The relationship between body shape, body size and locomotor mode in extant lepidosaurs

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Abstract

Despite historic work, the mechanisms and evolutionary drivers associated with the adoption of a facultatively bipedal locomotor mode in extant lepidosaurs are unclear. Recent work has provided insights into the biomechanical triggers of bipedal locomotion, but the associated anatomies are yet to be fully understood, particularly with regard to body size across Lepidosauria. Using a dataset derived from museum specimens, representing a range of lepidosaur body shapes, we highlight the differences between obligate quadrupeds and facultative bipeds within this group and demonstrate the value of non-caudal skeletal material in identifying facultative bipeds using osteology alone. We use multiple statistical approaches to identify trends across locomotor modes relative to body size. Body size has a significant effect upon body proportions across the two locomotor modes, especially in the hindlimbs. Forelimb lengths do not differ significantly across locomotor modes for animals of similar body size, but distal hindlimbs are significantly longer in facultative bipeds. Interestingly, femoral length does not differ across locomotor modes of a similar body size. Our findings contrast with historical tropes and are significant for future work attempting to identify the factors driving the evolution of a facultatively bipedal locomotor mode in Lepidosauria.

Introduction

The subclass Lepidosauria, comprising Squamata and Rhynchocephalia (*Sphenodon punctatus* – the tuatara – being the only living representative), is one of the most diverse and widespread extant terrestrial vertebrate groups. With a near-global continental distribution, excluding only Antarctica, and a plethora of ecologies, extant lepidosaurs exhibit a range of morphological adaptations suited to their environment. One of the most interesting aspects of squamate biology is their range and variety of locomotor behaviours, such as their iterative evolution of partial or total limb reduction (Wiens, Brandley & Reeder, 2006), extreme arboreality (Fischer, Krause & Lilje, 2010) and gliding (McGuire & Dudley, 2005). An equally distinctive locomotor behaviour that is well-represented in squamates is facultative bipedality, defined as the ability of ordinarily quadrupedal animals to adopt a bipedal gait for movement. This locomotor style has been observed in several squamate species (e.g. basilisks, scrub lizards and others (Hsieh, 2003; Kinsey & McBrayer, 2018)) and in other vertebrates, such as rodents and primates (Alexander, 2004).

In squamates, the mechanisms and evolutionary drivers associated with the adoption of a high-performance, bipedal and locomotor mode remain unclear, despite facultative bipedality having evolved at least 110 million years ago in this group (Lee *et al.*, 2018). Historically, it has been suggested that the

velocity at which these animals move, in combination with a posterior shift of the gravitational centre of mass resulted in the ability to rear up on the hindlimbs (Snyder, 1952). Modelling work suggests that the postural shift is linked to *acceleration* (a change in speed in a given direction) rather than to *velocity* (speed in a given direction) (Aerts *et al.*, 2003). Recent studies have further refined the identification of the trigger for bipedality to *angular acceleration* about the hip joint, rather than simply acceleration of the whole animal (Clemente & Wu, 2018). Paradoxically, bipedal locomotion appears to be neither faster nor more energetically efficient in lizards expressing this behaviour, raising more profound questions focused upon why this behaviour has evolved (Clemente *et al.*, 2008).

In addition to the poor, but growing, understanding of the biomechanical mechanisms and evolutionary drivers of this locomotor mode, there are deficiencies in our current understanding at a morphological level. Morphological data can be indicative of many behaviours, including locomotor mode. For example, a recent study revealed correlations between body centre of mass and locomotor mode in extant squamates (Clemente, 2014). Similar methods indicate feeding ecology in coral reef fishes (Bridge *et al.*, 2016), and linear body metrics have been used to predict locomotor mode in fossil archosaurs (Kubo & Kubo, 2012). Facultative bipedality has been associated with a long tail and a low intermembral index

([Humerus + Radius] ÷ [Femur + Tibia]) in the fossil record of Archosauria (Galton, 1973; Padian, 2008; Persons & Currie, 2017), Lepidosauria (Simões *et al.*, 2017) and in extant squamates (Snyder, 1962; Irschick & Jayne, 1999a). The use of tail dimensions for the identification of facultative bipedality presents a challenge when working with museum specimens alone, as many specimens are either missing tails or have regenerated tails not indicative of the original bony structure (Jacyniak, McDonald & Vickaryous, 2017). As such, using inaccurate tail length to identify anatomies associated with facultative bipedality can be misleading. Additionally, historic work investigating morphological relationships with locomotor mode has suffered from limited species diversity and mis-identification of facultative bipeds from behavioural data (Snyder, 1962). Due to the iterative nature of scientific observation, our understanding of squamate behaviour has improved over time. Modern field reports and experimental studies on a wider variety of animals promote confidence in correct identification of locomotor mode than that of years gone by (Greene *et al.*, 1978; Irschick & Jayne, 1999b; Clemente & Wu, 2018). For example, the green iguana (*Iguana iguana*) was identified as an obligate quadruped by Snyder (1962). This animal has since been identified, in multiple studies, as being capable of facultative bipedality (Greene *et al.*, 1978; Blob & Biewener, 2001). This updated knowledge and general improvement in our background understanding permit a renewed investigation into the relationship between skeletal morphology and locomotor mode in extant lepidosaurs.

Using a dataset characterizing a range of body shapes, we highlight the differences between obligate quadrupeds and facultative bipeds within this group, demonstrating the value of non-caudal skeletal material in identifying potential facultative bipeds. Most importantly, we investigate the differences in these animals relative to body size and whilst considering phylogenetic interrelatedness. It is anticipated that these findings will aid the identification of anatomies associated with facultative bipedality more consistently and will complement the understanding of the biomechanical aspects of this locomotor mode.

Materials and methods

A selection of lepidosaur specimens representing 10 species were chosen for analysis based on availability of museum specimens at the Natural History Museum (NHMUK, London, UK) and the University Museum of Zoology (UMZC, Cambridge, UK). These were supplemented by X-ray micro-computed tomography (µCT) scans of a further 18 species, obtained from open-source online databases and publication repositories (Schachner *et al.*, 2014; Regnault, Hutchinson & Jones, 2017) (Supporting Information Table S1, www.morphosource.org). Some species are represented by more than one specimen (predominantly facultative bipeds), and thus the mean values for these species were used. Use of the mean to represent multiple specimens of one species balanced the weighting of better-sampled species in the experimental dataset.

Specimen type varied, with some specimens being either disarticulated or articulated osteological material, whereas others were fluid-preserved whole body forms with soft tissue intact. For skeletal specimens, measurements were recorded using a pair of digital callipers (± 0.0100 mm). For fluid-preserved specimens, X-ray µCT scans were used to examine skeletal elements digitally. Scans were performed at the Cambridge Biotomography Centre using a Nikon XT H 225 ST CT scanner (Nikon Metrology, Brighton, MI, USA). Scanning parameters varied between 125–150 µA, 125–155 kV and voxel size 0.0358–0.118 mm. Full details of all specimens scanned for this study are available in Supporting Information Table S2. In total, we examined 38 adult specimens representing 28 species, including eight facultatively bipedal species.

MicroCT scans were visualized using open-source medical imaging software 3DSlicer (Version 4.10, www.slicer.org; (Fedorov *et al.*, 2012)). TIFF image stacks were imported and scaled using voxel size determined by the scan data. The Segmentation toolkit was used to segment out appropriate osteological material; then, 3D models were rendered from these segments. Body measurements were taken using the Ruler tool, which allows measurements to be taken in rendered 3D space. Linear measurements were based on a comparative literature survey of facultative bipedality in reptiles (Snyder, 1952, 1962; Irschick & Jayne, 1999a, 1999b; Aerts *et al.*, 2003; Alexander, 2004; Kubo & Kubo, 2012; Clemente & Wu, 2018). We use precaudal length as an osteological correlate of snout–vent length (SVL; the standard measure of body size in herpetology) as our metric for body size. Lepidosaurs commonly have the fourth toe of the pes as the longest, so the fourth toe defines the principal limb axis in our study. Measurements of the autopod are therefore derived from the fourth metacarpal/metatarsal. In the case of the manus, the third and fourth metacarpals are usually of approximately equal length, and therefore, choice of principal limb axis predominantly affects hindlimb length data. A full list of the measurements and their definitions are available in the supplementary file. All measurements were conducted by the same individual to avoid variation in method by different persons.

All statistical analyses were conducted in the R statistical environment (R Core Team, 2017) using the packages cited below, to a significance determination of P-value ≤ 0.05. We constructed all plots using the *ggplot2* package (Wickham, 2016). A recent phylogenetic tree (Zheng & Wiens, 2016) was pruned to match the specimens in the dataset using the *drop.tip* function in the ape package (Paradis et al., 2014). In instances where species in our dataset were not present in the phylogeny, the closest relative was substituted.

We conducted parametric tests (Shapiro–Wilk, F-test, two sample t-test) of the data using the *stats* function in R (R Core Team, 2017) to inform correct statistical procedure, favouring log-transformed data in the majority of tests (Supporting Information Table S4).

Using the *pgls* function in *caper* (Orme et al., 2012) and the pruned tree mentioned previously, we constructed maximum-likelihood phylogenetically corrected bivariate linear plots of body segments relative to SVL in both locomotor modes. These phylogenetic least-squares (PGLS) plots were constructed of these models using *ggplot2* (Figs. 1 and 2) and regression outputs recorded (Supporting Information Table S5).
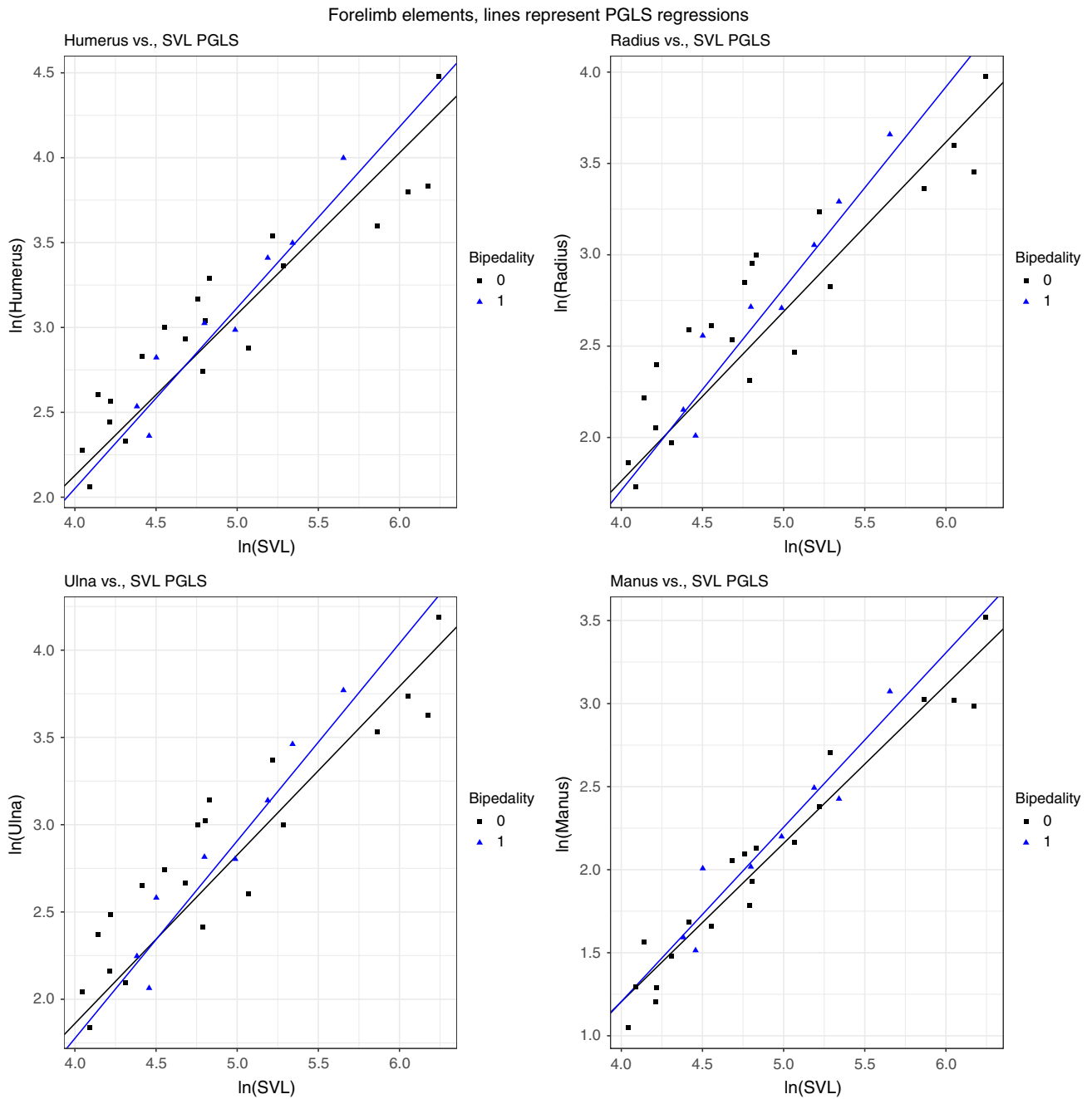
Figure 1 Bivariate linear regressions of forelimb segment lengths and snout–vent length. Lines represent phylogenetic least-squares regressions. Obligate quadrupeds (0) represented by black squares; facultative bipeds represented by blue triangles. These symbols remain consistent in all further plots. Regression line equations and segment definitions can be found in the supplementary file. Both x- and y-axes were log scaled.

Analyses of covariance (ANCOVA) in the data was performed using the *anova.pgls* function in *caper* on PGLS models in order to assess how differences in body metrics were linked to locomotor mode. These models used locomotor mode as a fixed effect and SVL as a covariate with maximum-

likelihood methods of phylogenetic correction in each instance. Models assessing the interaction between SVL and locomotor mode used the formula:

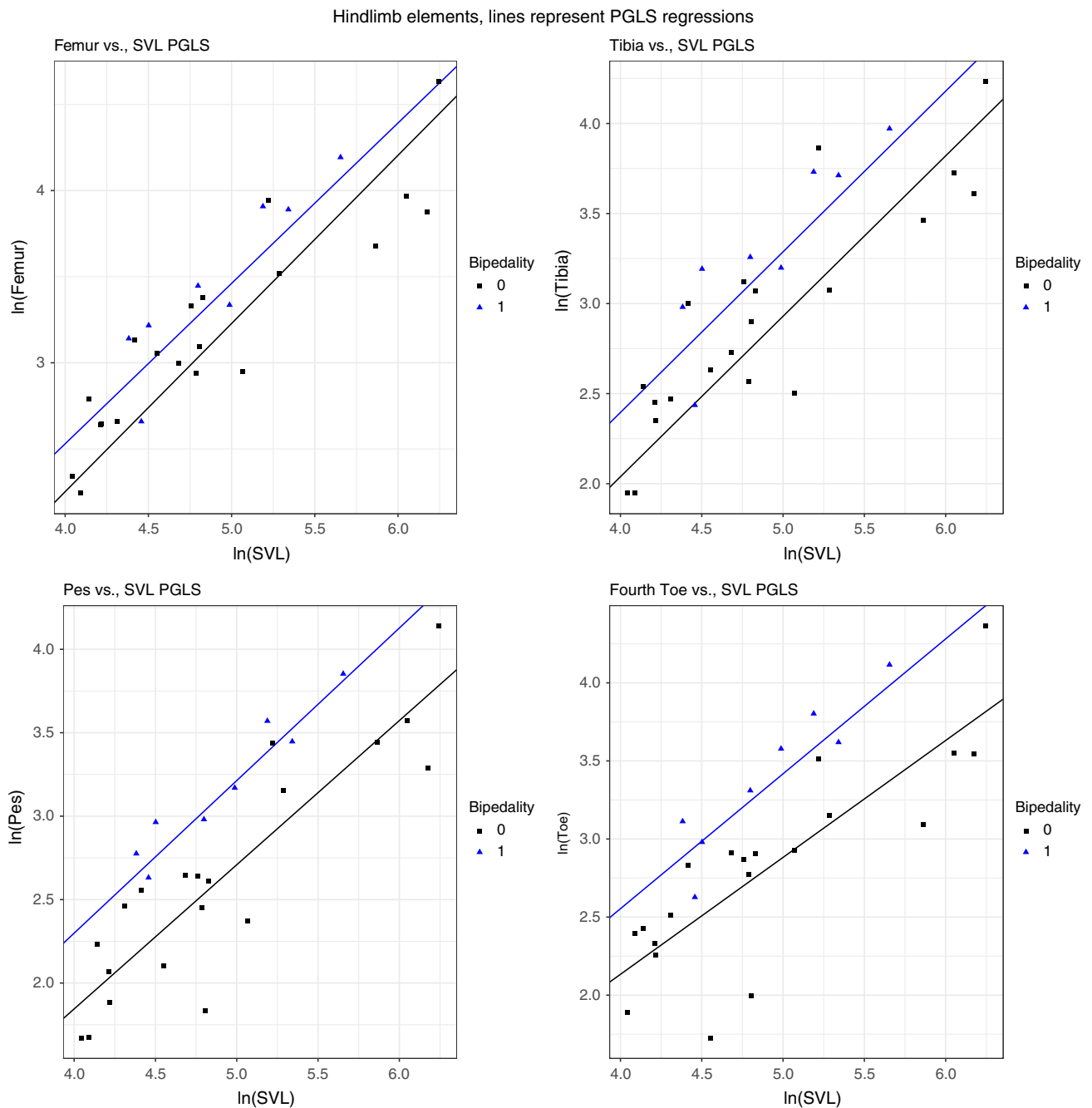$$\text{Body segment} \sim \text{SVL} \times \text{Bipedality}$$

Hindlimb elements, lines represent PGLS regressions

Figure 2 Bivariate linear regressions of hindlimb segment lengths and snout–vent length. Lines represent phylogenetic least-squares regressions. Regression line equations and segment definitions can be found in the supplementary file. Both x- and y-axes were log scaled.

We then constructed another batch of models to examine for a relationship between the variables themselves, using the formula:

$$\text{Body segment} \sim \text{SVL} + \text{Bipedality}$$

The first looks for differences in the gradients between body segment and SVL across locomotor modes. The second looks for differences in y-intercept, in the case that there is no difference in intercept. ANCOVA results are presented in Table 1.

We also performed a phylogenetically independent principal component analysis (PCA) alongside these linear regression approaches on all linear measurements (Supporting Information Table S3). PCA is an ordinate analytical technique designed to provide information regarding how much influence metrics have on overall variation within a dataset. This was performed using

Table 1 Results from PGLS ANCOVA analyses, using locomotor mode as a fixed effect, significance level $P < 0.05$

Predictor	Interaction term	Slope analysis (variable ~ SVL* Bipedality)		Intercept analysis (variable ~ SVL + Bipedality)	
		F value	P-value	F value	P-value
Cervical	0.0335	0.0198	0.88937	3.3356	0.07976
Femur_diameter	0.118	2.5893	0.1207	0.0529	0.8199
Femur	0.0368	0.0864	0.7714	2.2054	0.15
Humerus	0.105	2.7557	0.10992	1.3991	0.248
Humerus_diameter	0.06	0.2082	0.6522	1.1457	0.2947
Manus34	0.0659	2.593	0.6153	0.9779	0.3322
Pes34	0.00837	0.0011	0.9738233	**21.101**	**0.0001068**
Radius	0.171	2.5751	0.1216	0.0436	0.8363
Tibia	0.0181	0.0081	0.92901	**6.4889**	**0.01739**
Tibia_diameter	0.1654	**4.4909**	**0.04462**		
TLS	−0.00056	0.0001	0.99365	**5.0474**	**0.03374**
Toe34	0.218	0.547	0.4667048	**17.227**	**0.0003363**
Ulna	0.183	**4.7078**	**0.04015**		
Ulna_diameter	0.0267	0.366	0.85	2.5298	0.1243

Bold fields indicate a significant result.

the *prcomp* function in *stats* and visualized (Figs. 3 and 4) using the *fviz_pca* function in *factoextra* (Kassambara & Mundt, 2017). Performing this analysis allows us to examine the differences between obligate quadrupeds and facultative bipeds in both a directly correlative linear regression sense and an ordinate dimensionally reductive sense. The cumulative variance and loadings for the first five principal components can be found in the Supporting Information (Tables S6 and S7).

Finally, in order to investigate differences in long bone stoutness between locomotor modes, we used the *sma* function in the *smatr* package to test for gradients equal to the isometric scaling component in bone length and midshaft diameter (Supporting Information Table S9).

Results

Phylogenetic least-squares linear regressions are represented by Figs. 1 and 2, representing the forelimb and hindlimb elements, respectively, with further exploratory plots in the supplementary file. Equations for the regression lines may be found in the supplementary file (Table S5). For all PGLS regressions, adjusted R-square values range from 0.671 to 0.987, and, in 23 of 28 models, exceeded 0.9, indicating a high degree of variance accounted for in the models.

Differences in the forelimb elements relative to SVL are not particularly evident to the eye between locomotor modes, with PGLS regression lines following largely similar gradients and intercepts. Hindlimb element lengths appear to be more different between locomotor modes (Fig. 2). In particular, large differences in intercept between tibia, pes and fourth toe length intercepts can be observed, indicating that facultative bipeds have longer hindlimb elements relative to obligate quadrupeds.

To assess these observations more robustly, PGLS ANCOVAs with bipedality as a fixed effect were used. We identified a significant difference between the gradients of tibia diameter and ulna length with SVL (P-value < 0.05; Table 1), and a significant

difference in the intercept ranges for length of the pes, tibia, fourth toe and thoracic–lumbar–sacral lengths with SVL (TLS; P-value < 0.05; Table 1). No significant interactions were identified in parameters of the humerus, femur, radius or manus (Table 1). The diameter of the tibia increases at a faster rate with SVL in facultative bipeds than in obligate quadrupeds (Supporting Information Table S5). A similar relationship is seen in the length of the ulna, with smaller facultative bipeds having relatively a shorter ulna and larger facultative bipeds having a longer ulna than their obligately quadrupedal counterparts (Fig. 1). Regarding the lengths of the pes, tibia and fourth toe, the rate of change is consistent across both locomotor modes, but elements are generally longer in facultative bipeds, for a given SVL (Table 1; Supporting Information Table S5). Similar too is TLS length, though this is generally shorter in facultative bipeds (Supporting Information Table S5). Regressions of long bone length against diameter indicates that, across both locomotor modes, distal long bones (ulna and tibia) scale with isometry (scaling exponent not significantly different to 1 on length–length axes; Supporting Information Table S9). Femora and humeri of facultative bipeds scale with isometry, but these bones in obligate quadrupeds scale with positive allometry (scaling exponent significantly greater than 1 on length–diameter axes; Supporting Information Table S9).

In the principal component analysis, PC1 explains 90.6% of the variance in our data and represents body size, with all elements loading negatively on this principal component (Supporting Information Table S7; Fig. 3). PC2 and PC3 represent 3.7% and 2.0%, respectively, (Supporting Information Table S7; Figs. 3 and 4). All facultative bipeds score negatively on PC2, indicating that this principal component represents locomotor mode, with hindlimb elements (toe, pes, femur and tibia length, and tibia diameter) loading negatively and distal forelimb components (ulna and humerus diameter, ulna and radius length) loading positively, in addition to TLS length loading positively. PC3 has long bone lengths (radius, tibia,
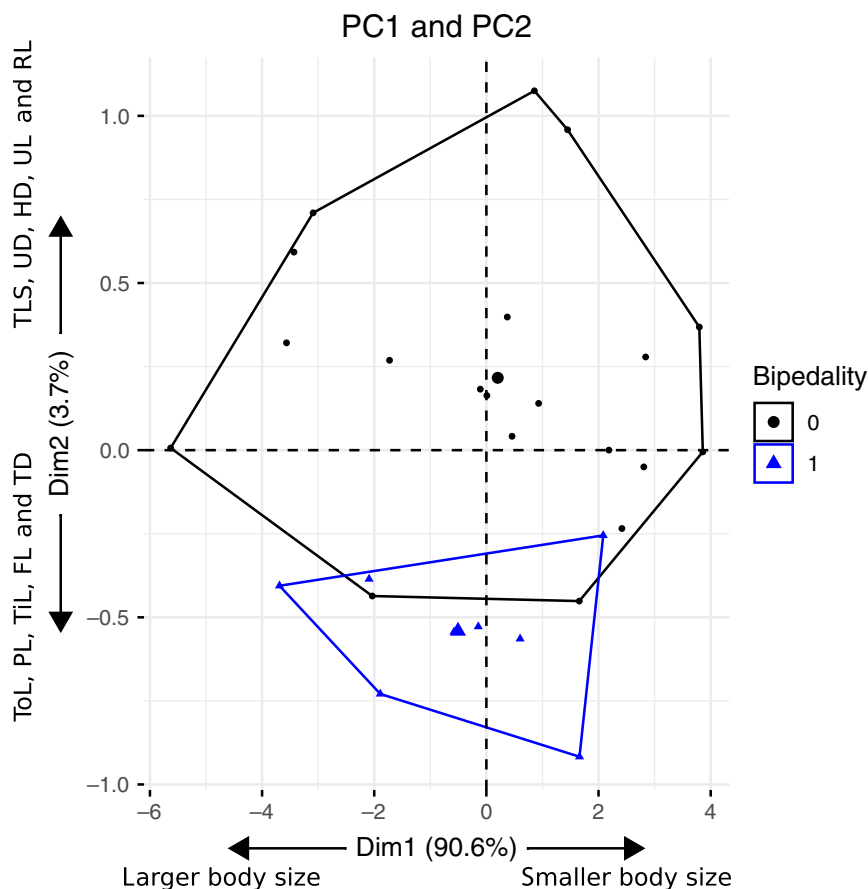
Figure 3 Principal component plot of PC1 and PC2. Each point represents a specimen. Larger symbols indicate centroids for the two locomotor modes. Loadings are represented on the relevant axes. PC1 represents body size; PC2 represents bipedality. TiL, tibia length; FL, femur length; HD, humerus diameter; PL, pes length; RL, radius length; TD, tibia diameter; TLS, thoracic-lumbar-sacral length; ToL, fourth toe length; UD, ulna diameter; UL, ulna length.

ulna, femur and humerus length) loading negatively and some axial skeletal measurements (skull length, skull width, tibia diameter, TLS and toe length) loading positively. Distribution across PC1 and PC3 is varied for both locomotor modes. Principal components beyond the third are not explored further in this analysis.

Discussion

Using a diverse dataset that accounts for phylogenetic relatedness across a wide range of lepidosaur species, we find that differences in the hindlimb relative to SVL are most significant for differentiating facultative bipeds from obligate quadrupeds. Longer elements are observed in the tibia, pes and fourth toe; no statistical differences relative to SVL are observed in the femur, as indicated by regression analyses. Interestingly, the relationship between ulna length and SVL differs between the two locomotor modes such that larger facultative bipeds have longer ulnae than obligately quadrupedal counterparts, but the reverse at shorter SVLs. Independent of SVL, using PCA, facultative bipeds are best differentiated by

the differences in the hindlimb, TLS length and the distal forelimb length. These factors differentiate our dataset into two distinct clusters represented by locomotor mode. Scaling analyses of long bone diameters relative to length indicate that whilst distal long bones (ulna and tibia) are equally stout between the two locomotor modes (isometric scaling gradients),and there is a difference in proximal long bones, such that the humeri and femora of facultative bipeds are less stout than those of obligate quadrupeds (isometry vs positive allometry). The literature regarding bone scaling in tetrapods is extensive, though frequently the orientation of the models constructed varies, resulting in a negative allometry in one study (e.g. x-axis diameter and y-axis length) being equivalent to a positive allometry in another (x-axis length and y-axis diameter), requiring care when comparing across studies. It has been noted that quadrupedal mammals scale their long bones with positive allometry (length on x-axis and diameter on y-axis), as is also seen in the proximal long bones of our obligate quadrupeds (Biewener, 1983, 2005). Similar results are seen in reptiles, showing that long bone length scales with negative allometry to diameter in pooled lizards and iguanians
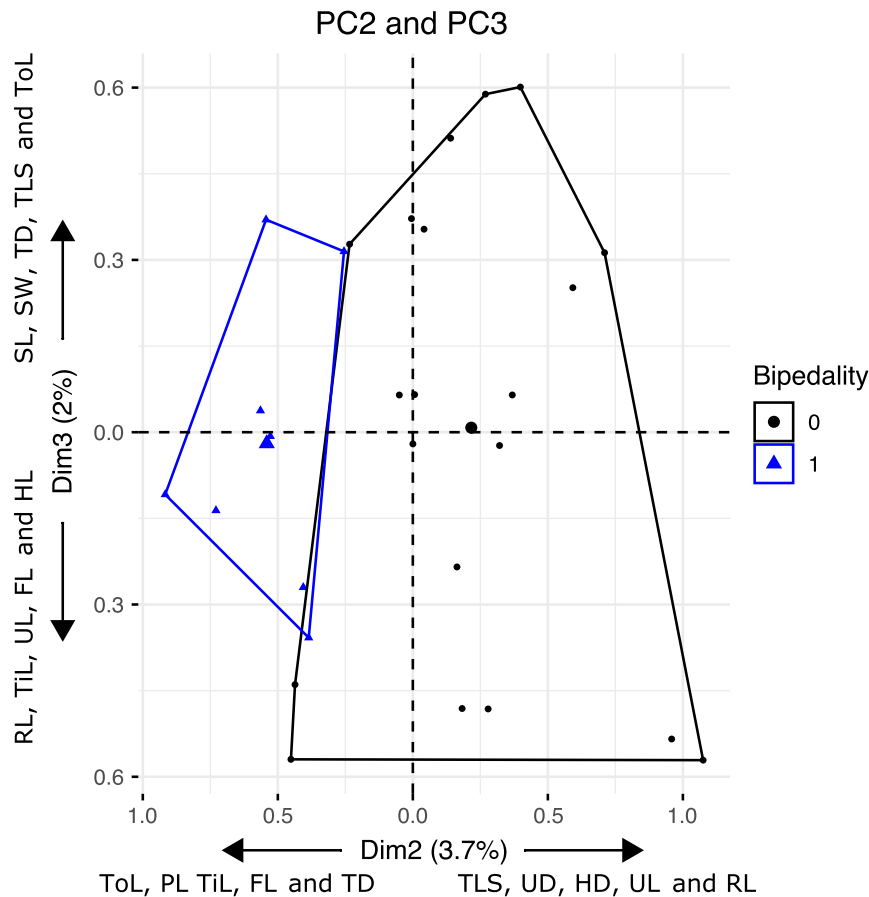
Figure 4 Principal component plot of PC2 and PC3. Each point represents a specimen. Larger symbols indicate centroids for the two locomotor modes. Loadings are represented on the relevant axes. PC2 represents bipedality. FL, femur length; HD, humerus diameter; HL, humerus length; PL, pes length; RL, radius length; SL, skull length; SW, skull width; TD, tibia diameter; TiL, tibia length; ToL, fourth toe length; TLS, thoracic-lumbar-sacral length; UD, ulna diameter; UL, ulna length.

(Blob, 2000) – reversing the regression to match the formula orientation of Biewener (1983) and our study (length on the x-axis and diameter on the y-axis), this equates to positive allometry. In both dinosaurs and mammals, long bone diameters scale with negative allometry in across all locomotor modes, with diameter on the x-axis (Carrano, 2001), a similar relationship to that observed in our obligate quadrupeds when the regression is reversed to match. In both reptiles and mammals broadly, the circumference of the femur has been identified as scaling isometrically with body mass (Campione & Evans, 2012). Interestingly, Carrano (2001) notes that the humeri of quadrupedal dinosaurs and bipedal mammals scale isometrically, whereas bipedal dinosaurs and quadrupedal mammals scale with negative allometry – equivalent to positive allometry under our model, which is intriguing given that bone stresses would be expected to be greater in the humeri of quadrupedal animals. The isometric scaling exponent of facultative biped proximal long bone diameters therefore becomes more intriguing and functionally relevant under the context presented here and warranting of future study into *in vivo* bone stresses in these animals.

Our assessment of locomotor mode and body segment lengths relative to body size (in the form of SVL) is novel with respect to the existing literature surrounding reptilian facultative bipedality. Much of the existing work regarding facultative bipedality generally is based on the use of ratios which, whilst informative, does not account for the body size-related constraints of an animal (Snyder, 1962; Berman *et al.*, 2000; Grinham, VanBuren & Norman, 2019). Updated locomotor classifications, such as for *Tropicagama temporalis* (Wilson & Swan, 2017) and *Iguana iguana* (Blob & Biewener, 2001), enable a more up-to-date assessment of the anatomy of facultative bipedality in extant squamates. Additionally, historic work does not typically correct at all for phylogenetic nonindependence, as should be done whenever considering evolutionarily related animals in biological studies (Felsenstein, 1985). As such, we must be cautious when comparing our results to those of the historic literature.

Snyder's conclusions regarding the anatomical differences between locomotor modes in squamates, long considered to be the authoritative work on the subject, indicate that facultative bipeds exhibit 'considerable reduction in the length of the

forelimb, and the degree of reduction is greatest in the manus' (Snyder, 1962, p. 195). It is also additionally stated that iguanids lengthen every segment of the hindlimb, whereas agamids exhibit no elongation of the hindlimb (summarized in the Supporting Information Table S8). Increased tail lengths are observed in all bipeds, as well as reduced precaudal lengths, though the tail is a factor that we do not investigate in our analysis, as previously stated. Interestingly, we do not find that the any elements of the forelimb appear significantly reduced in facultative bipeds when compared to their SVL. Rather, the hindlimb extension forms the majority of any morphological variation between the locomotor modes. We attribute this factor to our analysis of these segments relative to SVL, rather than comparable limb ratios. It should also be highlighted that some of the quadrupedal species sampled in the historic works have since been identified to be capable of facultative bipedality and also that we include a far more diverse range of species in our study and represent Lacertidae as a family exhibiting this locomotor mode (Edwards *et al.*, 2015; Savvides *et al.*, 2016).

The smallest, *Brookesia micra* (Glaw *et al.*, 2012), and largest, *Varanus komodoensis* (Dick & Clemente, 2016), known extant lepidosaurs are both obligate quadrupeds. Both of these species fall outside of the SVL range of taxa examined in this study. Thus, we can confidently state that the body size of facultative bipeds falls within the total range of extant lepidosaur body size, rather than existing as a discrete or partially overlapping zone. Examining how body proportions change across SVL therefore becomes even more crucial to enhancing our understanding of the anatomies associated with facultative bipedality.

Regarding the morphology of the forelimb across locomotor modes, any interpretations should be considered in line with studies into forelimb kinematics in facultative bipedality. Recently, it has been noted that forelimb positioning contributes significantly to stabilization of lizard bipedal locomotion (Kinsey & McBrayer, 2018). This is particularly interesting, given our observation of longer ulnae in larger facultative bipeds than their obligately quadrupedal counterparts. Additionally, the forelimb ground reaction forces of facultative bipeds are significantly different to those of obligate quadrupeds (Clemente & Wu, 2018). Interpreting the drivers of these shifts is difficult, and not explored in more detail here, though whether these shifts are by-products of acquiring a bipedal mode, or active changes to enable this behaviour, is intriguing. In tandem with these observations, it is noted that many large facultative bipeds are also partially arboreal (basilisks, frilled dragon and green iguana). A longer forelimb indicates longer muscles eliciting larger muscle moment arms, increasing the work potential and effective mechanical advantage of the limb (Biewener, 2003) – essential for behaviours such as pulling an arboreal animal up a tree trunk. It is possible, therefore, that at larger body sizes and forelimb proportions are influenced more by substrate preference than locomotor mode, and perhaps exapted to aid bipedal locomotion.

The differences in hindlimb anatomy across locomotor modes are more simply interpreted, based on the consistent differences across segments. At all values of SVL, hindlimb elements beyond the femur are consistently longer amongst facultative bipeds than in obligate quadrupeds. Functionally, this facilitates larger muscle moment arms, longer stride lengths and greater ground clearance, all of which contribute to more powerful strides and greater support for the body on two limbs. Values echoing these factors are consistently found in the literature (Snyder, 1954, 1962; Christian, Horn & Preuschoft, 1994; Hsieh, 2003; Clemente & Wu, 2018), contributing to work on musculoskeletal modelling of the facultative locomotor mode in these animals (Aerts *et al.*, 2003). Interestingly, the gradient of SVL to hindlimb segment length remains similar across locomotor modes, indicating a largely mechanical relationship between body size and locomotor demands in this group, that is the size of long bones is constrained by locomotor mode (differences in intercept between locomotor modes), as opposed to body size (signified by differences in gradient between the two modes). There is scope for further exploration regarding why differences in femoral length are not significant, with this metric perhaps being tied to other selective pressures. Though the length of the femur does not significantly differ between locomotor modes, we do observe a difference in midshaft diameter. The femora of facultative bipeds tend to be more slender than similarly sized obligate quadrupeds. This is functionally grounded in a femur that should be less resistant to external forces, which contrastingly are higher during bipedal locomotion than quadrupedal locomotion (Aerts *et al.*, 2003; Clemente & Wu, 2018). However, this may have trade-offs in the performance of the animal – a lower safety factor but more manoeuvrability of the femur, and thus the whole limb.

Historically, it has been noted that the torso section, equivalent to the skull and TLS regions in our analysis, is shorter in facultative bipeds than obligate quadrupeds (Snyder, 1954, 1962). We find that this difference is manifested as a significantly shorter TLS region for animals of equal SVLs, effectively caudally displacing the pectoral girdle. This caudad shift reduces the anterior body mass and ensures that the centre of mass (CoM) moves closer to the hips, a necessity for maintaining bipedal locomotion (Alexander, 2004; Hutchinson, 2004a, 2004b; Clemente & Wu, 2018).

Principal component analysis results corroborate the findings of the linear models, indicating that differences in hindlimb, TLS and distal forelimb lengths are sufficient to create reasonable separation between locomotor modes across PC2, whilst also indicating that facultative bipeds exist within the body size range of all lepidosaurs. The two species falling within the range of facultative bipeds are *Lophosaurus dilophus* and *Brochochela cristatella*, neither of which has been observed to employ facultative bipedality to date. However, both of these species are by preference arboreal (Diong & Lim, 1998; Mack, 1999) – an ecology shared by many facultative bipeds.

The implications of these findings extend beyond observations of extant lepidosaurs. The deep ancestral history of facultative bipedality within squamates provides cause to explore these anatomies beyond the living record (Lee *et al.*, 2018). By exploring the anatomies and mechanical demands associated with this locomotor mode, we can better understand the evolution of facultative bipedality that is exhibited in multiple

families throughout the clade Lepidosauria (Clemente *et al.*, 2008). Beyond lepidosaurs, facultative bipedality is hypothesized to form an intermediate stage in the evolution of bipedality in both hominins and archosaurs (Preuschoft, 2004; Maslin, Shultz & Trauth, 2015; Grinham *et al.*, 2019). We used this study to explore body metrics associated with facultative bipedality excluding the caudal vertebrae to increase the applicability of our results beyond the living record. It has been noted in dinosaurs that tail preservation is notoriously poor, very rarely does a caudal vertebral series appear complete and intact (Hone, 2012). In exploring our dataset, not including the tail, we have demonstrated that caudal vertebrae are not essential for indicating a facultatively bipedal mode, though long tails are undoubtedly associated with a bipedal mode in lizards (Snyder, 1962; Kinsey & McBrayer, 2018). Testing the predictive capabilities of the indicators presented here in a wider extant sample, such as hindlimb element length with respect to body size, will help form future research directions before application to the lepidosaur fossil record. Beyond that, we hope these findings help develop a further understanding of how differences in the lepidosaur and archosaur body plan contribute towards a bipedal locomotor mode in alternative ways.

Conclusion

Linear morphometric trends are identified in the precaudal skeleton across a range of extant lepidosaur species indicative of facultative bipedality. Contrary to past studies (Snyder, 1962; Christian *et al.*, 1994), we examine these metrics with respect to body size. We find that for animals of a similar size, locomotor mode has a minor effect upon the size of the forelimb, but a considerable effect on hindlimb bones distal to the femur. These differences are corroborated between phylogenetically corrected linear regressions and principal component analysis. Significant differences in anatomy between the two locomotor modes have been discussed in a functional framework in light of relevant and recent biomechanical studies. The wider implications for this work are highlighted, particularly in its use increasing our understanding of the identifications and evolution of bipedality in the fossil record of reptile groups.

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Author contributions

LRG conceived the study, designed the experiment, collected the data, carried out analyses, interpreted the results and wrote the paper. DN interpreted the results and helped draft the paper. Both authors gave final approval for publication.

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