

Cryptic complexity in felid vertebral evolution: shape differentiation and allometry of the axial skeleton

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Received 23 October 2015; revised 21 January 2016; accepted for publication 25 January 2016

Members of the mammalian family Felidae (extant and extinct cats) are grossly phenotypically similar, but display a 300-fold range in body size, from less than 1 kg to more than 300 kg. In addition to differences in body mass, felid species show dietary and locomotory specializations that correlate to skull and limb osteological measurements, such as shape or cross-sectional area. However, ecological correlates to the axial skeleton are yet untested. Here, we build on previous studies of the biomechanical and morphological evolution of the felid appendicular skeleton by conducting a quantitative analysis of morphology and allometry in the presacral vertebral column across extant cats. Our results demonstrate that vertebral columns of arboreal, scansorial and terrestrial felids significantly differ in morphology, specifically in the lumbar region, while no distinction based on dietary specialization was found. Body size significantly influences vertebral morphology, with clear regionalization of allometry along the vertebral column, suggesting that anterior (cervicals and thoracics) and posterior (lumbar) vertebrae may be independently subjected to distinct selection pressures.

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doi: 10.1111/zoj.12403

ADDITIONAL KEYWORDS: Felidae – locomotion – morphology – vertebral column.

INTRODUCTION

The carnivoran family Felidae (Mammalia, Placentalia) includes *c.* 37 living species of grossly morphologically similar animals (Ewer, 1973; Turner & Antón, 1996; Sunquist & Sunquist, 2002; Johnson *et al.*, 2006; MacDonald, Macdonald & Loveridge, 2010). With the exception of fur patterning, body size is the greatest gross anatomical difference observed between species, with the Felidae displaying a considerable body mass range from 1 kg in the rusty-spotted cat (*Prionailurus rubiginosus*) to over 300 kg in the tiger (*Panthera tigris*). In addition to their overall phenotypic similarity, felids are an exception to the general mammalian biomechanical trend of size-correlated limb posture. According to this trend,

increases in body size drive increased limb erectness (i.e. joint extension) to maintain safe levels of peak functional stresses acting on supportive tissues (Biewener, 1989, 2005; Bertram & Biewener, 1990). However, despite the 300-fold range in body mass in felids, limb posture is remarkably uniform throughout the clade and, instead, some bone allometry is observed in limb long bones' cross-sections (Day & Jayne, 2007; Doube *et al.*, 2009; Zhang *et al.*, 2012). Indeed, it has been hypothesized that the lack of correlation between body size and limb posture in felids may reflect a large-bodied ancestral condition for the clade (Mattern & McLennan, 2000; Johnson *et al.*, 2006; Day & Jayne, 2007; but see Cuff *et al.*, 2015).

Felids are also remarkably conservative in behavioural and ecological attributes, such as diet: all felids are hypercarnivores specialized in vertebrate prey, with species differing mainly in terms of prey

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size and prey-killing techniques (Ewer, 1973; Carbone *et al.*, 1999; Sunquist & Sunquist, 2002). Felid species are known to show different killing strategies in relation to prey size, with bigger cats usually applying a sustained bite to the prey's muzzle or neck, and smaller felids killing by faster nape or head bites (Ewer, 1973; Leyhausen, 1979; MacDonald *et al.*, 2010). Interestingly, unlike other carnivores such as canids, the forelimbs of felids present a duality in function between locomotion and prey-killing behaviour (Ewer, 1973; Gonyea, 1978; Leyhausen, 1979), and therefore, along with differences in skull, mandible and dental shape, the shape of the forelimbs also reflects diversification in prey size choice (Slater & Van Valkenburgh, 2008, 2009; Meachen-Samuels & Van Valkenburgh, 2009a,b; Meachen-Samuels, 2012).

Several recent studies have examined the shape, function and evolution of mammalian limbs, especially those of carnivores (Meachen-Samuels & Van Valkenburgh, 2009a; Meachen-Samuels, 2010; Walsley *et al.*, 2012; Alvarez, Ercoli & Prevosti, 2013; Samuels, Meachen & Sakai, 2013). These studies have demonstrated that osteological measurements of the entire limbs, and of their individual segments, are informative about locomotory habits, such that qualitative reconstructions of the ecology of fossil species are possible by comparing their morphology to better known living species. Within Felidae, these studies have additionally shown that the limb morphology is informative about prey size specialization and, furthermore, that limb shape is related to hunting strategies in extant and, by inference, extinct species (Meachen-Samuels & Van Valkenburgh, 2009b, 2010; Meachen-Samuels, 2012). However, to date, the vertebral column has been underrepresented in the morphological and biomechanical literature on felids and other species, and is often treated as one functional segment, with few functional studies considering the complexity and regionalization of this structure in detail [but see Halpert *et al.*, (1987), Macpherson & Ye (1998) and Jones (2015)].

The vertebral column has a critical role in body support against gravity, is connected to the limbs by means of bony, ligamentous and muscular components, and is composed of many consecutive articulations that take active participation in locomotion and prey procurement (Pridmore, 1992; Macpherson & Fung, 1998; Macpherson & Ye, 1998; Long, Adcock & Root, 2002; Schilling, 2011). Different degrees of torsion, flexion-extension and bending capacities of the vertebral column are important components of movement at different locomotor speeds and postures, and in the control of body deformations and manoeuvres (Carlson, Halbertsma & Zomlefer, 1979; Pridmore, 1992; Gál, 1993; Long *et al.*, 1997; Smit,

2002; Molnar, Pierce & Hutchinson, 2014). Changes in the size and angle of vertebral processes reflect differences in the size of muscles, tendons and ligaments inserting on those elements, and the relative length of centra is associated with the degree of movement between two consecutive vertebrae (Long *et al.*, 1997; Koob & Long, 2000; Pierce, Clack & Hutchinson, 2011). Thus, morphological specializations of vertebrae translate into functional modifications in the flexibility and range of motion of the whole spine, as well as its role in body support and general locomotor performance.

The vertebral column of placental mammals is largely constrained to a fixed number of presacral segments, relative to other amniotes (Muller *et al.*, 2010), with a few exceptions in 'southern' placental clades, Afrotheria and Xenarthra (Narita & Kuratani, 2005). Potentially due to this constraint in vertebral numbers, specialization into discrete niches has been accompanied by a diversification of vertebral shapes across placentals (Narita & Kuratani, 2005; Muller *et al.*, 2010; Pierce *et al.*, 2011; Buchholz *et al.*, 2012; Buchholz, 2014). Although studies are limited, identification of correlated changes between vertebral shape and various ecological attributes has extended our understanding of the behaviour of living animals and aided in reconstructing the behaviour and ecology of extinct species (Antón & Galobart, 1999; Argot, 2003; Shapiro *et al.*, 2005; Pierce *et al.*, 2011, 2013). Moreover, morphological specializations of vertebrae have been associated with body size changes across mammalian clades: for example, Smeathers (1981) suggested that small and large animals differ in the total length and flexibility of the lumbar column due to different metabolic costs required to maintain stability and posture, with larger animals having comparatively shorter, stiffer and therefore more stable lumbar columns (Gál, 1993).

To understand how extant felid ecomorphology and body mass have impacted the size and shape of the postcranium as a whole, detailed data from the vertebral column are required. Here, we investigate whether differences in ecological niche among felid species are reflected in their vertebral shape. Specifically, we test if differences in the whole vertebral column, or in discrete regions of the spine (i.e. cervical, thoracic and lumbar regions), discriminate the different locomotory styles and/or prey-size specializations observed in extant cats. We also examine the effect of body size on felid vertebral evolution through an analysis of scaling across a large suite of biomechanically relevant measurements. In accordance with Smeathers (1981), Gál (1993) and most recently Jones (2015), we predict that increases in felid body size are correlated with a decrease in the flexibility

of the vertebral column. Furthermore, based on these studies, we predict that this effect will be regionally heterogeneous, with increased robustness and decreased flexibility focused primarily at the posterior portion of the spine of larger species, while flexibility will be maintained more anteriorly, providing a wider range of motion to the neck and thorax associated with tackling prey. Combined, these analyses will allow us to assess the importance of the vertebral column in the evolution of felid size, ecology and locomotion.

MATERIAL AND METHODS

DATA COMPOSITION

Species and specimens

The data set is composed of 24 specimens representing 22 extant felid species, which is ~62% of the total number of species in the family (Fig. 1). The chosen species embody the full phylogenetic breadth of extant felids, with each of the eight identified clades (Johnson *et al.*, 2006) represented by at least one species. The sample also encompasses the full range of body sizes (e.g. *Leopardus colocolo* and *Leopardus wiedii*, both at the small body mass end at 2–4 kg, and *Panthera tigris* at the large body mass extreme of up to 325 kg) and ecologies (e.g. arboreal, scansorial and terrestrial) displayed by living felids (Sunquist & Sunquist, 2002; Meachen-Samuels & Van Valkenburgh, 2009b). Specimens were chosen based on completeness, being disarticulated (which allows

a greater number of anatomical features to be observed and measured) and, whenever possible, being wild caught (known captive-raised specimens are identified in Table 1). The specimens sampled are held in the zoological collections at the Natural History Museum in London (NHM), the University Museum of Zoology Cambridge (UMZC) and the Muséum National d'Histoire Naturelle in Paris (MNHN) (Table 1).

Anatomical measurements

All 27 pre-sacral vertebrae (seven cervicals 13 thoracics and seven lumbar) of one to two specimens per species were measured with digital callipers (accuracy of 0.01 mm) for linear measurements and with a goniometer for angular measurements (to the nearest degree). The measurements were particular to each of the three regions of the vertebral column (cervical, thoracic and lumbar) and only features present in all species were used in statistical analyses. Because different regions have unique vertebral features, different combinations of measurements were taken on separate sets of morphologically similar vertebrae (Fig. 2). In total, there were 28 measurement categories (i.e. centrum length, neural spine angle) with a sum total of 309 variables across the column, and an overall total of 6798 measurement values in the dataset. Missing values (e.g. where vertebrae were broken) were randomly imputed in R version 3.1.2 (R Foundation, 2013) by basing the new values on observed instances for each specific variable. This method also calculates

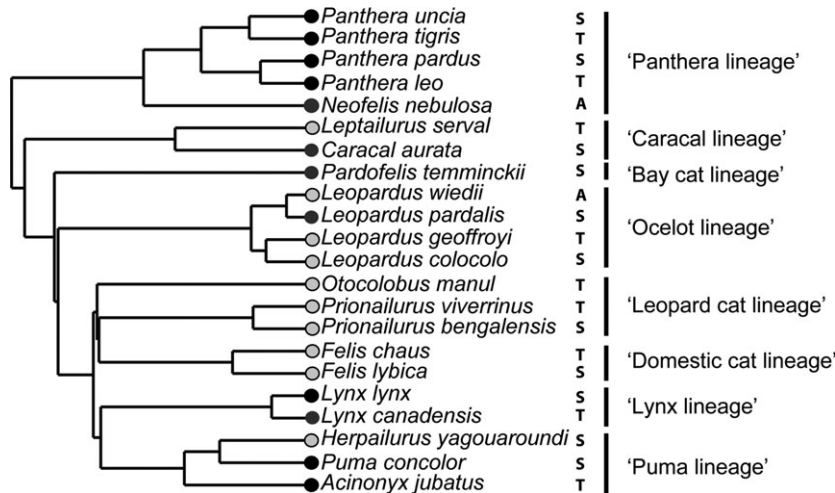


Figure 1. Felid phylogeny showing studied species, from a subset of Nyakatura & Bininda-Emonds (2012), with felid lineage designation according to Johnson *et al.* (2006), and locomotory (A, S, and T) and prey size specialization (circles at tip of phylogeny) according to Meachen-Samuels & Van Valkenburgh (2009b). Abbreviations: arboreal (A), scansorial (S) and terrestrial (T). Prey size symbols: black circles – large prey specialist; dark grey circles – mixed prey specialist; and light grey with black rim circles – small prey specialist.

Table 1. List of species studied with their corresponding specimen information, including sex, assigned locomotory group, prey size specialization and clade (Johnson *et al.*, 2006; Meachen-Samuels & Van Valkenburgh, 2009a,b), and museum specimen numbers

Species	Sex	Prey size	Phylogenetic lineage	Locomotory group	Museum identification number
<i>Acinonyx jubatus</i>	Unidentified	Large	'Puma'	Terrestrial	NHM 1940.1.20.17
<i>Caracal aurata</i>	Female	Mixed	'Caracal'	Terrestrial	NHM 1965.8.26.3
<i>Felis chaus</i>	Female	Small	'Domestic cat'	Terrestrial	NHM 1892.5.22.1
<i>Felis lybica</i>	Male	Small	'Domestic cat'	Scansorial	NHM 1940.1.20.12
<i>Herpailurus yagouarundi</i>	Male	Small	'Puma'	Scansorial	NHM 1932.2.14.1
<i>Leopardus colocolo</i>	Unidentified	Small	'Ocelot'	Scansorial	NHM 1848.6.26.8 – 126.B
<i>Leopardus geoffroyi</i>	Male	Small	'Ocelot'	Terrestrial	NHM 32.2.14.1
<i>Leopardus pardalis</i>	Unidentified	Mixed	'Ocelot'	Scansorial	UMZC K.6022 – 934A
<i>Leopardus wiedii</i>	Unidentified	Small	'Ocelot'	Arboreal	NHM 1846.4.21.8 – 123B
<i>Leopardus wiedii</i>	Unidentified	Small	'Ocelot'	Arboreal	NHM 1849.11.7.2 – 933a
<i>Leptailurus serval</i>	Unidentified	Small	'Caracal'	Terrestrial	NHM 1845.9.25.23 – 133c
<i>Leptailurus serval</i> *	Female	Small	'Caracal'	Terrestrial	NHM 2006.550
<i>Lynx canadensis</i>	Unidentified	Mixed	'Lynx'	Scansorial	UMZC K.6682 – 937 I
<i>Lynx lynx</i>	Male	Large	'Lynx'	Scansorial	MNHN 1973-83
<i>Neofelis nebulosa</i>	Female	Mixed	'Panthera'	Arboreal	MNHN 1961-217
<i>Otocolobus manul</i> *	Female	Small	'Leopard cat'	Terrestrial	MNHN 2009-251
<i>Panthera leo</i>	Male	Large	'Panthera'	Terrestrial	NHM 1931.1.13.1
<i>Panthera pardus</i>	Female	Large	'Panthera'	Scansorial	NHM 1938.4.21.11
<i>Panthera tigris</i>	Female	Large	'Panthera'	Terrestrial	NHM 1884.1.22.6
<i>Panthera uncia</i> *	Female	Large	'Panthera'	Scansorial	NHM 1967.6.29.1
<i>Pardofelis te mminckii</i>	Unidentified	Mixed	'Bay cat'	Scansorial	MNHN 1941-293
<i>Prionailurus bengalensis</i>	Unidentified	Small	'Leopard cat'	Scansorial	NHM 1860.4.23.18 – 1309B
<i>Prionailurus viverrinus</i>	Male	Small	'Leopard cat'	Terrestrial	NHM 75.2287
<i>Puma concolor</i>	Unidentified	Large	'Puma'	Scansorial	UMZC K.5745 – 936E

*Potentially captive-reared specimens.

NHM, London Natural History Museum; MNHN, Muséum National d'Histoire Naturelle; UMZC, University of Cambridge Museum of Zoology.

regression values for the missing data and imputation is continued until convergence (German & Hill, 2006; Ilin & Raiko, 2010). Approximately 2% of the total measurement values were imputed in the dataset. While the linear measurements were used in the statistical analyses presented here, all measurements, both linear and angular, were explored through visualization of vertebral profiles (see below).

Measurements were selected based on their relevance for the flexibility and range of motion of the vertebral column, their identification as important muscle attachment sites, and their potential relevance for understanding how the spine responds to differences in body size (e.g. presence of allometry). The measurements were grounded primarily on those by Pierce *et al.* (2011), and supplemented with additional measures to capture morphological attributes relevant for felids (Table 2). All measurements were taken by one observer (M.R.), repeated three times and averaged to produce the final dataset used

in further analyses. Measurements of the angles between the pre-zygapophyses and the accessory processes were removed from the original dataset due to high error.

DATA ANALYSES

Principal component analysis (PCA)

All linear measurements were \log_{10} -transformed prior to analysis. Measurements were then phylogenetically size-corrected using \log_{10} total vertebral column length as a proxy for body size in R with the phytools package (Revell, 2009). This procedure removes the effects of body size from the data by using phylogenetic regressions to calculate independent slopes for the clades. This is an important step when analysing families such as Felidae where a clear phylogenetic bias is found for body size, and larger-bodied species are concentrated in a few closely related genera (e.g. the *Panthera* clade; Cuff *et al.*, 2015; Ewer, 1973; Johnson *et al.*, 2006;

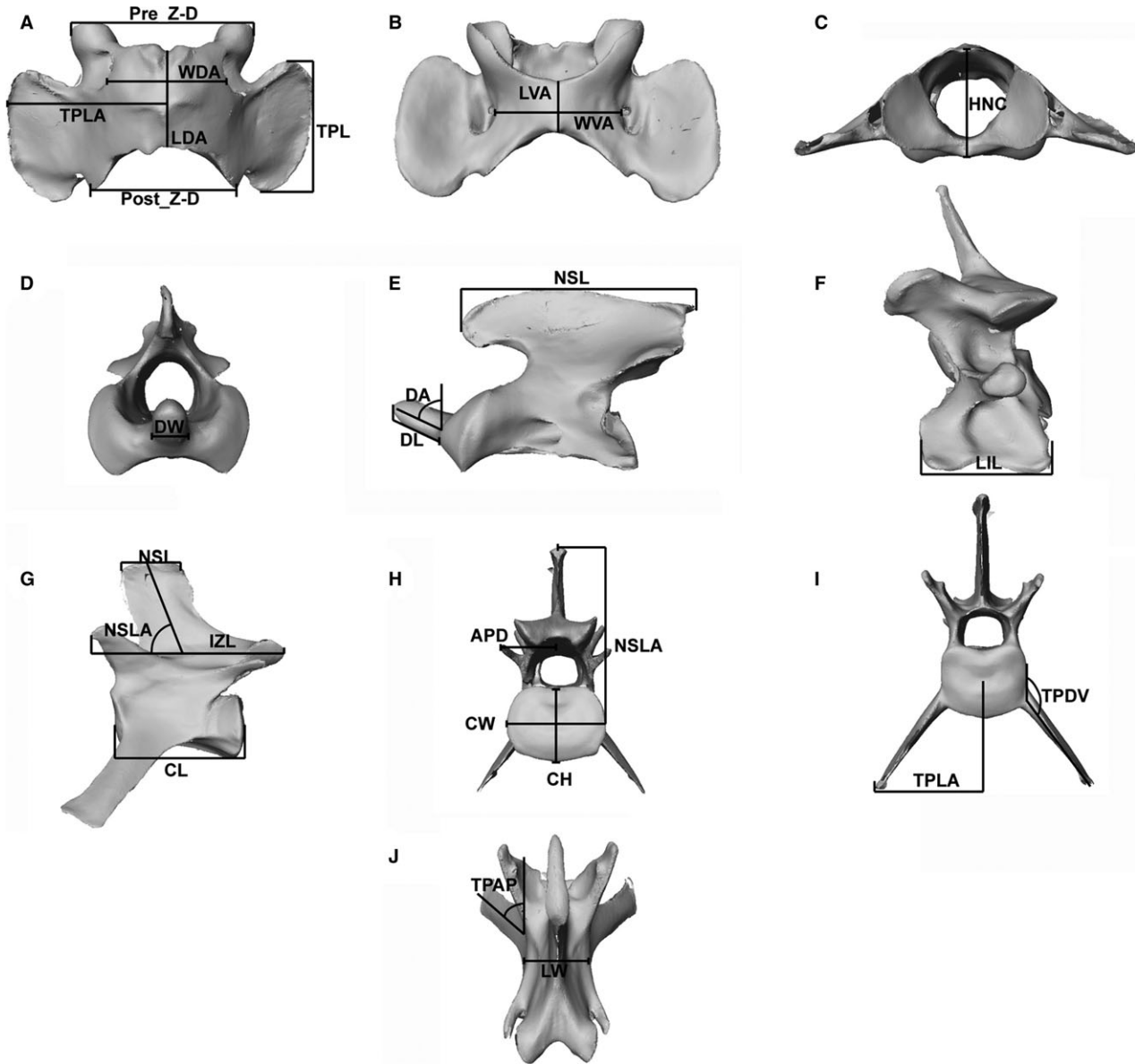


Figure 2. Vertebral measurements: A–C, atlas; D and E, axis; F, C6; G–J, L2. Abbreviations: LDA, length of dorsal arch; Pre_Z-D, prezygapophyseal distance; Post_Z-D, postzygapophyseal distance; TPLA, transverse process lever arm; WDA, width of dorsal arch. B. LVA, length of ventral arch; WVA, width of ventral arch. C. HNC, height of the neural canal. D. DW, dens width. E. DA, dens angle; DL, dens length; NSL, neural spine anteroposterior length at tip. F. LIL, length of inferior lamella. G. CL, centrum length; IZL, interzygapophyseal length; NSL, neural anteroposterior length at tip; NSLA, neural spine lever arm. H. APD, accessory process distance; CH, centrum height; CW, centrum width; NSLA, neural spine lever arm. I. TPDV, transverse process dorsoventral angle; TPLA, transverse process lever arm. J. LW, lamina width; TPAP, transverse process anteroposterior angle. Vertebral images are from of a CT scan of *Acinonyx jubatus* (cheetah).

Sunquist & Sunquist, 2002). Phylogenetic relationships were based on a recent supertree analysis of carnivorans (Nyakatura & Bininda-Emonds, 2012), which was cropped in Mesquite version 3.02 (Maddison & Maddison, 2014) to include only species represented in this study (Fig. 1). These measurements

were analysed with a PCA in PAST version 2.17c (Hammer, Harper & Ryan, 2001) for five subsets of the original dataset: all vertebrae (i.e. all 27 vertebrae), cervicals only (i.e. only the seven vertebrae of the cervical region), thoracics only (i.e. only the 13 vertebrae of the thoracic region), lumbar only (i.e.

Table 2. List of all measurements taken on each vertebra

Vertebra	Measurement	Abbreviation
Atlas	Length of ventral arch	LVA
	Width of ventral arch	WVA
	Length of dorsal arch	LDA
	Width of dorsal arch	WDA
	Transverse process lever arm	TPLA
	Interzygapophyseal length	IZL
	Pre-zygapophyseal distance	Pre-Z_D
	Post-zygapophyseal distance	Post-Z_D
	Height of neural canal	HNC
Axis	Length of centrum	CL
	Height of centrum	CH
	Width of centrum	CW
	Neural spine lever arm	NSLA
	Interzygapophyseal length	IZL
	Dens length	DL
	Dens width	DW
	Dens angle	DA
	Transverse process anteroposterior angle	TPAP
C3–C7	Length of centrum	CL
	Height of centrum	CH
	Width of centrum	CW
	Neural spine lever arm	NSLA
	Transverse process lever arm	TPLA
	Neural spine anteroposterior length at tip	NSL
	Length of inferior lamella	LIL
C3–C6 only	Interzygapophyseal length	IZL
	Width of lamina	LW
	Neural spine angle	NSA
C3–L7 only	<i>Pre-zygapophyseal angle</i>	<i>Pre-ZA</i>
C3–C6 only	Inferior lamella dorsoventral angle	ILDV
C3–C6 only	Inferior lamella anteroposterior angle	ILAP
C5–C7 only	Transverse process dorsoventral angle	TPDV
C5–C7 only	Transverse process anteroposterior angle	TPAP
T1–T13	Length of centrum	CL
	Height of centrum	CH
	Width of centrum	CW
	Neural spine lever arm	NSLA

Table 2. *Continued*

Vertebra	Measurement	Abbreviation
	Transverse process lever arm	TPLA
	Interzygapophyseal length	IZL
	Width of lamina	LW
	Neural spine angle	NSA
	Neural spine anteroposterior length at tip	NSL
T1–T10 only	Transverse process dorsoventral angle	TPDV
T1–T10 only	Transverse process anteroposterior angle	TPAP
T12–T13 only	Accessory process distance	APD
T12–T13 only	<i>Accessory process dorsoventral angle</i>	APDV
T12–T13 only	<i>Accessory process anteroposterior angle</i>	APAP
L1–L7	Length of centrum	CL
	Height of centrum	CH
	Width of centrum	CW
	Neural spine lever arm	NSLA
	Transverse process lever arm	TPLA
	Interzygapophyseal length	IZL
	Width of lamina	LW
	Neural spine angle	NSA
	Transverse process dorsoventral angle	TPDV
	Transverse process anteroposterior angle	TPAP
	Neural spine anteroposterior length at tip	NSL
L1–L5 only	Accessory process distance	APD
L1–L5 only	<i>Accessory process dorsoventral angle</i>	APDV
L1–L5 only	<i>Accessory process anteroposterior angle</i>	APAP

C, cervical vertebra; T, thoracic vertebra; L, lumbar vertebra.

Measurements in italics were not included in subsequent statistical analyses due to higher measurement error.

only the seven vertebrae of the lumbar region), and thoracics + lumbar combined (i.e. the 20 vertebrae composing the thoracic and lumbar regions, from T1 to L7).

To ensure that size had been removed prior to our PCA, and therefore that PCs were uncorrelated with

size, PC scores from significant PC axes (i.e. those with eigenvalues higher than the Jolliffe cut-off) in the 'all vertebrae' PCA were regressed against \log_{10} total vertebral column length as a proxy for body size. The scores were regressed both across the full 'all vertebrae' sample and per locomotory group (as this was the main trait influencing morphospace occupation; see Results). This same procedure was repeated for the full 'all vertebrae' sample while controlling for phylogeny, with independent contrasts (Felsenstein, 1985) calculated for the PC scores from significant axes and for total vertebral length using the R package 'ape' (Paradis, Claude & Strimmer, 2004). This further step was performed to ensure that size had been removed from our data even when phylogeny was taken into account. Independent contrasts (for scores of each PC axis against vertebral column length) were then subjected to reduced major axis (RMA) regression in R using the 'smatr' package (Warton *et al.*, 2012).

To test how locomotor specialization affects vertebral shape, species were categorized by three primary locomotor modes – arboreal, scansorial and terrestrial – and qualitatively evaluated in PCA morphospace (the full linear dataset and four regional linear subsets) using convex hulls. Species assignment to locomotory categories is detailed in Figure 1 and Table 1 and was based on the studies of Meachen-Samuels & Van Valkenburgh (2009b) and Sunquist & Sunquist (2002). Furthermore, to explore the impact of prey specialization on vertebral shape, the 'cervicals only' and the 'all vertebrae' subsets were qualitatively examined in PCA morphospace by grouping species by prey size (i.e. small, mixed and large) according to the study by Meachen-Samuels & Van Valkenburgh (2009a). Finally, to assess the effect of phylogenetic relatedness on vertebral morphology, species were also categorized according to clade ('Panthera', 'Bay cat', 'Caracal', 'Ocelot', 'Lynx', 'Puma', 'Leopard cat' and 'Domestic cat' lineages based on Johnson *et al.*, 2006; Fig. 1) in the resulting PCA morphospace. All qualitative assessments using PCA were followed by the confirmatory analyses detailed below.

MANOVA and phylogenetic MANOVA

Differences in the area of morphospace occupied by each of the locomotory, prey size and clade groupings were further assessed quantitatively using multivariate analysis of variance (MANOVA). Locomotory and prey size groupings were also analysed with phylogenetic MANOVAs (pMANOVAs) to account for the potentially confounding effect of phylogeny. These pMANOVAs address the issue of non-independence due to relatedness in species' phenotypes by correcting the overestimation of degrees of freedom in com-

parative cross-species tests (Garland *et al.*, 1993). Specifically, the significance of the standard test statistic is assessed using a Brownian motion model to simulate the distribution of the relevant dependent variables along a given phylogenetic tree. MANOVAs and pMANOVAs were performed on the PC scores of all axes that presented an eigenvalue equal to or higher than the Jolliffe cut-off (i.e. the first nine PCs for the 'all vertebrae' analysis, which were all higher than the cut-off value of 0.04595). The phylogenetic relationships used were identical to those used to conduct the phylogenetic size-correction (see above). All standard and phylogenetic MANOVAs were performed in R software (R Foundation, 2013) using the 'geiger' and 'stats' packages (Harmon *et al.*, 2014).

Vertebral profiles

To further examine variation along the vertebral column and identify aspects of individual vertebrae and vertebral regions associated with niche specialization, vertebral profiles were plotted for a subset of 12 measurements: centrum length, height and width, width of centrum lamina, lever arm and angle of the neural spine, anteroposterior length of the tip of neural spine, lever arm and angles (anteroposterior and dorsoventral projections) of the transverse process, length of interzygapophyseal distance, and accessory process distance. In addition, variation in centrum shape was examined by calculating the change in relative centrum length [$2 \times \text{centrum length} / (\text{centrum height} + \text{centrum width})$] throughout the vertebral column (Pierce *et al.*, 2011). This measure of centrum shape provides clearer information with regard to the flexibility and range of motion of intervertebral joints (Buchholtz, 2001a,b).

To generate niche-specific vertebral profiles, \log_{10} -transformed, phylogenetically size-corrected linear measurements and raw angles were averaged for all species in a corresponding group, and plotted against vertebral number. Only measurement variables that were found either on all vertebrae or on at least three or more consecutive vertebrae (e.g. accessory processes, from T12 to L5) were plotted and no imputed variables were used in this analysis. Statistical significance of the differences between vertebral profiles was evaluated by performing ANOVAs on vertebral bins composed of seven vertebrae each (except bin '3' which was composed of only six vertebrae, from T8 to T13), corresponding to four bins at 25% vertebral intervals: bin '1', atlas – C7; bin '2', T1–T7; bin '3', T8–T13; and bin '4', L1–L7.

Scaling regressions

Vertebral column length and body mass: To test if vertebral column length is a robust predictor of

specimen body size (see below), and to examine how the whole column scaled with body mass, generalized least squares (GLS) regressions of \log_{10} body mass (based on average species body mass from Cuff *et al.*, 2015) were made against \log_{10} total presacral vertebral column length (C1–L7) (based on the sum total of centrum lengths, without the intervertebral disc/space). The GLS regressions were carried out with and without phylogenetic correction under a Brownian motion model of evolution using the ‘pGLS’ package (Martins & Hansen, 1997; Mao & Ryan, 2013) within R. We also investigated regional scaling by performing phylogenetically corrected GLS regressions of \log_{10} body mass against each of the separate \log_{10} total lengths of the cervical, thoracic and lumbar regions. Analyses to test if vertebral column length scaled isometrically with body mass were made by comparing the obtained slopes to an isometry slope of 0.333 (i.e. $\text{length} \sim \sqrt[3]{\text{mass}}$).

Individual vertebrae and total length: In addition, we also tested for allometric changes within individual vertebrae. To control for phylogeny, independent contrasts of \log_{10} raw linear measurements and \log_{10} total vertebral column length were calculated using the same procedure cited above. Those independent contrasts (for scores of each individual linear vertebral measurement against vertebral column length) were then subjected to RMA regression in R using the ‘smatr’ package (Warton *et al.*, 2012). Analyses to test if these individual linear vertebral measurements scaled isometrically with total vertebral length were made by comparing the obtained slopes to an isometry slope of 1 (i.e. $\text{length} \sim \text{length}^1$).

RESULTS

PCA, MANOVA AND pMANOVA

The ‘all vertebrae’ PCA revealed nine PCs that were significant according to the Jolliffe cut-off value of 0.04595 (Table 3), and the sum of the variance explained by those reached almost 80% (i.e. 79.166%) of the total variance. Regressions of all significant PC scores from the ‘all vertebrae’ PCA on \log_{10} total vertebral column length, before and after phylogenetic correction and between locomotory groups, demonstrated that shape variables were statistically uncorrelated with size ($r^2 \ll 0.4$, and $P \gg 0.05$) and that the effects of size variation were removed prior to PCA.

PC1 \times PC2 showed a large area of overlap between the terrestrial and scansorial groups, but a clear clustering of arboreal species in a distinct area

Table 3. PCA results from the ‘all vertebrae’ analysis

PC	Eigenvalue	% Variance explained
1	0.341	24.747
2	0.160	11.610
3	0.138	9.974
4	0.106	7.656
5	0.088	6.384
6	0.074	5.393
7	0.073	5.265
8	0.058	4.241
9	0.054	3.896
10	0.044	3.218
11	0.041	2.993
12	0.037	2.691
13	0.036	2.620
14	0.032	2.317
15	0.026	1.883
16	0.022	1.574
17	0.017	1.214
18	0.012	0.885
19	0.012	0.864
20	0.008	0.575
21	0.000	0.000

PCs with an eigenvalue higher than the Jolliffe cut-off of 0.046 are marked in bold.

of morphospace (Fig. 3A). There was a much better separation of all three locomotory groups in PC1 \times PC3 (Fig. 3B), with only a very small overlap between the terrestrial and scansorial groups. The vertebral features which were most relevant to contributing to this result in terms of high correlation coefficients [i.e. $r > 0.6$, following Pierce *et al.* (2011)] are detailed in Table 4. While most variables exhibited high PC1 loading correlation values, PC3 was only highly correlated with measurements of neural spine anteroposterior length at tip in the thoracic and lumbar regions, and centrum height in the lumbar region.

Clade groupings in the ‘all vertebrae PCA’ were significant as a clustering factor when analysed with MANOVA, showing that among the species studied here, closely related taxa tended to be more similar in their axial skeletal morphology. The ‘all vertebrae PCA’ revealed that the clustering of species by their locomotory groups was indeed statistically significant, both with (phylogenetic $P \ll 0.05$) and without ($P \ll 0.05$) phylogenetic correction. Prey size groups in the ‘all vertebrae’ morphospace were non-significant ($P \gg 0.05$, and phylogenetic $P \gg 0.05$; Table 5).

The ‘thoracics only’, ‘lumbars only’ and ‘thoracics + lumbars’ subset analyses revealed clustering similar to the ‘all vertebrae’ PCA (data not shown).

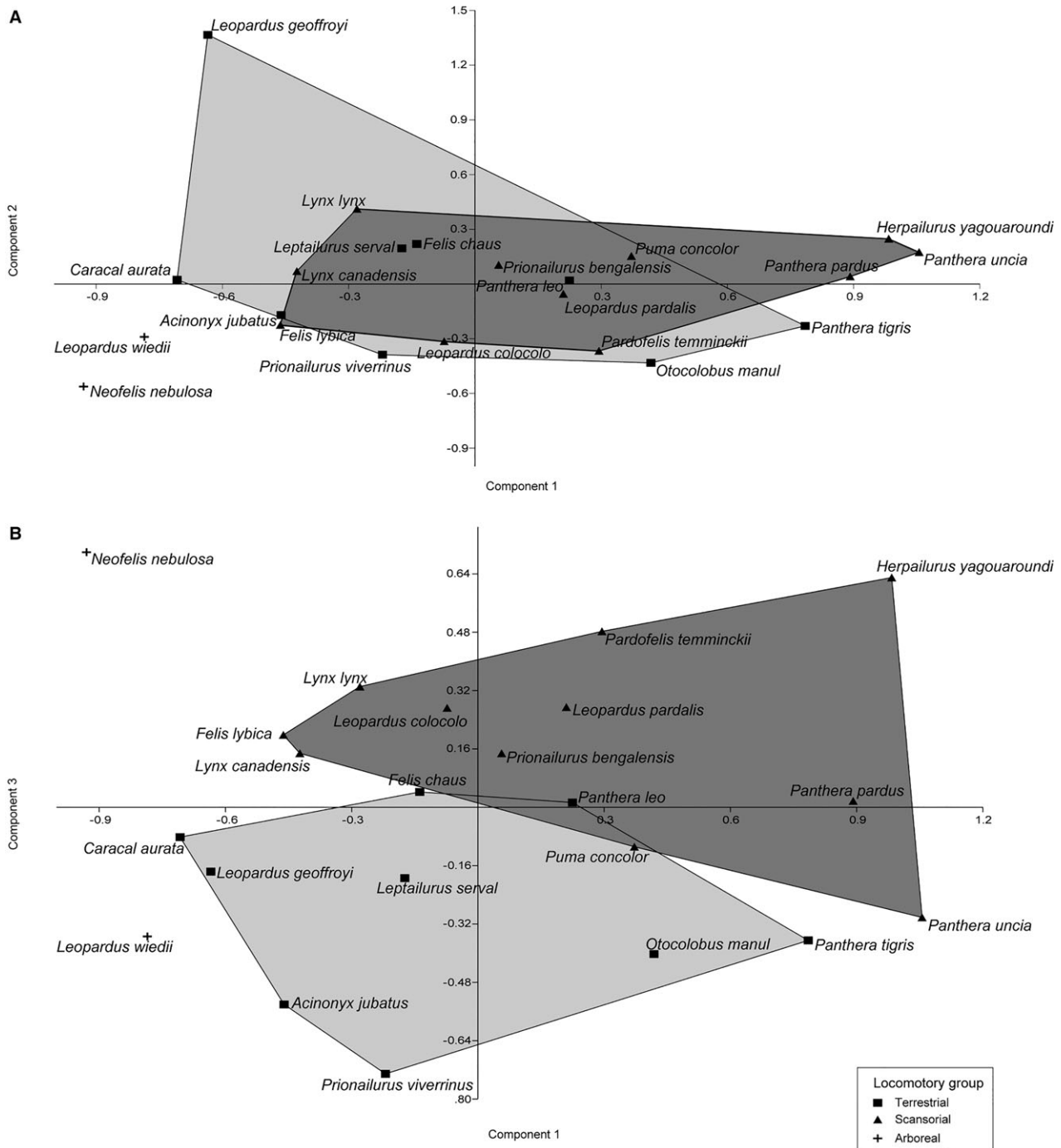


Figure 3. PCA plots of PC1 × PC2 (A) and PC1 × PC3 (B) showing species distribution in vertebral morphospace. Species are grouped according to their locomotory mode (i.e. cross: arboreal species; triangle: scansorial species; squares: terrestrial species).

MANOVA results calculated from the ‘lumbar only’ subset showed that locomotory groups occupied different areas of morphospace, both with and without phylogenetic correction ($P < 0.05$). However, for both the ‘thoracics only’ and the ‘thoracics + lumbar’ sub-

sets, a significant statistical difference between locomotory groups was only achieved when phylogeny was taken into account. However, comparison of all significant results with a Bonferroni-corrected $P = 0.0065$ resulted in only the ‘all vertebrae’ and

Table 4. Vertebral measurements that display high (i.e. >0.6) correlations on PC axes for the ‘all vertebrae’ PCA

Vertebra	Measurement with high pc loadings (i.e. correlation >0.6)	PC axes with high loadings correlations
Atlas	Length of ventral arch	PC1
	Length of dorsal arch	PC1
	Length of transverse process	PC1
Axis	Length of centrum	PC4
	Width of centrum	PC1
	Interzygapophyseal length	PC6
C3–C7	Height of centrum	PC1
	Width of centrum	PC1
	Transverse process lever arm	PC1
	Width of lamina	PC1
T1–T13	Neural spine length at tip	PC1
	Height of centrum	PC1, PC2
	Width of centrum	PC1, PC2
	Neural spine lever arm	PC1, PC4, PC5
	Transverse process lever arm	PC1, PC2, PC7
	Interzygapophyseal length	PC1, PC4
	Width of lamina	PC1, PC2
	Neural spine length at tip	PC1, PC2, PC3, PC4, PC5
L1–L7	Length of centrum	PC4
	Height of centrum	PC1, PC3
	Width of centrum	PC1
	Transverse process lever arm	PC1
	Width of lamina	PC1
*L1–L5 Only	Neural spine length at tip	PC1, PC3
	Accessory process distance	PC1

‘lumbar only’ subsets exhibiting significant separation between locomotory clusters.

The ‘cervicals only’ analyses did not reveal any clear association of taxa by locomotory or prey size groupings, and the respective phylogenetic MANOVA again confirmed the non-significance of these groups (locomotory groups: P and phylogenetic $P \gg 0.05$; prey-size groups: P and phylogenetic $P > 0.05$). Results for all MANOVAs and pMANOVAs are shown in Table 5.

VERTEBRAL PROFILES

As locomotory mode was the only examined ecological trait found to have a significant influence on morphospace occupation, average vertebral profiles were created for species designated arboreal, scansorial or terrestrial. The profiles revealed similar overall trends along the vertebral column, with some localized differences in the shape of individual vertebral features (Fig. 4). After Bonferroni correction, only the ANOVAs of four pairwise comparisons between group profiles were statistically significant (Table 6): centrum width (CW) between arboreal and terrestrial species at bin ‘2’, with terrestrial species having lower values for CW or more narrow vertebrae; centrum shape (CS) between arboreal and scansorial groups at bin ‘3’, with the scansorial group displaying smaller values for CS and therefore shorter and wider vertebrae; inter-zygapophyseal length (IZL) between arboreal and terrestrial groups at bin ‘2’, for which the terrestrial group presented the shortest IZL; and the transverse process dorsoventral projection (TPDV) between arboreal and scansorial categories at bin ‘3’, where the scansorial species had the lowest TPDV angle values (i.e. the least ventrally directed).

Table 5. Results of the MANOVA and phylogenetic MANOVA tests on PC scores from significant PCs as determined by the Jolliffe cut-off

Groups tested	MANOVA (P value)	Phylogenetic MANOVA (P value)
PC1–9 (‘all vertebrae’, locomotory groups)	<i>0.03043</i>	0.006
PC1–9 (‘all vertebrae’, prey size groups)	0.2811	0.6454
PC1–9 (‘all vertebrae’, clades: ‘panthera’ × ‘ocelot’ lineages)	0.0000	NA
PC1–9 (‘thoracics only’, locomotory groups)	0.0648	<i>0.0120</i>
PC1–9 (‘thoracics + lumbar’, locomotory groups)	<i>0.0662</i>	<i>0.0120</i>
PC1–9 (‘lumbar only’, locomotory groups)	<i>0.0083</i>	0.002
PC1–9 (‘cervicals only’, locomotory groups)	0.4293	0.2547
PC1–9 (‘cervicals only’, prey size groups)	0.3	0.6693

Significance at $P < 0.05$ is indicated in italics, while significance after Bonferroni correction (i.e. $P < 0.00625$) is shown in bold.

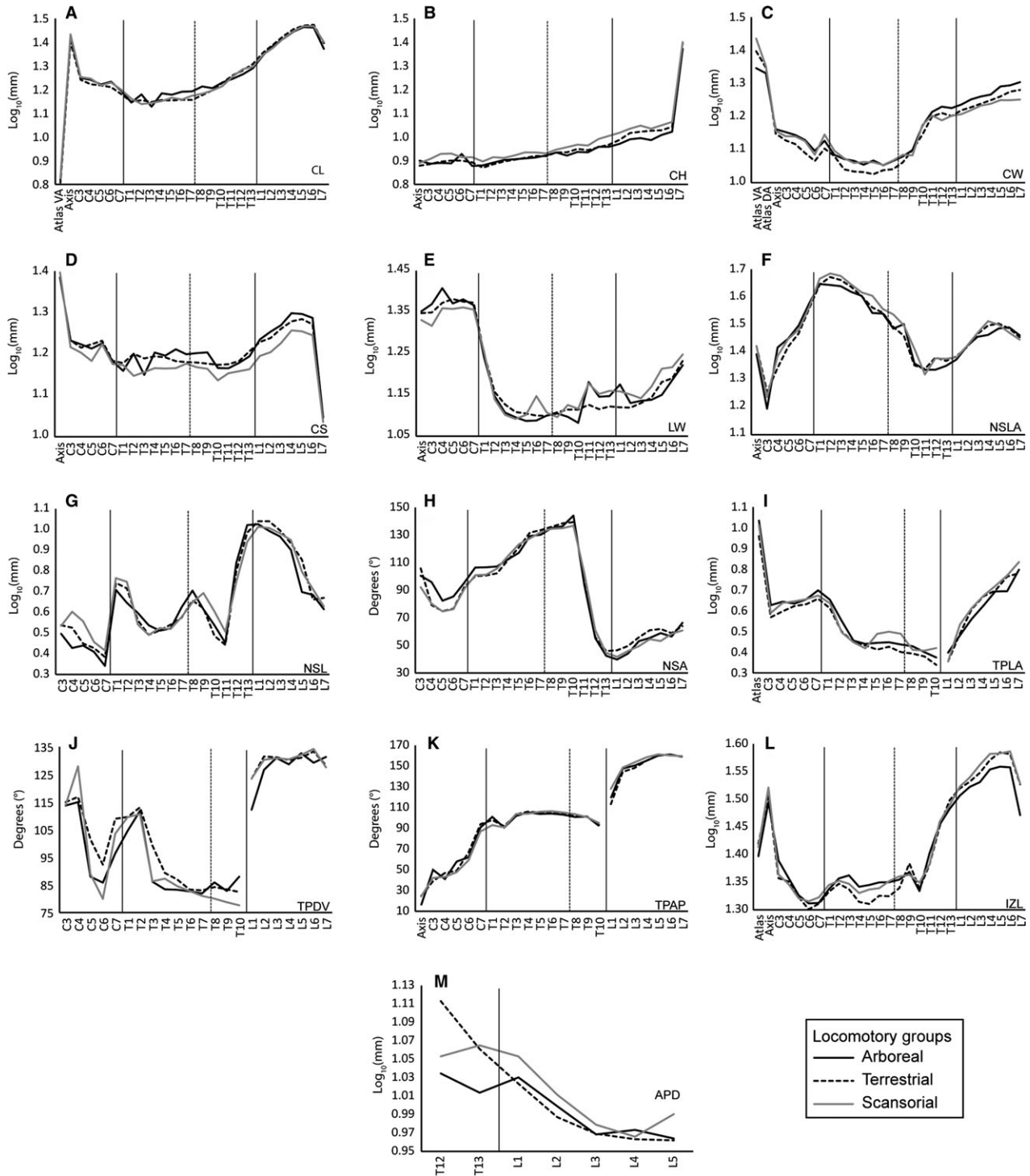


Figure 4. Vertebral profile plots of locomotory groups (i.e. arboreal, terrestrial and scansorial species) showing variation in vertebral measurements along the vertebral column number. A, centrum length (CL); B, centrum height (CH); C, centrum width (CW); D, centrum shape (CS); E, lamina width (LW); F, neural spine lever arm (NSLA); J, transverse process dorsoventral angle (TPDV); K, transverse process anteroposterior angle (TPAP); L, interzygapophyseal length (IZL); M, accessory process distance (APD). Regular vertical bars mark the boundaries between vertebral regions (i.e. cervical, thoracic and lumbar regions) and the corresponding analytical bins, while dotted vertical lines mark boundaries only related to vertebral bins.

Table 6. Results from the ANOVAs and Turkey pairwise tests on vertebral profile bins

	ANOVA <i>F</i> test <i>P</i> value	Turkey's pairwise comparison <i>P</i> -value		
		Arboreal × scansorial	Arboreal × terrestrial	Scansorial × terrestrial
CH				
BIN 1	0.0346	0.0840	0.928	<i>0.0423</i>
BIN 2	0.0573			
BIN 3	0.0162	<i>0.0167</i>	0.7482	0.0681
BIN 4	0.8472			
CL				
BIN 1	0.9747			
BIN 2	0.1148			
BIN 3	0.9901			
BIN 4	0.8993			
CW				
BIN 1	0.9258			
BIN 2	0.0051	0.9675	0.0086	<i>0.0146</i>
BIN 3	0.883			
BIN 4	0.0199	<i>0.0159</i>	0.4386	0.1798
CS				
BIN 1	0.9544			
BIN 2	0.01341	<i>0.0246</i>	0.999	<i>0.027</i>
BIN 3	0.0063	0.0096	0.941	<i>0.01851</i>
BIN 4	0.6848			
IZL				
BIN 1	0.9924			
BIN 2	0.00248	0.5606	0.0025	<i>0.0228</i>
BIN 3	0.9985			
BIN 4	0.1712			
NSLA				
BIN 1	0.9821			
BIN 2	0.4854			
BIN 3	0.8225			
BIN 4	0.9231			
NSL				
BIN 1	0.139			
BIN 2	0.9971			
BIN 3	0.9572			
BIN 4	0.8664			
TPLA				
BIN 1	0.8853			
BIN 2	0.6615			
BIN 3	0.1421			
BIN 4	0.9081			
LW				
BIN 1	0.0403	<i>0.0372</i>	0.7032	0.1606
BIN 2	0.9099			
BIN 3	0.4424			
BIN 4	0.41			
APD				
All as 1 BIN	0.7078			
BIN 3	0.1575			
BIN 4	0.5943			
NSA				
BIN 1	0.3712			

Table 6. *Continued*

	ANOVA <i>F</i> test <i>P</i> value	Turkey's pairwise comparison <i>P</i> -value		
		Arboreal × scansorial	Arboreal × terrestrial	Scansorial × terrestrial
BIN 2	0.9856			
BIN 3	0.9981			
BIN 4	0.4832			
TPAP				
BIN 1	0.9749			
BIN 2	0.9759			
BIN 3	0.9142			
BIN 4	0.8732			
TPDV				
BIN 1	0.753			
BIN 2	0.7959			
BIN 3	0.0081	0.0073	0.3255	<i>0.0416</i>
BIN 4	0.559			

Vertebrae were divided into four bins of seven vertebrae each, with the exception of 'bin 3' with only six vertebrae, representing 25% intervals (i.e. cervical, anterior thoracic, posterior thoracic and lumbar vertebrae). Significance at $P < 0.05$ is indicated in italics, while significance after Bonferroni correction (i.e. $P < 0.0125$) is shown in bold.

Table 7. Results from scaling analysis for vertebral column length against average body mass, with lower and upper confidence limits from the slope value

Vertebral column length	Slope	Lower limit	Upper limit	Coefficient of determination (r^2)	<i>P</i> -value
Total length	0.267	0.225	0.308	0.815	<0.001
(phyl.) total length	0.286	0.220	0.353	0.483	<0.001
(phyl.) cervical length	0.321	0.240	0.401	0.483	<0.001
(phyl.) thoracic length	0.286	0.222	0.350	0.483	<0.001
(phyl.) lumbar length	0.263	0.192	0.335	0.483	<0.001

Bold indicates the only correlation significantly different from isometry (i.e. a slope of 0.333), while the prefix '(phyl.)' marks GLS regressions with phylogenetic correction.

SCALING

Vertebral column length and body mass

The GLS for \log_{10} total pre-sacral vertebral column length against \log_{10} body mass showed a relationship significantly different from isometry (slope = 0.267; $r^2 = 0.815$, $P \ll 0.05$), but after phylogenetic correction, the relationship was weaker ($r^2 = 0.483$) and the regression slope was not significantly differently from isometry (Table 7). All individual vertebral column regional regressions (i.e. cervical, thoracic and lumbar lengths) had similarly weak correlation values ($r^2 = 0.483$) and possessed slopes that were not significantly different from an isometric relationship (Table 7).

Within individual vertebrae

Phylogenetically corrected scaling analyses of individual linear vertebral measurements revealed 64

cases of significant allometric scaling, i.e. with a regression slope different from 1 (Table 8, complete table in Supporting Information, Table S1): 61 positive and three negative. There was clear regionalization of vertebral allometry: of 64 instances, 19 (18 positive and one negative) were in the cervical region, 34 (33 positive and one negative) in the thoracic region and only 11 (ten positive and one negative) in the lumbar region. These allometric measurements could be further divided into five categories: centrum-related (30 instances), neural spine-related (25 instances), zygapophyseal-related (six instances), inferior lamella-related (two instances) and transverse process-related (one instance).

Of the 19 allometric instances in the cervical region, 16 were found in the five similarly shaped post-axis vertebrae (i.e. C3–C7). All of the post-axis cervical vertebrae exhibited a positive allometric relationship in terms of centrum length and height.

Table 8. Results from the phylogenetic scaling analyses showing the slope for the relationship between the variables on the first column and body size (i.e. total vertebral length), with lower and upper confidence limits from the slope value, and *P*-value for the null hypothesis of the slope being different from 1 (i.e. isometry). Variables from thoracic vertebrae are shown in bold, while variables from lumbar vertebrae are shown in italics.

Variable	Slope	Slope lower limit	Slope upper limit	Slope <i>P</i> -value	Regression <i>P</i> -value	Correlation (r^2)
Atlas_LVA	1.249	1.013	1.540	0.039	0.000	0.806
Atlas_PRE.Z_D	0.729	0.599	0.888	0.003	0.000	0.830
Axis_CH	1.292	1.096	1.522	0.004	0.000	0.882
C3_CL	1.162	1.019	1.326	0.028	0.000	0.924
C3_CH	1.215	1.037	1.422	0.018	0.000	0.891
C3_IZL	1.165	1.002	1.356	0.048	0.000	0.900
C4_CL	1.153	1.021	1.301	0.024	0.000	0.936
C4_CH	1.279	1.081	1.513	0.006	0.000	0.876
C4_LIL	1.312	1.068	1.612	0.012	0.000	0.813
C4_IZL	1.178	1.036	1.340	0.015	0.000	0.928
C5_CL	1.307	1.143	1.495	0.000	0.000	0.921
C5_CH	1.256	1.044	1.512	0.018	0.000	0.849
C5_LIL	1.548	1.054	2.272	0.027	0.007	0.328
C5_IZL	1.221	1.046	1.425	0.014	0.000	0.896
C6_CL	1.250	1.059	1.475	0.011	0.000	0.880
C6_CH	1.216	1.052	1.405	0.011	0.000	0.909
C7_CL	1.133	1.020	1.258	0.022	0.000	0.952
C7_CH	1.339	1.161	1.544	0.000	0.000	0.911
C7_DW	1.228	1.021	1.476	0.031	0.000	0.851
C7_IZL	1.158	1.064	1.261	0.002	0.000	0.969
T1_CH	1.274	1.107	1.466	0.002	0.000	0.914
T1_NSL	1.596	1.074	2.371	0.022	0.013	0.284
T2_CL	1.105	1.001	1.220	0.047	0.000	0.957
T2_CH	1.269	1.116	1.442	0.001	0.000	0.928
T2_NSL	1.555	1.084	2.231	0.018	0.002	0.410
T3_CL	1.119	1.003	1.250	0.045	0.000	0.947
T3_CH	1.308	1.141	1.500	0.001	0.000	0.918
T3_NSL	1.817	1.219	2.708	0.004	0.015	0.272
T4_CL	1.083	1.003	1.170	0.044	0.000	0.974
T4_CH	1.236	1.093	1.397	0.002	0.000	0.934
T4_NSL	1.338	1.022	1.752	0.035	0.000	0.677
T5_CH	1.209	1.061	1.378	0.007	0.000	0.925
T5_Calculated_NSLA	1.234	1.029	1.480	0.025	0.000	0.856
T6_CH	1.212	1.095	1.341	0.001	0.000	0.955
T6_Calculated_NSLA	1.292	1.078	1.548	0.008	0.000	0.857
T6_NSL	1.470	1.031	2.095	0.034	0.001	0.431
T7_CH	1.288	1.148	1.446	0.000	0.000	0.942
T7_Calculated_NSLA	1.221	1.078	1.383	0.003	0.000	0.933
T7_IZL	0.869	0.763	0.989	0.035	0.000	0.926
T7_NSL	1.492	1.007	2.209	0.046	0.011	0.297
T8_CH	1.240	1.123	1.369	0.000	0.000	0.957
T8_NSL	1.635	1.148	2.329	0.008	0.001	0.435
T9_CH	1.262	1.161	1.371	0.000	0.000	0.970
T9_Calculated_NSLA	1.249	1.035	1.508	0.023	0.000	0.844
T9_Calculated_TPLA	1.562	1.076	2.265	0.020	0.003	0.371
T10_CH	1.461	1.002	2.130	0.049	0.004	0.354
T10_Calculated_NSLA	1.574	1.095	2.263	0.016	0.002	0.403
T11_CH	1.167	1.050	1.296	0.006	0.000	0.952
T11_Calculated_NSLA	1.301	1.104	1.532	0.003	0.000	0.882

Table 8. *Continued*

Variable	Slope	Slope lower limit	Slope upper limit	Slope <i>P</i> -value	Regression <i>P</i> -value	Correlation (r^2)
T12_CH	1.288	1.042	1.592	0.021	0.000	0.803
T12_Calculated_NSLA	1.491	1.095	2.031	0.013	0.000	0.573
T13_Calculated_NSLA	1.310	1.068	1.608	0.012	0.000	0.816
T13_NSL	1.463	1.027	2.084	0.036	0.001	0.434
L1_Calculated_NSLA	1.294	1.141	1.469	0.000	0.000	0.930
L2_Calculated_NSLA	1.336	1.172	1.523	0.000	0.000	0.925
L3_Calculated_NSLA	1.253	1.092	1.438	0.003	0.000	0.917
L4_Calculated_NSLA	1.241	1.079	1.428	0.004	0.000	0.914
L4_WL	0.839	0.708	0.995	0.044	0.000	0.873
L5_Calculated_NSLA	1.220	1.004	1.484	0.046	0.000	0.832
L5_NSL	1.962	1.397	2.755	0.000	0.000	0.480
L6_Calculated_NSLA	1.277	1.099	1.483	0.003	0.000	0.902
L7_CH	1.195	1.043	1.369	0.013	0.000	0.919
L7_Calculated_NSLA	1.281	1.102	1.491	0.003	0.000	0.900
L7_NSL	1.664	1.275	2.172	0.001	0.000	0.685

Variables that have an apparent allometric relationship with body size are shown here; scaling results for all variables are shown in Table S1 (Supporting Information).

Whereas C4 and C5 displayed the exact same instances of allometric change (centrum length, centrum height, length of the inferior lamella and interzygapophyseal length), C6 showed the lowest number of instances (centrum length and centrum width only). The atlas had a unique combination of allometric changes, while the axis only presented positive allometric change in centrum height.

Within the thoracic region, allometry was observed in almost all vertebrae for two primary features: centrum height, which was positively allometric from T1 to T12; and neural spine lever arm, which was positively allometric from T5 to T13 (but absent on T8). Although a positively allometric relationship was also found for the neural spine anteroposterior length at its tip for most thoracic vertebrae, six of these had weak correlation values between the variables (i.e. $r^2 < 0.45$). Within the thoracic region, there appears to be two sub-groups of vertebrae that showed the same combination of allometric features: T2–T3 (centrum length and centrum height, both showing positive allometry), and T10–T12 (centrum height, and neural spine lever arm, both also showing positive allometry).

The presence of allometry was weakest in the lumbar region. Although all seven lumbar vertebrae presented instances of allometry, these were restricted to only one measurement in most cases: the neural spine lever arm, always demonstrating positive allometry with total vertebral column length. In addition to this, L5 and L7 also showed positive allometry on the length at the tip of the neural spine, L4 presented negative allometry on its lamina

width and L7 showed positive allometry with respect to centrum height.

DISCUSSION

SHAPE AND ECOLOGY

Here, we quantitatively analysed the morphology of the entire presacral vertebral column in felids to test whether morphological differentiation of the vertebral column across species is driven by body size and/or ecologically derived traits, such as locomotory mode and prey-hunting specialization, as has been previously demonstrated for felid limbs (Gonyea, 1978; Meachen-Samuels & Van Valkenburgh, 2009b; Meachen-Samuels, 2012). Our study shows that linear shape variation in the felid vertebral column significantly discriminated terrestrial, arboreal and scansorial species, demonstrating that locomotory specialization, but not prey size, has fashioned vertebral column evolution within felids. Locomotor differentiation was statistically significant only when phylogenetic relationships were taken into account, and only when either ‘all vertebrae’ were analysed together or when the analysis was restricted to the lumbar vertebrae. In a study comparing the relative lengths of limbs and axial skeletons of species of large-bodied felids, Gonyea (1976) suggested that locomotory specialization was reflected by changes in the length of the lumbar region (but see scaling results below). This result indicates that, although size-independent changes in shape are somewhat dispersed throughout the whole vertebral column,

widespread changes in the lumbar vertebra are particularly important for locomotor specialization.

Although there was significant differentiation of locomotory groups across all PCs, there was also clear overlap between scansorial and terrestrial species on most PCs (Fig. 3). Such morphological similarities between these locomotory groups may reflect a hypothesized scansorial ancestral condition for felids, as has been reconstructed for *Proailurus*, the earliest fossil felid (Turner & Antón, 1996; Peigné, 1999), or that all living species have the ability to climb (Ewer, 1973; Sunquist & Sunquist, 2002; MacDonald *et al.*, 2010). Only a few conspicuous locomotory specializations are observed in living cats, such as the cheetah, *Acinonyx jubatus*, which is more cursorial than other felids (Ewer, 1973; Sunquist & Sunquist, 2002; MacDonald *et al.*, 2010), and the highly arboreal margay, marbled cat and clouded leopard – *Leopardus wiedii*, *Pardofelis marmorata* and *Neofelis nebulosa*, respectively – with their broad feet and very flexible ankles (Sunquist & Sunquist, 2002; MacDonald *et al.*, 2010).

This relative similarity in the felid axial skeleton was also demonstrated by our vertebral column profile analyses (Fig. 4). The profile plots revealed a strong general resemblance between locomotory groups, with a few instances of significant statistical difference between them (Table 6), and primarily in the thoracic region. These instances were found in comparisons between the arboreal group's profile and the other two locomotory groups, suggesting that arboreality may require distinct morphological specialization of the axial skeleton. Our results indicate that arboreal species present greater passive stiffness in the thoracic region due to larger values of centrum width and shape (Fig. 4C, D) (Long *et al.*, 1997; Koob & Long, 2000; Shapiro, 2007; Pierce *et al.*, 2011). This may, however, be counterbalanced by a greater propensity for intervertebral mobility (i.e. *sensu* range of motion) granted by a larger IZL in the anterior thoracic region (Fig. 4L) (Jenkins, 1974; Pierce *et al.*, 2011). Contrary to the profile plots, our PC analyses recover the lumbar region as holding the majority of the locomotory signal. This discrepancy may indicate that unlike similar analyses (e.g. Pierce *et al.*, 2011; Jones & German, 2014; Molnar *et al.*, 2014), univariate measures are not sufficient to discriminate between felid locomotor specializations, and that such distinction is best achieved with more complex, multidimensional shape analyses.

Prey-killing techniques, which if reflective of prey size choice can subdivide species based on the morphological signal of the forelimbs and cranium (Leyhausen, 1979; Slater & Van Valkenburgh, 2008; Meachen-Samuels & Van Valkenburgh, 2009a,b,

2010). However, prey size was not significantly associated with vertebral shape in this study, counter to our expectations for the cervical vertebrae. This result may be a reflection of the measurements chosen in this study, which were based on biomechanical traits relevant for locomotory modes (Pierce *et al.*, 2011), or that variation in vertebral shape across felid evolution is not closely tied to variations in prey-killing techniques. To more fully understand the effect of prey specialization on the vertebral column of felids, most specifically on the cervical vertebrae, further investigation of vertebral shape using more sophisticated analytical techniques (e.g. geometric morphometrics) would be advantageous.

SHAPE AND BODY SIZE

Our analyses revealed widespread allometry in the vertebral column of extant felids, a pattern consistent with Doube *et al.* (2009), who found similar scaling in the appendicular skeleton. Therefore, body size, which is often the most conspicuous difference when grossly comparing the skeletons of distantly related felid species, has a great influence on the overall morphology of the vertebral column. In light of the suggestions of shorter and stiffer lumbar regions in larger mammals (Smeathers, 1981; Gál, 1993; Jones, 2015), and also taking into account the postural uniformity in felids through increases in body size (Day & Jayne, 2007; Doube *et al.*, 2009; Zhang *et al.*, 2012), we had initially hypothesized that, as felid species increase in size, there would be an increase in vertebral column stiffness. Furthermore, we hypothesized that this increase in stiffness would be particularly evident in the posterior column due to the necessity to support greater body mass. In keeping with this, the total length of the vertebral column in living felid species was shown to be highly correlated with body mass (Table 7), and there was a negative allometric relationship between the two variables (i.e. the vertebral column is relatively shorter in larger species). This result agrees with the recent findings of allometric shortening of the thoracolumbar region in felids by Jones (2015). However, the relationship found here was not maintained after phylogenetic correction, and the length of the whole vertebral column, or of discrete vertebral column regions, displayed a relationship with body mass that was not significantly different from what is expected from isometry. In contrast, Jones (2015) found that her evolutionary negatively allometric patterns were consistent prior to and after phylogenetic correction, both for total thoracolumbar length and for the individual thoracic and lumbar regions. The cause of this disagreement between analyses is unclear, but may lie in the different phylogenetic methods used

[i.e. independent contrasts in Jones (2015) vs. phylogenetic GLS here], or because here we use average species body mass rather than an estimate of body mass based on a regression equation from limb dimensions.

Compared to our whole vertebral column results, analyses of individual vertebral measurements showed extensive intravertebral allometry, with most vertebral dimensions being positively allometric when corrected for phylogeny (i.e. relatively larger in larger species), particularly in the thoracic region (Table 7). The most prevalent allometry was centrum height, being present in over two-thirds of the vertebral column (19 out of 27 vertebrae), from the atlas to T12 and L7. Increased height of the centrum in larger felid species suggests greater stability in the dorsoventral plane in the cervical and thoracic region. Jones (2015) also found centrum height to be positively allometric in the thoracic region; however, she also found this measurement to be positively allometric in the mid-lumbar region. Our analyses found no support for allometric scaling of centrum dimensions in the lumbar region, except for L7. The most prevalent allometry in the lumbar vertebrae was the neural spine lever arm; longer neural spines in larger animals will increase passive stiffness due to the presence of larger epaxial musculature (and ligaments), but it will also increase the leverage for dorsoventral bending capacity of the lumbar region (Long *et al.*, 1997; Pierce *et al.*, 2011), which may contribute to stride length. Therefore, our data imply that larger felid species increase passive stiffness in the lumbar region via acquisition of greater muscle mass and ligament leverage, rather than changes in centrum dimensions.

The three main groups of allometric variables – centrum-related, neural spine-related and zygapophyseal-related – appear to dominate in different regions of the column (i.e. before and after the anticlinal vertebra T11): whereas the neural spine-related allometries were almost equally spread throughout the vertebral column, the centrum- and zygapophyseal-related allometries were concentrated in the cervical and thoracic regions, with few instances in the lumbar vertebrae. Allometry has been suggested to be a strong factor contributing to morphological integration (Klingenberg, 2008; Klingenberg & Marugán-Lobón, 2013), and the pattern of regionalization of specific allometric trends would be consistent with the presence of modularity in the vertebral column [i.e. existence of sets of characters that covary more strongly between themselves due to shared function or proximity, and present some evolutionary independence from other traits (Olson & Miller, 1958)]. Morphological, developmental and functional modularity has been studied in the mam-

malian skeleton, with many examples focusing on the skull (Goswami, 2006; Goswami *et al.*, 2012; Meloro & Slater, 2012; Piras *et al.*, 2013) but also on the vertebral column and limbs (Polly, Head & Cohn, 2001; Goswami, Weisbecker & Sanchez-Villagra, 2009; Buchholtz *et al.*, 2012; Buchholtz, 2014; Fabre *et al.*, 2014).

Based on the distribution of allometries recovered here, we propose the hypothesis of the presence of two major functional modules in the felid vertebral column: an anterior module composed of the cervical and thoracic vertebrae, and a posterior or lumbar module. Moreover, our findings of similar allometric trends in cervicals C4 and C5 match the previously suggested diaphragmatic module for the mammalian column (Buchholtz, 2014), and we additionally propose a functional ‘anticlinal module’ composed of the anticlinal vertebra (T11) and the immediate surrounding vertebrae (T10 and T12). These hypothesized modules within the felid vertebral column are an interesting starting point for further analysis of morphological integration and morphological/functional regionalization of the felid vertebral column using more appropriate methodologies (e.g. Goswami & Polly, 2010; Klingenberg & Marugán-Lobón, 2013; Fabre *et al.*, 2014; Head & Polly, 2015).

CONCLUSION

Comparative functional studies on animals with similar musculoskeletal anatomy are important to understand the form–function relationship (e.g. Irschick, 2002; Nyakatura & Fischer, 2010), and such studies allow researchers to better understand the behaviour of living organisms and infer the habits of extinct species (Moon, 1999; Hutchinson, 2012). The work we present here provides a new perspective on how extant felids have adapted their postcranial skeleton to deal with ecological specializations over a wide range of body mass, irrespective of having a relatively conservative morphology. Specifically, our results show evidence for hitherto underappreciated differentiation in vertebral shape in Felidae, which reflects specialization for locomotion mode (arboreal, scansorial and terrestrial). Furthermore, there is evidence for extensive allometric scaling within individual vertebrae. In particular, evolutionary increases in body size have driven stabilization of the anterior axial skeleton (cervical and thoracic vertebrae) through widespread modification of vertebral form. In contrast, size-correlated stabilization of the lumbar region seems to be primarily accomplished by means of increases in epaxial muscle mass in felids. The heterogeneous effects of axial allometry within the felid vertebral column suggest the presence of modularity beyond traditional region-

alization boundaries, which will be tested in future studies.

ACKNOWLEDGEMENTS

We thank P. David Polly and an anonymous reviewer for their constructive reviews of the manuscript. For access to specimens, we thank M. Lowe and R. Asher at the University Museum of Zoology, Cambridge; R. Portela Miguez at the Natural History Museum, London; and C. Lefèvre at the Muséum National d'Histoire Naturelle, Paris. We thank Dr Mark Bell and Dr Claire Peart for their help with the R scripts. We also thank the Adaptive group at University College London for their helpful input during preparation of the manuscript. This work was supported by Leverhulme Trust grant RPG 2013-124 to A.G. and J.R.H. and the National Science Foundation award EAR-1524523 to S.E.P.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the supporting information tab for this article.

Table S1. Results from the phylogenetic scaling analysis showing the slope of the relationship between the variables in the first column and body size (i.e. total vertebral length), with lower and upper 95% confidence limits from the slope value, and *P*-value for the null hypothesis of the slope being different from 1 (i.e. isometry).