Is phylogeny driving tendon length in lizards?

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Abstract

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Tendons transmit tensile forces generated by muscles and are a crucial part of the musculoskeletal system in vertebrates. Because tendons and tendon cells respond to altered mechanical load by increasing collagen synthesis, we hypothesized that a correlation between tendon morphology and the loading regime imposed by locomotor style or habitat use exists. This makes tendons an interesting model for studying the relationship between morphology and environment. In this study, we compare the general morphology of the palmar flexor plate, the length of the digital tendons, and the length of the flexor carpi ulnaris tendon in species of lizards that exploit a variety of structural habitats. The results from statistical analyses show that phylogenetic relatedness has a major impact on our ability to detect differences between habitat groups, and no differences in tendon length could be detected between iguanian species occupying different habitats when taking into account the relatedness between species. Our data for lizards diverge from the general mammalian paradigm where variation in tendon is often associated with habitat use or locomotor style.

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Introduction

Tendons are crucial elements in the transmission of the tensile forces generated by muscles to the skeleton. Acute exercise has been shown to induce alterations in the molecular structure of tendons, and there is considerable evidence supporting a direct relationship between mechanical load and the tendon cellular response (Benjamin *et al.* 1986, 1992; Carter *et al.* 1998). *In vitro*, tendon cells have been shown to up-regulate collagen synthesis when subjected to tensile forces (Benjamin *et al.* 2008). The environment provides mechanical input to tissues through ground reaction forces, gravity, barometric pressure, vibration, and contact with objects. Therefore, we hypothesize that the overall morphology of the tendons of the appendicular skeleton would be related to the use of different structural habitats given the different mechanical constraints imposed by these habitats on locomotion.

Indeed, previous studies have noted some relationship between the length of the tendons of some limb muscles and the mechanical constraints imposed by the environments in which they are used. For example, the *flexor carpi ulnaris* tendon in didelphid mammals has been shown to be related to habitat use (Abdala *et al.* 2006). The forelimb muscles of some didelphid species possess extremely long tendons.

Phylogenetic mapping of relative tendon lengths suggested that long tendons in the forelimb are a convergent adaptation linked to yet unknown ecological requirements (Abdala *et al.* 2006). Abdala *et al.* (2008) also noted a similar trend in the limb tendons of turtles, which are generally better developed in the terrestrial forms relative to aquatic forms. Our observations of distal limb morphology in lizards also suggest a connection between tendon morphology (i.e. tendon length) and habitat use. More generally, previous authors (Losos 1990a,b; Garland and Losos 1994; Arnold 1998; Bonine and Garland 1999) have shown that the locomotor capacity of lizards is reflected in morphology, particularly in limb or limb segment length. Thus, variation in habitat use and locomotor performance is expected to be correlated with the morphology of the appendicular system (Lundelius. 1957; Collette 1961; Russell and Bauer 1989; Carrillo de Espinoza *et al.* 1990; Colli *et al.* 1992; Vrcibradic and Rocha 1996; Etheridge 2000; Irschick *et al.* 2000; Zani 2000; Mc Elroy 2008). Along these lines, Tulli *et al.* (2009) reported that a considerable fraction of the variability in claw morphology in Neotropical Iguanian lizards can be linked to habitat use.

The fore and hind limbs in lizards have differential roles during vertical versus horizontal movements (Zaaf *et al.* 2001a,b). Whereas the forelegs mainly function to support

body weight during level running, they provide propulsion and pull the body toward the surface during climbing (Cartmill, 1985; Zaaf *et al.* 2001a). Accordingly, the hind legs, which support body weight and provide most of the propulsion during level running, show a reduction in the normal forces by one-sixth during climbing (Autumn *et al.* 2006). Clearly, during level running, the hind limb is more important than the forelimb in determining stride length and in generating the propulsive forces, particularly in those lizard species in which the hind limb is longer than the forelimb. According to Goodman (2006), differences in limb length explain most of the variation in performance among skink species, with sprint speed being correlated with hind limb length. Climbing speed and clinging ability, on the other hand, are correlated with forelimb length (Goodman 2006). These data indicate a differential role of the forelimb during locomotion, with the forelimbs playing a crucial role during climbing.

In this study, we focus on the tendinous system of the forelimbs as this likely plays a role in allowing a secure grip on perches through digit flexion (Vanhooydonck and Van Damme 2001). Previous studies (Abdala *et al.* 2009) demonstrated a link between the tendinous pattern of the palm of the hand and movement abilities in lizards. Arboreal lizards have flexor tendons running independently to the tips of the digits, and ground dwellers have fused tendons forming a single tendinous plate (Moro and Abdala 2004; Abdala *et al.* 2009). In the present study, we first describe the tendinous patterns and the occurrence of tendon independence in the palm of the hand for a wide range of lizards of different families occupying different habitats. Next, we quantitatively analyze tendon length in 50 species of iguanian lizards occupying a variety of structural habitats in order to test for an association between morphology of the forelimb tendons and habitat use. For each species, we measured the length of the *flexor carpi ulnaris* muscle and its tendon. We selected this muscle because a substantial portion of its length is usually comprised of tendon and prior observations suggested a potential role of these tendons in arboreal locomotion (Abdala *et al.* 2006). We also compared the length of the digital flexor tendons among species. To account for the variation in the flexor tendon associated with the different tendinous patterns of the palm of the hand, we also measured the width of the palmar flexor plate in addition to the flexor tendon length (Haines 1950; Abdala *et al.* 2009).

We decided to focus our study on tendon length instead of the more conventional cross-sectional area measures because of its relevance in transmitting forces from the muscle to the structures interacting with the environment. Bobbert (2001) already called the attention to one intriguing design aspect of the musculoskeletal system in humans: distal muscle–tendon complexes span the large distance between origin and insertion with long tendinous structures and very short muscle fibers suggesting an important role for tendons in force transmission. This design is present also in lizards (VA, personal observation). In this context, we predict that (1) climbing

lizards that utilize narrow substrates such as twigs or branches have more individualized tendons in the palm of the hand than ground-dwelling lizards; (2) ground-dwelling and climbing lizards show significant differences in tendon length, with climbing species having relatively longer *flexor carpi ulnaris* tendons than ground-dwelling species because this may provide a more effective transmission of motion and force in animals with short limbs; and (3) given that limb length is constrained in climbing lizards, effective force transmission to the claws by relatively short muscles may be achieved by relatively longer tendons. Thus, we predict that climbing lizards have relatively longer digital flexor tendons.

Materials and Methods

Three hundred and thirty-four adult specimens of both sexes, belonging to 71 species of Neotropical lizards were used in our descriptive study of tendon morphology (Table 1; Appendix S1). These specimens were selected to provide a range of species from different families varying widely in habitat use. From these, we obtained data on digit length, muscle length, and tendon length for 240 adult specimens belonging to 50 species (see supporting information Table S1) which were used for our quantitative analyses. Specimens used in the analysis were obtained from the herpetology collections of the American Museum of Natural History (AMNH), the Fundación Miguel Lillo (FML), the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', the Museum of Comparative Zoology (MCZ), and the National Museum of Natural History (USNM).

Morphology

The muscles and tendons of the forearm and hand were dissected using a binocular scope. Dissections of the right forearm were performed on all specimens investigated. The tendons of each hand were photographed in ventral view with a high-resolution digital camera (SONY DSC-H 5; Sony Corp., Tokyo, Japan) at the same magnification and from the same distance (Fig. 1). Tendons were outlined in Corel Draw 12 (Corel Corp., Ottawa, Canada). The number of adult specimens per species varied with availability and ranged between 1 and 10.

Body size snout-vent length, (SVL) and 13 traits related to the forearm and internal hand morphology (described below) were measured in a subset of lizards (supporting information Table S1). Snout-vent length and all measurements of the forearm and hand were performed by MJT using digital calipers (Mitutoyo CD-15B; ± 0.01 mm). For the forearm, we measured the following: the length of the tendons of all digits (LTD1-5, Figs 1A and 2) from the base of the tendon where it separates from the palmar flexor plate (L and G patterns), or from the m. *flexor digitorum longus* tendon (P-pattern) to its insertion at the distal phalanx (Fig. 2); the length of the *flexor carpi ulnaris* muscular fibers (LMF, Fig. 1A,B; Haines 1950;

Table 1 Habitat use of the species examined in qualitative part of this study. Habitats were assigned based on literature data

Species	Author	Function	Habits
Ameiva ameiva	Vitt and Colli (1994)	Ground-dwelling	Terrestrial
Anolis cuvieri	Schwartz and Henderson (1991)	Clinging ability	Arboreal
Anolis cupreus	Schwartz and Henderson (1991)		
Anolis cybotes	Schwartz and Henderson (1991)	Ground-dwelling	Terrestrial
Anolis distichus	Schwartz and Henderson (1991)	Clinging ability	Arboreal
Anolis equestris	Schwartz and Henderson (1991)		
Anolis garmani	Schwartz and Henderson (1991)		
Anolis graham	Schwartz and Henderson (1991)		
Anolis gundlachi	Schwartz and Henderson (1991)	Ground-dwelling	Terrestrial
Anolis humilis	Talbot (1979)		
Anolis limifrons	Talbot (1979)	Clinging ability	Arboreal
Anolis mestrei	Schwartz and Henderson (1991)	Clinging ability	Saxicolous
Anolis monensis	Schwartz and Henderson (1991)	Ground-dwelling	Terrestrial
Anolis pentaprion	Savage (2002)	Clinging ability	Arboreal
Anolis pulchellus	Schwartz and Henderson (1991)		
Anolis sagrei	Schwartz and Henderson (1991)	Ground-dwelling	Terrestrial
Basiliscus basiliscus	Frost *et al.* (2001a, 2001b)	Clinging ability	Arboreal
Basiliscus plumifrons	Savage (2002)		
Basiliscus vitattus	Savage (2002)		
Cnemidophorus longicaudus	Scolaro (2005)	Ground-dwelling	Terrestrial
Diplolaemus bibronii	Scolaro (2005)		
Diplolaemus darwinii	Scolaro (2005)		
Diplolaemus sexcintus	Cei *et al.* (2003)	Ground-dwelling	Terrestrial
Urostrophus gallardoi	Frost *et al.* (2001a,b)	Clinging ability	Arboreal
Hemidactylus mabouia	Genise and Montanelli (1991)		
Homonota fasciata	Scolaro (2005)	Ground-dwelling	Terrestrial
Iguana iguana	Hirth (1963)	Clinging ability	Arboreal
Liolaemus albiceps	Abdala (2007)	Ground-dwelling	Terrestrial
Liolaemus bibronii	Cei (1986)		
Liolaemus canqueli	Etheridge (2000)	Bury in the sand	Arenicolous
Liolaemus ceii	Cei (1986)	Clinging ability	Saxicolous
Liolaemu coereleus	Pincheira-Donoso *et al.* (2007)		
Liolaemus crepuscularis	Abdala and Diaz Gomez (2006)	Ground-dwelling	Terrestrial
Liolaemus cuyanus	Halloy *et al.* (1998)	Bury in the sand	Arenicolous
Liolaemus darwinii	Etheridge (2000)	Ground-dwelling	Terrestrial
Liolaemus dorbignyi	Abdala and Quinteros (2008)	Clinging ability	Saxicolous
Liolaemus elongatus	Cei (1986)		
Liolaemus fitzingeri	Etheridge (2000)	Bury in the sand	Arenicolous
Liolaemus irregularis	Abdala (2007)	Ground-dwelling	Terrestrial
Liolaemus koslowskyi	Etheridge (2000)		
Liolaemus laurenti	Etheridge (2000)	Bury in the sand	Arenicolous
Liolaemus multimaculatus	Halloy *et al.* (1998)	Bury in the sand	Arenicolous
Liolaemus olongasta	Etheridge (2000)		
Liolaemus ornatus	Abdala (2007)	Ground-dwelling	Terrestrial
Liolaemus petrophilus	Avila *et al.* (2004)	Clinging ability	Saxicolous
Liolaemus pictus	Medel *et al.* (1988)		Arboreal
Liolaemus poecilochromus	Abdala field observations	Ground-dwelling	Terrestrial
Liolaemus riojanus	Halloy *et al.* (1998)	Bury in the sand	Arenicolous
Liolaemus salinicola	Halloy *et al.* (1998)		
Liolaemus scapularis	Halloy *et al.* (1998)		
Liolaemus tenuis	Medel *et al.* (1988)	Clinging ability	Arboreal
Liolaemus xanthoviridis	Etheridge (2000)	Bury in the sand	Arenicolous
Leiosaurus belli	Laspiur *et al.* (2007)	Ground-dwelling	Terrestrial
Leiosaurus catamarcensis	Laspiur *et al.* (2007)		
Mabuya frenata	Vrcibradic and Rocha (1998)		Terrestrial
Mabuya dorsivitatta	Ávila-Pires (1995)		

Table 1 (*Continued*)

Species	Author	Function	Habits
Phymaturus antofagastensis	Pereyra (1985)	Clinging ability	Saxicolous
Phymaturus somuncurensis	Lobo and Quinteros (2005)		
Phymaturus spectabilis	Lobo and Quinteros (2005)		
Phyamturus tenebrosus	Lobo and Quinteros (2005)		
Polychrus acutirostris	Frost *et al.* (2001a,b)		Arboreal
Polychrus marmoratus	Frost *et al.* (2001a,b)		
Pristidactylus achalensis	Frost *et al.* (2001a,b)	Ground-dwelling	Terrestrial
Pristidactylus scapulatus	Frost *et al.* (2001a,b)		
Teius teyou	Cei (1993)		Terrestrial
Teius occulatus	Scolaro (2005)		
Tropidurus hispidus	Frost (1992)	Clinging ability	Saxicolous
Tropidurus etheridgei	Frost (1992)	Ground-dwelling	Terrestrial
Tropidurus oreadicus	Frost (1992)	Clinging ability	Arboreal
Tropidurus plica	Frost (1992)		
Tupinambis rufescens	Scolaro (2005)	Ground-dwelling	Terrestrial

Abdala *et al.* 2009); and the length of the tendon of the *flexor carpi ulnaris* muscle when present (Fig. 1B). This corresponds to the length of the visible tendon of this muscle, from the myotendinous junction to its insertion onto the distal extreme of the ulna. In addition, we measured the length of digits one through five (DI-DV) (Fig. 2). Species means, standard deviations and the number of individuals per species used are included in the supporting information Table S1.

To test whether tendon length differed among species living in different habitats, we classified each species as belonging to one of the following habitat groups: generalized terrestrial, sand-dwelling, saxicolous, and arboreal. The assignment of habitat use in the lizards studied here is based on literature data (Table 1) and follows Tulli *et al.* (2009).

Statistical analysis

Mean values of all morphometric traits were $\log_{10}$-transformed prior to analyses to meet the requirements of normality and homoscedascity (Zar 1999). Because species are not independent data points but related by their phylogenetic history, we used both traditional and phylogenetically informed statistical analyses. To test for correlations between tendon and digit lengths with overall body size (SVL), we ran regression analyses on the raw species means as well as on the independent contrasts. Independent contrasts were calculated using the PDAP package (Garland *et al.* 1999), and all regressions were forced through the origin. The phylogenetic framework for these analyses was constructed using the topologies
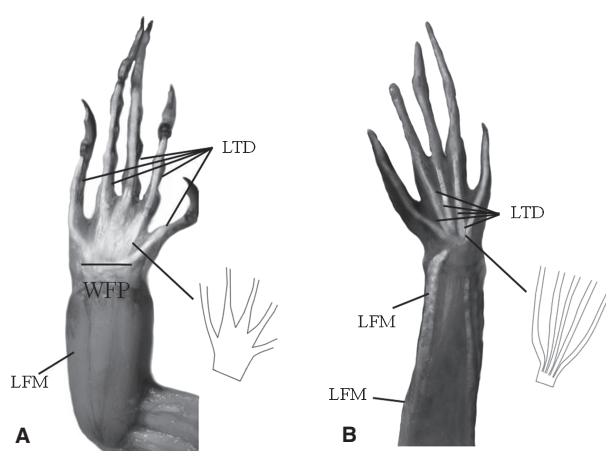

Fig. 1—Measurements of the forearm considered in the statistical analysis: —**A**. LTD: length of the tendons of all digits; LMF: length of the *flexor carpi ulnaris* muscular fibers; WFP: width of the flexor plate of the hand; —**B**. LTM: length of the tendon of the *flexor carpi ulnaris* muscle.

Fig. 2—Measurements of the digits and digit tendons considered in the statistical analysis: 1 corresponds to digit length and 2 corresponds to tendon length in both, L (left) and P (right) patterns.

provided in Frost *et al.* (2001a,b), Nicholson (2002), Avila *et al.* (2004), Morando *et al.* (2004), Lobo and Quinteros (2005) and Abdala (2007); Fig. 3). All branch lengths were set to unity because branch lengths were not available for all taxa included in our analysis. To test whether branch lengths of unit length were indeed appropriate for our analyses, we used the diagnostics options in the PDTREE program and correlated the absolute values of the standardized contrasts with their standard deviations (Garland *et al.*, 1992). The resulting correlations were not significant, thus suggesting that these branch lengths are indeed appropriate. Residual contrasts of tendon and digit lengths were calculated based on regressions of each variable on SVL and used in a regression analyses to test for associations between the length of the digit and its tendon, independent of variation in body size. All regression contrasts were forced through the origin (Garland *et al.*, 1992).

Simulation analyses were performed using the PDSIMUL and PDANOVA programs (Garland *et al.* 1993). In the PDSIMUL program, we used Brownian motion as our model for evolutionary change and ran 1000 unbounded simulations to create an empirical null distribution against which the *F*-value from the original data could be compared (note that simulations with the lower limit constrained to 0 gave qualitatively similar results). In the PDANOVA program, habitat use was entered as factor, and tendon and digit lengths as dependent variables, and SVL as a covariate. However, because of our independent contrast analysis indicated a significant and positive association between the residual standardized contrasts of the lengths of the tendons and the lengths of their respective digits, we also ran analyses of co-variance on the tendon lengths with the length of the corresponding digit as covariate. We considered differences between categories significant if the original *F*-value was higher than the F_{95}-value derived from the empirical distribution. All traditional analyses were performed with SPSS v.15 (SPSS Inc., Chicago, USA).

We also calculated the phylogenetic signal present in our data using the randomization test described by Blomberg *et al.* (2003) and computed the K-statistic (an indicator of phylogenetic signal) for the morphometric traits included in our analysis. This analysis was implemented in MATLAB version 6.1.0 for PC (The MathWorks Inc., Natick, MA, USA) using the module 'PHYSIG' (available upon request from Dr. T. Garland Jr., University of California at Riverside). Finally, we ran an ancestral character state reconstruction using parsimony on the habitat use data in Mesquite (Maddison and Maddison 2004) to visualize how habitat use is clustered within the phylogeny and may affect our ability to detect evolutionary patterns.

Results

Morphological patterns

The flexor plate adopted different architectures in the lizards examined here. Typically, it is composed of four triangular surfaces, each continuing distally as a thick tendon that runs within the fibrous digital flexor tunnels and inserts onto the terminal phalanx of its respective digit. The belly of the m. *flexor carpi ulnaris* originating from the distal end of the ulna also inserts onto the flexor plate but is slightly separated from the other two parts and persists as a bulky, divided tendon. This conventional L pattern (Moro and Abdala 2004) was present in all leiosaurid species, *Phymaturus*, all *Liolaemus* species analyzed except for *L. tenuis, Iguana iguana*, all teiids analyzed, and *Mabuya* (Fig. 4A). The P-pattern (Moro and Abdala 2004) occurred in all *Polychrus* and *Anolis* species (Fig. 4B). In this pattern, the tendons of the m. *flexor digitorum longus* pass across the palmar surface and insert directly onto the terminal phalanges. Developed flexor plates are absent in the P-pattern; however, a reduced flexor plate and palmar sesamoid bones can be observed in some *Anolis* specimens. The geckos possess a G-pattern (Abdala *et al.* 2009), by which each tendon is distributed to its respective digit from a palmar flexor plate without sesamoid; however, the tendon of digit V is slightly independent of the main flexor plate (Fig. 4C).

There were discernible interspecific differences within representatives of the L-pattern. All *Basiliscus* species possess a flexor plate with deeply embedded sesamoid bones. Additionally, the large tendon of the m. *flexor digitorum longus* bifurcates and reinforces the flexor tendons of digits II and III in this genus (Fig. 4D). In *Liolaemus tenuis*, the tendons are interlaced in the palmar region and continue distally as separate tendons (Fig. 4E). Tropidurids have a flexor plate with a marginal sesamoid bone embedded. Individual tendons in the flexor plate are distinguishable and independently course distally along their respective digits (Fig. 4F). *Tropidurus plica* presents a noticeable small flexor plate with considerably longer digital flexor tendons (Fig. 4G).

Is tendon length correlated with digit length?

Comparative analyses using phylogenetically independent contrasts show significant correlations between tendon length, digit length, and overall body size (all $P < 0.001$). Moreover, a regression of the residual independent contrasts of tendon length on the residual independent contrasts of digit length forced through the origin indicates significant correlations between the length of the tendon and the length of the corresponding digit for digits 2-4 (T2-D2: $r = 0.52$, $P < 0.01$; T3-D3: $r = 0.44$, $P < 0.01$; T4-D4: $r = 0.39$, $P < 0.01$). Thus, the evolution of longer digits 2–4 is associated with the evolution of longer flexor tendons.

Do species that utilize different habitats differ in the length of the digital flexor tendons?

An ANOVA testing for differences in snout-vent length in species occupying different habitats indicated significant differences ($F_{3,47} = 4.51$; $P = 0.007$). Post hoc tests showed that
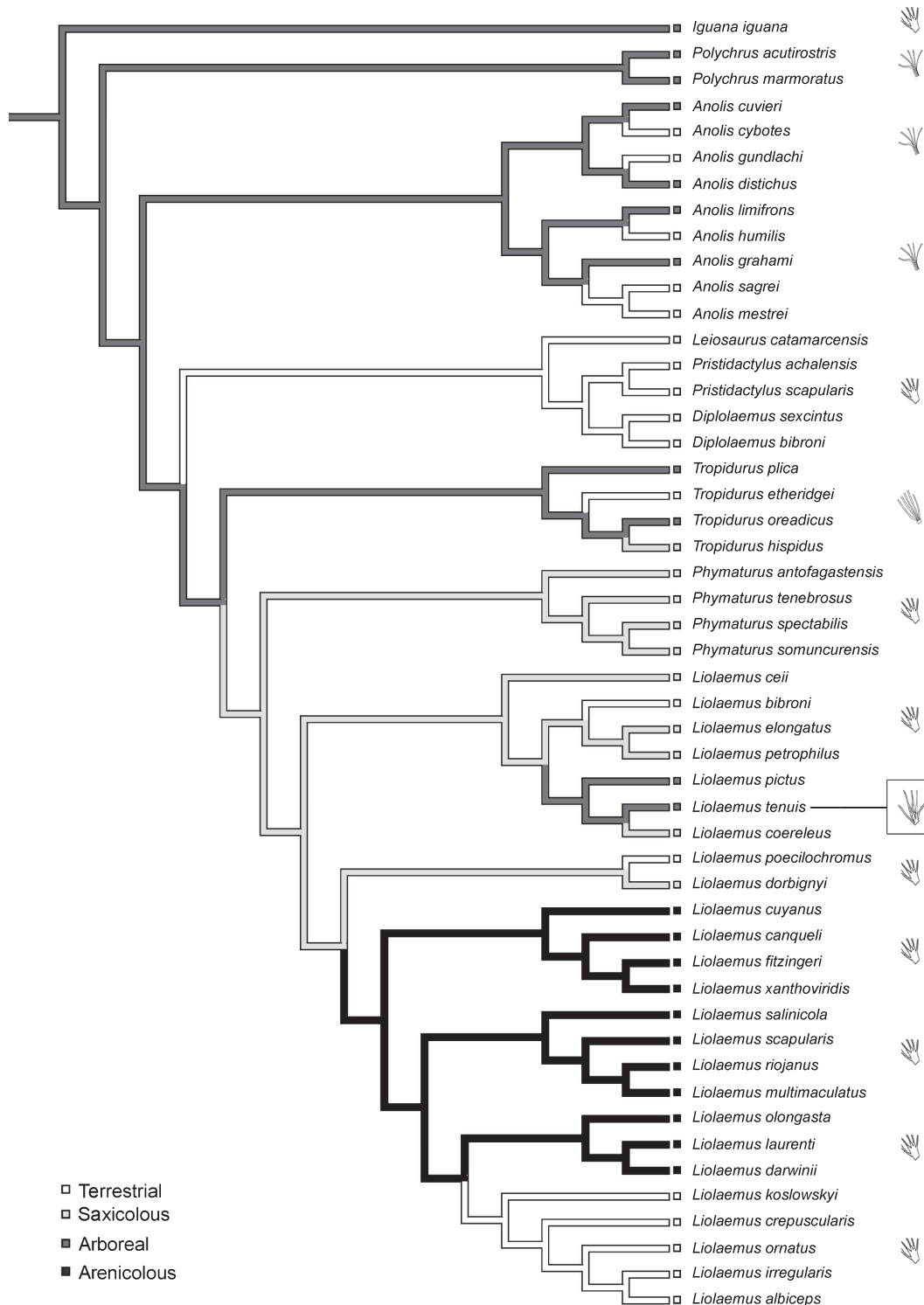
Fig. 3—Ancestral character state reconstruction illustrating how habitat use is clustered within specific clades in the phylogeny, especially within the *Liolaemus* group. To the right of the tree, the tendinous patterns observed are indicated by schematic drawings. The tree topology is based on Frost *et al.* (2001a,b), Nicholson (2002), Avila *et al.* (2004), Morando *et al.* (2004), Lobo and Quinteros (2005), Abdala (2007) and was used as a phylogenetic framework for the statistical analysis. White = terrestrial, light gray = saxicolous, dark gray = arboreal, black = arenicolous.
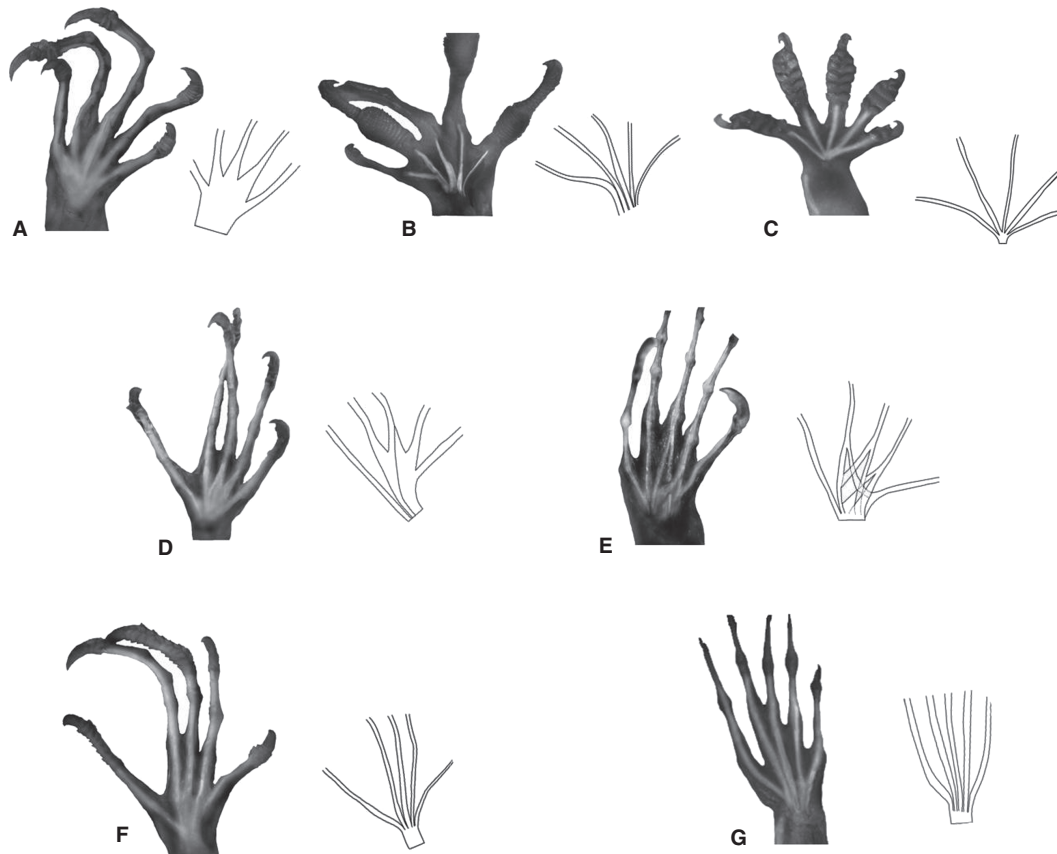
Fig. 4—Illustration of the different patterns of the tendinous structures observed on the palmar surface of the hand. —**A**. Conventional L-pattern as described by Moro and Abdala (2004); —**B**. P-pattern (Moro and Abdala 2004) with a trend to total independence of the tendons running to the digits; —**C**. G-pattern present in the gecko analyzed as described in Abdala *et al*. (2009); —**D**. Flexor plate as in *Basiliscus*, with a reinforcing of the flexor tendons of digits II and III; —**E**. *Liolaemus tenuis* incomplete flexor plate, comprised of separated but interweaved tendons; —**F**. Tropidurid flexor plate with a marginal sesamoid bone embedded and some degree of independence of the tendons; —**G**. *Tropidurus plica* showing a reduced flexor plate.

sand dwellers were significantly smaller than arboreal and saxicolous species. Terrestrial and sand-dwelling species did not differ in body size. The difference in body size between sand-dwelling and climbing species did not remain, however, after taking into account the phylogenetic relationships ($F_{crit} = 9.95$; $P = 0.29$).

Traditional analyses of co-variance indicate no differences in digit length between species utilizing different habitats (Table 2). In contrast, differences in the lengths of tendons of digits 3–5 were significant (Table 2) with sand-dwelling species having the shortest tendons and arboreal species having the longest tendons for their body size. Terrestrial and saxicolous species were intermediate. However, when taking into account the phylogenetic relationships between species, none of these differences remained significant (Table 2). An analysis of co-variance on the length of the *flexor carpi ulnaris* muscle indicated no differences between habitat groups in either traditional ($F_{3,46} = 0.01$; $P = 0.99$) or phylogenetically informed analyses ($F_{crit} = 9.81$; $P = 1$).

Table 2 Results of ANCOVAS with snout-vent length as co-variate testing for differences between habitat groups in tendon and digit length

	d.f.	F	P	F_{phyl}	P_{phyl}
Digit I	1,46	0.28	0.84	9.22	0.96
Digit II	1,46	1.73	0.18	9.6	0.67
Digit III	1,46	2.27	0.09	9.2	0.55
digit IV	1,46	2.30	0.09	9.71	0.54
Digit V	1,46	1.54	0.22	9.62	0.71
Tendon DI	1,46	1.21	0.32	9.64	0.77
Tendon DII	1,46	1.19	0.33	9.40	0.78
Tendon DIII	1,46	3.22	0.031	9.46	0.54
Tendon DIV	1,46	4.18	0.011	9.94	0.31
Tendon DV	1,46	4.78	0.006	9.47	0.25

D, digit; d.f., degrees of freedom.

Analyses of co-variance testing for differences in tendon length correcting for differences in digit length indicated significant differences between species occupying different

habitats for the tendons of all digits except digit 2 (Table 3). Again, sand-dwelling species had the shortest and arboreal species the longest tendons for a given digit length with terrestrial and saxicolous species being intermediate. However, none of these differences remained significant after taking into account the relationships between species (all $P > 0.05$).

Phylogenetic signal and habitat clustering

Our calculations indicate significant phylogenetic signal in the data ($K > 1$) for all traits with the exception of the length of digit one (Table 4). Moreover, our ancestral character state reconstructions indicate only few transitions between habitat types within clades (Fig. 3). This suggests that clades have radiated within specific habitats while retaining their morphology, especially among *Liolaemus* lizards.

Discussion

The different morphological patterns of the palmar tendon in lizards described here are modified versions of the previously described L-pattern (Moro and Abdala 2004; Abdala *et al.* 2009). The P-pattern of *Anolis* or *Polychrus*, however, represents a distinguishable shift to independence in the digital flexor tendons (and a concomitant reduction or absence of

Table 3 Results of ANCOVAS testing for differences in tendon length between habitat groups with respective digit lengths as covariates

	d.f.	F	P	F_{phyl}	P_{phyl}
Tendon DI	1,46	4.39	0.008	9.23	0.26
Tendon DII	1,46	1.56	0.21	8.80	0.69
Tendon DIII	1,46	2.73	0.055	9.66	0.47
Tendon DIV	1,46	3.45	0.024	8.90	0.39
Tendon DV	1,46	4.81	0.005	9.39	0.25

D, digit; d.f., degrees of freedom.

Table 4 Results of the calculation of phylogenetic signal

	K	P
Length digit 1	0.705	0.051
Length digit 2	2.353	<0.0001
Length digit 3	2.058	<0.0001
Length digit 4	2.341	<0.0001
Length digit 5	1.472	<0.0001
Length tendon digit 1	1.932	<0.0001
Length tendon digit 2	2.385	<0.0001
Length tendon digit 3	2.461	<0.0001
Length tendon digit 4	2.444	<0.0001
Length tendon digit 5	1.565	<0.0001
Length flexor muscle	2.83	<0.0001
Snout-vent length	1.887	<0.0001

the palmar tendon, i.e. the flexor plate) relative to the L-pattern of most species of *Liolaemus* and *Iguana*. Minimal or absent tendon independence results in a more rigid palmar structure, which may be beneficial during terrestrial locomotion, as it may allow the animal to effectively use its entire hand as a pivot (Abdala *et al.* 2009).

Interestingly, the distribution of the L-pattern across the different species indicates that almost all modifications of this pattern are present in taxa considered as arboreal (Fig. 3). Yet, some climbing taxa, such as *Iguana iguana*, *Urostrophus gallardoi*, and *Liolaemus pictus*, have palmar tendons corresponding to the conventional, relatively rigid, L-pattern (Fig. 4A). These results reject our hypothesis that arboreal lizards possess only independent flexor tendons and illustrate how climbing lizards can have any of the previously described arrangements of palmar tendons. However, most of the arboreal groups were characterized by having a narrow flexor plate and long digital flexor tendons, e.g. *Tropidurus plica* (Fig. 4G). Here, we propose that at least part of this incongruence may be linked to the definition of what an arboreal habitat actually is and suggest that perch diameter should be considered when defining arboreal habitat categories. Abdala *et al.* (2009), for example, showed that tendon independence improves the capacity to flex the hand in lizards, allowing them to move effectively on narrow substrates (i.e. perches of small diameter). Although often overlooked in ecomorphological analysis, perch diameter has been used extensively in defining *Anolis* ecomorphs (Collette 1961; Losos and Sinervo 1989; Losos 1990a,b), indicating the importance of this structural habitat variable. If we subcategorize the arboreal habitat into one comprising large branches and the other narrow ones, our data would support most of our hypotheses. In this case, *Iguana iguana*, *Urostrophus gallardoi*, *Liolaemus pictus*, *L. tenuis*, *Tropidurus plica*, and *T. oreadicus* would be ranked in the first subcategory comprised of animals utilizing principally wide branches, while lizard species possessing a P-pattern (more elongate, independent palmar flexor tendons) would be ranked in the second subcategory (Fig. 3). Interestingly, most tropidurids posses a flexor plate with a marginal sesamoid bone embedded and some degree of independence of the tendons, a morphology that we suggest may allow them to utilize different habitats. For example, whereas different populations of *Tropidurus etheridgei* have specialized in occupying different microhabitats (Cruz *et al.* 1998), no differences can be observed in their hand tendon morphology (MJT and VA personal observation.).

Despite the significant differences in tendon length observed among habitat groups in the traditional analyses, phylogenetically informed analyses indicated that these cannot be interpreted as being adaptive (Table 2). Indeed, these analyses indicated a very strong effect of phylogeny on the distribution of the characters, leading to significant phylogenetic clustering (Vanhooydonck and Van Damme 1999; see Fig. 3) and thus reducing our power to detect differences. Interestingly, Grizante *et al.* (2010) also found significant

phylogenetic signal in some forelimb traits in their analysis of limb morphology in tropidurid lizards. More generally, lizard anatomical structures appear to be rather conservative in many clades of Neotropical lizards such as Liolaemini. Indeed, lizards of the genus *Liolaemus* display far less morphological variation when compared with members of the highly species-rich *Anolis* clade (Medel *et al.* 1988; Tulli *et al.* 2009). Interestingly, with respect to tendon morphology, our sample of *Anolis* lizards is as conservative as the *Liolaemus* lizards examined. This is particularly noticeable because this lizard group has always been reported as having a morphology that varies strikingly in relation to habitat use (Collette 1961; Losos 1990a,b,c; Losos 2009; Irschick *et al.* 1997). However, it should also be noted that our sample does not encompass the entire morphological and ecological variability known to exist in *Anolis* lizards (e.g. we did not include any twig anoles in our analysis that are known to be divergent from other *Anolis* ecomorphs), and thus we may not be capturing some of the variation in the group.

Our data suggest that *Liolaemus* lizards retain an all-purpose morphology that allows them to use a variety of habitats (see also Medel *et al.* 1988). This all-purpose morphology may also lie at the basis of the over-arching effect of phylogeny observed in our analyses. Indeed, not only did our analyses detect significant phylogenetic signal in the data set, ancestral character state reconstructions indicate that habitat use is strongly nested within the phylogeny with only a limited number of transitions between habitat types within clades. Possibly, an analysis comparing similar morphometric traits across lizard families or even across different groups of tetrapods may provide more insights into the adaptive nature of variation in the length of the tendons of the distal forelimb. Alternatively, our data may simply reflect varying levels of constraint on the evolution of internal structures such as tendons versus the evolution of limb dimensions often documented in animals such as *Anolis* lizards (Collette 1961; Losos 1990a,b,c, 2009; Irschick *et al.* 1997). In summary, our data suggest that in contrast to what is observed for mammals (McNeill Alexander 1984; Abdala *et al.* 2006), tendon length is determined more by phylogeny than by habitat use at least in neotropical iguanian lizards.

Acknowledgements

Supporting Information

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

Appendix S1. Institutional Abbreviations.
Table S1. Morphological measurements of the tendon of the forelimb reported as mean ± SE. All measurements in mm unless otherwise indicated.

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References

Abdala, C. S. and Díaz Gómez, J. M. 2006. A new species of the Liolaemus darwinii group (Iguania: Liolaemidae) from Catamarca Province, Argentina. *Zootaxa* **1317**: 21–33.

Abdala, C. S. 2007. Phylogeny of the boulengeri group (Iguania: Liolaemidae, *Liolaemus*) based on morphological and molecular characters. – *Zootaxa* **1538**: 1–84.

Abdala, V. and Moro, S. 2006. Comparative myology of the forelimb of *Liolaemus* sand lizards (Liolaemidae). – *Acta Zoologica (Stockholm)* **87**: 1–12.

Abdala, C. S. and Quinteros, S. 2008. Una nueva especie de *Liolaemus* (Iguania: Liolaemidae) endémica de la Sierra de Fiambalá, Catamarca, Argentina. – *Cuadernos de Herpetología* **22**: 35–47.

Abdala, V., Moro, S. and Flores, D. 2006. The flexor tendons in the didelphid manus. – *Mastozoología Neotropical* **13**: 193–204.

Abdala, V., Manzano, A. S. and Herrel, A. 2008. The distal forelimb musculature in aquatic and terrestrial turtles: phylogeny or environmental constraints? – *Journal of Anatomy* **213**: 159–172.

Abdala, V., Manzano, A. S., Tulli, M. J. and Herrel, A. 2009. The tendinous patterns in the palmar surface of the lizard manus: functional consequences for grasping ability. – *The Anatomical Record* **292**: 242–253.

Arnold, E. N. 1998. Structural niche, limb morphology and locomotion in lacertid lizards (Squamata, Lacertidae); a preliminary survey. – *Bulletin British Museum (Natural History) Zoology* **64**: 63–89.

Autumn, K., Hsieh, S. T., Dudek, D. M., Chen, J., Chitaphan, C. and Full, R. J. 2006. Dynamics of geckos running vertically. – *Journal of Experimental Biology* **209**: 260–272.

Ávila-Pires, T. C. S. 1995. Lizards of Brazilian Amazonia. *Zoologische Verhandelingen* **299**: 1–706.

Avila, L. J., Morando, M., Perez, C. H. F. and Sites, J. W. Jr 2004. Phylogenetic relationships of lizards of the *Liolaemus petrophilus* group (Squamata, Liolaemidae), with description of two new species from western Argentina. – *Herpetologica* **60**: 187–203.

Benjamin, M., Evans, E. J. and Copp, L. 1986. The histology of tendon attachments to bone in man. – *Journal of Anatomy* **149**: 89–100.

Benjamin, M., Newell, R. L. M., Evans, E. J., Ralphs, J. R. and Pemberton, D. J. 1992. The structure of the insertions of the tendons of biceps brachii, triceps and brachialis in elderly dissected room cadavers. – *Journal of Anatomy* **180**: 327–332.

Benjamin, M., Kaiser, E. and Milz, S. 2008. Structure–function relationships in tendons: a review. – *Journal of Anatomy* **212**: 211–228.

Blomberg, S. P., Garland, T., Jr. and Ives, A. R. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57: 717–745.

Bobbert, M. 2001. Dependence of human squat jump performance on the series elastic compliance of the triceps surae: a simulation study. – *Journal of Experimental Biology* 204: 533–542.

Bonine, K. E. and Garland, T. Jr. 1999. Sprint performance of phrynosomatid lizards, measured on a high-speed treadmill, correlates with hindlimb length. – *Journal of Zoology (London)* 248: 255–265.

Carrillo de Espinoza, N., Rothenstein, D., Salas, A. and Werner, Y. L. 1990.Radiation and convergence among desert geckos: *Phyllodactylus* species resembling both *Ptyodactylus* and *Stenodactylus*. – *Amphibia-Reptilia* 11: 275–284.

Carter, D. R., Mikić, B. and Padian, K. 1998. Epigenetic mechanical factors in the evolution of long bones epiphyses. – *Zoological Journal of the Linnean Society* 123: 163–178.

Cartmill, M. 1985. Climbing. In: Hildebrand, M., Bramble, D. M., Liem, K. F. and Wake, D. B. (Eds): *Functional Vertebrate Morphology*, pp. 73–88. Belknap Press, Cambridge.

Cei, J. M. 1986. *Reptiles del Centro, Centro-oeste y Sur de la Argentina. Herpetofauna de las Zonas Áridas y Semiáridas*. Monografia 4, Museo Regionali Scienci Naturali, Torino, Italy.

Cei, J. M. 1993. *Reptiles del Noroeste, Nordeste y Este de la Argentina*. Museo Regionale di Scienze Naturali, Torino, Monografie, **XIV**: pp. 949.

Cei, J. M., Scolaro, J. A. and Videla, F. 2003. A taxonomic revision of recognized argentine species of the leiosaurid genus *Diplolaemus* (Reptilia, Squamata,Leiosauridae). – *Facena* 19: 87–106.

Collette, B. B. 1961.Correlation between ecology and morphology in anoline lizards of Havana, Cuba and southern of Florida. – *Bulletin of the Museum of Comparative Zoology* 125: 137–162.

Colli, G. R., Araujo, A. F. B., Silveira, R. and Roma, F. 1992. Niche partitioning and morphology of two syntopic *Tropidurus* (Sauria: Tropiduridae) in Mato Grosso, Brazil. – *Journal of Herpetology* 26: 66–6.

Cruz, F. B., Silva, S. and Scrocchi, G. J. 1998. Ecology of the lizard *Tropidurus etheridgei* (Squamata: Tropiduridae) from the dry Chaco of Salta, Argentina. – *Herpetological Natural History* 6: 23–31.

Etheridge, R. E. 2000. A review of the *Liolaemus wiegmannii* group (Squamata, Iguania, Tropiduridae), and a history of morphological change in the sand-dwelling species. – *Herpetological Monographs* 14: 293–352.

Frost, D. R. 1992. Phylogenetic analysis and taxonomy of the *Tropidurus* group of lizards (Iguania: Tropiduridae). – *American Museum Novitates* 3033: 1–68.

Frost, D. R., Etheridge, R. E., Janies, D. and Titus, T. A. 2001a. Total evidence, sequence alignment, evolution of polychrotid lizards and a reclassification of the Iguania (Squamata, Iguania). – *American Museum Novitates* 3343: 1–38.

Frost, D. R., Rodrigues, M. T., Grant, T. and Titus, T. A. 2001b. Phylogenetics of the lizard genus *Tropidurus* (Squamata: Tropiduridae: Tropidurinae): direct optimization, descriptive efficiency, and sensitivity analysis of congruence between molecular data and morphology. – *Molecular Phylogenetics and Evolution* 21: 352–371.

Garland, T., Jr., Harvey, P. H. and Ives, A. R. 1992. Procedures for the analysis of comparative data using phylogenetic independent contrasts. *Systematic Biology* 41: 18–32.

Garland, . T. Jr and Losos, J. B. 1994. Ecological morphology of locomotor performance in squamate reptiles. In: Wainwright, P. C. and Reilly, S. M., (Eds): *Ecological Morphology: Integrative Organismal Biology*, pp. 240–302. University Chicago Press, Chicago.

Garland, T. Jr, Dickerman, A. W., Janis, C. M. and Jones, J. A. 1993. Phylogenetic analysis of covariance by computer simulation. – *Systematic Biology* 42: 265–292.

Garland, T. Jr, Midford, P. E. and Ives, A. R. 1999. An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral values. – *American Zoologist* 39: 374–388.

Genise, J. F. and Montanelli, S. B. 1991. Primer hallazgo de Hemidactylus mabouia (Moreau de Jones) en la Argentina. *Boletín de la Asociación Herpetológica Argentina* 7: 23.

Goodman, B. 2006. *Ecomorphology, Microhabitat Use, Performance and Reproductive Output in Tropical Lygosomine lizards*. PhD Thesis, James Cook University, Queensland.

Grizante, M. B., Navas, C. A., Garland, T. Jr and Kohlsdorf, T. 2010. Morphological evolution in Tropidurinae squamates: an integrated view along a continuum of ecological settings. – *Journal of Evolutionary Biology* 3: 98–111.

Haines, R. W. 1950. The flexor muscles of the forearm and hand in lizards and mammals. – *Journal of Anatomy* 84: 13–29.

Halloy, M., Etheridge, R. and Burghardt, G. M. 1998. To bury in sand: phylogenetic relationships among lizard species of the boulengeri group, *Liolaemus* (Reptilia: Squamata: Tropiduridae), based on behavioral characters. – *Herpetological Monographs* 12: 1–37.

Hirth, H. F. 1963. Some aspects of the natural history of Iguana iguana on a tropical strand. – *Ecology* 44: 613–615.

Irschick, D. J., Vitt, L., Zani, P. and Losos, J. B. 1997. A comparison of evolutionary radiations in mainland and Caribbean *Anolis* lizards. – *Ecology* 78: 2191–2203.

Irschick, D. J., Macrini, T. E., Koruba, S. and Forman, J. 2000. Ontogenetic differences in morphology, habitat use, behaviour, and sprinting capacity in two West Indian *Anolis* lizards. – *Journal of Herpetology* 34: 444–451.

Laspiur, A., Acosta, J. C. and Abdala, C. S. 2007. A new species of *Leiosaurus* (Iguania: Leiosauridae) from central-western Argentina. – *Zootaxa* 1470: 47–57.

Lobo, F. and Quinteros, S. 2005. A morphology-based phylogeny of *Phymaturus* (Iguania: Liolaemidae) with the description of four new species from Argentina. – *Papeis Avulsos Zoologia (São Paulo)* 45: 143–177.

Losos, J. B. 1990a. The evolution of form and function: morphology and locomotor performance in west indian anolis lizards. – *Evolution* 44: 1189–1203.

Losos, J. B. 1990b. Ecomorphology, performance capability and scaling of West Indian *Anolis* lizards: an evolutionary analysis. – *Ecological Monographs* 60: 369–388.

Losos, J. B. 1990c. Concordant evolution of locomotor behaviour, display rate and morphology in *Anolis* Lizards. – *Animal Behaviour* 39: 879–890.

Losos, J. B. 2009. *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation in Anoles*. California University Press, Berkeley.

Losos, J. B. and Sinervo, B. 1989. The effects of morphology and perch diameter on sprint performance in *Anolis* lizards. – *Journal of Experimental Biology* 145: 23–30.

Lundelius., E. Jr 1957. Skeletal adaptations in two species of *Sceloporus*. – *Evolution* 11: 65–83.

Maddison, W. P. and Maddison, D. R. 2009. Mesquite: a modular system for evolutionary analysis. Version 2.6. Available at: http://mesquiteproject.org.

Mc Elroy, J. 2008. *The Functional Morphology of Lizard Locomotion: Integrating Biomechanics, Kinematics, Morphology, and Behavior*. PhD Thesis, University of Ohio.

McNeill Alexander, R. 1984. Elastic energy stored in running vertebrates. – *American Zoologist* 24: 85–94.

Medel, R. G., Marquet, P. A. and Jaksic, F. M. 1988. Microhabitat shifts of lizards under different contexts of sympatry: a case study with South American *Liolaemus*. – *Oecología* **76**: 567–569.

Morando, M., Avila, L. J., Baker, J. and Sites, J. W. Jr 2004. Phylogeny and phylogeography of the *Liolaemus darwinii* complex (Squamata: Liolaemidae): evidence for introgression and incomplete lineage sorting. – *Evolution* **58**: 842–861.

Moro, S. and Abdala, V. 2004. Análisis descriptivo de la miología flexora y extensora del miembro anterior de *Polychrus acutirostris* (Squamata, Polychrotidae). – *Papeis Avulsos Zoologia (São Paulo)* **44**: 81–89.

Nicholson, K. E. 2002. Phylogenetic analysis and a test of the current infrageneric classification of Norops (beta *Anolis*). – *Herpetological Monographs* **16**: 93–120.

Pereyra, E. A. 1985. Nuevo iguánido del género Phymaturus del noroeste Argentino. *Boletín de la Asociación Herpetológica Argentina* **2**: 4.

Pincheira-Donoso, D., Scolaro, J. A. and Schulte, J. A. II 2007. The limits of polymorphism in Liolaemus rothi: Molecular and phenotypic evidence for a new species of the *Liolaemus boulengeri* clade (Iguanidae, Liolaemini) from boreal Patagonia of Chile. – *Zootaxa* **1452**: 25–42.

Russell, A. P. and Bauer, A. M. 1989. The morphology of the digits of the golden gecko, *Calodactylodes aureus* and its implications for the occupation of rupicolous habitats. – *Amphibia-Reptilia* **10**: 125–140.

Schwartz, A. and Henderson, R. W. 1991. *Amphibians and Reptiles of the West Indies: Descriptions, Distributions, and Natural History*. Florida Univ. Press, Gainesville, pp. 720.

Scolaro, J. A. 2005. *Reptiles patagónicos sur. Guía de campo*. Univ. Nac. Patagonia Ed, Trelew.

Talbot, J. J. 1979. Time Budget, niche overlap, inter- and intraspecific aggression in *Anolis humilis* and *A. limifrons* from Costa Rica. – *Copeia* **3**: 472–481.

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

Vanhooydonck, B. and Van Damme, R. 1999. Evolutionary relationships between body shape and habitat use in lacertid lizards. – *Evolutionary Ecology Research* **1**: 785–805.

Vanhooydonck, B. and Van Damme, R. 2001. Evolutionary trade-offs in locomotor capacities in lacertid lizards: are splendid sprinters clumsy climbers?. – *Journal of Evolutionary Biology* **14**: 46–54.

Vitt, L. J. and Colli, G. R. 1994. Geographical ecology of a Neotropical lizard: Ameiva ameiva (Teiidae) in Brazil. *Canadian Journal of Zoology* **72**: 1986–2008.

Vrcibradic, D. and Rocha, C. F. D. 1996. Ecological differences in tropical sympatric skinks (*Mabuya macrorhyncha* and *Mabuya agilis*) in southeastern Brazil. – *Journal of Herpetology* **30**: 60–67.

Vrcibradic, D. and Rocha, C. F. D. 1998. The ecology of the skink Mabuya frenata in an area of rock outcrops in Southeastern Brazil. *Journal of Herpetology* **32**: 229–237.

Zaaf, A., Van Damme, R., Herrel, A. and Aerts, P. 2001a. Spatio-temporal gait characteristics of level and vertical locomotion in a ground-dwelling and a climbing gecko. – *Journal of Experimental Biology* **204**: 1233–1246.

Zaaf, A., Van Damme, R., Herrel, A. and Aerts, P. 2001b. Limb joint kinematics during vertical climbing and level running in a specialist climber: *Gekko gecko* Linneus, 1758 (Lacertilia: Gekkonidae). – *Belgian Journal of Zoology* **131**: 173–182.

Zani, P. A. 2000. The comparative evolution of lizard claw and toe morphology and clinging performance. – *Journal of Evolutionary Biology* **13**: 316–325.

Zar, J. H. 1999. *Biostatistical Analysis*. Prentice Hall, Upper Sadle River.