

Article

Anuran forelimb muscle tendinous structures and their relationship with locomotor modes and habitat use

Silvia DE OLIVEIRA-LAGÔA^a, Félix B. CRUZ^{B,*}, Débora L. Moreno AzóCAR^b, Esteban O. LAVILLA^c, and Virginia ABDALA^{D,*}

^aFacultad de Ciencias Exactas y Naturales - Universidad Nacional de Asunción, San Lorenzo, Paraguay, ^bInstituto de Investigaciones en Biodiversidad y Medioambiente INIBIOMA (CONICET-UNCOMA) Quintral Bariloche, Rio Negro, Argentina, ^cInstituto de Herpetología, UEL (Fundación Miguel Lillo - CONICET), Tucumán, Argentina, and ^dInstituto de Biodiversidad Neotropical (UNT-CONICET) Horco Molle s/n Yerba Buena, Tucumán. Cátedra de Biología General, Facultad de Ciencias Naturales, UNT, Tucumán, Argentina

*Address correspondence to Félix B Cruz and Virginia Abdala. E-mail: felix.cruz@crub.uncoma.edu.ar; virginia@webmail.unt.edu.ar.

Handling Editor: Rudiger Riesch

Received on 30 June 2018; accepted on 11 November 2018

Abstract

The interaction between organisms and their environment is central in functional morphology. Differences in habitat usage may imply divergent morphology of locomotor systems; thus, detecting which morphological traits are conservative across lineages and which ones vary under environmental pressure is important in evolutionary studies. We studied internal and external morphology in 28 species of Neotropical anurans. Our aim was to determine if internal morphology (muscle and tendons) shows lower phylogenetic signal than external morphology. In addition, we wanted to know if morphology varies in relation to the habitat use and if there are different functional groups. We found differences in the degree of phylogenetic signal on the groups of traits. Interestingly, postaxial regions of the forelimb are evolutionarily more labile than the preaxial regions. Phylomorphospace plots show that arboreal (jumpers and graspers) and swimmer frogs cluster based on length of fingers and the lack of sesamoid, also reflected by the use of habitat. These functional clusters are also related to phylogeny. Sesamoid and flexor plate dimensions together with digit tendons showed to be important to discriminate functional groups as well as use of habitat classification. Our results allow us to identify a “grasping syndrome” in the hand of these frogs, where palmar sesamoid and flexor plate are absent and a third metacarpal with a bony knob are typical. Thus, a lighter skeleton, long fingers and a prensile hand may be key for arboreality.

Key words: comparative analyses, ecomorphology, neotropical frogs, function, habits, phylogeny

How an organism interacts with its environments has important implications for the selective forces shaping the phenotypes of the species. The potential association between morphology and functional performance at different levels (individual, population and species) may yield differences in fitness providing the raw material

for natural selection to act upon (Arnold 1983; Kingsolver and Huey 2003). In this sense, the relationship between morphology, locomotor performance and habitat use is one of the most studied aspects of evolutionary phenotypic variation (Losos and Sinervo 1989; Losos 1990a, 1990b; Bonine and Garland 1999; Van Damme

and Vanhooydonck 2002; Goodman et al. 2008; Irschick et al. 2008). It was observed that differences in habitat occupation underlie divergent evolution of the morphology of locomotor systems in numerous taxa (Irschick et al. 2005; Calsbeek and Irschick 2007). Among the studies on this issue, research on reptiles certainly outnumber those in other groups; for example, many studies have investigated the relationship between limb morphology and habitat use in *Anolis*, liolaemids and tropidurine lizards (Losos 1990a, 1990b, 1990c; Kohlsdorf et al. 2001; Irschick et al. 2005; Calsbeek and Irschick 2007; Grizante et al. 2010; Tulli et al. 2009, 2011, 2012a, 2012b). However, these associations are not always clear (Tulli et al. 2012b, 2016). In a broad evolutionary context, convergent evolution of traits in species from different regions can lead to similar body shape among them, although they could be phylogenetically unrelated (Donley et al. 2004; Moen et al. 2013). Alternatively, despite the dispersal of lineages, relevant ecological traits may be conserved during and after separation (Losos, 1990a, 1990b, 1990c; Stephens and Wiens 2004; Moen et al. 2013). For these reasons, detecting which morphological traits are conserved across lineages and which ones vary under environmental pressure is an important aspect of evolutionary studies.

Several studies made interesting observations on the variation of muscles across different taxonomic groups of amphibians (Dunlap 1960; Davies and Burton 1982; Burton 1983, 1996a, 1996b, 1998, 2001, 2004; Liem 1970; Manzano and Lavilla 1995; Manzano 2000; Faivovich 2002; Manzano et al. 2008; Salgar et al. 2009; Hoyos et al. 2014; Hoyos and Salgar 2016; Blotto et al. 2017). In addition, it has been observed that in the palmar surface of the hand of some lizard and anuran taxa, the flexor tendon connecting the forearm muscles with the digits present an embedded palmar sesamoid (Abdala et al. 2009; Ponssa et al. 2010) that prevents the palmar flexion of the hand and consequently restricts its movement (Abdala et al. 2009; Sustaita et al. 2013; Fontanarrosa and Abdala 2014, 2016). Some studies on the palmar sesamoid and myotendinous structures in Squamata (Haines 1950; Moro and Abdala 2004; Abdala et al. 2009; Tulli et al. 2012b; Fontanarrosa and Abdala 2014, 2016), marsupials (Abdala et al. 2006) and placental mammals (Carrizo et al. 2014) showed that these structures can be associated with ecological aspects of the studied animals, such as habitat use or locomotor modes (Abdala et al. 2006; Tulli et al. 2012b; Carrizo et al. 2014).

Anuran morphology has been studied in association with locomotor mode in several cases (Rand 1952; Zug 1972, 1978; Emerson 1978, 1988; Gomes et al. 2009; Jorgensen and Reilly 2013; Vidal-García et al. 2014). The remarkable abilities of frogs to swim, hop, walk, climb, dig and even glide allow them to occur in almost all available environments and make them an interesting group to highlight relationships between morphology and ecology (Soliz and Ponssa 2016; Soliz et al. 2017). In the past, hind limb morphology has been studied in relation to the mentioned locomotor modes, because of the biomechanical role of the limbs as the propulsive agent (Zug 1972). As such, strong jumpers are known to exhibit proportionately longer hind limbs and longer tibio-fibulae (Zug 1972; Emerson 1978, 1985). Comparatively, variations in the forelimb anatomy of frogs has received less attention (but see Gillis et al. 2014).

Here, we studied the comparative anatomy of the forelimb in 28 species of Neotropical anurans focusing on the muscle-tendinous system. We postulate that muscle-tendinous structures can also reflect the evolutionary history of a group, acting as anatomical descriptors and showing adaptive changes to lifestyle (Burton 1998). Considering the relevance of the palmar sesamoid in the manual abilities of tetrapods (Abdala et al. 2009; Sustaita et al. 2013;

Fontanarrosa and Abdala 2014, 2016), we also included this structure in our study. We test whether the external and muscle-tendinous morphologies of anuran forelimbs differ among taxa that exhibit different locomotor modes or habitat use. We also test whether such patterns can be explained solely by the phylogenetic relationships between the species examined or by another underlying factor. It should be noted that the muscle-tendinous structures have been seldom considered in ecomorphological studies of vertebrates (Abdala et al. 2008, 2014; Tulli et al. 2012b; Fabrezi et al. 2014; Carrizo et al. 2014; Fratani et al. 2018). We hypothesize that: a) internal morphology (muscle and tendons) will show a higher phylogenetic signal pattern than external morphology as was shown in previous studies on other tetrapod taxa (Tulli et al. 2012a; Carrizo et al. 2014); b) morphological traits of terrestrial species will differ from arboreal ones because climbing ability is associated with the position of the center of gravity; thus, arboreal species need different morphological arrangements to compensate the potential problem of a displaced center of gravity (Cartmill 1985; Tulli et al. 2009) and c) arboreal graspers and jumpers will show different morphology compared with walker jumpers and swimmer frogs that tend to exhibit a morphological continuum (Soliz et al. 2017). Specifically, we predict that the grasping anurans will exhibit a “grasping syndrome” as described by Fontanarrosa and Abdala (2016).

Material and Methods

We dissected the forearm and manus of 156 adult specimens belonging to 28 species of 7 anuran families: *Bufo*idae, *Leptodactylidae*, *Hylidae*, *Phyllomedusidae*, *Telmatobiidae*, *Odontophrynidae*, and *Microhylidae* (Figure 1, Supplementary Material) that occur in Northwestern Argentina, Paraguay, South of Bolivia and Brazil. The choice of the species aimed to maximize representation of habitat use and locomotor modes in the sample of species occurring in similar habitats, such as Chaco (arid, semiarid and wet) and Monte (Cabrera and Willink 1980). In addition, we aimed for a balanced phylogenetic representation of different clades within each studied family, with the exception of one *Telmatobius* species. Sample sizes for each species ranged from 3 to 9 specimens according to availability (Supplementary Material). Because sample size might be small and variable, we measured the variation coefficient (VC, given in percentage) for snout-vent length within each species (as this is the most variable trait) obtaining variation coefficients from 3% to 11% (mean VC for the entire sample was 6.17% and set point 4.24–8.87%). A broad intraspecific variation may be an important source of uncertainty that can influence our results and may lead to misinformed conclusions (Garamszegi and Møller 2010); however, our data did not show a broad dispersion. For each individual we made dissections of the palmar sesamoid and myological and tendinous traits related to the forelimb and manus under a binocular microscope (Nikon SMZ645), for details see Abdala et al. (2006, 2008). Muscle-tendinous variables were measured with a digital caliper (Mitutoyo CD-15B; ± 0.01 mm, Japan). Species means and the number of individuals per species used are included as supporting information (Supplementary Material).

Following the protocol of Abdala et al. (2006), Tulli et al. (2012b), and Carrizo et al. (2014), we studied the variability between muscle and tendon dimensions of the structures directly implicated in manual burrowing, grasping, walking, jumping, and swimming. External characters are shown in Figure 2A, B; muscles and tendons measured are shown in Figure 2C, D. All of the muscles analyzed exhibit a parallel-fibered arrangement. In addition to

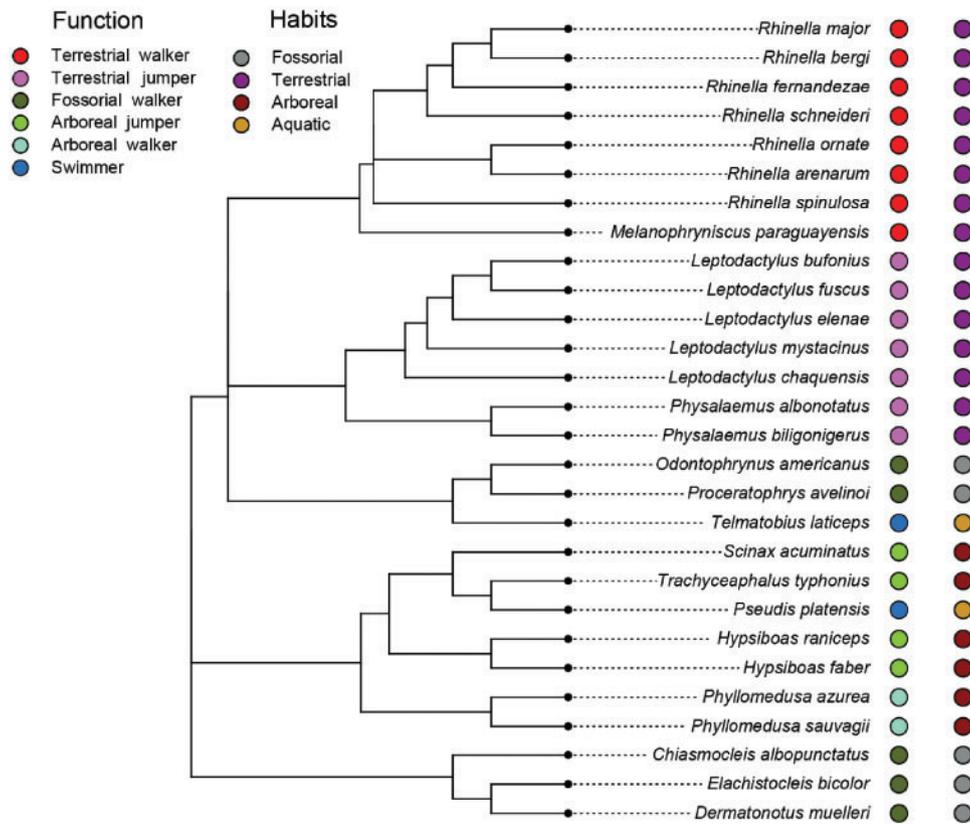


Figure 1. Composite tree of the species studied here. Colored circles on the right show the functional groups (left) and habits categories (right) these species have.

length, maximum width of muscles was also recorded to obtain an estimate of the morphometric variation of each muscle as a whole to allow testing if there exists correlation between aponeurosis and tendon dimensions that are associated with these muscles.

The different categories of habitat use and locomotor modes were considered after [Ceï \(1980\)](#), [Wells \(2007\)](#), [Jorgensen and Reilly \(2013\)](#) and personal observations. Therefore, we obtained 4 habitat use categories (fossorial, terrestrial, arboreal and aquatic), and 6 locomotor mode categories (terrestrial walker, terrestrial jumper, terrestrial burrower, arboreal jumper, arboreal grasper-walker and swimmer; [Figure 1](#)).

Because species have a shared history ([Figure 1](#)), data of traits corresponding to these species cannot be considered as independent data-points ([Harvey and Pagel 1991](#)) and phylogenetically informed statistical analyses are required. For this purpose, we used a composite tree of the phylogenetic relationship based on [Pyron and Wiens \(2011\)](#) tree and because branch lengths (BL) were not available, we used arbitrary branch lengths (e.g., all BL equal to one, BL transformed with Grafen method and BL transformed with Pagel method). We tested the adequacy of each one of these 3 arbitrary branch lengths following [Garland et al. \(1992\)](#), by plotting the absolute value of each standardized independent contrast versus its standard deviation. We used Mesquite v2.74 ([Maddison and Maddison 2015](#)) and the PDAP PDTREE v1.15 modules ([Midford et al. 2009](#)) and searched for significant differences. After plotting trees and BL for all traits, we found that the tree with BL equal to one was the more adequate arrangement (BL = 1 showed 4 out of 26 significant plots, while Pagel BL transformation showed 8/26 and Grafen 11/26 significant relationships).

Mean values of all morphological variables ([Supporting Information Supplementary Material](#)) were \log_{10} transformed for further analyses. We then tested the data for phylogenetic signal (i.e., the consistency in trait values with the phylogeny); for this purpose, we used the Pagel's λ value estimator for each variable ([Pagel 1999](#)) that varies between 0 and 1, where 0 means no phylogenetic signal and 1 means that a variable is highly dependent of the phylogenetic structure. In addition, a randomization analysis was performed for each variable to determine the probability of the estimator.

As the morphological variables studied here showed to be highly correlated with body size ($r^2 > 0.8$), and to consider the phylogenetic context of this study, we proceeded with phylogenetic size correction ([Revell 2009](#)). Through this, we obtained the residuals of each target morphological trait (least squares regression analysis trait vs. body size - snout-vent length, SVL) while simultaneously controlling for phylogenetic independence. Then, we used the residuals in subsequent analyses as size-corrected morphological measurements.

We studied 23 different morphological variables ([Supplementary Material, Table S2](#)). Since this amount of information is difficult to analyze, we reduced the number of variables by running a phylogenetically based principal component analysis (phyl.pca). We collected the scores of each principal component from the phylogenetically informed PCA. We considered the first 4 principal components that attained for 84% of the accumulated variance of the total analyses. We also plotted the phylomorphospace and the contribution of all morphological variables; for this descriptive purpose, we used the command phylomorphospace from the program Phytools ([Revell 2012](#)).

We tested if internal or external variables showed variability in relation to the 6 functional groups considered here (fossorial walkers, terrestrial walkers, terrestrial jumpers, arboreal jumpers, arboreal walker or graspers and aquatic swimmers) as well as for the type of habitats these frogs use (e.g., burrows, terrestrial, arboreal and aquatic). In this case we used phylogenetic MANOVA by running Geiger program (Harmon et al. 2008), we concatenated the internal or external variables in relation to the different factors (functional groups or habitat types) as a formula to compute the analyses. Finally, for testing if there were differences in each one of the different morphological traits (in total 23 external and internal traits) among species as a function of their habitat use (aquatic, terrestrial, arboreal or fossorial) a phylogenetically based analysis of variance was performed (phylANOVA of Phytools, Revell 2012) with 1000 iterations. Similarly, we used the same 23 traits to determine whether there were differences due to the species' locomotor

modes; these analyses were conducted through a phylogenetically informed ANOVA too. All these analyses were performed including phylogenetic information through the following functions; *phylosig*, *phyl.resid*, *phyl.pca*, *phylomorphospace*, *phylANOVA* of the Phytools package version 6.0 (Revell 2012) in the open access environment R (Version 3.4.0, R Core team 2017).

Results

In all dissected specimens the *m. flexor digitorum communis* originates from the humerus distal condyle, through a wide and short tendon from the aponeurosis covering the elbow. The *m. flexor carpi ulnaris* originates on the medial epicondylus of the humerus, with some fibers connected to the *m. flexor digitorum communis*. All specimens present a superficial and a deep fascia that can be colored (Figure 2C, D). Bufonidae, Leptodactylidae, Telmatobiidae,

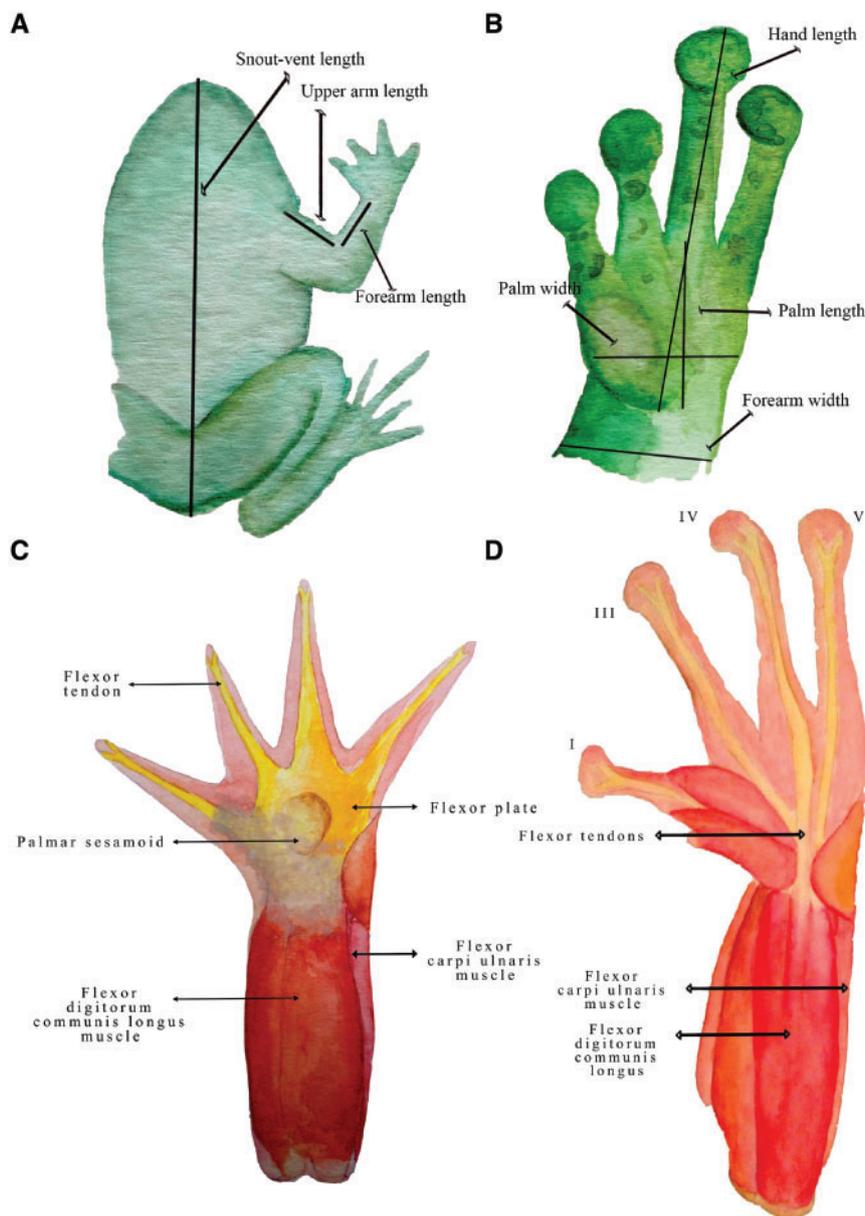


Figure 2. External characters. (A) body and arm external measurements, (B) manus external measurements (C) muscle and tendons showing sesamoid and flexor plate, (D) tendons and muscles where no flexor plate or sesamoid are present (drawings courtesy S. De Oliveira Lagôa).

Odontophryniidae, and most of Microhylidae present a flexor plate with an embedded palmar sesamoid, over which a palmar aponeurosis is distinguishable (Figure 2C); conversely, hylids, Phyllomedusidae and some microhylids lack a palmar sesamoid (Figure 2D). From the flexor plate originate the flexor tendons of digits III, IV, and V that insert onto the basal portion of the distal phalanx of each digit (Figure 2D).

In the case of bufonids, a tendinous sheet between the mm. flexor digitorum longus and mm. opponens of digit II are distinguishable. In leptodactylids, telmatobiids, and odontophryniids, there is an aponeurosis between the flexor plate and the muscles of digit II. In *Leptodactylus fuscus*, the flexor tendon of digit II is visible and originates from the flexor plate inserting into the basal portion of the distal phalanx. The tendon is covered by fascias and the mm. opponens and flexor digitorum II longus. Some microhylids, such as *Dermatonotus muelleri* present a flexor tendon of digit II. In Hylidae and Phyllomedusidae, the m. flexor digitorum communis is where the flexor tendons of digits III, IV and V originate, then they insert in the basal portion of the distal phalanx of each digit. The pennation angle is lower than 45° in all analyzed species.

Depending on the source of the data (external or internal morphology), we found different degrees of phylogenetic signal on the group of traits. Snout-vent length (SVL) showed no significant phylogenetic signal, whereas among the external morphology traits, only arm length showed a significant phylogenetic signal (Table 1). For the internal morphology traits (muscles and tendinous systems), 8 out of 16 traits showed significant phylogenetic signal based on the values of the λ estimate (Table 1). Finally, the distribution of species in different types of habitats and their locomotor modes also

showed high values of phylogenetic signal estimators (0.999, Table 1).

Phylogenetic PCA showed that the following variables contributed most (based on higher absolute eigenvalues) to the principal components functions (Table 2): sesamoid length, sesamoid width, digit IV and V tendon length and width (respectively), flexor carpi ulnaris tendon length and width (internal morphology variables; Table 2); similarly, forearm width and hand length show high loadings in the PCA (Table 2). In addition, phylomorphospace plots considering external and internal morphology characters show that all arboreal (jumpers and graspers) and one swimmer species cluster according to the length of finger tendons and sesamoid (Figure 3A), which is reflected by the use of habitat where aquatic (partially) and arboreal species clearly differ from terrestrial and burrower species (Figure 3B). These clusters are probably due to phylogeny, since Figure 3C shows the same pattern and also because of the high phylogenetic signal observed in the most informative traits of PC1 (see Table 2). With respect to the habitat use groups, both arboreal jumpers and walkers (Hylidae and Phyllomedusidae) are different from other functional groups in their flexor plates (Figure 3A). However, it must be taken into account that there is an important component of phylogeny in these results as the phylogenetic signal and phylomorphospace analyses show (see Table 1 and Figure 3C).

The phylogenetic MANOVAs with locomotor modes as factor revealed that, according to the Wilks' statistic, only internal traits differed in response to habitat use (Table 3). However, phylogenetic MANOVA of external traits showed significant differences when considering functional groups only (Table 3). The detailed phylogenetic ANOVA revealed that external traits do not show significant variation in any of the cases (functional groups or habitat types).

Table 1. Phylogenetic signal using Pagel's lambda estimate

	λ	P
External Measures		
Snout-vent length	0.152	0.653
Arm length	0.587	0.047
Forearm length	0.126	0.697
Forearm width	<0.001	1
Palm length	0.171	0.584
Palm width	0.272	0.375
Hand length	0.335	0.301
Internal Measures		
Sesamoid length	0.958	<0.001
Sesamoid width	0.931	<0.001
Flexor plate length	0.999	<0.001
Flexor plate width	0.999	<0.001
Finger III tendon length	0.682	0.042
Finger IV tendon length	0.694	0.026
Finger V tendon length	0.999	<0.002
Finger III tendon width	0.446	0.045
Finger IV tendon width	0.120	0.613
Finger V tendon width	0.291	0.663
Flexor carpi ulnaris muscle length	0.484	0.145
Flexor carpi ulnaris muscle width	0.335	0.149
Flexor digitorum communis muscle length	0.275	0.367
Flexor digitorum communis muscle width	<0.001	1
Flexor carpi ulnaris tendon length	0.317	0.105
Flexor carpi ulnaris tendon width	0.314	0.123
Ecology		
Function	0.999	<0.001
Habitat	0.999	<0.001

Boldface denotes the presence of phylogenetic signal in the variable.

Table 2. Phylogenetic PCA analysis on external and internal morphology variables

Variable	PC1	PC2	PC3	PC4
External measures				
Arm length	0.078	0.122	-0.465	0.799
Forearm length	-0.206	0.246	-0.466	0.515
Forearm width	-0.047	0.275	0.342	0.424
Palm length	-0.315	0.196	-0.112	-0.049
Palm width	-0.045	0.116	0.003	0.219
Hand length	-0.095	0.235	0.663	0.112
Internal Measures				
Sesamoid length	-0.880	0.365	-0.101	-0.040
Sesamoid width	-0.946	0.091	-0.149	-0.001
Flexor plate length	-0.954	0.202	-0.072	-0.022
Flexor plate width	-0.975	0.014	0.011	-0.044
Finger III tendon length	0.200	-0.338	-0.804	-0.185
Finger IV tendon length	0.243	0.057	-0.767	-0.216
Finger V tendon length	0.433	-0.199	-0.780	-0.043
Finger III tendon width	-0.405	-0.194	0.428	0.087
Finger IV tendon width	-0.172	-0.346	0.122	0.137
Finger V tendon width	0.070	-0.171	0.545	0.010
Flexor carpi ulnaris muscle length	-0.042	0.026	-0.854	0.186
Flexor carpi ulnaris muscle width	0.028	0.301	0.641	0.575
Flexor digitorum communis m. length	-0.001	0.053	-0.787	0.291
Flexor digitorum communis m. width	-0.543	0.077	0.303	0.096
Flexor carpi ulnaris tendon length	-0.238	-0.958	0.049	0.034
Flexor carpi ulnaris tendon width	-0.252	-0.957	0.008	0.103
Cumulative contribution to variance	35.6	63.6	78.31	84.42

Boldface denotes most informative variables in the morphospace after absolute values of eigenvalues.

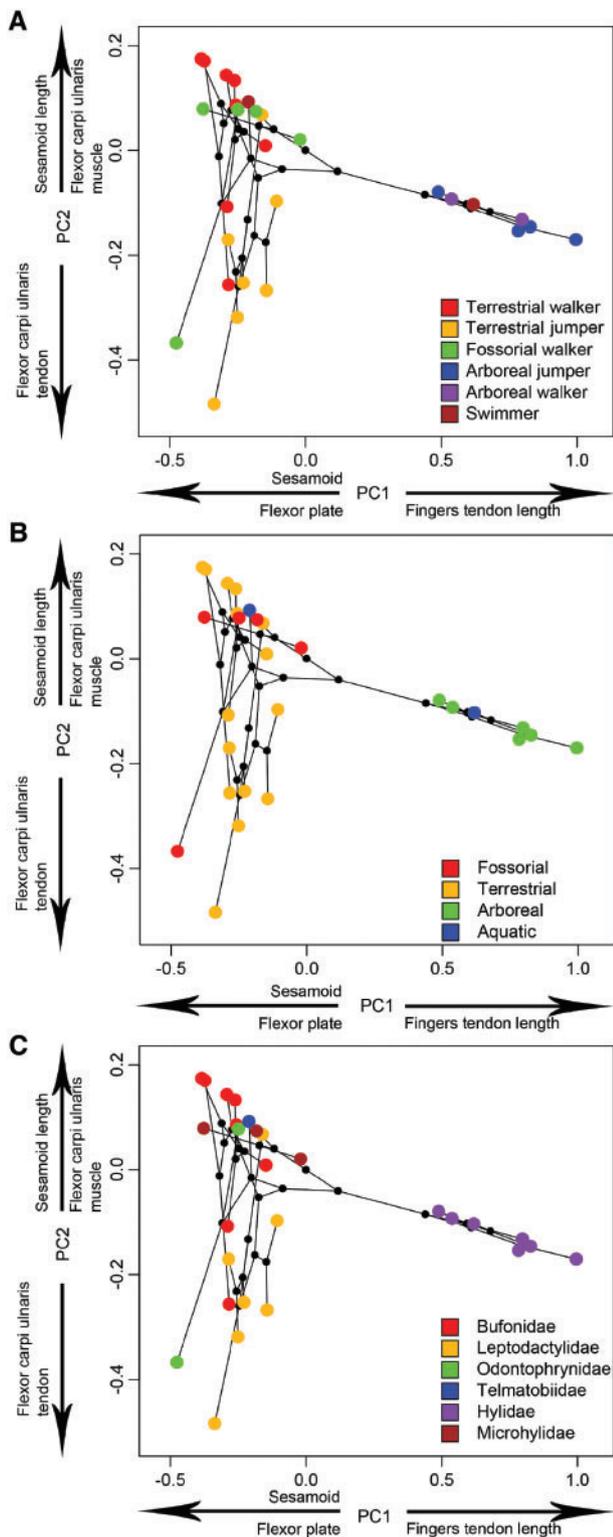


Figure 3. Phylomorphospace plots of (a) functional groups (terrestrial walker, jumper, and burrower; swimmer and arboreal walker and jumper); (b) habitat use (terrestrial; fossorial, swimmer, and arboreal) and (c) family level (Microhylidae, Hylidae, Leptodactylidae, Odontophrynidae, Bufonidae, Telmatobiidae) among the 28 anuran species. Axes correspond to PC1 35.6% of variance (increase of finger tendon [III, IV, and V] length, decrease of sesamoid and flexor plate size) and PC2 28.0% of variance (increase of flexor carpi ulnaris—FCU muscle and sesamoid length and decrease of FCU tendon size).

Table 3. Phylogenetic MANOVA of external and internal variables grouped as a function of locomotor mode (terrestrial walker, terrestrial jumper, fossorial walker, arboreal jumper, arboreal grasper or walker, and aquatic swimmer) and habitat use (fossorial, terrestrial, arboreal, aquatic)

	Locomotor mode			
	<i>df</i>	Wilk's	<i>F</i>	<i>P</i>
External	5 22	0.111	1.707	0.034
Internal	5 22	<0.001	3.926	<0.001
	Habitat use			
	<i>df</i>	Wilk's	<i>F</i>	<i>P</i>
External	3 24	0.405	1.132	0.349
Internal	3 24	0.005	2.692	0.003

Boldface denotes significant differences.

When examining internal traits, sesamoid width and length showed significant differences, as well as flexor plate dimensions. Terrestrial and burrowing species formed a homologous group, separated from the aquatic and arboreal species after Holm post hoc test (Table 4). The results for functional groups after the phylogenetic ANOVA of sesamoid and flexor plate dimensions show that terrestrial (jumpers and walkers), burrowers and swimmers species form a homogeneous group different from the arboreal species (walker and jumpers, see Table 4) that exploit vertical habitats. Tendon width also shows significant differences in the functional groups: swimmers, arboreal jumpers, and arboreal walkers or graspers form a group separated from terrestrial (burrowers, jumpers, and walkers) species (Table 4). When considering habitat use, phylogenetic ANOVA showed that for sesamoid and flexor plate, tendon width of finger V and tendon length of finger VI, terrestrial and burrower species form a group and arboreal and aquatic species were segregated (Table 4). Noticeably, Hylidae and Phyllomedusidae species mostly belong to the arboreal habitat use category (except for *Pseudis platensis* segregated by Holm post hoc test (Figure 3B, C, Table 4). Finally, the flexor digitorum communis muscle width showed significant differences in habitat type used, although no homogeneous group was formed (Table 4). These results highlight the importance of the palmar sesamoid and the flexor plate in the exploitation of vertical dimensions of the habitat.

Discussion

Our data show an important pattern of phylogenetic signal: half of the internal morphology traits show high phylogenetic signal estimator values (Table 1). The effect of the phylogeny on the internal morphology was also verified in other tetrapod groups, such as lizards and anurans (Tulli et al. 2012a; Abdala et al. 2014; Fratani et al. 2018). Interestingly, digit tendons length was additionally an important trait that segregated arboreal and swimming species showing that this morphology–lifestyle relationship probably arose from features clustered in phylogenetic groups with the same habitat use (Vanhooydonck and Van Damme 1999). This niche conservatism (Wiens and Graham 2005) was also apparent among microhylids that exhibit a strong conservatism in ecology, morphology and performance (although, not in body size). This is especially remarkable, given that these species have been separated for more than 65 million years (Moen et al. 2013). Taken together these data

Table 4. Phylogenetic ANOVA of external and internal variables as a function of locomotor mode (Tw = terrestrial walker, Tj = terrestrial jumper, Bw = fossorial walker, Aj = arboreal jumper, Aw = arboreal grasper or walker, and S = aquatic swimmer) and habitat use (F = fossorial, T = terrestrial, Ar = arboreal, Aq = aquatic)

	Functional group			Habitat type		
	<i>F</i>	<i>P</i>	posthoc	<i>F</i>	<i>P</i>	posthoc
External Measures						
Arm length	1.138	0.429		1.244	0.768	
Forearm length	4.081	0.549		0.192	0.98	
Palm length	4.084	0.545		0.974	0.808	
Palm width	0.874	0.941		0.735	0.868	
Hand length	1.631	0.848		2.851	0.549	
Internal Measures						
Sesamoid length	21.012	0.009	<u>TwTjBwS AjAw</u>	31.285	0.005	BT Aq Ar
Sesamoid width	19.37	0.022	<u>TwTjBwS AjAw</u>	29.282	0.008	BT Aq Ar
Flexor plate length	28.184	0.007	<u>TwTjBwS AjAw</u>	47.078	0.001	BT Aq Ar
Flexor plate width	26.408	0.007	<u>TwTjBwS AjAw</u>	46.606	0.001	BT Aq Ar
Finger III tendon length	8.664	0.211		14.114	0.061	
Finger IV tendon length	5.193	0.451		9.168	0.16	
Finger V tendon length	13.318	0.071		23.448	0.019	BT Aq Ar
Finger III tendon width	19.914	0.021	<u>TwTjBw S AjAw</u>	30.836	0.007	BT Aq Ar
Finger IV tendon width	4.898	0.454		5.878	0.262	
Finger V tendon width	3.173	0.651		1.675	0.712	
Flexor carpi ulnaris muscle length	1.561	0.866		0.864	0.828	
Flexor carpi ulnaris muscle width	3.774	0.587		1.424	0.707	
Flexor digitorum communis m. length	0.327	0.995		0.186	0.975	
Flexor digitorum communis m. width	10.579	0.14		18.326	0.036	BT Aq Ar
Flexor carpi ulnaris tendon length	2.982	0.67		2.064	0.613	
Flexor carpi ulnaris tendon width	2.835	0.701		1.833	0.647	

Boldface denotes significant differences and post hoc (Holm method) shows the homogeneous groups formed between groups (when underlined together).

support our first hypothesis, since internal morphology traits tended to have higher phylogenetic signal than external ones.

Among external morphology traits, only arm length exhibited a phylogenetic pattern indicating that this trait is somehow more unique to each lineage among the species studied here. Traits exhibiting strong phylogenetic signal suggest gradual changes over time (e.g., Brownian Motion model of evolution), and traits with no phylogenetic signal may be highly labile on the time scale of phylogeny, or may not change at all (Revell 2008).

Other traits that showed low phylogenetic signal were the width and length of the tendon of the flexor carpi ulnaris that also played an important role in our phylomorphospace figures. These traits may segregate the jumper species from the rest (particularly among the terrestrial species), but we need more detailed information to corroborate this assumption. The low phylogenetic signal in the flexor tendons width of digits IV and V, and flexor carpi ulnaris muscle suggests that the postaxial regions of the forelimb are evolutionarily more prompted to vary than the preaxial regions. These results are interesting as the postaxial region corresponds to the primary axis that passes through digit IV in early limb development (Fabrezi et al. 2007) and it would be expected a more stable configuration to this crucial limb zone. However, it should be considered that those variables related to length of the postaxial (ulnar) structures show a strong phylogenetic pattern, decoupled thus of their width, which exhibit a lower phylogenetic signal, contrasting with the observations of Herrel et al. (2014) for hind limb kinematics of *Xenopus tropicalis*. These authors found that hind limb length in females were good descriptors of the peak resultant jumping force, compared with longer iliums and the length of the longest toe in males. This probably indicates the different roles fore and hind limb

may play in locomotion (Wang et al. 2014). Previous studies have shown that toads use their forelimbs almost exclusively to decelerate and control the body at the moment of impact (Gillis et al. 2014), our results suggest that the postaxial region of the forelimbs could exert a differential role in landing deserving special consideration, particularly in jumping species (all leptodactylid frogs and some hylids).

Our phylogenetic informed MANOVA and ANOVA show that some of the selected morphological variables are clearly related to the arboreal locomotion mode and may be characterized by the morphological pattern of the forelimb, such as lack of sesamoid bone and flexor plate. Forearm structures in arboreal anurans show that they present in their manus the same pervasive trend exhibited for all lineages of grasping tetrapods (Sustaita et al. 2013; Manzano et al. in press). A vertical position in terrestrial environments requires that the animals stay in balance by pulling their fore-feet toward the substrate, while their hind feet push on the substrate (Tulli et al. 2011). This ability allows organisms to balance over a vertical substrate and requires that the organisms maintain their center-of-mass aligned with their support. This can be achieved by developing an adhesive or suction force between their body and the contact surface (Cartmill 1985) or grabbing a narrow stem of the surface, as most arboreal anurans and other grasping tetrapods do (Sustaita et al. 2013; Manzano et al. in press). These morphological descriptors of the arboreal habitat use in the studied frogs allow us to uphold our second and third predictions. Previous studies across many organisms have shown a strong relationship between ecology (e.g., microhabitat use and diet) and morphology (see reviews in Wainwright 2007). Far fewer have considered performance (e.g., Losos 1990a, 1990b, 1990c; Herrel et al. 2014; Tulli et al. 2011,

2012a), which forms the link between ecology and morphology (Wainwright 1991). In addition, Moen et al. (2013) found that frog species using the same microhabitat had both similar morphology and performance, regardless of geographical location.

A recent study on morphology and habitat use also found that frogs that live in the bush and swim and jump, exhibit modifications in the postcranial skeleton such as broad proximal sacral diapophyses, broad vertebral bodies, and longer urostyles that relate their morphology and habits (Soliz and Ponssa 2016). These authors also suggest that a slender body would be better adjusted to face any challenging locomotor modes, which is typical of hylid frogs that possess slender hands too. In addition, arboreal and walker species have smaller and shorter vertebral bodies in their column, weaker coracoids and clavicles compared with jumper and swimmer species (Soliz and Ponssa 2016). Likewise, some features found in Odontophoridae frogs suggest a functional relationship of some morphological features, as they present a typical configuration of burrowing frogs (Reilly and Jorgensen 2011). However, our data are not strong and clear enough to support a specific hypothesis related to this burrowing group of frogs (Blotto et al. 2017), despite the fact that we studied 5 species belonging to 2 different families.

A nested clade (Hylidae and Phyllomedusidae) showed a particular and different composition of limb anatomy, which caused them to be clustered together in a separate group from the rest of the species in our phylomorphospace figure. Our results also showed that the traits that differentiate these anuran species exhibiting an arboreal locomotor mode are the palmar sesamoid and the flexor plate (Table 3). These traits allow us to identify a “grasping syndrome” (Fontanarrosa and Abdala 2016) in the hand skeleton of these frogs, characterized by no palmar sesamoid, no flexor plate and the third metacarpal bone with a bony knob (Dang et al. 2018); supporting our third hypothesis. Previous studies have shown that a large palmar sesamoid embedded in a large flexor plate is a clear impairment for flexing the hand and, consequently, prevents the acquisition of manual dexterity (Abdala et al. 2009; Sustaita et al. 2013; Fontanarrosa and Abdala 2014, 2016). In addition, a large palmar sesamoid appears to obstruct tendon travel, thereby preventing flexion of the digital joints (Sustaita et al. 2013).

Reduction or loss of the palmar sesamoid allows other possibilities to exploit the habitat, for example, arboreality, as the hands can hold narrow branches through grasping. This ability has been reported in hylids and in all members of the Phyllomedusidae family (Manzano et al. 2008, 2018; Sustaita et al. 2013) and the anatomical requirements of a grasping hand were extensively reported in several tetrapod species (Manzano et al. 2008, 2018; Abdala et al. 2009; Sustaita et al. 2013; Fabre et al. 2013). Similarly, it was shown that in hylids, the bony knob of the third metacarpal has evolved independently from phylogeny resulting in a significant correlation with the arboreal habitat use (Dang et al. 2018). These authors also show that a muscle is adhered to this bony knob, suggesting that this could be an additional attachment point for muscles related with grasping (Dang et al. 2018).

The absence of the palmar sesamoid in *Pseudis* indicates that distribution of this character is mainly linked to the phylogenetic history in these taxa, since they belong to the Hylidae clade (Pyron and Wiens 2011), as the phylogenetic signal clearly shows. In fact, sesamoid absence is common for species with different locomotor modes (walkers and jumpers and swimmers) and habitat use (arboreal and aquatic), allowing the interpretation that the lack of this bone and flexor plate in hylids and phyllomedusa (Sustaita et al. 2013) is an exaptation (Gould and Vrba 1982) for grasping of

narrow branches or twigs (Manzano et al. in press). Aquatic frogs such as *Xenopus* with a large palmar sesamoid suggest that their presence is owed more to phylogeny than to ecological reasons. However, recent studies suggest that *Xenopus*, a genus lacking grasping syndrome, exhibits also considerable manual abilities, including an intermediate or scissor grip (Anzeraey et al. 2017). This report defied the pervasive link between arboreality and manual grasping and requires a new perspective to explain the genesis of grasping among anurans (Manzano et al. in press). In addition, it has to be noted that the palmar sesamoid may be ambiguously present or absent (Ponssa et al. 2010); consequently, our results should be taken with caution because of a possible taxon sampling problem, probably as a consequence of choosing species that may coexist as they occur in Chaco and Monte biogeographic provinces (Cabrera and Willink 1980).

Supplementary Material

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

Acknowledgments

We thank Instituto de Investigación Biológica del Paraguay (IIBP), Museo Nacional de Historia Natural del Paraguay (MNHNP), Fundación Miguel Lillo and Colección Zoológica de FaCEN, for providing access to specimens.

Funding

Funding for this study was provided by Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina (Grant PIP-CONICET 389 to VA), Agencia Nacional de Promoción Científica y Tecnológica, Argentina (Grant PICT 2015-2471 to FBC, PICT 2016-2772 to VA).

References

- Abdala V, Moro S, Flores D, 2006. The flexor tendons in the didelphid manus. *Mastozool Neotrop* 13:193–204.
- Abdala V, Manzano AS, Herrel A, 2008. The distal forelimb musculature in aquatic and terrestrial turtles: phylogeny or environmental constraints? *J Anat* 213:159–172.
- Abdala V, Manzano AS, Tulli MJ, Herrel A, 2009. The tendinous patterns in the palmar surface of the lizard manus: functional consequences for grasping ability. *Anat Rec* 292:242–253.
- Abdala V, Tulli MJ, Russell AP, Powell GL, Cruz F, 2014. Anatomy of the crus and pes of Neotropical iguanian lizards in relation to habitat use and digitally based grasping capabilities. *Anat Rec* 297:397–409.
- Anzeraey A, Aumont M, Decamps T, Herrel A, Pouydebat E, 2017. The effect of food properties on grasping and manipulation in the aquatic frog *Xenopus laevis*. *J Exp Biol* 220:4486–4491.
- Arnold SJ, 1983. Morphology, performance and fitness. *Am Zool* 23:347–361.
- Blotto B, Pereyra MO, Faivovich J, Dos Santos Dias PH, Grant T, 2017. Concentrated evolutionary novelties in the foot musculature of Odontophrynidae (Anura: neobatrachia), with comments on adaptations for burrowing. *Zootaxa* 4258:425–442.
- Bonine KE, Garland T Jr, 1999. Sprint performance of phrynosomatid lizards, measured on a high-speed treadmill, correlates with hind limb length. *J Zool* 248:255–265.
- Burton TC, 1983. The musculature of the Papuan frog *Phrynomantis stictogaster* (Anura, Microhylidae). *J Morphol* 175:307–324.
- Burton TC, 1996a. Adaptation and evolution in the hand muscles of Australo-Papuan Hylid Frogs (Anura: hylidae: pelodyadinae). *Australian J Zool* 44:611–623.
- Burton TC, 1996b. Variation in the hand and superficial throat musculature of neotropical leptodactylid frogs. *Herpetologica* 54:53–72.

- Burton TC, 1998. Are the distal extensor muscles of the fingers of anurans an adaptation to arboreality. *J Herpetol* 32:611–617.
- Burton TC, 2001. Variation in the foot muscles of frogs of the family Myobatrachidae. *Australian J Zool* 49:539–559.
- Burton TC, 2004. Muscles of the pes of hylid frogs. *J Morphol* 260:209–233.
- Cabrera A, Willink A, 1980. *Biogeografía de América Latina*. Secretaría General OEA, Serie Biológica, Monografía.
- Calsbeek R, Irschick DJ, 2007. The quick and the dead: correlational selection on morphology, performance, and habitat use in island lizards. *Evolution* 61:2493–2503.
- Carrizo LV, Tulli MJ, Dos Santos DA, Abdala V, 2014. Interplay between postcranial morphology and locomotor types in Neotropical sigmodontine rodents. *J Anat* 224:469–481.
- Cartmill M, 1985. Climbing. In: Hildebrand M, Bramble DM, Liem KF, Wake DB, editors. *Functional Vertebrate Morphology*. Cambridge: Belknap Press of Harvard University. 73–88.
- Cei JM, 1980. *Amphibians of Argentina*. Monitore Zoologico Italiano, New Series, Monographs, Milan.
- Dang NX, Wang JS, Liang J, Jiang DC, Liu J et al., 2018. The specialization of the third metacarpal and hand in arboreal frogs: adaptation to arboreal habitat? *Acta Zool* 99:115–125.
- Davies M, Burton TC, 1982. Osteology and myology of the gastric brooding frog *Reobatrachus silus* Liem (Anura: leptodactylidae). *Australian J Zool* 30:503–521.
- Donley JM, Sepulveda CA, Konstantinidis P, Gemballa S, Shadwick RE, 2004. Convergent evolution in mechanical design of lamnid sharks and tunas. *Nature* 429:61–65.
- Dunlap DG, 1960. The comparative myology of the pelvic appendage in the Saliencia. *J Morphol* 106:1–76.
- Emerson SB, 1978. Allometry and jumping in frogs: helping the twain to meet. *Evolution* 32:551–564.
- Emerson SB, 1985. Jumping. In: Hildebrand M, Bramble DM, Liem KF, Wake DB, editors. *Functional Vertebrate Morphology*. Cambridge: Belknap Press of Harvard University. 58–72.
- Emerson SB, 1988. Convergence and morphological constraint in frogs: variation in postcranial morphology. *Fieldiana Zool* 43:1–19.
- Fabre AC, Cornette R, Slater G, Argot C, Peigné S et al., 2013. Getting a grip on the evolution of grasping in musteloid carnivores: a three-dimensional analysis of forelimb shape. *J Evol Biol* 26:1521–1535.
- Fabrezi M, Abdala V, Martínez Oliver MI, 2007. Developmental basis of limb homology in lizards. *Anat Rec* 290:900–912.
- Fabrezi M, Manzano A, Abdala V, Lobo F, 2014. Anuran locomotion: ontogeny and morphological variation of a distinctive set of muscles. *Evol Biol* 41:308–326.
- Faivovich J, 2002. A cladistic analysis of *Scinax* (Anura: hylidae). *Cladistics* 18:367–393.
- Fontanarrosa G, Abdala V, 2014. Anatomical analysis of the lizard carpal bones in terms of skilled manual abilities. *Acta Zool* 95:249–263.
- Fontanarrosa G, Abdala V, 2016. Bone indicators of grasping hands in lizards. *PeerJ* 4: e1978.
- Fratani J, Ponsa ML, Abdala V, 2018. Tendinous framework of anurans reveals an all-purpose morphology. *Zoology* 126:172–184.
- Garamszegi LZ, Møller AP, 2010. Effects of sample size and intraspecific variation in phylogenetic comparative studies: a meta-analytic review. *Biol Rev* 85:797–805.
- Garland T Jr, Harvey PH, Ives AR, 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst Biol* 41:18–32.
- Gillis GB, Ekstrom L, Azizi E, 2014. Biomechanics and control of landing in toads. *Integr Comp Biol* 54:1136–1147.
- Gomes FR, Rezende EL, Grizante MB, Navas CA, 2009. The evolution of jumping performance in anurans: morphological correlates and ecological implications. *J Evol Biol* 22:1088–1097.
- Goodman BA, Miles DB, Schwarzkopf L, 2008. Life on the rocks: habitat use drives morphological and performance evolution in lizards. *Ecology* 89:3462–3471.
- Gould SJ, Vrba E, 1982. Exaptation: a missing term in the science of form. *Paleobiology* 8:4–15.
- Grizante MB, Navas CA, Garland T Jr, Kohlsdorf T, 2010. Morphological evolution in *Tropidurinae squamates*: an integrated view along a continuum of ecological settings. *J Evol Biol* 3:98–111.
- Haines RW, 1950. The flexor muscles of the forearm and hand in lizards and mammals. *J Anat* 84:13–29.
- Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W, 2008. GEIGER: investigating evolutionary radiations. *Bioinformatics* 24:129–131.
- Harvey PH, Pagel MD, 1991. *The Comparative Method in Evolutionary Biology*. Oxford: Oxford University Press.
- Herrel A, Vasilopoulou-Kampitsi M, Bonneaud C, 2014. Jumping performance in the highly aquatic frog *Xenopus tropicalis*: sex-specific relationships between morphology and performance. *PeerJ* 2: e661.
- Hoyos JM, Mantilla C, Galindo D, Salgar L, 2014. Phylogenetic analysis within the *Pristimantis unistrigatus* (Anura, Craugastoridae) group based on morphological characters. *Caldasia* 36:107–124.
- Hoyos JM, Salgar L, 2016. New conditions and intraspecific variation of some muscles of hands and feet of *Dendropsophus labialis* (Peters, 1863) (Anura, Hylidae). *Acta Zool* 97:143–153.
- Irschick DJ, Vanhooydonck B, Herrel A, Jay Meyers J, 2005. Intraspecific correlations among morphology, performance, and habitat use within a green anole lizard *Anolis carolinensis* population. *Biol J Linn Soc* 85:223–234.
- Irschick DJ, Meyers JJ, Husak JF, Le Galliard JF, 2008. How does selection operate on whole-organism functional performance capacities? A review and synthesis. *Evol Ecol Res* 10:177–196.
- Jorgensen ME, Reilly SM, 2013. Phylogenetic patterns of skeletal morphometrics and pelvic traits in relation to locomotor mode in frogs. *J Evol Biol* 26:929–943.
- Kingsolver JG, Huey RB, 2003. Introduction: the evolution of morphology, performance, and fitness. *Integr Comp Biol* 43:361–366.
- Kohlsdorf T, Garland T Jr, Navas CA, 2001. Limb and tail lengths in relation to substrate usage in *Tropidurus* lizards. *J Morphol* 248:151–164.
- Liem SS, 1970. The morphology, systematics, and evolution of the Old World treefrogs (Rhacophoridae and Hyperoliidae). *Fieldiana Zool* 57:1–145.
- Losos JB, 1990a. Ecomorphology, performance capability and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecol Monogr* 60:369–388.
- Losos JB, 1990b. Concordant evolution of locomotor behaviour, display rate and morphology in *Anolis* lizards. *Anim Behav* 39:879–890.
- Losos JB, 1990c. The evolution of form and function: morphology and locomotor performance in Indian *Anolis* lizards. *Evolution* 44:1189–1203.
- Losos JB, Sinervo B, 1989. The effects of morphology and perch diameter on sprint performance in *Anolis* lizards. *J Exp Biol* 145:23–30.
- Maddison W, Maddison D, 2015. Mesquite: a modular system for evolutionary analysis. Version 3.03 (7 May 2015) [cited 2016 April 22]. Available from: <http://mesquiteproject.org>.
- Manzano AS, 2000. Miología pectoral de algunos Centrolenidae (Amphibia: anura). *Cuad Herp* 14:27–45.
- Manzano AS, Lavilla EO, 1995. Notas sobre la miología apendicular de *Phyllomedusa hypocondrialis* (Anura, Hylidae). *Alytes* 12:169–174.
- Manzano AS, Abdala V, Herrel A, 2008. Morphology and function of the forelimb in arboreal frogs: specializations for grasping ability? *J Anat* 213:296–307.
- Manzano AS, Fontanarrosa G, Abdala V, in press. Manual and pedal grasping among anurans: a review of relevant concepts with empirical approaches. *Biol J Linn Soc*.
- Midford PE, Garland T Jr, Maddison W, 2009. PDAP:PDTREE package for Mesquite, version 1.15. http://mesquiteproject.org/pdap_mesquite/.
- Moen DS, Irschick DJ, Wiens JJ, 2013. Evolutionary conservatism and convergence both lead to striking similarity in ecology, morphology and performance across continents in frogs. *Proc R Soc B* 280:20132156.
- Moro S, Abdala V, 2004. Análisis descriptivo de la miología flexora y extensora del miembro anterior de *Polychrus acutirostris* (Squamata, Polychrotidae). *Pap Avulsos Zool* 44:81–89.
- Pagel M, 1999. Inferring the historical patterns of biological evolution. *Nature* 401:877–884.
- Ponsa ML, Goldberg J, Abdala V, 2010. Sesamoids in anurans: new data, old issues. *Anat Rec* 293:1646–1668.

- Pyron RA, Wiens JJ, 2011. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Mol Phylog Evol* 61:543–583.
- R Core Team. 2017. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. Available from: <http://www.R-project.org/>
- Rand AS, 1952. Jumping ability of certain anurans, with notes on endurance. *Copeia* 1952:15–20.
- Reilly SM, Jorgensen ME, 2011. The evolution of jumping in frogs: morphological evidence for the basal anuran locomotor condition and radiation of locomotor system in crown group anurans. *J Morphol* 272:149–168.
- Revell LJ, 2008. On the analysis of evolutionary change along single branches in a phylogeny. *Am Nat* 172:140–147.
- Revell LJ, 2009. Size-correction and principal components for interspecific comparative studies. *Evolution* 63:3258–3268.
- Revell LJ, 2012. Phytools: an R package for phylogenetic comparative biology (and other things). *Meth Ecol Evol* 3:217–223.
- Salgar L, Hoyos JM, Acosta A, 2009. New conditions and intraspecific variations in hand and foot muscles in *Pristimantis bogotensis* (Peters, 1863) (Amphibia: anura: strabomantidae). *Zootaxa* 2025:56–68.
- Soliz MC, Ponsa ML, 2016. Development and morphological variation of the axial and appendicular skeleton in Hylidae (Lissamphibia, Anura). *J Morphol* 277:786–813.
- Soliz M, Tulli MJ, Abdala V, 2017. Osteological postcranial traits in hylid anurans indicate a morphological continuum between swimming and jumping locomotor modes. *J Morphol* 278:403–417.
- Stephens PR, Wiens JJ, 2004. Convergence, divergence, and homogenization in the ecological structure of emydid turtle communities: the effects of phylogeny and dispersal. *Am Nat* 164:244–254.
- Sustaita D, Pouydebat E, Manzano A, Abdala V, Hertel F et al., 2013. Getting a grip on tetrapod grasping: form, function, and evolution. *Biol Rev* 88:380–405.
- Tulli MJ, Cruz FB, Herrel A, Vanhooydonck B, Abdala V, 2009. The interplay between claw morphology and microhabitat use in neotropical iguanian lizards. *Zoology* 112:379–392.
- 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, 2012a. Effects of different substrates on the sprint performance of lizards. *J Exp Biol* 215:774–784.
- Tulli MJ, Herrel A, Vanhooydonck B, Abdala V, 2012b. Is phylogeny driving tendon length in lizards? *Acta Zool* 93:319–329.
- Tulli MJ, Cruz FB, Kohlsdorf T, Abdala V, 2016. When a general morphology allows many habitat uses. *Integr Zool* 11:473–489.
- Van Damme R, Vanhooydonck B, 2002. Speed versus maneuverability: association between vertebral number and habitat structure in lacertid lizards. *J Zool* 258:327–334.
- Vanhooydonck B, Van Damme R, 1999. Evolutionary relationships between body shape and habitat use in lacertid lizards. *Evol Ecol Res* 1:785–805.
- Vidal-García M, Byrne PG, Roberts JD, Keogh JS, 2014. The role of phylogeny and ecology in shaping morphology in 21 genera and 127 species of Australo-Papuan myobatrachid frogs. *J Evol Biol* 27:181–192.
- Wainwright PC, 1991. Ecological morphology: experimental functional anatomy for ecological problems. *Am Zool* 31:680–693.
- Wainwright PC, 2007. Functional versus morphological diversity in macroevolution. *Annu Rev Ecol Evol Syst* 38:381–401.
- Wang Z, Ji A, Endlein T, Samuel D, Yao N et al., 2014. The role of fore- and hindlimbs during jumping in the Dybowski's frog *Rana dybowskii*. *J Exp Zool* 321A:324–333.
- Wells KD, 2007. *The Ecology and Behavior of Amphibians*. Chicago (IL): The University of Chicago press.
- Wiens JJ, Graham CH, 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. *Annu Rev Ecol Evol Syst* 36:519–539.
- Zug GR, 1972. Anuran locomotion: structure and function. I. Preliminary observations on relation between jumping and osteometrics of appendicular and postaxial skeleton. *Copeia* 1972:613–624.
- Zug GR, 1978. Anuran locomotion: structure and function II. Jumping performance of semiaquatic, terrestrial, and arboreal frogs. *Smithson Contrib Zool* 276:1–31.