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2   Title: Cryptic complexity in felid vertebral evolution: shape differentiation and allometry of

3   the axial skeleton.

4   Short running head: Vertebral evolution in cats

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1   Abstract:

2   Members of the mammalian family Felidae (extant and extinct cats) are grossly

3   phenotypically similar, but display a 300-fold range in body size, from less than 1kg to more

4   than 300kg. In addition to differences in body mass, felid species show dietary and

5   locomotory specialisations that correlate to skull and limb osteological measurements, such

6   as shape or cross-section area. However, ecological correlates to the axial skeleton are yet

7   untested. Here, we build on previous studies of the biomechanical and morphological

8   evolution of the felid appendicular skeleton by conducting a quantitative analysis of

9   morphology and allometry in the presacral vertebral column across extant cats. Our results

10   demonstrate that vertebral columns of arboreal, scansorial, and terrestrial felids significantly

11   differ in morphology, and more so specifically in the lumbar region, while no distinction

12   based on dietary specialisation was found. Body size significantly influences vertebral

13   morphology, with clear regionalization of allometry along the vertebral column, suggesting

14   that anterior (cervicals and thoracics) and posterior (lumbar) vertebrae may be independently

15   subjected to distinct selection pressures.

16   Keywords: Allometry – Felidae – locomotion – morphology – vertebral column

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1   Introduction:

2   The carnivoran family Felidae (Mammalia, Placentalia) includes *ca.* 37 living species of

3   grossly morphologically similar animals (Ewer, 1973; Johnson et al., 2006; MacDonald et al.,

4   2010; Sunquist & Sunquist, 2002; Turner & Antón, 1996). With the exception of fur

5   patterning, body size is the greatest gross anatomical difference observed between species,

6   with the Felidae displaying a considerable body mass range from 1kg in the rusty-spotted cat

7   (*Prionailurus rubiginosus*) to over 300kg in the tiger (*Panthera tigris*). In addition to their

8   overall phenotypic similarity, felids are an exception to the general mammalian

9   biomechanical trend of size-correlated limb posture. According to this trend, increases in

10   body size drive increased limb erectness (i.e. joint extension) in order to maintain safe levels

11   of peak functional stresses acting on supportive tissues (Bertram & Biewener, 1990;

12   Biewener, 1989; Biewener, 2005). However, despite the 300-fold range in body mass in

13   felids, limb posture is remarkably uniform throughout the clade and, instead, some bone

14   allometry is observed in limb long bones' cross-sections (Day & Jayne, 2007; Doube et al.,

15   2009; Zhang et al., 2012). Indeed, it has been hypothesized that the lack of correlation

16   between body size and limb posture in felids may reflect a large-bodied ancestral condition

17   for the clade (Day & Jayne, 2007; Johnson et al., 2006; Mattern & McLennan, 2000; but see

18   Cuff et al., 2015).

19   Felids are also remarkably conservative in behavioural and ecological attributes, such as diet:

20   all felids are hypercarnivores specialised in vertebrate prey, with species differing mainly in

21   terms of prey size and prey-killing techniques (Carbone et al., 1999; Ewer, 1973; Sunquist &

22   Sunquist, 2002). Felid species are known to show different killing strategies in relation to

23   prey size, with bigger cats usually applying a sustained bite to the prey's muzzle or neck, and

24   smaller felids killing by faster nape or head bites (Ewer, 1973; Leyhausen, 1979; MacDonald,

1   Macdonald & Loveridge, 2010). Interestingly, unlike other carnivorans such as canids, the

2   forelimbs of felids present a duality in function between locomotion and prey-killing

3   behaviour (Ewer, 1973; Gonyea, 1978; Leyhausen, 1979), and therefore, along with

4   differences in skull, mandible and dental shape, the shape of the forelimbs also reflect

5   diversification in prey size choice (Meachen-Samuels & Van Valkenburgh, 2009a; Meachen-

6   Samuels & Van Valkenburgh, 2009b; Meachen-Samuels, 2012; Slater & Van Valkenburgh,

7   2008; Slater & Van Valkenburgh, 2009).

8   Several recent studies have examined the shape, function, and evolution of mammalian limbs,

9   especially those of carnivorans (Alvarez, Ercoli & Prevosti, 2003; Meachen-Samuels, 2010;

10   Meachen-Samuels & Van Valkenburgh, 2009a; Samuels, Meachen & Sakai, 2013; Walmsley

11   et al., 2012). These studies have demonstrated that osteological measurements of the entire

12   limbs and of their individual segments are informative about locomotory habits, such that

13   qualitative reconstructions of the ecology of fossil species are possible by comparing their

14   morphology to better known living species. Within Felidae, these studies have additionally

15   shown that the limb morphology is informative about prey size specialisation and,

16   furthermore, that limb shape is related to hunting strategies in extant and, by inference,

17   extinct species (Meachen-Samuels & Van Valkenburgh, 2009b; Meachen-Samuels, 2012;

18   Meachen-Samuels & Van Valkenburgh, 2010). However, to date, the vertebral column has

19   been underrepresented in the morphological and biomechanical literature on felids and other

20   species, and is often treated as one functional segment, with few functional studies

21   considering the complexity and regionalisation of this structure in detail (but see Macpherson

22   and Ye (1998) and Jones (2015)).

23   The vertebral column has a critical role in body support against gravity, is connected to the

24   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

(Long, Adcock & Root, 2002; Macpherson & Fung, 1998; Macpherson & Ye, 1998;

Pridmore, 1992; 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; Gál, 1993b; Long et al., 1997; Molnar,

Pierce & Hutchinson, 2014; Pridmore, 1992; Smit, 2002). 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 (Koob & Long, 2000; Long et al., 1997; Pierce, Clack &

Hutchinson, 2011). Thus, morphological specialisations 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 and Kuratani (2005).

Potentially due to this constraint in vertebral numbers, specialisation into discrete niches has

been accompanied by a diversification of vertebral shapes across placentals (Buchholtz,

2014; Buchholtz et al., 2012; Muller et al., 2010; Narita & Kuratani, 2005; Pierce et al.,

2011). Although studies are limited, identification of correlated changes between vertebral

shape and various ecological attributes have extended our understanding of the behaviour of

living animals and aided in reconstructing the behaviour and ecology of extinct species

(Ánton & Galobart, 1999; Argot, 2003; Pierce et al., 2013; Pierce et al., 2011; Shapiro et al.,

2005). Moreover, morphological specialisations 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, 1993b).

In order 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 analysis of scaling across a large suite of biomechanically relevant measurements. In accordance with Smeathers (1981), Gál (1993b) 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:

1. *Data composition:*

1   *Species and specimens.* – The data set is composed of 24 specimens representing 22

2   extant felid species, which is ~62% of total number of species in the family (Figure 1). The

3   chosen species embody the full phylogenetic breadth of extant felids, with each of the eight

4   identified clades (Johnson et al., 2006) represented by at least one species. The sample also

5   encompasses the full range of body sizes (e.g. *Leopardus colocolo* and *Leopardus wiedii*,

6   both at the small body mass end at 2 – 4kg, and *Panthera tigris* at the large body mass

7   extreme of up to 325kg) and ecologies (e.g. arboreal, scansorial, and terrestrial) displayed by

8   living felids (Meachen-Samuels & Van Valkenburgh, 2009b; Sunquist & Sunquist, 2002).

9   Specimens were chosen based on completeness, being disarticulated (which allows a greater

10   number of anatomical features to be observed and measured) and, whenever possible, being

11   wild caught (known captive-raised specimens are identified in Table 1). The specimens

12   sampled are held in the zoological collections at the Natural History Museum in London

13   (NHM), the University Museum of Zoology Cambridge (UMZC), and the Muséum National

14   d'Histoire Naturelle in Paris (MNHN) (Table 1).

15   *Anatomical measurements.* – All 27 pre-sacral vertebrae (seven cervicals, 13 thoracics,

16   and seven lumbars) of one to two specimens per species were measured with digital callipers

17   (accuracy of 0.01mm) for linear measurements and with a goniometer for angular

18   measurements (to the nearest degree). The measurements were particular to each of the three

19   regions of the vertebral column (cervical, thoracic, and lumbar) and only features present in

20   all species were used in statistical analyses. Because different regions have unique vertebral

21   features, different combinations of measurements were taken on separate sets of

22   morphologically similar vertebrae (Figure 2). In total, there were 28 measurement categories

23   (i.e. centrum length, neural spine angle) with a sum total of 309 variables across the column,

24   and an overall total of 6798 measurement values in the dataset. Missing values (e.g. where

25   vertebrae were broken) were randomly imputed in R version 3.1.2 (R Foundation, 2013) by

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basing the new values on observed instances for each specific variable. This method also

calculates 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 measurement, 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 (MR), 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.

 2. *Data analyses:*

 Principal Component Analyses (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; Sunquist & Sunquist, 2002).

1   Phylogenetic relationships were based on a recent supertree analysis of carnivorans

2   (Nyakatura & Bininda-Emonds, 2012), which was cropped in Mesquite version 3.02

3   (Maddison & Maddison, 2014) to only include species represented in this study (Figure 1).

4   These measurements were analysed with a Principal Components Analysis (PCA) in PAST

5   version 2.17c (Hammer, Harper & Ryan, 2001) for five subsets of the original dataset: all

6   vertebrae (i.e. all 27 vertebrae), cervicals only (i.e. only the seven vertebrae of the cervical

7   region), thoracics only (i.e. only the 13 vertebrae of the thoracic region), lumbars only (i.e.

8   only the seven vertebrae of the lumbar region), and thoracics + lumbars combined (i.e. the 20

9   vertebrae composing the thoracic and lumbar regions, from T1 to L7).

10   In order to ensure that size had been removed prior to our PCA, and therefore that PCs were

11   uncorrelated with size, PC scores from significant PC axes (i.e. those with eigenvalues higher

12   than the Jollife cut-off) in the 'all vertebrae' PCA were regressed against $\log_{10}$ total vertebral

13   column length as a proxy for body size. The scores were regressed both across the full 'all

14   vertebrae' sample and per locomotory group (as this was the main trait influencing

15   morphospace occupation; see Results). This same procedure was repeated for the full 'all

16   vertebrae' sample while controlling for phylogeny, with independent contrasts (Felsenstein,

17   1985) calculated for the PC scores from significant axes and for total vertebral length using

18   the R package 'ape' (Paradis, Claude & Strimmer, 2004). This further step was performed in

19   order to ensure that size had been removed from our data even when phylogeny was taken

20   into account. Independent contrasts (for scores of each PC axis against vertebral column

21   length) were then subjected to Reduced Major Axis (RMA) regression in R using the 'smatr'

22   package (Warton et al., 2012).

23   To test how locomotor specialization affects vertebral shape, species were categorised by

24   three primary locomotor modes - arboreal, scansorial, and terrestrial - and qualitatively

1   evaluated in PCA morphospace (the full linear dataset and four regional linear subsets) using

2   convex hulls. Species assignment to locomotory categories are detailed in Figure 1 and Table

3   1 and were based on the studies of Meachen-Samuels and Van Valkenburgh (2009b) and

4   Sunquist and Sunquist (2002). Further, to explore the impact of prey specialization on

5   vertebral shape, the 'cervicals only' and the 'all vertebrae' subsets were qualitatively

6   examined in PCA morphospace by grouping species by prey size (i.e. small, mixed, and

7   large) according to the study by Meachen-Samuels and Van Valkenburgh (2009a). Finally, to

8   assess the effect of phylogenetic relatedness on vertebral morphology, species were also

9   categorised according to clade ('Panthera', 'Bay cat', 'Caracal', 'Ocelot', 'Lynx', 'Puma',

10   'Leopard cat', and 'Domestic cat' lineages based on Johnson et al., 2006; Figure 1) in the

11   resulting PCA morphospace. All qualitative assessments using PCA were followed by the

12   confirmatory analyses detailed below.

13   *MANOVA and Phylogenetic MANOVA.* – Differences in the area of morphospace

14   occupied by each of the locomotory, prey size, and clade groupings were further assessed

15   quantitatively using MANOVA. Locomotory and prey size groupings were also analysed

16   with phylogenetic MANOVAs (pMANOVAs) to account for the potentially confounding

17   effect of phylogeny. These pMANOVAs address the issue of non-independence due to

18   relatedness in species' phenotypes by correcting the overestimation of degrees of freedom in

19   comparative cross-species tests (Garland et al., 1993). Specifically, the significance of the

20   standard test statistic is assessed using a Brownian motion model to simulate the distribution

21   of the relevant dependent variables along a given phylogenetic tree. MANOVAs and

22   pMANOVAs were performed on the PC scores of all axes that presented an eigenvalue equal

23   to or higher than the Jolliffe cut-off (i.e. the first nine PCs for the 'all vertebrae' analysis,

24   which were all higher than the cut-off value of 0.04595). The phylogenetic relationships used

25   were identical to those used to conduct the phylogenetic size-correction (see above). All

standard and phylogenetic MANOVA analyses 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
specialisation, 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*centrum length/(centrum height +
centrum width)] throughout the vertebral column (Pierce et al., 2011). This measure of
centrum shape provides clearer information in regards to the flexibility and range of motion
of intervertebral joints (Buchholtz, 2001a; Buchholtz, 2001b).

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 – 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. –

1   a) *Vertebral column length and body mass* – To test if vertebral column length is a

2   robust predictor of specimen body size (see below), and to examine how the whole

3   column scaled with body mass, generalised least squares (GLS) regressions of $\log_{10}$

4   body mass (based on average species body mass (from Cuff AR et al., 2015) were

5   made against $\log_{10}$ total presacral vertebral column length (C1-L7) (based on the sum

6   total of centrum lengths, without the intervertebral disc/space). The generalised least

7   squares regressions were carried out with and without phylogenetic correction under a

8   Brownian motion model of evolution using the 'pGLS' package (Martins & Hansen,

9   1997; Mao & Ryan, 2013) within R. We also investigated regional scaling by

10   performing phylogenetically-corrected GLS regressions of $\log_{10}$ body mass against

11   each of the separate $\log_{10}$ total lengths of the cervical, thoracic and lumbar regions.

12   Analyses to test if vertebral column length scaled isometrically with body mass were

13   made by comparing the obtained slopes to an isometry slope of 0.333 (i.e. length ~

14      $\sqrt[3]{mass}$).

15   b) *Individual vertebrae and total length* – In addition, we also tested for allometric

16   changes within individual vertebrae. To control for phylogeny, independent contrasts

17   of $\log_{10}$ raw linear measurements and $\log_{10}$ total vertebral column length were

18   calculated using the same procedure cited above. Those independent contrasts (for

19   scores of each individual linear vertebral measurement against vertebral column

20   length) were then subjected to Reduced Major Axis (RMA) regression in R using the

21   'smatr' package (Warton et al. 2012). Analyses to test if these individual linear

22   vertebral measurements scaled isometrically with total vertebral length were made by

23   comparing the obtained slopes to an isometry slope of 1 (i.e. length ~ length[1]).

24   Results:

1   *Principal Component Analysis, MANOVA and Phylogenetic MANOVA.* – The 'all

2 vertebrae' PCA revealed nine PCs which were significant according to the Jolliffe cut-off

3 value of 0.04595 (Table 3), and the sum of the variance explained by those reached almost

4 80% (i.e. 79.166%) of the total variance. Regressions of all significant PC scores from the

5 'all vertebrae' PCA on $\log_{10}$ total vertebral column length, before and after phylogenetic

6 correction and between locomotory groups, demonstrated that shape variables were

7 statistically uncorrelated with size ($r^2 \ll 0.4$, and $p \gg 0.05$) and that the effects of size

8 variation were removed prior to PCA.

9 PC1xPC2 showed a large area of overlap between the terrestrial and scansorial groups, but a

10 clear clustering of arboreal species in a distinct area of morphospace (Figure 3A). There was

11 a much better separation of all three locomotory groups in PC1xPC3 (Figure 3B), with only a

12 very small overlap between the terrestrial and scansorial groups. The vertebral features which

13 were most relevant to contributing to this result in terms of high correlation coefficients (i.e. r

14 > 0.6, following Pierce et al. (2011)) are detailed in Table 4. While most variables exhibited

15 high PC1 loading correlation values, PC3 was only highly correlated with measurements of

16 neural spine anteroposterior length at tip in the thoracic and lumbar regions, and centrum

17 height in the lumbar region.

18 Clade groupings in the 'all vertebrae PCA' were significant as a clustering factor when

19 analysed with MANOVA, showing that among the species studied here, closely related taxa

20 tended to be more similar in their axial skeletal morphology. The 'all vertebrae PCA'

21 revealed that the clustering of species by their locomotory groups was indeed statistically

22 significant, both with (phylogenetic $p \ll 0.05$) and without ($p \ll 0.05$) phylogenetic

23 correction. Prey size groups in the 'all vertebrae' morphospace were non-significant ($p \gg$

24 0.05, and phylogenetic $p \gg 0.05$; Table 5).

The 'thoracics only', 'lumbars only', and the 'thoracics + lumbars' subset analyses revealed clustering similar to the 'all vertebrae' PCA (not shown). MANOVA results calculated from the 'lumbars 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 'thoracics + lumbars' subsets, 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 'lumbars 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 localised differences in the shape of individual vertebral features (Figure 4A-M). 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).

Scaling:

a) *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).

b) *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 Supp
Table 1): 61 positive and three negative. There was clear regionalisation of vertebral
allometry: out 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).

Out 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 exhibit a positive allometric relationship in terms of centrum length and height. 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-T12; and neural spine lever arm, which was positively allometric from T5-T13 (although 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 shows positive allometry with respect to centrum height.

Discussion:

1   *Shape and ecology.–*

2   Here, we quantitatively analysed the morphology of the entire pre-sacral vertebral column in

3   felids to test whether morphological differentiation of the vertebral column across species is

4   driven by body size and/or ecologically derived traits, such as locomotory mode and prey-

5   hunting specialization, as has been previously demonstrated for felid limbs (Gonyea, 1978;

6   Meachen-Samuels & Van Valkenburgh, 2009b; Meachen-Samuels, 2012). Our study shows

7   that linear shape variation in the felid vertebral column significantly discriminated terrestrial,

8   arboreal, and scansorial species, demonstrating that locomotory specialization, but not prey

9   size, has fashioned vertebral column evolution within felids. Locomotor differentiation was

10   statistically significant only when phylogenetic relationships were taken into account, and

11   only when either 'all vertebrae' were analysed together or when the analysis was restricted to

12   the lumbar vertebrae. In a study comparing the relative lengths of limbs and axial skeletons of

13   species of large-bodied felids, Gonyea (1976) suggested that locomotory specialisation was

14   reflected by changes in the length of the lumbar region (but see scaling results below). This

15   result indicates that, although size-independent changes in shape are somewhat dispersed

16   throughout the whole vertebral column, wide-spread changes in the lumbar vertebra are

17   particularity important for locomotor specialization.

18   Although there was significant differentiation of locomotory groups across all principal

19   components, there was also clear overlap between scansorial and terrestrial species on most

20   PCs (Figure 3). Such morphological similarities between these locomotory groups may

21   reflect a hypothesized scansorial ancestral condition for felids, as has been reconstructed for

22   *Proailurus*, the earliest fossil felid (Peigné, 1999; Turner & Antón, 1996), or that all living

23   species have the ability to climb (Ewer, 1973; MacDonald et al., 2010; Sunquist & Sunquist,

24   2002). Only a few conspicuous locomotory specialisations are observed in living cats, such as

1   the cheetah, *Acinonyx jubatus*, which is more cursorial than other felids (Ewer, 1973;

2   MacDonald et al., 2010; Sunquist & Sunquist, 2002), and the highly arboreal margay,

3   marbled cat, and clouded leopard; *Leopardus wiedii, Pardofelis marmorata*, and *Neofelis*

4   *nebulosa*, respectively; with their broad feet and very flexible ankles (MacDonald et al.,

5   2010; Sunquist & Sunquist, 2002).

6   This relative similarity in the felid axial skeleton was also demonstrated by our vertebral

7   column profile analyses (Figure 4). The profile plots revealed a strong general resemblance

8   between locomotory groups, with a few instances of significant statistical difference between

9   them (Table 6), and primarily in the thoracic region. These instances were found in

10   comparisons between the arboreal group's profile and the other two locomotory groups,

11   suggesting that arboreality may require distinct morphological specialisation of the axial

12   skeleton. Our results indicate that arboreal species present greater passive stiffness in the

13   thoracic region due to larger values of centrum width and shape (Figure 4C-D) (Koob &

14   Long, 2000; Long et al., 1997; Pierce et al., 2011; Shapiro, 2007). This may, however, be

15   counterbalanced by a greater propensity for intervertebral mobility (i.e. *sensu* range of

16   motion) granted by a larger interzygapophyseal length (IZL) in the anterior thoracic region

17   (Figure 4L) (Jenkins, 1974; Pierce et al., 2011). Contrary to the profile plots, our PC analyses

18   recover the lumbar region as holding the majority of the locomotory signal. This discrepancy

19   may indicate that unlike similar analyses (e.g. Jones & German, 2014; Molnar et al., 2014;

20   Pierce et al., 2011), univariate measures are not sufficient to discriminate between felid

21   locomotor specialisations, and that such distinction is best achieved with more complex,

22   multidimensional shape analyses.

23   Prey-killing techniques, which if reflective of prey size choice, can subdivide species based

24   on the morphological signal of the forelimbs and cranium (Leyhausen, 1979; Meachen-

1   Samuels & Van Valkenburgh, 2009a; Meachen-Samuels & Van Valkenburgh, 2009b;

2   Meachen-Samuels & Van Valkenburgh, 2010; Slater & Van Valkenburgh, 2008). However,

3   prey size was not significantly associated with vertebral shape in this study, counter to our

4   expectations for the cervical vertebrae. This result may be a reflection of the measurements

5   chosen in this study, which were based on biomechanical traits relevant for locomotory

6   modes (Pierce et al., 2011) or that variation in vertebral shape across felid evolution is not

7   closely tied to variations in prey-killing techniques. To more fully understand the effect of

8   prey specialization on the vertebral column of felids, most specifically on the cervical

9   vertebrae, further investigation of vertebral shape using more sophisticated analytical

10   techniques (e.g. geometric morphometrics) would be advantageous.

11   *Shape and body size.–*

12   Our analyses revealed widespread allometry in the vertebral column of extant felids, a pattern

13   consistent with Doube et al. (2009), who found similar scaling in the appendicular skeleton.

14   Therefore, body size, which is often the most conspicuous difference when grossly

15   comparing the skeletons of distantly related felid species, has a great influence on the overall

16   morphology of the vertebral column. In light of the suggestions of shorter and stiffer lumbar

17   regions in larger mammals (Smeathers 1981, Gál 1993b, and recently Jones 2015), and also

18   taking into account the postural uniformity in felids through increases in body size (Day &

19   Jayne, 2007; Doube et al., 2009; Zhang et al., 2012), we had initially hypothesized that, as

20   felid species increase in size, there would be an increase in vertebral column stiffness.

21   Further, we hypothesized that this increase in stiffness would be particularly evident in the

22   posterior column due to the necessity to support greater body mass. In keeping with this, the

23   total length of the vertebral column in living felid species was shown to be highly correlated

24   with body mass (Table 7), and there was a negatively 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 2/3 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 mammalian 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 (Buchholtz, 2014;

Buchholtz et al., 2012; Fabre et al., 2014; Goswami, Weisbecker & Sanchez-Villagra, 2009;

Polly, Head & Cohn, 2001).

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 'anticlinality 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. Fabre et al., 2014; Goswami & Polly, 10; Head & Polly, 2015; Klingenberg & Marugán-Lobón, 2013)

Conclusion:

Comparative functional studies on animals with similar musculoskeletal anatomy are important to understand the form-function relationship (e.g. Irschick (2002) and Nyakatura and Fischer (2010)), and such studies allow researchers to better understand the behaviour of living organisms and infer the habits of extinct species (Hutchinson, 2012; Moon, 1999). The work we present here provides a new perspective on how extant felids have adapted their postcranial skeleton to deal with ecological specialisations 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 specialisation 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 stabilisation of the anterior axial skeleton (cervical and thoracic vertebrae) through widespread modification of vertebral form. In contrast, size-correlated stabilisation 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 regionalisation boundaries, which will be tested in future studies.

Acknowledgements

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Figure legends:

Figure 1: Felid phylogeny showing studied species, from a subset of Nyakatura and 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.
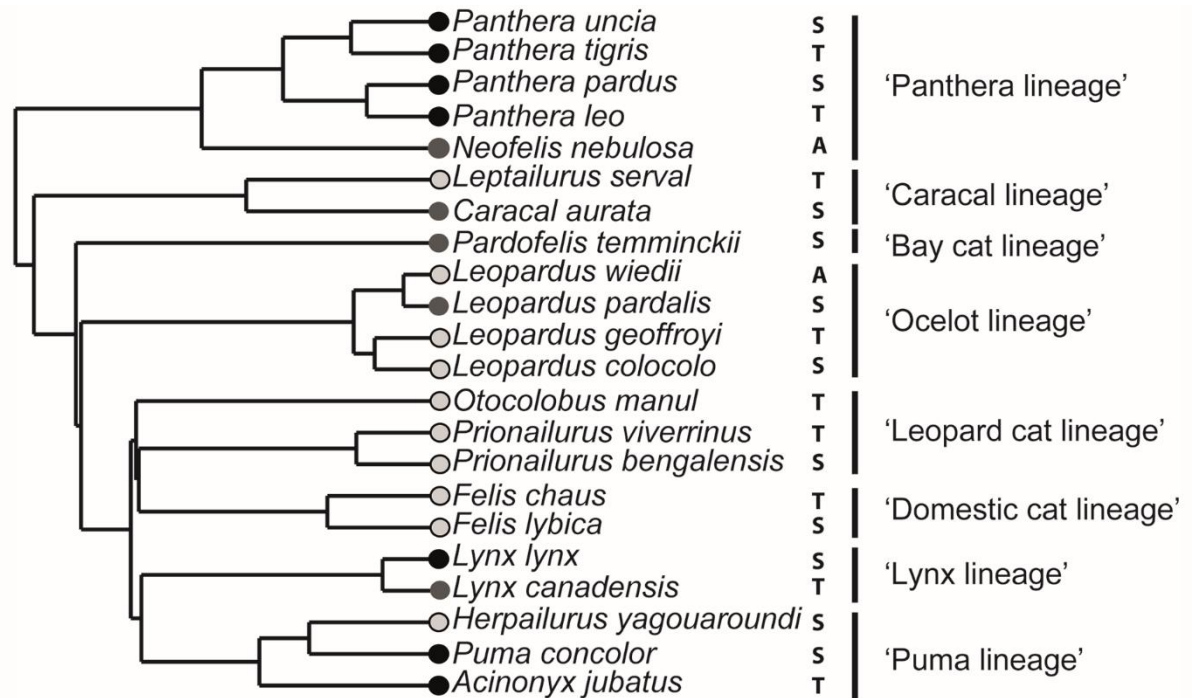
Figure 2: Vertebral measurements: (A-C) atlas, (D-E) axis, (F) C6, and (G-J) L2 Abbreviations. LDA: Length of dorsal arch; Pre_Z-D: Prezygapophyseal distance; Post_Z-D: Postzygapophyseal distance; TPLA: Transverse process lever arm; WDA: Width of dorsal arch. B. LVA: Length of ventral arch; WVA: Width 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)
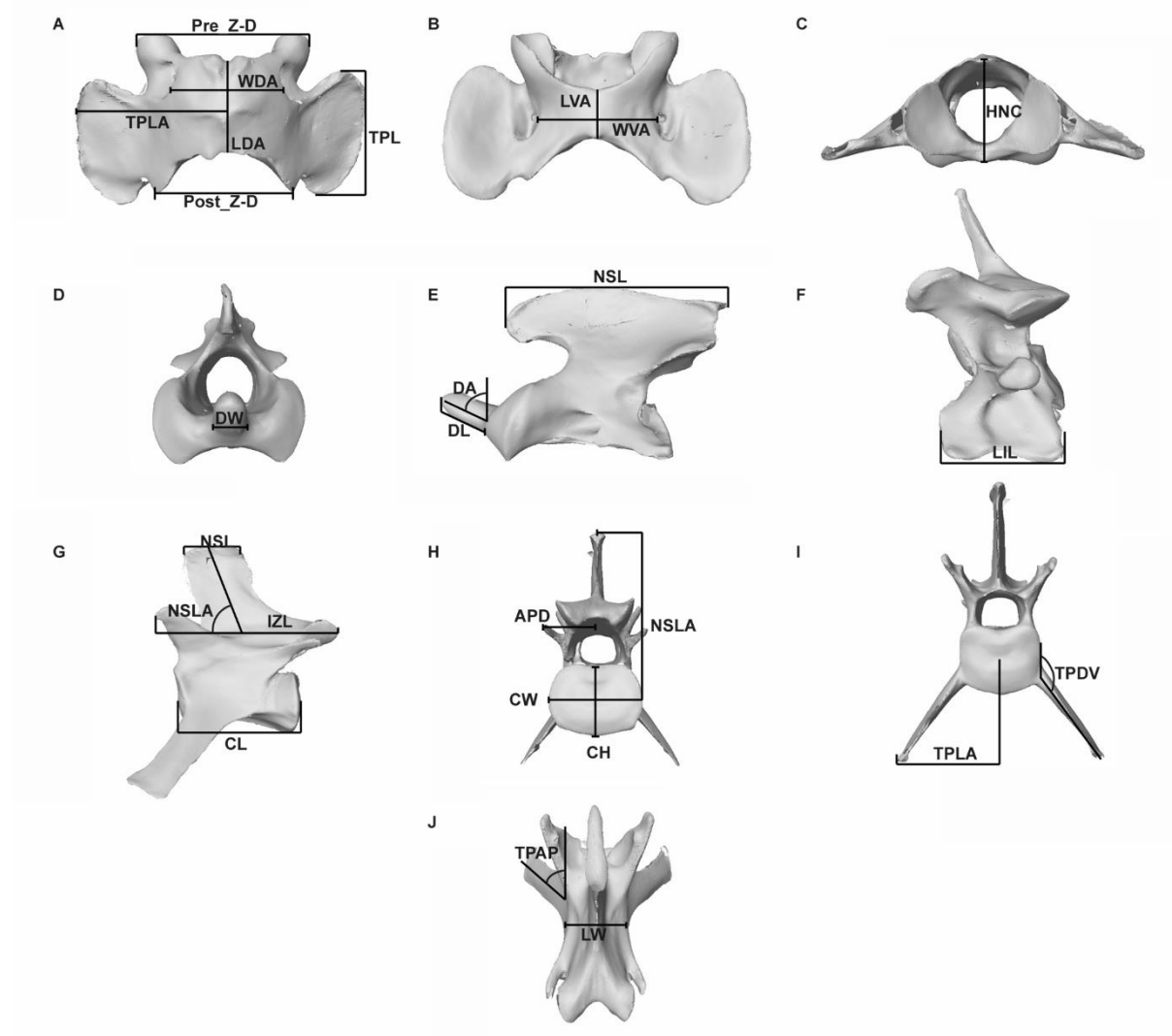
Figure 3: PCA plots of PC1 x PC2 (A) and PC1 x PC3 (B) showing species distribution in vertebral morphospace. Species are grouped according to their locomotory mode (i.e. cross: arboreal species; triangle: scansorial species, and squares: terrestrial species).
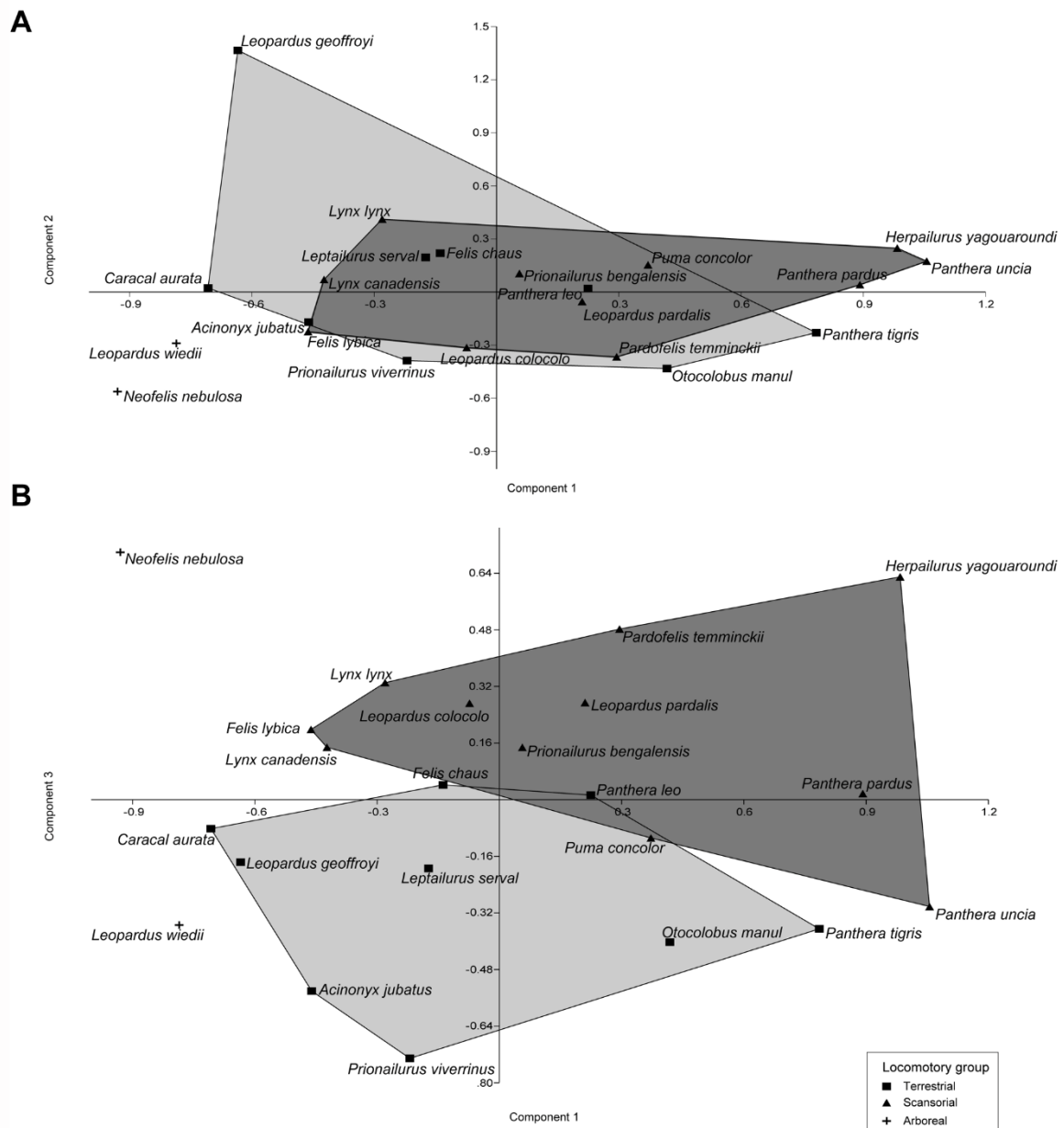
Figure 4: Vertebral profile plots of locomotory groups (i.e., arboreal, terrestrial, and scansorial species) showing variation in vertebral measurements along the vertebral column number. A. Centrum length (CL); B. Centrum height (CH); C. Centrum width (CW); D. Centrum shape (CS); E. Lamina width (LW); 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.
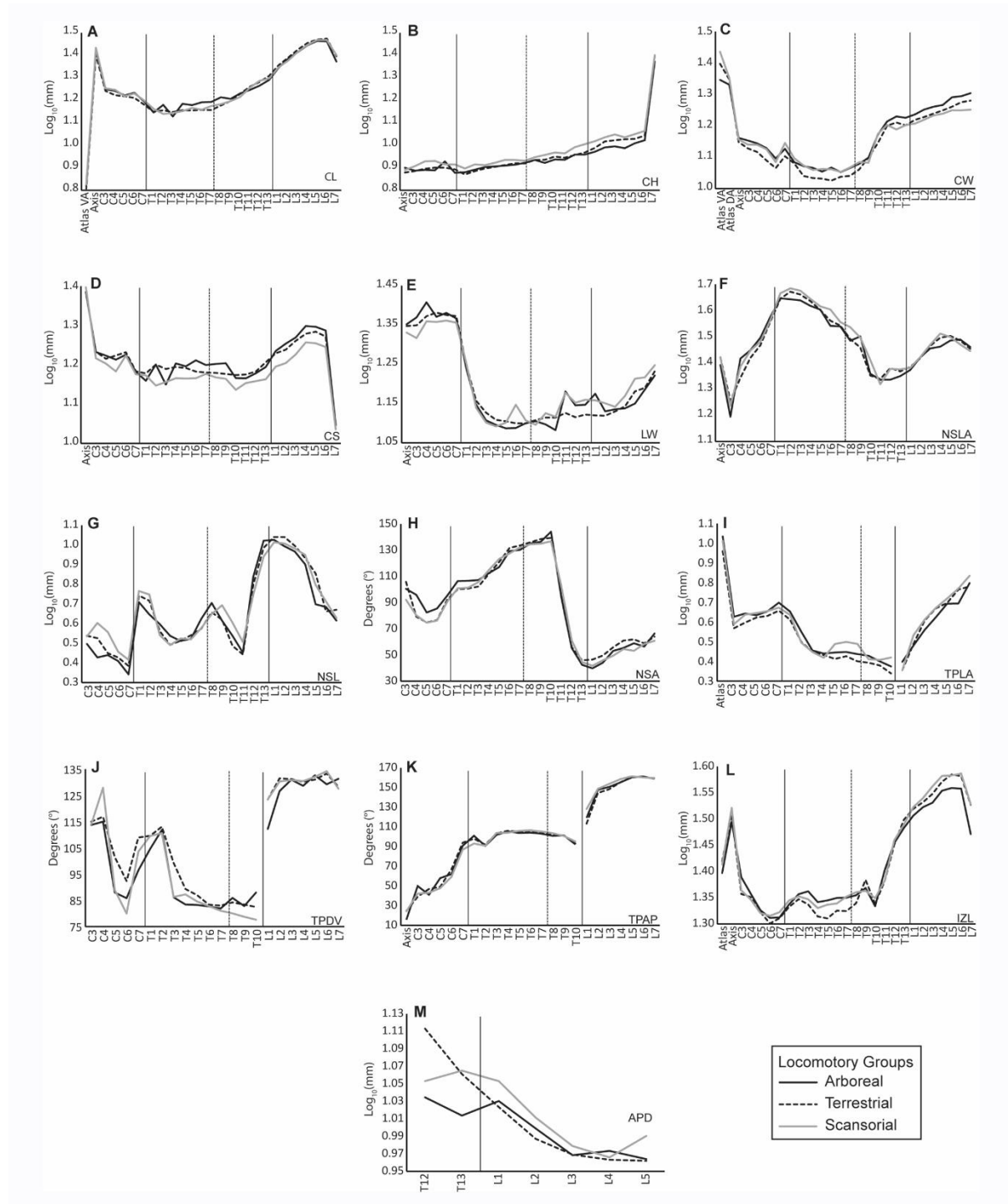
Tables:

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

Table 2. List of all measurements taken on each vertebra. C, cervical vertebra; T, thoracic vertebra; L, lumbar vertebra. Measurements in italics were not included in subsequent statistical analyses due to higher measurement error.

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
*C3-C6 ONLY	Length of inferior lamella	LIL
	Interzygapophyseal length	IZL
	Width of lamina	LW
	Neural spine angle	NSA
*C3-L7 ONLY	*Pre-zygapophyseal angle*	*Pre-ZA*
*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
	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	*Accessory process dorsoventral angle*	APDV
T12 – T13 ONLY	*Accessory process anteroposterior angle*	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	*Accessory process dorsoventral angle*	APDV
*L1-L5 ONLY	*Accessory process anteroposterior angle*	APAP

Table 3: PCA results from the 'all vertebrae' analysis. PCs with an eigenvalue higher than the Jolliffe cut-off of 0.046 are marked in bold.

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

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
	Neural spine length at tip	PC1
T1 – T13	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
	Neural spine length at tip	PC1, PC3
*L1-L5 ONLY	Accessory process distance	PC1

Table 5: Results of the MANOVA and phylogenetic MANOVA tests on PC scores from significant PCs as determined by the Jolliffe cut-off. Significance at P < 0.05 is indicated in italics, while significance after Bonferroni correction (i.e. P < 0.00625) is shown in bold.

GROUPS TESTED	MANOVA (P VALUE)	PHYLOGENETIC MANOVA (P VALUE)
PC1-9 ('ALL VERTEBRAE', LOCOMOTORY GROUPS)	*0.03043*	***0.006***
PC1-9 ('ALL VERTEBRAE', PREY SIZE GROUPS)	0.2811	0.6454
PC1-9 ('ALL VERTEBRAE', CLADES: 'PANTHERA' X 'OCELOT' LINEAGES)	***0.0000***	N.A.
PC1-9 ('THORACICS ONLY', LOCOMOTORY GROUPS)	0.0648	*0.0120*
PC1-9 ('THORACICS + LUMBARS', LOCOMOTORY GROUPS)	*0.0662*	*0.0120*
PC1-9 ('LUMBARS ONLY', LOCOMOTORY GROUPS)	*0.0083*	***0.002***
PC1-9 ('CERVICALS ONLY, LOCOMOTORY GROUPS')	0.4293	0.2547
PC1-9 ('CERVICALS ONLY', PREY SIZE GROUPS)	0.3	0.6693

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

	ANOVA	TUKEY'S PAIRWISE COMPARISON P-VALUE		
	F TEST P VALUE	**ARBOREAL x SCANSORIAL**	**ARBOREAL x TERRESTRIAL**	**SCANSORIAL x TERRESTRIAL**
CH				
BIN 1	0.0346	0.0840	0.928	*0.0423*
BIN 2	0.0573			
BIN 3	0.0162	*0.0167*	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***	*0.0146*
BIN 3	0.883			
BIN 4	0.0199	*0.0159*	0.4386	0.1798
CS				
BIN 1	0.9544			
BIN 2	0.01341	*0.0246*	0.999	*0.027*
BIN 3	0.0063	***0.0096***	0.941	*0.01851*
BIN 4	0.6848			
IZL				
BIN 1	0.9924			
BIN 2	0.00248	0.5606	***0.0025***	*0.0228*
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	*0.0372*	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			
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	*0.0416*
BIN 4	0.559			

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

VERTEBRAL COLUMN LENGTH	SLOPE	LOWER LIMIT	UPPER LIMIT	COEFFICIENT OF DETERMINATION (R^2)	P-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

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

VARIABLE	SLOPE	SLOPE LOWER LIMIT	SLOPE UPPER LIMIT	SLOPE P-VALUE	REGRESSION P-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**
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