The ecomorphology of facultative bipedality in Lepidosauria: implications for the evolution of reptilian bipedality

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Declaration

I hereby declare that except where specific reference is made to the work of others, the contents of this dissertation are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other university. This dissertation is my own work and contains nothing which is the outcome of work done in collaboration with others, except as specified in the text and Acknowledgements. This dissertation contains fewer than 275 numbered pages of which not more than 225 pages are text, appendices, illustrations and bibliography.

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Abstract

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Bipedality is a distinctive locomotor characteristic of some of the most noteworthy animals of all time, including dinosaurs and humans. However, the evolution of a bipedal locomotor mode is poorly understood in reptiles. It has been repeatedly hypothesised that a facultative locomotor mode, where an animal moves both bipedally and quadrupedally under different conditions, forms an intermediate stage in the evolution of obligate bipedality. I demonstrate that the evidence supporting this hypothesis is lacking, recovering facultative bipedality as an intermediate stage only once in multiple independent evolutions of bipedality, under two different topologies. In order to better understand facultative bipedality and the associated anatomies, I performed multiple studies into the ecomorphology and evolution of this behaviour in a modern clade: Lepidosauria. Linear morphometric studies accounting for variation in body size indicate that forelimb segment lengths across locomotor modes do not differ for lepidosaurs of the same size, but that distal hindlimbs segments differ greatly, contrasting with historical tropes. Using 3D landmark-based geometric morphometrics, I demonstrate that arboreal and facultatively bipedal species share many characteristics in the bony elements of the pelvis, including a straight-to-concave iliac blade and large ischial base. These shared anatomies are functionally qualified based on anatomical studies of lepidosaur pelvic girdle myology, and indicate a similarity in mechanical demands of both arboreality and facultative bipedality. Finally, I tested for the correlated evolution of facultative bipedality with substrate preference in a derived clade of squamates: Episquamata. Findings suggest that there is no correlation between substrate and a facultative locomotor mode, instead indicating that facultative bipedality is an exaptation of anatomies associated with vertically diverse environments. This echoes the evolution facultative bipedality in hominin, macropods and rodents, and is distinct from any current hypotheses concerning the evolution of archosaurian bipedality.

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-Luke

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Chapter 1

Introduction

Bipedality as a locomotor mode is characteristic of many of the most successful species to walk the Earth, most notably hominins, dinosaurs, and birds. A bipedal locomotor mode enables varied functionality of the forelimbs in animals because they are no longer required to play a role in body-weight support during terrestrial locomotion. The most striking examples of the release of the forelimb weight-support constraint are seen in the evolution of avian flight (Gatesy and Dial, 1996; Segre and Banet, 2018), and tool use in primates (Napier, 1962; Van Lawick-Goodall, 1971).

The evolution of a bipedal locomotor mode from a primitive quadrupedal locomotor mode has, in many clades, been assumed to involve an intermediate facultative locomotor mode (Persons and Currie, 2017; Senut et al., 2018). A facultative biped, herein, is defined as an animal or species that ordinarily moves using all four of its limbs for substrate locomotion, but is capable of progressing on just two limbs under certain conditions. This type of locomotor behaviour can be observed directly in many extant species, including squamates (Clemente et al., 2008; Irschick and Jayne, 1999a), kangaroos (Kram and Dawson, 1998), some rodents (Rankin et al., 2018) as well as some insect species (Alexander, 2004). The inverse could be stated for species exhibiting facultative quadrupedality, reversing both the mechanical and evolutionary polarity.

The types of bipedality exhibited by the clades above are distinctly different. In hominins, bipedality is characterised by a vertical body posture, S-shaped spinal curvature, and the absence of a tail (Alexander, 2004). In archosaurs, such as dinosaurs and birds, key characters include a horizontal body posture enabled by a cantilevering tail, and vertically oriented hindlimbs (Alexander, 2006, 2004; Hancock et al., 2007; Jones et al., 2000); similar structural adaptations are also exhibited by macropod marsupials such as kangaroos (Alexander, 2004). When extant squamates exhibit facultative bipedality, they do so with limbs that are not positioned directly beneath the body, as seen in other bipeds (Hsieh, 2003; Irschick and Jayne, 1999b), and exhibit variable degrees of inclination of the body relative to the horizontal axis (Clemente and Wu, 2018; Irschick and Jayne, 1999a). A long cantilevering tail is also conserved, as seen in all non-primate vertebrate representatives of this locomotor mode.

While the advantages of obligate bipedality are quite clear, with forelimbs gaining capacity to be used in display, combat and environmental manipulation; these do not necessarily apply in taxa that exhibit facultative bipedality. Thus, understanding the drivers or advantages associated with facultative bipedality is challenging. In particular, facultative bipedality in extant squamates is neither more efficient nor faster than quadrupedal motion (Clemente et al., 2008). In fact, in lizards it appears that rotational acceleration around the hip joint initiates bipedality, momentarily destabilising quadrupedal locomotion prior to the subsequent adoption of a dynamically stable bipedal style (Clemente and Wu, 2018). In this new light, facultative bipedality can be reasoned to be a reflection of other aspects of these animals' biology, rather than conferring any particular mechanical or efficiency gains upon the species exhibiting this style of movement. In other reptile clades (such as Archosauria), analyses of locomotor style are compromised by the fragmentary nature of the fossil record; there is also a lack of observable biomechanical correlates among the extant representatives of this clade. Extant crocodilians are obligate quadrupeds (Gatesy and Middleton, 1997; Hutchinson and Gatesy, 2000), whereas birds are obligate bipeds (Daley, 2006; Hancock et al., 2007). Each of these examples is adapted for quite distinct locomotor styles, and neither exhibit at any stage during their life-cycles a facultatively bipedal phase that would represent the hypothetical pathway leading to obligate bipedality. Therefore, facultative bipedality among reptiles remains a functional, ecological and evolutionary enigma.

The anatomical understanding of facultative bipedality in lizards is based largely on the pioneering work of Richard Snyder (Snyder, 1949, 1952, 1954, 1962). The findings derived from these investigations can be summarised broadly as demonstrating that the ability of some lizards to run bipedally correlated with a set of specific morphological adaptations in the skeleton: an extended tail, shortened torso, and a lower intermembral index (ratio of forelimb to hindlimb). In the years since these publications, an improved understanding of the biomechanical and functional aspects of this locomotor mode has developed. Various studies have considered the potential advantages conferred by adopting facultative bipedality (Clemente et al., 2008), some have constructed biomechanical models (Aerts et al., 2003); much of this has built on the foundations provided by Snyder's ground-breaking work. However, direct observations of the behaviour of various lizard species over the last 70 years have provided a more comprehensive understanding of the expression of bipedality in this group. Species once considered to be obligate quadrupeds have been observed moving bipedally (Blob and Biewener, 2001; Greene et al., 1978); these have rendered the historic understanding of the anatomies associated with facultative bipedality in squamates as less clear-cut than previously established by Snyder.

This thesis aims to reassess the anatomies associated with facultative bipedality in reptiles and its significance in our understanding and determination of the evolution of obligate bipedality. The majority of this thesis focuses on extant Lepidosauria, using modern analytical methods in both shape analysis and phylogenetic inference, to develop a comprehensive understanding of facultative bipedality in extant representatives of this clade. Additionally, the ecological significance of facultative bipedality in extant lepidosaurs has been considered.

The content of each chapter is outlined below:

Chapter Two: The first data chapter of this thesis is a study published in *Royal Society Open Science* (Grinham et al., 2019). In this study, I tested for presence of facultative bipedality as an intermediate locomotor mode in the evolution of archosaur bipedality, using currently published diagnostics of the locomotor mode among archosauriform taxa and ancestral state reconstruction methods. Contrary to expectations, facultative bipedality did not form an intermediary state in the majority of transitions. This study shaped the remainder of the thesis because it suggested that our understanding of facultative bipedality in reptiles was either poor or entirely lacking, particularly with regard to identifying this locomotor mode from skeletal material alone.

Chapter Three: This chapter, published in *Journal of Zoology* (Grinham and Norman, 2020a), begins this thesis' assessment of facultative bipedality in extant Lepidosauria. Compared to the fossil archosaurs examined in Chapter One, which offer limited and imperfect anatomical data, and no behavioural data, extant lepidosaurs provide unambiguous anatomical and behavioural data. This study provides a novel reassessment of the linear body metrics associated with observed locomotor mode in extant lepidosaurs, particularly with regard to body size. These findings contrast strikingly with those observed by previous researchers: for animals of a similar body size, locomotor mode has little effect on forelimb segment lengths, but significant effects on distal hindlimb element lengths. It is particularly notable that I do not include tail dimensions in my analyses. Caudal vertebral characteristics are not essential for the identification of facultative bipedality and are unreliable in museum specimens—however they remain integral to facultatively bipedal locomotion in multiple clades.

Chapter Four: Published in *Biological Journal of the Linnean Society* (Grinham and Norman, 2020b). This study elaborates on that undertaken in the previous Chapter. Due to the functional connection of the pelvis to the tail and hindlimbs, both important factors in locomotor mode capability, the pelvis was subjected to detailed analysis using landmark-based 3D geometric morphometrics. I also included substrate preference in this analysis, based on direct observations made of many species exhibiting facultative bipedality. This study indicates that species preferring arboreal habitats exhibit common features of their pelvic morphology: a large preacetabular process, a straight-to-concave iliac blade, and an anteriorly projecting pubis; when compared with the range of squamate taxa capable of adopting a facultatively bipedal locomotor mode. Functional interpretations of this suite of morphologies are suggested, based on published musculoskeletal anatomy, biomechanical analyses and new exploratory dissections.

Chapter Five: The final data chapter of this thesis tests for a correlation between the evolution of substrate preference and facultative bipedality in extant squamates. The analysis focuses on the clade Episquamata (Toxicofera + Laterata), to the exclusion of Serpentes. I conclude that the hypothesised correlation between arboreality and facultative bipedality does not exist as a co-evolutionary linkage, but rather that facultative bipedality commonly occurs as an exaptation of body forms living in ‘vertically diverse’ environments, such as forests as well as rocky substrates. In a far broader evolutionary context, this pattern echoes that seen in the evolution of facultative bipedality among hominins, rodents and macropod marsupials. It needs to be stressed that this is general evolutionary pattern seems to be entirely distinct from any of the current hypotheses concerning the evolution of facultative bipedality in Archosauriformes. These findings may promote discussions regarding the ecology of basal archosaurs with regard to the evolution of bipedality or other locomotor transitions, such as in agile archosauromorphs or vertically diverse habitat preferences in pterosaurs.

Chapter 2

Testing for a facultative locomotor mode in the evolution of archosaur bipedality

This chapter has been published as:

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Author contributions are as follows:

L.R.G. conceived of the study, designed the study, collected the data, carried out the statistical analyses, interpreted the results, and drafted the manuscript; C.S.V.B. conceived of the study, designed the study, helped with statistical analyses and helped draft the manuscript; D.B.N. conceived of the study and helped draft the manuscript.

2.1 Abstract

Bipedal locomotion is a key characteristic of humans and birds and has a profound effect on how these groups interact with their environment. Results from extensive hominin research indicate that there exists an intermediate stage in hominin evolution—facultative bipedality—between obligate quadrupedality and obligate bipedality that uses both forms of locomotion. It is assumed that archosaur locomotor evolution followed this sequence of functional and hence character-state evolution. However, this assumption has never been tested in a broad phylogenetic context. We test whether facultative bipedality is a transitionary state of locomotor mode evolution in the most recent early archosaur phylogenies using maximum-likelihood ancestral state reconstructions for the first time. Across a total of seven

independent transitions from quadrupedality to a state of obligate bipedality, we find that facultative bipedality exists as an intermediary mode only once, despite being acquired a total of 14 times. We also report more independent acquisitions of obligate bipedality in archosaurs than previously hypothesized, suggesting that locomotor mode is more evolutionarily fluid than expected and more readily experimented with in these reptiles.

2.2 Background

Bipedal locomotion is one of the most distinguishing characters of humans and birds—some of the most widely distributed vertebrate species alive today—as well as many ricochetal mammals. There are various hypotheses that attempt to explain why bipedal locomotion is evolutionarily advantageous. In humans, for example, it has been proposed that a shift toward savannah-like aridity encouraged tree-dwelling populations of hominins on to the ground, where bipedal locomotion was demonstrably more energetically efficient for moving between increasingly distant arboreal habitats (Maslin et al., 2015; Preuschoft, 2004). The most obvious advantage of using only hindlimbs to locomote is the freeing of forelimbs for use in functions other than those associated with support and locomotion. These functions can be broadly categorised as social use (communication, combat), micro-mechanical use (tool and object manipulation) and macro-mechanical use (flight, or environmental manipulation such as digging). The enhanced capacity to interact with other organisms and the surrounding environment undoubtedly contributes to the success of modern humans and birds relative to other vertebrate groups (Brusatte et al., 2015). However, there is limited understanding of how bipedality evolved in non-human and non-avian animals.

Hominin bipedality is suspected to have evolved first around 4.4 Ma with *Ardipithecus ramidus* (Alexander, 2004), though there are some indicators of potential bipedal capability as far back as 7 Ma in *Sahelanthropus* (Brunet et al., 2002). It is widely accepted that the achievement of bipedality was not the consequence of a single event, but rather represented a progressive acquisition of anatomical features that enabled an upright posture and two-legged locomotion (Harcourt-Smith and Aiello, 2004; Hatala et al., 2016).

Species exhibiting a tendency to employ both bipedal and quadrupedal locomotor modes are referred to herein as facultative bipeds. Among reptiles, this locomotor mode is seen today in modern squamates, such as basilisk lizards or frilled lizards (Snyder, 1952). It can be argued that the facultative locomotor mode exists in two states: facultative bipedality and facultative quadrupedality, depending upon the predominant style of movement based on behavioural observation. This is a matter of evolutionary polarity: facultative quadrupedality is commonly used when describing secondarily quadrupedal animals having evolved from obligately bipedal ancestors; this contrasts with bipedal animals evolving from ancestral quadrupeds, the case that we are investigating in this study.

For birds, the origin of bipedal locomotion is rooted much deeper in their evolutionary history. Bipedality is plesiomorphic for birds, as it is for all dinosaurs (Sereno et al., 1993), and its evolutionary origin is currently hypothesised to lie within dinosauromorph archosaurs (Persons and Currie, 2017). In 2012, Kubo and Kubo proposed that bipedality arose up to six times within archosaurs, by correlating limb proportions indicative of cursoriality with bipedalism. In 2017, Persons and Currie re-iterated the hypothesis that facultative bipedality represented a transitional stage in the acquisition of bipedality in dinosauromorphs (as in hominins), although no quantitative evidence was offered. The latter authors predicted that taxa interpreted as obligate bipeds (e.g. the first dinosaurs) should have ancestors that are facultative bipeds. However, no large-scale taxon-level assessment of locomotor mode across Archosauria and their direct ancestors and descendants (Archosauriformes and Dinosauria) has been attempted within a phylogenetic framework, making it difficult to assess the validity of this prediction. Examining the sequence of character evolution across clades provides a framework to test the robustness of adaptive evolutionary hypotheses in the fossil record (VanBuren et al., 2015).

Here, we test the sequence in which locomotor states evolved across the transition between quadrupedal and bipedal locomotor modes using two recently published phylogenies focused on the relationships of early archosaurs and their ancestors.

2.3 Methods

Two recent phylogenies of early archosaurs have yielded insights into patterns of morphological evolution in this clade (Nesbitt et al., 2017), generated from two independent character matrices created by Ezcurra (2016) and Nesbitt (2011). The terms “Ezcurra tree” and “Nesbitt tree” will be used in this article. We used the strict consensus trees from the authors’ analyses that were derived from four most parsimonious trees in the case of the Ezcurra analysis, and 36 most parsimonious trees for the Nesbitt analysis. The Ezcurra tree comprises mostly early archosauriforms, their proximate ancestors and descendants, ranging from the earliest known Carboniferous diapsid Petrolacosaurus through to early herrerasaurids of the Upper Triassic, with a notably large representation of Lower Triassic taxa. The Nesbitt tree focuses greater attention upon Upper Triassic archosaurs and their immediate descendants (including early dinosaurs and crocodylomorphs). The phylogenies include 107 and 83 taxa respectively.

To determine whether each taxon was classified as an obligate quadruped (OQ), facultative biped (FB), or obligate biped (OB), we conducted a literature survey of all taxa included in the two matrices and recorded the most recent interpretation of locomotor mode for each taxon, along with the evidence thereof (Appendix A). The methods used by authors to determine locomotor mode varied considerably. Taxa diagnosed as primarily or semi-aquatic were classified as obligate quadrupeds, because of their lifestyles and morphofunctional convergence upon that seen in modern crocodilians. Semi- or obligate aquatic archosaurs exhibit a range of morphological features not suited for high velocity, bipedal terrestrial locomotion including modified paddle-like limbs, changes in intervertebral joint stiffness (initially lesser but becoming greater as lineages become more aquatic), and reduced limb length relative to trunk length (Molnar et al., 2015; Nesbitt et al., 2009; Osburn, 1905). Paddle-shaped limbs are self-evidently less effective at supporting upright body positions. Overly limber or overly stiff vertebral columns do not offer either the stability or flexibility necessary for the maintenance of a horizontal, balanced posture during bipedal movement. And, reduced limb lengths would be insufficient for achieving the necessary speed or ground clearance.

Figured reconstructions in publications were considered to be indicative of the authors' determination of locomotor mode and of equal merit to textual determination. In instances where only diagrams were presented as the basis for determining locomotor style, the reconstructed posture of the animal was considered to be indicative of the determination. In instances where both quadrupedal and bipedal diagrams were presented, taxa were determined to be facultative bipeds. We consider this to be justifiable because diagrams only come to exist in the literature as the consequence of a cascade of decisions: firstly, authors have made an intellectual assessment of an animal's posture based on their understanding of the osteological material that is available; secondly, that figure has been produced by the authors themselves or on the authors' behalf (and approved by them); thirdly, the peer-review process has deemed that figure appropriate for publication in a scientific journal. Therefore, the reconstruction must be considered representative of a reasonable scientific understanding of the animal at the time of publication. Taxa with no published locomotor mode were pruned from the dataset because the methods used in this study cannot accommodate unknown character states.

In total, 108 taxa were included in these analyses after pruning, 15 of which were diagnosed on the basis of diagrams alone (Appendix A.1). Locomotor mode was treated as a discrete variable with character states 0, 1 or 2 to represent OQ, FB, and OB, respectively. We did not impose any directional preference on transitioning from one mode to another, as this would bias the analysis towards finding a certain result and not provide an objective assessment of the evolutionary variability of bipedality. To assess the sensitivity of our analytical approach, we replicated the following analyses using a dataset that excluded 15 taxa for which there was only diagrammatic data available.

The topologies of the two phylogenetic trees were redrawn in Mesquite (version 3.51) (Maddison and Maddison, 2018) and imported into the R statistical environment (version 3.4.3) (R Core Team, 2017). Polytomies were randomly resolved into bifurcations using the *multi2di* function in R package *ape* (Paradis et al., 2014), as character states cannot be optimised on polytomies using these methods. Random polytomy resolution had no effect on any of the patterns observed in these analyses, because all taxa included in each polytomy were assigned the same locomotor mode. Trees and their branches were dated by first and last appearance in the fossil record using the *DatePhylo* function in the *strap* R package (Bell and Lloyd, 2014), using equal share dating. First and last occurrence data were taken from the Paleobiology Database (www.paleobiodb.org).

Ancestral state reconstructions were performed on each tree using the *ace* function in *ape* (Paradis et al., 2014). We opted for a maximum likelihood ancestral state estimation with discrete character states and an equal rates model of transition rather than a parsimony-based analysis. This reflects the highly variable branch lengths between taxa, whereas parsimony-based analyses assume that each branch of the tree is of equal time length. Maximum likelihood can account for varied branch lengths by using a rate of evolution algorithm (Paradis et al., 2014). We used a joint estimation procedure, which incorporates information from all nodes to calculate the maximum likelihood ancestral state at each node, rather than just the tips and branches descending from that node, as is done in a marginal estimation procedure. This approach gives the most likely combination of ancestral likelihood states (Paradis et al., 2014; Pupko et al., 2000). Maximum likelihoods were graphically represented as proportional pie charts at each node in the trees, and were plotted using the *geoscalePhylo* function in *strap* (Bell and Lloyd, 2014) (Figures 2.1 and 2.2). Using the most likely character state at each node, we then determined if the acquisition of OB from OQ involved an intermediate FB stage for each independent evolution of OB. The sensitivity analyses were conducted and presented using the same procedures (Figures 2.3 and 2.4). The R code used in this analysis is available in Appendix B.

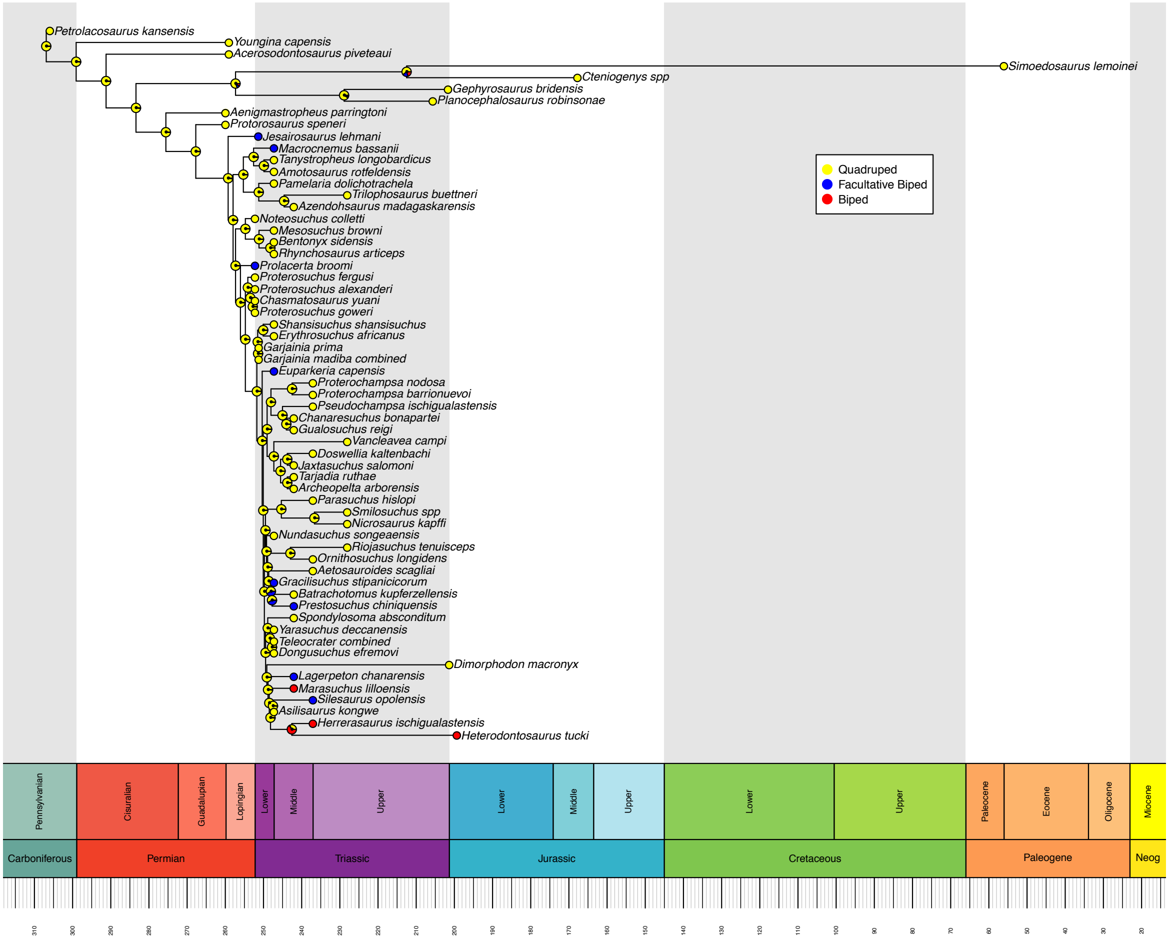

Fig. 2.1 Maximum likelihood reconstruction of ancestral states for locomotor mode based on the time-calibrated Ezcurra tree. Likelihoods are represented by graphical pie charts.

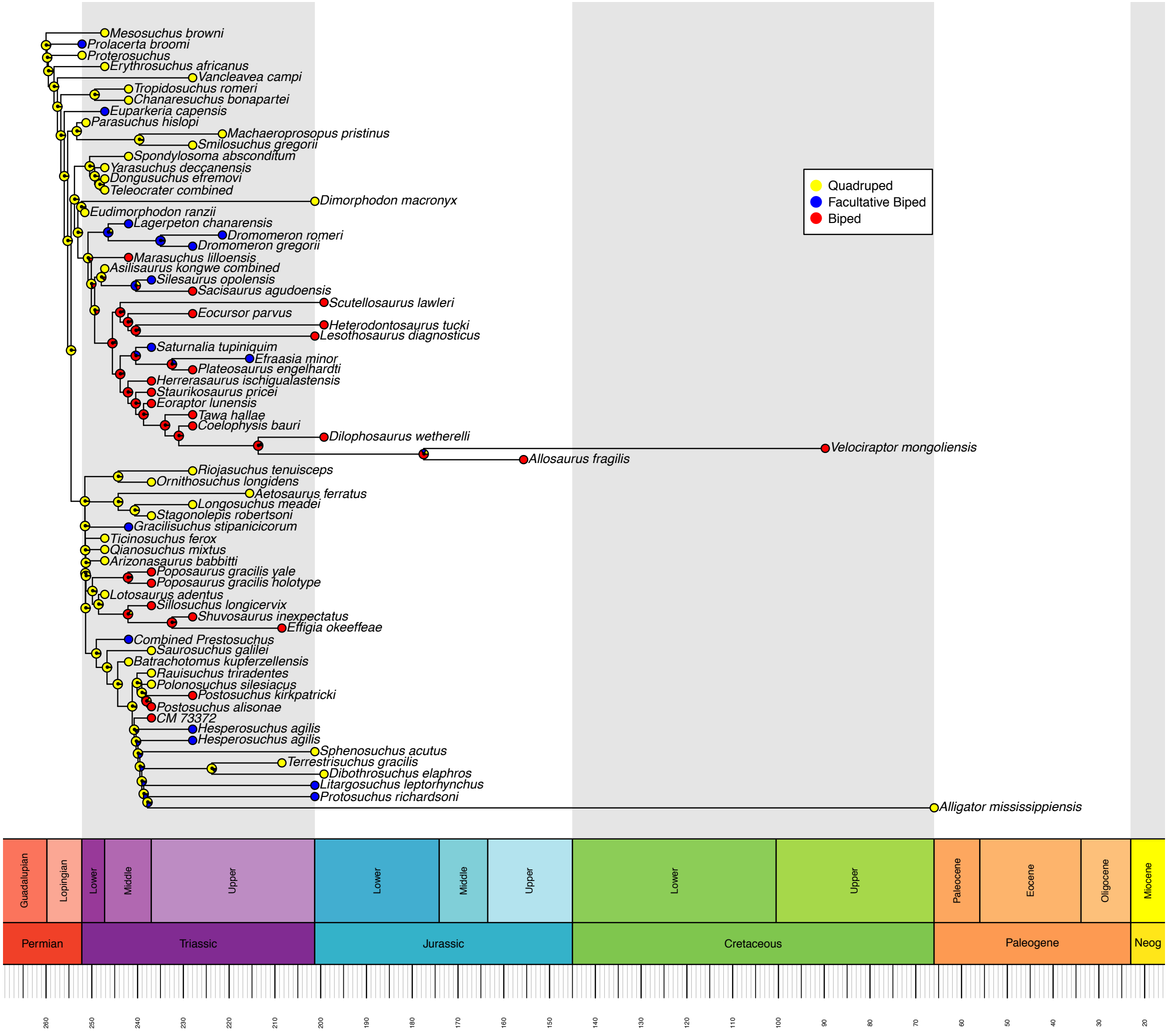

Fig. 2.2 Maximum likelihood reconstruction of ancestral states for locomotor mode based on the time-calibrated Nesbitt tree. Likelihoods are represented by graphical pie charts.

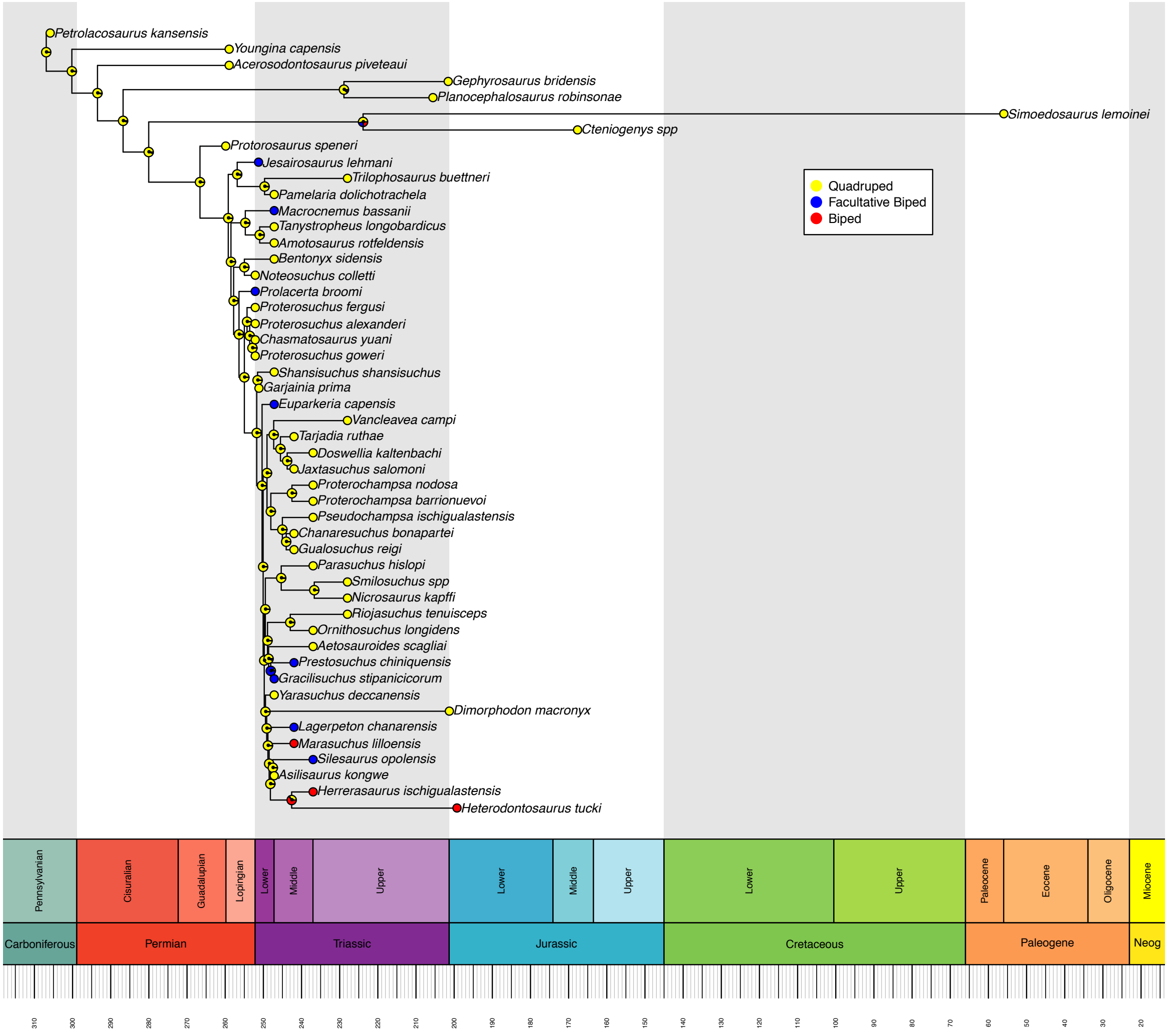

Fig. 2.3 Sensitivity analysis, with diagrammatically diagnosed species removed, for maximum likelihood reconstruction of ancestral states for locomotor mode based on the time-calibrated Ezcurra tree. Likelihoods are represented by graphical pie charts.

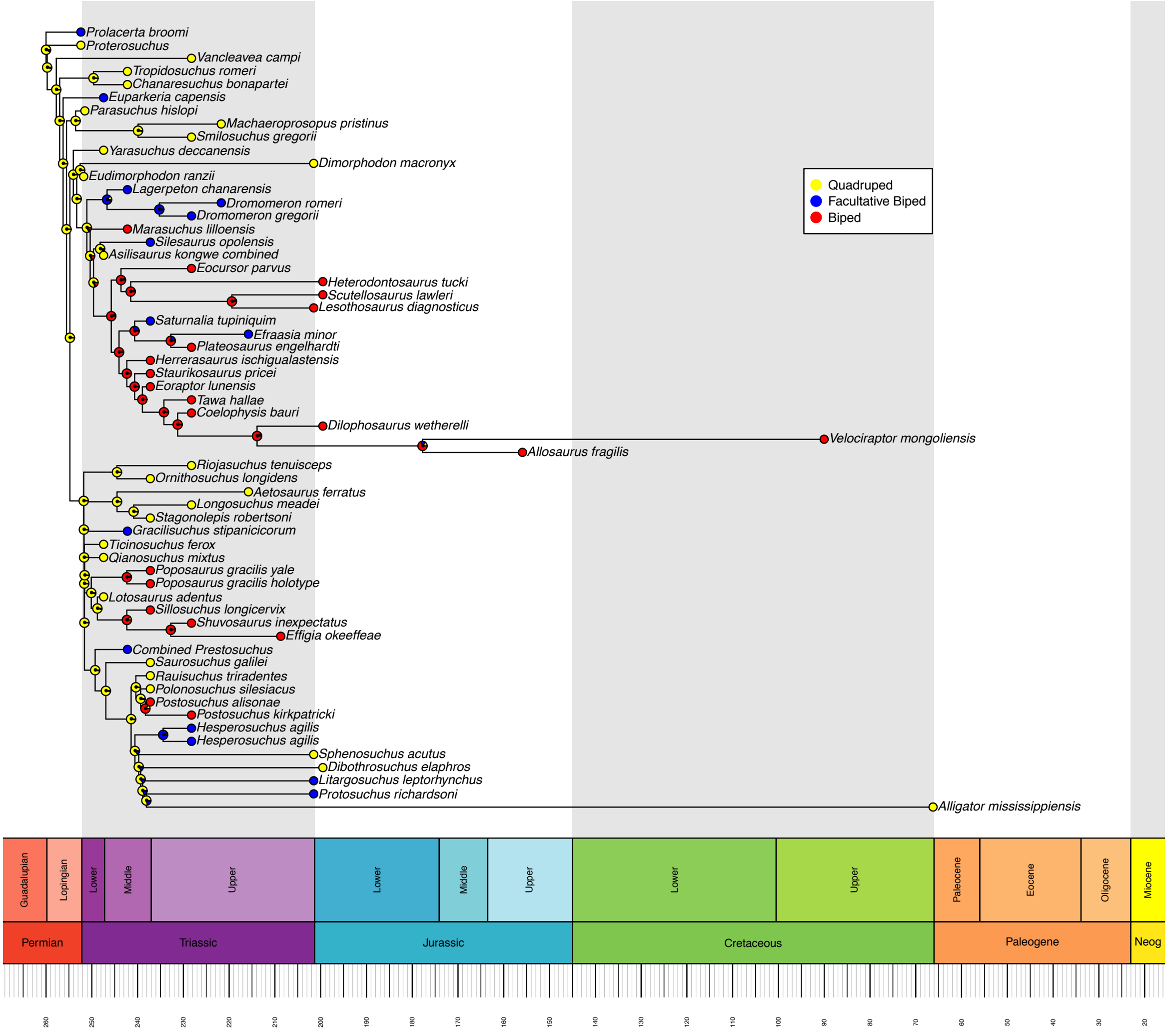

Fig. 2.4 Sensitivity analysis, with diagrammatically diagnosed species removed, for maximum likelihood reconstruction of ancestral states for locomotor mode based on the time-calibrated Nesbitt tree. Likelihoods are represented by graphical pie charts.

2.4 Results

The Ezcurra tree is focused primarily upon early archosauriforms. In this tree (Figure 2.1), FB is only ever recovered evolving from an OQ ancestor. Likewise, OB is only ever recovered evolving from OQ. Ancestral states within dinosauriforms remain quadrupedal, despite the end nodes being either facultative or obligate bipeds. Within the Ezcurra tree, we recover eight instances of the independent acquisition of FB, and two instances of OB acquisition. In the sensitivity analysis of the Ezcurra tree, we recover seven independent acquisitions of FB, and two of OB.

The Nesbitt tree (Figure 2.2) includes a wider range of taxa including later archosaurs and early Dinosauria. In this tree, we do recover the expected transition from OQ through FB to OB, but this only occurs once, within Silesauridae. The silesaurid ancestral node (*Asilisaurus* (*Silesaurus* + *Sacisaurus*)) is recovered as quadrupedal, with its descendant node recovered as a facultative biped, and finally *Sacisaurus* is determined to be an obligate biped. Throughout this tree, there are no other instances of OB emerging from a FB ancestral state, though there are two instances of FB evolving from an OB state. In total, we recover 12 independent acquisitions of FB and seven of OB. In the sensitivity analysis, we no longer recover the OQ-FB-OB sequence because the locomotor mode of *Sacisaurus* was determined on the basis of an anatomical diagram rather than a textual description. Here, we recover a total of 12 independent acquisitions of FB, and five of OB.

Accounting for the overlapping of some taxa across both trees, we recover a total of 14 independent acquisitions of FB and seven of OB across the two trees (Figures 2.1 and 2.2), reduced to 13 of FB and five of OB in the sensitivity analysis (Figures 2.3 and 2.4). All instances of OB acquisition are found within Archosauria, and the single full transition from OQ through FB to OB occurs in Silesauridae.

Some nodes, such as the ancestor to *Simoedosaurus* and *Cteniogenys* (Figures 2.1 and 2.3) show OB and FB components despite being deeply located within an OQ dominated section of the tree and having OQ tips. This is an artefact of long branch lengths coupled with the rate of evolution model used in calculating the most likely ancestral state, resulting in likelihoods that entertain the possibility of OB and FB evolving by chance in the ancestor to those species. A similar artefact is also seen the node ancestral to *Allosaurus* and *Velociraptor* (Figures 2.2 and 2.4).

2.5 Discussion

We inferred patterns of locomotor mode evolution across two recent early archosaur phylogenies to test whether FB exists as a consistent transitional locomotor mode between the conditions of OQ and OB. We identify just a single instance in which FB forms an intermediate locomotor mode in the evolution of archosaur bipedality from quadrupedality, out of a total of 14 instances of FB evolution and seven instances of OB evolution. This single example of the OQ-FB-OB transition occurs within the clade Silesauridae, which has a basal sister-group relationship to Dinosauria and does not therefore contribute directly to the origin or emergence of bipedality within Dinosauria.

In the past, a maximum of six independent acquisitions of archosaur bipedality have been hypothesised. That total figure includes instances determined by the authors to be possible, but unconfirmed (Kubo and Kubo, 2012). Our finding of seven independent acquisitions of obligate bipedality in archosaurs exceeds all other estimates made to date. Considered alongside the 14 acquisitions of facultative bipedality, it implies that the adoption of particular locomotor modes in these reptiles was far more evolutionarily plastic than previously hypothesised. This is strongly supported by the pervasive distribution of facultative bipedality in the Nesbitt tree, which focuses on a more derived range of archosaurs than the Ezcurra tree. These findings contrast markedly with the classic, and perfectly plausible, hypothesis that facultative bipedality played an important transitional role in archosaur locomotor evolution.

There are two common issues in palaeontological research that may affect our results: phylogenetic topology and morphological proxies for behaviour. Data quality is known to affect the robustness of phylogenetic hypotheses (Benton et al., 2011), and there may not be enough well-known early archosaurs described to establish robust, stable topologies. In this work, we have used the current understanding of the fossil record to investigate evolutionary transitions between diagnosed locomotor modes (Appendix A). It is possible that facultatively bipedal taxa not yet described and lying on the dinosauriform stem of Dinosauria will provide support for the existence of a transitional locomotor mode in future. Despite these limitations, advances have been made in studying locomotor transitions in extinct diapsids.

Kubo and Kubo (2012) found a significant correlation between their indices for bipedality (humerus plus radius length divided by femur plus tibia length) and cursoriality (metatarsal to femur length) in Triassic archosaurs, suggesting that bipedal archosaurs were also more cursorial, adding a layer of complexity to the evolution of bipedality. Maidment and Barrett (2012, Barrett and Maidment (2017)) explored the full scope of traits associated with the evolution of quadrupedal locomotion in Ornithischia. Alongside whole-body traits such as a more cranial centre of mass distribution; this included five readily identifiable osteological correlates relating to muscle attachments or postural shifts. Based on the apparent co-evolution of cursoriality and bipedality, and the multifaceted nature of quadrupedality evolution, we infer that the emergence of a bipedal locomotor mode would be similarly mosaic.

It is therefore clear that the identification of locomotor mode in the fossil record has always been challenging, and this uncertainty undoubtedly influences our results. This is especially important when evaluating the potential capacity for a facultative locomotor mode to exist as an intermediary stage in the evolution of bipedality. The means used by different authors to determine locomotor mode have been extremely variable across the history of archosaur research (Padian, 2008; Piechowski and Dzik, 2010; Romer, 1972; Schachner et al., 2011). For some species a robust determination has been made using biomechanical models and in-depth musculoskeletal reconstructions (Fechner, 2009; Schachner et al., 2011). Many studies, particularly older studies, use a deterministic methodology that lack such a rigorous mechanistic approach and they are thus inconsistent with each other. In some cases, little-to-no justification was given by the author, e.g. relying solely on longer distal limb elements to diagnose facultative bipedality, regardless of other anatomical features (Gow and Kitching, 1988). This last methodological approach, although widely used, stems from data compiled using mammalian limb proportions, rather than a diapsid or multi-taxon dataset; this latter approach commonly relies on forelimb-to-hindlimb ratios as an indicator of bipedality (Carrano, 1999). It should be noted that a musculoskeletal modelling approach does not equate to accuracy, though by the nature of its multidisciplinary methodology it does demand more rigour than inference alone.

When considered in the context of the results presented here, we must entertain the possibility that current interpretations of archosauriform locomotor mode are unlikely to be accurate and under- or mis-identify facultative bipeds in the fossil record. Despite this uncertainty, we do find evidence for FB existing as a transitional mode in this study, as has been hypothesised as widely accepted by the paleontological research community for some time. However, we do not find evidence for this in the direct ancestors of Dinosauria. Our results find only one example of the predicted evolutionary sequence, which occurred when taxa determined on the basis of anatomical reconstructions alone were included. This result highlights issues regarding the identification of locomotor mode, particularly FB, in the archosaur fossil record. Ultimately, the literature-based determinations of locomotor mode used in this analysis have been made by experts in their respective fields using their own anatomical knowledge, inference and understanding of the biomechanics of archosaurs. Therefore, the analyses presented here are based on the most current interpretations of the archosaur fossil record.

If it transpires that we are currently identifying facultatively bipedal archosaurs at the correct frequency in the fossil record, further investigation is warranted into the mechanisms of acquiring an obligate bipedal locomotor mode directly from an obligate quadrupedal one. In light of these results, a systematic review of archosaur locomotor mode is required to more accurately test the hypothesis of FB forming a necessary intermediate mode in the acquisition of dinosaur bipedality. Following a rigorous analysis of traits emerging among the first bipedal archosaurs, in a similar vein to Maidment & Barrett in recent years (2012, Barrett and Maidment (2017)), a thorough analysis of the emergence of these traits across the phylogenies presented here should be conducted. A particular focus of such work should be on the instances of bipedal evolution recovered in the analyses presented here. The primary difficulty that we anticipate in such a programme of work lies in the rarity of good-quality osteological material from exclusively Late Triassic and Early Jurassic locations. The previous worked example used what appears to be a better-quality (exclusively ornithischian) fossil record based on almost exclusively on large dinosaurs, and had the benefit of spanning a considerably greater time-range.

2.6 Conclusion

Using the most recent phylogenetic hypotheses and a range of rationales for locomotor mode determination, we recover seven independent evolutionary origins of obligate bipedality among archosaurs. Of these, only a single complete evolutionary transition via a facultative locomotor mode from an obligate quadrupedal one exists, although this result is not recovered in our sensitivity analysis. Our results therefore suggest that facultative bipedality is not a necessary transitional locomotor state in the evolution of archosaur bipedality (as hypothesised repeatedly in the past) and does not form an essential stage in the evolution of dinosaurian bipedality. We suggest that this unexpected result might be attributable to inconsistent interpretation of the morphology associated with facultative bipedality in a wide range of archosaur studies, or alternatively that archosaurs are acquiring an obligately bipedal locomotor mode via unexplored anatomical mechanisms.

A renewed assessment and interpretation of the morphological traits associated with locomotor mode, particularly facultative bipedality, in early archosaurs seems to be necessary if we are to more accurately interpret the evolutionary transition to bipedality in this group and properly test the novel hypothesis implicit in this analysis.

Chapter 3

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

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Author contributions are as follows:

L.R.G. conceived of the study, designed the study, collected the data, carried out the statistical analyses, interpreted the results, and drafted the manuscript; D.B.N. helped draft the manuscript.

3.1 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.

3.2 Background

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 et al., 2006), extreme arboreality (Fischer et al., 2010) and gliding (McGuire and 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 and 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 and 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 and 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 and Currie, 2017), Lepidosauria (Simões et al., 2017) and in extant squamates (Irschick and Jayne, 1999a; Snyder, 1962). 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 et al., 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 (Clemente and Wu, 2018; Greene et al., 1978; Irschick and Jayne, 1999b). 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 (Blob and Biewener, 2001; Greene et al., 1978). 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.

3.3 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 (Regnault et al., 2017; Schachner et al., 2014) (Appendix C Table C.1, 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 Appendix C Table C.2. 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 (Aerts et al., 2003; Alexander, 2004; Clemente and Wu, 2018; Irschick and Jayne, 1999a,b; Kubo and Kubo, 2012; Snyder, 1952, 1962). 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 Appendix C Table C.3. 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 and Wiens, 2016) was pruned to match the specimens in the dataset using the *drop.tip* function in the *ape* package (Paradis and Schliep, 2019). 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 package in R (R Core Team, 2017) to inform correct statistical procedure, favouring log-transformed data in the majority of tests (Appendix C Table C.4). 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* (Figures 3.1 and 3.2) and regression outputs recorded (Appendix C Table C.5).

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:

Body segment $\sim$ SVL $\times$ Bipedality

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

Body segment $\sim$ SVL + 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 3.1.

We also performed a phylogenetically independent principal component analysis (PCA) alongside these linear regression approaches on all linear measurements (Appendix C Table C.3). 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 the *prcomp* function in *stats* and visualized (Figures 3.3 and 3.4) using the *fviz_pca* function in *factoextra* (Kassambara and 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 Appendix C Tables C.6 and C.7.

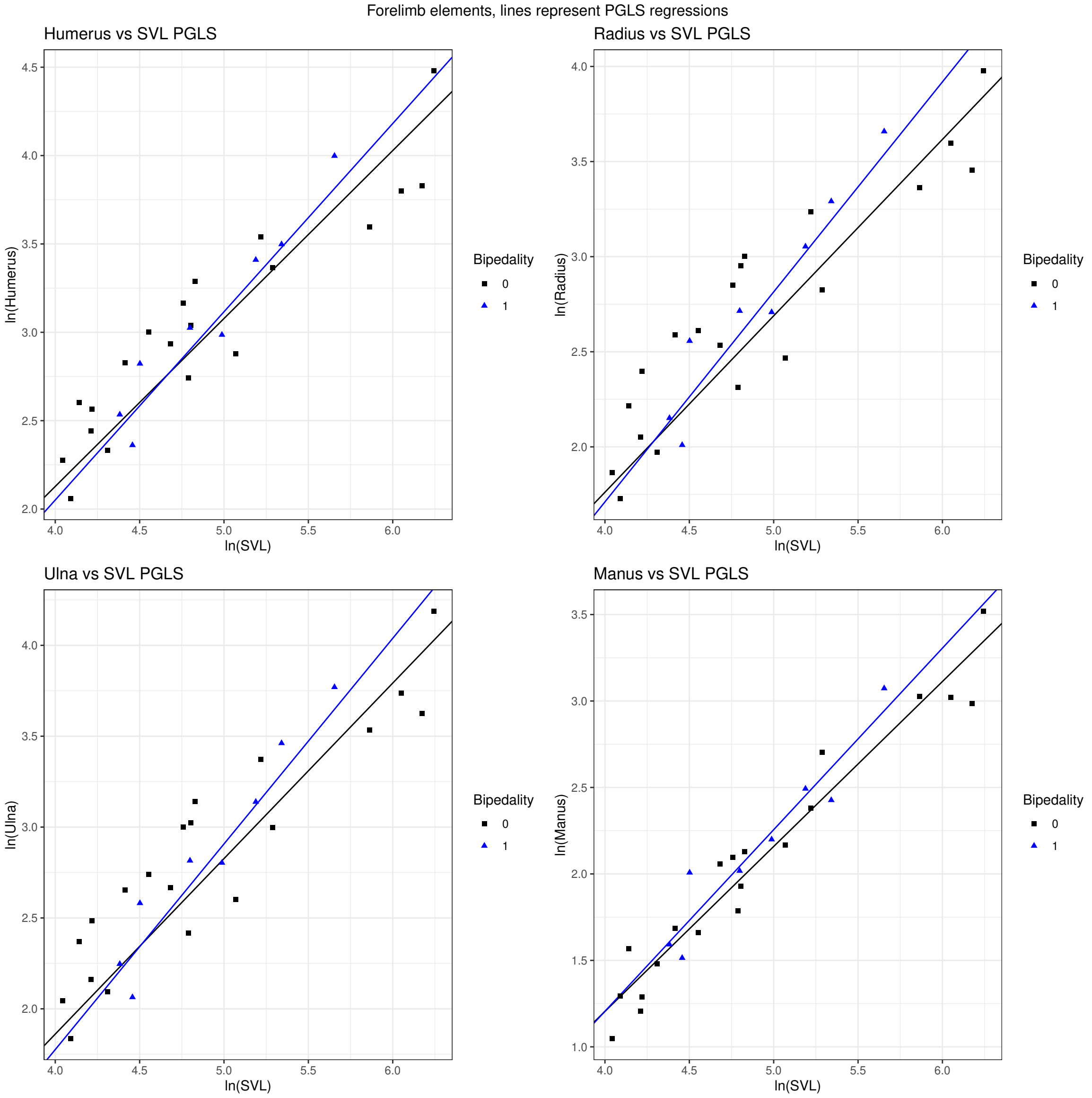

Fig. 3.1 Bivariate linear regressions of forelimb segment lengths and snout–vent length. Lines represent phylogenetic least-squares regressions. Obligate quadrupeds (0) represented by black squares; facultative bipeds represented by blue triangles. These symbols remain consistent in all further plots. Regression line equations and segment definitions can be found in Appendix C. Both x- and y-axes were log scaled.

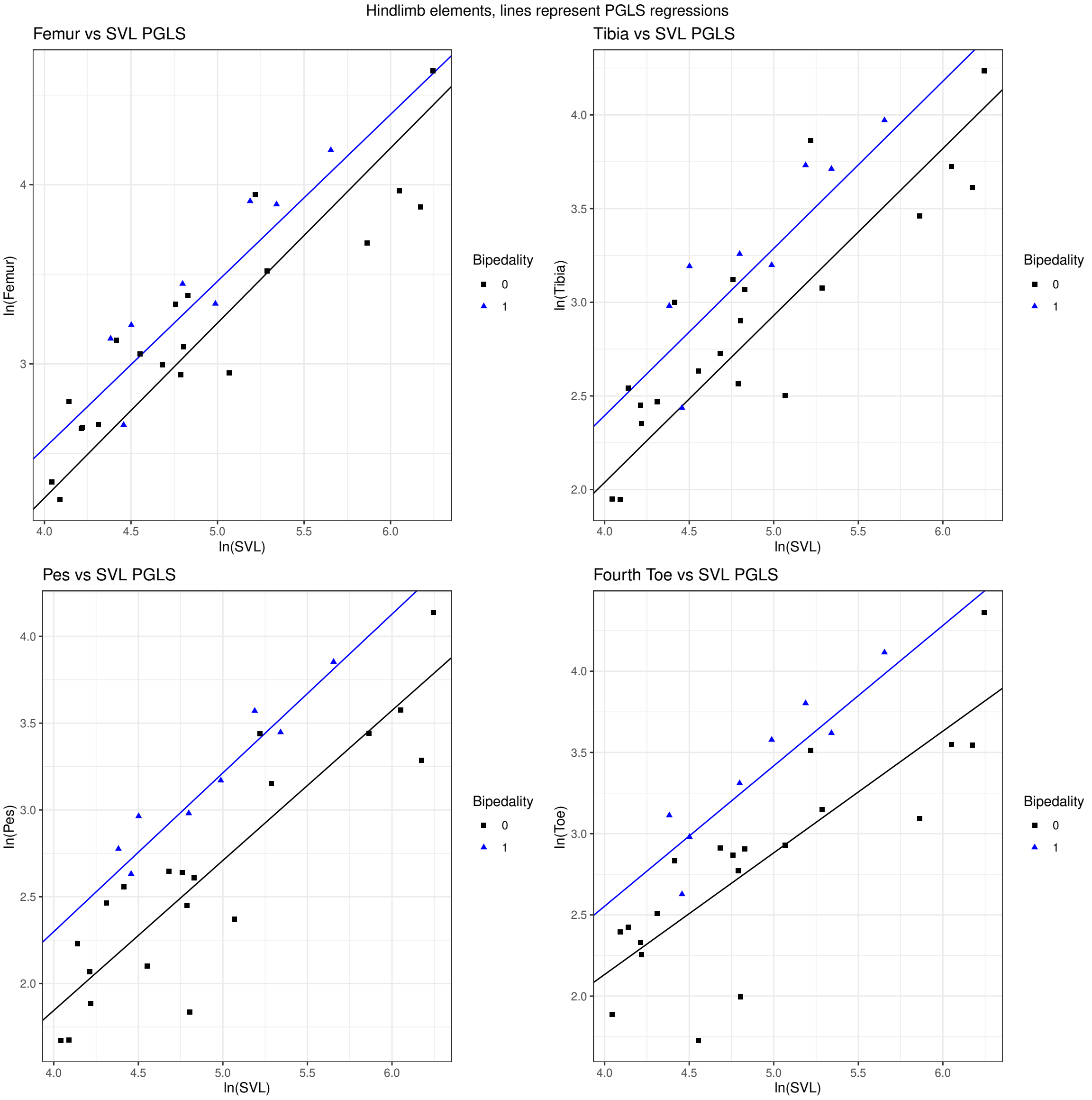

Fig. 3.2 Bivariate linear regressions of hindlimb segment lengths and snout–vent length. Lines represent phylogenetic least-squares regressions. Regression line equations and segment definitions can be found in Appendix C. Both x- and y-axes were log scaled.

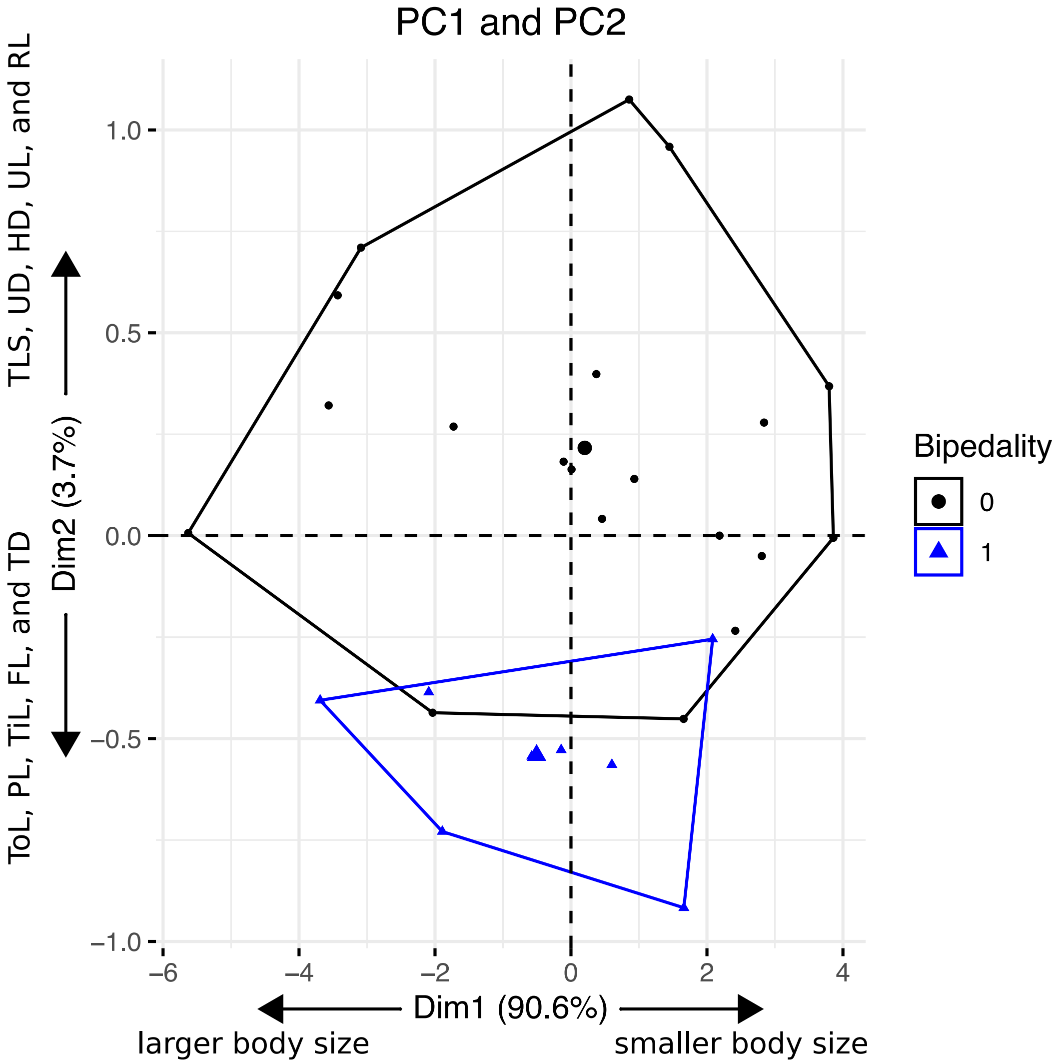

Fig. 3.3 Principal component plot of PC1 and PC2. Each point represents a specimen. Larger symbols indicate centroids for the two locomotor modes. Loadings are represented on the relevant axes. PC1 represents body size; PC2 represents bipedality. TiL, tibia length; FL, femur length; HD, humerus diameter; PL, pes length; RL, radius length; TD, tibia diameter; TLS, thoracic-lumbar-sacral length; ToL, fourth toe length; UD, ulna diameter; UL, ulna length.

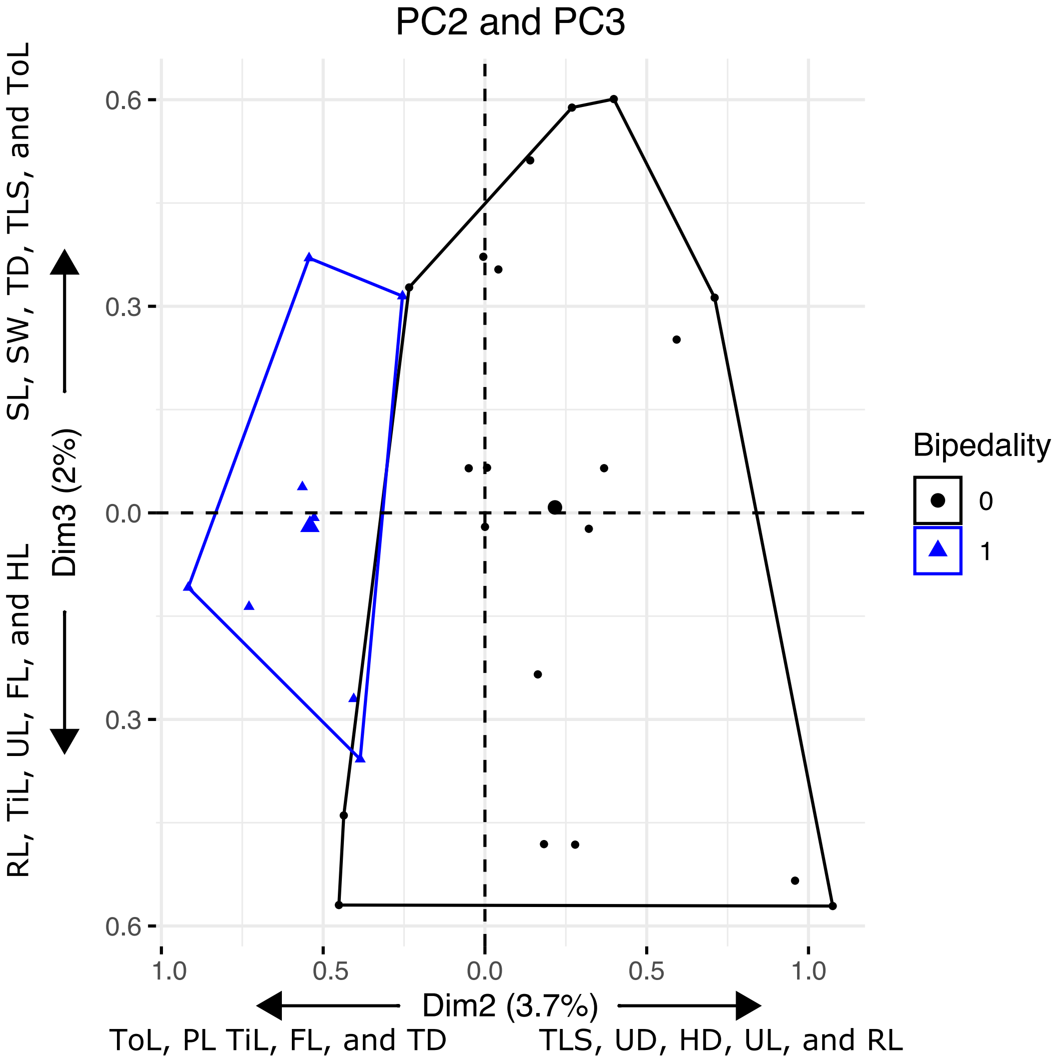

Fig. 3.4 Principal component plot of PC2 and PC3. Each point represents a specimen. Larger symbols indicate centroids for the two locomotor modes. Loadings are represented on the relevant axes. PC2 represents bipedality. FL, femur length; HD, humerus diameter; HL, humerus length; PL, pes length; RL, 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.

Table 3.1 Results from PGLS ANCOVA analyses, using locomotor mode as a fixed effect, significance level $P < 0.05$. Bold fields indicate a significant result.

	Slope analysis (variable $\sim$SVL * Bipedality)			Intercept analysis (variable $\sim$SVL + Bipedality)	
Predictor	Interaction term	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

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 (Appendix C Table C.8).

3.4 Results

Phylogenetic least-squares linear regressions are represented by Figures 3.1 and 3.2, representing the forelimb and hindlimb elements, respectively, with further exploratory plots in the supplementary file. Equations for the regression lines may be found in Appendix C Table C.5. 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 (Figure 3.2). 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 3.1), 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 3.1). No significant interactions were identified in parameters of the humerus, femur, radius or manus

(Table 3.1). The diameter of the tibia increases at a faster rate with SVL in facultative bipeds than in obligate quadrupeds (Appendix C Table C.5). 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 (Figure 3.1). 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 3.1; Appendix C Table C.5). Similar too is TLS length, though this is generally shorter in facultative bipeds (Appendix C Table C.5). 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; Appendix C Table C.9). 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; Appendix C Table C.9).

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 (Appendix C Table C.7; Figure 3.3). PC2 and PC3 represent 3.7% and 2.0%, respectively, (Appendix C Table C.7; Figures 3.3 and 3.4). 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, 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.

3.5 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 (Blob, 2000) – reversing the regression to match the formula orientation of (Biewener, 1983) 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 and 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 the 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 concerning 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 (Berman et al., 2000; Grinham et al., 2019; Snyder, 1962). Updated locomotor classifications, such as for *Tropicagama temporalis* (Wilson and Swan, 2017) and *Iguana iguana* (Blob and 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 Appendix C Table C.9). 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 and 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 and 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 and 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 (Christian et al., 1994; Clemente and Wu, 2018; Hsieh, 2003; Snyder, 1954, 1962), 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 and 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; Clemente and Wu, 2018; Hutchinson, 2004a,b).

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 and 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 extant 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 (Grinham et al., 2019; Maslin et al., 2015; Preuschoft, 2004). 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 (Kinsey and McBrayer, 2018; Snyder, 1962). 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.

3.6 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 (Christian et al., 1994; Snyder, 1962), 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. Within extant Lepidosauria, facultative bipeds are shown to occupy a niche region within the overall lepiodosaur morphospace. The wider implications for these results are highlighted, particularly in their use as a way of increasing our understanding of the identifications and evolution of bipedality in the fossil record of reptile groups. Understanding the relationships between limb segment lengths and body size in living reptilian facultative bipeds will inform the analysis and identification of this locomotor mode in extinct representatives of the reptile clade.

Chapter 4

The pelvis as an anatomical indicator for facultative bipedality and substrate use in lepidosaurs

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Author contributions are as follows:

L.R.G. conceived of the study, designed the study, collected the data, carried out the statistical analyses, interpreted the results, and drafted the manuscript; D.B.N. helped draft the manuscript.

4.1 Abstract

Facultative bipedality is regarded as an enigmatic middle ground in the evolution of obligate bipedality and is associated with high mechanical demands in extant lepidosaurs. Traits linked with this phenomenon are largely associated with the caudal end of the animal: hindlimbs and tail. The articulation of the pelvis with both of these structures suggests a morphofunctional role in the use of a facultative locomotor mode. Using a three-dimensional geometric morphometric approach, we examine the pelvic osteology and associated functional implications for 34 species of extant lepidosaur. Anatomical trends associated with the use of a bipedal locomotor mode and substrate preferences are correlated and functionally in-

terpreted based on musculoskeletal descriptions. Changes in pelvic osteology associated with a facultatively bipedal locomotor mode are similar to those observed in species preferring arboreal substrates, indicating shared functionality between these ecologies.

4.2 Background

Lepidosauria represents one of the most diverse groups of modern tetrapods, comprising >10 000 species inhabiting every continent except Antarctica. In addition to a near global distribution, they exhibit a variety of adaptations reflective of the ecological niches they occupy in their preferred environments. These can relate to diet, differing reproductive strategies and, of most interest to this study, their varied locomotor capabilities. The adaptability of the lepidosaur body plan to locomotor styles appropriate to different environments is evident across the group: iterative evolution of a limb-reduced, snake-like body plan (M. Woltering, 2012); specializations for arboreal lifestyles (Anzai et al., 2014; Higham and Jayne, 2004), including an ability to glide between trees (McGuire and Dudley, 2005); and even pachyostotic postcranial skeletons to facilitate diving in the case of the marine iguana (*Amblyrhynchus cristatus*) (Hugi and Sánchez-Villagra, 2012). One of the most intriguing locomotor styles represented in this group is facultative bipedality, a locomotor mode during which ordinarily quadrupedal animals temporarily move using only two limbs. Multiple avenues of research into vertebrate evolution and anatomy have shown that animal behaviour can have an impact on morphology, particularly that related to navigating their environment. Phenomic integration of anatomy and behaviour, amongst other traits, are essential to the survival of animals within ecological niches and the understanding of evolutionary transitions. A classical example is the transition from a quadrupedal to a bipedal locomotor mode in hominins; structural changes are observed in the pelvic girdle, the vertebral column and the limbs as more upright postures are adopted (Senut et al., 2018). However, the observable phenome in the fossil record is distinct from that in the modern world because of the lack of behavioural observations. Forging an understanding of fossil species requires a thorough understanding of the observable features in living taxa that create a plausible framework for comparison with, and interpretation of, extinct forms. For example, a recent study of inner ear morphology in canids enabled plausible inferences to be made concerning the hunting behaviour of their fossil relatives (Schwab et al., 2019). The connection between modern phenomic observations and palaeontological data, based solely upon anatomy, has a substantial history dating back to early 19th century observations by Georges Cuvier and the perceptive commentaries by authors such as George Lauder (1991). Recent technological advances have done much to improve this connectivity. The facultatively bipedal locomotor mode observed in some extant squamate species is of particular interest because of its potential application to the interpretation of locomotor modes among taxa in the fossil record (Irschick and Jayne, 1999a). It has been hypothesized that the adoption of facultative bipedality represents an intermediate locomotor mode in the evolution of hominin bipedality (Senut et al., 2018). In contrast, the role of facultative bipedality in the evolution of archosaurian bipedality is far less well understood (Grinham et al., 2019). Many studies have examined the kinematics and performance ranges of this locomotor mode in modern squamates (Clemente et al., 2008; Clemente and Wu, 2018; Irschick and Jayne, 1999b). However, the core anatomical understanding is far less extensively explored. Our current understanding of the morphology linked to locomotor mode in squamates is based almost exclusively on classical examples of what may now be termed 'historic' research (Snyder, 1952, 1954, 1962). These articles were based upon dated methods of observations and interpretative modelling and would now benefit from the broadening of such analyses to incorporate a wider range of species and the use of recently developed shape analysis methods. Fresh research in this area has the potential to provide improved insights into the mechanical understanding of facultative bipedality in Lepidosauria and, ultimately, to the evolution of bipedality in reptiles broadly (Persons and Currie, 2017). In this study, we address the current understanding of morphologies associated with facultative bipedality in extant Lepidosauria and test a novel hypothesis that locomotor mode and substrate use in these reptiles can be linked causally to the morphology of the lizard pelvis. By examining both the locomotor mode (obligate quadruped or facultative biped) and the preferred substrate (arboreal, semi-aquatic, terrestrial or rock- climbing) of the species, we will attempt to identify which aspects of ecological behaviour influence pelvic morphology most significantly in these animals.

4.3 Methods

The pelvis was selected as an anatomical structure of interest based on several key factors. Firstly, the articulation of the pelvis with both the axial and appendicular skeleton, particularly the tail and hindlimbs, means that it plays a central role in locomotor function. Secondly, it is readily identifiable in microcomputed X-ray tomography (µCT) scans, making it a good target for segmentation. Finally, owing to its link to locomotor function, it should be subject to evolutionary pressures based on the use of the axial and hindlimb appendicular skeletal structures. Landmark-based three-dimensional (3D) geometric morphometrics was used to analyse the shape of the three bones of the pelvis (ilium, ischium and pubis) in 34 different species of extant lepidosaur. Before specimen analysis, the pelvises of 36 extant lepidosaur species representing all major extant four-legged groups were examined using µCT. These species inhabit a wide range of substrates and include representatives that are either obligate quadrupeds or facultative bipeds. These examinations revealed that the pelvises of chamaeleon species were so dissimilar to those of other lepidosaurians in terms of osteological morphology that their inclusion in this dataset would compromise the analytical protocols used in this study (see Appendix D Figure D.1; (Fischer et al., 2010; Higham and Jayne, 2004)). As a result, representatives of Chamaeleonidae were removed from the dataset, leaving a final dataset of 34 specimens. Scans were either collected as primary data and scanned at the Cambridge Biotomography Centre or collected from online databases and publication repositories (Table 4.1; (Regnault et al., 2017; Schachner et al., 2014)). In these specimens, the locomotor mode was determined based on literature sources or personal observations, and substrate use was determined based on a large-scale ecological database of extant squamates (Meiri, 2018). For species known to use multiple substrates, the most preferred was also noted, based on this database and an extensive literature review.

Table 4.1 Sources for specimens and scans used in this study

Species	Museum code (if applicable)	Museum	Source
Ablepharus pannonicus	fmnh-amphibians and reptiles-83533	Field Museum of Natural History	Morphosource.org
Acanthocercus cyanogaster	fmnh-amphibians and reptiles-12522	Field Museum of Natural History	Morphosource.org
Acanthodactylus boskianus	fmnh-amphibians and reptiles-68769	Field Museum of Natural History	Morphosource.org
Acanthodactylus schreiberi	BMNH 1888.11.3.7	Natural History Museum, London	Primary
Adolfus africanus	fmnh-amphibians and reptiles-154745	Field Museum of Natural History	Morphosource.org
Agama agama	fmnh-amphibians and reptiles-188910	Field Museum of Natural History	Morphosource.org
Amblyrhynchus cristatus	uf-herp-41558	Florida Museum of Natural History	Morphosource.org
Ameiva ameiva	ummz-herps-245032	University of Michigan Museum of Zoology	Morphosource.org

Anolis carolinensis	ummz-herps-245698	University of Michigan Museum of Zoology	Morphosource.org
Basiliscus basiliscus	BMNH 1914.5.21 16	Natural History Museum, London	Primary
Basiliscus vittatus	R.19025	University Museum of Zoology, Cambridge	Primary
Bronchocela cristatella	uf-herp-112989	Florida Museum of Natural History	Morphosource.org
Cachryx defensor	uf-herp-41534	Florida Museum of Natural History	Morphosource.org
Chlamydosaurus kingii	R.8429 1	University Museum of Zoology, Cambridge	Primary
Cophotis ceylanica	uf-herp-86474	Florida Museum of Natural History	Morphosource.org
Cordylus cordylus	uf-herp-63302	Florida Museum of Natural History	Morphosource.org
Crotaphytus collaris	BMNH 1889.7.3.14	Natural History Museum, London	Primary
Ctenosaura similis	uf-herp-181929	Florida Museum of Natural History	Morphosource.org
Cyclura carinata	uf-herp-32820	Florida Museum of Natural History	Morphosource.org
Dracaena guianensis	uf-herp-129938	Florida Museum of Natural History	Morphosource.org
Draco dussumieri	uf-herp-19920	Florida Museum of Natural History	Morphosource.org
Egernia whitii	uf-herp-133957	Florida Museum of Natural History	Morphosource.org
Heloderma suspectum	uf-herp-167975	Florida Museum of Natural History	Morphosource.org
Iguana iguana	uf-herp-181922	Florida Museum of Natural History	Morphosource.org

Lacerta trilineata	uf-herp-65017	Florida Museum of Natural History	Morphosource.org
Lyriocephalus scutatus	uf-herp-126295	Florida Museum of Natural History	Morphosource.org
Moloch horridus	uf-herp-126296	Florida Museum of Natural History	Morphosource.org
Shinisaurus crocodilurus	UF-H-60925	Florida Museum of Natural History	Morphosource.org
Sphenodon punctatus	QMBC 0614	Queen Mary University London	https://osf.io/bds35/
Tiliqua rugosa	uf-herp-87304	Florida Museum of Natural History	Morphosource.org
Tropicagama temporalis	ummz-herps-245428	University of Michigan Museum of Zoology	Morphosource.org
Tropiocolotes tripolitanus	cas-herp-123433	California Academy of Sciences	Morphosource.org
Varanus exanthematicus	V3 (live scan)		https://datadryad.org/resource/doi:10.5061/dryad.v1d30
Zonosaurus haraldmeieri	uf-herp-72878	Florida Museum of Natural History	Morphosource.org

We tested for a phylogenetic signal in substrate use and locomotor mode within our dataset using the packages *phytools*, *ace* and *phylobase* (Hackathon et al., 2011; Paradis and Schliep, 2019; Revell, 2012). A recently published molecular-based time-calibrated phylogeny for Squamata was pared down to match the species in our dataset (Zheng & Wiens, 2016). For species featured in our database, but not the phylogeny, the most closely related species was substituted (see Appendix D Table D.1). Tuatara (Sphenodon punctatus) was also added to the base of the modified tree, expanding the analysis to include extant Lepidosauria. Using the phylo4d function, we mapped traits associated with substrate use and locomotor mode on to our tree. The phyloSignal function was used to perform Pagel's λ test for phylogenetic signal (Table 4.2). Figure 4.1 was produced using the *gridplot.phylo4d* function and serves to illustrate the relationships between phylogeny and trait values.

Table 4.2 Pagel's λ test for phylogenetic signal

Trait	Lambda	p-value Lambda
Bipedal	0.0000717	1
Arboreal	0.6830000	0.00982
Saxicolous	0.0000411	1
Semi.Aquatic	0.0000582	1
Terrestrial	0.4370000	0.227

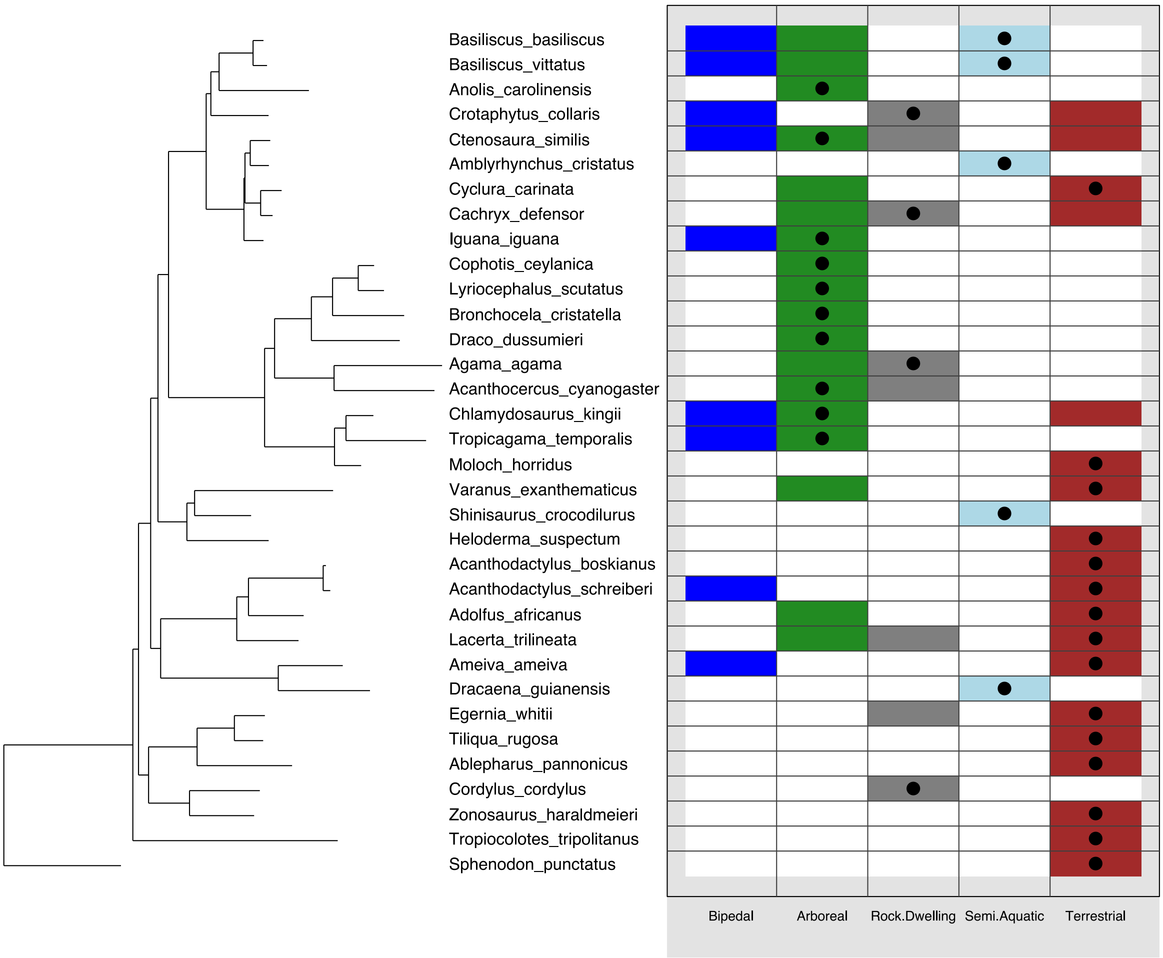

Fig. 4.1 The phylogenetic relationships of the species in the sample, along with locomotor mode and substrates used. A black dot represents the preferred substrate. Colour correspondence is maintained in all subsequent figures in this chapter.

Microcomputed X-ray tomography scans of specimens were processed using the open-source medical imaging software 3DSlicer (v.4.10; www. slicer.org; (Fedorov et al., 2012)). TIFF image stacks were scaled using voxel size. The three major bones of one half of the pelvis (pubis, ilium and ischium) were then segmented using the inbuilt Segmentation toolkit. Bones were segmented individually when possible, then combined as a composite structure. In species where the three elements were fused into a single unit, such as in Chinese crocodile lizard (Shinisaurus crocodilurus), bones were segmented as a single unit (Costelli and Hecht, 1971). Three- dimensional models were generated with a surface smoothing parameter of 10–20% dependent on scan resolution, with the intention of preserving maximum shape detail and increasing workability. These models were saved and then exported for use in landmarking software. Lateral profiles for a selection of these models are shown in Figure 4.2, in order to represent the morphological variation encompassed by species in this study, and key morphologies are highlighted in Figure 4.3. All specimens were fluid preserved at the time of scanning, except the savannah monitor (Varanus exanthematicus), which was scanned during live medical trials (Schachner et al., 2014). Methods of preservation are expected to have had no impact on scans because only hard tissue is examined here.

Three-dimensional models were imported into IDAV Landmark (Wiley et al., 2005) software for application of landmarks. We used a mixture of 25 type II/III landmarks as defined in Table 4.3. Using the LaSEC function (Watanabe, 2018), the effectiveness of this number of landmarks was tested and confirmed to account for over 95% of shape variation (Figure 4.4). The multi-scaled forest lizard (*Adolfus africanus*) was used as the atlas species in Landmark.

Landmark data were imported into the R programming environment (R Core Team, 2017) for analysis using the readland.nts function in geomorph (Adams et al., 2018) These data were then subjected to a general Procrustes analysis (GPA) using the gpagen function. General Procrustes analysis enables mathematical comparison of 3D point clouds (landmarks previously mentioned) by scaling and rotating all point clouds to match the same orientation and centroid size. The mathematical output from GPA can then be examined statistically with principal components analysis (PCA) using the gm.prcomp function (Table 4.4). The outputs of PCAs are displayed in Figures 4.5-4.8 and represent the morphospace of the pelvic structure.

After examining the results of the shape analysis, functional interpretations regarding the variable structures present in the pelvises of the species in our dataset were explored. Using historical and modern works regarding the musculoskeletal architecture of the lepidosaur pelvis, we infer functional interpretations pertaining to the limb and axial skeleton relative

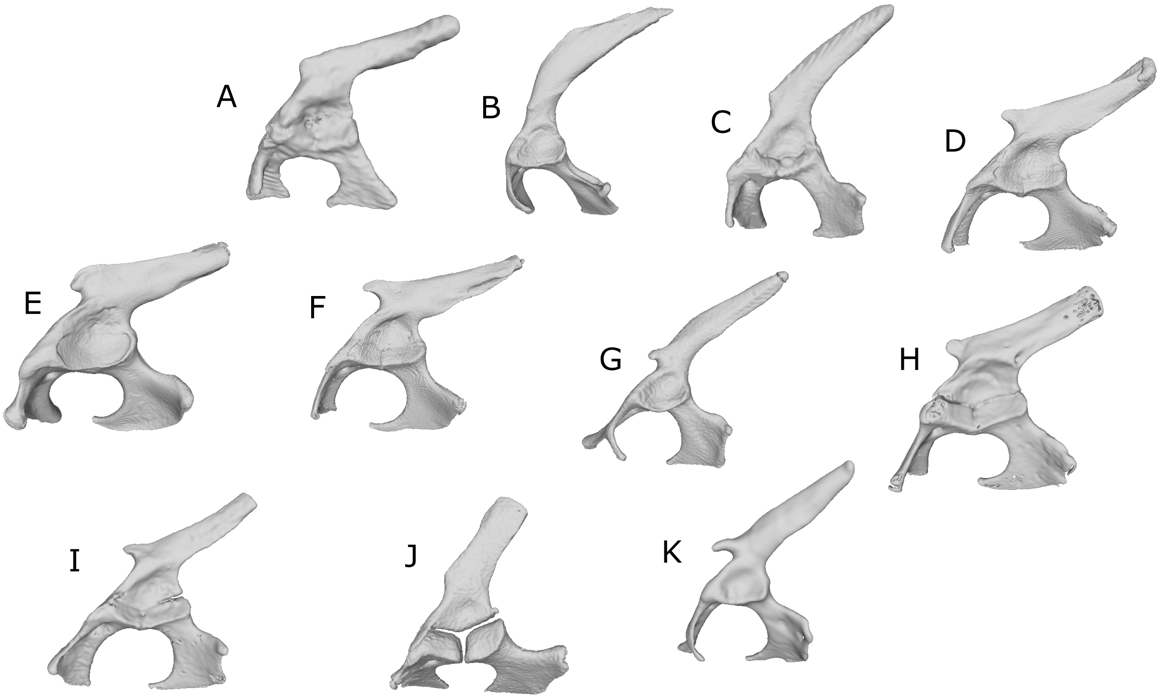

Fig. 4.2 Representative morphology, in side profile and not to scale, for a range of species in the study. Species are as follows: A, *Varanus exanthematicus*; B, *Tiliqua rugosa*; C, *Cachryx defensor*; D, *Iguana iguana*; E, *Amblyrhynchus cristatus*; F, *Ctenosaura similis*; G, *Lacerta trilineata*; H, *Chlamydosaurus kingii*; I, *Basiliscus basiliscus*; J, *Sphenodon punctatus*; K, *Acanthodactylus schreiberi*.

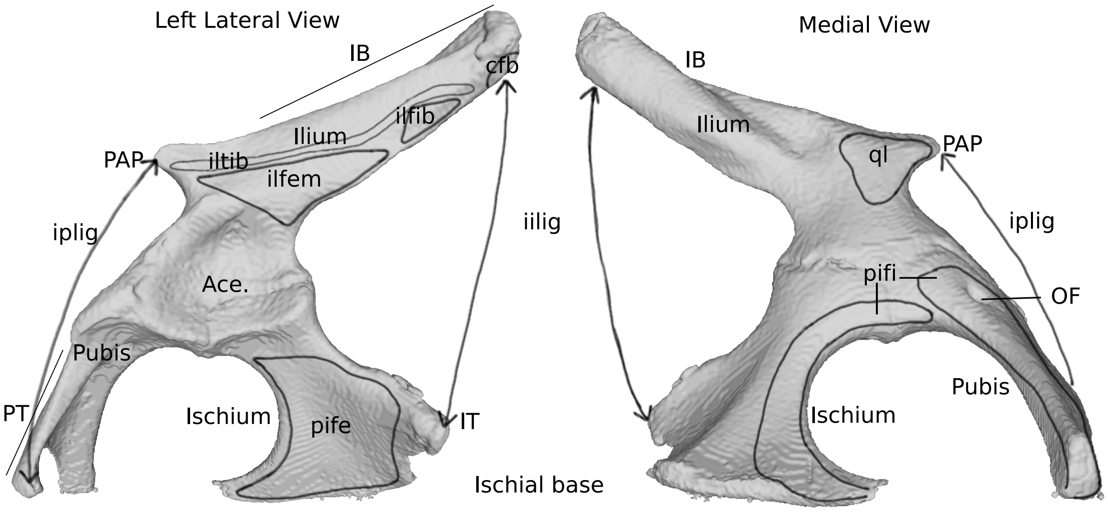

Fig. 4.3 Green iguana pelvis in left lateral and medial view. Key hard and soft tissue osteological features are highlighted. Modified from Snyder (1954) and Russell and Bauer (2008). Abbreviations: Ace., acetabulum; cfb, m. caudofemoralis brevis; IB, iliac blade; iilig, ilioischiadic ligament; ilfem, m. ilifemoralis; ilfib, m. iliofibularis; iltib, m. iliotibialis; iplig, iliopubic ligament; IT, ischidiac tuberosity; OF, obturator foramen; PAP, preacetabular process; pife, m. puboischiofemoralis externus; pifi, m. puboischiofemoralis internus; PT, pubic tuberosity; ql, m. quadratus lumborum.

Table 4.3 Landmark locations and definitions

Landmark	Type	Location
1	II	Distal tip of iliac blade
2	III	Apex of iliac blade dorsal surface curve
3	III	Dorsal aspect prior to preacetabular process
4	II	Tip of preacetabular process
5	III	Ventral aspect prior to preacetabular process
6	II	Pectineal process, near acetabulum
7	II	External edge of pubis perpendicular to obturator foramen
8	II	Processus lateralis pubis
9	II	Inward apex between landmarks 9 and 10
10	II	Pubic tubercle
11	II	Anterior-most point of obturator foramen
12	II	Posterior-most point of obturator foramen
13	II	Puboischidiac symphysis, most ventral point
14	II	Articulation between ischium and proischidac cartilage
15	II	Articulation between ischium and hypoischium
16	II	Most posterior point of ischidiac tuberosity
17	II	Most posterior point of the second portion of the ischidiac tuberosity
18	II	Articulation between ischium and ilium, on acetabular rim
19	II	Lateral entre acetabulum
20	III	Opposite preacetabular porcess, on ventral aspect of iliac blade
21	III	Opposite landmark 2, on ventral aspect of iliac blade
22	III	Inner ischium, perpendicular to the narrowest portion of the ischium
23	III	Top external rim of acetabulum, just below supracetabular process
24	III	Bottom of external rim of acetabulum
25	II	Medial centre acetabulum, approximately opposite 19

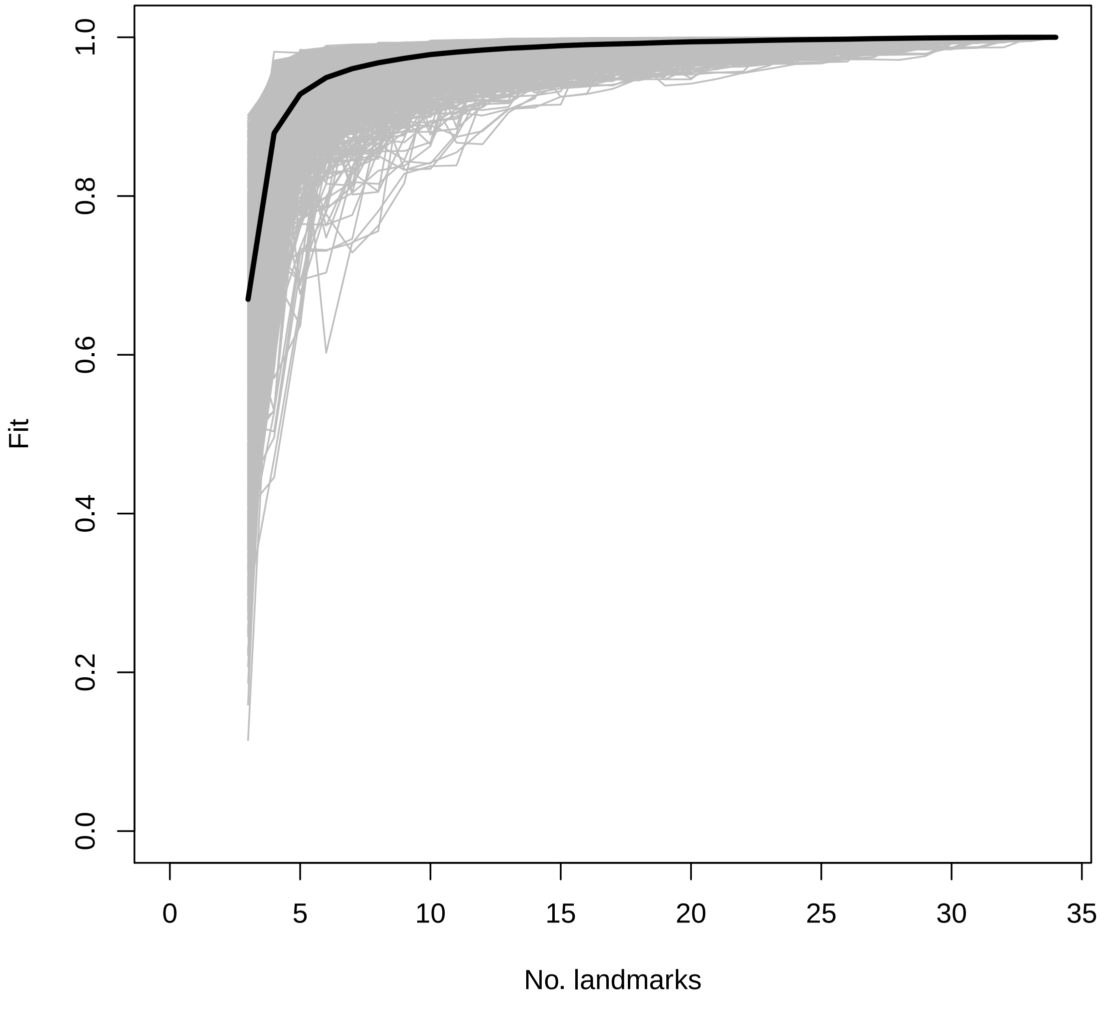

Fig. 4.4 *LaSEC* output indicating the percentage of variation in shape captured by different numbers of landmarks, based on iterative models.

Table 4.4 Principal components analysis scores for each species

Species	PC1	PC2	PC3	PC4	PC5	PC6
A. africanus	0.129	0.054	-0.041	0.014	0.002	0.033
A. ameiva	-0.033	0.028	0.052	-0.024	0.067	-0.046
A. boskianus	0.051	0.033	0.064	-0.003	0.037	-0.001
A. carolinensis	-0.005	0.086	-0.047	-0.006	-0.038	-0.060
A. cristatus	-0.071	-0.037	-0.019	0.074	0.027	0.038
A. cyanogaster	-0.064	-0.019	0.046	0.037	0.019	-0.018
A. pannonicus	0.252	-0.005	0.020	-0.021	-0.040	-0.030
A. schreiberi	0.065	0.036	0.070	0.003	0.023	-0.003
A. wagneri	-0.051	0.001	0.082	0.083	-0.026	-0.023
B. basiliscus	-0.089	0.023	0.025	-0.004	-0.041	-0.020
B. cristatella	0.002	-0.005	0.025	0.053	-0.046	-0.028
B. vittatus	-0.091	0.066	0.051	-0.045	-0.056	-0.020
C. carinata	-0.058	-0.011	-0.020	0.020	0.039	-0.021
C. ceylanica	-0.074	0.015	-0.076	-0.025	-0.039	0.003
C. collaris	-0.053	-0.003	0.022	0.023	0.087	0.060
C. cordylus	0.094	-0.069	-0.016	0.071	-0.018	0.030
C. defensor	-0.018	-0.036	-0.052	-0.044	0.027	-0.003
C. kingii	-0.127	0.033	0.033	-0.010	0.017	0.019
C. similis	-0.078	0.037	0.010	0.017	0.081	0.016
D. dussumieri	-0.060	0.080	-0.002	0.097	-0.125	0.026
D. guianensis	0.000	-0.064	-0.018	-0.061	0.033	-0.032
E. whitii	0.199	0.036	0.013	-0.020	-0.020	-0.021
H. suspectum	-0.034	0.060	-0.192	-0.033	0.007	0.051
I. iguana	-0.066	0.011	0.028	0.025	0.021	0.019
L. scutatus	-0.023	-0.023	-0.032	-0.053	0.011	-0.065
L. trilineata	0.109	0.051	0.031	-0.016	-0.013	0.027
M. horridus	-0.041	-0.195	-0.001	-0.003	-0.018	-0.037
S. crocodilurus	0.012	-0.039	-0.103	-0.012	-0.033	0.040
S. punctatus	-0.106	-0.102	0.096	-0.109	-0.079	0.064
T. rugosa	0.139	-0.101	-0.035	0.079	0.052	-0.001
T. temporalis	-0.015	0.038	0.042	0.005	0.025	-0.026
T. tripolitanus	0.058	-0.080	-0.016	-0.022	-0.043	0.020
V. exanthematicus	-0.050	0.039	-0.111	-0.011	0.022	-0.057
Z. haraldmeieri	0.100	0.060	0.071	-0.078	0.040	0.065

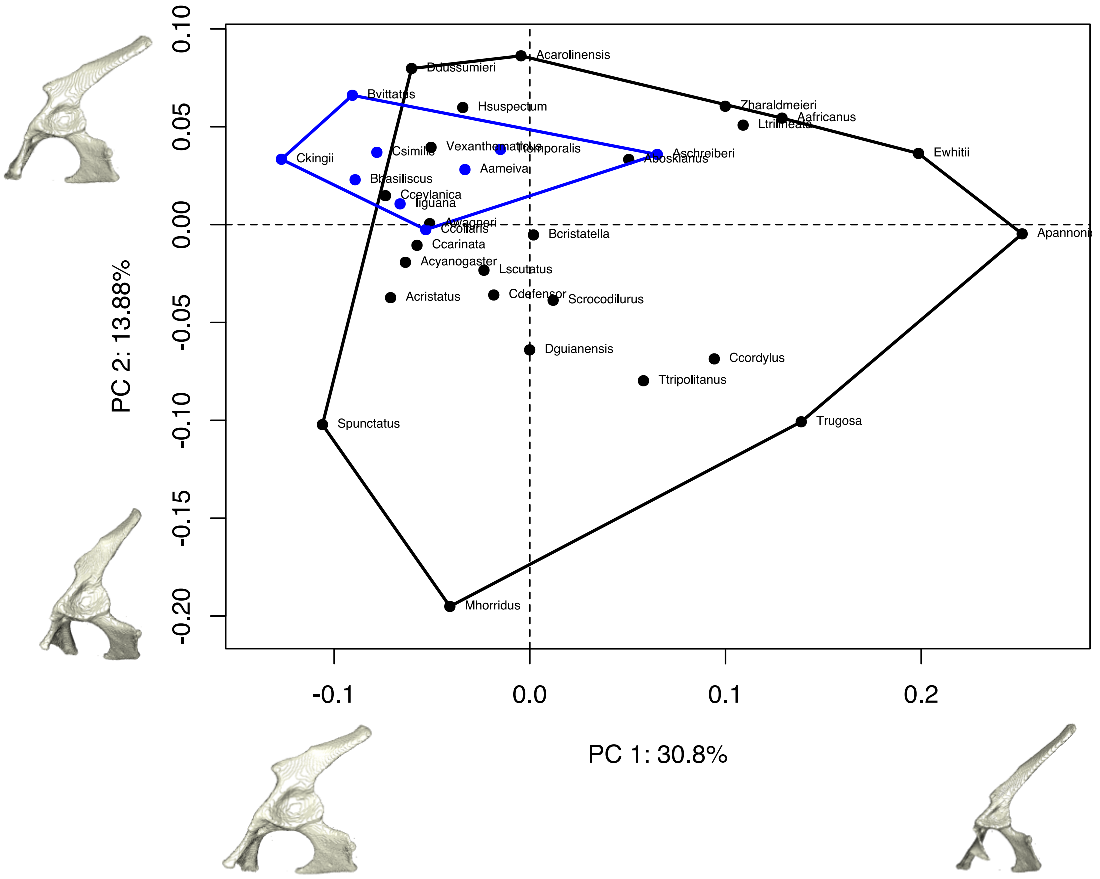

Fig. 4.5 Distribution of locomotor mode across principal components (PCs) 1 and 2. Black indicates obligate quadrupeds, blue indicates facultative bipeds. Warped meshes of the green forest lizard indicate the range of morphologies present on each PC.

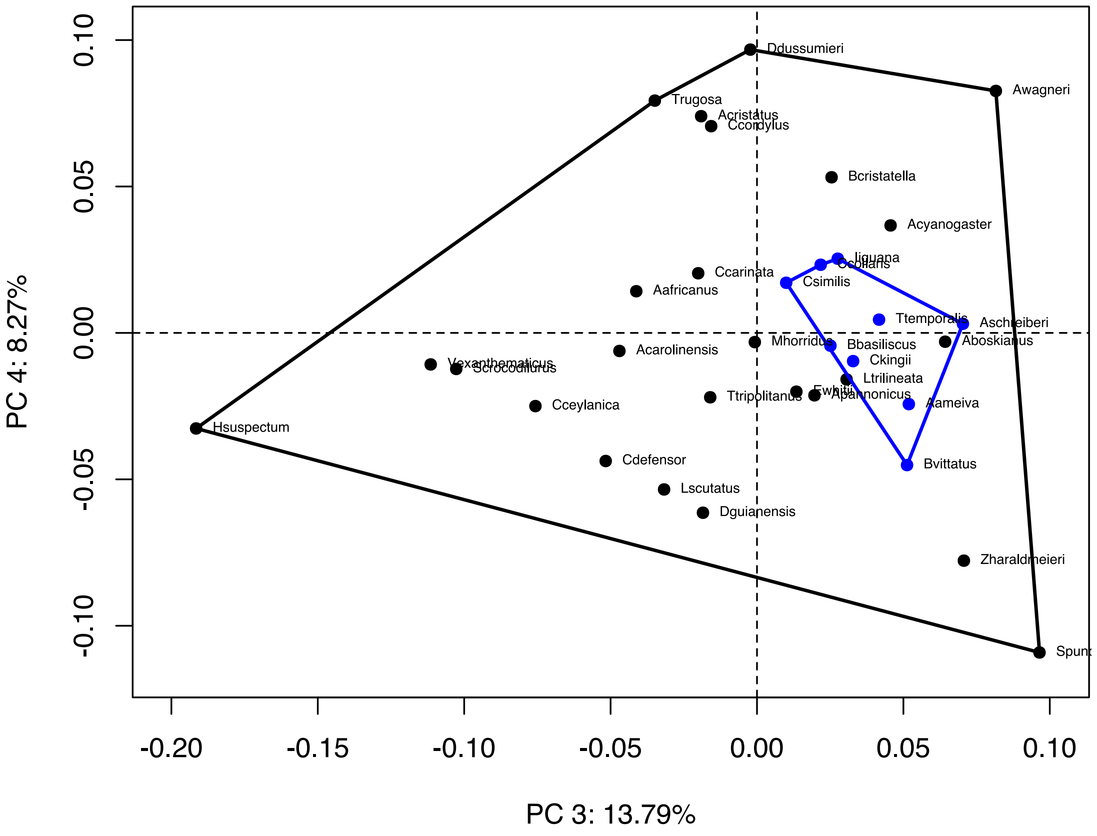

Fig. 4.6 Distribution of locomotor mode across principal components (PCs) 3 and 4. Black indicates obligate quadrupeds, blue indicates facultative bipeds.

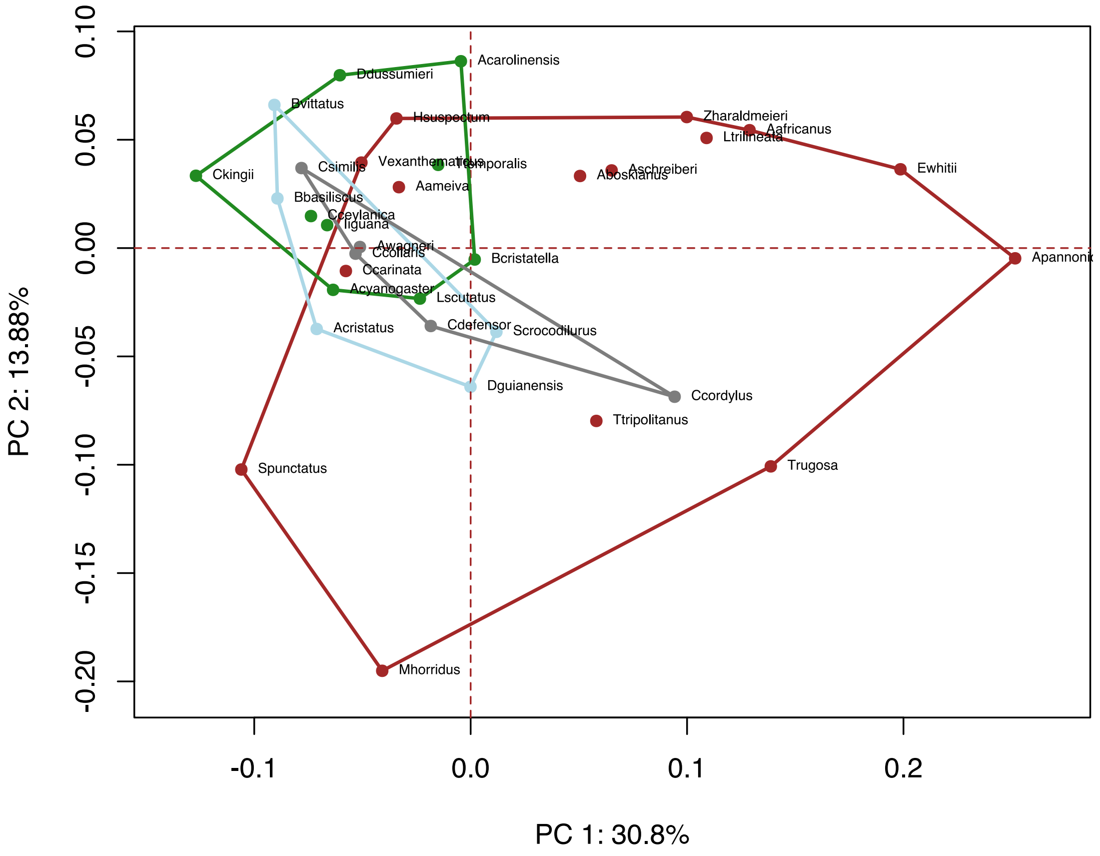

Fig. 4.7 Distribution of preferred substrate use across principal components (PCs) 1 and 2. Colours indicate substrate preference: brown, terrestrial; green, arboreal; sky blue, semi-aquatic; grey, rock-dwelling.

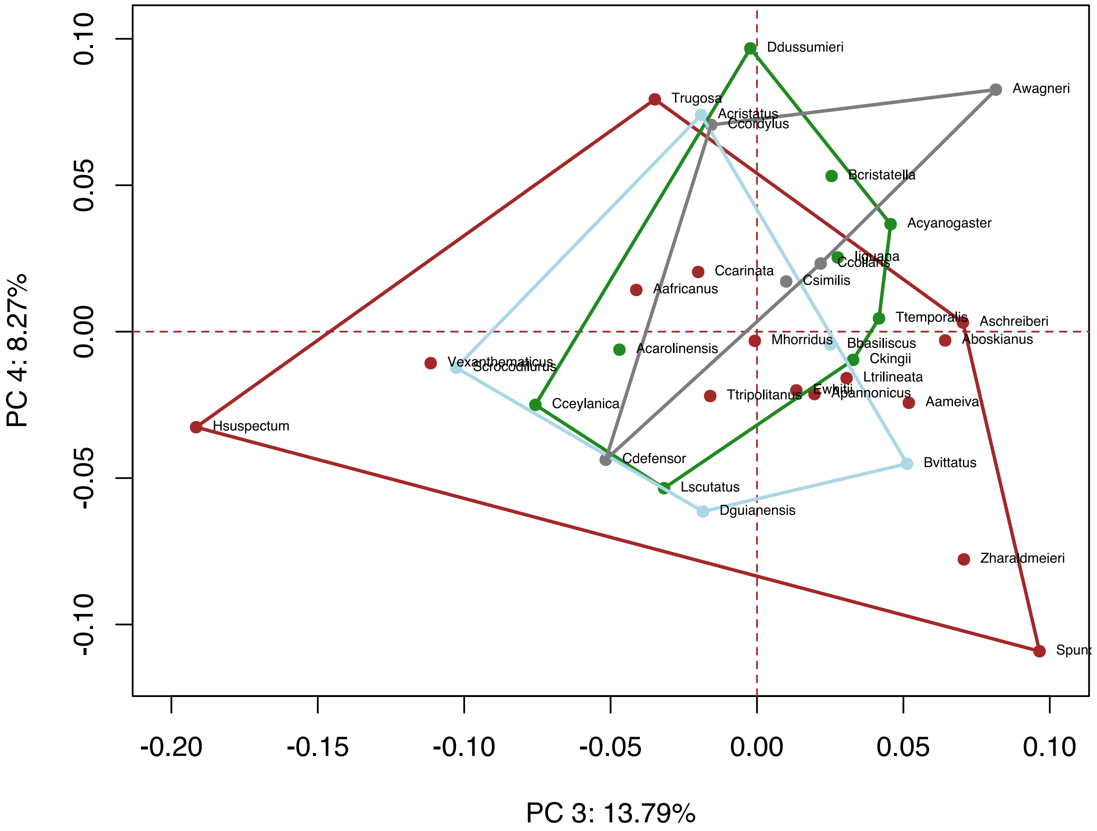

Fig. 4.8 Distribution of preferred substrate use across principal components (PCs) 3 and 4. Colours indicate substrate preference: brown, terrestrial; green, arboreal; sky blue, semi-aquatic; grey, rock-dwelling.

to locomotor mode and substrate preference (Dick and Clemente, 2016; Diogo and Molnar, 2014; Diogo et al., 2018; Fischer et al., 2010; Snyder, 1954, 1962).

4.4 Results

We find that there is no phylogenetic signal present for the traits of bipedality, rock-climbing, semi-aquatic or terrestrial substrate use (P-values > 0.05; Table 4.2). There is a signal present for arboreal substrate use (P-values < 0.05; Table 4.2). Given that this study focuses primarily on the use of a bipedal locomotor mode and a signal is present in only one of the five substrates tested, we decided not to conduct a phylogenetic correction for subsequent analyses. All PCA results are presented on two-axis scatter graphs, with each species represented by a single point in morphospace (Figures 4.5–4.8). The colour of the point is based on either locomotor mode or primary substrate, dependent on the result presented. Convex hull polygons were used to represent the range of morphospace used by species in each category. Large empty spaces within convex hulls can be indicative of disparate morphotypes occupying a similar ecological niche. Scores for each specimen on principal components (PCs) and cumulative variance are recorded in Tables 4.4 and 4.5. Morphospace maximum and minimum approximations for PC1 and PC2 are indicated by warped meshes of the green forest lizard (Bronchocela cristatella) pelvis on the axis of Figure 4.5.

Table 4.5 Cumulative variance represented by each principal component

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalues	0.0081	0.0037	0.0036	0.0022	0.0021	0.0013
Proportion of variance	0.3080	0.1388	0.1379	0.0827	0.0802	0.0492
Cumulative Proportion	0.3080	0.4468	0.5847	0.6675	0.7477	0.7970

Principal component 1 (30.8% variance) largely accommodates a general robustness in the pelvis, differences in the size of the acetabulum relative to the whole structure, the length of the iliac blade and the size of the preacetabular process (Figures 4.5 and 4.7). Additionally, the angle of the iliac blade relative to the ischial base is notably shallower in negative PC1 values. Species with highly positive scores for PC1 are skink species, which tend to have a gracile pelvis, whereas the negatively scoring species represent a variety of iguanid and agamid species, alongside tuatara. Facultative bipeds tend to score negatively on PC1, with the exception of Schreiber's fringe-fingered lizard (*Acanthodactylus schreiberi*; Figure 4.5). Principal component 2 (13.88% variance) accommodates less of a gracility gradient than PC1, instead accounting for variation in specific pelvic osteologies. The preacetabular process is hypertrophied and more defined in positive PC2 values. The angle of the iliac blade remains relatively constant to the ischial base. The orientation of the pubis is more anterior in positive PC2 values. A longer ischidiac base is also observed in positive PC2 values. Facultative bipeds tend to score positively on PC2 (Figure 4.5). Principal component 3 (13.79% variance) constrains a large amount of variation in shape in the ilium, in particular (Figure 4.6). Negative PC3 values indicate a convexly curving iliac blade extending well beyond the ischidiac tuberosity, with a lack of any distinguishable preacetabular process. Positive PC3 values, on the contrary, suggest a short and concave iliac blade, ending vertically short of the ischidiac process, with a well-defined preacetabular process. Facultative bipeds all score positively on PC3 (Figure 4.6). Principal component 4 (8.75% variance) accounts for more variance in the iliac blade, but also in the morphology of the acetabulum (Figure 4.6). Positive PC4 values indicate a slightly dorsoventrally flattened acetabulum, along with an ilium morphology extending well beyond the ischidiac tuberosity, with a defined preacetabular process. Negative PC4 values indicate a sharply angled ilium and a short pubic tuberosity. Facultative bipeds score across a wide range of PC4 scores (Figure 4.6). Regarding substrate use on the PC1 and PC2 plot (Figure 4.7), the largest region of the morphospace is represented by the terrestrial substrate. These reptiles spend their time primarily on the ground. There is significant overlap between the terrestrial substrate and all other substrates. The next largest region on this plot is arboreality, with almost all arboreal species scoring negatively on PC1 and positively on PC2, suggesting a more constrained pelvic shape relative to terrestrial dwellers. The regions of morphospace occupied by facultative bipeds and arboreal dwellers are very similar. Semi-aquatic species occupy the next largest region of morphospace, and this includes semi-arboreal species, such as the basilisks (*Basiliscus vittatus* and *Basiliscus basiliscus*). They occupy a mostly negative PC1 region and a variable PC2 region, overlapping to a degree with arboreal species. Rock-climbing species occupy the smallest region of the morphospace of our investigated substrates, occupying a range of both PC1 and PC2, but in a very narrow band. On the PC3 and PC4 axes (Figure 4.8), terrestrial substrate use again occupies the largest area of the morphospace. No particular substrate use separates distinctly from any other, with significant overlap between all substrates and a varied PC distribution, scoring both positively and negatively on PC3 and PC4.

4.5 Discussion

The value of phylogenetic correction in datasets such as the one used here is contentious. For studies examining the relationships between groups and evolutionary trends, the use of a phylogenetic correction is undeniably of value. However, when the study examines functional traits over a relatively short ecological timescale, a phylogenetic correction can affect the perception of results ((de Bello et al., 2015); this situation describes the study presented here. Regardless, we tested for a signal using Pagel's λ. This test was chosen because it is frequently the most robust with suboptimal branch length information (Molina-Venegas and Rodríguez, 2017), as may be the case where we have substituted closest relatives for species in our dataset. Owing to the absence of a signal for a facultatively bipedal locomotor mode, which was the trait we were primarily investigating, we concluded that a phylogenetic signal correction was unnecessary and risked obscuring any results regarding this locomotor mode. The presence of a signal in one of the four substrate traits alludes to a scope for application of phylogenetic correction in some of this study, but to allow comparisons between substrates and locomotor mode we opted to continue the study without correction. Additionally, given that GPA outputs are not a form of raw anatomical data, they are not subject to the same concerns regarding phylogenetic non-independence as other types of data (Felsenstein, 1985).

The shape of the pelvis in facultatively bipedal extant lepidosaurs falls within the overall morphospace of lepidosaurs generally. However, it is generally found in a very concentrated area of that morphospace (Figure 4.3). This suggests that the changes in shape, although not necessarily indicative of bipedal capacity in their entirety, are very much enabling factors. Based on the results presented here, facultative bipeds generally possess the following features: iliac blades that are straight to concave, generally robust pelvises, large and pronounced preacetabular processes, anteriorly extended pubic tubercles and a long ischial base. In some texts, particularly regarding fossil lepidosaur species, the preacetabular process is called the anterior iliac tubercle or the preacetabular spine. These morphologies, along with other key anatomies discussed later, are visible in the pelvic profile of the green iguana (*Iguana iguana*; Figure 4.3), a facultatively bipedal species. This is the observed case across the majority of the facultatively bipedal taxa in this study.

The sexes of the majority of specimens used in this study have not been identified, and thus we cannot comment on sexual distribution within a dataset where species are represented by a single specimen. However, previous works have not identified sexual dimorphism in the pelvis of the green iguana, and thus we infer there to be no sexually dimorphic effect in our dataset (Prieto-Marquez et al., 2007).

Conclusions can be drawn regarding pelvic morphology and substrate use, although not with the same clarity as for locomotor mode. Preferential terrestrial substrate use is both the most widely distributed in the morphospace and the most numerous in our dataset. It occupies a range of PC1 and PC2 values, having several tighter clusters of points and several isolated ones. This indicates a variety of different terrestrial morphs (e.g. the thorny devil (*Moloch horridus*) at maximum negative PC2, skinks at maximum positive PC1, lacertid species at PC1 values of 0.1; Figure 4.7). This is apparent in the lifestyles of these animals: the thorny devil is regarded as a particularly slow-moving lizard (Clemente et al., 2004), skinks commonly burrow and have relatively short limbs, and lacertids tend to express similar ecologies to one another, hence their grouping. It is difficult to make particularly detailed inferences regarding the effect of different niches on these morphs owing to the umbrella term ‘terrestrial’ encompassing a range of more specific substrates, such as sand, leaf-litter, burrowing, urban and grassland (Meiri, 2018).

The arboreal subset of the dataset is far more concentrated with regard to PC distribution, occupying primarily negative PC1 and positive PC2 values (Figure 4.7). There is a significant overlap between the arboreal morphospace and that of the facultatively bipedal species, despite only four of the eight facultative bipeds using a primarily arboreal substrate, implying shared functionality of the pelvis between these two groups. Many of the species defined as ‘semi-aquatic’ by Meiri (2018) can also be considered semi-arboreal or rock-climbing [e.g. the marine iguana (*Amblyrhynchus cristatus*) is considered semi-aquatic, but spends a significant portion of its life living and climbing on rocky shores]. To remain objective and reproducible, this research has retained the classifications from the study above. When considering rock-climbing species (saxicolous according to Meiri (2018)), the overall morphospace for these species forms an almost linear ‘y = x’ distribution across PC1 and PC2, heavily overlapping with semi-aquatic species. At a species- level comparison, there appears to be a subset of these arboreal species sharing the specific morphologies seen in our facultatively bipedal species, forming what we will refer to as the ‘common morph’ for the remainder of this study.

Principal components 3 and 4 provide little to no useful information for identifying differences between our substrate choices, owing to significant overlap of all species, and thus are not discussed in further detail here (Figures 4.6, 4.8).

These osteological morphologies in the common morph are likely to have a significant functional impact on the environmental demands of these animals (Figure 4.7). In particular, negative PC1 values are dominantly for non-terrestrial species, such as those that swim and climb, in addition to facultative bipeds. Based on works on the musculoskeletal anatomy of the lepidosaur pelvis (Bergmann and Hare-Drubka, 2015; Dick and Clemente, 2016; Diogo et al., 2018; Fischer et al., 2010; Lunn, 1948; Russell and Bauer, 2008; Snyder, 1954), the functional implications of the osteology defining this morph and its differences relative to other pelvic morphologies are discussed herein. It is noted that, at least in varanid lizards, the ranges of femoral abduction, rotation and ankle extension are all restricted in climbing species, such as those that use primarily arboreal or rocky substrates (Clemente et al., 2013). Additionally, we expect rapid limb recovery during the stride (swing phase musculature) to be especially important in bipedal species, but also that this is less important in climbing species that spend more time maintaining a braced position on surfaces. For reasons of succinctness, we address a selection of these morphologies we infer to be impactful, rather than an exhaustive list of all morphological elements observed. The functional implications of an altered ilium must be considered. In the common morph, the iliac blade tends to be relatively shallow in angle and is straight to concave. The iliac blade is the origin site for multiple important muscles used during locomotion, including the m. iliofemoralis (ilfem) m. iliotibialis (iltib) and m. iliofibularis (ilfib). These muscles serve as a swing phase femoral abductor, stance phase knee extensor and swing phase knee flexor, respectively (Dick and Clemente, 2016). Additionally, there is the ilioischiadic ligament (iilig) connecting the tip of the iliac blade to the ischiadic tuberosity, which serves as the muscle origin for m. flexor tibialis internus (fti), a knee flexor during the stance phase (Dick and Clemente, 2016). A shallower angle on the iliac blade has a significant effect on the moment arms of these muscles. In particular, as a relatively distal muscle, the ilfib should be subject to a marked change in muscle moment arm significantly altering the effective mechanical advantage of this muscle (Biewener, 1989). This is of note for bipedal species, which rely on powerful limb musculature to support their body weight on only one limb at a time; should that limb not be ready to support the animal, the stride would fail. Incidentally, this is often observed in facultatively bipedal lizards that are unable to maintain bipedal locomotion for extended periods of time and is a matter of fatigue linked to their ability to meet metabolic demand. Regarding stance phase musculature, the iltib is responsible, alongside the m. femorotibialis (ftib), for extending the knee and driving forward locomotion of the animal, both in two- and four-legged terrestrial locomotion and during climbing. This muscle extends along the majority of the length of the iliac blade, attaching dorsally to the ilfem and ilfib. The patella is imbedded in the distal tendon of this sheet-like muscle (Dick and Clemente, 2016). Modification of the iliac blade results in a significantly different moment arm angle for this structure which, along with increased ftib muscle mass, may increase the efficacy of knee flexion (Snyder, 1954).

Additionally, the ilium has a preacetabular process (Figure 4.3; PAP), a structure on which two important soft tissue elements originate. The first of these is the m. quadratus lumborum (ql), equivalent to the psoas muscle in mammals (Diogo et al., 2018). This muscle originates on the medial aspect of the preacetabular process and inserts on the posterior dorsal ribs, connecting the pelvis to the axial skeleton, forming a more anterior soft tissue attachment between the pelvis and the axial skeleton. This muscle appears to serve little function during quadrupedal locomotion in varanid lizards (Ritter, 1995, 1996), certainly not contributing to the lateral trunk bending used to augment stride length in many lepidosaurs.

Instead, this muscle serves as an axial skeletal dorsoventral flexor, particularly involved in antigravitational dorsal flexion (Russell and Bauer, 2008). This elevation of the anterior body is essential to both branch-to-branch arboreal locomotion and bipedal locomotion, perhaps making the enlarged preacetabular process the most functionally significant aspect of the common morph. The second soft tissue structure of importance here is the iliopubic ligament (iplig), connecting the preacetabular process to the pubic tubercle. This ligament has a restrictive function, encapsulating a variety of nerves and vessels, in addition to the m. puboischiofemoralis internus (pifi), in a vacuity formed between the two osteological processes it connects. The pifi is a muscle originating on the pubis and ischium, inserting into both sides of the femoral trochanter (Clemente et al., 2011). The increase in the size of the preacetabular process and the anteriorly projecting pubic tubercle create a larger vacuity beneath this ligament, enabling a larger pifi (an important femoral protractor), greater innervation and vascular flow to the area, all of which may contribute to hindlimb-intensive activities, including bipedal locomotion and hindlimb-driven vertical climbing. The presence of a large preacetabular process has been noted in bipeds (Russell and Bauer, 1992; Snyder, 1962), but has previously not been correlated with arboreality independent of facultative bipedality. Additionally, the extended ischial base provides a larger attachment site for the pifi in species presenting the common morph, thus making up a larger portion of the pelvic girdle musculature. This further highlights the important role of this muscle as a femoral protractor during the swing phase (Dick and Clemente, 2016). Many of the anatomical features highlighted here relate, in particular, to movement of the femur and flexion–extension of the knee joint, which are deemed important in lepidosaur locomotion (Bergmann and Hare-Drubka, 2015; Snyder, 1952). Naturally, the pelvis is involved in these functions regardless of the morphology presented, but the functional significance of modifications to these specific osteologies allows us to infer similarities between the hindlimb functions of facultatively bipedal and arboreal species.

Some studies in recent years have investigated the differences between terrestrial, quadrupedal-running and branch-climbing anole species (Anzai et al., 2015, 2014). These studies found that running species exhibited greater muscle masses in the hindlimb extensors (m. ambiens, ftib and iltib) and that climbing species tended to have greater muscle mass in their hindlimb retractors [m. caudofemoralis longus (cfl) and m. caudofemoralis brevis (cfb)]. Many studies on the evolution of bipedal locomotion have noted the significance of the cfl in enabling this mode (Gatesy et al., 1990; Hutchinson, 2004a,b; Persons and Currie, 2017). Combining these findings with our own enables us to identify an association between the use of arboreal substrates and a facultative bipedal locomotor mode in extant lepidosaurs that is observable in the osteology of the pelvis. Our findings demonstrate that the same pelvic morphologies are present in facultative bipeds as are found in arboreal species (enlarged preacetabular process, iliac blade morphology and large ischial base). Based on findings from modern (Anzai et al., 2015, 2014; Russell and Bauer, 2008) and historical literature (Snyder, 1952), we expect that the muscle architecture of these animals will follow similar trends and that these might be distinct from those animals that tend to run quadrupedally. Further analysis of the variation in muscle mass in a wide range of facultative bipeds would enable more thorough testing of this hypothesis.

There are several different whole-body morphs present in the lepidosaur group that prefer an arboreal substrate and climbing locomotion: the chamaeleonid morph (highly specialized girdle, autopodial and tail morphologies), the gekkonid morph (specialized toe pads in many species) and the less specialized morph common to most other arboreal dwellers (iguanids, corytophanids, anolids, lacertids, etc.). These less specialized forms tend to rely on limb proportions, gripping claws, balancing tails and often less than graceful movement through branches. These less specialized forms are those expressing the common pelvic morph that we have highlighted in this study. We propose that these groups might be predisposed towards the acquisition of a facultatively bipedal locomotor mode when moving out of their usual arboreal environment, aided by commonalities in the musculoskeletal adaptations for an arboreal, climbing lifestyle and a facultatively bipedal locomotor mode. This general pattern echoes the most widely supported model of hominin bipedality evolution, whereby a facultatively bipedal locomotor mode was adopted in response to a shift from arboreal to terrestrial habitats.

Owing to the nature the taphonomic process and the complicated 3D structure of the pelvis, it is not uncommon for pelvises to be poorly preserved or unpreserved in lizard fossils. We have included an assessment of an exemplar fossil specimen from Lepidosauria and provided our inference regarding their locomotor ecology, demonstrating the potential value of this work in the fossil record.

The Maastrichtian *Polyglyphandon* (Gilmore, 1942) has been hypothesized to lie nested within Macroteiida, a clade within Teiidae. Some species of teiid, such as *Ameiva ameiva*, are known to exhibit facultative bipedality (Urban, 1965). The pelvis of *Polyglyphanodon sternbergi* bears a striking resemblance to that of the northern caiman lizard (*Dracaena guianensis*), a semi-aquatic and semi-arboreal species featured in this study (Appendix D Figure D.2), and thus would be likely to score similarly in a PCA. Shared anatomical features are a stout iliac blade with a midshaft swelling, a small but distinct preacetabular process, a rounded iliac tip and a reasonably short ischium with a distinct ischial tuberosity. The pubis is similar in the two species, as are other parts of the pelvis. The northern caiman lizard is not placed within the range of facultative bipeds on any PC, thus we predict that *P. sternbergi* was probably an obligate quadruped; it most probably used a terrestrial substrate, potentially climbing low-level rockery or shrubbery. We do not predict a semi-aquatic lifestyle, as is observed in the northern caiman lizard, based on shared pelvic morphology, because there are many other whole-body osteological features that are more informative for such a behaviour, such as bone density and limb morphology.

4.6 Conclusion

In conclusion, we have used 3D landmark-based geometric morphometrics to demonstrate that the overall morphospace for the lepidosaur pelvis is broad and wide- ranging. Within this overall morphospace, a small region is occupied by facultative bipeds. The vast majority of this smaller morphospace overlaps that occupied by species that show a preference for arboreal habitats. Pelvic morphological adaptations relevant for living in an arboreal environment are similar to those necessary to facilitate facultative bipedality. These include a large preactebular process, straight-to-concave iliac blade and anteriorly projecting pubis, amongst others. These anatomies are functionally quantified relative to facultatively bipedal and arboreal locomotor behaviours. In light of these similarities in anatomy and functionality, we propose a hypothesis that a particular morphotype of arboreal species is naturally predisposed for facultative bipedality. Using the pelvis of *Polyglyphandon sternbergi*, we demonstrate how the morphological features highlighted in this study may be of use in predicting the locomotor mode or substrate preference of fossil taxa from pelvis osteology.

Chapter 5

Testing for the correlated evolution of facultative bipedality and arboreality in extant squamates using stochastic mapping, Bayesian and maximum likelihood methods, and the implications for evolving bipedality in lizards and other groups.

5.1 Abstract

The evolution of facultative bipedality is understood in varying degrees across vertebrates. Whereas detailed evolutionary models are established for primate and marsupial bipedality, the understanding of how this locomotor mode is acquired across reptiles in general is less clear. Previous chapters have identified similarities in osteology and body form between arboreal and facultatively bipedal species of extant squamate. Using stochastic mapping, Bayesian and maximum likelihood methods, I assess the correlated evolution of facultative bipedality with susbtrate preference in Episquamata, the most inclusive clade of squamates expressing facultative bipedality. I identified a systematic lack of correlation between arboreality and facultative bipedality in representatives of this clade. However, I did identify that in facultative bipeds, a cladistic history of arboreal substrate preference was present. A

similar pattern was present for substrates. This general proposition indicates that facultative bipedality is an exaptation of a preexisting anatomical form, rather than a selected-for trait within lizards. Additionally, this follows a similar model of evolution that hominins and macropod marsupials appear to exhibit, whereby arboreality and forested environments precede the acquisition of a bipedal locomotor mode. This promotes more detailed discussion and research into how environments affect locomotor transitions, particularly in other reptile groups such as early archosaurs.

5.2 Background

In the previous two chapters of this thesis (Chapters Three (Grinham and Norman, 2020a) and Four (Grinham and Norman, 2020b)), I have demonstrated a tendency for arboreal and known facultatively bipedal extant lepidosaurs to share a suite of morphological characteristics, including body proportions and osteological features of the pelvis. Deepening the understanding of how these similar body forms relate to each other in an evolutionary context will provide valuable insight into the mechanisms of acquiring a bipedal locomotor mode in these reptiles.

Answering biological questions of an evolutionary nature is challenged by our understanding of the observable natural world. In the context of this thesis, identifying key factors in the acquisition of facultative bipedality throughout deep time is not possible without first identifying where these bipedal acquisitions occur. With previous chapters highlighting anatomical and behavioural correlations between facultative bipedality and arboreality in extant lepidosaurs, we are better equipped than ever to examine the phylogenetic relevance, if any, of these two ecological traits.

Assessing the correlated evolution of traits poses several challenges. In this study, a correlated evolution indicates two binary traits transitioning while being dependent on each other within a phylogeny. For example, acquisition of either trait is followed by the other, closely or over a time period. This can be tested for using statistical measures, such as Bayesian or maximum likelihood analyses of evolutionary models, or can be examined using qualitative methods. Alternatives to correlated evolution of traits could be repeated instances of convergence, or multiple instances of an exaptation of existing features to a single common solution. In this instance, this would present itself as animals from multiple different substrate preferences exapting certain aspects of their biology into expression of a facultatively bipedal locomotor mode.

The use of phylogenetic comparative methods in this way is not a novel venture. The correlated evolution of a variety of traits has been conducted in the literature, across a range of species. Sexual systems in moss (Crawford et al., 2009), flowering systems and seed number (Bawa et al., 2019), and even behavioural traits in zebrafish (Kern et al., 2016) have all been examined under a correlated evolution framework using a variety of the methods mentioned above, in addition to morphological correlative methods. There are similarities in these assessments of correlated evolution to the model of correlated progression published by Kemp (2007) after many years of development. In essence, Kemp suggests that phenotypic evolution is subject to multidimensional selective pressures acting on various traits, subject to varying rates of evolution.

The aim of this study is to identify the evolutionary relationship between substrate use and locomotor mode in squamates. Previous work (Grinham and Norman, 2020a,b) suggests shared morphological features between facultatively bipedal and arboreal species. Identifying whether or not this shared body type is homoplastically convergent or a result of truly correlated evolution will help understand the evolution of facultative bipedality in lepidosaurs, and may provide valuable context regarding the evolution of bipedality in other reptile groups. Here, I use phylogenetic comparative methods to test for correlated evolution in traits of substrate use and locomotor mode.

5.3 Methods

A molecular-based supermatrix phylogeny for Squamata was sourced from the literature (Zheng and Wiens, 2016). Data for substrate use was sourced from a recent database of lizard ecological traits (Meiri, 2018). The tree and substrate data matrix were then both imported into the *R* statistical environment (R Core Team, 2017) and twice parsed using the *match* (base R; ((R Core Team, 2017)) and *drop.tip* (*ape* package; (Paradis and Schliep, 2019)) functions to eliminate those species missing from one dataset or the other. For every species in this reduced database, I collected data concerning locomotor mode from the literature or personal communications with observers, for a total of 2786 species. A binary character was constructed for expression of a facultatively bipedal locomotor mode, such that both obligate quadrupeds and legless lizards scored a '0' for quadrupedality, and facultative bipeds scored a '1' for facultative bipedality (note that Serpentes are not included in the Meiri database). For further analyses, the dataset was reduced to Episquamata, excluding clades Scincidae and Gekkota, which had the effect of reducing data processing demands on clades expressing no facultative bipedality. This resulted in a final dataset of 1379 species of which 88 were facultative bipeds.

Qualitative analyses of correlated evolution were conducted within *R*. Character matrices for each substrate featured in the Meiri database (arboreal, terrestrial, rock-dwelling, semi-aquatic) were combined with the Episquamata phylogeny using the *make.Simmap* function in *phytools* (Revell, 2012) to create stochastic trait maps under an equal rates of evolution model, known as SIMMAP modelling. A total of 100 maps were generated for each trait. The *densityMap* function in *phytools* was then used to generate a probability density map of these 100 maps for each trait. This density map was then plotted on the Episquamata tree, visualising facultative bipedality opposite a map for each substrate, with colours representing positive presence of each trait. By contrasting across each tree, we can compare cladistic histories of substrate use or facultative bipedality expression throughout the tree.

Quantitative analysis of correlated evolution was performed in *BayesTraits* computer software package (Version 3.0.2; http://www.evolution.rdg.ac.uk/BayesTraitsV3.0.2/BayesTraits V3.0.2.html). A nexus file of the Episquamata tree was exported from R using the *write.nexus* function in *ape* (Paradis and Schliep, 2019), along with character matrices for facultative bipedality and each substrate. *BayesTraits* was used to perform Markov Chain Monte Carlo tests of character correlation using the *Discrete* module. Each substrate was paired with presence of facultative bipedality and subject to both dependent and independent model tests. The independent model (*I*) assumed that the characters were capable of varying independent of each other, whereas the dependent model (*D*) allowed one character to vary based on the value of the other. ‘Priors’ were set to a standard exponential mean of 10, and the ‘stepping stones’ function used over 100 stones and 1000 iterations. The first 10,000 iterations were used as burn-in for the model and discarded. Determining the better-fitting model, dependent or independent was done using the log Bayes Factor (logBF; *log BF=2x[marginal likelihood complex [dependent] model – marginal likelihood simple [independent] model]*). Bayes Factor values in excess of two indicate evidence of a better dependent model fit, greater than five indicates strong evidence of a better fit, and greater than ten indicates very strong evidence of a better fit (Gilks et al., 1996). Negative values indicate a total lack of support for the dependent model. Additionally, maximum likelihood (ML) models were constructed, using the same substrate and bipedality combinations. Models were compared using a likelihood ratio test, (LR; *LR = 2*[log-likelihood better fitting model – log likelihood worse fitting model]*). Likelihood ratio values were compared to a χ^2 distribution table with four degrees of freedom (Pagel, 1994), and p-value <0.05 was used to indicate significant support for the better fitting model.

5.4 Results

5.4.1 SIMMAP models

Four comparable figures of facultative bipedality expression were created based on the stochastic model density maps (Figures 5.1-5.4). The left-hand side of each of these four Figures is identical, representing the distribution of facultative bipedality throughout the tree, with the right-hand side representing the different substrates. These Figures enable qualitative comparison of the emergence of facultative bipedality in association with substrate use.

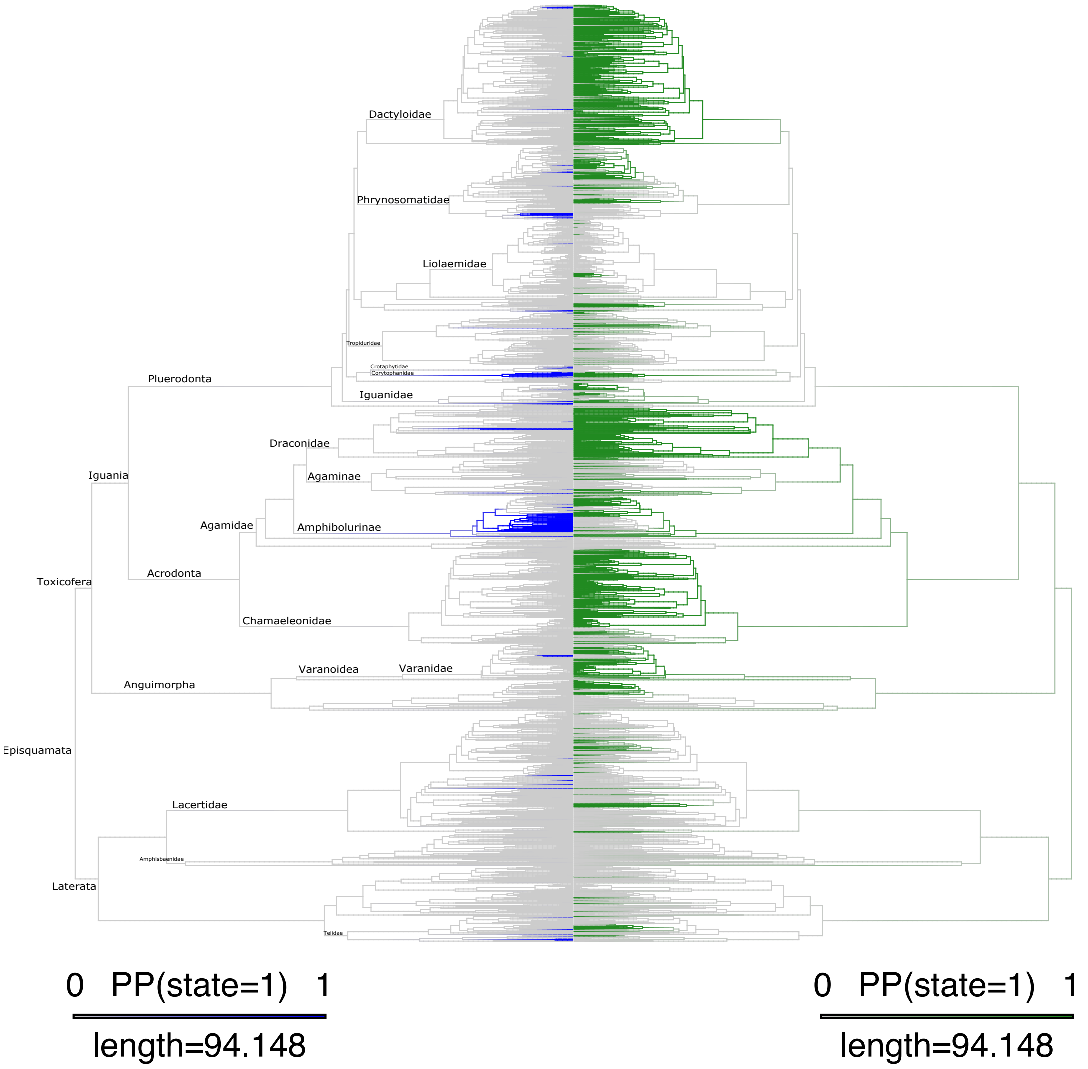

Fig. 5.1 Topologies indicating SIMMAP results for bipedality (left) and arboreality (right). Darker colours indicate more frequent presence of the character in model output.

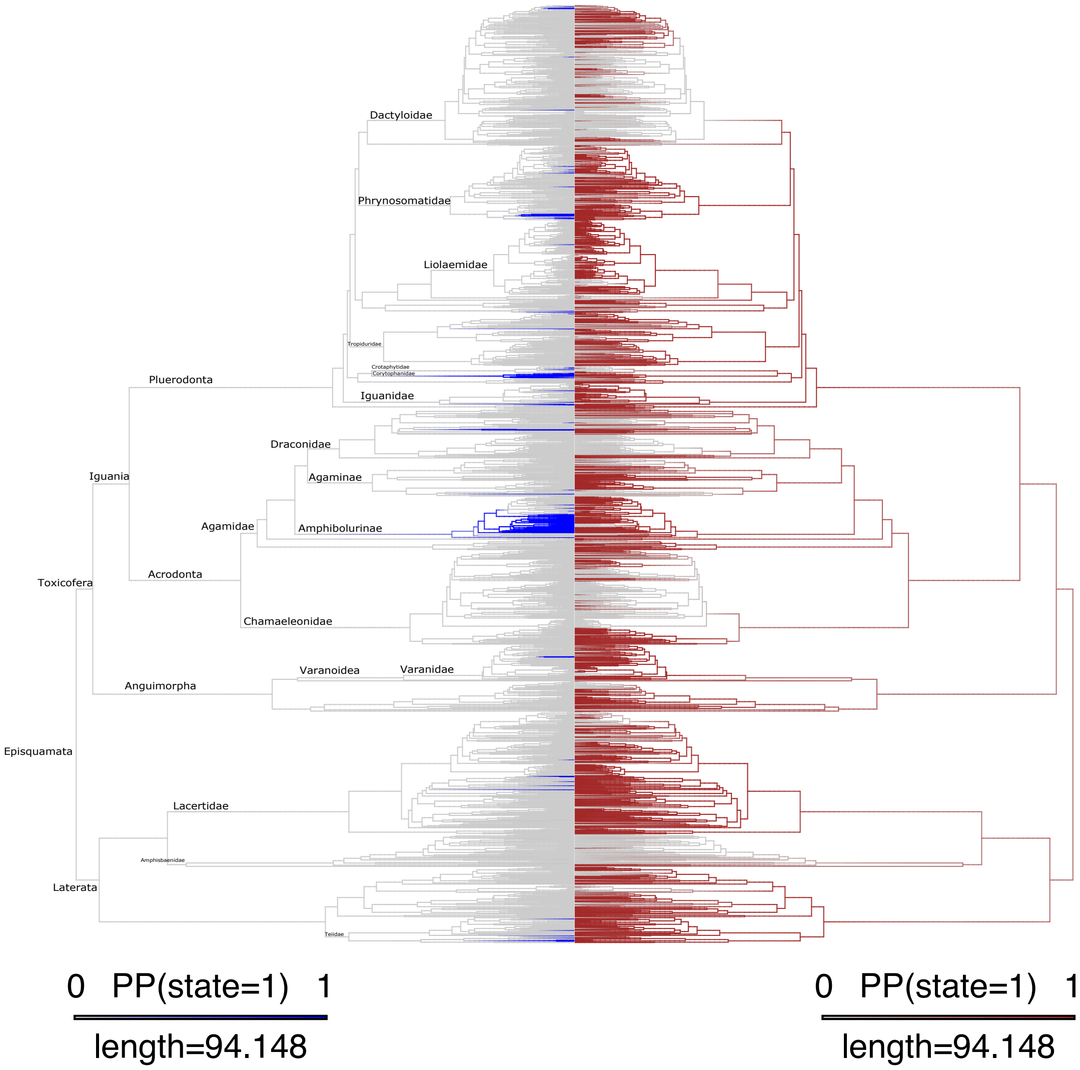

Fig. 5.2 Topologies indicating SIMMAP results for bipedality (left) and terrestriality (right). Darker colours indicate more frequent presence of the character in model output.

Facultative bipedality is distributed fairly pervasively, but sporadically, throughout Episquamata, present in species of anole (Dactyloidae) and spiny lizards (Phrynosomatidae), as well as many more iguanian and agamid species that are well documented (Greene et al., 1978; Hsieh, 2003; Irschick and Jayne, 1999b). This locomotor mode is also observed in species of teids and lacertids (Clemente, 2014). Several clades appear to adopt facultative bipedality as a frequently expressed behaviour, most notably collared lizards (Crotaphytidae), basilisk lizards (Corytophanidae), and comb-bearing dragons (*Ctenophorus*).

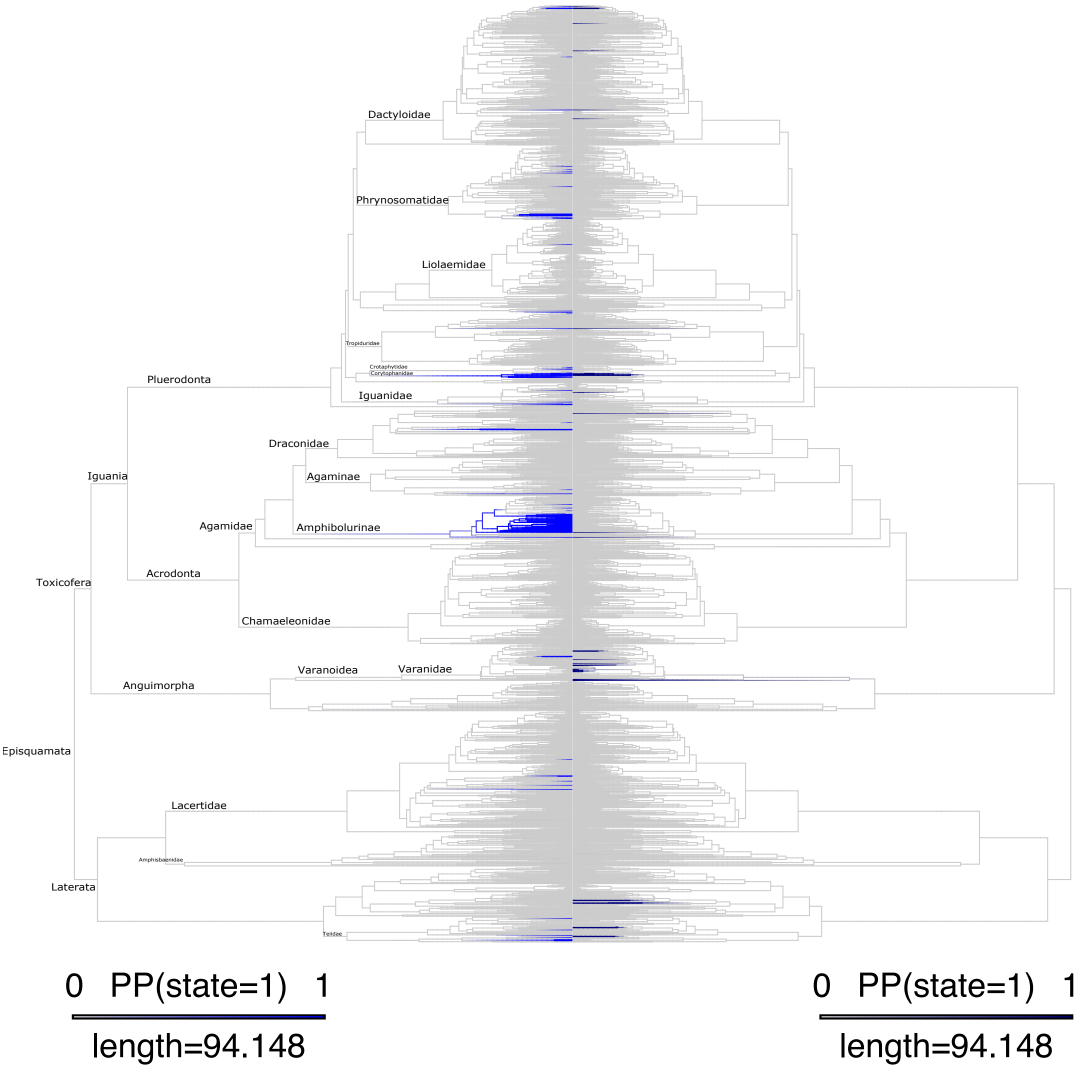

Fig. 5.3 Topologies indicating SIMMAP results for bipedality (left) and semi-aquatic use (right). Darker colours indicate more frequent presence of the character in model output.

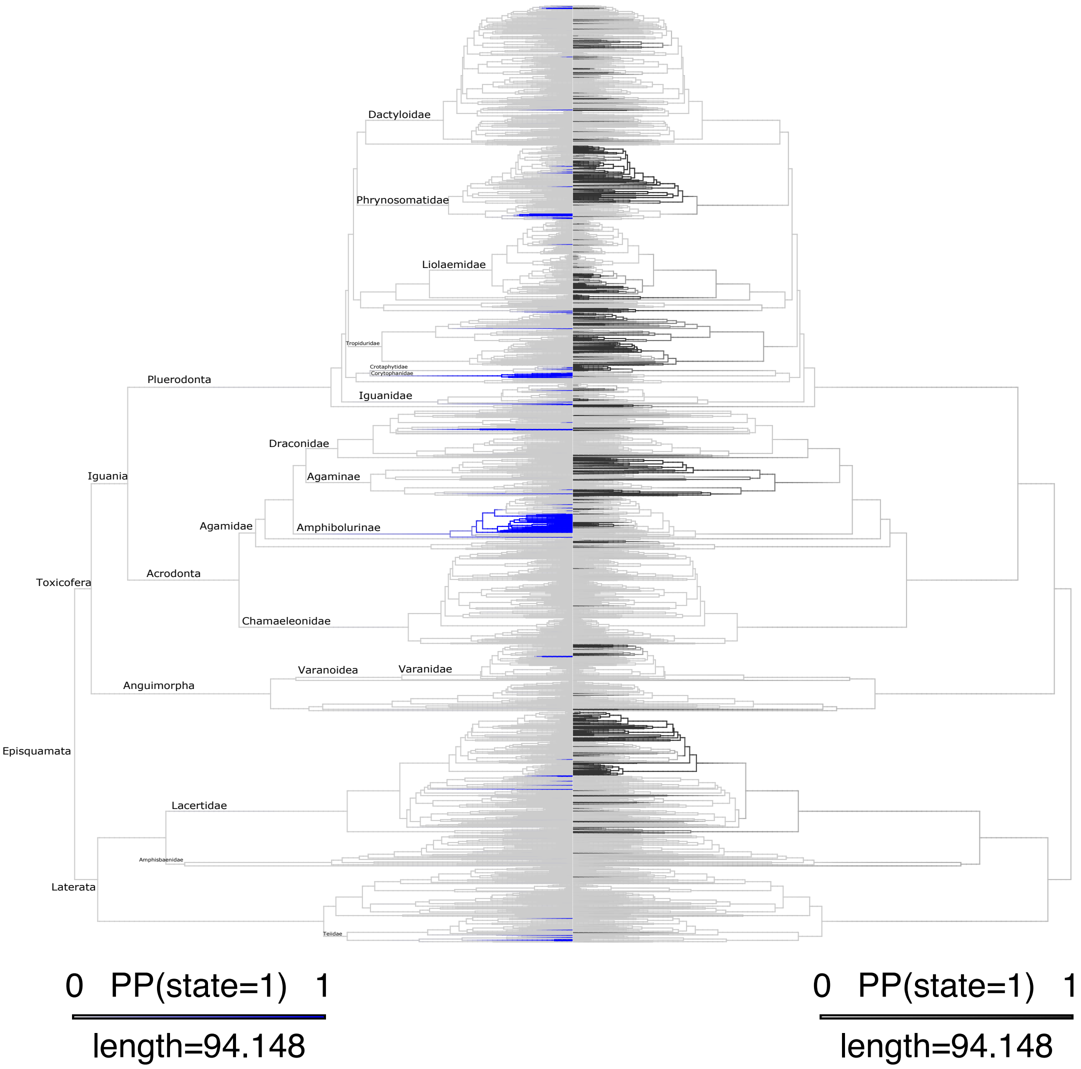

Fig. 5.4 Topologies indicating SIMMAP results for bipedality (left) and rocky substrate use (right). Darker colours indicate more frequent presence of the character in model output.

Contrastingly, arboreality is pervasive, being distributed across many clades. Arboreality is a commonly expressed within Episquamata, frequently adopted in a cladewise fashion, rather than individual species as observed in the cases of adoption of facultative bipedality.

Comparing these two density maps side-by-side (Figure 5.1), it is observed that the majority of species exhibiting facultative bipedality have a cladistic history of arboreality, even if use of an arboreal substrate has been abandoned in more recently derived species. In Amphibolurinae, adopting a facultatively bipedal locomotor mode often occurs in tandem with abrupt abandonment of arboreal substrate use. This pattern is also seen in some species of iguanid, varanid, and dactyloid species. By contrast, some clades retain arboreality as a primary substrate even after developing a bipedal locomotor mode, such as Corytophanidae. This association of arboreality and facultative bipedality holds reasonably well throughout Episquamata, with the exception of Laterata (Teiidae + Lacertidae), some species of which are facultative bipeds without a clade-derived history of arboreality.

Terrestrial substrate use is pervasive throughout the Episquamata tree (Figure 5.2), being present in every major clade – note that a terrestrial substrate is considered distinct from a fossorial one, as is common in Amphisbaenidae. Almost all facultative bipeds use a terrestrial substrate as a major component of their lifestyle.

A semi-aquatic capacity is expressed by several species of Episquamata (Figure 5.3), most notably the basilisk lizards (Corytophanidae). Within Iguania, many semi-aquatic species exhibit a facultatively bipedal locomotor mode. This correlation does not exist in non-iguanian clades, including Laterata and Anguimorpha.

Finally, a saxicolous lifestyle involving a significant rock-based substrate, based on boulders or a rock face, is reasonably common (Figure 5.4). In fact, within Amphibolurinae, a large portion of those facultative bipeds not exhibiting arboreality instead prefer a rock-based substrate. A similar pattern is observed in collared lizards (Crotaphytidae) and some anole species (Dactyloidae).

5.4.2 BayesTraits models

The BayesTraits MCMC modelling indicated no support for a dependent model of evolution between substrate and locomotor mode. Log Bayes Factor values were negative for all models (Table 5.1). Therefore, MCMC modelling indicates that facultative bipedality evolved independently of substrate use.

For ML models, the LR test indicated support for dependent models of evolution between rocky, terrestrial and semi-aquatic substrates ($p<0.05$, Table 5.2), but not for arboreal substrates ($p>0.99$, Table 5.2). These models, therefore, imply that locomotor mode and substrates that are not arboreality are evolutionarily linked.

Table 5.1 Markov Chain Monte Carlo analyses of substrate coevolution with facultative bipedality.

Substrate	Dependent log marginal likelihood	Independent log marginal likelihood	Log BF	Significance
Arboreal	-925.076	-909.819	-30.514	-
Rock	-905.924	-902.611	-6.626	-
Semi-aquatic	-403.33	-398.203	-10.254	-
Terrestrial	-1097.822	-1091.824	-11.996	-

Table 5.2 Maximum likelihood analyses of substrate coevolution with facultative bipedality.

Substrate	Dependent log-lik	Independent log-lik	Likelihood Ratio	Significance (p value)
Arboreal	-967.795	-967.903	0.216	>0.99
Rock	-951.687	-961.905	20.436	<0.05***
Semi-aquatic	-394.247	-411.7	34.906	<0.05***
Terrestrial	-1135.412	-1179.199	87.574	<0.05***

5.5 Discussion

The results presented here indicate several different key concepts. Firstly, we can see that facultative bipedality does not correspond consistently with use of an arboreal substrate in extant Episquamata (Figure 5.1). However, observing character mapping throughout Episquamata indicates a history of many clades exhibiting ancestral arboreality in the presence of facultative bipedality. It is possible that, in a large proportion of modern facultative bipeds, we are observing exaptation of the arboreal body form into something capable of facultative bipedality. The tendency of arboreal history in clades exhibiting facultative bipedality is indicative of this exaptation, rather than multiple independent instances of convergent evolution on this body form.

The results presented in this chapter indicate several different important results. Firstly, and most importantly, there is no consensus result across the two different methods. These are in discussed in due course.

Regarding the qualitative analysis, using stochastic mapping, the results indicate a lack of correlated evolution between arboreality and facultative bipedality in the extant taxa. However, facultative bipeds that do not use an arboreal substrate as a significant part of their lifestyle often have a cladistic history, or ancestral state, of arboreality. In the multiple observed instances of facultative bipedality acquisition occurring at a similar time to arboreal relinquishment, e.g. Dactyloidae and some Amphibolurinae species, it is reasonable to suggest that the arboreal body form becomes exapted for facultative bipedality. Given the similar body forms of facultative bipeds and arboreal dwellers, and the distribution of these characters throughout Episquamata, it does not appear that multiple instances of independent convergence on the same body form for two different purposes occur. Moreover, it can be argued that the arboreal body form is convergently acquired across multiple clades, and then exapted for other functions such as facultative bipedality in the majority of instances. The exception to this general pattern is Laterata, and further analysis into the employment of a facultative locomotor mode for those species in this clade would be fruitful in dissecting out subtler nuances associated with evolving facultative bipedality without an arboreal body form *per se*.

Terrestrial substrate use is commonly exhibited by facultative bipeds (Figure 5.2). This is unsurprising, since the majority of facultative bipeds perform this way on land (Clemente and Wu, 2018; Kinsey and McBrayer, 2018), though some basilisks and anoles are known to move bipedally through, or across the surface of, water bodies (Glasheen and Mcmahon, 1996; Hsieh, 2003). Given the pervasive distribution of terrestrial substrate use, by far the most common throughout sample used here and among reptiles generally, it is difficult to draw more meaningful conclusions regarding this substrate and the acquisition of the facultatively bipedal mode in an evolutionary context.

Those species exhibiting semi-aquatic substrate use, whilst few and far between (Figure 5.3), are of some interest regarding the evolution of a facultatively bipedal locomotor mode. Typically, semi-aquatic species are also arboreal and live in temperate forest or rainforest climes, with the most notable exceptions being marine iguanas and crocodile lizards. Semi-aquatic substrate use does not typically have a cladistic history throughout Episquamata, being distributed far too sparsely. As such, evolution of a semi-aquatic preference does not appear to be reliably associated with a facultatively bipedal locomotor mode.

Finally, for the stochastic mapping, we can consider the effect of rock-dwelling species (Figure 5.4), recorded as ‘saxicolous’ in Meiri (2018). Rocky substrates share many features as arboreal ones – primarily uneven terrain (rocks vs branches) with distinctive levels of elevation and scalable, vertical faces. Outside Laterata, arboreality and rock-dwelling substrates encompass the majority of facultatively bipedal species. Biomechanically, the demands between arboreal and rocky substrates are similar due to these shared features, which results in anatomies useful for bipedal locomotion (Grinham and Norman, 2020b).

Based on the four reconstructions presented here, there is evidence to suggest that the evolution of facultative bipedality in extant squamates is not correlated with any substrate. Instead, it appears that generalised transitions from vertical to more level habitats, such as arboreal or rock-dwelling to more terrestrial, results in locomotor transitions occurring. This pattern of vertical shifts in habitat being linked to locomotor mode is observed in the evolution of bipedality in hominin primates, macropodan marsupials, as well as in the evolution of flight among avian and potentially pterosaurian archosaurs.

Perspective shifts under consideration of the quantitative analyses performed in this chapter. There are several challenges associated with using advanced statistical phylogenetic comparative models in this study. The most prevalent of these lies in sample size: of 1379 species in the total analysed dataset, only 88 of these exhibit facultative bipedality (6.4%). Using statistical methods to investigate the trends associated with such a small percentage of cases is challenging. This contrasts with other studies examining instances of correlated evolution that is immediately apparent when simply observing a character map (Bawa et al., 2019). Nevertheless, I discuss the results presented below.

Under MCMC models, I recover no support for a dependent model of evolution of any substrate in combination with facultative bipedality (Table 5.1). This indicates that under a continuous time Markov model, where traits and evolutionary rates are allowed to vary independently, a correlation between a substrate and facultative bipedality is not recovered. This implies that there is no clearly supportable correlation between the evolution of substrate preference and facultative bipedality. The absence of a correlation tends to corroborate the conclusions derived from the stochastic analyses above.

The final analysis, using maximum likelihood evolution models, indicates that a correlation can be identified between bipedality and each of terrestrial, semi-aquatic and rock-dwelling substrates ($p<0.05$; Table 5.2). This contrasts with the two prior reported methods. Though these results are presented here for transparency, I do not have confidence in this analysis, for the reasons discussed below.

Maximum likelihood models of analysis and Bayesian inference MCMC models should output similar results, given the absence of missing data in our final dataset (Brooks et al., 2007). The recovery of significant support for the dependent models of correlated evolution of bipedality with multiple substrates, despite MCMC not supporting correlation is intriguing. A recent study comparing the two methods indicated that MCMC methods are typically more robust under the presence of a weak phylogenetic signal, such as is present in this study (Nagy et al., 2010). Additionally, some studies that do employ maximum likelihood methods can be observed to alter the LR formula away from the advised form in Pagel (1994). For example, in (Crawford et al., 2009), the standard formula for LR is subject to a negative sign multiplier, resulting in negative terms being made positive, which could turn negative results positive under some circumstances. Additionally, the term order is designed to be flexible, such that the independent or dependent model may come first (Pagel, 1994), which is not followed in the cited work. The variation in application of this test in the literature reduces the confidence I hold in the application of this method, and for this reason I do not consider the maximum likelihood analysis as robust as the MCMC method.

In light of the evidence presented here, being the consensus seemingly shared by MCMC and SIMMAP methods, I reject the hypothesis that the acquisition of a facultatively bipedal locomotor mode is correlated with substrate use in extant squamates. Instead, based on the results above, facultative bipedality appears to be enabled by exaptation of body form changes required for vertical elevation trends in substrate use. Hence, a greater prevalence of bipedal locomotor capacity in clades with a history of arboreal or rocky substrate preference. Understanding the instances of facultative bipedality exhibition outside of these instances, such as in Laterata, will require more detailed analysis drawing heavily on the specific biomechanics of animals in these groups (Aerts et al., 2003; Urban, 1965).

5.5.1 Wider implications

Within Lepidosauria, facultative bipedality has been recorded as far back as 110Mya (Lee et al., 2018), and is therefore a deeply rooted feature of their evolutionary history. However, unlike some groups known to have exhibited facultative bipedality (hominins, archosaurs), an obligately bipedal evolutionary "end state" has not yet been reached. Instead, better comparative groups for understanding the relationship of environment to bipedal capacity can be found within Metatheria—Macropodidae. Within this clade, which includes wallabies, kangaroos and tree-kangaroos, many species exhibit facultative bipedality. Now, notably, the biomechanical differences between squamates and kangaroos are profound, as are their extensive biological characters. For example, squamates do not use an elastic tendon-driven hopping system as kangaroos do (Kram and Dawson, 1998). However, the current hypothesis of the evolution of hopping within this group centres around a forested ancestral habitat (McGowan and Collins, 2018). Similarities can also be seen in bipedal rodents, such as kangaroo rats and jerboas (Rankin et al., 2018), despite living in primarily arid desert environments in their extant representatives, ancestrally they appear to have inhabited humid forested environments, and are hypothesised to have been facultatively bipedal at this time (McGowan and Collins, 2018). As explored in some detail in Chapter Two (Grinham et al., 2019), the hominin model of bipedality evolution also shows a similar trend, beginning in an arboreal, forested environment, then employing facultative bipedality alongside a semi-arboreal lifestyle involving extended periods on the ground (Senut et al., 2018).

There is, therefore, a trend evident. Facultative bipedality is acquired, in three of the major exhibiting clades, during habitat transitions out of densely forested environments. This appears to happen when a group of arboreally specialised animals becomes more dependent on a terrestrial substrate, either as a result of forest-thinning or habitat transition. Interestingly, this hypothesis does not translate to the other major clade exhibiting facultative bipedality: Archosauriformes.

Through the Late Permian to Late Triassic, during which facultative bipedality appears to have emerged most frequently in archosaur-line reptiles (Grinham et al., 2019), there is little to suggest the animals in question meet the aforementioned conditions. Whilst forested habitats, comparable to those present in the transitions of mammalian and lepidosaurian groups, were indeed present (Brea et al., 2008), their use in Archosauriformes and their immediate ancestors is vastly different from the mid-to-late Cenozoic groups. Outside Dinosauria, the basal representatives of which were bipedal, arboreality is poorly represented within Archosauriformes, and even less so within those taxa hypothesised to be facultatively bipedal. Even widening the search for all of Archosauromorpha, only *Megalancosaurus* (Renesto, 1994), drepanosaurs, and *Trilophosaurus* (Spielmann et al., 2005) have been hypothesised to be arboreal. Additionally, the capacity for an equivalent level of arboreality to the modern lepidosaur is unlikely. Permo-Triassic floras differ significantly from the modern rainforest equivalent most common in today's facultatively bipedal representatives. Whereas modern forests are predominantly woody trees with branching structures, Late Permian and Triassic forests were formed of ferns and gymnosperms (Nowak et al., 2019), these arboreal equivalents lack the range and variety of horizontal platforms throughout the vertical extent (i.e. boughs branching from trunks) that are used by lizard species today. Additionally, modern and extinct archosaurs have typically occupied a much larger size average than seen in modern lizards, exhibiting a far less agile and "tree friendly" body form than we observe in modern representatives of the lizard group.

Thus the question of the evolutionary drivers of bipedality in the ancestors to dinosaurs remains unclear. Neither fossil nor palaeobotanical evidence points towards a similar set of ecological conditions being present in the evolution of archosaur facultative bipedality compared to that of hominins, squamates and macropod marsupials. Natural discussion-points to follow in the future relate to archosauriform joint motility compared to those of lepidosaurian and mammalian species, particularly in the hip, knee and ankle. Arboreal behaviour has been observed in extant crocodilians (Dinets et al., 2014), despite not bearing typical hallmarks of arboreality in their body form—small bodies, long distal hindlimbs and sharp claws. As such, further investigation into the ecological conditions of Permo-Triassic archosauriforms is necessary to further assess the conditions under which facultative bipedality evolved and contributed to the bipedality that characterised Dinosauria as a clade.

5.6 Conclusion

In summary, facultative bipedality does not appear to evolve in tandem with any particular substrate preference in extant squamates. However, a cladistic history of rocky or arboreal habitat preferences does appear to precede the expression of a facultatively bipedal locomotor mode in many cases, particularly within Iguania. This pattern of evolution is also observed in the evolution of hominin and macropod bipedality, though is not hypothesised in the evolution of archosaur bipedality. The data presented in this study provokes further investigation into the ecological setting and biomechanical capacity of Permo-Triassic archosauriformes. Better understanding the palaeoecological and functional capacity of early archosaurs and their predecessors will enable better evolutionary models regarding their locomotor transitions to be constructed, and shed light on the origins of dinosaurian bipedality.

Chapter 6

Conclusions

6.1 Summary

Despite many years of research being dedicated to the evolution and mechanisms of bipedality in Archosauria, this thesis has demonstrated that facultative bipedality is not as comprehensively understood as previously indicated within living reptile species. The evolution of bipedality in Diapsida remains one of the most intriguing features of the clades within. Bipedality is a hallmark characters of Dinosauria in the first instance and has enabled the evolution of powered flight in avian dinosaurs. Despite many years of research utilising a variety of advanced methods (Alexander, 2006; Hutchinson, 2004b; Persons and Currie, 2017), our understanding of the evolution of bipedality in reptiles is still lacking. In this thesis, I have demonstrated how our understanding of the anatomies associated with a facultatively bipedal locomotor mode were less resolved than previously thought, and have provided a basis on which to further investigate facultative bipedality within reptiles. Identifying a fundamental lack of support for the classical hypothesis of archosaur bipedality evolution shaped the direction of this thesis (Chapter Two). Being unable to identify facultative bipedality as a consistent intermediary stage from obligate quadrupedality to obligate bipedality indicated a necessity to better understand facultative bipedality as a locomotor mode within reptiles. I decided to approach this from the extant record, using anatomical, ecological and behavioural data in a reptilian clade exhibiting facultative bipedality to better understand the demands and drivers of this so-called intermediate stage. These studies revealed that facultative bipedality in extant Lepidosauria was similarly misunderstood. A re-examination of the relationship between body segment lengths, body size and locomotor mode (Chapter Three) contrasted with historic tropes indicating differences in forelimb lengths and femoral lengths between obligate quadrupeds and facultative bipeds (Snyder, 1962). Additionally, this study indicated the value of non-caudal skeletal material in predicting locomotor mode, especially with regards to body size. As long tails are unquestionably indicative of bipedal capacity, understanding that other body metrics are of similar indicative power is relevant for the fossil record applications of this work. Interestingly, there appear to be many functional similarities between use of an arboreal substrate and the use of a facultative locomotor mode. These were explored in Chapter Four, wherein shared morphological aspects of the pelvises of facultatively bipedal and arboreal dwelling lizards were highlighted. Previous studies have identified the link between arboreal substrate use and locomotor variation in many clades (Granatosky, 2018; McGowan and Collins, 2018; Rankin et al., 2018; Senut et al., 2018), and Lepidosauria appears to be no exception to this trend, as further explored in Chapter Five using a phylogenetic comparative framework. The findings of this thesis make an exciting contribution toward the understanding of lepidosaur locomotor ecology and evolution. As in many other tetrapod groups, preferences for vertically diverse habitats, such as tree- or rock-based substrates, appears to promote diversity in locomotor function. Here, I have only considered facultative bipedality at length, but capacity to swim and glide are often also represented in tandem with preference for an arboreal lifestyle. However, beyond this, the results presented here also promote a renewed assessment of locomotor evolution and the associated anatomies with transitionary locomotor modes in Archosauria.

6.2 Limitations

This thesis has primarily conducted a multi-analytical assessment of the ecomorphology of facultative bipedality in extant Lepiodsauria. Throughout the studies presented here, species diversity has been high and representative of a wide range of facultatively bipedal and obligately quadrupedal animals. Where appropriate, phylogenetic corrections have been applied to correct for interspecific similarity. One of the most significant limitations challenging the results shown here can be attributed to ontogenetic scaling throughout Lepidosauria. Due to the challenges associated with obtaining museum specimens representing a varied growth stages for a wide range of species, the studies presented here only dealt with adult specimens. When considering the relationship between shape and function, ontogenetic shape variation should be considered. Ontogenetic data cannot be included alongside adult data in studies such as Chapter Three as differences in locomotor mode may then be hidden by the ontogenetic allometry that species typically express (Esquerré et al., 2017; Irschick and Jayne, 2000). This is, however, an area that should be considered in future work. As Irschick and Jayne (2000) demonstrated in a facultatively bipedal lizard, ontogenetic scaling can have a significant effect on kinematic performance during locomotion. Ontogenetic studies must therefore be constructed to compare growth curves of multiple species with equal ontogenetic representation, relative to locomotor mode. Additionally, analyses of substrate use in Chapters Four and Five could be further categorised into microhabitats rather than preferred substrates, as done in a recently published study on squamate tail spines correlations with microhabitat use (Ramm et al., 2020). Whereas in these analyses species could score in multiple substrates, e.g. significant terrestrial use scored positively for terrestriality, categorising by microhabitat would enable higher resolution grouping of species into similar substrate preferences. This may reveal correlations between locomotor mode and substrate use not identified in the analyses presented here. However, I do not believe this will be significantly more informative than the presented results, given the associations between substrate and locomotor mode identified in Chapter Four.

6.3 Future directions

Future work should focus on two key directions. Firstly, functionally quantifying the biomechanical similarities between arboreal and facultatively bipedal locomotion in lizard species exhibiting both is essential. Some studies have considered aspects of arboreal locomotion and its comparison to terrestrial locomotion (Higham et al., 2011), but few multi-specific kinematic and biomechanical analyses have been conducted. These studies would require multiple specimens of species that exhibit both arboreal locomotion and terrestrially bipedal locomotion, such as frilled lizards and basilisks, and high speed kinematic footage of these lizards performing under both natural and maximally performative conditions. Additionally, comparing *in vivo* bone stresses during these two different locomotor styles would allow an even better comparison of the varied demands of moving in these ways.

Secondly, additional methods of ancestral state reconstruction could be applied, particularly with regards to the second chapter of this thesis. Additional models constraining evolutionary rates and directions could be constructed, as opposed to only equal rates transition models. For example, exploring where transitions are recovered in the Nesbitt and Ezcurra phylogenies under a model preferring obligate bipedality evolving only from facultative bipedality, as opposed to obligate quadrupedality, may be informative and help direct anatomical study of specific taxa.

Thirdly, and following from the second point, this thesis raises a clear case for renewed assessment of archosaur facultative bipedality broadly. Reconsidering the necessary anatomy to enable a facultative mode is essential, especially in light of the results presented here indicating a divergence from the historically perceived requirements in lizards. Even with behavioural data available, it can be challenging to resolve the finer aspects of anatomy enabling this mode, and this becomes even more problematic in the fossil record. This proposed future research should also address wider aspects of the palaeoecology associated with the reptiles that are experimenting with a facultatively bipedal locomotor mode. Naturally, an arboreal locomotor mode is not expected of these experimental reptiles, given the lack of hypothesised arboreality within Permian and Triassic archosaurs. However, understanding the niches filled by facultative bipeds will aid an understanding of how bipedality was effective in the earliest stages of dinosaurian evolution. This is essential information required to improve an understanding of how a characterising feature of one of the most studied animal groups to have ever lived, the dinosaurs, came into use.

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Appendix A

Locomotor mode classifications and sources for all original taxonomic units in Chapter Two, as published in Royal Society Open Science.

Table A.1 Locomotor mode classifications and sources for all original taxonomic units in Chapter Two.

Taxon	Source (Description where applicable; Diagnostic paper if different to original)	Locomotor mode	Text or diagram based diagnosis and supporting statement
Acerosodontosaurus piveteaui	(Currie, 1980); (Bickelmann et al., 2009)	OQ	Text – “aquatic” therefore quadruped based on assumptions in the manuscript.
Aenigmastropheus parringtoni	(Ezcurra et al., 2014)	OQ	Diagram – text states “k value … higher than those observed in aquatic and semi-aquatic animals, supporting a terrestrial mode of life”.
Aetosauroides scagliai	(Casamiquela, 1960); (Desojo et al., 2013)	OQ	Text – “a clade of obligately quadrupedal, heavily armoured pseudosuchians”.
Aetosaurus ferratus	(Schoch, 2007); (Desojo et al., 2013)	OQ	Text – as above.
Alligator mississippiensis	(Daudin 1801); (Allen et al., 2010)	OQ	Text – “Extant crocodylians are the only known living tetrapods to use nearly the full range of recognized quadrupedal terrestrial locomotion patterns”.
Allosaurus fragilis	(Marsh, 1877); (Farlow et al., 2000)	OB	Text – “Theropods were erect, digitigrade, striding bipeds”.

Amotosaurus rotfeldensis	(Fraser and Rieppel, 2006)	OQ	Text – semi-aquatic “juveniles of T. antiquus were at least partly terrestrial. By contrast, they became fully marine when adult”. Juvenile *T. antiquus* specimens were reassigned to *Amotosaurus rotfeldensis*. Also, “clearly a tanystropheid”, a group which exhibits an unusual, long-necked morphology.
Archeopelta arborensis	(Desojo, Ezcurra and Schultz, 2011)	OQ	Diagram – within the text, a possible aquatic lifestyle is alluded to, or a terrestrial foraging lifestyle.
Arizonasaurus babbitti	(Welles, 1947); (Nesbitt, 2005)	OQ	Diagram – no caudal vertebrae or limb bones. Diagnosed based on a reconstruction.
Asilisaurus kongwe combined	(Nesbitt et al., 2010)	OQ	Text – “a member of the Silesauridae”, “Silesaurids were diverse … with … a quadrupedal stance”.
Azendohsaurus madagaskarensis	(Flynn et al., 2010); (Nesbitt et al., 2015)	OQ	Diagram – the text contains very thorough descriptions of many specimens that suggest quadrupedality without explicitly stating it. “an elongated neck, a short, stocky tail, robust limbs”.
Batrachotomus kupferzellensis	(Gower, 1999); (Gower and Schoch, 2009)	OQ	Diagram – largely complete post-crania with a complete skull. Diagram is in a quadrupedal pose.
Bentonyx sidensis	(Langer et al., 2010); (Ezcurra, Montefeltro and Butler, 2016)	OQ	Text – “rhynchosaurs were bulky, herbivorous and quadrupedal animals”

Chanaresuchus bonapartei	(Romer, 1971b); (Romer, 1972a)	OQ	Text – “I have restored the animal as a quadruped”. However, Romer tentatively debates facultative bipedality as a consequence of an amphibious lifestyle. As this animal is a terminal taxa deep in a OB section of the tree, a character state change here would not affect the rest of the analysis.
Chasmatosaurus yuani	(Young, 1936); (Charig and Reig, 1970)	OQ	Text – “they were undoubtedly quadrupedal, despite the typical archosaurian limb disparity”.
CM 73372	(Weinbaum, 2013)	OB	Text – CM-73372 is used for informing the anatomy of *Postosuchus*, deemed “an obligate biped” based on “limb proportions ... the size of the manus ... and the highly reduced nature of the digits and vertebral measurements”, thus is considered a biped here, though this is contentious. Removed in sensitivity analysis.
Coelophysis bauri	(Cope, 1887)	OB	Text – also in many studies since. Unequivocal derived theropod, thus bipedal.
Cteniogenys spp	(Gilmore, 1928); (Evans, 1990)	OQ	Text – many aquatic features, thus diagnosed as quadrupedal.
Dibothrosuchus elaphros	(Simmons, 1965); (Wu, 1986; Wu and Chatterjee, 1993)	OQ	Text – “The limbs are long, slender and adapted for quadrupedal terrestrial gait”.
Dilophosaurus wetherelli	(Welles, 1954)	OB	Text – theropod dinosaur, bipedal.

Dimorphodon macronyx	(Buckland, 1829); (Frigot, 2018)	OQ	Text – "*Dimorphodon* was an obligate quadruped." However, it should be noted that pterosaur posture and gait is contentious, with support for both bipedal and quadrupedal stances prevalent in the literature (Padian, 2008; Witton, 2015)
Dongusuchus efremovi	(Sennikov, 1988); (Nesbitt et al., 2017)	OQ	Diagram – however, the text states that Aphanosaurs were "long-necked, non-cursorial" and "so more like stem-archosaurs than later avemetatarsalians", which all suggest obligate quadrupedality.
Doswellia kaltenbachi	(Weems, 1980), (Dilkes and Sues, 2009); (Sues, Desojo and Ezcurra, 2013)	OQ	Text – in Weems (1980): "front limbs almost certainly functioned as strong walking structures", facultative bipedality is mentioned and dismissed as a possibility based on pelvic osteology. Sues et al (2013) suggests semi-aquatic lifestyle, hence OQ.
Dromomeron gregorii	(Sterling J. Nesbitt, Irmis, et al., 2009); (Fechner, 2009)	FB	Text – anatomical analysis, identifies *Dromomeron* as facultatively bipedal
Dromomeron romeri	(Irmis et al., 2007); (Fechner, 2009)	FB	Text – anatomical analysis, identifies *Dromomeron* as facultatively bipedal
Effigia okeeffeae	(Nesbitt and Norell, 2006); (Nesbitt, 2007)	OB	Text – "The convergences suggest that a 'theropod dinosaur body plan' developed in a group of crocodile-line archosaurs before it appeared in theropod dinosaurs.". This indicates bipedality, supported by a diagram in a bipedal pose.

Efraasia minor	(Huene, 1908); (Galton, 1973; Kubo and Kubo, 2012)	FB	Text – anatomical analysis indicated *Efraasia* exists in a facultatively bipdal region of Kubo and Kubo's Quadrupedality Index
Eoraptor lunensis	(Sereno et al., 1993); (Sereno, Mar Inez and Alcober, 2013)	OB	Text – basal sauropodomorph, limb proportions "consistent with bipedal posture at speed", but does not mention quadrupedality, therefore classified bipedal. Additionally, represented as a biped in the skeletal reconstruction.
Erythrosuchus africanus	(Broom, 1905); (Ezcurra, Butler and Gower, 2013)	OQ	Diagram – text states that no detailed locomotor studies have been performed, previous studies have alluded to a possible semi-aquatic lifestyle.
Eudimorphodon ranzii	(Zambelli, 1973); (Witton, 2015)	OQ	Text – See *Dimorphodon* for discussion regarding pterosaur posture
Euparkeria capensis	(Broom, 1913); (Ewer, 1965)	FB	Text – various morphological analyses in the same vein as "this contrast between fore and hind limbs is strongly suggestive of facultative bipedalism".
Garjainia madiba combined	(Gower et al., 2014)	OQ	Diagram – life reconstruction by paleontologist and paleoartist Mark Witton. *Garjainia prima* is diagnosed by text in (Ezcurra et al., 2018), which is a close relative.
Garjainia prima	(Ochev, 1958); (Ezcurra et al., 2018)	OQ	Text – "The erythrosuchids were quadrupedal, probably sprawling and bulky, predatory archosauromorphs".
Gephyrosaurus bridensis	(Evans, 1980); (Evans, 1981)	OQ	Text – *Gephyrosaurus* shows no structural changes in this direction and it was probably a swift quadruped" .

Gracilisuchus stipanicicorum	(Romer 1972)	FB	Text – “It is possible that the normal pose of *Gracilisuchus* was a quadrupedal one; however I feel sure that, if pressed, this animal was able to run in the bipedal manner in which I have restored it.”. This has been reiterated in works completed since, e.g. (Kubo and Kubo, 2012).
Gualosuchus reigi	(Romer, 1971b)	OQ	Text – Semi-aquatic: “The general structure of these four genera is strongly suggestive of amphibious habits”.
Herrerasaurus ischigualastensis	(Reig, 1963); (Sereno and Novas, 1992)	OB	Text – “*Herrerasaurus*, a primitive theropod, was an agile, bipedal predator”.
Hesperosuchus agilis	(Colbert, 1952)	FB	Text – “It is probable that *Hesperosuchus* … also utilised both the quadrupedal and bipedal types of locomotion.”
Heterodontosaurus tucki	(Crompton and Charig, 1962); (Pontzer, Allen and Hutchinson, 2009)	OB	Text – “… in the small, presumably active bipeds, *Hesperosuchus*, *Compsognathus* and *Velociraptor*”.
Jaxtasuchus salomoni	(Schoch and Sues, 2014)	OQ	Text – semi-aquatic: “*Jaxtasuchus* has an elongate body form with a long tail and proportionately small limbs, which, together with its extensive dorsal armour, lent it a rather crocodile-like appearance that, in turn, may reflect an at least semi-aquatic mode of life”.

Jesairosaurus lehmani	(Jalil, 1997)	FB	Text – “The elongation of the hindlimb associated with the shortening of the neck , in comparison with other prolacertiforms, might be correlated with facultative bipedalism in *Jesairosaurus*.”.
Lagerpeton chanarensis	(Romer, 1971a); (Fechner, 2009)	FB	Text – anatomical analysis indicates facultative bipedalism, “The transversely broad pelvis, the adductor-controlled postural support and especially the intermediate hindlimb posture clearly indicate that obligate bipedal locomotion has to be ruled out for *Lagerpeton*.”
Lesothosaurus diagnosticus	(Galton, 1978); (Bates et al., 2012, 2015; Baron, Norman and Barrett, 2016)	OB	Text – anatomical, 3D computational modelling. Correction paper (2015) confirms that major findings from original study (2012) are not changed.
Litargosuchus leptorhynchus	(Clark and Sues, 2002); (Gow and Kitching, 1988)	FB	Text – previous analysis when the specimen was assigned to *Pediticosaurus* state that “*Pediticosaurus* was unquestionably bipedal at speed”. Though the specimen has been reassigned, the available material has not changed, and locomotor mode has not been reassessed.
Longosuchus meadei	(Sawin, 1947); (Desojo et al., 2013)	OQ	Text – “All aetosaurs were obligate quadrupeds”. *Longosuchus* is alternatively named *Typothorax*.
Lotosaurus adentus	(Zhang, 1975); (Hagen et al., 2018)	OQ	Text – assortment of anatomical features “indicative of quadrupedality”.

Machaeroprosopus pristinus	(Mehl, 1928); (Stocker and Butler, 2013)	OQ	Text – “They were quadrupedal” in reference to phytosaurs generally, this animal is included in their list of phytosaurs.
Macrocnemus bassanii	(Nopcsa, 1930); (Rieppel, 1989)	FB	Text – “A functional analysis of the appendicular skeleton suggests predominantly terrestrial habits, perhaps even facultative bipedalism during rapid locomotion”.
Marasuchus lilloensis	(Romer 1972b); (Sereno and Arcucci, 1994)	OB	Text – “Two small bipedal archosaurs in the Middle Triassic Los Chanares fauna, *Lagerpeton chanarensis* and *Marasuchus lilloensis*, provide the best available evidence of ornithodirans that predate the Late Triassic America radiation of dinosaur”. Based on anatomical analysis and reconstruction of a near complete specimen.
Mesosuchus browni	(Watson, 1912b); (Dilkes, 1998)	OQ	Diagram – skeletal reconstruction in quadrupedal stance based on a number of different specimens
Nicrosaurus kapffi	(Meyer, 1860); (Kimmig, 2013; Stocker and Butler, 2013)	OQ	Text – As for all phytosaurs, “They were quadrupedal” and *Nicrosaurus* is included in the list of phytosaurs in the Stocker and Butler (2013) analysis
Noteosuchus colletti	(Watson, 1912a); (Ezcurra, 2016; Ezcurra, Montefeltro and Butler, 2016)	OQ	Text – “rhynchosaurs were bulky, herbivorous and quadrupedal animals”.
Nundasuchus songeaensis	(Nesbitt et al., 2014)	OQ	Diagram – relatively complete hindlimb, partial forelimb, partial postcrania.

Ornithosuchus longidens	(Huxley, 1877); (von Baczko and Ezcurra, 2013; von Baczko and Desojo, 2016)	OQ	Text – “*Ornithosuchus* might have been facultative bipedal animals, acquiring an upright posture during fast gaits.” was a previous interpretation, but this has been more recently overwritten by “*Ornithosuchids* are terrestrial quadrupedal carnivorous archosaurs”. There is scope for considering *Ornithosuchus* a facultative biped.
Pamelaria dolichotrachela	(Sen, 2003)	OQ	Text – “The reconstructed skeleton … suggests that it was a quadrupedal walker.”.
Parasuchus hislopi	(Lydekker, 1885); (Chatterjee, 2001; Stocker and Butler, 2013)	OQ	Text – As for all phytosaurs, “They were quadrupedal” and *Parasuchus* is included in the list of phytosaurs in the Stocker and Butler (2013) analysis
Petrolacosaurus kansensis	(Lane, 1945); (Peabody, 1952)	OQ	Text – “*Petrolacosaurus* was terrestrial, fully quadrupedal in gait, perhaps arboreal, and that it was capable of rapid locomotion.”.
Planocephalosaurus robinsonae	(Fraser, 1982); (Fraser and Walkden, 1984)	OQ	Text – “The small limb disparity in quadrupedal eosuchians, such as *Planocephalosaurus*”
Plateosaurus engelhardti	(Meyer, 1837); (Mallison, 2010)	OB	Text – “Here, I present evidence, derived from a detailed mounting of a 3D digital skeleton and a computer-aided engineering assessment of a digital 3D model of the living animal, that *Plateosaurus* was indeed an obligate biped.”.

Polonosuchus silesiacus	(Sulej, 2005)	OQ	Text – “The new species was probably similar to *P. kirkpatricki* in general view. *P. kirkpatricki* was a medium-sized (3 m long) heavily built rauisuchid, a large- skulled and short-necked, non-cursorial quadruped”.
Poposaurus gracilis holotype	(Mehl, 1915); (Gauthier et al., 2011; Schachner, Manning and Dodson, 2011)	OB	Text – “This animal represents the most complete poposauroid skeleton known to date, and one of the most complete bipedal basal archosaurs yet discovered”.
P*oposaurus gracilis* yale	(Mehl, 1915); (Gauthier et al., 2011; Schachner, Manning and Dodson, 2011)	OB	Text - “This animal represents the most complete poposauroid skeleton known to date, and one of the most complete bipedal basal archosaurs yet discovered”
Postosuchus alisonae	(Peyer et al., 2008); (Weinbaum, 2013)	OB	Text – “evidence … suggests that *Postosuchus* may have been habitually bipedal”, “Analysis of the postcranial skeleton of *Postosuchus* suggests it may have been an obligate biped” based on anatomical features. The analysis in this study is based on mulitiple *Postosuchus* specimens, so we apply it to both *P. alisonae* and *P. kirkpatricki* here.
Postosuchus kirkpatricki	(Chatterjee, 1985); (Weinbaum, 2013)	OB	Text – As above.
Prestosuchus chiniquensis	(Huene, 1942); (Liparini and Schultz, 2013)	FB	Text – “*Prestosuchus chiniquensis* may be a quadruped, facultative biped, with semi-erect to parasagittal limb postures.”.

Prolacerta broomi	(Parrington, 1935); (Gow, 1975)	FB	Text – "*Prolacerta* was clearly a bipedal runner with a large tail to counter- balance the weight of the body.". MS makes it clear that this is a running adaptation throughout and is reconstructed diagrammatically as a quadruped, so facultative bipedality is used.
Proterochampsa barrionuevoi	(Reig, 1959); (Trotteyn, Arcucci and Raugust, 2013)	OQ	Text – semi-aquatic, stated in the Paleobiology section of this work. Also reconstructed diagrammatically as a quadruped. We use this analysis for both species of *Proterochampsa*.
Proterochampsa nodosa	(Barberena, 1982); (Trotteyn, Arcucci and Raugust, 2013)	OQ	Text – As above.
Proterosuchus	(Broom, 1903)	OQ	Text – Semi-aquatic: "*Proterosuchids* are diapsids that probably had a predominantly sprawling gait", "mostly aquatic, predacious reptiles living in ponds, lakes and rivers, using swimming as their main form of locomotion", "similar lifestyle to that of extant crocodiles".
Proterosuchus alexanderi	(Hoffman, 1965); (Ezcurra, Butler and Gower, 2013)	OQ	Text – As above
Proterosuchus fergusi	(Broom, 1903); (Ezcurra, Butler and Gower, 2013)	OQ	Text – As above
Proterosuchus goweri	(Ezcurra and Butler, 2015); (Ezcurra, Butler and Gower, 2013)	OQ	Text – As above
Protorosaurus speneri	(Meyer, 1832); (Gottman-Quesada and Sander, 2009)	OQ	Text – "*Protosaurus* is a quadrupedal reptile."

Protosuchus richardsoni	(Brown, 1933); (Colbert, Mook and Brown, 1951)	FB	Text – "*Protosuchus* probably had a varied mode of locomotion, partially bipedal and partially quadrupedal.".
Pseudochampsa ischigualastensis	(Trotteyn, Arcucci and Raugust, 2013); (Trotteyn and Ezcurra, 2014)	OQ	"Proterochampsids are crocodile-like, probably semi-aquatic, quadrupedal archosauriforms".
Qianosuchus mixtus	(Li et al., 2006)	OQ	Text – semi-aquatic: "*Q. mixtus* is unique among Triassic archosaurians in having a mosaic of specializations for both aquatic and terrestrial ways of life.".
Rauisuchus triradentes	(Huene, 1942); (Nesbitt et al., 2013; Lautenschlager and Rauhut, 2015)	OQ	Text – "Mid- to large-sized quadrupedal predators" in reference to Rauisuchidae, which *Rauisuchus* is member of. Also a quadrupedal diagram in Lautenschlager & Rahut (2015).
Rhynchosaurus articeps	(Owen, 1842); (Benton, 1990)	OQ	Diagram – however, with a lot of text describing the posture as "semi-erect hindlimb" and "partly sprawling forelimb" in a "'fast walking' pose"
Riojasuchus tenuisceps	(Bonaparte, 1969); (von Baczko and Desojo, 2016)	OQ	Text – "Ornithosuchids are terrestrial quadrupedal carnivorous archosaurs". Previous arguments applied to *Ornithosuchus* could apply here. A detailed biomechanical analysis of this largely complete specimen would be insightful.

Sacisaurus agudoensis	(Ferigolo and Langer, 2007; Langer and Ferigolo, 2013)	OB	Diagram – However, this animal was first described as an early ornithischian rather than a non-dinosaurian dinosauromorph, so this classification is contentious. Given how this animal's bipedality is an end result in a complete transition OQ > FB > OB, the lack of clarity surrounding this animal is paramount to examining the transition in detail.
Saturnalia tupiniquim	(Langer et al., 1999); (Bronzati et al., 2017)	FB	Text – "*Saturnalia tupiniquim* is a facultative biped".
Saurosuchus galilei	(Reig, 1959); (Nesbitt et al., 2013)	OQ	Text – "Mid- to large-sized quadrupedal predators" in reference to Rauisuchidae, which *Saurosuchus* is member of.
Scutellosaurus lawleri	(Colbert, 1981); (Breeden, 2016)	OB	Text – "A small (1 m) bipedal thyreophoran ornithischian dinosaur"
Shansisuchus shansisuchus	(Young, 1964); (Ezcurra et al., 2018)	OQ	Text – Erythrosuchid, "The erythrosuchids were quadrupedal, probably sprawling and bulky, predatory archosauromorphs".
Shuvosaurus inexpectatus	(Chatterjee, 1993); (Nesbitt, Irmis and Parker, 2007)	OB	Text – "The convergences suggest that a 'theropod dinosaur body plan' developed in a group of crocodile-line archosaurs before it appeared in theropod dinosaurs.". Indicative of a bipedal locomotor mode.
Silesaurus opolensis	(Dzik, 2003); (Piechowski and Dzik, 2010)	FB	Text – "This suggests a greater load on the pelvic girdle and the ability of *Silesaurus* to run bipedally on occasion".

Sillosuchus longicervix	(Alcober and Parrish, 1997); (Parker and Nesbitt, 2013)	OB	Text – “We therefore hypothesize that *S. longicervix* was also bipedal”.
Simoedosaurus lemoinei	(Gervais, 1877); (Matsumoto and Evans, 2010)	OQ	Text – aquatic therefore OQ, mentioned repeatedly throughout this paper.
Smilosuchus gregorii	(Camp, 1930); (Stocker and Butler, 2013)	OQ	Text – “They were quadrupedal” in reference to phytosaurs generally, this animal is included in their list of phytosaurs.”.
Sphenosuchus acutus	(Haughton, 1915); (Walker, 1990)	OQ	Text – “There is little doubt that *Sphenosuchus* was quadrupedal.”.
Spondylosoma absconditum	(Huene, 1942); (Nesbitt et al., 2017)	OQ	Diagram – in a diagram with other Aphanosaurs.
Stagonolepis robertsoni	(Agassiz, 1844); (Desojo et al., 2013)	OQ	Text – “a clade of obligately quadrupedal, heavily armoured pseudosuchians”.
Staurikosaurus pricei	(Colbert, 1970)	OB	Text – “A bipedal saurischian of small size, with strong hind limbs and small forelimbs.”.
Tanystropheus longobardicus	(Bassani, 1886); (Renesto, 2005)	OQ	Text – semi-aquatic or shoreline taxa with an extreme neck morphology. Plenty of discussion about counterbalancing the body around the pectoral girdle, thus quadrupedal. There is also a diagram showing a quadrupedal pose.
Tarjadia ruthae	(Arcucci and Marsicano, 1998); (Sues, Desojo and Ezcurra, 2013)	OQ	Text – Sues et al (2013) suggests semi-aquatic lifestyle, hence OQ.
Tawa hallae	(Sterling J Nesbitt et al., 2009)	OB	Text – Theropod dinosaur, reasonably complete. Also has a diagram of a bipedal animal.

Teleocrater combined	(Nesbitt et al., 2017)	OQ	Diagram – based on a number of specimens, reconstructed in a quadrupedal pose.
Terrestrisuchus gracilis	(Crush, 1984); (Irmis, Nesbitt and Sues, 2013)	OQ	Diagram – also discussed to be a cursorial terrestrial animal at length, but a mode is never explicitly stated in any literature
Ticinosuchus ferox	(Krebs, 1965); (Lautenschlager and Desojo, 2011)	OQ	Text – "large, quadrupedal taxa (such as … *Ticinosuchus* …)".
Trilophosaurus buettneri	(Case, 1928); (Spielmann et al., 2008)	OQ	Text – Arboreal, "used both its forelimbs and hindlimbs for propulsion". Classifed as obligately quadrupedal. Also represented by a quadrupedal diagram.
Vancleavea campi	(Long and Murry, 1995); (Nesbitt et al. 2009)	OQ	Text – aquatic, therefore OQ.
Velociraptor mongoliensis	(Osborn, Kaisen and Olsen, 1924)	OB	Text – Theropod dinosaur, reconstructed as a biped multiple times.
Yarasuchus deccanensis	(Sen, 2005)	FB	Text – "probably with a facultatively bipedal gait".
Youngina capensis	(Broom, 1914); (Gow, 1975)	OQ	Text – "This points towards a terrestrial quadrupedal existence".

Appendix B

R code for analysis in Chapter Two, as published in Royal Society Open Science

```
#### Testing for a facultative locomotor mode in the acquisition of archosaur bipedality ####
# Author: Luke R. Grinham
# Version: March 2019

#### Pre-requisites ####
setwd("~/Dropbox/PhD/Data␣collection/Analysis") #set working directory
#load required packages
library("phangorn", lib.loc="/Library/Frameworks/R.framework/Versions/3.4/Resources/library")
library("ape", lib.loc="/Library/Frameworks/R.framework/Versions/3.4/Resources/library")
library("strap", lib.loc="/Library/Frameworks/R.framework/Versions/3.4/Resources/library")
library("phytools", lib.loc="/Library/Frameworks/R.framework/Versions/3.4/Resources/library")
library("phylotools", lib.loc="/Library/Frameworks/R.framework/Versions/3.4/Resources/library")
library("phylobase", lib.loc="/Library/Frameworks/R.framework/Versions/3.4/Resources/library")
#### Analysis and plots for Ezcurra matrix ####
```

```
agesEz<-read.delim(file="fadladsEz.txt") #read in first and last appearance based on paleobiodb.org
treeEz<-read.nexus(file="Ezbpd.txt") #read in matrix modified from Ezcurra 2016
ditreeEz<-multi2di(treeEz,random=TRUE) #randomly break polytomies
datedEz<-DatePhylo(ditreeEz,agesEz,rlen=1,method="equal",add.terminal=FALSE) #date tree using DatePhylo() using equal share dating
LdatedEz<-ladderize(datedEz, right= TRUE) #ladderize for clarity
# geoscalePhylo(LdatedEz,cex.ts=0.6,cex.tip=0.6) #plot time calibrated tree if desired, for visualisation
plot(datedEz)
nodelabels()
dataEz<-read.delim(file="matrixEz.txt")
charEz<-dataEz #create character matrix to modify
charEz<-charEz[match(datedEz$tip.label,charEz$Taxa),]#make sure char order matches tree order after break
missingEz<-which(is.na(charEz[,2])) #taxa missing diagnosis
culledEz<-drop.tip(datedEz,missingEz)#cull non-diagnosed taxa
LculledEz<-ladderize(culledEz, right=TRUE) #ladderize for clarity
culcharEz<-charEz[-missingEz,2]#reduced character matrix
acemodelEz<-ace(culcharEz,culledEz,type="discrete",model="ER") #perform maximum likelihood ancestral character reconstruction on discrete chars with ace()
co<-c("yellow","blue","red") #yellow quad, blue facbi, red bi - colour palette
#create plot
pdf("Figure␣1.pdf",width=11,height=9)
geoscalePhylo(LculledEz,cex.ts=0.6,cex.tip=0.7,x.lim=25,units=c("Period","Epoch"),boxes=c("Period")) #plot with geoscalePhylo
```

```
nodelabels(pch=19,pie=acemodelEz$lik.anc,piecol=co,cex=0.2) #add ACR likelihoods as pie charts on nodes
#legend
legend("topright",
        legend=c("Quadruped", "Facultative␣Biped", "Biped"),
        col=c("yellow","blue","red"),
        pch=19,
        bty="o",
        cex=0.7,
        pt.cex=1.2,
        inset=0.2,
        bg="white")
tiplabels(pie=to.matrix(culcharEz,sort(unique(culcharEz))),
            piecol=c("yellow","blue","red"),cex=0.17)
dev.off()
#### Analysis and plots for Nesbitt matrix ####
agesNes<-read.delim(file="fadladsNes.txt") #read in first and last appearance based on paleobiodb.org
treeNes<-read.nexus(file="Nesbpd.txt") #read in matrix modified from Nesbitt 2017
ditreeNes<-multi2di(treeNes,random=TRUE) #randomly break polytomies
datedNes<-DatePhylo(ditreeNes,agesNes,rlen=1,method="equal",add.terminal=FALSE) #date tree using DatePhylo() using equal share dating
LdatedNes<-ladderize(datedNes, right= TRUE) #ladderize for clarity
geoscalePhylo(LdatedNes,cex.ts=0.6,cex.tip=0.6) #plot time calibrated tree if desired, for visualisation
plot(datedNes)
nodelabels()
dataNes<-read.delim(file="matrixNes.txt")
charNes<-dataNes #create character matrix to modify
charNes<-charNes[match(datedNes$tip.label,charNes$Taxa),]# make sure char order matches tree order after break
```

```
missingNes<-which(is.na(charNes[,2])) #taxa missing diagnosis
culledNes<-drop.tip(datedNes,missingNes)#cull non-diagnosed taxa
LculledNes<-ladderize(culledNes, right=TRUE) #ladderize for clarity
culcharNes<-charNes[-missingNes,2]#reduced character matrix
acemodelNes<-ace(culcharNes,culledNes,type="discrete",model="ER") #perform maximum likelihood ancestral character reconstruction on discrete chars with ace()
co<-c("yellow","blue","red") #yellow quad, blue facbi, red bi - colour palette
#create plot
pdf("Figure␣2.pdf",width=10,height=9)
geoscalePhylo(LculledNes,cex.ts=0.6,cex.tip=0.7,x.lim=25,units=c("Period","Epoch"),boxes=c("Period")) #plot with geoscalePhylo
nodelabels(pch=19,pie=acemodelNes$lik.anc,piecol=co,cex=0.2) #add ACR likelihoods as pie charts on nodes
#legend
legend("topright",
       legend=c("Quadruped", "Facultative␣Biped", "Biped"),
       col=c("yellow","blue","red"),
       pch=19,
       bty="o",
       cex=0.7,
       pt.cex=1.2,
       inset=0.2,
       bg="white")
tiplabels(pie=to.matrix(culcharNes,sort(unique(culcharNes))),
          piecol=c("yellow","blue","red"),cex=0.17)
dev.off()
#### SENSITIVITY Analysis and plots for Ezcurra matrix ####
agesEzSENS<-read.delim(file="fadladsEz.txt") #read in first and last appearance based on paleobiodb.org
```

```
treeEzSENS<-read.nexus(file="EzbpdSENS.txt") #read in matrix modified from Ezcurra 2016
ditreeEzSENS<-multi2di(treeEzSENS,random=TRUE) #randomly break polytomies
datedEzSENS<-DatePhylo(ditreeEzSENS,agesEzSENS,rlen=1,method="equal",add.terminal=FALSE) #date tree using DatePhylo() using equal share dating
LdatedEzSENS<-ladderize(datedEzSENS, right= TRUE) #ladderize for clarity
geoscalePhylo(LdatedEzSENS,cex.ts=0.6,cex.tip=0.6) #plot time calibrated tree if desired, for visualisation
plot(datedEzSENS)
nodelabels()
dataEzSENS<-read.delim(file="matrixEzSENS.txt")
charEzSENS<-dataEzSENS #create character matrix to modify
charEzSENS<-charEzSENS[match(datedEzSENS$tip.label, charEzSENS$Taxa),]#make sure char order matches tree order after break
missingEzSENS<-which(is.na(charEzSENS[,2])) #taxa missing diagnosis
culledEzSENS<-drop.tip(datedEzSENS,missingEzSENS)#cull non-diagnosed taxa
LculledEzSENS<-ladderize(culledEzSENS, right=TRUE) #ladderize for clarity
culcharEzSENS<-charEzSENS[-missingEzSENS,2]#reduced character matrix
acemodelEzSENS<-ace(culcharEzSENS,culledEzSENS,type="discrete",model="ER") #perform maximum likelihood ancestral character reconstruction on discrete chars with ace()
co<-c("yellow","blue","red") #yellow quad, blue facbi, red bi - colour palette
#create plot
pdf("Figure␣3.pdf",width=10,height=9)
```

```r
geoscalePhylo(LculledEzSENS,cex.ts=0.6,cex.tip=0.7,x.lim=25,units=c("Period","Epoch"),boxes=c("Period")) #plot with geoscalePhylo
nodelabels(pch=19,pie=acemodelEzSENS$lik.anc,piecol=co,cex=0.2) #add ACR likelihoods as pie charts on nodes
#legend
legend("topright",
       legend=c("Quadruped", "Facultative␣Biped", "Biped"),
       col=c("yellow","blue","red"),
       pch=19,
       bty="o",
       cex=0.7,
       pt.cex=1.2,
       inset=0.2,
       bg="white")
tiplabels(pie=to.matrix(culcharEzSENS,sort(unique(culcharEzSENS))),
          piecol=c("yellow","blue","red"),cex=0.17)
dev.off()
#### SENSITIVITY Analysis and plots for Nesbitt matrix ####
agesNesSENS<-read.delim(file="fadladsNes.txt") #read in first and last appearance based on paleobiodb.org
treeNesSENS<-read.nexus(file="NesbpdSENS.txt") #read in matrix modified from Nesbitt 2017
ditreeNesSENS<-multi2di(treeNesSENS,random=TRUE) #randomly break polytomies
datedNesSENS<-DatePhylo(ditreeNesSENS,agesNesSENS,rlen=1,method="equal",add.terminal=FALSE) #date tree using DatePhylo() using equal share dating
LdatedNesSENS<-ladderize(datedNesSENS, right= TRUE) #ladderize for clarity
geoscalePhylo(LdatedNesSENS,cex.ts=0.6,cex.tip=0.6) #plot time calibrated tree if desired, for visualisation
plot(datedNesSENS)
nodelabels()
dataNesSENS<-read.delim(file="matrixNesSENS.txt")
```

```
charNesSENS<-dataNesSENS #create character matrix to modify
charNesSENS<-charNesSENS[match(datedNesSENS$tip.label, charNesSENS$Taxa),]#make sure char order matches tree order after break
missingNesSENS<-which(is.na(charNesSENS[,2])) #taxa missing diagnosis
culledNesSENS<-drop.tip(datedNesSENS, missingNesSENS)#cull non-diagnosed taxa
LculledNesSENS<-ladderize(culledNesSENS, right=TRUE) #ladderize for clarity
culcharNesSENS<-charNesSENS[-missingNesSENS,2]#reduced character matrix
acemodelNesSENS<-ace(culcharNesSENS, culledNesSENS, type="discrete", model="ER") #perform maximum likelihood ancestral character reconstruction on discrete chars with ace()
co<-c("yellow","blue","red") #yellow quad, blue facbi, red bi - colour palette
#create plot
pdf("Figure␣4.pdf",width=10,height=9)
geoscalePhylo(LculledNesSENS,cex.ts=0.6,cex.tip=0.7,x.lim=25,units=c("Period","Epoch"),boxes=c("Period")) #plot with geoscalePhylo
nodelabels(pch=19,pie=acemodelNesSENS$lik.anc,piecol=co,cex=0.2) #add ACR likelihoods as pie charts on nodes
#legend
legend("topright",
       legend=c("Quadruped", "Facultative␣Biped", "Biped"),
       col=c("yellow","blue","red"),
       pch=19,
       bty="o",
       cex=0.7,
       pt.cex=1.2,
       inset=0.2,
       bg="white")
```

```
tiplabels(pie=to.matrix(culcharNesSENS,sort(unique(
    culcharNesSENS))),
           piecol=c("yellow","blue","red"),cex=0.17)
dev.off()
```

Appendix C

Supplementary information for Chapter Three, as published in Journal of Zoology

Table C.1 Specimens included in this study, including locomotor mode, preservation method and source. Specimens without a scan source were measured physically using digital calipers. Scans obtained from published sources are cited in the manuscript.

Species	Specimen ID	Bipedality	Preservation	Scan source	Collection
Acanthocercus cyanogaster	fmnh-amphibians and reptiles-12522	0	Soft tissue	Morphosource.org	Field Museum, Chicago, USA
Acanthodactylus boskianus	fmnh-amphibians and reptiles-68769	0	Soft tissue	Morphosource.org	Field Museum, Chicago, USA
Acanthodactylus schreiberi	BMNH 1888.11.3.7 & BMNH 1888.11.3.14	1	Soft tissue	Primary collection	Natural History Museum, London, UK
Adolfus africanus	fmnh-amphibians and reptiles-154745	0	Soft tissue	Morphosource.org	Field Museum, Chicago, USA
Agama agama	fmnh-amphibians and reptiles-188910	0	Soft tissue	Morphosource.org	Field Museum, Chicago, USA
Amblyrhynchus cristatus	BMNH 76.6.21.7	0	Osteological	-	Natural History Museum, London, UK

Ameiva ameiva	ummz-herps-245032	1	Soft tissue	Morphosource.org	University of Michigan Museum of Zoology, Ann Arbor, USA
Anolis carolinensis	ummz-herps-245698	0	Soft tissue	Morphosource.org	University of Michigan Museum of Zoology, Ann Arbor, USA
Basiliscus basiliscus	BMNH 1914.5.21 16 & BMNH 1914.5.21 17 & R.8746	1	Soft tissue	Primary collection	Natural History Museum, London, UK; University Museum of Zoology, Cambridge, UK
Basiliscus vittatus	R.19025.1 & R.19025.2 &R.19025.1	1	Soft tissue	Primary collection	Natural History Museum, London, UK
Bronchocela cristatella	uf-herp-112989	0	Soft tissue	Morphosource.org	Florida Museum of Natural History, Gainesville, USA

Cachryx defensor	uf-herp-41534	0	Soft tissue	Morphosource.org	Florida Museum of Natural History, Gainesville, USA
Chamaeleo chamaeleon	BMNH 1156	0	Osteological	-	Natural History Museum, London, UK
Chlamydosaurus kingii	BMNH Vol.1.P.401	1	Osteological	-	Natural History Museum, London, UK
Cophotis ceylanica	uf-herp-86474	0	Soft tissue	Morphosource.org	Florida Museum of Natural History, Gainesville, USA
Crotaphytus collaris	BMNH 1889.7.3.14 & BMNH 1889.7.3.16	1	Soft tissue	Primary collection	Natural History Museum, London, UK
Draco dussumieri	uf-herp-19920	0	Soft tissue	Morphosource.org	Florida Museum of Natural History, Gainesville, USA

Heloderma horridum	BMNH 1975.995	0	Osteological	-	Natural History Museum, London, UK
Iguana iguana	BMNH 1974.2486 & BMNH 1974.2487	1	Osteological	-	Natural History Museum, London, UK
Lacerta trilineata	uf-herp-65017	0	Soft tissue	Morphosource.org	Florida Museum of Natural History, Gainesville, USA
Lophosaurus dilophus	BMNH 1910.4.26.23	0	Osteological	-	Natural History Museum, London, UK
Lyriocephalus scutatus	uf-herp-126295	0	Soft tissue	Morphosource.org	Florida Museum of Natural History, Gainesville, USA
Moloch horridus	uf-herp-126296	0	Soft tissue	Morphosource.org	Florida Museum of Natural History, Gainesville, USA

Shinisaurus crocodilurus	UF-H-60925	0	Soft tissue	Morphosource.org	Florida Museum of Natural History, Gainesville, USA
Sphenodon punctatus	QMBC 0614 & R.2595 & R.2596	0	Soft tissue	Open Science Framework	Queen Mary's Univerisity, London, UK; University Museum of Zoology Cambridge, Cambridge, UK
Tropicagama temporalis	ummz-herps-245428	1	Soft tissue	Morphosource.org	University of Michigan Museum of Zoology, Ann Arbor, USA
Varanus exanthematicus	V1 & V3	0	Soft tissue	Dryad	-
Varanus griseus	BMNH 1974.2481	0	Osteological	-	Natural History Museum, London, UK

Table C.2 Scanning parameters for all specimens scanned at the Cambridge Biotomography Centre.

Specimen	Voxel size (mm)	kV	µA
BMNH 1888.11.3.7	0.066991493	125	125
BMNH 1888.11.3.14	0.055554867	125	125
BMNH 1889.7.3.14	0.062120657	125	150
BMNH 1889.7.3.16	0.054108437	125	150
BMNH 1914.5.21 16	0.117876887	125	150
BMNH 1914.5.21 17	0.111402837	125	150
R.8746	0.115257413	145	135
R.19025.1	0.075755022	140	140
R.19025.2	0.059488066	140	140
R.19025.3	0.049141283	140	140

Table C.3 Definitions of all measurements taken in this study.

Segment	Definition
Humerus	Maximal length of the humerus along the long axis
Humerus_diameter	Maximal diameter at the radial mid-shaft, perpendicular to the long axis
Radius	Maximal length of the radius along the long axis
Ulna	Maximal length of the ulna along the long axis
Ulna_diameter	Maximal diameter at the ulnar mid-shaft, perpendicular to the long axis
Manus34	Distal end of metacarpal 4 to the proximal end of carpals
Femur	Maximal length of the femur along the long axis
Femur_diameter	Maximal diameter at the femoral mid-shaft, perpendicular to the long axis
Tibia	Maximal length of the tibia along the long axis
Tibia_diameter	Maximal diameter at the tibial mid-shaft, perpendicular to the long axis
Pes34	Proximal end of tarsals to the distal end of metatarsal 4
Toe34	Proximal end of the 4th phalange to the claw tip on the 4th toe
Skull_length	Length of the skull, from tip of the premaxilla to the occipital condyle.
Skull_depth	Depth of the skull, including the jaw when closed.
Skull_width	Width of the skull, including the jaw when closed.
Cervical	Maximal distance along the vertebral column from anterior articulation of first cranial vertebral element to the posterior articulation of the final cervical vertebral element. Cervical count variable across species.
TLS	Maximal distance along the vertebral column from anterior articulation of first thoracic vertebral element to the posterior articulation of the final sacral vertebral element.
SVL	Sum of Skull_length, Cervical and TLS. Osteological proxy for snout-vent length

Table C.4 Results from the Shapiro-Wilk normality tests (raw and log-transformed data), F test for variance (log-transformed data) and Two Sample t-test for equal means (log-transformed data). $p<0.05$ for Shapiro-Wilk indicates non-normality, $p<0.05$ for F tests indicates non-equal variance, $p<0.05$ for two Sample t-test indicates a difference in means between the two groups.

Shapiro-Wilk normality test			
	W	p-value	
Raw data, obligate quadrupeds	0.74537	0.0001473	
Raw data, facultative bipeds	0.89981	0.2878	
log-data, obligate quadrupeds	0.8933	0.03094	
log-data, facultative bipeds	0.93735	0.5852	
F test to compare two variances			
data: SVL by Bipedality			
F = 2.3946	num df = 29	denom df = 7	p-value = 0.2412
alternative hypothesis: true ratio of variances is not equal to 1			
95 percent confidence interval:	0.5341566	7.3055278	
ratio of variances	2.394574		
Two Sample t-test			
t = -0.095969	df = 26	p-value = 0.9243	
alternative hypothesis: true difference in means is not equal to 0			
95 percent confidence interval:			
-0.5892847 0.5367139			
sample estimates:			
mean in group 0 mean in group 1			
4.887691 4.913976			

Table C.5 Phylogenetic least squares regressions. Lines of format y=ax+b. Locomotor mode: 0 = obligate quadruped; 1 = facultative biped. Bold indicates significant results in PGLSANVOCA.

y	Locomotor mode	a	Standard Error	b	Standard Error	R-squared
Cervical	0	1.14	0.0749	-2.69	0.37	0.924
	1	1.17	0.296	-2.65	1.46	0.678
Femur	0	0.977	0.072	-1.66	0.397	0.906
	1	0.93	0.0716	-1.2	0.383	0.9601
Femur_diameter	0	1.02	0.0517	-4.34	0.293	0.953
	1	1.1	0.132	-4.6	0.649	0.909
Humerus	0	0.95	0.0524	-1.67	0.291	0.945
	1	1.07	0.0613	-2.21	0.328	0.977
Humerus_diameter	0	1.01	0.0675	-4.39	0.356	0.921
	1	1.15	0.0952	-5.08	0.47	0.954
Manus34	0	0.954	0.0532	-2.61	0.263	0.944
	1	1.05	0.135	-2.99	0.664	0.895
Pes34	0	0.864	0.104	-1.61	0.512	0.782
	1	0.914	0.052	-1.36	0.275	0.978
Radius	0	0.928	0.0671	-1.95	0.365	0.909
	1	1.1	0.0677	-2.71	0.362	0.974
Tibia	0	0.891	0.101	-1.53	0.53	0.802
	1	0.892	0.087	-1.17	0.466	0.937
Tibia_diameter	0	0.967	0.06	-4.17	0.341	0.932
	1	1.08	0.108	-4.65	0.0534	0.934
TLS	0	1.03	0.0267	-0.59	0.132	0.987
	1	1.03	0.0542	-0.66	0.268	0.981
Toe34	0	0.749	0.119	-0.862	0.586	0.671
	1	0.864	0.0874	-0.902	0.468	0.933
Ulna	0	0.966	0.0609	-2	0.335	0.93
	1	1.13	0.064	-2.75	0.34	0.978
Ulna_diameter	0	1.04	0.0717	-4.82	0.0382	0.9171
	1	1.09	0.11	-5.18	0.545	0.9328

Table C.6 Principal components analysis cumulative variance.

	PC1	PC2	PC3	PC4	PC5
Standard deviation	2.4714	0.49637	0.36673	0.32131	0.2061
Proportion of Variance	0.9064	0.03657	0.01996	0.01532	0.0063
Cumulative Proportion	0.9064	0.94302	0.96298	0.9783	0.9846

Table C.7 PCA loadings

	PC1	PC2	PC3	PC4	PC5
Humerus	-0.2277736	0.11233797	-0.26960477	0.14203619	-0.06806293
Humerus_diameter	-0.2621321	0.20965105	0.10194551	0.15325133	-0.09581034
Radius	-0.2261549	0.13004082	-0.42131474	0.07677133	-0.10984474
Ulna	-0.2337347	0.1620451	-0.40121404	0.09012268	-0.1205523
Ulna_diameter	-0.26282	0.24770063	0.14350105	0.14936637	0.03297537
Manus34	-0.2565614	0.07923945	0.131999	0.05723148	-0.03222061
Femur	-0.227965	-0.1618144	-0.30762752	0.06492614	0.07037668
Femur_diameter	-0.2673138	0.01080619	0.17077273	0.1074159	-0.08621388
Tibia	-0.2286283	-0.28971052	-0.40218109	0.05283584	0.16118165
Tibia_diameter	-0.2553461	-0.02602143	0.20539265	0.06142528	0.05995535
Pes34	-0.2498781	-0.47776982	0.08045103	-0.098761	0.12209944
Toe34	-0.2259127	-0.62476786	0.18162548	0.03773944	-0.43474839
Skull_length	-0.2022229	0.04173519	0.26141896	-0.14207494	0.05830208
Skull_height	-0.2049639	0.04194571	0.08613135	0.15879087	0.43802811
Skull_width	-0.2212436	0.03944278	0.24355827	0.24444116	0.45289255
Cervical	-0.2950335	0.11467349	-0.05799986	-0.87911047	0.16946933
TLS	-0.25585	0.29138811	0.18651597	-0.0724931	-0.53149785

Table C.8 Long bone stoutness tests, using *sma* in the *smatr* package. Scaling exponents with confidence intervals not different to 1 indicate isometry.

x	y	Locomotor mode	Exponent	Lower CI	Upper CI	Intercept	Lower CI	Upper CI	R-squared	p-value	Scaling
Humerus length	Humerus diameter	0	1.186223	1.038747	1.354638	-3.067337	-3.556376	-2.578298	0.9278	<0.001	Positive allometry
		1	1.0058215	0.7863308	1.2865794	-2.530868	-3.311451	-1.750286	0.938	<0.001	Isometry
Femur length	Femur diameter	0	1.191605	1.016202	1.397283	-3.162311	-3.780836	-2.543786	0.896	<0.001	Positive allometry
		1	1.0644135	0.7184182	1.5770426	-2.864491	-4.369817	-1.359165	0.837	0.00145	Isometry
Tibia length	Tibia diameter	0	1.1099854	0.8903182	1.3838506	-2.73208	-3.46639	-1.99777	0.798	<0.001	Isometry
		1	1.0510027	0.7657559	1.4425049	-2.799174	-3.930207	-1.66814	0.896	<0.001	Isometry
Ulna length	Ulna diameter	0	1.1691972	0.9862649	1.3860596	-3.069786	-3.649859	-2.489713	0.881	<0.001	Isometry
		1	0.8936911	0.6344535	1.2588531	-2.360249	-3.26966	-1.450838	0.878	<0.001	Isometry

Table C.9 Summary of Snyder's assessment of extant squamate body proportion differences, based on historical studies (Snyder, 1954, 1962). + indicates a greater value than in obligate quadrupeds, - indicates a lesser value, ? indicates instances where some variation is seen. For example, -? Indicates that values are less than or equal to those in obligate quadrupeds.

	Humerus	Radius	Manus	Femur	Tibia	Pes	Toe	TLS	Tail
Agamid bipeds	-	-	–	-	-	+?	+?	-	+
Iguanid bipeds	-?	-?	-	++	+	+	+	-	+
Overall summary	-	-	-	+		+	+	-	+

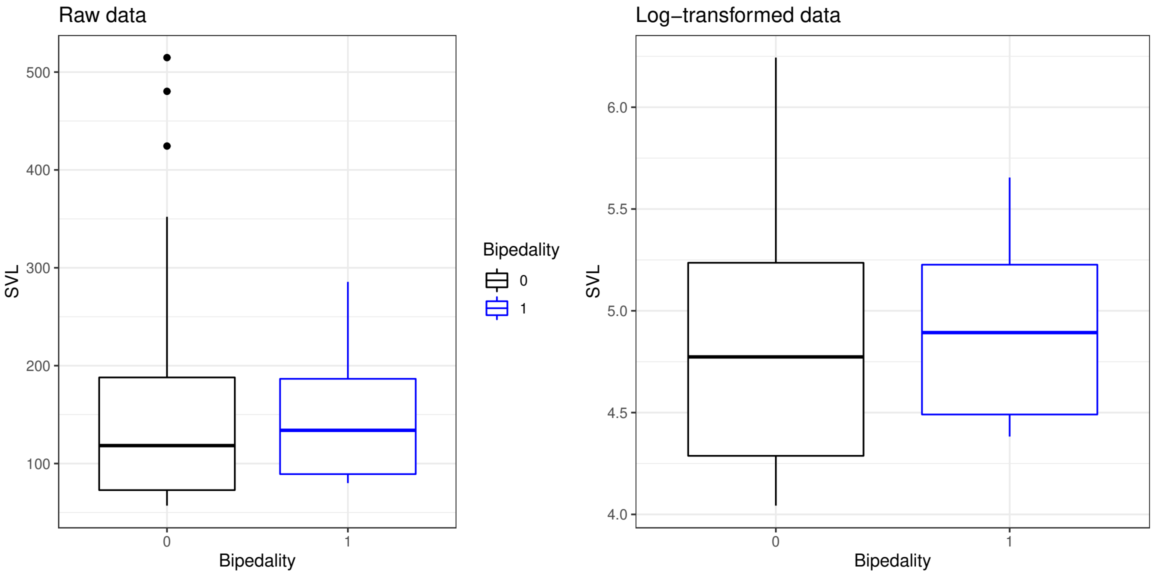

Fig. C.1 Box plots of distribution across body size.

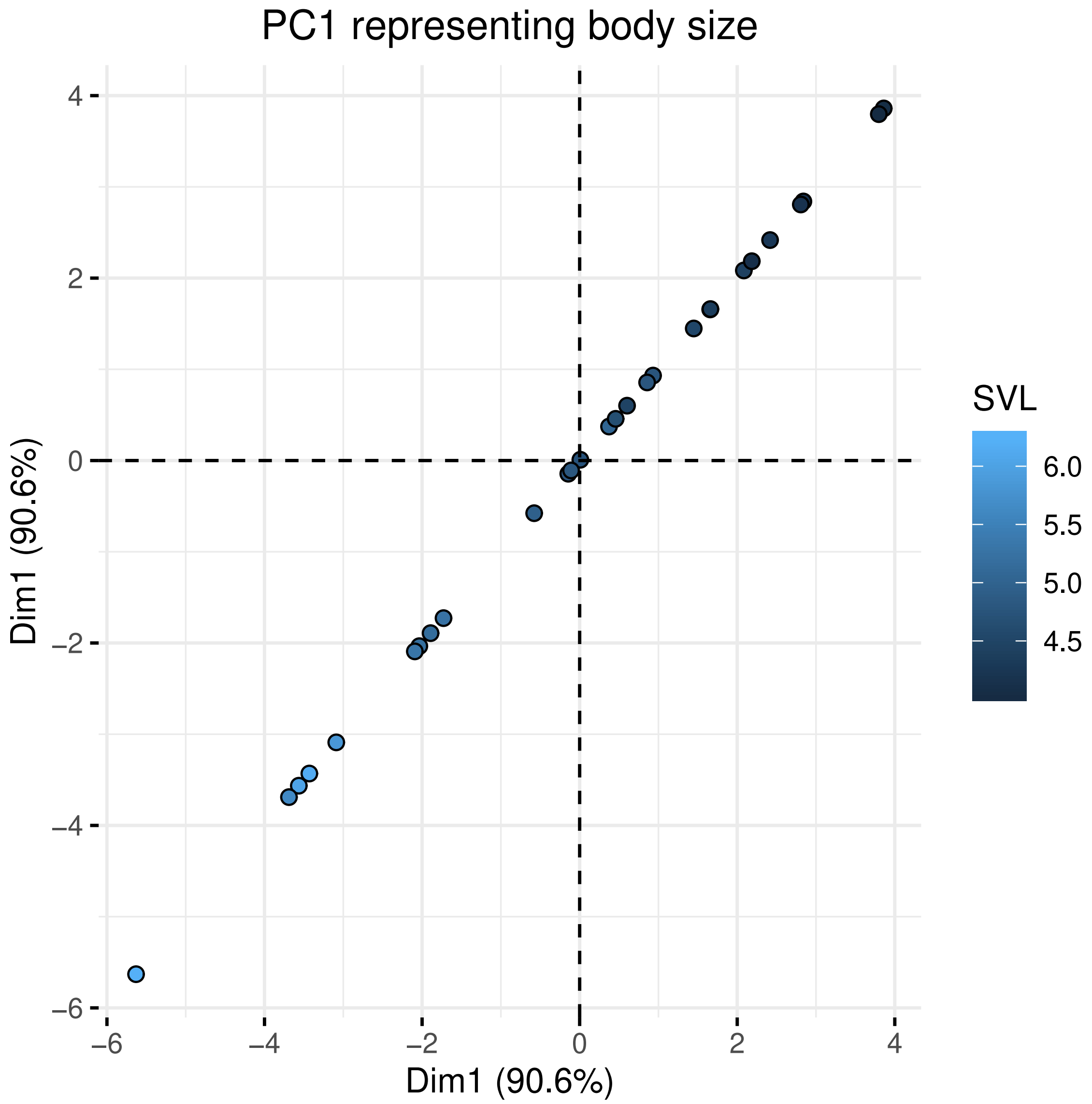

Fig. C.2 Box plots of distribution across body size.

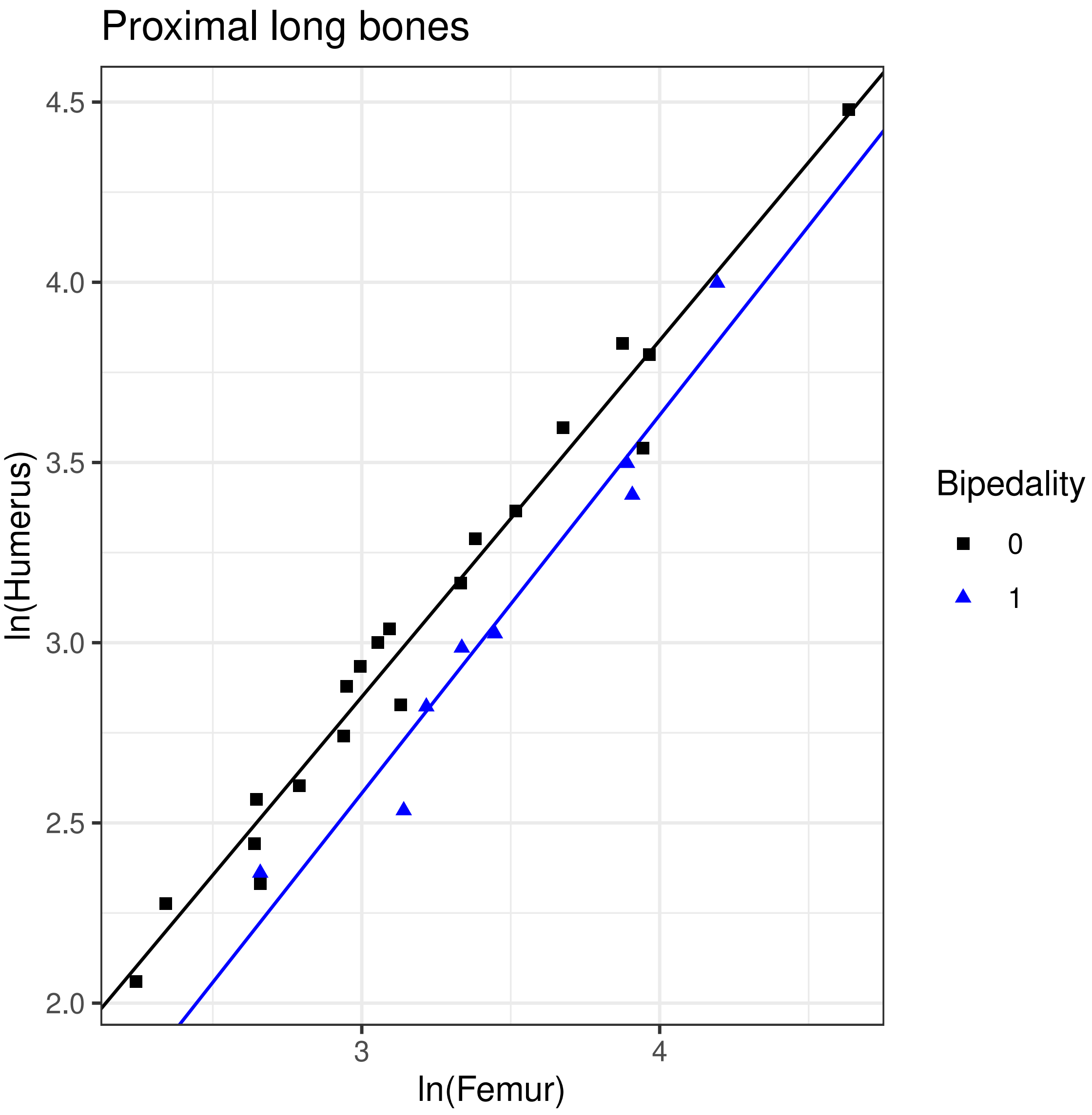

Fig. C.3 Proximal long bones relationships. Obligate quadruped: Gradient: 0.989 (0.039 standard error). Intercept: -0.117 (0.126 standard error). Facultative biped: Gradient: 1.05 (0.995 standard error). Intercept: -0.566 (0.349 standard error).

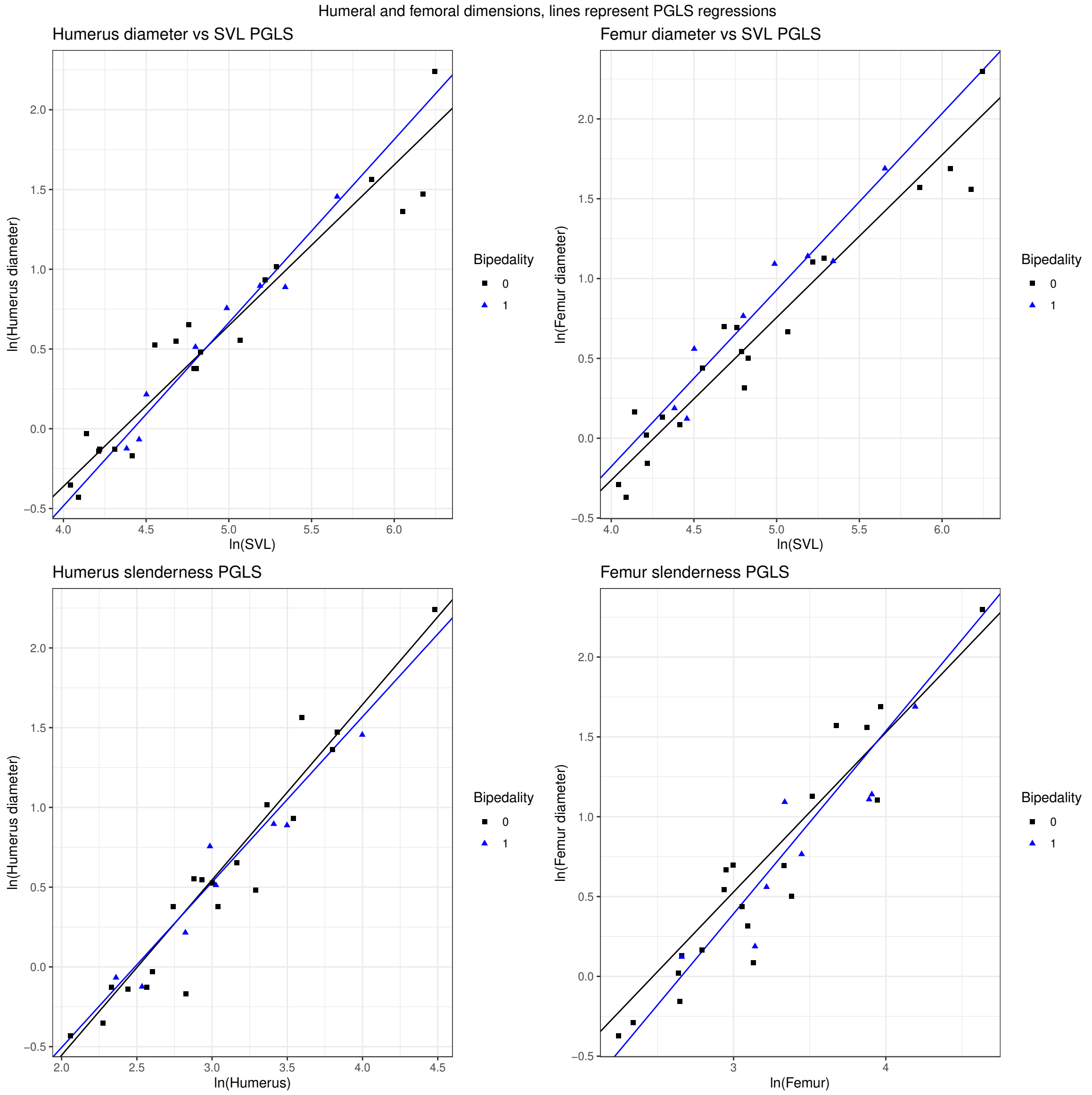

Fig. C.4 Proximal long bone slenderness. Humerus slenderness: Obligate quadruped: Gradient: 1.10 (0.068 standard error). Intercept: -2.75 (0.232 standard error). Facultative biped: Gradient: 1.03 (0.0785 standard error). Intercept: -2.58 (0.262 standard error). Femur slenderness: Obligate quadruped: Gradient: 0.998 (0.0662 standard error). Intercept: -2.47 (0.256 standard error). Facultative biped: Gradient: 1.14 (0.107 standard error). Intercept: -3.04 (0.0.422 standard error).

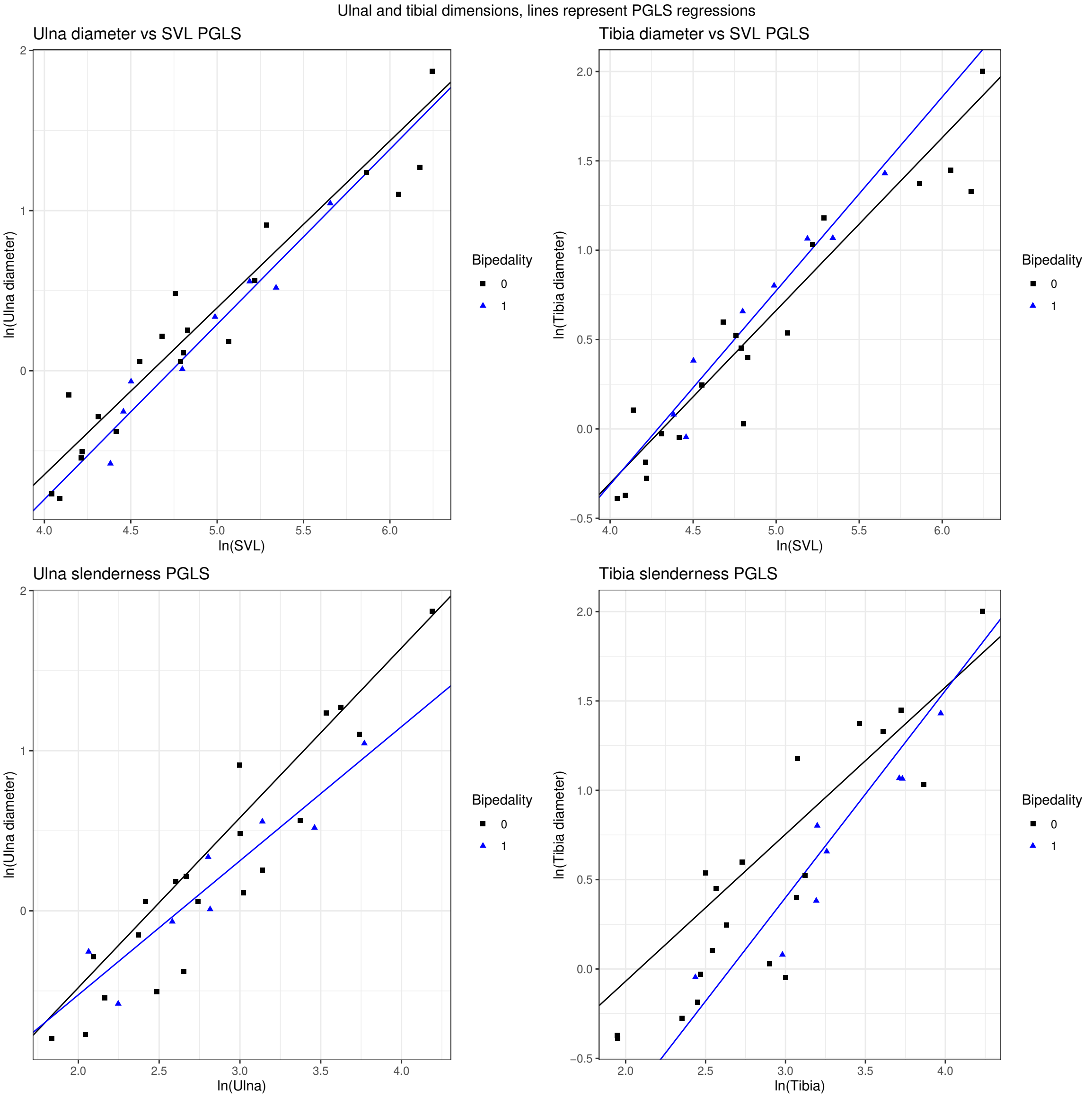

Fig. C.5 Distal long bone slenderness.

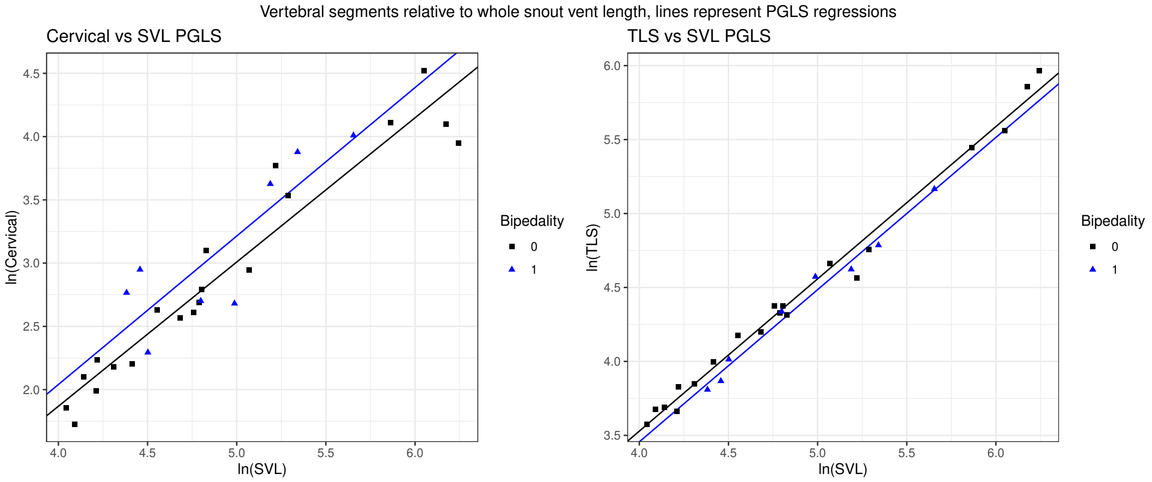

Fig. C.6 Vertebral segment lengths.

Appendix D

Locomotor mode classifications and sources for all original taxonomic units in Chapter Two.

D.1 Chamaeleonidae exclusion

Chamaeleon species are stated in the manuscript as having pelvis osteology extremely different to those of all other lepidosaurs. The pelvises from two species, *Chamaeleo chamaeleon* and *Chamaeleo gracilis* are pictured below.

In contrast to the pelvises in the main article figure 4.2, many differences are apparent which interfere with the landmarks chosen for this study. Firstly, a large scalloped ilium is present. The is a complete absence of a preacetabular process. The orientation of the acetabulum is not perpendicular to the pelvis, in fact appearing to be posteriorly angled in *C. gracilis*. The pubic tubercle exists as a branch at the end of the pubis, rather than a structure running the length of the pubis as is usually the case in other lepidosaurs. There is not a clear iliac tuberosity. And more generally, the body form within which these pelves function differs greatly from their close relatives. Prehensile tails, a laterally compressed body, and very upright posture (Fischer et al., 2010; Higham and Jayne, 2004) are very different functions linked to the pelvis than in other species we examined, perhaps explaining the difference in shape.

The differences in the morphology of the chamaeleon pelvis make comparison between themselves and other lepidosaurs challenging under the GPA and PCA framework, as we cannot accurately place homologous landmarks on these species' pelvises. It is for these reasons that chamaeleon species were excluded from the analysis presented here.

Fig. D.1 Pelvises in side profile of *C. chamaeleo* (left) and *C. gracilis* (right).

There has recently been work developing advanced methods for using landmark based geometric morphometrics to compare disparate morphology, but that is beyond the scope of this study (Bardua et al., 2019; Goswami et al., 2019; Watanabe et al., 2019).

D.2 Species substitutions

Table D.1 Species substitution for phylogenetic signal analysis

Species in dataset	Species in tree (substituted)	Source	Reasoning
Acanthocercus cyanogaster	*Acanthocercus atriocolis*	(Leaché et al., 2014)	Same genus
Cachryx defensor	*Ctenosaura pinguis*	(Malone et al., 2017)	Earlier than *Amblyrhynchus*, still later than *Iguana*. As accurate as possible with conflicting phylogenies
Tropicagama temporalis	*Lophognathus temporalis*	(Melville et al., 2018)	Updated classification

D.3 Pelvis comparison from description

Dracaenea guianensis exhibits similar morphology to *Polyglyphanodon sternbergi* (Figure D.2. Iliac blade morphology, preacetabular process and ischium all share many features and shape similarities. These details are elaborated upon more in the Discussion section of the main article.

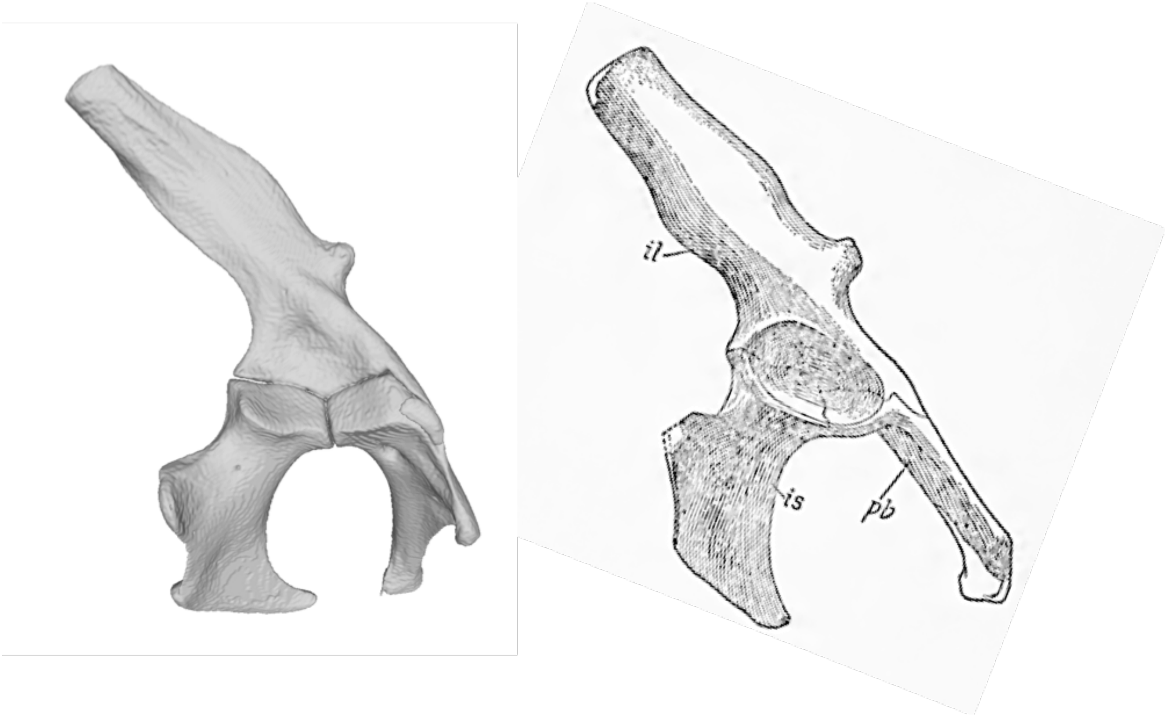

Fig. D.2 *Dracaenea guianensis* (left) compared to *Polyglyphanodon sternbergi* (right, from Gilmore (1942)).