All results including non-significant patterns are provided in Supplementary Information.

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

Table 3. Significant SMA (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**	**slope p**	**intercept**	**r^2**	**r^2 p**	**n**
Before phylogenetic correction								
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								

Table 4. Significant SMA (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 shown in bold. Column headings as in Table 2. “Flexor 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
Before phylogenetic correction								
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**
After phylogenetic correction								
None								

Table 5. Significant SMA (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 shown in bold. Column headings as in Table 2.

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

Table 6. Significant SMA (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 shown in bold. Column headings as in Table 2.

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

Table 7. Significant SMA (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 shown in bold. Column headings as in Table 2. “Extensor digitorum (c)” is M. extensor digitorum complex, “Flexor carpi ulnaris (u)” is the ulnar head of that muscle.

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

Table 8. Significant SMA (before and after phylogenetic correction) scaling results for log muscle body lengths plotted against log body mass, 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**	**slope p**	**intercept**	**r^2**	**r^2 p**	**n**
Before phylogenetic correction								
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**
After phylogenetic correction								
None								

Table 9. Significant SMA (before and after phylogenetic correction) scaling results for log tendon lengths plotted against log body mass, 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**	**slope p**	**intercept**	**r^2**	**r^2 p**	**n**
Before phylogenetic correction								
Serratus dorsalis thor.	-1.53	-6.00	-0.389	0.032	0.484	0.001	0.953	5
After phylogenetic correction								
None								

Table 10. Significant SMA (before and after phylogenetic correction) scaling results for log 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 shown in bold. Column headings as in Table 2.

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

Table 11. Significant SMA (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 shown in bold. Column headings as in Table 2.

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

Figure legends

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

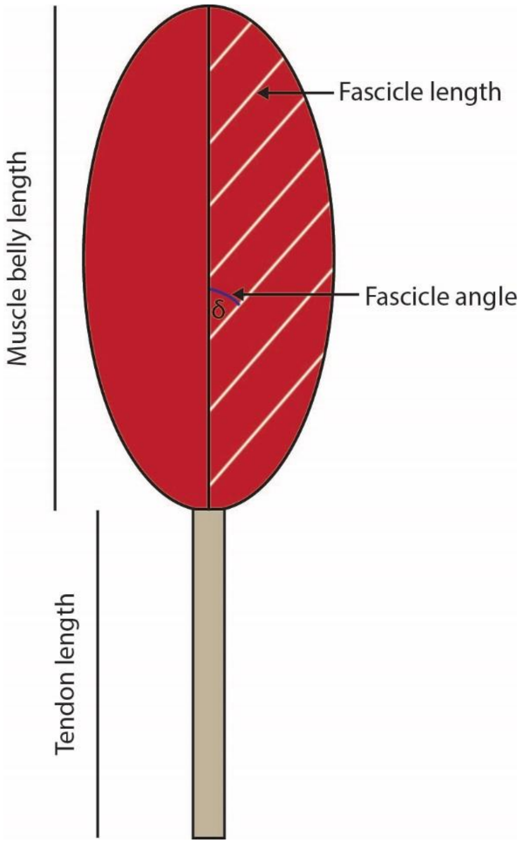

Figure 2. Muscles displaying potential allometry (prior to phylogenetic analysis) in the studied felid species are shown in colour; others as white; for a representative left forelimb. A) Lateral superficial muscles of the shoulder; B) Lateral muscles of the lower forelimb; C) Medial muscles of the lower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

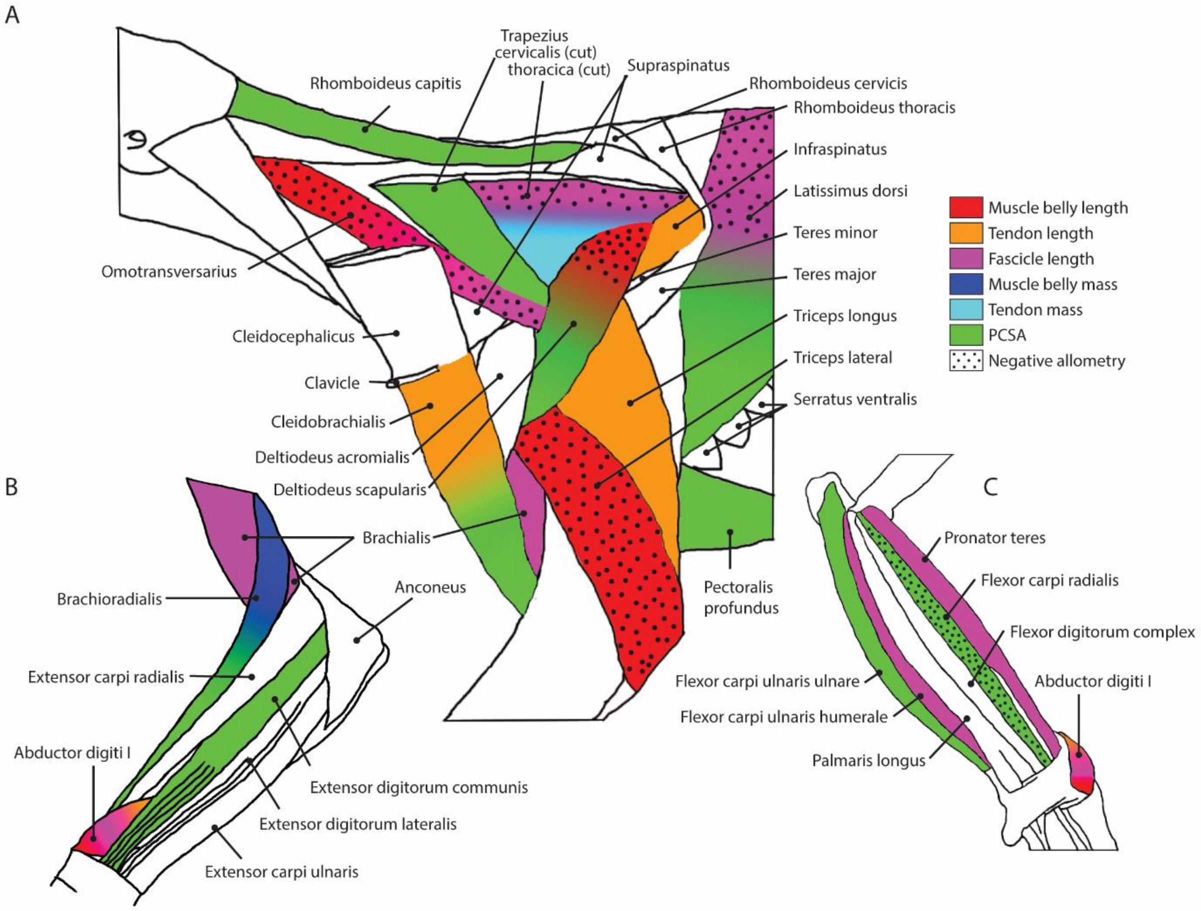

Figure 3 Principal component analysis of the forelimb architectural metrics, grouped by body size and locomotory mode. A) and B) body size, with blue for small felids, orange for large felids (Cuff et al., 2015); C) and D) locomotory mode, with red for terrestrial, pink for scansorial. A) and C) show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

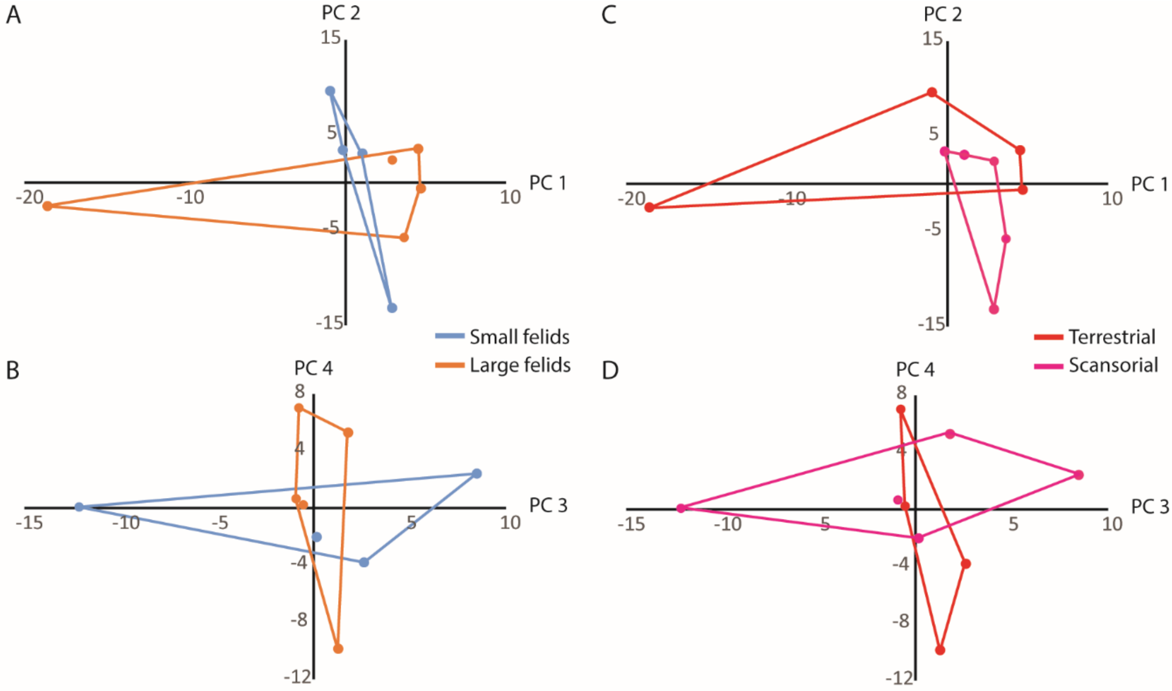

Figure 4. Principal component analysis of the forelimb and vertebral architectural metrics grouped by body size and locomotory mode. A) and B) body size with blue for small felids, orange for large felids (Cuff et al., 2015); C) and D) locomotory mode with red for terrestrial, pink for scansorial. A)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