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When a general morphology allows many habitat uses

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Abstract

During the last decades the study of functional morphology received more attention incorporating more detailed data corresponding to the internal anatomy that together contribute for a better understanding of the functional basis in locomotion. Here we focus on 2 lizard families, Tropiduridae and Liolaemidae, and use information related to muscle-tendinous and external morphology traits of hind legs. We investigate whether the value of the traits analyzed tend to exhibit a reduced phenotypic variation produced by stabilizing selection, and whether species showing specialization in their habitat use will also exhibit special morphological features related to it. As a result, we identified that evolution of hind limb traits is mainly explained by the Ornstein–Uhlenbeck model, suggesting stabilizing selection. Liolaemids and tropidurids show clear ecomorphological trends in the variables considered, with sand lizards presenting the most specialized morphological traits. Some ecomorphological trends differ between the 2 lineages, and traits of internal morphology tend to be more flexible than those of external morphology, restricting the ability to identify ecomorphs shared between these 2 lineages. Conservative traits of external morphology likely explain such restriction, as ecomorphs have been historically defined in other lizard clades based on variation of external morphology.

Key words: external morphology, Lioalemidae, muscles, tendon, Tropiduridae

INTRODUCTION

Organisms move in the environment during prey capture, predator escape and territory maintenance,

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and the performance exhibited by an individual when performing a given ecological task is intimately related to its morphology (Hildebrand 1985; Biewener 2003; Polly 2007). Squamata has been a lineage particularly well studied in terms of ecomorphological relationships due to the outstanding diversity of locomotor modes that have allowed lizards to exploit a wide range of habitats (for examples, see Losos & Sinervo 1989; Losos 1990a,b; Bonine & Garland 1999; Herrel *et al*. 2002; Van Damme & Vanhooydonck 2002; Goodman *et*

al. 2008).

Evolution of morphological traits in close association with ecological parameters has been described in many squamate families (Losos 2009), but most of the classical studies focus on external morphology of the group of Caribbean *Anolis*. These lizards exhibit limb proportions strongly associated with habitat use on each of the islands they colonized, where twig anoles moving on narrow branches evolved shorter legs (Losos 2009). A recent study of 2 species of *Anolis* (*A. valencienni* and *A. sagrei*) combined external with muscle anatomy, and showed that lizards with longer limbs and heavier gastrocnemius muscle run faster than species having an opposite conformation (Herrel *et al*. 2008). The association between morphology and ecology is so evident in *Anolis* lizards that it is possible to recognize the so-called "ecomorphs," which are defined by Williams (1972, p. 72) as "species with the same structural habitat/niche, similar in morphology and behavior, but not necessarily close phylogenetically." The recognition of ecomorphs is mostly based on morphological traits likely associated with locomotion in different microhabitats (Wegener *et al*. 2014). However, Herrel *et al*. (2008) show that morphology of the musculoskeletal system is related not only to habitat use, but also to the evolution of locomotor performance, suggesting that ecomorph differences go beyond the external morphological traits. Ecomorphs seem to not be restricted only to *Anolis* lizards. For example, Grizante *et al*. (2010) identify associations that suggest adaptive changes in foot shape and hind limb size involved in the colonization of several habitats by Tropiduridae lizards. Although they do not use the term "ectomorphs," some of the associations described in their study may actually fit into this definition coined by Williams (1972). However, in other lizard groups the ecomorph concept is not so easily applicable. For example, in iguanian lizards, such as Liolaemidae, Tropiduridae and *Anolis*, Tulli *et al*. (2009, 2012a,b) do not find clear associations between morphology and habitat use. In this lizard group, it was suggested that some traits evolved early and then maintained along the evolutionary process (Schulte *et al*. 2004; Cruz *et al*. 2009, 2011; Tulli *et al*. 2009, 2012a,b), proposing a pattern recently synthesized in the "early burst" evolutionary model (Harmon *et al*. 2010). These observations suggest an existing contrast between the strong ecomorphological associations present in Tropiduridae (Kohlsdorf *et al*. 2001, 2008; Grizante *et al*. 2010) and the morphological conservation of Liolaemidae (Cruz *et al*. 2009, 2011; Tulli *et al*. 2009, 2012a,b), a disparity particularly

unexpected given that the 2 lineages are phylogenetically relatively close, exhibit similar foraging modes (sit-and-wait), and have sympatric representatives in several geographic areas with similar environments.

In the present study we compile a large database composed of hind limb myo-tendinous and external morphological traits from species belonging to these 2 diverse neotropical lizard families (Tropiduridae and Liolaemidae), encompassing a wide geographical distribution and broad ecological preferences. We test whether these apparently highly-contrasting trends emerging from previous studies implemented separately for each family are confirmed when data are analyzed through an ecomorphological analysis applied to the 2 lizard families together. We combine published information with new data for habitat use and morphology, and construct combined matrices for both lizard families to find the evolutionary model that fits our data better. We also investigate whether the species showing specialization in their habitat use also exhibit special morphological features related to it. Based on previous results (Abdala *et al*. 2014), we expect to find a conserved morphological configuration versatile enough to allow exploitation of almost all of the available habitats.

This work can be considered a starting point for ecomorphological studies based on internal morphology traits that opened a new perspective following other papers in lizards (Tulli *et al*. 2012b; Abdala *et al*. 2014), frogs (Gomes *et al*. 2009; Jorgensen & Reilly 2013; Enriquez *et al*. 2015) and rodents (Carrizo *et al*. 2014a,b). In all these papers it has been demonstrated that internal morphology plays a main role in the evolution of the locomotor performance and habitat use of tetrapods.

MATERIAL AND METHODS

The dataset analyzed combines ecological information compiled from the literature with morphometric traits on external morphology and hind limb myo-tendinous anatomy obtained from our examination of lizard specimens. Specifically, we dissected the crus and pes of 165 adult specimens belonging to 21 species of Liolaemidae and 10 species of Tropiduridae lizard families. Voucher specimens of the species studied were deposited at the Herpetology Collection of the Fundación Miguel Lillo, Tucumán, Argentina and Coleção Herpetológica de Ribeirão Preto (CHRP-USP), at the University of São Paulo, Brazil (Table S1). Species' choice aimed to maximize representation of habitat use and locomotor modes in the sample, as well as phylogenet-

ic representation of different clades within each family. Dissections of the myological and tendinous traits related to the hind limb and foot (see Abdala *et al.* 2014) were performed under a binocular microscope (Nikon SMZ645). Muscle-tendinous variables were measured with digital calipers (Mitutoyo CD-15B; ±0.01 mm, Japan). Species mean values of traits and the number of individuals per species used are detailed in the Supporting Information (Table S2).

Morphological data

Here we present an analysis that includes already published data. Because of this, we fixed our new data collected to datasets constructed for analysis of pedal grasping (Abdala *et al.* 2014) that were based on crus and pes traits, for example. Following the protocol of Abdala *et al.* (2014), we included data related to body size (snout–vent length [SVL]) and hind limb sections: femur and tibia length, width and length of the foot (distance from the wrist to the end of the digit IV, which is the longest digit in these animals), and digit lengths of all 5 digits. We also studied the variability between muscle and tendon dimensions of the structures directly implicated in pedal rotation, plantar flexion, and stabilization of the ankle joint (Russell & Bauer 2008). Muscle and tendon measurements (Fig. 1) are listed on Table 1. All muscles analyzed exhibit a parallel-fibered arrangement. In addition to length, the maximum width of each muscle was also recorded to obtain an estimate of the morphometric variation of each muscle as a whole,

and to permit correlation with aponeurosis and tendon dimensions that are associated with these muscles, for which we report width and length (Table 1).

Ecological settings

The ecological data considered was based on the relative use of 5 habitat categories: ground, sand, rocks, trunks and branches (Table 2). Because many species use more than one habitat type, we followed Grizante *et al.* (2010) and estimated the proportion of substrate usage based on the number of individuals of each species described in each substrate. Specifically, each habitat use category was treated as a continuum based on how many individuals were captured in a given substrate in the papers checked for gathering ecological information on Tropiduridae and Liolaemidae. Thus, we worked with a value ranging from 0 (none of the individuals sampled in a given substrate type) to 1 (all individuals sampled using that substrate type). Almost one-third of the species are considered habitat specialists, as 100% of the observations were made in a single substrate category (Table 2; see species with values equal to 1.00).

Phylogenetic context

Phylogenetic comparative analyses were performed using a composite tree (Fig. 2) based on Pyron *et al.* (2013), Frost *et al.* (2001) and Lobo *et al.* (2010); the last 2 were used to fill the gaps of species not considered in Pyron *et al.* (2013). Branch lengths are not available and our composite tree is based on the topologi-
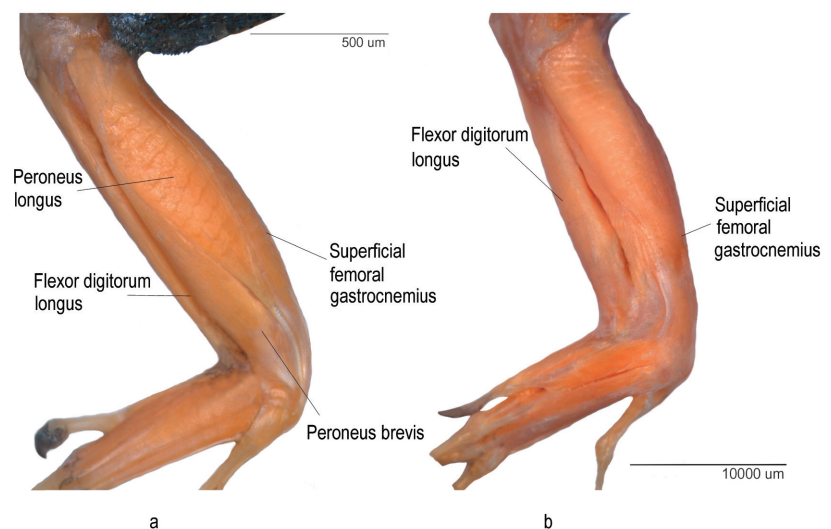

Figure 1 General scheme of some of the muscles and tendons examined for muscle-tendinous traits. a dorsal view, b ventral view.

Table 1 List of the muscles and tendons of the hind limb examined in this study

Muscles	Abbreviations	Tendon
Peroneus longus length	Pll	
Peroneus longus width	Plw	
Peroneus longus origin tendon length	Plol	x
Peroneus longus insertion tendon length	Plil	x
Peroneus brevis length	Pbl	
Peroneus brevis width	Pbw	
Peroneus brevis tendon length	Pbtl	x
Superficial femorotibial aponeurosis length	Sfal	
Superficial femorotibial aponeurosis width	Sfaw	
Superficial femoral gastrocnemius length	Sfgl	
Superficial femoral gastrocnemius width	Sfgw	
Femorotibial gastrocnemius length	Fgl	
Femorotibial gastrocnemius width	Fgw	
Flexor digitorum longus length	Fdll	
Flexor digitorum longus width	Fdlw	
Flexor digitorum longus aponeurosis length	Fdlal	
Flexor digitorum longus aponeurosis width	Fdlaw	
Digital flexor tendon digit 1 length	Dfl1	x
Digital flexor tendon digit 2 length	Dfl2	x
Digital flexor tendon digit 3 length	Dfl3	x
Digital flexor tendon digit 4 length	Dfl4	x
Digital flexor tendon digit 5 length	Dfl5	x

cal relationships among species; thus, we used arbitrary branch lengths (all branch lengths equal to 1 and branch length transformations using the methods of Pagel, Nee and Grafen [Grafen 1989; Pagel 1992]). For testing the adequacy among the 3 branch lengths (which topology and branch lengths better standardized the traits), we followed the method of Garland *et al.* (1992) that consists of plotting the absolute value of each standardized independent contrast versus the square root of the sum of its branch lengths, which represents its standard deviation. For this, we used diagnostic plots of independent contrasts corresponding to the different trees and branch lengths using Mesquite v2.74 (Maddison & Maddison 2015) and the PDAP PDTREE v1.15 module (Midford *et al.* 2009). Nee transformations and branch length equal to 1 showed the lower number of significant trends (2 out of 30 plots), so we deemed the tree with Nee branch length transformation method as the more appropriate for our study (following Garland *et al.* 1992).

Statistical analysis

Mean values of morphological variables were log$_{10}$ transformed prior to analyses to meet requirements of
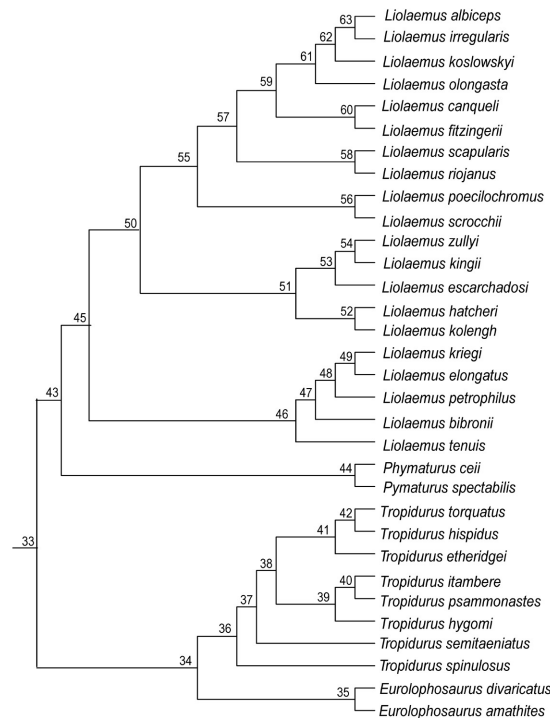

Figure 2 Tree topology based on Pyron *et al.* (2013), Frost *et al.* (2001) and Lobo *et al.* (2010); the last 2 to include species that not considered in Pyron *et al.* (2013). Numbers indicate nodes for a reference.

Table 2 Ecological indexes estimated for the habitat used by the selected species (values indicate the proportion of lizards usually found in each habitat type)

Species	Sand	Rock	Trunk	Branch	Ground	Literature source
Eurolophosaurus amathites	1.00	0.00	0.00	0.00	0.00	Rodrigues (1984, 1996)
Eurolophosaurus divaricatus	1.00	0.00	0.00	0.00	0.00	Rodrigues (1986)
Liolaemus albiceps	0.00	0.00	0.00	0.02	0.98	Abdala (2007)
Liolaemus bibroni	0.10	0.00	0.00	0.00	0.90	Schulte *et al.* (2000)
Liolaemus canqueli	0.60	0.00	0.00	0.00	0.40	Personal observation
Liolaemus elongatus	0.00	0.90	0.00	0.00	0.10	Personal observation
Liolaemus escarchadosi	0.00	0.20	0.00	0.00	0.80	Scolaro and Cei (1997)
Liolaemus fitzingerii	0.70	0.00	0.00	0.00	0.30	Personal observation
Liolaemus scrocchii	0.00	0.90	0.00	0.00	0.10	Quinteros *et al.* (2008)
Liolaemus hatcheri	0.00	0.20	0.00	0.00	0.80	Etheridge (2000)
Liolaemus irregularis	0.00	0.00	0.00	0.00	1.00	Abdala (2007)
Liolaemus kingii	0.00	0.90	0.00	0.00	0.10	Personal observation.
Liolaemus kolengh	0.00	0.40	0.00	0.00	0.60	Abdala and Lobo (2006)
Liolaemus koslowskyi	0.60	0.00	0.00	0.00	0.40	Etheridge (2000)
Liolaemus kriegi	0.00	1.00	0.00	0.00	0.00	Personal observation
Liolaemus olongasta	0.80	0.00	0.00	0.00	0.20	Etheridge (2000)
Liolaemus petrophilus	0.00	1.00	0.00	0.00	0.00	Avila *et al.* (2004)
Liolaemus poecilochromus	0.00	0.60	0.00	0.00	0.40	Personal observation
Liolaemus riojanus	0.98	0.00	0.00	0.00	0.02	Halloy *et al.* (1998)
Liolaemus scapularis	0.98	0.00	0.00	0.00	0.02	Halloy *et al.* (1998)
Liolaemus tenuis	0.00	0.30	0.20	0.20	0.30	Medel *et al.* (1988)
Liolaemus zullyi	0.00	0.20	0.00	0.00	0.80	Personal observation
Phymaturus ceii	0.00	1.00	0.00	0.00	0.00	Lobo and Quinteros (2005)
Phymaturus spectabilis	0.00	1.00	0.00	0.00	0.00	Lobo and Quinteros (2005)
Tropidurus etheridgei	0.00	0.00	0.50	0.00	0.50	Vitt (1991)
Tropidurus hispidus	0.95	0.03	0.00	0.00	0.02	Rodrigues (1988); Vitt (1995); Vitt *et al.* (1996); Van Sluys *et al.* (2004)
Tropidurus hygomi	0.90	0.00	0.00	0.10	0.00	Vanzolini and Gomes (1979)
Tropidurus itambere	0.00	1.00	0.00	0.00	0.00	Van Sluys (1993, 1998)
Tropidurus psammonastes	1.00	0.00	0.00	0.00	0.00	Rodrigues (1988, 1996)
Tropidurus semitaeniatus	0.00	1.00	0.00	0.00	0.00	Vitt (1995); Rodrigues (1996)
Tropidurus spinulosus	0.00	0.00	0.97	0.00	0.03	Colli *et al.* (1992); Vitt (1991)
Tropidurus torquatus	0.45	0.50	0.00	0.05	0.00	Rodrigues (1981, 1988); Araujo (1991); Bergallo and Rocha (1993); Rocha and Bergallo (1997)

normality. Because habitat use is expressed in proportions, ecological data were transformed to the arcsin of the square root of each value (Martin & Bateson 1999). All statistical analyses were implemented in the R statistical environment (R Core Development Team 2011). Morphological traits need body size correction besides the phylogenetic context, so we performed the phylogenetic size-correction analysis described by Revell (2009). We calculated residuals from least squares regression analyses of morphological traits on body size (SVL), while controlling non-independence due to phylogeny by using phylo.resid (a module of Phytools for R

developed by Revell 2012). The resultant residuals were then used in the subsequent analyses.

To reduce the number of variables and at the same time identify correlated evolution among traits, we ran a phylogenetically-based principal component analysis of the morphological residual variables using a Varimax rotation, implemented with the module Phyl.PCA from the Phytools package for R (Revell 2012). From these analyses, we obtained the species scores and morphological loadings corresponding to the first 3 principal components in relation to approximately 72% of accumulated variance. From the morphological loadings we considered those vectors with higher absolute values (negative or positive) in order to detect those variables that contributed more to each principal component (PC).

Next we studied the evolutionary processes through which morphological traits may have evolved in Tropiduridae and Liolaemidae, testing phylogenetic evolutionary models to discriminate among 3 different evolutionary hypotheses. In the first model, evolutionary change in a trait would result from random fluctuations through time (Felsenstein 1988; Harmon *et al.* 2010), with a better fit of the Brownian motion evolutionary model (BM). The second model refers to cases when a trait varies in relation to an optimum or stabilizing selection to a state for this trait in part of the lineage (Butler & King 2004; Harmon *et al.* 2010); this evolutionary model is known as the Ornstein–Uhlenbeck model. Finally, the third hypothesis predicts trait changes early in the evolutionary tree followed by gradual deceleration of the rate of evolution using the early burst model (Harmon *et al.* 2010). To test which evolutionary model better fits each variable, we ran the fitContinuous analysis using "Geiger" (Harmon *et al.* 2010) and "ape" (Analysis of Phylogenetics and Evolution; Paradis *et al.* 2004) packages for R. The command "fitContinuous" (implemented in the package Geiger for R, Harmon *et al.* 2010) describes the rate of change of a trait under the 3 evolutionary models aforementioned, and also provides an Akaike value to each procedure. Then, the best fit among candidate evolutionary models is obtained using the Akaike information criterion (Burnham & Anderson 2002; Angilletta 2006). For this purpose, we used Akaike weights (wAICc) as a measure of strength for each model, indicating the probability that a given model is the best among a series of candidate models (Burnham & Anderson 2002).

Because species cannot be considered as independent data points given their phylogenetic relationships (Harvey & Pagel 1991), we estimated Pagel's phylogenetic signal (λ) from the residual errors simultaneously on the regression parameters of phylogenetic generalized least squares models (PGLS) analyses. These analyses were performed in "caper" (Orme *et al.* 2012) and "ape" (Paradis *et al.* 2004) packages for R. Ecological data given by arcsin of the proportions of habitat use were entered as independent variables, and models were built using either a single habitat variable (e.g. rocks) or by one of their possible combinations as determined from principal component analysis (e.g. PC~ sand + ground + rocks + trunk + branches), and morphological information was entered as dependent variables. In addition, we ran PGLS analyses for these morphological traits that showed loads higher than 0.65 within each PC and the corresponding habitat use. The model's choice was based on the model's fit using the Akaike information criterion as mentioned above.

RESULTS

Our PC analyses (PCA) from residual values of different morphological variables (muscle-skeletal and external morphology) under phylogenetic analysis show that the first 3 PCs account for the 72% of accumulated variance. PC1 loads show that peroneus longus insertion tendon length (Plil), peroneus brevis tendon length (Pbl), superficial femoral gastrocnemius length (Sfgl) and flexor digitorum longus aponeurosis length (Fdlal) all contributed importantly; all these variables show negative values (Table 3). In the case of PC2, peroneus longus origin tendon length (Plol) and femorotibial gastrocnemius width (Fgw) were the morphological traits with higher loads, the first with negative value and the second positive (Table 3). Finally, PC3 show that the highest load was achieved by flexor digitorum longus aponeurosis length (Fdlal) width with positive load (Table 3).

Morphological evolution in Tropiduridae and Liolamidae seems to have followed different evolutionary processes, as the studied traits were not explained by a single evolutionary model (Table 4). Some traits had a better fit for the Ornstein–Uhlenbeck model of evolution (OU): for example, peroneus longus width, peroneus brevis width, femorotibial gastrocnemius width, digital flexor tendons of digits 3–5, foot length and width (Table 4). The BM model, which predicts a random rate of change, better explained the evolution of flexor digitorum longus length (Fdll), peroneus longus length (Pll), femur length (Fel) and tibia length (Til) (Table 4). Unfortunately, methodological limitations (species sample size and

Table 3 Component scores resulting from a principal component analysis (PCA) performed on the morphometric traits

Variables	PC1	PC2	PC3
Peroneus longus length	−0.227	−0.641	−0.06
Peroneus longus width	−0.405	−0.227	−0.095
Peroneus brevis length	−0.343	0.667[†]	−0.048
Peroneus longus origin tendon length	−0.847[†]	−0.172	0.295
Peroneus longus insertion tendon length	−0.435	0.527	0.201
Peroneus brevis width	−0.038	0.43	−0.226
Peroneus brevis tendon length	−0.732	0.156	−0.61
Sup femorotibial aponeurosis length	−0.299	−0.302	−0.174
Sup femorotibial aponeurosis width	0.115	−0.109	−0.522
Femoral gastrocnemius sup length	−.798	−0.428	0.371
Femoral gastrocnemius sup width	−0.602	−0.629	0.104
Femorotibial gastrocnemius length	−0.68	−0.317	0.159
Femorotibial gastrocnemius width	0.161	−0.705[†]	−0.153
Flexor dig longus length	−0.559	−0.365	−0.058
Flexor dig longus width	−0.341	−0.427	−0.134
Flexor dig longus aponeurosis length	−0.841[†]	0.111	−0.239
Flexor dig longus aponeurosis width	−0.185	0.252	0.66
Digital flexor tendon digit 1 length	0.159	−0.413	0.06
Digital flexor tendon digit 2length	0.308	−0.562	0.032
Digital flexor tendon digit 3length	0.357	−0.571	−0.021
Digital flexor tendon digit 4length	0.236	−0.587	−0.129
Digital flexor tendon digit 5length	0.017	−0.546	−0.285
Digit 3 length	−0.308	−0.116	−0.004
Digit 4length	−0.418	−0.221	−0.197
Digit 5length	−0.143	−0.38	−0.016
Foot length	−.497	0.473	0.085
Foot width	−0.52	0.613	0.177
Femur length	0.353	−0.771[†]	−0.201
Tibia length	0.358	0.827[†]	−0.256
% variance explained	36.03	25.58	9.88
Eigenvalue	0.131	0.03	0.036

All traits were log-transformed, and effects of body size were removed prior to analysis by phylogenetically computing residuals from regressions on snout–vent length. [†]Traits contributing most to each component. The total variance of the data explained by these first 3 principal components is 72%.

arbitrary branch lengths) do not allow us to determine the number of regimes and mean values of theses regimes for the models. None of the morphological variables showed a best fit under the early burst evolutionary model. Moreover, for some variables there was not a single evolutionary model explaining the variation, as BM and the OU model (Butler & King 2004; Harmon *et al.* 2010) were equally possible under the Akaike criterion (Table 4).

We tested a total of 155 possible PGLS models, from which 9 were considered the most informative after the Akaike criterion, and provided evidence for associations between morphology and ecology (Table S3). These models describe significant slopes for some

Table 4 Values of AICc and log likelihood (LogL) that correspond to the evolutionary models tested (Brownian motion [BM], Ornstein–Uhlenbeck [OU] and Early Burst [EB]) for all the morphological variables. wAICc is the weight of the different models. BeMo indicates the evolutionary model that best fitted the data based on the wAICc values.

Morphological variables	LogL	AICc	wAICc	LogL	AICc	wAICc	LogL	AICc	wAICc	BeMo
Pll	53.91	−103.39	0.627[†]	53.92	−100.99	0.188	53.91	−100.95	0.185	BM
Plw	46.17	−87.93	0.212	48.62	−90.37	0.725[†]	46.17	−85.48	0.063	OU
Plol	12.15	−19.88	0.447[†]	13.31	−19.76	0.421[†]	12.15	−17.44	0.132	BM-OU
Plil	14.63	−24.84	0.345[†]	16.34	−25.82	0.555[†]	14.63	−22.39	0.1	BM-OU
Pbl	48.03	−91.65	0.288	50.03	−93.2	0.627[†]	48.03	−89.2	0.085	OU
Pbw	51.51	−98.61	0.541[†]	52.14	−97.43	0.3[†]	51.51	−96.17	0.159	BM-OU
Pbtl	1.86	0.78	0.037	6.28	−5.7	0.952[†]	1.86	3.23	0.011	OU
Sfal	42.14	−79.86	0.408[†]	43.51	−80.16	0.472[†]	42.14	−77.42	0.12	BM-OU
Sfaw	38.87	−73.33	0.03	43.55	−80.24	0.961[†]	38.87	−70.88	0.009	OU
Sfgl	2.7	−0.98	0.06	6.65	−6.45	0.923[†]	2.7	1.45	0.017	OU
Sfgw	19.29	−34.17	0.039	23.69	−40.52	0.949[†]	19.29	−31.72	0.012	OU
Fgl	10.7	−16.98	0.009	16.67	−26.49	0.989[†]	10.7	−14.74	0.002	OU
Fgw	41.06	−77.71	0.272	43.14	−79.43	0.642[†]	41.06	−75.27	0.086	OU
Fdll	42.75	−81.09	0.625[†]	42.78	−78.71	0.19	42.75	−78.64	0.185	BM
Fdlw	43.79	−83.16	0.167	46.55	−86.25	0.784[†]	43.79	−80.72	0.049	OU
Fdlal	20.08	−35.75	0.606[†]	20.26	−33.67	0.215	20.08	−33.31	0.179	BM
Fdlaw	33.19	−61.97	0.061	37.12	−67.39	0.921[†]	33.19	−59.53	0.018	OU
Dfl1	48.03	−91.66	0.446[†]	49.2	−91.54	0.422[†]	48.03	−89.21	0.132	BM-OU
Dfl2	25.01	−45.61	0.422[†]	26.3	−45.75	0.453[†]	25.01	−43.17	0.125	BM-OU
Dfl3	46.91	−89.41	0.557[†]	47.44	−88.03	0.279[†]	46.91	−86.96	0.164	BM-OU
Dfl4	39.19	−73.96	0.283	41.22	−75.59	0.634[†]	39.19	−71.52	0.083	OU
Dfl5	29.51	−44.62	0.002	32.2	−57.54	0.997[†]	29.51	−42.17	0.001	OU
Digit 3 length	58.69	−112.97	0.271	60.78	−114.91	0.649[†]	58.69	−110.53	0.08	OU
Digit 4 length	57.85	−111.3	0.418[†]	59.17	−111.48	0.458[†]	57.85	−108.86	0.124	BM-OU
Digit 5 length	63.21	−122.02	0.289	65.21	−123.56	0.626[†]	63.21	−119.57	0.085	OU
Foot length	27.62	−50.82	0.256	29.8	−52.74	0.668[†]	27.62	−48.38	0.076	OU
Foot width	13.71	−23.02	0.283	15.74	−24.63	0.633[†]	13.71	−20.58	0.084	OU
Femur length	23.74	−43.07	0.595[†]	24.01	−41.16	0.23	23.74	−40.62	0.175	BM
Tibia length	14.29	−24.17	0.569[†]	14.74	−2263	0.264	14.29	−21.73	0.167	BM

[†]variables selected with high wAICc

of the habitat use variables (Fig. 3; Table 5), as, for example, a significant association between PC1 and use of sand (Fig. 3a). Sandy lizards tend to have lower values for the lengths of peroneus longus insertion tendon, peroneus brevis tendon, femoral gastrocnemius superficial, femorotibial gastrocnemius, and aponeurosis of the flexor digitorum longus (Fig. 3a, Table 3). The particular PGLS analysis for the morphological traits with loads higher than 0.65 with sand as the environmental independent variable showed that peroneus longus insertion tendon length and superficial femoral gastrocnemius length contributed significantly (Plil~sand, $\lambda = 0.472$, slope = 0.101, $P = 0.019$; Sfgl~sand, $\lambda = 0$, slope = 0.111, $P = 0.05$), whereas the other variables (Pbtl, femorotibial gastrocnemius length Fgl, Fdlal; see Table 1) slopes (between 0.024

Table 5 Summary of the best fitting PGLS models for 4 principal components (PC1, PC2, PC3 and PC4) that explained nearly 80% of variance of the morphology and the proportion of habitat used (sand, ground, rock and branches)

Model	λ	Adjusted r^2	Intercept	Variables	Slope	*Pp*	*Pt*	AICc	Wi
PC1~sand	0	0.14	0.08	Sand	-0.207	0.188*	0.188*	14.017	0.189
PC1~sand+rock	0	0.15	0.18	Sand	-0.280	0.013*	0.036	15.137	0.180
				Rock	-0.116	0.227			
PC2~branches	0.44	0.16	0.04	Branches	-1.044	0.012*	0.012	2.999	0.134
PC3~ground+branches	0	0.25	-0.008	Ground	0.169	0.072	0.005	2.196	0.200
				Branches	-1.132	0.008*			
PC3~trunk	0.70	0.02	0.01	Trunk	-0.126	0.188	0.188	-24.370	0.064
PC3~rock	0.72	0.04	-0.04	Rock	0.074	0.126	0.126	-25.040	0.090
PC3~ground	0.81	0.07	0.04	Ground	-0.127	0.069	0.069	2-5.719	0.126
PC3~ground+trunk	0.79	0.08	0.05	Ground	-0.114	0.103	0.114	-24.604	0.072
				Trunk	-0.103	0.274			
PC3~sand+rock	0.78	0.10	-0.12	Sand	0.097	0.102	0.081	-25.423	0.109
				Rock	0.128	0.029*			

See Table S2 for all models. λ (Pagel's phylogenetic signal), adjusted r^2 (Adj r^2), intercept and slopes were considered for those informative variables based on the Akaike criterion (AICc and Wi). *Pp* means the partial *P*-value for each variable; *Pt* is the *P*-value for the complete model. *Significant results.

and 0.069) were not significant ($P > 0.130$). Another association identified occurred between PC2 and the use of branches, where branch lizards tend to have longer tibia and femur, wider femorotibial gastrocnemius but shorter peroneus longus origin tendon (Fig. 3b, Table 3). In the case of the particular analysis for the above of 0.65 loads in the PCA, none of the morphological traits (Plol, Fgw, Fel, Til) showed significant slopes (between 0.057 and 0.436; $P > 0.69$), although we may consider that Fel and Til are marginally significant (Fel~branches, λ = 0.839, slope = 0.314, $P = 0.079$; Til~branches, λ = 0.788, slope = 0.436, $P = 0.069$). We have also identified an association between PC3 and the concomitant use of rock and sand, where lizards having such ecology tend to exhibit wider aponeurosis of the flexor digitorum longus muscle (Fig. 3c, Table 3). However, none of the morphological traits showed significant ($P > 0.099$) slopes (between −0.046 and 0.032) in our particular PGLS analyses.

DISCUSSION

We studied the evolutionary processes through which morphological traits may have evolved in Tropiduridae and Liolaemidae, testing phylogenetic evolutionary models to discriminate which one of the evolutionary hypotheses better fits the morphological data and establishing whether identified differences between models reflect ecomorphological associations. We found that there is not a unique evolutionary model for the different morphological variables. Some of them fitted better for the Brownian Motion evolutionary model (Felsenstein 1985; Blomberg *et al.* 2003), suggesting a random path of evolution, while other variables fitted better in the Ornstein–Uhlembeck evolutionary model (Hansen 1997; Butler & King 2004), suggesting a regime of variation or directional selection. None of the morphological variables showed the best fit under the early burst evolutionary model.

Our results partially recover the trend described by Harmon *et al.* (2010) for squamates in general and for liolaemids in particular, where the Ornstein–Uhlenbeck model best fits most of the morphological variables. It is worth mentioning that Harmon *et al.* (2010) evaluated body size and body shape based exclusively on external morphology, while our dataset also incorporates traits of myo-tendinous anatomy. Interestingly, in Tropiduridae and Liolaemidae, only 5 morphological variables fitted better in the Brownian motion model, in accordance with previous studies on Liolaemidae, suggesting these lizards as morphologically and ecologically conserved (Schulte *et al.* 2004; Tulli *et al.* 2009, 2012a,b). It has
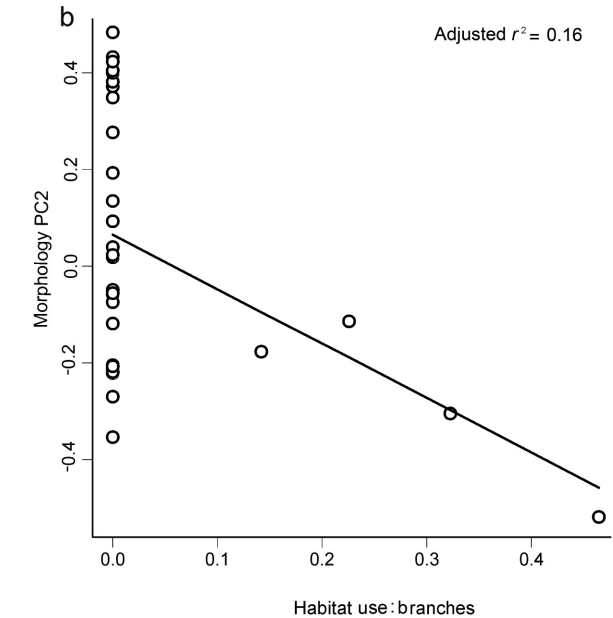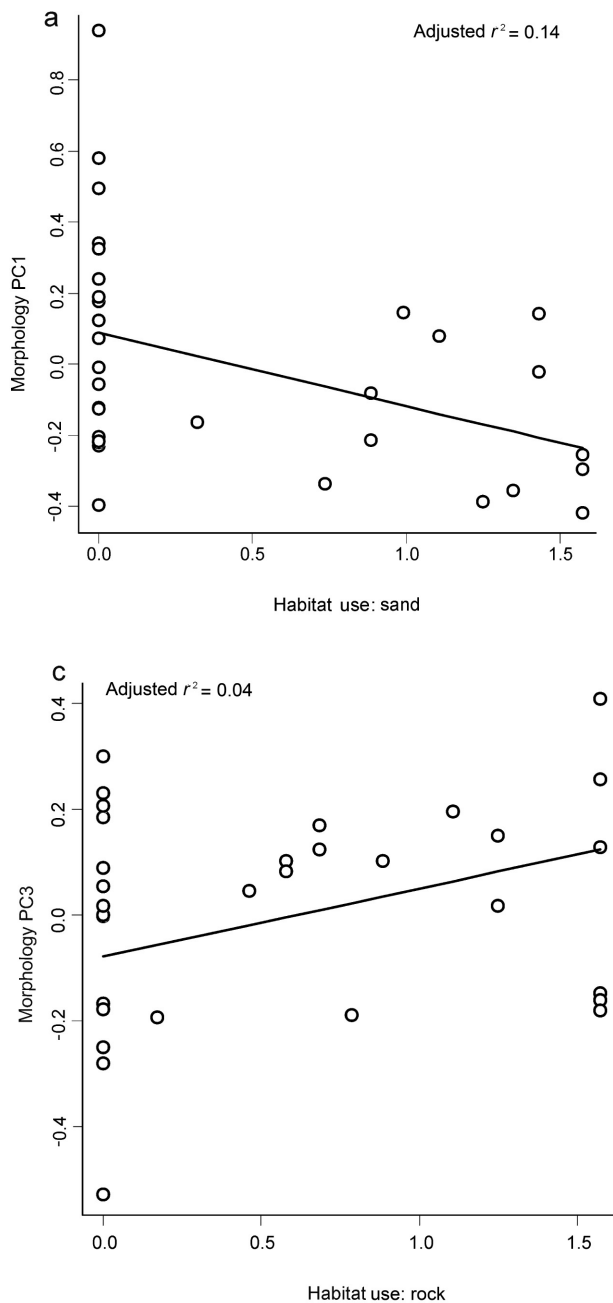
Figure 3 Product-moment relationships between habitat use and morphology (principal components) according to PGLS models. Each panel shows on the x axis the habitat use and on the y axis (morphology) the contribution of each principal component (PC) for those relationships that were significant. Panel a) association between PC1 (with high contribution for peroneus longus insertion tendon length, peroneus brevis tendon length, femoral gastrocnemius sup length and flexor dig longus aponeurosis length) and use of sand; b) the relationship between PC2 (peroneus longus origin tendon length and femorotibial gastrocnemius width) and the use of branches and c) relationship between PC3 (flexor dig longus aponeurosis width) and the use of rock and sand partially. The orientation of arrows along the morphology axis indicates if the contribution of the variable increases (positive load contribution) or decreases (negative load contribution) with habitat use. For details see Table 4.

to be said that none of these papers tested the models of evolution. There is, however, enough recent evidence favoring the hypothesis that Liolaemidae consists of a mixture of conservative and adaptive traits, where phylogenetic clustering hampers adaptive responses (Pincheira-Donoso *et al.* 2009; Tulli *et al.* 2009,

2012a,b). Recently, Pincheira-Donoso *et al.* (2015) found that *Liolaemus* lizards' body size diversification conforms to an Ornstein–Uhlenbeck model with multiple trait optima. In the case of Tropiduridae, a broader ecological diversification in habitat use seems to be related to morphological variation and specialization

(Kohlsdorf *et al.* 2001, 2008; Grizante *et al.* 2010; Kohlsdorf & Navas 2012), despite specific indications of some morphological constraints too (see Kohlsdorf *et al.* 2004). Apparently both lineages show some degree of morphological conservatism, although it seems stronger in liolaemids.

From a functional perspective, our results suggest that the evolution of important tendon traits that play a main role in the rotation and flexion of the metatarsus (Brinkman 1980) follows a pattern that deviates from random evolution (e.g. digital flexor tendons of digits 3–5). The metatarsus design seems related to the speed running flexibility because in most lizards a relatively longer metatarsus is associated with higher sprint speeds (Russell & Bels 2001). Enhancement of functional possibilities elicited by morphological variation has also been suggested by our dataset in regards to the fifth pedal digit, which is articulated by the digital flexor tendon 5, the femoral gastrocnemius superficial muscle and the peroneus longus complex. These structures are also involved in the abduction and flexion of this digit during grasping (Russell & Rewcastle 1979; Brinkman 1980), permitting exertion of a clasp grip around a branch (Robinson 1975; Abdala *et al.* 2014), which as a consequence very likely favors the invasion of spatial niches having narrow branches. Variations in lizard foot morphology, especially in relation to foot size, femur and tibia lengths, and fifth toe length, seem particularly relevant during evolutionary processes involving the colonization of arboreal habitats. Our data indicate that length of digits 4 and 5 and foot length and width show a trend to directional selection, concurring with Butler and King (2004) and Harmon *et al.* (2010); this evolutionary model is known as the Ornstein–Uhlenbeck evolutionary model. Interestingly, tibia and femur lengths follow a BM model, reinforcing the hypothesis settled on developmental biology that suggests a modular evolution of autopod and zeugopod in tetrapods (Huang *et al.* 2015) by showing that these modules might evolve following different models in the lizards we studied.

Lizards that exploit branches tend to have proportionally longer tibias, which contribute to elongated hind limbs that enhance sprint-speed (Bonine & Garland 1999; Bonino *et al.* 2011; Tulli *et al.* 2012a), while in slower species the tarsals and metatarsals tend to be shorter than the rest of the limb (Irschick & Jayne 1999). Interestingly, in our dataset the branch habitat use is exclusively associated with external morphology variables, while the associations with rock and sand involve both external and myo-tendinous traits.

Our results for species using sandy habitats suggest ecomorphological associations involving longer superficial femoral gastrocnemius muscle in comparison with species that move mainly over trunks, rocks and ground. The gastrocnemius muscle is a plantar flexor that also bends the leg at the knee joint (Russell & Bauer 2008). Interestingly, this muscle tends to be wider in lizards exhibiting pedal grasping (Abdala *et al.* 2014). We observe that in iguanid lizards from sandy habitats the leg segment tends to be elongated, as do the associated tendons. This variation, represented only by the tendinous system, is also recovered in saxicolous lizards that exhibit wider flexor digitorum longus aponeurosis. It should be considered that when muscles are longer, the force generated might be lower because muscles produce force over a narrower range of lengths (Higham & Nelson 2008). For example, aponeurotic and tendinous tissues can change in length with little or no variation in muscle fascicle lengths (Higham & Nelson 2008), a change produced in a way that most of the variation is settled in the tendon rather than in the muscle, reducing the need for extra work by muscle fibers (Wilson & Lichtwark 2011). Elongation of hind limb tendons has been reported by Snyder (1954) in bipedal lizards, where the distal ends of limb segments are lightened by elongated tendons and can be moved through the locomotor arc with less energy expenditure. We propose that this arrangement is also found in sandy lizards and likely explains the high sprint speeds they usually attain (Tulli *et al.* 2012a).

The identified correlation between muscle-tendinous morphology and habitat use suggests that strict saxicolous lizards tend to exhibit wider flexor digitorum longus aponeurosis. This pattern of tendon/muscle relationship contrasts the prediction that distal limb muscles should have longer tendons and very short muscle fascicles (Bobbert 2001; Tulli *et al.* 2012b). Deviation from such prediction in saxicolous lizards might be explained by the higher resistance imposed by clinging to rocks (Tulli *et al.* 2011).

The discussion about ecomorphological evolution in squamates is centered on the concept of "ecomorphs" (see Williams 1972), and one of the principal characteristics used to recognize ecomorphs is limb length (Losos 2009). Collins *et al.* (2013) also found morphological variables related to limb length to be ecomorphologically relevant in terrestrial lizards. These parameters were also included in our dataset for

Tropiduridae and Liolaemidae, and clear and significant trends involving limb lengths have not been identified when these 2 families have been evaluated together. Our analyses suggest that this type of external morphology evolves only in association with branch-using species, although we recognize that the number of species from such habitat use in our dataset is low. In contrast, evolution of the internal muscle-tendinous morphology seems associated to all types of habitat usage in these lizard families. Thus, external morphology traits seem mostly conservative, except for species that exploit branches, a result that seems to explain why it is hard to visually recognize ecomorphs shared by tropidurid and liolaemid lizards, as ecomorphs are usually defined using patterns of external morphology (Losos 1990a,b, 2009; Irschick & Losos 1996; Beutell & Losos 1999). The only ecomorphological study considering muscular traits of *Anolis* (Herrel *et al.* 2008) detected a tendency to differentiation in muscular mass between *A. sagrei* and *A. valencienne*. The *Anolis* ecomorphs, in particular, exist in clear association with arboreal habitats and islands characterized by high microhabitat structure complexity (e.g. in the same tree several species of *Anolis* may be observed in different parts of the tree), and each island in the Greater Antilles presents many specific niches, with the morphological diversity of *Anolis* being a factor that permits occupation of the different existing niches (Losos *et al.* 1998). In contrast, most of liolaemid and tropidurid species inhabit open areas, such as savannas and deserts (Cei 1986; Vitt 1991), which are characterized by increased distances between shelters to be covered when escaping from predators (see Goodman 2009), which probably prevents extreme morphological diversification. It has been previously suggested that conservation of gross morphology may represent a mechanism to accommodate demands of a wide array of environmental challenges by permitting adequacy in all such circumstances (Gans 1993; Abdala *et al.* 2014). This is the functionally intermediate morphology (Arnold 1998) already described for Liolaemid lizards, which have the ability to perform relatively well (or similarly badly) at several tasks and can be considered as "jack of all trades and master of none" (Tulli *et al.* 2012a), retaining an all-purpose morphology allowing them to use a variety of habitats. Thus, natural selection is acting on several traits and allows lizards to exploit several surfaces (Sathe & Husak 2015); therefore, a generalized morphology could represent a morphological optimum. It should be noted that this generalized morphology seems absent in *Tropidurus* lizards, which have demonstrated to be more versatile (Grizante *et al.* 201.0). The lineage, however, also includes generalist species such as the lizard *Tropidurus torquatus* and *Tropidurus etheridgei*, which use a broad variety of environments and exhibit suitable performance in physically contrasting surfaces (Vitt 1991; Cruz *et al.* 1998; Brandt *et al.* 2015). A next step, therefore, may consist of incorporating locomotor performance of tropidurids and liolaemids running along a wide range of surface types in order to directly access the functional implications of generalized morphologies in different ecological settings.

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SUPPLEMENTARY MATERIALS

Additional supporting information may be found in the online version of this article.

Table S1 Voucher specimens of the species studied

Table S2 Species means and the number of individuals per species used are included

Table S3 A total of 155 possible PGLS models described significant slopes for some of the habitat use variables. In boldface we illustrate 9 of the most informative models after Akaike criterion and provided evidence for associations between morphology and ecology. The best models after considering adjusted r^2 (Adj r^2), AICc and wAICc values. λ, phylogenetic signal (Pagel's); AICc, Akaike information criterion. Morphology is represented by 4 principal components (PC1, PC2, PC3 and PC4). Sand is sandy soils; rock is rock boulders as microhabitat, trunk is mean trunk of trees; branch is branches of trees; and ground is horizontal and general ground soil. Slopes for each habitat portion used are represented

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