

Skink ecomorphology: forelimb and hind limb lengths, but not static stability, correlate with habitat use and demonstrate multiple solutions

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Interspecific variation in animal form, function and behaviour is often associated with habitat use, implying co-adaptation. Numerous studies of the ‘ecomorphs’ of Greater Antillean anoles support this generality, but no other lizard group has shown unambiguous, consistent relationships between limb length and habitat use. We tested for such relationships in lygosomine skinks, a speciose and geographically widespread group that exhibits tremendous variation in relative limb length, has repeatedly invaded terrestrial, saxicolous and arboreal habitats, and uses a narrow range of substrates within these habitats. We combined new morphometric measurements of museum specimens and data from the literature ($N = 101$ total species) to determine if biomechanically founded ecomorphological predictions could successfully describe relationships of habitat with body size and with size-adjusted limb size, while also testing for differences among clades and for interactions between habitat and clade. In phylogenetically informed statistical analyses, both body size and size-adjusted hind limb length had a significant clade-by-habitat interaction and this interaction approached statistical significance for size-adjusted forelimb lengths. The ratio of forelimb to hind limb length varied among clades. However, size-adjusted limb spans, stance area and static stability were unrelated to either habitat or clade. Overall, although limbs tend to be longer in climbing than in terrestrial skinks, the clade-dependent nature of this relationship suggests that lygosomine skinks have achieved multiple solutions to similar selective regimes. We propose that longer limbs are probably more important for active climbing than for static clinging, and suggest that climbing and clinging ability may be somewhat independent.

ADDITIONAL KEYWORDS: arboreal – behaviour – co-adaptation – comparative method – functional morphology – habitat – limb length – locomotion – saxicolous – terrestrial.

INTRODUCTION

Ecomorphology seeks to find matches (but see [Diogo, 2017](#)) between the morphology of organisms and their environments or life histories ([Leisler & Winkler, 1985](#)). However, the interface between lower-level or subordinate morphological traits and the selective regime occurs through organismal performance, behaviour and energetics (e.g. see [Arnold, 1983](#); [Careau & Garland, 2012](#); [Lailvaux & Husak, 2014](#); [Foster *et al.*, 2015](#); [Storz *et al.*, 2015](#)). In particular, locomotor ability has a profound impact on the expression of many behaviours essential for survival, such as capturing

prey and evading predators ([Husak *et al.*, 2006](#)). Among measures of locomotor performance, sprint speed is the most commonly studied ([Husak, 2006a, b](#); [Husak, 2015](#)), and has been found to correlate positively with social dominance, as measured in laboratory arenas, in two species of sceloporine lizards ([Garland *et al.*, 1990](#); [Robson & Miles, 2000](#)). Additionally, higher sprint speed predicted territory area and number of offspring sired in collared lizards ([Husak *et al.*, 2006](#)). Therefore, sprint speed permeates almost every aspect of locomotor-based behaviours.

Many elements contribute to high sprint speed, including morphological, physiological and motivational factors ([Jones & Lindstedt, 1993](#); [Foster *et al.*, 2015](#)). Hind limb length is the most commonly

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studied aspect of morphology (usually adjusted for variation in body size) used to predict sprint speed. When moved through comparable arcs, longer limbs increase the distance over which the body travels in a given stride (i.e. stance phase) compared to shorter limbs, and thus increase the maximal sprint speed the animal is able to achieve if all else remains equal (Garland & Janis, 1993; Garland & Losos, 1994). However, to achieve high levels of performance, animals must interact successfully with environments that are often highly variable, which introduces a number of extrinsic variables that can affect the intrinsic factors mentioned above (Foster *et al.*, 2015). In particular, properties of the substrate, such as compliance, incline and grain diameter, can profoundly affect locomotor performance, irrespective of an animal's inherent ability (Losos & Sinervo, 1989; Losos & Irschick, 1996; Gilman *et al.*, 2012; Birn-Jeffery & Higham, 2014). Therefore, intrinsic factors that affect locomotor abilities are expected to co-adapt (evolve in concert with) other aspects of the organism, including behaviour and habitat usage (e.g. Losos, 1990b; Bauwens *et al.*, 1995; Foster & Higham, 2017).

The relationship between limb length and habitat use is often used to link morphology to behaviour and ecology in lizards. Intraspecific studies have identified differences in locomotor morphology and performance that correlate with variation in habitat use among populations of the same species (Malhotra & Thorpe, 1997; Macrini & Irschick, 1998; Herrel *et al.*, 2001, 2011; Gifford *et al.*, 2008; Hopkins & Tolley, 2011). In addition to providing evidence for local adaptation (Garland & Adolph, 1991), such studies are valuable for generating broader evolutionary hypotheses about how selection for habitat specialization might drive interspecific differences in body shape. However, these ecomorphological expectations have rarely been met in broad interspecific comparisons within lizards.

The classic studies in lizard ecomorphology compared *Anolis* species from the Greater Antilles islands. Ecomorphs (Williams, 1972) that commonly use open, broad, terrestrial surfaces have longer hind limbs, lower forelimb/hind limb ratios, and greater sprinting and jumping performance whereas ecomorphs that use closed, arboreal habitats dominated by narrow perch diameters have shorter limbs, which aid stability, but reduce sprinting and jumping performance (Losos, 1990a, b; Beuttell & Losos, 1999; Mattingly & Jayne, 2004). However, mainland anole species do not show a clear relationship between limb length and habitat structure (Losos, 2009).

Few other lizard taxa exhibit a clear relationship between limb length and habitat structure. Of the 29 studies (see Supporting Information, Table S1) attempting to link limb morphology and habitat structure in non-anoline lizards, only six found statistically significant relationships when comparing

more than two species (for a discussion of two species comparisons, see Garland & Adolph, 1994) through use of phylogenetically informed statistical analyses: in Phrynosomatinae (Herrel *et al.*, 2002b; females only: Olberding *et al.*, 2016), Tropicurinae (Grizante *et al.*, 2010), and Scincomorpha (*Cryptoblepharus*: Blom *et al.*, 2016; five other skink genera: Goodman *et al.*, 2008; *Niveoscincus*: Melville & Swain, 2000a). However, several methodological challenges probably limited the ability of certain studies to detect morphology–habitat relationships, including the absence of a well-supported phylogeny (e.g. Bickel & Losos, 2002), ambiguous habitat data (e.g. Miles, 1994; Herrel *et al.*, 2002b) and a low number of transitions between habitats (e.g. Vanhooydonck & Van Damme, 1999; Kohlsdorf *et al.*, 2001).

A shift away from exclusively morphometric traits to biomechanically informed measures may prove a more fruitful avenue to test ecomorphological hypotheses, because such traits may have more direct performance consequences. Many simple morphometric traits, such as limb length, can be used to calculate traits that are more functionally relevant and hence should have a more direct relationship to performance. For example, one can create a measure of static stability by calculating the distance from the animal's centre of mass to the edge of the stance boundary (Ting *et al.*, 1994). All else being equal, inclusion of these sorts of functionally motivated variables should increase the likelihood of detecting morphology–habitat associations. In addition, these may be more relevant than limb length in groups that do not have fast sprinting species.

We tested for associations between habitat use and limb lengths or derived variables in skinks (family Scincidae), which are remarkably speciose (the largest lizard family with 1613 species) and include terrestrial, fossorial, arboreal and semi-aquatic species. Geographically, skinks range from Amazonian lowlands to African and Australian deserts, and from cool montane habitats to Brazilian cerrado (Pianka & Vitt, 2003). Skinks offer two advantages that should increase the probability of detecting ecomorphological relationships, if they exist. First, different skink lineages have repeatedly and independently invaded a wide range of habitats (Fig. 1), which should increase statistical power to detect ecomorphological relationships (Garland *et al.*, 1993, 2003; Vanhooydonck & Van Damme, 1999; Rezende & Diniz-Filho, 2012). Second, the skink subfamilies we sampled seem to have less variation in within-habitat substrate use than occurs in some other lizard taxa (e.g. compare Miles, 1994). For example, the 'arboreal' skinks in our study are known to use primarily broad vertical tree trunks rather than branches. Such ecological data, on the microhabitat scale, are essential to avoid miscategorization of species (Herrel *et al.*, 2002b).

Our hypotheses were founded on the basic functional demands of the terrestrial, saxicolous and arboreal

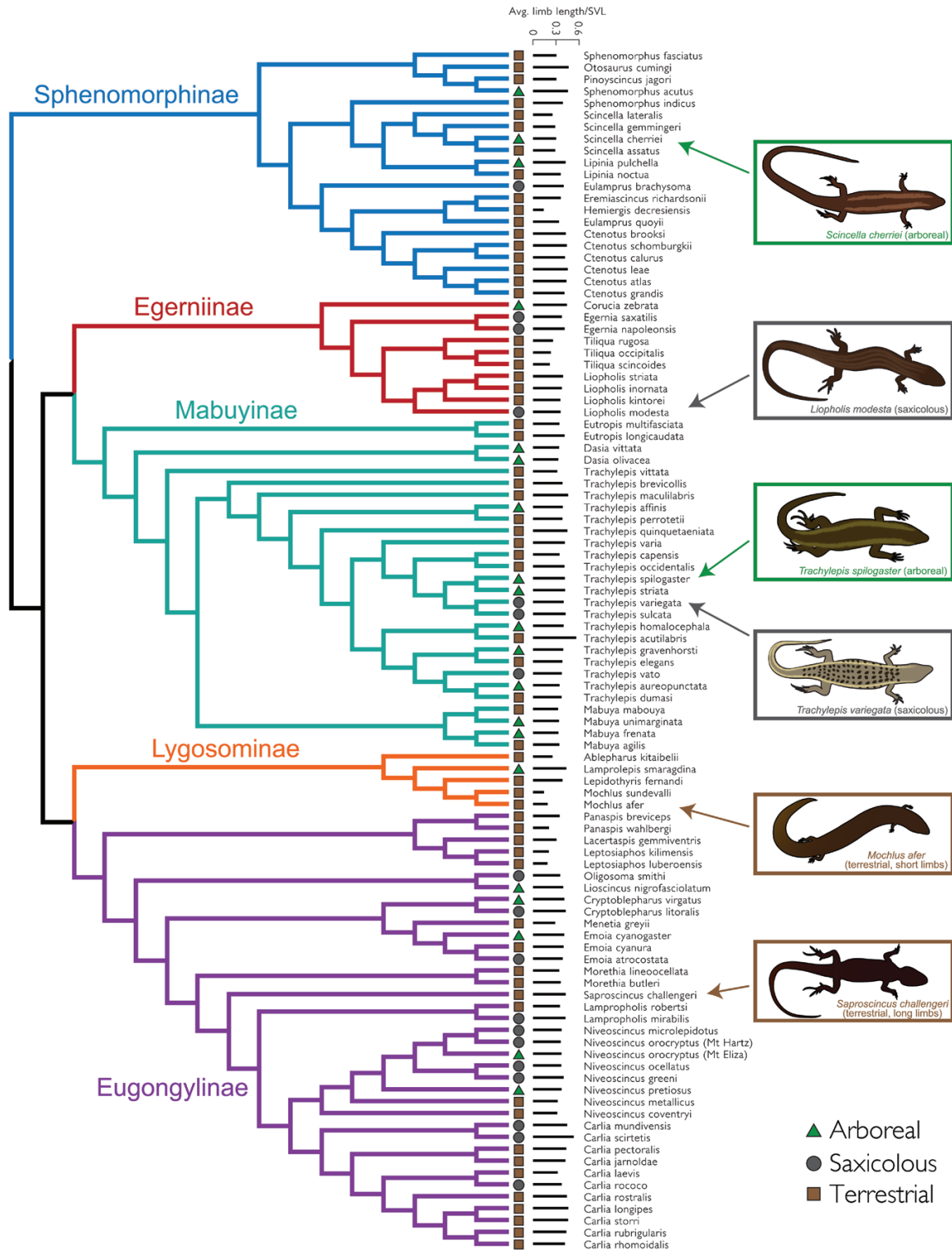


Figure 1. Hypothesized phylogeny for 101 species of skinks used in this study. Composite topology was derived from Melville & Swain (2000b), Goodman & Isaac (2008) and Pyron *et al.* (2013). Branch lengths are arbitrary following Pagel's (1992) method. Colours of branches represent the five clade groupings used in analyses. Symbols next to species names indicate terrestrial (brown square), saxicolous (grey circle) and arboreal (green triangle) conditions. See Methods for further details. Bars next to each species represent average limb length (average of forelimb and hindlimb) divided by snout-vent

habitats. Animals moving on cliff faces and broad tree trunks not only have to combat the negative effects of gravity, but also must overcome the tendency to topple out and away from the surface and roll laterally along the long axis of the body (i.e. rotation in the transverse plane; [Cartmill, 1985](#); [Preuschoft, 2002](#); [Revell *et al.*, 2007](#)). Furthermore, moving in such open habitats (rather than beneath and among leaf litter) may increase exposure to potential predators, necessitating fast bursts of movement ([Revell *et al.*, 2007](#); [Goodman *et al.*, 2008](#)). Therefore, longer limbs, higher forelimb/hind limb ratios, greater limb span and greater static stability (achieved by increasing sprawl to increase the distance from the centre of mass to the edge of the stance area) should be advantageous for remaining stable and moving faster by increasing stride length ([Losos, 1990b](#); [Arnold, 1998](#); [Beuttell & Losos, 1999](#); [Melville & Swain, 2000a](#); [Zaaf & Van Damme, 2001](#); [Zaaf *et al.*, 2001](#); [Herrel *et al.*, 2002a](#); [Revell *et al.*, 2007](#); [Goodman *et al.*, 2008](#); [Grizante *et al.*, 2010](#)). In contrast, many terrestrial skinks inhabit a more closed habitat, with obstacles that may impede locomotion (e.g. low-lying bushes, fallen leaves and branches; [Olberding *et al.*, 2012](#)), and thus benefit from shorter limbs ([Pianka, 1969](#); [Jaksić & Núñez, 1979](#); [Melville & Swain, 2000a](#)). Therefore, as compared with terrestrial species, we predicted that ‘climbing’ species (combined arboreal and saxicolous) would have longer limbs ([Fig. 2A](#)), a more equal forelimb/hind limb ratio, greater limb spans, greater stance area and greater static stability ([Fig. 2B](#)). Furthermore, because tree trunks and cliff faces are expected to pose similar challenges for these skinks, we did not expect differences between arboreal and saxicolous skinks ([Fig. 2B](#)). This prediction was tested by comparing the fit of statistical models that split habitat into three categories (terrestrial, arboreal, saxicolous) vs. two (terrestrial, climbing).

Our statistical analyses also tested for possible differences among the five subfamilies (clades) represented in our sample, as well as possible interactions between habitat and clade, the latter of which could indicate multiple adaptive responses to similar selective regimes (multiple solutions: [Alfaro *et al.*, 2004](#); [Garland *et al.*, 2011](#); [Losos, 2011](#)). Although many comparative studies have examined differences among phylogenetic lineages and/or among habitats or other ecologically defined categories (e.g. [Perry & Garland, 2002](#); [Herrel *et al.*, 2002b](#); [Revell *et al.*, 2007](#); [Gartner *et al.*, 2010](#); [Blankers *et al.*, 2013](#); [Tingle *et al.*, 2017](#)), and some have tested for interactions between body size and clade or ecological factors (e.g. [Lavin *et al.*,](#)

[2008](#)), few have tested for lineage-specific responses (but see [Collar *et al.*, 2010](#)). In addition, our initial analyses revealed much higher variability among terrestrial species than among the climbing species for several size-corrected traits. This heteroscedasticity violates an important assumption of ANOVA- or ANCOVA-type analyses, making *P* values unreliable (e.g. see [Cleasby & Nakagawa, 2011](#)). Therefore, we wrote new Matlab programs (see Supporting Information) to allow the use of established phylogenetic analyses ([Lavin *et al.*, 2008](#)) while allowing for heterogeneous variances in the residuals. Because we did not have a priori hypotheses regarding the heteroscedasticity, we used the phylogenetic simulation approach ([Garland *et al.*, 1993](#)) to test hypotheses regarding differences in group means and did not attempt to test for among-group differences in variability (cf. [Hutcheon & Garland, 2004](#); [O’Meara *et al.*, 2006](#); [Beaulieu *et al.*, 2012](#)).

MATERIAL AND METHODS

SPECIES SAMPLING

We studied the Lygosominae for three reasons: (1) this lineage has terrestrial, saxicolous and arboreal representatives; (2) we could not address questions about limb length in species with no limbs, which excludes many skink genera; and (3) Lygosominae is the largest (in terms of number of genera) subfamily within Scincidae based on recent phylogenetic analyses ([Pyrone *et al.*, 2013](#)). Species sampling was dictated by the availability of phylogenetic and detailed habitat data in the literature. To maximize our sample size, in cases where both LACM specimens and habitat data were available for species that were absent from the [Pyrone *et al.* \(2013\)](#) phylogeny, the locations of genera on that phylogeny were used to place up to two species (the maximum number of species that could be unambiguously inserted without additional knowledge of phylogenetic hierarchy). This procedure resulted in an evenly distributed sampling of species belonging to 45% of the genera within the Lygosominae subfamily, the largest subfamily within Scincidae ([Pyrone *et al.*, 2013](#)).

MORPHOMETRIC DATA

Limb and body linear dimensions were measured with Mitutoyo digital calipers (error \pm 0.01 mm) for 61 species of skinks from specimens at the Natural History Museum of Los Angeles County (LACM; [Table S2](#)). Measurements recorded were: body mass of the preserved specimens, total length (including

length (SVL). Importantly, both habitat usage and relative limb lengths have evolved multiple times within this group of skinks. Lizard illustrations are of representative specimens belonging to each of the three habitat conditions, and also show the greater diversity in relative limb length of terrestrial species (see also [Figs 5, 6](#)).

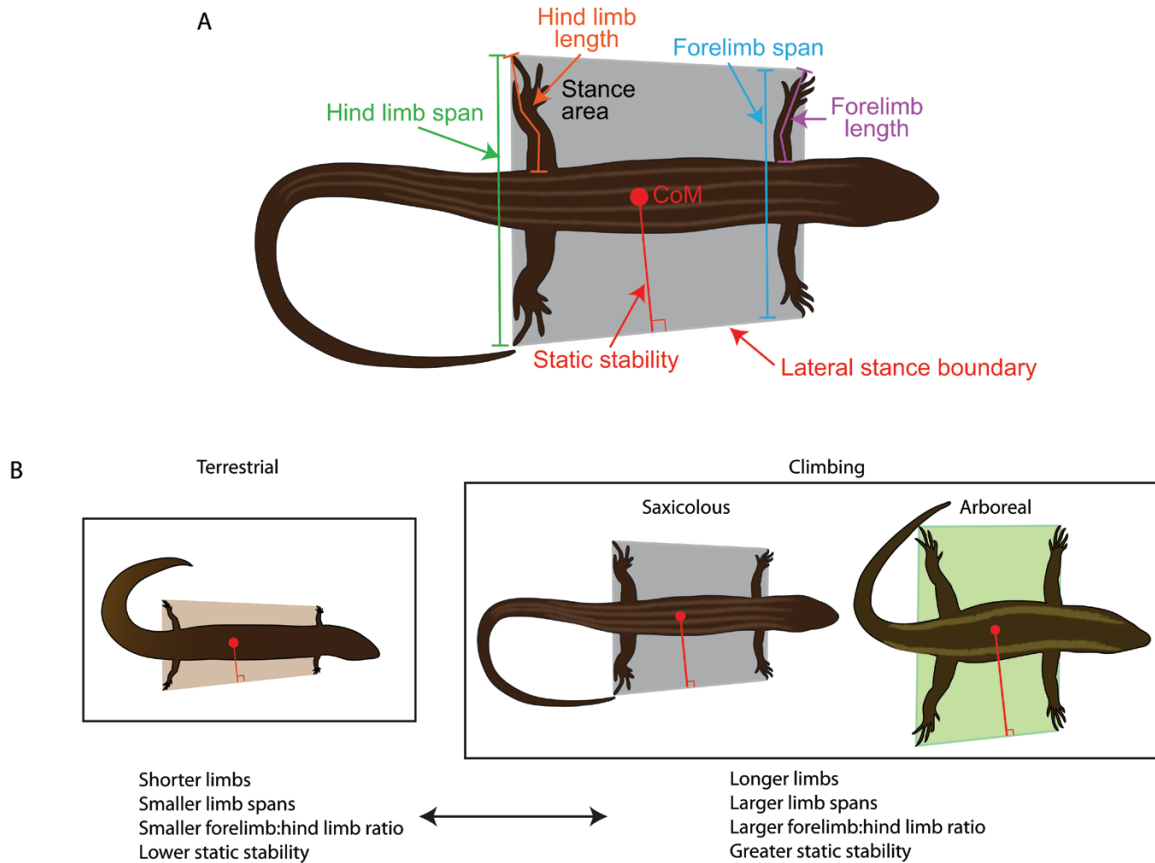


Figure 2. A, illustration of the measured and calculated dependent variables used in analyses. Static stability is defined as the minimum distance between the centre of mass (CoM) and the lateral stance boundary. B, illustration of the differences in dependent variables that we predicted among habitat categories, based on our biomechanically inspired hypotheses.

tail), snout–vent length (SVL), inter-girdle length (IGL; measured as the distance between the centre of each girdle), body width at the pelvis (BW, measured from the ventral side as the distance between where the hind limbs leave the body, at the middle of the limbs), proximal forelimb length, distal forelimb length (excluding manus), manus length (from wrist to tip of third digit), number of digits on the forelimb, proximal hind limb length, distal hind limb length (excluding pes), pes length (from ankle to tip of fourth digit) and number of digits on the hind limb. These measurements were then used to calculate the dependent variables used in all analyses (illustrated in Fig. 2): total forelimb length (FL), total hind limb length (HL), the ratio of forelimb to hind limb length (FL:HL ratio), stance area (SA), static stability (*sensu* Ting *et al.*, 1994), calculated as the minimum distance between the centre of mass (CoM; calculated as 72.57% of SVL, based on Clemente *et al.*, 2008) and the lateral stance boundary (defined by length of limbs), and three measures of limb span (FL span, HL span and average limb span), which represent the maximum distance between the distal-most tips of the longest

digits of each limb in a completely sprawled posture in which all limb segments are splayed in a horizontal plane (equal to the sum of body width and $2 \times$ limb length). Note that although Clemente *et al.* (2008) measured CoM position in agamid lizards, this was the best resource available to calculate CoM position in our skinks. Furthermore, our use of body width at the pelvic girdle for our calculation of forelimb span assumes that widths of the pectoral and pelvic girdles are similar. This is a justifiable assumption in our specimens, which have a largely cylindrical trunk shape with differences in girdle width estimated at less than 0.5 mm. In addition to these data, SVL, FL, HL and FL:HL ratio for 40 additional species were compiled from the literature and added to the dataset, resulting in a sample size of 101 species for these variables. Data for individuals were averaged to obtain single values for each species, as analysis of intraspecific morphological variation across populations within a species was beyond the scope of this study. These means were then \log_{10} -transformed (to obtain linear relationships and homoscedasticity) prior to all analyses.

HABITAT

Species were classified as terrestrial ($N = 63$), saxicolous (rock-dwelling; $N = 18$) or arboreal ($N = 20$) based on field observations from the literature (Table S2). These categories may, at first glance, seem broad. Theoretically, species classified as terrestrial could use primarily open, closed, fossorial or semi-fossorial habitats, arboreal species may specialize on leaves, narrow branches or tree trunks, and saxicolous species may use cliff faces or boulders. This within-habitat variation, if present, could alter or affect the detection of ecomorphological patterns. However, habitat usage within each category was actually quite limited for the majority of species in our study and, therefore, we did not classify our species into narrower habitat categories. Most terrestrial species were primarily fossorial or semi-fossorial, living primarily in leaf litter or under logs, although some were found in more open habitats. We found no descriptions of any of the terrestrial species included in this study burrowing into the ground (i.e. into packed soil). The majority of arboreal species utilized tree trunks rather than narrow branches, meaning that these species primarily use broader, vertical surfaces. These surfaces are similar to the broad, vertical surfaces common in the saxicolous habitat and that must be utilized, at least some of the time, by saxicolous species, regardless of whether they are found primarily on cliff faces or boulders. Given the similarity of some of the challenges faced by arboreal and saxicolous species, statistical models examining differences in morphology included habitat either divided into these three categories or divided into two categories, with saxicolous and arboreal habitats grouped into a single 'climbing' category for comparison with terrestrial species. Importantly, although it is common for saxicolous species in other lizard groups to have a dorsoventrally flattened body, all saxicolous species in this study had cylindrical trunk shapes similar to terrestrial and arboreal species. For this reason, we do not believe that a uniquely saxicolous body form will confound the utility of combining arboreal and saxicolous species into a single habitat category. Species defined as semi-arboreal in the literature were classified as arboreal for our analyses, and *Egernia napoleonsis*, which was described as both saxicolous and semi-arboreal in the literature, was classified as saxicolous in this study.

The skink species in this study represent numerous independent exploitations of arboreal, saxicolous and terrestrial habitats (Fig. 1). With the exception of Lygosominae, which contains a single arboreal species, three terrestrial species and no saxicolous species, all of the other subfamilies have representatives living in each of the three habitats (Sphenomorphinae: 3

arboreal, 1 saxicolous, 17 terrestrial; Egerniinae: 1 arboreal, 3 saxicolous, 6 terrestrial; Mabuyiinae: 10 arboreal, 3 saxicolous, 15 terrestrial; Eugongylineae: 5 arboreal, 11 saxicolous, 22 terrestrial).

PHYLOGENY AND ANCESTRAL RECONSTRUCTIONS OF HABITAT

The phylogeny used in this study (Fig. 1) was compiled from three published phylogenies. The majority of the topology was derived from Pyron *et al.* (2013), with additional detail for *Carlia* and *Niveoscincus* from Goodman & Isaac (2008) and Melville & Swain (2000b), respectively. In addition, in a few cases (*Cryptoblepharus*, *Leptosiphos*, *Mochlus*, *Morethia*, *Oligosoma* and *Panaspis*), the placement of the genus in the Pyron phylogeny was used to insert one or two congeneric species. Although combining multiple trees is not ideal, due to differences in characters and methods used for phylogenetic reconstruction, adding these species to the Pyron phylogeny allowed for an increased sample size. Furthermore, it should be noted that although a more recent phylogeny exists for this group (Tonini *et al.*, 2016), it, too, lacks some of the species measured in this study, in addition to having several unresolved polytomies that would hamper our phylogenetic analyses. For this reason, we elected to use the slightly older (Pyron *et al.*, 2013) phylogeny.

Because estimates of divergence times could not be determined for this composite tree, Pagel's (1992) arbitrary branch lengths, in which all tip branches are contemporaneous and equal in length, were used. Diagnostic plots [PDTREE module of Mesquite (v.2.75, <http://mesquiteproject.org>)] of the absolute values of the standardized independent contrasts vs. the standard deviations of the contrasts suggested that these branch lengths were statistically adequate for the traits we included (Garland *et al.*, 1992).

A note on nomenclature: although all the taxa analysed belong to what Pyron *et al.* (2013) referred to as the subfamily Lygosominae, this subfamily contains five higher-level taxon groupings that are currently considered subfamilies within Scincomorpha and are useful for testing clade-level transitions in morphology. These subfamilies within Scincomorpha are: Sphenomorphinae, Egerniinae, Mabuyiinae, Lygosominae and Eugongylineae. To test for differences at higher-level clade transitions, rather than just a relationship with the hierarchical structure within the clades, the species were grouped into these five clades (Fig. 1).

We reconstructed the ancestral habitat condition using the unordered maximum-parsimony method and performed 1000 habitat randomizations to test for the presence of phylogenetic signal using Mesquite (Rezende *et al.*, 2004; Tulli *et al.*, 2012). In addition, we inferred the evolutionary history of habitat with

stochastic character mapping (Bollback, 2006), implemented in the R-package *phytools* (Revell, 2012) using R 3.4.4 (R Core Team, 2018). In contrast to parsimony or likelihood methods of ancestral state reconstruction, stochastic character mapping allows for changes to occur along branches, not just at the nodes. The incorporation of branch lengths is therefore potentially more realistic from a biological perspective, because long branches, representing more time, will have a greater chance to feature a transition than a short branch. We applied stochastic character mapping by allowing the Q matrix, which describes the instantaneous rates of change from one state to another along branches, to take the form of three different models. The simplest model, ER, assumes equal rates across all transitions, in all directions. A transition from terrestrial to saxicolous habitat would therefore be assumed to occur at the same rate as a transition from saxicolous to arboreal, and vice versa. The symmetrical model, SYM, is more flexible and allows different rates of transitions between states, but forces the rates of trait reversals (= pairwise rates) to be equal. A transition from terrestrial to saxicolous habitat would therefore be allowed to be different from the rate of a transition from saxicolous to arboreal. However, the corresponding character state reversals, saxicolous to terrestrial, and arboreal to saxicolous, are forced to be equal to the initial transition rates. The resulting transition rate matrix is symmetrical, hence the name of the model. Finally, the third model, ARD, allows all rates to be different, in all directions. All three models (ER, SYM or ARD) were generated in two ways, one in which we forced the root node to be terrestrial, because the habitats of extant skink outgroups strongly suggest a terrestrial origin of skinks (Taylor, 1956; Duellman, 1963; Pyron *et al.*, 2013), and one in which we did not predefine the root state, instead allowing the ancestral state to be determined by the model. Each model of ancestral state reconstructions was iterated 1000 times and results were summarized in the form of pie charts for each internal node, which represent the proportions of states reconstructed for each node. We used Akaike information criterion (AIC) scores and the corresponding Akaike weights, the relative likelihoods of the models, to select the best fitting ancestral state reconstruction model.

STATISTICAL ANALYSES

We performed univariate analyses of phylogenetic signal following Blomberg *et al.* (2003), implemented in *phytools* (Revell, 2012), *phylosig()*-function. We report the K statistic as an indicator of the amount of phylogenetic signal and we performed randomizations on the mean squared error to test for statistical significance (1000 randomizations) vs. the null

hypothesis of no signal. We analysed log SVL and the ratios HL/SVL and FL/SVL. Use of these ratios assumes that HL and FL scale isometrically with SVL, which seemed reasonable based on initial analyses (for a more sophisticated way of adjusting for body size, see Blomberg *et al.*, 2003).

For each dependent variable, we tested two ANCOVA-style models. Each model included SVL as a covariate to remove the effect of body size from the variables of interest (referred to as SVL-adjusted variables). Each model also included two categorical explanatory variables: the clade variable, to test for differences among the five major lineages represented in our data set (Fig. 1), and habitat. However, the models differed in our incorporation of the habitat variable. The first model tested the effect of habitat defined based on three categories (terrestrial, saxicolous, arboreal), whereas the second combined the saxicolous and arboreal groups into a single 'climbing' group and compared that group against the terrestrial group (i.e. habitat was divided into two categories). In this latter model, we used one-sided *t*-tests to test the prediction that all dependent variables would be larger in climbing species than in terrestrial species. In addition, we analysed SVL as a dependent variable to determine if body size differed among habitats and/or clades.

All analyses were performed in Matlab (v.R2014a, The MathWorks, Natick, MA, USA) and R (R Core Team, 2018) using phylogenetic generalized least-squares (PGLS) methods that were modified to incorporate Ornstein–Uhlenbeck (OU)-transformed branch lengths (see appendix in Lavin *et al.*, 2008 regarding terminology). PGLS is implemented in different software packages with several ways to parameterize the phylogenetic variance–covariance matrix. We chose RegOU for Matlab, which uses the *d*-parameter to model an OU process (Lavin *et al.*, 2008) as our principal method, and it is explained thoroughly in the appendix of the published paper. PGLS can also be conducted in R, for example with the *nlme* (Pinheiro *et al.*, 2017) and *caper* (Orme *et al.*, 2018) packages. *Caper* is limited in that it only supports the Pagel tree transformation parameters λ , κ and δ , but not the biologically more meaningful α (Martins & Hansen, 1997) or the *d*-parameter of RegOU.

RegOU simultaneously estimates regression coefficients and the best-fitting transformation of the branch lengths, i.e. the branch lengths that result in the lowest mean squared error (Lavin *et al.*, 2008). The transformed branch lengths estimated with RegOU models range from those on a star-like phylogeny with no hierarchical structure, as is assumed in ordinary least-squares (OLS) models (values of the OU transformation parameter, *d*, at the lower limit of 0), and the hierarchical structure of PGLS models (values

of d near 1; Lavin *et al.*, 2008; Gartner *et al.*, 2010). Values of d greater than 1 indicate a topology with a more hierarchical structure than that of the original tree. If the restricted maximum likelihood (REML) estimate of d is statistically greater than 0, then the residuals of the model contain phylogenetic signal (Blomberg *et al.*, 2003; Lavin *et al.*, 2008; Rezende & Diniz-Filho, 2012).

For all SVL-adjusted variables, considerably more variation was found among species belonging to the terrestrial habitat category than the other two categories (see Results). This unequal variance among habitat categories (one type of heteroscedasticity) violates assumptions of ANCOVA-style analyses. Although this heteroscedasticity could be accounted for by modelling it as an inherent part of the statistical analysis (e.g. see Ho & Ané, 2014; Adams & Collyer, 2018), it was not something about which we had a priori hypotheses and so we took a simulation approach that allowed us to perform robust tests of our primary hypotheses regarding effects of habitat and clade on the mean values of the measured traits. The simulation approach involved three primary steps: (1) generating 2000 simulated datasets with the same overall characteristics of our real dataset, (2) adjusting these simulated datasets so that the variances within the habitat categories reflected the same variances in our real dataset, and (3) analysing all simulated datasets using the same ANCOVA-style RegOU analyses to generate F and t -distributions against which the F and t -values from our real dataset could be compared. This general procedure follows Garland *et al.* (1993) but adds the step of adjusting variances for a particular category. Details of each of these steps are described below.

To begin, we generated 2000 Monte Carlo simulations using the PDSIMUL program (Garland *et al.*, 1993). In all cases, the following options in PDSIMUL were employed. First, simulations were created to match the (non-phylogenetic) mean, variance, and correlation between the dependent variable of interest and SVL from the real dataset (always on the log scale). Second, the maximum and minimum values of the simulated dataset were defined as equal to the maximum and minimum values of the real dataset plus or minus 10%, respectively, and any values generated outside of these limits were truncated to the minimum or maximum value. Thus, PDSIMUL produced 2000 simulations with the same overall average characteristics as the real dataset.

Next, because PDSIMUL does not provide the option of simulating different variances among categories of an independent variable, a custom Matlab code (transformSimulData_v3.m, written by K.L.F., see Supporting Information) was used to transform the variance of each of the habitat categories in all the simulations to approximately match the variance of the

habitat categories of the real dataset. This was done without affecting the mean of the habitat categories or the mean and variance of the entire simulated dataset. Specifically, the variance in the groups in the simulated dataset was compared with that of the groups in the real dataset. If the difference in the variance was greater than 2% of the variance of the real dataset, then a transformation factor was calculated that would reduce or increase the variance of the simulated group to help it match the variance of the real dataset. This transformation factor was calculated as the square-root of the ratio of the simulated group variance to the real group variance, divided by the mean of the simulated group dataset. This process was done iteratively, with adjustments made to ensure that means of the groups in the simulated dataset remained equal to the means of the groups in the real dataset, and the new values were truncated if they exceeded the 10% upper or lower bounds of the initial simulation parameters.

Finally, all 2000 transformed simulation datasets were run through the RegOU models described above using regressionV2MultiProcess.m, an automated version of REGRESSIONv2.m (Lavin *et al.*, 2008) written by K.L.F. (see Supporting Information). The results of these analyses were used to generate a null F distribution for the effect of habitat and/or clade on each of the dependent variables, using simulRegOutSummaryV2.m, written by K.L.F. (see Supporting Information). Each F value from the real dataset (i.e. for tests of a given trait) was compared to the corresponding F distribution generated from the simulations to determine the probability that the real F value for a clade or habitat could have been achieved by chance (e.g. see Garland *et al.*, 1993). For the climbing vs. terrestrial comparison, which requires a one-tailed test, we instead used t statistics. Finally, to test whether all clades evolved climbing behaviour using similar morphological shifts, we performed the above analyses again, using F -statistics, with the addition of a clade-by-habitat interaction variable. However, there were several instances of singularity in this interaction variable for the datasets for SA, minimum distance between the CoM and lateral stance boundary, FL span, HL span and average limb span because they consisted of a subset of the species for the other variables. For this reason, we were unable to perform analyses including the interaction term for those traits.

In addition to RegOU (Lavin *et al.*, 2008), we performed the same analyses with a different parameterization of the OU process (Felsenstein, 1988; Garland *et al.*, 1993) where the α parameter (Hansen, 1997; Martins & Hansen, 1997) is used to transform the starter branch lengths and hence specify the residual correlation structure of the PGLS model. The α value is a parameter that describes the strength of stabilizing selection, with large α suggesting strong selection that has led to a loss of phylogenetic

signal, and small α indicating weak selection that has not erased the phylogenetic signal in the residuals of the dependent variable. If $\alpha = 0$, the correlation structure of the residuals is the same as in a Brownian motion process along the original phylogenetic tree. We used the `gls()` function in the `nlme`-package (Pinheiro *et al.*, 2017) for R to implement these models. The `gls()` function accepts correlation structure objects, and we used the `corMartins()` function of the `ape`-package (Paradis *et al.*, 2004) to generate a correlation structure corresponding to the OU process. `corMartin()` is one of several functions written to supply various phylogenetic correlation structures to `nlme`, a procedure explained in the accompanying online tutorial of Symonds & Blomberg (2014). We estimated α with REML, with $\alpha = 0.1$ as starting value of the search, except for the clade-by-habitat interaction models which were started at $\alpha = 0.01$. As before, SVL was added to the models as covariate, with habitat and clade as factors. Although the additive habitat and clade models caused no computational problems, all models that included the habitat-by-clade interaction term failed with singularity issues when habitat was scored with three discrete traits. We tracked these singularity issues to the lack of a saxicolous state in the Lygosominae clade, which R could not handle, in contrast to Matlab. All two-state clade-by-habitat interactions completed without issues. We did not attempt to perform computer simulations with these analyses, as they would be largely redundant with the RegOU analyses, so the nominal P -values cannot be trusted due to heteroscedasticity among the habitat categories (see above). Therefore, these results are only presented in the online Supporting Information (Tables S3 and S4).

RESULTS

Although we predefined a terrestrial root state in some of our ancestral reconstruction models based on

the literature (Taylor, 1956; Duellman, 1963; Pyron *et al.*, 2013), all models (maximum parsimony and stochastic character mapping) in which we did not predefine a terrestrial root state indicated that the ancestor of the skink lineage analysed here was indeed terrestrial, even though the ARD model support for a terrestrial origin was weak in comparison to the ER and SYM models (Table 1; Figs 3, S1). The maximum parsimony model suggested the presence of significant phylogenetic signal; 30 steps were required to evolve the existing habitat distribution, which is low compared with the distribution for 1000 randomized data sets (mean = 34.7, median = 35, range = 29–38; $P = 0.007$). Of the stochastic character mapping models, the ARD model, which allowed different rates of evolution among the three habitat categories, was the best supported (Table 1). The ARD model with predefined terrestrial root indicated an average of 164.73 changes between habitat states along all branches across the entire tree. On average, saxicolous arose 26.27 times (8.55 and 17.72 times from terrestrial and arboreal conditions, respectively), arboreality arose 78.40 times (52.00 and 26.39 times from terrestrial and saxicolous conditions, respectively), and a reversion to terrestriality occurred 60.07 times, exclusively from the arboreal condition. The mean proportion of time spent in the terrestrial, saxicolous and arboreal states was 64%, 17% and 19%, respectively (Fig. 3, Table 1). The minimum number of transitions, if we ignore changes along a branch and only consider changes that occurred from node-to-node, is 31, including 13 transitions from terrestriality to arboreality, 12 transitions from terrestriality to saxicolous, but only five transitions from saxicolous to arboreality and one transition from saxicolous back to terrestriality. Transitions away from arboreality were not observed when only considering node-to-node changes.

Statistically significant phylogenetic signal was detected for log SVL ($P < 0.001$) and for the ratios HL/SVL and FL/SVL (both $P = 0.001$). The K statistics

Table 1. Summary of results for stochastic character mapping performed with the `phytools` package for R (Revell, 2012)

Transition rate model	Root state pre-defined?	Probability of terrestrial root state	Mean number of habitat state changes	AIC	Akaike weight*
ER	No	A: 0.197, S: 0.198, T: 0.605	67.75	202.90	0.0002
ER	Yes	Fixed at A: 0, S: 0, T: 1	58.65	201.45	0.0004
SYM	No	A: 0.096, S: 0.038, T: 0.866	88.39	197.70	0.0024
SYM	Yes	Fixed at A: 0, S: 0, T: 1	84.47	195.78	0.0062
ARD	No	A: 0.336, S: 0.318, T: 0.346	167.07	185.62	0.9975
ARD	Yes	Fixed at A: 0, S: 0, T: 1	164.73	185.61	0.9935

We performed two sets of analyses: one with a terrestrial prior for the root state, strongly supported by outgroup comparisons, and one without prior. For both sets of analyses, we allowed the transition rate matrix to take the form of all rates being equal (ER), equal pairwise rates (SYM) and all rates different (ARD), and compared results via AIC scores. All models without a pre-defined root state support a terrestrial root state (bold text). A, arboreal; S, saxicolous; T, terrestrial. See text for details.

*Akaike weights were calculated separately for models with and without fixed root state.

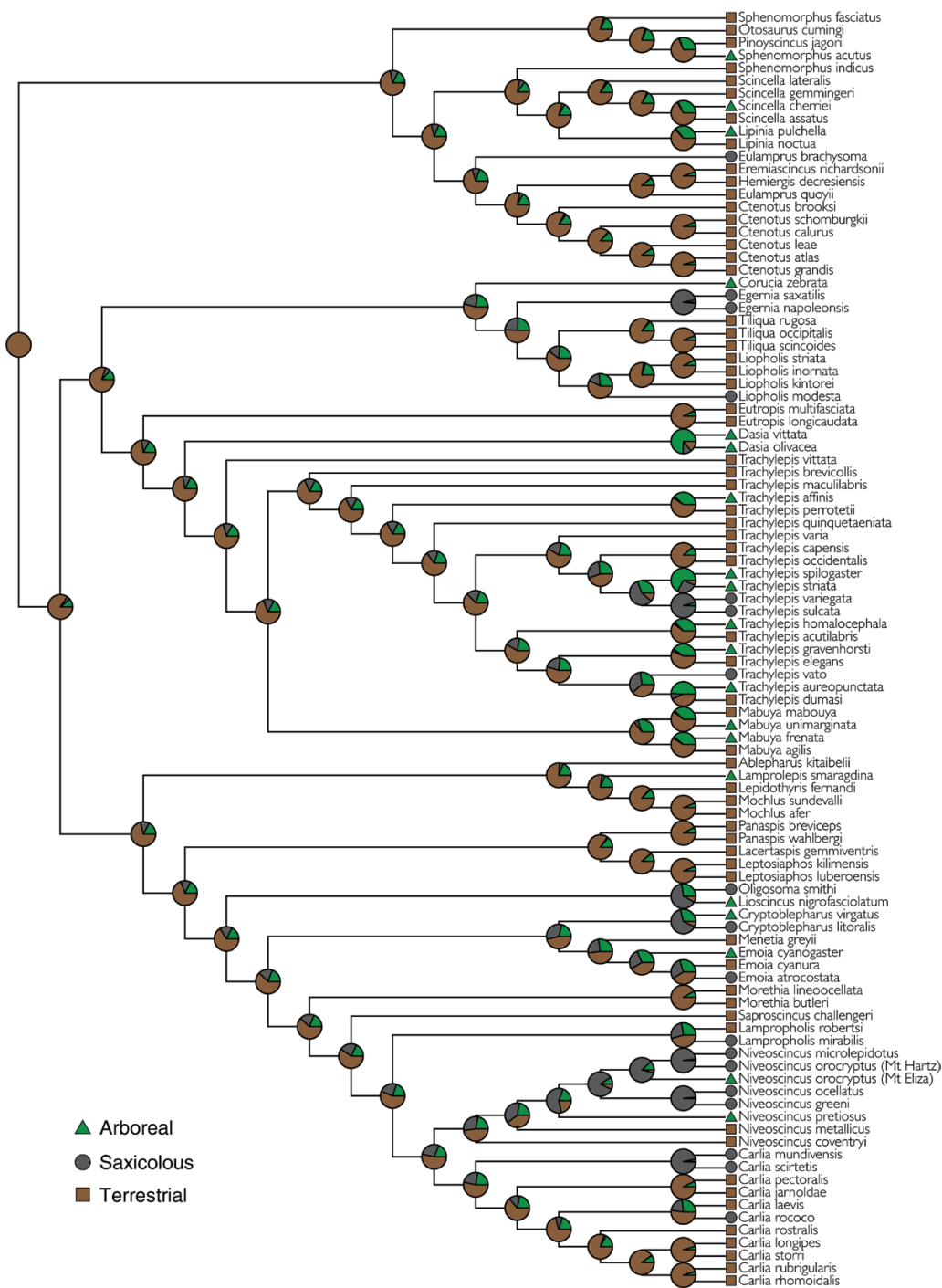


Figure 3. Ancestral state reconstructions of habitat preference in skinks summarized from 1000 iterations of stochastic character mapping performed in phytools for R (Revell, 2012). Shown here is the summary for the transition rate model that allowed for all rates to be different (ARD) and the root state locked in as terrestrial on the basis of outgroup comparisons. The pie charts indicate the probability for each of the three states (arboreal, saxicolous and terrestrial) at the given node. Most common are transitions from a terrestrial lifestyle to arboreality (at least 13) and saxicolous (at least 12). For the ER and SYM models, please refer to the online Supporting Information.

were, respectively, 0.452, 0.277 and 0.293. Differences among clades are also indicative of phylogenetic signal (Gartner *et al.*, 2010), and we detected clade effects or clade-by-habitat interactions for these traits as well.

For the traits with a sample size of 101 species, we first examined models that included the clade-by-habitat interaction term, which was statistically significant for SVL and HL, and marginally non-significant for FL, based on the simulated null distributions of P -values (Table 2). For SVL, the ln maximum likelihood of the model with three habitat categories (68.48) was substantially higher than that with two habitat categories (61.78: ln maximum likelihood ratio test, $\chi^2 = 13.4$, d.f. = 1, $P = 0.0003$). Figure 4A illustrates the interaction: body size varies with habitat in some clades but not in others, and no consistent pattern exists across clades.

For relative forelimb and hind limb lengths, as well as FL/HL ratio, the three-category models did not offer any improvement in fit, so the simpler models with two habitat categories are preferred. For these models (Table 2), the clade-by-habitat interaction was marginally non-significant for FL ($P = 0.0610$; Fig. 4B) and significant for HL ($P = 0.0230$; Fig. 4C). The FL/HL ratio analysed in the preferred two-category habitat model showed significant variation among clades (Fig. 4D).

For completeness, we also show models without the clade-by-habitat interaction term in Table 3.

As predicted, the SVL-adjusted forelimbs and hind limbs of climbing (arboreal + saxicolous) species were longer than in terrestrial species (Fig. 5; one-tailed $P = 0.0175$ and $P = 0.0480$, based on comparisons with phylogenetically simulated t -distributions).

Perhaps surprisingly, given previous studies (see Introduction and Table S1), none of the other morphometric traits considered, including the derived indices, showed statistically significant differences among habitats, based on the simulations (Table 3). For example, habitat did not affect forelimb span, hind limb span or static stability (Fig. 6).

DISCUSSION

MULTIPLE SOLUTIONS IN RESPONSE TO SIMILAR SELECTIVE REGIMES

For the traits with larger sample size, we detected statistically significant clade-by-habitat interactions for two of four traits, and a marginally non-significant interaction for another (Table 2). Moreover, for our measure of body size, testing for a clade-by-habitat interaction revealed a habitat effect that was missed in statistical models that excluded the interaction term (see analyses of SVL in Tables 2 and 3). Although numerous ecomorphological studies have tested

Table 2. Results of phylogenetically informed statistical analyses, with interactions between the clade and habitat variables included

Dependent variable	N	Habitat coding	RegOU analyses (Lavin <i>et al.</i> , 2008) (d parameterization)					RegOU simulation analyses		
			ln max likelihood	REML d	Habitat P -value	Clade P -value	Clade \times Habitat P -value	Habitat P -value	Clade P -value	Clade \times Habitat P -value
SVL	101	AST	68.48	0.4307	0.6590	0.0001	0.0030	0.4705	0.0955	<0.0001
SVL	101	CT	61.78	0.4562	0.8481	0.2322	0.0181	0.8285	0.5095	0.0135
FL	101	AST	102.39	0.2344	0.2239	0.0459	0.2389	0.0835	0.5380	0.0765
FL	101	CT	102.03	0.2359	0.0794	0.7090	0.0610	0.0565	0.7885	0.0610
HL	101	AST	104.90	0.2992	0.3883	0.0251	0.1659	0.1885	0.4330	0.0365
HL	101	CT	104.60	0.2992	0.1643	0.9351	0.0339	0.1100	0.9690	0.0230
FL/HL ratio	101	AST	181.65	0.1027	0.4947	0.0022	0.3436	0.6685	0.1130	0.6510
FL/HL ratio	101	CT	181.61	0.0944	0.2274	0.0176	0.0872	0.5840	0.0150	0.5840

Analyses compare F statistics with conventional distributions ('RegOU analyses' in the table) or with distributions based on 2000 phylogenetic simulations generated with the PDSIMUL.EXE program (Garland *et al.*, 1993), then modified to have greater variance for the terrestrial habitat category ('RegOU simulation analyses' in the table; see text for details), and finally analysed with RegOU. P -values for 'original' analyses were obtained from phylogenetic regressions in which the residuals are assumed to follow a distribution consistent with an Ornstein-Uhlenbeck (OU) model of evolution, by use of the REGRESSIONv2.m Matlab program (RegOU models described in Lavin *et al.*, 2008). P -values for 'simulation' analyses represent the proportion of F -values from simulations that were greater than the F -value from the real dataset, with all analyses again conducted under the OU model of residual trait evolution (obtained from the new simulRegOutSummaryV2.m). Model 1 (labelled 'AST' in the table) incorporates habitat divided into three categories (arboreal, saxicolous and terrestrial) and model 2 (labelled 'CT' in the table) incorporates habitat divided into two categories (climbing and terrestrial). Snout-vent length (SVL, log transformed) was included as a covariate in all models, with the exception of the first two models where log SVL was analysed as a dependent variable. SVL was highly significant ($P < 0.0001$) in all models where it was included as a covariate (results not shown). Both models contain clade, with five divisions as indicated in Figure 1. P values < 0.05 are in bold type: only those from the simulation analyses should be considered reliable. FL, forelimb length; HL, hind limb length; SA, stance area; static stability, distance from CoM to lateral stance boundary; Clade \times Habitat, clade-by-habitat interaction. Results for PGLS implemented in the nlme-package in R, which do not account for heterogeneous variances among habitat categories, are given in the Supporting Information, Table S3.

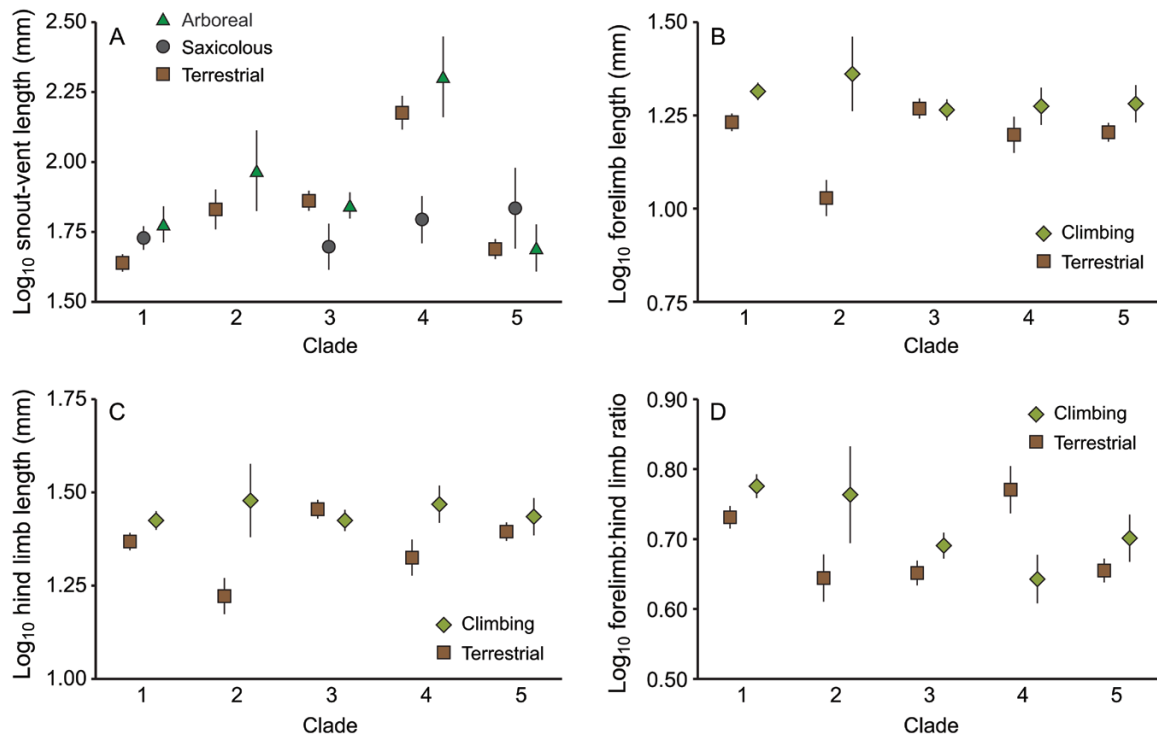


Figure 4. A, means and standard errors for \log_{10} snout–vent length (SVL) (our measure of body size), illustrating the interaction between clade and habitat: body size varies with habitat in some clades but not others, and no consistent pattern exists across clades. Results from the three-habitat model are shown as this is the preferred model for \log_{10} SVL (based on likelihoods shown in Table 2; see text). Relative forelimb (B) and hind limb (C) also showed clade-by-habitat interactions, as illustrated by estimated marginal means from conventional two-way ANOVAs (SPSS) that include the clade-by-habitat interaction term (as in Table 2). For relative forelimb and hind limb lengths (with body size as a covariate), the two-habitat models are shown based on the likelihoods shown in Table 2 (see text). The FL/HL ratio (D) varied significantly among clades, based on the simulated null distributions (Table 2). Clade 1, Eugongylinae; clade 2, Lygosominae; clade 3, Mabuyinae; clade 4, Egerniinae; clade 5, Sphenomorphinae. See Table 2 for phylogenetically informed statistical analyses.

for effects of clade or habitat, few have tested for interactions. Indeed, the study by Collar *et al.* (2010: fig. 4), also on lizard ecomorphology, is the only other example of which we are aware. We encourage future comparative studies of ecomorphology, physiology, behaviour and life history traits to implement explicit tests for such lineage-specific effects.

FORELIMBS VS. HIND LIMBS

Both relative forelimb length and hind limb length covaried with habitat among the 101 species of skinks considered here. Specifically, forelimbs and hind limbs were longer in climbing (i.e. arboreal and saxicolous) species than in terrestrial species (Fig. 5). However, inspection of Figures 5 and 6 shows that these differences are driven by about ten terrestrial species that have short limbs for their body size; for the remaining species, relative forelimb length is similar for the three habitat categories. Importantly, these short-limbed terrestrial species are not from a single

branch of the phylogenetic tree, but rather occur in several separate areas (Fig. 1). Furthermore, although severe limb reduction suggests a fossorial lifestyle, the majority of the terrestrial species included in this study were described in the literature as being fossorial or semi-fossorial, as noted above. Thus, we do not believe that these ten or so species are ecologically distinct from the remaining terrestrial species.

Parsimony reconstruction and stochastic character mapping suggest that the ancestral condition is terrestrial, a finding supported by the terrestrial condition of the closest sister genera (Pyron *et al.*, 2013), *Ophiomorus* (limbless) and *Mesoscincus* (Taylor, 1956; Duellman, 1963), to the group analysed here. Furthermore, all models of stochastic character mapping suggest that arboreality and saxicolity arose independently many times in this group. Therefore, our analyses suggest that elongating both forelimbs and hind limbs is important for the evolution of climbing behaviour from a terrestrial ancestor. Interestingly, this contrasts with the ecomorphological relationship

Table 3. Results of phylogenetically informed statistical analyses comparing dependent variables among habitat categories and clades, without interactions between habitat and clade

Dependent variable	N	Habitat coding	RegOU analyses (Lavin <i>et al.</i> , 2008) (d parameterization)				RegOU simulation analyses	
			ln max likelihood	REML <i>d</i>	Habitat <i>P</i> -value	Clade <i>P</i> -value	Habitat <i>P</i> -value	Clade <i>P</i> -value
SVL	101	AST	56.33	0.4256	0.3875	0.0006	0.3960	0.2250
SVL	101	CT	55.40	0.4402	0.2872	0.0007	0.2995	0.2410
FL	101	AST	97.12	0.2318	0.0477	0.2427	0.0605	0.7655
FL	101	CT	97.03	0.2360	0.0074	0.2412	0.0175	0.7645
HL	101	AST	98.93	0.2983	0.2042	0.2810	0.1640	0.7695
HL	101	CT	98.80	0.3038	0.0422	0.2953	0.0480	0.7780
FL/HL ratio	101	AST	176.95	0.1293	0.2404	0.0018	0.5875	0.0915
FL/HL ratio	101	CT	176.92	0.1317	0.0470	0.0017	0.2360	0.0900
SA	61	AST	92.89	0.0024	0.5618	0.0119	0.3395	0.2170
SA	61	CT	92.59	0.0003	0.2422	0.0093	0.8190	0.2035
Static stability	61	AST	76.40	0.0228	0.7732	0.0423	0.6495	0.4175
Static stability	61	CT	76.23	0.0143	0.3707	0.0365	0.3755	0.4020
FL span	61	AST	59.18	0.0653	0.5200	0.1757	0.3540	0.6415
FL span	61	CT	59.15	0.0579	0.1335	0.1581	0.0995	0.6310
HL span	61	AST	57.39	0.1452	0.7669	0.0914	0.6590	0.5435
HL span	61	CT	57.39	0.1394	0.2392	0.0823	0.2070	0.5405
Avg. limb span	61	AST	59.34	0.1121	0.6688	0.1205	0.5215	0.5840
Avg. limb span	61	CT	59.32	0.1052	0.1928	0.1085	0.1540	0.5770

Models are as explained in Table 2. *P*-values for ‘simulation’ analyses represent the proportion of *F* or *t*-values from simulations that were greater than the *F* or *t*-value from the real dataset, with all analyses again conducted under the Ornstein–Uhlenbeck (OU) model of residual trait evolution (obtained from the new simulRegOutSummaryV2.m). Model 1 (labelled ‘AST’ in the table) incorporates habitat divided into three categories (arboreal, saxicolous and terrestrial) and *P*-values for simulation analyses for habitat were calculated from tests of *F*-value distributions. Model 2 (labelled ‘CT’ in the table) incorporates habitat divided into two categories (climbing and terrestrial) and *P*-values for simulation analyses for habitat were calculated from one-tailed tests with *t*-value distributions, testing the hypothesis that climbing species have larger values for all traits. For both models, *P*-values for simulation analyses for clade were calculated from tests of *F*-value distributions. *P* values < 0.05 are in bold type: only those from the simulation analyses should be considered reliable. Snout–vent length (SVL, log-transformed) was included as a covariate in all models, with the exception of the first two rows where SVL was analysed as a dependent variable. SVL was highly significant ($P < 0.0001$) in all models where it was included as a covariate (results not shown). FL, forelimb length; HL, hind limb length; SA, stance area; static stability, distance from CoM to lateral stance boundary. Results for PGLS implemented in the nlme-package in R, which do not account for heterogeneous variances among habitat categories, are given in the Supporting Information, Table S4.

found across 90 species of agamids, in which increased forelimb length, but not hind limb length, correlated with increased arboreality (see Table S1 for details; Collar *et al.*, 2010). However, those relationships were based on qualitative observations of principal components analysis results, and were not statistically tested (Collar *et al.*, 2010). The length of the hind limb typically correlates positively with sprint speed (e.g. Bonine & Garland, 1999), whereas forelimb length correlates negatively (Losos, 1990a, b; Beuttell & Losos, 1999; Mattingly & Jayne, 2004). However, climbing increases the relative propulsive role of the forelimb, and this can approach, or in some cases surpass, the propulsive role of the hind limb (Arnold, 1998; Zaaf *et al.*, 1999; Autumn *et al.*, 2006; Goldman *et al.*, 2006; Lammers, 2007; Foster & Higham, 2012, 2014). In such cases, elongation of the forelimb may contribute equally or even more to increasing locomotor performance than increasing hind limb length. Additionally, climbing

lizards will naturally go downhill frequently, which can increase the reliance on the forelimb for braking (Birn-Jeffery & Higham, 2014). Thus, longer forelimbs may represent the increased role of braking. Our finding that climbing skinks have significantly longer forelimbs and hind limbs than terrestrial skinks is consistent with these studies, although additional investigation into the kinematics and kinetics of these species moving on different substrates is required to establish the relative importance of fore- and hind limbs for propulsion.

CLIMBING ABILITY VS. STABILITY

Although climbing skinks had longer forelimbs and hind limbs (relative to body size) compared with terrestrial species (Fig. 5), this elongation did not appear to result in significant increases in our measure of static stability (see Methods and Fig. 2), forelimb span or

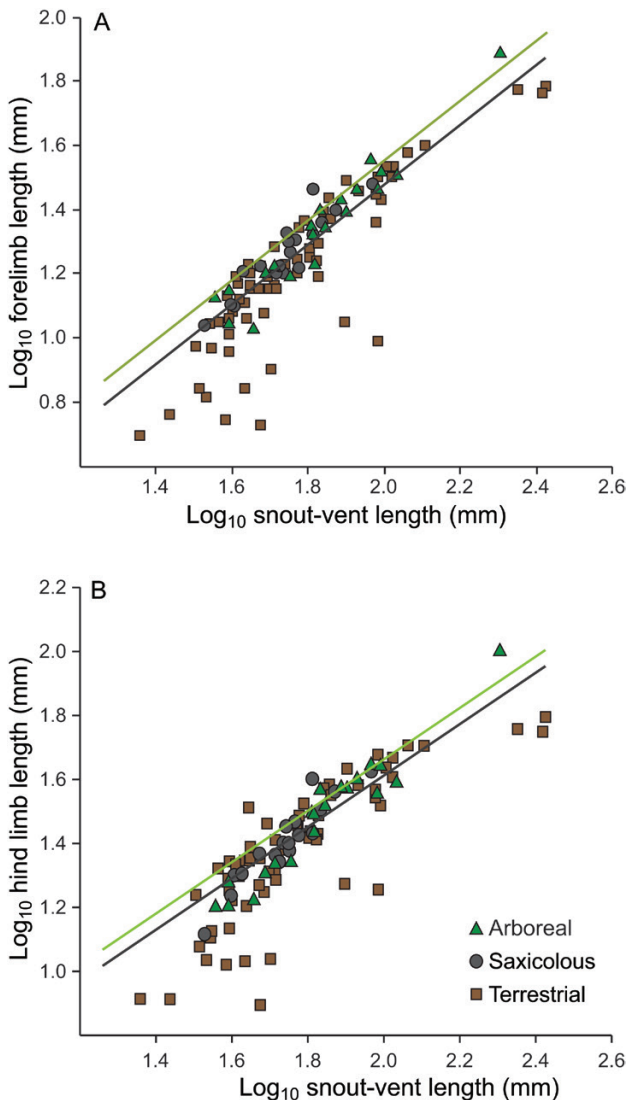


Figure 5. Log-transformed forelimb length (A) and log-transformed hind limb length (B), vs. log-transformed snout–vent length. Climbing species are shown as medium grey circles (saxicolous) and green triangles (arboreal), terrestrial species as brown squares, and their parallel regression lines are shown in pale green (climbing) and dark grey (terrestrial). All data points represent mean values for species, as presented in Table S2. Regression lines are from phylogenetically informed (RegOU) analyses. $N = 101$. For climbing vs. terrestrial species, in models that did not include the clade-by-habitat interaction, the P -value for one-tailed comparison of real vs. simulated data was 0.0175 for the forelimb and 0.0480 for the hind limb (Table 3).

hind limb span (Fig. 6). This seemingly contradictory result was surprising because of the mathematical relationship between these variables and limb length; in particular, limb spans (Fig. 6A, B) were calculated

as the sum of the length of both limbs (Fig. 5), on either side of the body, and body width. Furthermore, there appeared to be greater variation in limb span (Fig. 6A, B) than in static stability (Fig. 6C) relative to body size. A drop in statistical power may partially explain the discrepancy in significance between limb length and limb span, as data from the literature did not have sufficient information to allow us to calculate these variables for 40 of our 101 species. Restricting our dataset for forelimb length, and for hind limb length to the 61 species that comprised the remaining variables increased the P -values for limb length variables above the critical value (data not shown). However, there may be another, more functional explanation. Although body width is a component of the maximum toe-to-toe distance that the limbs can encompass, and thus directly relates to static clinging ability to a broad, vertical surface (Cartmill, 1985), it may be considerably less important for active propulsion on a vertical surface, and certainly less important than other traits that contribute to sprint speed. In general, a longer limb allows for higher maximal sprint speeds because moving a longer limb through a given angle at the same rate of speed results in a greater displacement of the body for a given stance phase, resulting in a greater stride length than would occur for a shorter limb (Bonine & Garland, 1999; Foster *et al.*, 2015). Using the same principle, body width can increase stride length via rotation of the girdle because a wider girdle moving through a given degree of rotation would result in a greater displacement of the limb (Peterson, 1971, 1984; Jenkins & Goslow, 1983; Fischer, Krause & Lilje, 2010). However, a number of factors, including the greater potential range of motion of limb joints compared to girdle rotation and the fact that limbs are longer than the body is wide, mean that the relative contribution of the body width to forward propulsion should be far surpassed by the contribution of limb length (except, perhaps, for species with extremely short limbs, such as some of those in the present data set, or possibly species that are bipedal at high speed and have a long airborne phase). Therefore, we believe that the habitat differences for limb length, but not limb span, may indicate historically stronger selection for active climbing ability than for static clinging ability in arboreal and saxicolous skinks.

OTHER CONSIDERATIONS

This study is the first to demonstrate statistically significant ecomorphological relationships between size-adjusted limb length and habitat use in such a large number of lizard species, excluding island-dwelling anoles. This success may be attributable to a number of factors. First, our relatively large sample size (101 species; larger than any previous study) was

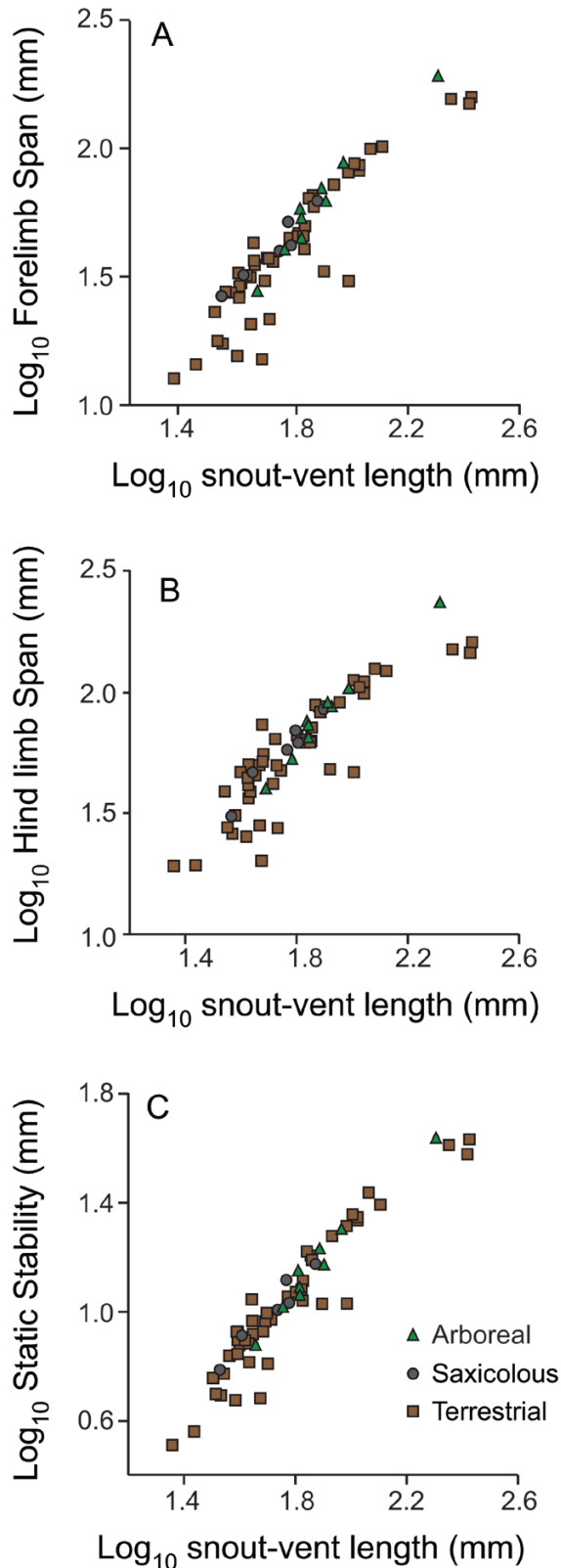


Figure 6. Log-transformed forelimb span (A), hind limb span (B) and static stability (i.e. distance from the centre of

within a single clade of lizards, which gave us more thorough sampling for this group. In contrast, other studies that had the next largest sample sizes (90 species in Blankers *et al.*, 2013; 49 species in Revell *et al.*, 2007) examined species across a broader range of lizard clades and thus had a more sparse sampling. Additionally, the vast majority of the 25 non-anoline papers to examine the relationship between limb length and habitat structure used far fewer species (Table S1). Perhaps a more thorough sampling of lizards in other groups will reveal ecomorphological relationships that have, as of yet, passed undetected.

High-quality ecological data are essential for the correct classification of species into habitat groups; without this, measurement error may obscure ecomorphological relationships. The vast majority of ecological sources used in this study reported detailed microhabitat use rather than just the substrate on which species were caught. This level of ecological detail also exists for Greater Antillean anoles and may help to explain why ecomorphological relationships have been so well established in that group (Losos, 1990a, b, 2009; Beuttell & Losos, 1999; Mattingly & Jayne, 2004). Additionally, microhabitat usage studies may reveal a degree of variation within habitat categories that acts to mask the relationships researchers expect. Studies that assess microhabitat use within a single species often find considerable differences in habitat use among populations (e.g. Collins *et al.*, 2015; Higham *et al.*, 2015), highlighting the potential to undermine a single category for a species. For example, morphs of the chameleon *Bradypodion pumilium* will occupy perches of varying diameter and incline, which results in divergent hind limb kinematics and function (Higham *et al.*, 2015). This appears to be less of an issue in our study, as the majority of our species appeared to use a relatively limited range of the surfaces within their habitat category. The arboreal skink species used in this study used tree trunks almost exclusively, eliminating a lot of the potential variation within an ‘arboreal’ classification. Most of the terrestrial species in our study primarily lived in closed habitats consisting of leaf litter and fallen logs and thus appeared to use a relatively small range of the microhabitats available to them rather than specializing on any number of the wide range of complex microhabitats that can fall within

mass to the lateral stance boundary) (C) vs. log-transformed snout–vent length. Climbing species are shown as green triangles (arboreal) and medium grey circles (saxicolous). Terrestrial species (squares) are shown as brown squares. All data points represent mean values for species. $N = 61$. No statistically significant differences among habitat types were found for any of the traits (see Table 3).

the terrestrial habitat classification. Regardless, for situations in which variation in habitat structure or behaviour is considerable, additional subdivision of the traditional ‘arboreal’, ‘saxicolous’ and ‘terrestrial’ categories into microhabitat categories may be more appropriate (e.g. [Kohlsdorf *et al.*, 2001](#)). Although we found such subdivision inappropriate given our current knowledge of these species, we have published our complete raw dataset to facilitate future re-analyses as new behavioural/ecological data become available.

In addition to simple linear measurements of limb segment or total limb length, derived variables that have clear biomechanical implications can be important. For example, studies of ‘cursorial’ locomotion in mammals have routinely considered the metatarsal/femur ratio (e.g. [Garland & Janis, 1993](#)). This biomechanically inspired variable is derived from basic morphometric measurements but has biomechanical implications because elongation of distal elements relative to proximal ones frequently leads to a more proximal concentration of muscle mass, resulting in less inertia in the distal limb, greater stride frequencies and greater locomotor efficiencies, leading to a positive relationship between running speed and metatarsal/femur ratio ([Hildebrand, 1985](#); [Garland & Janis, 1993](#); [Carrano, 1999](#)). However, derived, biomechanically inspired variables are rarely examined in studies of evolutionary morphology (for examples, see [Alfaro *et al.*, 2005](#); [Samuels & Van Valkenburgh, 2008](#); [Slater & Van Valkenburgh, 2009](#); [Castro & Garland, 2018](#)). Although we found that static clinging ability and static stability, here indexed as limb span, stance area and distance from the CoM to lateral stance boundary, were not important for distinguishing the skinks from different habitats, these may be important for other groups. In fact, it would not be surprising to find higher-level composite variables differing among species, with a lack of difference for simple limb measurements. For example, minor shifts in hind limb length could contribute to a significant shift in stance area, distance from the CoM to the stance boundary, or even hind limb span.

Much more work remains to be done to understand the ecomorphology of the locomotor system of skinks (see also [Blom *et al.*, 2016](#)). Our data suggest that arboreal and saxicolous species have repeatedly adapted to life in their respective habitats by evolving longer forelimbs and hind limbs, which we hypothesize provide greater propulsive ability, rather than enhanced static clinging ability. Although this makes intuitive sense, at least in hindsight, especially given the greater exposure to predation that might be expected on a cliff face, side of a boulder or tree trunk in comparison with crawling amongst leaf litter ([Revell *et al.*, 2007](#); [Goodman *et al.*, 2008](#)), this hypothesis is based solely on morphology. Morphology is just one of several levels of biological organization

that intervene between the genetic material and the primary targets of selection in nature (e.g. see [Foster *et al.*, 2015](#): fig. 11.1; [Storz *et al.*, 2015](#): fig. 1). Thus, future work should explore the behaviour, at both gross (e.g. foraging styles, anti-predator strategies; [Samia *et al.*, 2016](#)) and detailed levels (kinematic and kinetic), as well as physiology (particularly muscle function) and internal morphology (e.g. [Tulli *et al.*, 2016](#)) of these species to better understand the roles of the forelimbs and hind limbs in skink locomotion, how these roles shift with locomotion on different substrates, and how these species might be adapted for their particular habitats. At the same time, we must recognize that ‘etho-eco-morphological mismatches’ may be more common than is typically presumed by the adaptationist programme ([Diogo, 2017](#)).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. Ancestral state reconstructions of habitat preference in skinks for models that allow all rates of evolution to be different (ARD), equal rates of evolution (ER), and different rates of evolution but symmetric reversals (SYM). See text for details.

Table S1. Summary of previous studies examining ecomorphological relationships between limb length and habitat in lizards. See “Literature cited in supplementary tables.docx” in supplementary material for full bibliographic information.

Table S2. Complete raw dataset, along with citations for ecological and morphological sources, when applicable. See “Literature cited in supplementary tables.docx” in supplementary material for full bibliographic information.

Table S3. Results of nlme analyses for models that include clade by habitat interactions. See text for details.

Table S4. Results of nlme analyses for models without clade by habitat interactions. See text for details.