Current Zoology, 2025, **XX**, 1–12
https://doi.org/10.1093/cz/zoaf003
Advance access publication 31 January 2025
Original Article

Evolutionary adaptations in the limb morphology of tropidurid lizards in response to sandy environments

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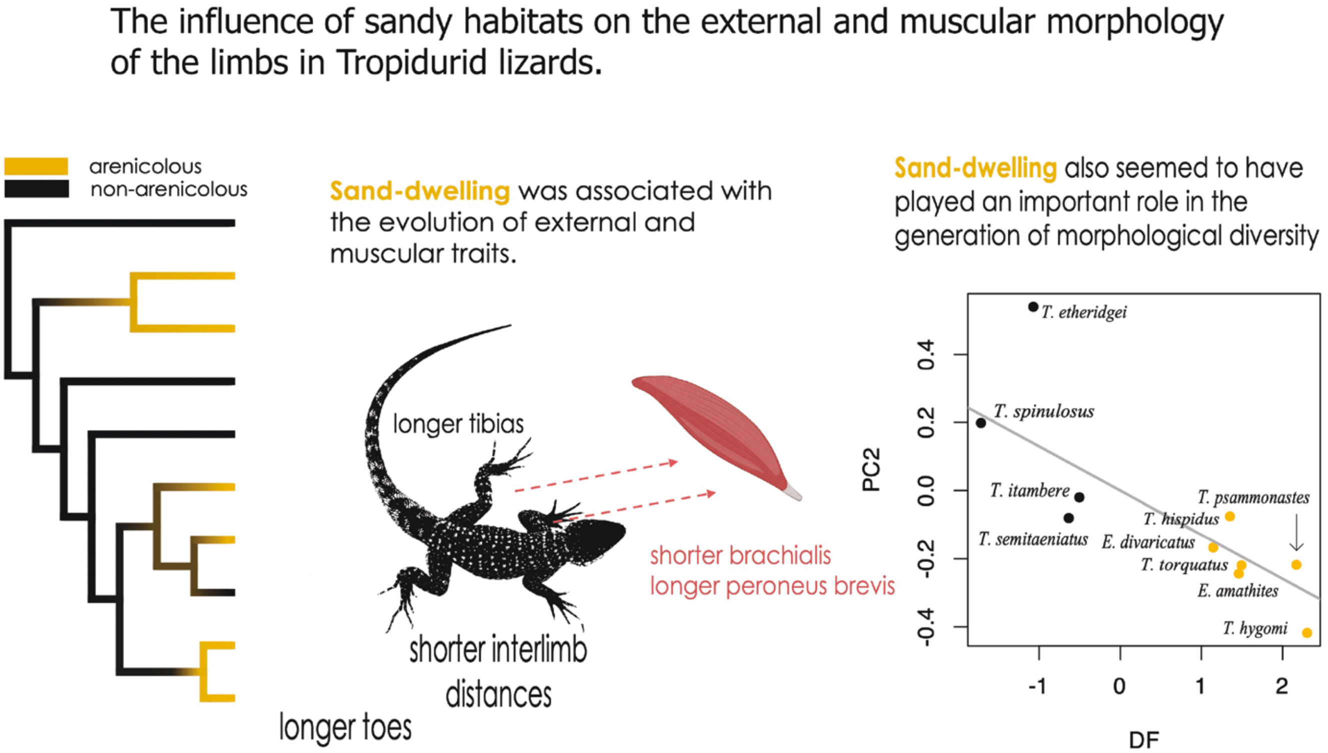
Handling editor: Zu-Shi Huang

Abstract

Different habitat types exert particular challenges to ecological performance, ultimately having a strong influence on the evolution of morphology. Although it is well known that external morphology can evolve under the selective pressure of habitat structure, the evolutionary response of internal morphological traits remains vastly unexplored. Here, we test for morphological divergence between arenicolous and nonarenicolous species in a clade of tropidurid lizards, considering external morphological proportions and limb muscle dimensions. We found that arenicolous species seem to have evolved internal and external morphological adaptations that separate them from other habitat specialists. Moreover, comparative analyses suggested that the traits that differed the most between arenicolous and nonarenicolous lizards might have evolved divergently towards different optima. Additionally, the axis of higher morphological divergence between arenicolous and nonarenicolous species represented an important proportion of the morphological diversity within our sample, indicating that the hypothetical adaptive divergence of internal and external traits has contributed significantly to phenotypic diversity. Our results show that evolutionary associations between morphology and habitat use can be detected on both external body proportions and muscle morphology. Moreover, they highlight the emergent importance of internal anatomical traits in ecomorphological studies, especially when such traits are directly involved in determining functional performance.

Key words: body shape, ecomorphology, muscles, sand substrates, Tropiduridae.

Graphical Abstract



The influence of sandy habitats on the external and muscular morphology of the limbs in Tropidurid lizards.

Sand-dwelling was associated with the evolution of external and muscular traits.

Sand-dwelling also seemed to have played an important role in the generation of morphological diversity

Received 23 July 2024; accepted 16 January 2025

The diversity of animal locomotor structures reflects the crucial role of specialized morphology in facilitating survival under specific ecological conditions (Wainwright and Reilly 1994). Efficient movement is a requirement for life in most habitats, thus morphological adaptations for locomotion, including both external (e.g., limb proportions) and internal (e.g., bone, muscle, and tendon characteristics) traits, have repeatedly evolved in nature (Currey 1984, 2003; Zaaf et al. 1999; Grizante et al. 2010; Collins et al. 2013; Abdala et al. 2014; Anzai et al. 2014; Tulli et al. 2016; Toyama et al. 2024), illustrating the central paradigm of ecomorphology: the relationship between morphology and fitness through the intermediate step of performance (Arnold 1983; Wainwright 1991; Wainwright and Reilly 1994).

Lizards are an excellent model system for studying ecomorphological relationships between habitat structure and locomotor morphology (Vanhooydonck et al. 2000; Schulte et al. 2004; Losos 2009), and how these associations evolve as a response to changing environments (Herrel et al. 2008; Stuart et al. 2014; Donihue et al. 2018). Previous ecomorphological studies in lizards have mostly focused on external traits (i.e., body part proportions), while the examination of internal morphology has been comparatively scarce. Nonetheless, studies on the muscular system of different lizard species have hinted at the importance of internal morphology for the adaptation to different structural environments (Garland and Losos 1994; Zani 1996; Herrel et al. 2008; Brandt et al. 2016; Ríos-Orjuela et al. 2020).

Sandy environments are among the most challenging habitat types for locomotion since granular substrates change their properties under given amounts of yield stress (Vanhooydonck et al. 2015; Bergmann et al. 2017). Sand-dwelling is known to have triggered the evolution of morphological adaptations in different groups of taxa (Jayne 1986; Gidmark et al. 2011; Hosoi and Goldman 2015; Astley et al. 2020). Among lizards, arenicolous species are thought to have evolved various adaptations to cope with the challenges imposed by sandy environments, demonstrating a range of behavioral, physiological, and morphological strategies to enhance locomotion, burrowing capabilities, and endurance on loose substrates (Carothers 1986; Maladen et al. 2009; Higham and Russel 2010; Kohlsdorf and Navas 2012; Vanhooydonck et al. 2015). For example, some authors have found that arenicolous lizard species tend to have long feet, and some have evolved toe fringes that enhance locomotor performance traits like speed and acceleration (Carothers 1986; Kohlsdorf et al. 2001; Tulli et al. 2012; Zheng et al. 2024). These adaptations aid locomotion in granular substrates by incrementing the surface area of each foot when in contact with the sand, thus distributing the yield stress and decreasing slippage in fluid-like substrates (Carothers 1986; Garland and Losos 1994). Moreover, other studies have suggested that arenicolous lizards present both external and myotendinous adaptations that favor movement in sandy environments; particularly, longer hindlimb muscles and tendons (Tulli et al. 2016). For instance, a common locomotion strategy among arenicolous lizards is bipedalism, which provides high speeds when escaping from predators (Mosauer 1932; Irschick and Jayne 1999; Rocha-Barbosa et al. 2008). Energy expenditure during bipedal locomotion is thought to be reduced through the elongation of the muscle-tendon system due to the decrease of the relative weight of the distal hindlimb segments (Snyder 1954). Arenicolous species could thus benefit from the modification of internal structures. However, despite the strong selective pressures acting on arenicolous lizards, no studies to date have formally tested for morphological divergence in both internal and external traits between these and other lizard species using different habitat types.

Tropiduridae is a diverse family of Neotropical lizards with more than 140 described species (Tonini et al. 2016), and it exemplifies the remarkable diversity of habitat types used by these animals. Tropidurid species range from generalist ground dwellers that live in open habitats such as semiarid caatingas, cerrado, and Chaco (Rodrigues 1987, 1988; Avila-Pires 1995; Grizante et al. 2010; Ribeiro and Freire 2011; Carvalho 2013; Santana et al. 2014; Cacciali and Köhler 2018), to highly specialized forms that live in Restinga (coastal dunes), savanna and rock outcrops (Vitt 1991; Colli et al. 1992; Vitt et al. 1997; Van Sluys et al. 2004; Meira et al. 2007; Grizante et al. 2010; Gomes et al. 2015; Maia-Carneiro and Rocha 2020, 2022; Maia-Carneiro et al. 2021). The diversity of habitat types used by Tropiduridae species makes it possible to investigate how different aspects of locomotor morphology evolve to fit different structural environments (Zaaf et al. 1999; Goodman et al. 2008; Tulli et al. 2009, 2011; Grizante et al. 2010; Anzai et al. 2014). Specifically, the use of sandy environments has repeatedly evolved within Tropiduridae (see Supplementary Figure S1), making it an ideal system in which to study the evolution of possible adaptations for sand-dwelling. However, while extensive research performed in tropidurid lizards has focused on the relationships between habitat use and external morphology (Zaaf et al. 1999; Goodman et al. 2008; Tulli et al. 2009, 2011; Grizante et al. 2010; Anzai et al. 2014; Toyama 2017), ecomorphological studies involving internal morphological traits remain scarce (Tulli et al. 2016).

In this study, we aimed to identify macroevolutionary signatures of morphological adaptation to sandy environments within a clade of tropidurid lizards, using both internal and external traits. At the macroevolutionary level, the evolution of ecomorphological relationships can leave traces of evidence that point towards a hypothetical adaptive origin. For example, patterns of low phylogenetic signal are often shown by functional traits with a central role in processes of adaptive radiation (Blomberg et al. 2003; Kamilar and Cooper 2013). Moreover, such traits might show evidence of having followed evolutionary trajectories towards different optima defined by distinct selective regimes (e.g., Burns and Sidlauskas 2019; Moen 2019). In this context, we hypothesized that tropidurid species inhabiting sandy environments have diverged morphologically from other habitat specialists through the evolution of both muscular and external traits and consequently expected the inferred patterns of evolutionary history of such traits to be compatible with processes of adaptation to different ecological requirements. Lineage evolution towards the use of novel resources often results in the use of previously unoccupied morphospaces, potentially increasing phenotypic diversity within groups of closely related taxa (Wainwright and Reilly 1994; Schluter 2000). Although radiations of cryptic species can comprise relatively high numbers of morphologically similar taxa (Bickford et al. 2007), previous studies on lizards seem to suggest that morphological evolution in response to diverging habitat structures should increase morphological diversity (e.g., Losos 2009; Blom et al. 2016; Toyama 2017). Thus, we also hypothesized that morphological adaptations to sandy environments should contribute significantly to phenotypic diversity. Overall, our research aims to shed light on the role of external and internal morphology in the evolution of ecomorphological relationships in the context of the use of different habitats. By focusing on both muscular features and external morphology, this study offers a novel perspective that addresses a critical gap in the study of lizard ecomorphology (Herrel et al. 2008; Tulli et al. 2012, 2016; Abdala et al. 2014).

Materials and Methods

Study species

For this study, 115 male adult individuals belonging to 10 species of Tropiduridae lizards were examined (Figure 1). These species belong to a clade that includes species of the genera *Tropidurus* and *Eurolophosaurus* (Supplementary Figure S1), whose members are adapted to arboreal, saxicolous, arenicolous, and generalist lifestyles (e.g., Vitt 1991; Rodrigues 1996). Although sand-dwelling is known to have triggered the evolution of convergent morphological traits even across different lizard families (Mosauer 1932; Luke 1986), we acknowledge that focusing solely on this clade might limit our ability to interpret our results when applied to other tropidurid genera. Nonetheless, the diversity of habitats used by species in this particular group (which reflects the diversity shown by the entire family) could provide useful insights regarding the ecomorphological divergence shown by arenicolous species and other habitat specialists across Tropiduridae and other families. Lizard species were categorized as arenicolous or nonarenicolous (Figure 1), with nonarenicolous species in our sample including arboreal and saxicolous specialists. This categorization was based on their preferred habitat types following literature records and personal observations. The number of individuals measured per species ranged from three to 25, depending on the availability. These specimens were obtained from the Herpetological Collection at Fundación Miguel Lillo, Tucumán, Argentina; Coleção Herpetológica de Ribeirão Preto (CHRP-USP) at the University of São Paulo, Brazil; and the Museum of Zoology of the University of São Paulo (MZUP) (Supplementary Appendix S1).

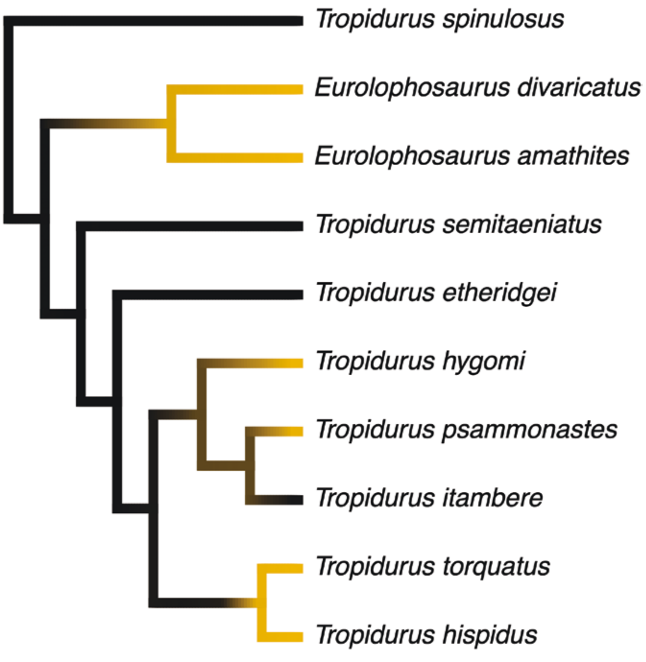


Figure 1. Phylogenetic relationships of the study species showing the posterior density map from stochastic mapping of habitat use (arenicolous = yellow, nonarenicolous = black) based on 1000 simulations.

Data collection

External morphology

Ten morphological measurements were taken directly from the specimens using digital calipers (Mitutoyo CD-15B, ± 0.01 mm) on the right side of the body: body size (snout-vent length—SVL), the distance between limbs [interlimb length: measured between the insertion of the arm and the insertion of the thigh (ILL)], and elements of fore- and hindlimbs [arm length: from the insertion of the arm to the tip of the humerus (ArmL), antebrachium length: from the tip of the proximal end of brachium to carpals (AL), thigh length: from the insertion of the leg to the distal end of the thigh (TL), crus length: from the proximal end of the crus to the heel (CL), foot length: from heel until the base of the toes (FL), and the length of the third (T3), fourth (T4) and fifth toe (T5): from the base of the toes until the tip without including the claw] (Supplementary Table S1).

Muscular morphology

We selected sixteen parallel-fiber muscles from the right fore- and hindlimbs for analysis. These muscles are involved in forearm extension, wrist extension, wrist flexion, and digit movement (Carrizo et al. 2014; Kępa et al. 2023) as well as pedal rotation, plantar flexion, and ankle joint stabilization (Russell and Bauer 2008; Tulli et al. 2016) (Table 1; Figure 2). The flexor muscles play a role in the medial direction, allowing for the bending of the entire hand. Muscles were dissected under a binocular microscope (Nikon SMZ645) and removed intact. We measured the length of each muscle between its corresponding origin and insertion points in millimeters using digital calipers (±0.01 mm; Mitutoyo CD-15B, Mitutoyo Corp., Kure, Japan). Since all muscles had parallel fibers aligned with the medial body axis, we assumed consistent measurement conditions. To minimize measurement error, we took three replicates of each measurement with digital calipers and reported the mean values. The terminology used follows Diogo and Abdala (2010). Mean muscle lengths and body size are detailed as necessary in the Supplementary Materials (Supplementary Table S1).

Phylogenetic context

The common evolutionary history of closely related species results in the nonindependence of individual observations when examining species-level data (Felsenstein 1985; Blomberg et al. 2003). By incorporating phylogenetic information, we can account for these relationships and gain a clearer understanding of the processes underlying observed patterns (Blomberg et al. 2003). In this study, we used a pruned phylogenetic tree based on the squamate phylogeny presented by Tonini et al. (2016) to encompass all the Tropiduridae species analyzed (Figure 1).

Data analyses

Species averages were calculated for each external and internal trait. The data were then log-transformed to improve

Table 1. Fore- and hindlimb musculature with muscle names, abbreviations and assigned functional group

Muscle	Abbreviations	Functional group
Fore limb		
Extensor		
Triceps brachii, scapularis	(TSc)	
Triceps brachii, lateral humeral	(TLat)	Extensor of the elbow
Triceps brachii, medial humeral	(TMh)	
Extensor antebrachii et carpi radialis	(ECR)	Extensor of the forearm
Extensor antebrachii et carpi ulnaris	(ECU)	Extensor of the forearm
Extensor digitorum communis	(EDC)	Extensor of the forearm
Flexor		
Biceps brachii	(BB)	Flexor of the arm
Brachialis	(B)	Flexor of the arm
Flexor carpi radialis	(FCR)	Flexor of the forearm
Flexor carpi ulnaris	(FCU)	Flexor of the forearm
Flexor digitorum communis	(FDC)	Flexor of the forearm
Hindlimb		
Extensor		
Femorotibial Gastrocnemius	(FemG)	Extensor of the knee
Peroneus longus	(Plong)	Extensor of the ankle
Peroneus brevis	(Pbrev)	Extensor of the ankle
Superficial Femoral Gastrocnemius	(SupFG)	Extensor of the ankle
Flexor		
Flexor longus	(FL)	Flexor of the digits

normality and analyzed using Kolmogorov–Smirnov tests. Homoscedasticity, or equal variance across groups, was assessed by visually inspecting the spread of the data. To account for the influence of body size, phylogenetic generalized least square (PGLS) regressions were performed between each considered trait and SVL (function “gls” from the R package *nlme*, Pinheiro et al. 2018), and the obtained residuals were recorded for each species to be used in posterior analyses. The absolute dimensions of limb muscles might be related to the absolute dimensions of the limbs. Thus, to avoid redundancy when including size-corrected external and muscle traits in our analyses, we tested for relationships between the residuals of each muscle length and the residuals of the external trait to which they were more closely associated (e.g., we tested the relationship between crus length and the superficial femoral gastrocnemius [SupFG]). We found no significant relationship for any of the tested relationships, except for that between superficial peroneus brevis and crus length (Supplementary Materials, Figure S2). However, all the associations were far from a 1:1 relationship, indicating that size-corrected muscle lengths cannot be predicted accurately by size-corrected limb lengths, suggesting the two trait types are not redundant.

Has the morphology of arenicolous species diverged from that of other habitat specialists?

To test whether arenicolous and nonarenicolous species exhibited different multivariate morphological phenotypes (including both internal and external morphology), we used the R package *mvMORPH* (Clavel et al. 2015) to perform a type II phylogenetic multivariate analysis of variance (MANOVA) with the entire set of size-corrected external and internal morphological traits as dependent variables and habitat use category (arenicolous versus nonarenicolous) as a predictor variable. Then, to identify which traits are more strongly associated with a hypothetical phenotypic difference between arenicolous and nonarenicolous species, we performed a phylogenetic discriminant function analysis (DFA, function “mvgls.dfa,” package *mvMORPH*; Clavel et al. 2015), again considering the entire set of size-corrected morphological traits as dependent variables and habitat use categories as the discriminating groups. Additionally, we performed phylogenetic pairwise comparisons between arenicolous and nonarenicolous species for each size-corrected morphological trait using the function ‘lm.rrpp’ from the R package *RRPP* (Collyer and Adams 2018). We expected the traits contributing the most to the difference between groups (based on the DFA) to differ when compared individually between habitat use categories.

Next, we calculated the *K*-statistic as an estimate of phylogenetic signal (Blomberg et al. 2003). A *K* value equal to 1 indicates that a trait shows an amount of phylogenetic signal expected under a Brownian motion (BM) model of evolution. *K* values lower than 1 indicate that a trait is less similar between species than expected, while *K* values greater than 1 suggest that a trait is more similar among species than expected under a BM model of evolution. We estimated the phylogenetic signal in all traits using the function “phylosig” from *phytools* (Revell 2012). Additionally, we tested whether the *K* value obtained for each trait exceeded the value expected by chance (Revell and Harmon 2022). We expected

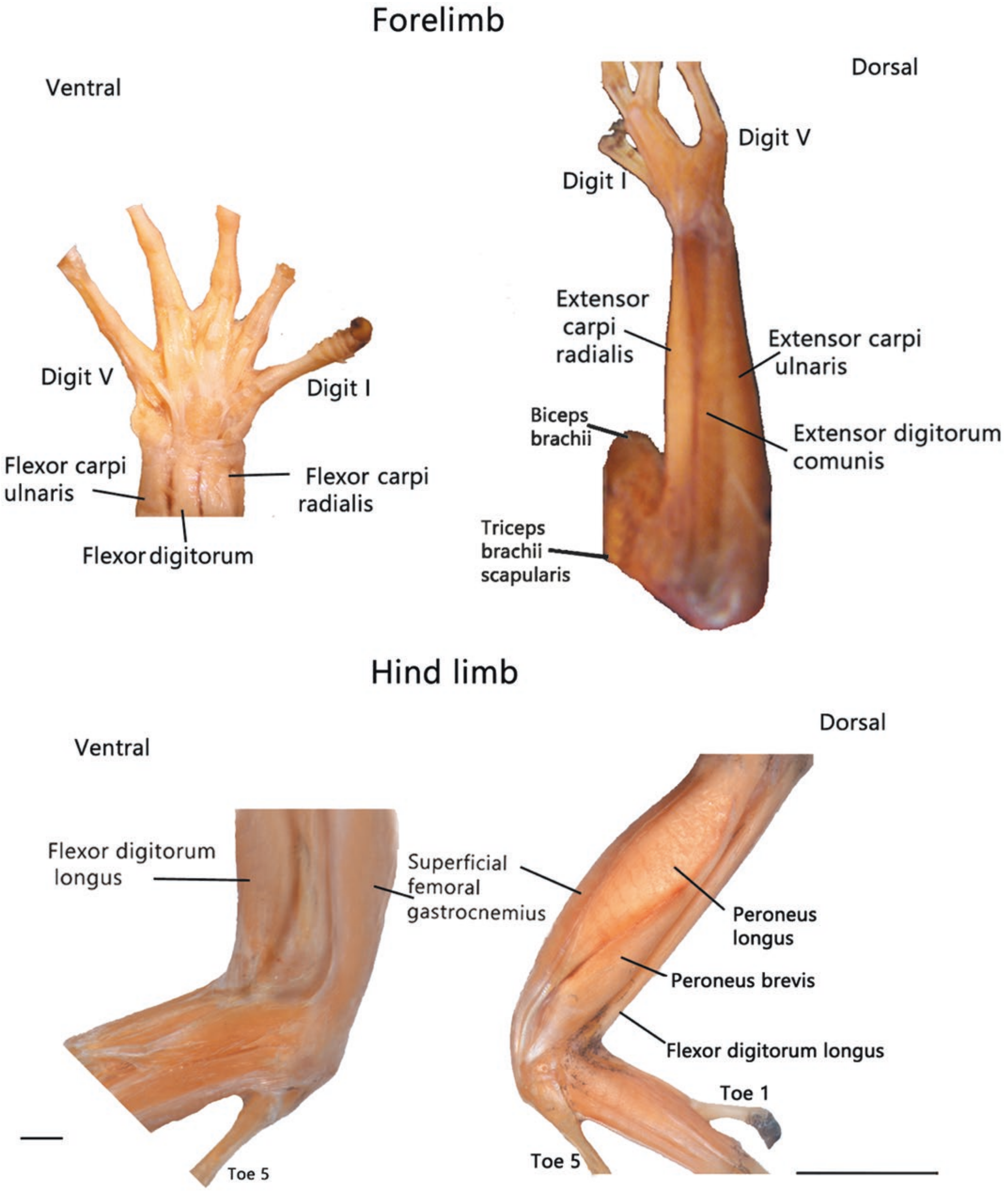


Figure 2. Ventral and dorsal views of forelimb and hindlimb muscles analyzed in this study. Scale bars for ventral and dorsal views correspond to 500 um and 1000 um, respectively.

the result of this test to be nonsignificant when applied to the traits that differed the most between habitat use categories, as this would suggest that phylogenetic relatedness has not had a stronger-than-expected effect on the evolution of these traits. When the phylogenetic signal of a trait was significant, we tested whether the *K* value was significantly different from 1. Then, to further investigate the evolutionary trajectories of the studied traits, we tested three different evolutionary models: BM and Ornstein-Uhlenbeck with a single (OU1) and multiple selective regimes (OUM). The BM model assumes that traits evolve randomly over time through stochastic fluctuations (Felsenstein 1985; Harmon et al. 2010), while in OU models traits evolve towards one or more optimal trait values under stabilizing or diversifying selection (Butler and King 2004; Harmon et al. 2010). We expected the OUM model to be a better fit for the most diverging morphological traits in our dataset, supporting the presence of hypothetical diverging morphological optima between arenicolous species and other habitat specialists. To do this, we utilized the “mvBM” and “mvOU” functions implemented in the R package *mvMORPH* (Clavel et al. 2015) and calculated Akaike weights as a way to estimate the proportional evidence supporting each model. The multiple selective regime model (OUM) requires character states to be mapped in the phylogeny. Thus, to map the estimated evolutionary history of habitat use categories (i.e., arenicolous vs nonarenicolous) as accurately as possible, we pruned the squamate phylogeny of Tonini et al. (2016) such that it included only lizards from the family Tropiduridae. Then, using habitat use information from Toyama (2017) and personal observations, we mapped the estimated history of transitions between both states in the Tropiduridae phylogeny using the *phytools* function “simmap” (Revell 2012). Finally, we pruned this phylogeny to include only the species of our study (Figure 1). We used the resulting tree to fit different evolutionary models of trait evolution to our set of morphological traits. Due to the inherent uncertainty of character state reconstructions, we repeated this process 100 times for each trait, and reported the mean and standard deviation of the Akaike weights obtained across all iterations for each of the fitted evolutionary models.

Do morphological adaptations to sandy environments contribute significantly to phenotypic diversity among tropidurid lizards?

We performed a phylogenetic principal component analysis (PCA) of size-corrected traits (function "phyl.pca," package *phytools*; Revell 2012, 2024) and retained the first principal components (PC) that together explained more than 75% of the morphological variation among species. As done with individual traits, we also estimated levels of phylogenetic signal and tested the fit of evolutionary models in these PCs. We used the "phylomorphospace" function from *phytools* to visualize the evolutionary relationships between species in a bivariate plot showing species scores for PC1 and PC2 (Figure 3A). Then, we tested PGLS models that regressed DFA scores (see previous section) on PC scores. We repeated this for each of the retained PCs. Assuming morphological adaptations for sand-dwelling have contributed significantly to the phenotypic diversity of our sample, we expected DFA scores to be significantly associated with the PC scores of at least one of the retained PCs. All statistical analyses were conducted in the R environment (version 4.2.1, R Development Core Team 2022).

Results

A phylogenetic MANOVA indicated that arenicolous tropidurid species were morphologically different from nonarenicolous species (test statistic = 0.88, $P = 0.04$). A subsequent DFA showed that this difference was driven by arenicolous species tending to have a relatively longer crus and fourth toe, and also a relatively longer flexor digitorum communis, peroneus brevis (Pbrev), superficial femoral gastrocnemius (SupFG), and flexor longus (FL) (Table 2). On the other hand, nonarenicolous species, which included arboreal and saxicolous lizards, tended to show relatively longer forelimb muscles, including the triceps brachii medial humeral (TMh), biceps brachii (BB), the brachialis (B), extensor digitorum communis, and the flexor carpi radialis (FCR), and also longer thighs and interlimb distances (Table 2). Phylogenetic pairwise comparisons indicated that five size-corrected traits differed significantly between arenicolous and nonarenicolous species: the lengths of the fourth toe, crus, brachialis (B), peroneus brevis (Pbrev), and FL (Table 2). Most traits; including the relative lengths of the fourth toe, crus, interlimb distance, peroneus brevis (Pbrev), and FL; did not show K values significantly higher than expected by chance (Table 3). The ones that did showed no evidence of $K \neq 1$ (Table 3). Several of the traits that seemed to differ between habitat groups (e.g., crus, interlimb distance, triceps brachii medial humeral [TMh], brachialis [B], peroneus brevis [Pbrev]) showed relatively strong evidence of having followed a OUM model of evolution (Table 3). Most of the rest of traits showed either evidence of following a BM model of evolution or inconclusive evidence for any model. OUM and BM were the strongest supported models for PC2 and PC3 scores, respectively (Table 3).

The first three PCs from a phylogenetic PCA performed on size-corrected traits represented 79.56% of the total variance (Table 2; Figure 3A). PC1 explained 39.85% of the variation among species and was positively associated with almost all morphological traits except the extensor digitorum muscles, which showed a weak negative association. We found no relationship between PC1 and the discriminant function (DF) described above ($t = 1.07$, $P = 0.317$). PC2 explained 27.32% of the total variation among species and was negatively and strongly associated with the relative length of most external limb traits and with internal traits associated with the hindlimb (Table 2). PC2 was also strongly and

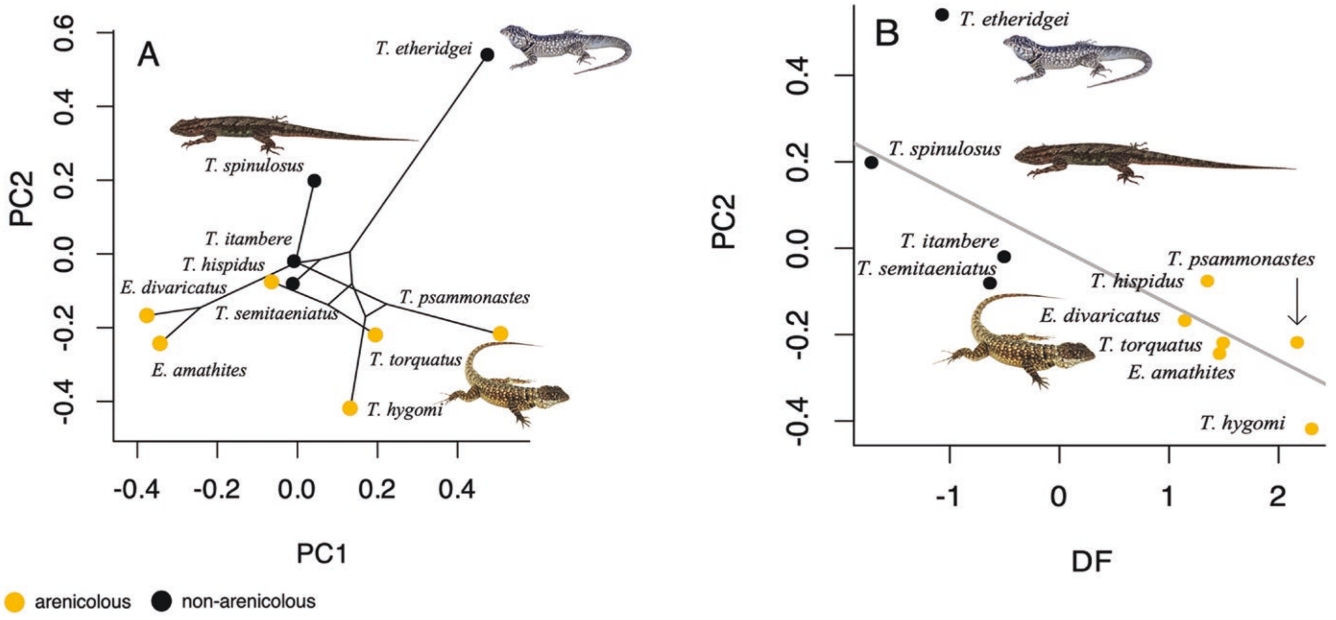


Figure 3. (A) Scatterplot showing PC1 and PC2 scores for arenicolous (yellow) and nonarenicolous species (black). Phylogenetic relationships between species are plotted in the figure. (B) A scatterplot shows the relationship between the scores of PC2 and a DF separating arenicolous and nonarenicolous species. The solid gray line represents the fit of a phylogenetic linear regression.

Table 2. The left part of the table shows the coefficients representing the association of each morphological variable with the DF and the first three PC. The right part shows the results from pairwise comparisons between arenicoulous and non-arenicolous species for each trait. *Z* = Z-scores, *P* = *P*-values, "arenicolous" and "non-arenicolous" columns indicate coefficients associated with each category. In bold P-values < 0.05 indicate differences significatives.

					Pairwise comparisons			
Variable	DF	PC1	PC2	PC3	*Z*	*P*	Arenicolous	Nonarenicolous
Arm L	0.72	0.20	0.20	0.00	−0.48	0.704	0.01	0.00
Antebrachium	−0.42	0.34	−0.75	0.06	0.61	0.280	0.03	−0.01
Toe 3	−0.33	0.05	−0.61	0.68	−0.43	0.665	0.02	−0.01
Toe 4	2.87	0.28	−0.77	0.45	1.64	**0.047**	0.17	−0.03
Toe 5	−0.73	0.01	−0.34	0.71	−0.76	0.775	−0.02	0.03
Foot L	−0.68	0.67	−0.46	0.43	0.10	0.477	0.04	−0.02
Thigh	−1.07	0.16	−0.49	0.04	−0.95	0.805	0.01	0.00
Crus	2.63	0.26	−0.53	−0.11	2.04	**0.014**	0.11	−0.07
Interlimb	−1.97	0.26	0.55	0.36	1.46	0.080	−0.07	0.08
TSc	0.01	0.78	0.52	0.00	−0.89	0.807	−0.01	0.01
Tlat	−0.12	0.73	0.45	0.21	−0.81	0.786	−0.02	0.01
TMh	−1.34	0.87	0.18	0.08	−0.93	0.810	−0.01	0.01
BB	−2.56	0.30	−0.04	0.43	0.41	0.353	−0.04	0.01
B	−3.38	0.17	0.64	0.27	2.11	**0.012**	−0.09	0.04
EDC	−1.78	−0.02	0.43	0.76	1.24	0.097	−0.06	0.03
ECU	−1.42	0.76	0.38	0.39	−0.12	0.556	−0.04	0.02
ECR	−0.08	0.31	0.37	0.54	−0.36	0.639	−0.02	0.01
FCU	−0.76	0.29	0.85	0.26	1.31	0.095	−0.09	0.04
FCR	−1.64	0.34	0.82	0.31	0.92	0.184	−0.10	0.04
FDC	1.18	0.79	0.52	0.02	−1.59	0.940	0.01	0.00
Plong	0.32	0.77	−0.27	0.23	0.58	0.288	0.05	−0.02
Pbrev	2.73	0.60	−0.75	0.16	2.22	**0.011**	0.15	−0.10
FemG	0.81	0.87	−0.11	−0.32	0.52	0.310	0.11	−0.07
SupFG	1.99	0.88	−0.10	−0.31	1.01	0.166	0.13	−0.08
FL	1.51	0.63	−0.62	−0.22	2.01	**0.023**	0.13	−0.07

Table 3. Phylogenetic signal and models of trait evolution. The left columns show *K*-statistic estimates of phylogenetic signal and corresponding *P*-values from significance tests (values of $P < 0.05$ indicate that the estimated signal is higher than expected by chance; boldface denotes significant results). The right columns show mean Akaike weights and standard deviations (in parentheses) corresponding to the evolutionary models (BM [Brownian Motion], single-regime [OU1], and multiple-regime Ornstein-Uhlenbeck [OUM]) tested on 100 simulated instances of stochastic mapping of habitat use on all traits

Traits	*K*	*P*-value	Akaike weights		
			BM	OU1	OUM
External traits					
SVL	0.73	0.206	0.69 (± 0.01)	0.22 (± 0.00)	0.09 (± 0.01)
Interlimb	0.59	0.402	0.08 (± 0.01)	0.07 (± 0.01)	0.84 (± 0.02)
Arm L	0.86	0.109	0.84 (± 0.00)	0.11 (± 0.00)	0.04 (± 0.00)
Antebrachium	1.13	**0.026**	0.79 (± 0.07)	0.13 (± 0.01)	0.08 (± 0.09)
Thigh	0.83	0.186	0.76 (± 0.02)	0.16 (± 0.00)	0.08 (± 0.02)
Crus	0.46	0.612	0.12 (± 0.01)	0.27 (± 0.03)	0.61 (± 0.04)
Foot L	0.46	0.588	0.22 (± 0.02)	0.53 (± 0.05)	0.24 (± 0.08)
Toe 3	0.79	0.153	0.80 (± 0.01)	0.14 (± 0.00)	0.07 (± 0.02)
Toe 4	0.41	0.746	0.12 (± 0.01)	0.39 (± 0.03)	0.49 (± 0.04)
Toe 5	0.51	0.519	0.20 (± 0.00)	0.57 (± 0.01)	0.24 (± 0.01)
Internal traits					
TSc	1.00	**0.047**	0.43 (± 0.20)	0.06 (± 0.03)	0.51 (± 0.23)
TLat	1.26	**0.023**	0.45 (± 0.23)	0.06 (± 0.03)	0.49 (± 0.26)
TMh	0.58	0.386	0.23 (± 0.12)	0.13 (± 0.07)	0.64 (± 0.19)
BB	1.22	**0.008**	0.44 (± 0.19)	0.06 (± 0.03)	0.50 (± 0.22)
B	1.12	**0.037**	0.02 (± 0.08)	0.00 (± 0.01)	0.97 (± 0.10)
ED	1.20	**0.010**	0.31 (± 0.17)	0.04 (± 0.02)	0.65 (± 0.19)
ECU	1.00	0.060	0.49 (± 0.18)	0.07 (± 0.02)	0.44 (± 0.20)
ECR	1.27	**0.005**	0.52 (± 0.19)	0.07 (± 0.03)	0.40 (± 0.22)
FCU	1.11	0.087	0.68 (± 0.16)	0.09 (± 0.02)	0.22 (± 0.18)
FCR	1.07	0.065	0.47 (± 0.24)	0.07 (± 0.03)	0.46 (± 0.28)
FDC	0.79	0.267	0.72 (± 0.04)	0.11 (± 0.01)	0.17 (± 0.04)
Plong	1.00	**0.042**	0.78 (± 0.03)	0.15 (± 0.01)	0.08 (± 0.03)
Pbrev	0.35	0.817	0.04 (± 0.00)	0.31 (± 0.01)	0.65 (± 0.02)
FemG	0.58	0.412	0.37 (± 0.03)	0.43 (± 0.03)	0.20 (± 0.04)
SupFG	0.66	0.276	0.48 (± 0.01)	0.36 (± 0.01)	0.16 (± 0.01)
FL	0.64	0.309	0.42 (± 0.04)	0.34 (± 0.03)	0.24 (± 0.07)
PC1	0.62	0.329	0.45 (± 0.10)	0.23 (± 0.05)	0.32 (± 0.16)
PC2	0.79	0.197	0.20 (± 0.12)	0.04 (± 0.02)	0.76 (± 0.14)
PC3	1.07	**0.039**	0.69 (± 0.10)	0.09 (± 0.01)	0.21 (± 0.11)

See Table 1 for abbreviations.

positively associated with almost all forelimb muscles except for BB (Table 2). We found a significant relationship between PC2 and DF ($t = -3.47$, $P = 0.008$, Figure 3B). Finally, PC3 explained 12.38 % of the total variation and was positively associated with the relative length of foot and toes and the relative length of several muscles like the flexor carpi ulnaris, FCR, and brachialis (B) (Table 2). Simultaneously, PC3 was negatively associated with the relative length of some hindlimb muscles and crus (Table 2). We found no relationship between PC3 and DF ($t = -0.90$, $P = 0.394$).

Discussion

The adaptation of closely related species to different ecological contexts often leads to the evolution of divergent morphological traits, which, in turn, enhance organismal performance and ultimately increase fitness (Arnold 1983; Wainwright and Reilly 1994; Kingsolver and Huey 2003). Although the study of ecomorphological relationships is rich regarding the examination of external morphological characteristics (i.e., body part proportions, e.g., Wainwright and Reilly 1994; Losos 2009), recent studies indicate that internal anatomical traits can also reflect hypothetical processes of adaptation to different ecological settings (e.g., Tulli et al. 2016; Alfieri et al. 2021; Toyama et al. 2024). In line with such studies, our results show how the examination of internal and external morphological traits can both contribute to ecomorphological studies as well as shed light on the functional significance of hypothetical processes of adaptation to different selective regimes.

Living in sandy environments presents significant challenges for locomotion, driving the evolution of a variety of morphological adaptations in different taxa (Jayne 1986; Gidmark et al. 2011; Hosoi and Goldman 2015; Astley et al. 2020). Accordingly, we found that arenicolous lizards seem to have evolved external and internal morphological traits likely to enhance locomotion in sandy substrates. Regarding external traits, species that exploit sandy substrates in our sample tended to have a relatively longer crus and fourth toe, as well as a relatively shorter interlimb distance. These characteristics presumably help arenicolous species navigate in irregular and sloping surfaces while maneuvering effectively on loose sand by increasing surface area and enhancing grip (Kohlsdorf et al. 2001; Teixeira-Filho et al. 2001; Goodman et al. 2008; Tulli et al. 2009, 2011; Grizante et al. 2010). Specifically, increasing the contact area between the surface and a lizard's feet might be especially important for locomotion in sandy environments, as this enhances traction and adhesion, increasing stability and minimizing the risk of slipping or losing balance (Qian et al. 2015; Bergmann et al. 2017).

Besides external body shape, previous studies have described how the muscle morphology of both fore- and hindlimbs are linked to the use of particular habitats in diverse lizard families, including Tropiduridae (Tulli et al. 2016; Lowie et al. 2018; Ríos-Orjuela et al. 2020). These studies suggest that specific muscle groups evolve in response to the locomotor challenges exerted by particular habitats, resulting in enhanced locomotor performance. For example, Abdala et al. (2014) and Tulli et al. (2016) have shown that muscle length is evolutionarily labile, suggesting that tropidurid lizards using similar habitat types could exhibit similar patterns regarding muscular morphology. Indeed, we found that diverse crus muscle groups such as peroneus brevis, FL, and femoral superficial gastrocnemius were relatively longer in arenicolous lizards when compared to other habitat specialists. It is known that the force and speed generated by muscles are affected by their length and arrangement, resulting in functional trade-offs: shorter muscles that generate greater force may impair speed, and longer muscles allow faster movement but may not generate as much force (Kardong, 2009). Our results thus suggest that arenicolous lizards benefit more from speed rather than force, following previous biomechanical and ecological studies (Qian et al. 2015; Tulli et al. 2016; Bergmann et al. 2017).

The nonarenicolous species in our sample were found to possess relatively longer forelimb muscles, such as BB, brachialis, and FCR, which indicates the importance of wrist and digit flexion in climbing (Cartmill 1985). These muscles likely play a role in resisting the movement of the animal's body past its forefeet during downward vertical locomotion (Taylor 1974; Biewener 1989) and can also act as effective stabilizers of the elbow joint during flexed positions. This is particularly crucial when the elbow is abducted, and the forelimb's distal parts are pressed against a tree or rocky substrates (Argot 2001; Carrizo et al. 2014). As so, the muscles biceps and FCR in tropidurids could be pivotal in enabling proficient vertical movement, in addition to contributing towards limb propulsion and stabilization mechanisms during various stages of vertical motion. The importance of the flexor muscles in vertical substrates such as trees or rocks is noteworthy due to their crucial role in several movements related to climbing, grasping, and digging (Lowie et al. 2018). These characteristics are likely to confer a selective advantage to lizards relying on vertical movement, improving their locomotor efficiency and ultimately contributing to their survival and reproductive success.

Most of the internal and external traits that differed the most between arenicolous and nonarenicolous species showed some evidence of having evolved towards different phenotypic optima by showing nonsignificant levels of phylogenetic signal and showing OUM as their most likely model of evolution (Table 3). Although the inference of evolutionary processes based on comparative methods like the estimation of phylogenetic signal should be exercised cautiously (Revell et al. 2008), these results are at least consistent with an adaptive hypothesis for the evolution of internal and external limb traits in response to sandy environments within the *Tropidurus-Eurolophosaurus* clade. Previous studies have shown that external morphological traits related to habitat use seem to be evolutionarily labile within Tropiduridae (Grizante et al. 2010; Tulli et al. 2016; Toyama 2017). Our results seem to follow these trends, given both the evolutionary lability of morphological traits within Tropiduridae and the concordance of our findings with previous ecomorphological studies in arenicolous lizards (e.g., Kohlsdorf et al. 2001; Higham and Russell 2010). Nonetheless, our results are among the first to show that muscle traits also exhibit evolutionary patterns that point towards adaptive explanations in lizards.

An important proportion of the total morphological variation in our sample seemed to be explained by the divergence between arenicolous and nonarenicolous species: we found a strong and significant relationship between a DF maximizing the separation between both groups and a principal component that explained more than 27% of the total morphological variability (Figure 3). This suggests that the hypothetical adaptation to sandy substrates has significantly increased the morphological diversity among the species in our sample, which is similar to what has been found by previous studies in tropidurid lizards (Vitt et al. 1997; Grizante et al. 2010; Tulli et al. 2016). Adaptation to different habitats is known to be a major driver of morphological novelties in nature (Wainwright et al. 2002; Losos 2009; Schwab et al. 2023), thus we expected the morphological differences between arenicolous and nonarenicolous species to represent an important axis of morphological divergence. Nonetheless, the axis of variation that separates arenicolous and nonarenicolous species the most was not related to the main axis of morphological variation in our sample (PC1). This suggests that, although habitat types might be important in driving morphological evolution, other factors might have had an equal or even stronger influence in the shaping of phenotypic diversity (Toyama 2017). The main axis of morphological diversity in our sample seemed to represent a continuum of shapes going from relatively short to relatively long limbs (and associated muscles), including both fore- and hindlimbs (Table 2). This result could be pointing towards other processes that might have influenced the evolution of morphology within this group. For example, relative limb length has been associated with climatic and geographical variables in macroecological studies due to the influence of body size and shape in energy acquisition and conservation (Nudds and Oswald 2007; Symonds and Tattersall 2010; Bidau et al. 2011; Jin and Liao 2015). Since temperature-related variables are especially important for ectotherms it might be possible for axes of interspecific morphological diversity describing relative limb elongation to be related to the different climates or microclimates experienced by a set of species, independently of their preferred habitat types.

Through the study of a clade of tropidurid lizards, we have shown how the evolution of external and internal morphological traits seems to have followed the colonization of sandy environments. Nonetheless, we expect other hypothetical ecomorphological relationships between internal morphology and habitat use to exist in other lizard species, as well as in other taxa. As methodological techniques and tools to measure these traits become more easily available, we expect ecomorphological studies involving aspects of the internal morphology to become more common, facilitating large-scale studies that require large quantities of data. Overall, our results highlight the emergent importance of internal anatomical traits in ecomorphological studies, especially when such traits are directly involved in determining functional performance.

Acknowledgments

We thank M. Cánepa and S. Ktretschmar (Fundación Miguel Lillo) for kindly providing the specimens used in this study and H. Zaher (MZUSP) and Tiana Kohlsdorf (CHRP-USP) for the loan of specimens. We thank Nicolás Pelegrin for allowing us to use his photos of species shown in Figure 3.

Authors' Contributions

M.J.T. conceived and designed the study and collected the data. M.J.T. and K.S.T. performed data analysis, interpreted the results, and wrote the manuscript. All authors contributed substantially to revisions and gave final approval for publication.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Statement

Our data collection was conducted entirely on museum specimens. Therefore, no permits from ethics committees or special permits were required.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

References

Abdala V, Tulli MJ, Russell AP, Powell LG, Cruz F, 2014. Anatomy of the crus and pes of Neotropical Iguanian lizards in relation to digitally based grasping capabilities. *Anat Records* **297**:297–409.

Alfieri F, Nyakatura JA, Amson E, 2021. Evolution of bone cortical compactness in slow arboreal mammals. *Evolution* **75**:542–554.

Anzai W, Omura A, Diaz AC, Kawata M, Endo H, 2014. Functional morphology and comparative anatomy of appendicular musculature in Cuban *Anolis* lizards with different locomotor habits. *Zool Sci* **31**(7):454–463.

Argot C, 2001. Functional–adaptive anatomy of the forelimb in the Didelphidae, and the paleobiology of the Paleocene marsupials *Mayulestes ferox* and *Pucadelphys andinus*. *J Morphol* **247**:51–79.

Arnold SJ, 1983. Morphology, performance and fitness. *Am Zool* **23**:347–361.

Astley HC, Mendelson JR III, Dai J, Gong C, Chong B et al., 2020. Surprising simplicities and syntheses in limbless self-propulsion in sand. *J Exp Biol* **223**(5):jeb103564.

Avila-Pires TCS, 1995. Lizards of Brazilian Amazonia (Reptilia: Squamata). *Zool Verhand* **299**:1–706.

Bergmann PJ, Pettinelli KJ, Crockett ME, Schaper EG, 2017. It's just sand between the toes: How particle size and shape variation affect running performance and kinematics in a generalist lizard. *J Exp Biol* **220**(20):3706–3716.

Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R et al., 2007. Cryptic species as a window on diversity and conservation. *Trends Ecol Evol* **22**:148–155.

Bidau CJ, Martí DA, Medina AI, 2011. A test of Allen's rule in subterranean mammals: The genus *Ctenomys* (Caviomorpha, Ctenomyidae). *Mammalia* **75**:311–320.

Biewener AA, 1989. Scaling body support in mammals: Limb posture and muscle mechanics. *Science* **245**:45–48.

Blom MP, Horner P, Moritz C, 2016. Convergence across a continent: Adaptive diversification in a recent radiation of Australian lizards. *Proc Biol Sci* **283**:20160181.

Blomberg SP, Garland TJR, Ives AR, 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**:717–745.

Brandt R, De Barros FC, Noronha C, Tulli MJ, Kohlsdorf T, 2016. Sexual differences in locomotor performance in *Tropidurus catalanensis* lizards (Squamata: Tropiduridae) – Body shape, size and limb musculature explain variation between males and females. *Biol J Linn Soc* **118**(3):598–609.

Burns MD, Sidlauskas BL, 2019. Ancient and contingent body shape diversification in a hyperdiverse continental fish radiation. *Evolution* **73**:569–587.

Butler MA, King AA, 2004. Phylogenetic comparative analysis: A modeling approach for adaptive evolution. *Am Nat* **164**:683–695.

Cacciali P, Köhler G, 2018. Diversity of *Tropidurus* (Squamata: Tropiduridae) in Paraguay an integrative taxonomic approach based on morphological and molecular genetic evidence. *Zootaxa* **4375**(4):511–536.

Carothers JH, 1986. An experimental confirmation of morphological adaptation: Toe fringes in the sand-dwelling lizard *Uma scoparia*. *Evolution* **40**(4):871–874.

Carrizo LV, Tulli MJ, Abdala V, 2014. An ecomorphological analysis of the forelimb musculo-tendinous system in sigmodontine rodents (Rodentia: Cricetidae: Sigmodontinae). *J Mammal* **95**:843–854.

Cartmill M, 1985. Climbing. In: Hildebrand M, Bramble DM, Liem KF, Wake DB, editors. *Functional Vertebrate Morphology*. Cambridge: Harvard University Press, 73–88.

Carvalho ALG, 2013. On the distribution and conservation of the South American lizard genus *Tropidurus* Wied-Neuwied, 1825 (Squamata: Tropiduridae). *Zootaxa* **3640**:42–56.

Clavel J, Escarguel G, Merceron G, 2015. mvmorph: An r package for fitting multivariate evolutionary models to morphometric data. *Methods Ecol Evol* **6**:1311–1319.

Colli GR, Araújo AFB, Silveira R, Roma F, 1992. Niche partitioning and morphology of two syntopic *Tropidurus* (Sauria: Tropiduridae) in Mato Grosso, Brazil. *J Herpetol* **26**(1):66–69.

Collins CE, Self JD, Anderson RA, McBrayer LD, 2013. Rock-dwelling lizards exhibit less sensitivity of sprint speed to increases in substrate rugosity. *Zoology (Jena, Germany)* **116**:151–158.

Collyer ML, Adams DC, 2018. RRPP: An r package for fitting linear models to high-dimensional data using residual randomization. *Methods Ecol Evol* **9**:1772–1779.

Currey JD, 1984. *The Mechanical Adaptations of Bones*. Princeton, NJ: Princeton University Press.

Currey JD, 2003. The many adaptations of bone. *J Biomech* **36**(10):1487–1495.

Donihue CM, Herrel A, Fabre AC, Kamath A, Geneva AJ et al., 2018. Hurricane-induced selection on the morphology of an island lizard. *Nature* **560**(7716):88–91.

Diogo R, Abdala V, 2010. *Muscles of Vertebrates: Comparative Anatomy, Evolution, Homologies and Development* (1st ed.). Boca Raton, FL: CRC Press.

Felsenstein J, 1985. Phylogenies and the comparative method. *Am Nat* **125**:1–15.

Garland T Jr, Losos JB, 1994. Ecological morphology of locomotor performance in squamate reptiles. In: Wainwright PC, Reilly SM, editors. *Ecological Morphology: Integrative Organismal Biology*. Chicago: University of Chicago Press, 240–302.

Gidmark NJ, Strother JA, Horton JM, Summers AP, Brainerd EL, 2011. Locomotory transition from water to sand and its effects on undulatory kinematics in sand lances (Ammodytidae). *J Exp Biol* **214**(4):657–664.

Gomes FFA, Caldas FLS, Santos RA, Silva BD, Santana DO et al., 2015. Patterns of space, time and trophic resource use by *Tropidurus hispidus* and *T. semitaeniatus* in an area of Caatinga, northeastern Brazil. *Herpetol J* **25**:27–39.

Goodman BA, Miles DB, Schwarzkopf L, 2008. Life on the rocks: Habitat use drives morphological and performance evolution in lizards. *Ecology* **89**(12):3462–3471.

Grizante MB, Navas CA, GarlandTJr, KohlsdorfT, 2010. Morphological Evolution in Tropidurinae squamates: An integrated view along a continuum of ecological settings. *J Evol Biol* **23**:98–111.

Harmon LJ, Losos JB, Davies J et al, 2010. Early bursts of body size and shape evolution are rare in comparative data. *Evolution* **64**:2385–2396.

Herrel A, Vanhooydonck B, Porck J, Irschick D, 2008. Anatomical basis of differences in locomotory behavior in *Anolis* lizards: A comparison between two ecomorphs. *Bull Mus Comp Zool* **159**:213–238.

Higham TE, Russell AP, 2010. Divergence in locomotor performance, ecology, and morphology between two sympatric sister species of desert-dwelling gecko. *Biol J Linn Soc* **101**(4):860–869.

Hosoi AE, Goldman DI, 2015. Beneath our feet: strategies for locomotion in granular media. *Annu Rev Fluid Mech* **47**:431–453.

Irschick DJ, Jayne BC, 1999. A field study of the effects of incline on the escape locomotion of a bipedal lizard, *Callisaurus draconoides*. *Physiol Biochem Zool* **72**:44–56.

Jayne BC, 1986. Kinematics of terrestrial snake locomotion. *Copeia***1986**(4):915–927.https://doi.org/10.2307/1445288.

Jin Y, Liao P, 2015. An elevational trend of body size variation in a cold-climate agamid lizard, *Phrynocephalus theobaldi*. *Curr Zool* **61**(3):444–453.

Kamilar JM, Cooper N, 2013. Phylogenetic signal in primate behaviour, ecology and life history. *Philos Trans R Soc London Ser B* **368**:20120341.

Kardong KV, 2009. *Vertebrates: Comparative Anatomy, Function, Evolution*. Boston, USA: McGraw-Hill Higher Education.

Kępa M, Tomańska A, Staszewska J, Tarnowska M, Klećkowska-Nawrot J et al., 2023. Functional anatomy of the thoracic limb of the Komodo dragon (*Varanus komodoensis*). *Animal* **13**(18):2895.

Kingsolver JG, Huey RB, 2003. Introduction: the evolution of morphology, performance, and fitness. *Integr Comp Biol* **43**:361–366.

Kohlsdorf T, GarlandTJr, Navas CA, 2001. Limb and tail lengths in relation to substrate usage in Tropidurus lizards. *J Morphol* **248**:151–164.

Kohlsdorf T, Navas CA, 2012. Evolution of form and function: Morphophysiological relationships and locomotor performance in tropidurine lizards. *J Zool* **288**:41–49.

Losos JB, 2009. *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation Of Anoles*. Los Angeles, CA: University of California Press.

Lowie A, Herrel A, Abdala V, Mazano AS, Fabre AC, 2018. Does the morphology of the forelimb flexor muscles differ between lizards using different habitats? *Anat Records* **301**:424–433.

Luke C, 1986. Convergent evolution of lizard toe fringes. *Biol J Linn Soc* **27**:1–16.

Maia-Carneiro T, Langie-Santos S, Navas CA, 2021. Defensive behaviors and microhabitat use *Tropidurus catalanensis* (Squamata, Tropiduridae): Body sizes and habitat openness/vegetation cover affect prediction of risk and flight distances. *North-West J Zool* **17**(2):204–211.

Maia-Carneiro T, Rocha CFD, 2020. Diverging temporal and thermal niche dimension favor syntopy of *Tropidurus hispidus* and *Tropidurus semitaeniatus* (Squamata: Tropiduridae). *Biologia* **76**:133–146.

Maia-Carneiro T, Rocha CFD, 2022. Diverging structures, perch heights, temperatures, and levels of sunlight of spatial niche dimensions ease the syntopic life of *Tropidurus hispidus* and *Tropidurus semitaeniatus* (Squamata: Tropiduridae). *J Nat Hist* **55**(43–44):2801–2821.

Maladen RD, Ding Y, Li C, Goldman DI, 2009. Undulatory swimming in sand: Subsurface locomotion of the sandfish lizard. *Science* **325**(5938):314–318.

Meira KTR, Faria RG, Silva MDM, Miranda VT, Zahn-Silva W, 2007. História natural de*Tropidurus oreadicus* em uma área de cerrado rupestre do Brasil Central. *Biota Neotrop* **7**(2):155–163.

Moen DS, 2019. What determines the distinct morphology of species with a particular ecology? The roles of many-to-one mapping and trade-offs in the evolution of frog ecomorphology and performance. *Am Nat* **194**:E81–E95.

Mosauer W, 1932. Adaptive convergence in the sand reptiles of the Sahara and of California: A study in structure and behavior. *Copeia* **1932**:72–78.

Nudds RL, Oswald SA, 2007. An interspecific test of Allen's rule: Evolutionary implications for endothermic species. *Evolution* **61**(12):2839–2848.

Pinheiro J, Bates D, DebRoy S, Sarkar D; R Core Team, 2018. nlme: Linear and Nonlinear Mixed Effects Models. R Package Version 3.1-137. http://CRAN.R-project.org/package=nlme

Qian F, Zhang T, Korff W, Umbanhowar PB, Full RJ et al., 2015. Principles of appendage design in robots and animals determining terradynamic performance on flowable ground. *Bioinspir Biomim* **10**(5):056014.

R Development Core Team, 2022. *R: A Language and Environment for Statistical Computing*. Vienna (Austria): R Foundation for Statistical Computing. Available at http://www.R-project.org.

Revell LJ, 2012. Phytools: An R package for phylogenetic comparative biology (and other things). *Methods Ecol Evol* **3**:217–223.

Revell LJ, Harmon LJ, 2022. *Phylogenetic Comparative Methods in R*. Princeton, USA: Princeton University Press.

Revell L J, 2024. Phytools 2.0: an updated R ecosystem for phylogenetic comparative methods (and other things). *PeerJ* **12**:e16505. doi:10.7717/peerj.16505

Revell LJ, Harmon LJ, Collar DC, 2008. Phylogenetic signal, evolutionary process, and rate. *Syst Biol* **57**:591–601.

Ribeiro LB, Freire EMX, 2011. Trophic ecology and foraging behavior of *Tropidurus hispidus* and *Tropidurus semitaeniatus* (Squamata, Tropiduridae) in a caatinga area of northeastern Brazil. *Iheringia. Série Zoologia* **101**:225–232.

Ríos-Orjuela JC, Camacho-Bastidas JS, Jerez A, 2020. Appendicular morphology and locomotor performance of two morphotypes of continental anoles: *Anolis heterodermus* and *Anolis tolimensis*. *J Anat* **236**(2):252–273.

Rocha-Barbosa O, Loguercio MFC, Velloso ALR, Bonates ACC, 2008. Bipedal locomotion in *Tropidurus torquatus* (Wied, 1820) and *Liolaemus lutzae* Mertens, 1938. *Braz J Biol* **68**:649–655.

Rodrigues MT, 1987. Sistemática, ecologia e zoogeografia dos *Tropidurus* do grupo *torquatus* ao sul do Rio Amazonas (Sauria, Iguanidae). *Arq Zool* **31**:105–230.

Rodrigues MT, 1988. Distribution of lizards of the genus *Tropidurus* in Brazil (Sauria, Iguanidae). In: Heyer WR, Vanzolini PE, editors. *Proceeding of a Workshop on Neotropical Distribution Patterns*. Rio de Janeiro: Academia Brasileira de Ciência, 305–315.

Rodrigues MT, 1996. Lizards, snakes, and amphisbaenians from the quaternary sand dunes of the middle Rio São Francisco, Bahia, Brazil. *J Herpetol* **30**:513–523.

Russell AP, Bauer AM, 2008. The appendicular locomotor apparatus of Sphenodon and normal-limbed squamates. In: Gans C, Gaunt AS, Adler K, editors. *Biology of the Reptilia, vol. 21, Morphology*

I: The Skull and Appendicular Locomotor Apparatus of Lepidosauria. Ithaca, NY: Society for the Study of Amphibians and Reptiles, 1–465.

Santana DO, Caldas FLS, Gomes FFA, Santos RA, Silva BD et al., 2014. Aspectos da História Natural de *Tropidurus hispidus* (Squamata: Iguania: Tropiduridae) em área de Mata Atlântica, nordeste do Brasil. *Neotrop Biol Cons* **9**(1):55–61.

Schluter D, 2000. *The Ecology of Adaptive Radiation*. Oxford: Oxford University Press.

Schulte JA, Losos JB, Cruz FB, Núñez H, 2004. The relationship between morphology, escape behaviour and microhabitat occupation in the lizard clade *Liolaemus* (Iguanidae: Tropidurinae: Liolaemini). *J Evol Biol* **17**(2):408–420.

Schwab JA, Figueirido B, Martín-Serra A, van der Hoek J, Flink T et al., 2023. Evolutionary ecomorphology for the twenty-first century: Examples from mammalian carnivores. *Proc Biol Sci* **290**:20231400. doi:10.1098/rspb.2023.1400.

Snyder RC, 1954. The anatomy and function of the pelvic girdle and hindlimb in lizard locomotion. *Am J Anat* **95**:1–45.

Stuart YE, Campbell TS, Hohenlohe PA, Reynolds RG, Revell LJ et al., 2014. Rapid evolution of a native species following invasion by a congener. *Science* **346**(6208):463–466.

Symonds MR, Tattersall GJ, 2010. Geographical variation in bill size across bird species provides evidence for Allen's rule. *Am Nat* **176**(2):188–197.

Taylor ME, 1974. The functional anatomy of the forelimbs of some African Viverridae (Carnivora). *J Morphol* **143**:307–335.

Teixeira-Filho P, Rocha-Barbosa O, Paes V, Ribas SC, Almeida JR, 2001. Ecomorphological relationships in six lizard species of Restinga da Barra de Maricá, Rio de Janeiro, Brazil. *Ver Chil Anat* **19**(1):45–50.

Tonini JFR, Beard KH, Barbosa Ferreira R, Jetz W, Pyron RA, 2016. Fully-sampled phylogenies of squamates reveal evolutionary patterns in threat status. *Biol Conserv* **204A**:23–31.

Toyama KS, 2017. Interaction between morphology and habitat use: A large-scale approach in tropidurinae lizards. *Breviora* **554**(1):1–20.

Toyama KS, Tinius A, Mahler DL, 2024. Evidence supporting an evolutionary trade-off between material properties and architectural design in *Anolis* lizard long bones. *Evolution* **78**:315–328.

Tulli MJ, Abdala V, Cruz FB, 2011. Relationships among morphology, clinging performance and habitat use in Liolaemini lizards. *J Evol Biol* **24**:843–855.

Tulli MJ, Abdala V, Cruz FB, 2012. Effects of different substrates on the sprint performance of lizards. *J Exp Biol* **215**:774–784.

Tulli MJ, Cruz FB, Herrel A, Vanhooydonck B, Abdala V, 2009. The interplay between claw morphology and habitat use in neotropical iguanian lizards. *Zoology* **112**:379–392.

Tulli MJ, Cruz FB, Kohlsdorf T, Abdala V, 2016. When a general morphology allows many habitat uses. *Integr Zool* **11**:483–499.

Van Sluys M, Rocha CFD, Vrcibradic D, Galdino CAB, Fontes AF, 2004. Diet, activity and microhabitat use of two syntopic *Tropidurus* species (Lacertilia: Tropiduridae) in Minas Gerais, Brazil. *J Herpetol* **38**:606–611.

Vanhooydonck B, Measey J, Edwards S, Makhubo B, Tolley KA et al., 2015. The effects of substratum on locomotor performance in lacertid lizards. *Biol J Linn Soc* **115**(4):869–881.

Vanhooydonck B, Van Damme R, Aerts P, 2000. Ecomorphological correlates of habitat partitioning in Corsican lacertid lizards. *Funct Ecol* **14**(3):358–368.

Vitt LJ, 1991. An introduction to the ecology of Cerrado lizards. *J Herpetol* **25**(1):79–90.

Vitt LJ, Caldwell JP, Zani PA, Titus TA, 1997. The role of habitat shifts in the evolution of lizard morphology: Evidence from tropical *Tropidurus*. *Proc Nat Acad Sci USA* **94**(1):3828–3832.

Wainwright PC, 1991. Ecomorphology: Experimental functional anatomy for ecological problems. *Am Zool* **31**(4):680–693.

Wainwright PC, Bellwood DR, Westneat MW, 2002. Ecomorphology of locomotion in labrid fishes. *Environ Biol Fishes* **65**:47–62.

Wainwright PC, Reilly SM, 1994. *Ecological Morphology: Integrative Organismal Biology*. Chicago: The University of Chicago Press.

Zaaf A, Herrel A, Aerts P, Vree FD, 1999. Morphology and morphometrics of the appendicular musculature in geckoes with different locomotor habits (Lepidosauria). *Zoomorphology* **119**:9–22.

Zani PA, 1996. Patterns of caudal autotomy evolution in lizards. *J Zool* **240**(2):201–220.

Zheng P, Liang T, Shi L, 2024. Are toe fringes important for lizard burying in highly mobile sand? *Front Zool* **21**:25.