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Evolution of morphology and locomotor performance in anurans: relationships with microhabitat diversification

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Abstract

The relationships between morphology, performance, behavior and ecology provide evidence for multiple and complex phenotypic adaptations. The anuran body plan, for example, is evolutionarily conserved and shows clear specializations to jumping performance back at least to the early Jurassic. However, there are instances of more recent adaptation to habit diversity in the post-cranial skeleton, including relative limb length. The present study tested adaptive models of morphological evolution in anurans associated with the diversity of microhabitat use (semi-aquatic arboreal, fossorial, torrent, and terrestrial) in species of anuran amphibians from Brazil and Australia. We use phylogenetic comparative methods to determine which evolutionary models, including Brownian motion (BM) and Ornstein-Uhlenbeck (OU) are consistent with morphological variation observed across anuran species. Furthermore, this study investigated the relationship of maximum distance jumped as a function of components of morphological variables and microhabitat use. We found there are multiple optima of limb lengths associated to different microhabitats with a trend of increasing hindlimbs in torrent, arboreal, semi-aquatic whereas fossorial and terrestrial species evolve toward optima with shorter hindlimbs. Moreover, arboreal, semi-aquatic and torrent anurans have higher jumping performance and longer hindlimbs, when compared to terrestrial and fossorial species. We corroborate the hypothesis that evolutionary modifications of overall limb morphology have been important in the diversification of locomotor performance along the anuran phylogeny. Such evolutionary changes converged in different phylogenetic groups adapted to similar microhabitat use in two different zoogeographical regions.

Introduction

The relations between morphology, behaviour and ecology have been studied in several phylogenetic groups (e.g. primates: Crompton, 1984; birds: Moreno & Carrascal, 1993; squamates: Losos, 1990a; Martins *et al.*, 2001; Harrison *et al.*, 2015; and amphibians: Zug, 1972; Gomes *et al.*, 2009; Vidal-García *et al.*, 2014) to understand how different selective pressures have moulded the evolution of morphological patterns (Irschick & Losos, 1999). Locomotion and its morphological correlates have received a great deal of attention (Zug, 1972, 1978; Emerson, 1979; Losos, 1990b; Gomes *et al.*, 2009; Jorgensen & Reilly, 2013; Moen *et al.*, 2013) because many ecologically relevant activities, such as escaping from predators, foraging and searching for reproductive partners, depend on locomotion (Meylan & Clobert, 2004; Husak *et al.*, 2006). Therefore, locomotor performance has been widely regarded as being associated with fitness (Christian & Tracy, 1981; Arnold, 1983; Garland, 1999).

An interesting group to address the relations between morphological specializations and locomotor

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performance are the Anurans. Anuran body shape presents specializations associated with jumping performance that are old and phylogenetically conserved, being present in their most basic aspects in fossils from the Jurassic period (Shubin & Jenkins, 1995). Ascarrunz *et al.* (2016) re-assessed saltatorial performance of the basalmost and oldest stem-anuran *Triadobatrachus massinoti*, from early Triassic. These authors suggested that the trunk of these animals mostly reflects the nonelongated ancestral condition and confirmed previous hypotheses that they were probably not specialized jumpers (Ascarrunz *et al.*, 2016). The origin and maintenance of adaptations to jumping performance in anuran amphibians are probably related to selective pressures associated with prey capture and escape from predators (Gans & Parsons, 1966; Zug, 1972; Emerson, 1978; Gomes *et al.*, 2002). Despite the constituent basic aspects of body shape in anurans being highly conserved, this group displays a wide variety of microhabitat occupation, including leaf litter, underground chambers, edges of ponds, reservoirs, streams and rivers, inside phytotelmata such as bromeliads, and on bush vegetation and tree branches (Bertoluci & Rodrigues, 2002; Haddad *et al.*, 2008).

The diversity in microhabitat use is linked to the evolution of locomotor performance and hindlimb morphology. Terrestrial and fossorial species tend to show lower jumping performance and shorter hindlimbs than semi-aquatic and arboreal species (Zug, 1972, 1978; Gomes *et al.*, 2009; Enriquez-Urzelai *et al.*, 2015;). Some morphological adaptations such as rotund shape, short limbs and well-developed metatarsal tubercles (spade-shaped) are related to species with burrowing habits (Duellman & Trueb, 1986; Wells, 2007; Vidal-García *et al.*, 2014). Also distinctive morphology and performance is associated with the microhabitat diversity, regardless of clade position or geographic precedence (Moen *et al.*, 2013). Even though ecomorphological comparative investigations on anurans have been conducted on large phylogenetic scales (Zug, 1972, 1978; Emerson, 1979; Marsh, 1994; Gomes *et al.*, 2009; Moen *et al.*, 2013; Enriquez-Urzelai *et al.*, 2015), studies that incorporate measurements of locomotor performance are still scarce in the literature (Zug, 1972, 1978; Gomes *et al.*, 2009; Herrel *et al.*, 2016). It is also noteworthy that these studies have been particularly biased in terms of zoogeographical areas, with poor representation of Neotropical and Ethiopic fauna, for example. Furthermore, the relations between morphology, locomotion performance and microhabitat use are rarely considered into an integrated evolutionary context.

Here, we use phylogenetic comparative methods to determine which evolutionary models, including Brownian motion and Ornstein–Uhlenbeck, are consistent with morphological variation observed across anuran species from Brazilian and Australian faunas. Specifically, we hypothesize that current variation in morphology across anuran species is consistent with stabilizing selection related to distinct phenotypic optima in different categories of microhabitat use. We predict that fossorial and terrestrial species have shorter hindlimbs than semi-aquatic, arboreal and torrent species. Short hindlimbs are thought to improve burrowing performance in fossorial species and facilitate walking/hopping ability in terrestrial species (Emerson, 1978; Zug, 1978), whereas long hindlimbs provide locomotory benefits in more aquatic habitats by improving propulsion when swimming in high-flow stream systems in aquatic and torrent species, respectively (Laurent, 1964; Emerson, 1978). Long hindlimbs may also improve arboreal agility in species that climb trees (Zug, 1978; Emerson, 1985, 1991).

To test this hypothesis, we compared the fit of species' morphology estimates to models that approximate evolution according to either Brownian motion or Ornstein–Uhlenbeck (OU) processes. Better fit of Brownian motion models would indicate that contemporary variation in morphology is consistent with the absence of consistent selection associated with microhabitat use. Alternatively, better fit of OU models would incorporate selection pressures that lead the adaptations towards different evolutionary optima across selective regimes (e.g. microhabitat use). Furthermore, we hypothesize that interspecific variation in anuran locomotor performance is associated with variation in morphology and microhabitat use. Particularly, we predict that fossorial and terrestrial species have shorter hindlimbs and lower maximum jumping distance than semi-aquatic, arboreal and torrent species. Accordingly, we employed phylogenetic regressions to investigate the relationship of maximum distance jumped as a function of components of morphological variables and microhabitat use.

Materials and methods

Morphological data

Species studied and measurements in museum collections

This study was conducted using 64 anuran species (44 species from Brazil and 20 species from Australia, such as shown in Table S1). The Brazilian and Australian measured specimens were available, respectively, at the Museum of Zoology from the University of São Paulo (MZUSP) and the South Australian Museum in Adelaide (SAM). The choice of species was carried out to cover the maximum morphological diversity and occupation of structural niches within each phylogenetic group. With this sampling approach, we intended to obtain the maximum number of evolutionary transitions along the phylogenetic tree, increasing the power to detect patterns of morphological evolution. We also considered the specimens' availability in the museum collections.

The number of individuals measured from each species ranged from three to 10, and all measurements were performed using digital callipers to the nearest 0.1 mm. Only adult males were measured, and sex was identified by external morphological characters, such as presence of vocal sacs, inguinal spots, nuptial pads, spines on fingers, darkened throats and differences in colour generally present in males. In the absence of external characters, sex determination occurred through the presence of vocal slits in adult males (Pombal & Izecksohn, 2011).

Morphological variables

Eight morphometric traits were obtained for each individual: humerus length (HUM, measured from tip of elbow to posterior insertion point of forelimb at the body wall); radioulnar length (RAD, measured from distal edge of outer palmar tubercle to tip of elbow); hand length (HAND, measured from distal edge of outer palmar tubercle to distal tip of third finger); femur length (FEM, measured from posterior tip of ischium to knee); tibiofibular length (TBFB, measured from tip of knee to tip of heel/proximal end of the tarsus); tarsus length (TAR, measured from tip of heel to proximal edge of inner metatarsal tubercle); foot length (FOOT, measured from proximal edge of inner metatarsal tubercle to tip of outstretched fourth toe); and the snout-to-vent length (SVL, measured from proximal edge of inner metatarsal tubercle to tip of outstretched fourth toe tip of snout to posterior end of the ischium). The same measurements were performed in animals used in the locomotor performance tests, and they were all included in the phylogenetic analysis.

Phylogenetic tree

A composite phylogenetic tree with 64 species was compiled (Fig. S1), mainly based on topology and divergence times proposed by Pyron (2014), which is the most comprehensive current phylogenetic hypothesis for anurans. Phylogenetic information was available for 31 preserved Brazilian species and for all 20 preserved Australian species in Pyron (2014). Some adjustments were necessary to include another 13 Brazilian species as follows: for inclusion of *Rhinella ornata,* a species from the *R. crucifer* group of species (Baldissera *et al.*, 2004), the topological position and divergence time for *Rhinella crucifer* in Pyron (2014) were assumed. For *Melanophryniscus moreirae*, the divergence time between the genus *Melanophryniscus* and the other clade formed by species of the family Bufonidae in Pyron (2014) was assumed. *Scinax rizibilis* were inserted in the topology at the base of the cladistic divergence between the clades *S. catharinae* and *S. ruber* according to Faivovich (2002). *Pseudis platensis* and *P. paradoxa* were inserted in the phylogeny considering the basal most divergence within the genus in Pyron (2014). *Hylodes asper* and *H. phyllodes*, as well as *Physalaemus olfersii* and *P. cuvieri,* were also inserted in the phylogeny considering the basal most divergence within the genus in Pyron (2014). To include *Leptodactylus fuscus*, the topological position of *L. longirostris* was assumed (Pyron, 2014), given that *Leptodactylus fuscus* is a sister species of *L. longirostris* according by Pyron & Wiens (2011). To include *Leptodactylus latrans,* the topological position of *L. leptodactyloides* was assumed (Pyron, 2014), given that *Leptodactylus latrans* (former *L. ocellatus*) is a sister group of *L. leptodactyloides* by Pyron & Wiens (2011). *Chiasmocleis leucosticta* and *C. carvalhoi* were inserted in the phylogeny considering the basal most divergence within the genus in Pyron (2014). *Elachistocleis ovalis* was inserted in place of *E. bicolor* (Pyron, 2014). *Odontophrynus americanus* was inserted in the phylogeny occupying the topological position of *O. cultripes*, given that they are considered sister groups by Pyron & Wiens (2011). Given that information about the topological position and divergence time is unavailable for *Cycloramphus dubius*, this species was depicted in a polytomy at the base of the *Cycloramphus* clade (Pyron, 2014). The systematic nomenclature followed Frost (2015).

Another phylogenetic tree with 19 species for locomotor performance data was compiled (Fig. S2), based on topology and divergence times proposed by Pyron (2014). Phylogenetic information was available for all Brazilian and Australian species in Pyron (2014).

Species and collection localities for the study of locomotor performance

Males from 19 species of anurans were collected in several localities from Brazil (15 species) and Australia (four species). They are represented in Table S3.

Animal maintenance in the laboratory

Brazilian species were placed in plastic boxes and transported to the laboratory in the University of São Paulo (Brazil), where they were housed individually, in terraria of 13 × 30 × 28 cm for the smaller species and in terraria of 27 × 45 × 32 cm for species larger than 80 mm SVL. Each terrarium contained fragments of vegetation, some stones and fresh water permanently available, and animals were fed weekly with small live cockroaches. The terraria were maintained in a climate-controlled room at 25 °C (± 1 °C) and 12-h: 12-h dark: light cycle, with the darkness starting at 7:40 pm. The collections were performed under approved permission of Ministério do Meio Ambiente, ICMBio, SISBio (licence number: 49250-1), and laboratory procedures were performed under the approval of the Comissão de Ética no Uso de Animais (CEUA), Instituto de

Biociências da Universidade de São Paulo (Protocol number: 192/2013). Fieldwork at Parque Estadual Intervales was conducted under authorization of the 'Coordenadoria de informações técnicas, documentação e pesquisa ambiental' (COTEC, process number 611/2015), Instituto Florestal, Secretaria do Meio Ambiente. For the other localities, no specific authorizations were required.

Australian species were placed in cloth bags and transported to the laboratory to the University of South Australia (Australia), where they were housed individually, in terraria of 13 × 13 × 16 cm for the small species (*L. ewingii*, *L. tasmaniensis* and *C. signifera*) and in terraria of 28 × 19 × 36 cm for the larger species (*L. caerulea*). Each terrarium contained a sandy substrate, some stones and fragments of vegetation, fresh water permanently available, and animals were fed weekly with small live crickets. The terraria were maintained in a climate-controlled room at 25 °C (± 1 °C) and 12-h: 12-h dark: light cycle, with the darkness starting at 12 midnight, this adjustment was made to collect the data during the day, and the individuals were acclimatized to these conditions for over 1 week prior to the experiments. The inverse light–dark cycle had no discernible effects on any measure of behaviour or physiological variables. Animals were collected, housed and studied under a permit from the University of South Australia Animal Ethics Committee (protocol no 33636). For these localities, no specific authorizations were required. Although terrariums had different dimensions in Brazil and Australia, they were sufficient to allocate the frogs comfortably in both locations. In the same way, frogs were fed on cockroaches and crickets in Brazil and Australia, respectively, but the insects were equally consumed and frogs maintained body condition equally well in both locations. In this way, we are confident that the differences in maintenance of frogs between locations did not affect the results.

Locomotor Performance tests in the laboratory

Individuals were maintained in plastic containers in a climate-controlled room at 25 °C for 1 h before locomotor tests. Locomotor tests were performed in an arena (120 cm width and 285 cm length) at constant temperature room (25 °C). The animals were stimulated to jump on a substrate with a good grip (ethylene vinyl acetate sheets (EVA) by tapping gently in the posterior region of the individuals for six consecutive times. Starting and landing points were marked on the floor, and later, the distance between marks was measured. The longest jump of the series was used as the best estimate of maximum jumping performance. To conduct the data collection, the observer used a headlamp with red light (500 Lumens), and data collection was performed within 2 weeks of the animals being captured. All data were collected during darkness, when frogs are fully active. In the laboratory, this condition was between 08:00 pm and 00:00 am in Brazil and between 08:00 am and 12:00 am in Australia. The only exception was the diurnal species *Hylodes asper (*Brazilian torrent frog), which had their locomotor performance tests carried out during the day (02:00–04:00 pm).

Comparative analysis

Eight morphometric variables, based on body size (snout–vent length – SLV), forelimb and hindlimb, were used in the statistical analyses: humerus length, radioulnar length, hand length, femur length, tibiofibular length, tarsus length and foot length. All mean values were log10-transformed, and we performed descriptive statistics for all morphological variables per species. The variables related to limbs were phylogenetically regressed against SVL, and the residuals were used in the analyses, in order to remove the effects of body size (Revell, 2009). The size-free variables were summarized with a phylogenetic principal component analyses (pPCA) in which components with eigenvalues larger than 1.0 were saved for later analyses (Revell, 2009). We also used phylogenetic regression to investigate the relationship between the maximum jumping distance and body size (Revell, 2009) and saved the residuals for later analyses. To test the hypothesis that interspecific variation in anuran locomotor performance is associated with morphological variation (body size) and microhabitat use, phylogenetic regressions were used to investigate the relationship between the locomotor performance and body size. Multiple phylogenetic linear models were implemented with the residuals of locomotor performance on body size (size-free jump performance) as the dependent variable, the scores from morphological components as independent variables and microhabitat use as a factor. We fitted phylogenetic generalized least squares (PGLS) and performed model selection by sequentially removing variables explaining the least variation on each model tested (Purvis *et al.*, 2000; Stuart-Fox & Ord, 2004). We always started by fitting a model that included the interaction between the scores from morphological components and microhabitat use. We always considered these interactions as our first removal from the models if not significant (Engqvist, 2005). All variables included in the preferred model had a significance level of $P < 0.05$ via partial-F tests. We reported results from all models tested together with Akaike information criterion corrected for small sample size (AICc), and considered as the preferred model the one showing higher AICc weight, according to Burnham & Anderson (2002). Burnham & Anderson (2002) note as a rule of thumb that models with AIC within two units of the preferred model are considered to have substantial support. We conducted procedures for evolutionary models and PGLS with the software R version 3.0.2. Evolutionary analyses of morphology were conducted within a phylogenetic context using Brownian motion (BM) and Hansen's (1997) multiple optimum Ornstein–Uhlenbeck (OU) models (more information in Martins & Hansen, 1997; Butler & King, 2004). We used BM model here to represent stochastic evolution with no assumptions about adaptation with respect to the selective pressures associated with microhabitat use. The adaptive models of evolution were constructed by assigning adaptive regimes to the terminal branches of the phylogeny based on independent ecological data or descriptions for the extant species with ancestral regimes reconstructed based on linear parsimony using Mesquite version 2.75 (build 564) (Maddison & Maddison, 2011).

Our adaptive model is based on microhabitat use and contains five optima: arboreal, semi-aquatic, terrestrial, fossorial and torrent. We considered that (1) semi-aquatic species live predominantly in or near water; (2) arboreal species live above the ground on vegetation; (3) fossorial species live predominantly amid leaf litter or in burrows; (4) terrestrial species live on the ground; and (5) torrent species live closely associated with rocky, fast-flowing streams. We assigned species into habitat regimes based on published descriptions of microhabitat use (Haddad *et al.*, 2008; Tyler & Walker, 2011) and personal observations from specialists: Prof. Miguel T. Rodrigues (University of São Paulo) and Prof. Michael Tyler (The University of Adelaide).

We used OUwie software package (Beaulieu *et al.*, 2012) in the R statistical computing environment (R Core Team 2010) to fit each morphological aspect considered and the selective regime into an evolutionary model, assuming our phylogeny. For this, we used four evolutionary models: (1) BM1 = single-rate Brownian motion; (2) BMS = Brownian motion with different rate parameters for each state on a tree; (3) OU1 = Ornstein–Uhlenbeck model with a single optimum for all species; and (4) OUM = Ornstein–Uhlenbeck model with different optimum associated with distinct selective regimes. The OUwie package offers a set of unique models allowing the trait optimum, rate of stochastic motion and strength of selection to vary across selective regimes. We compared the fits of each model using the Akaike information criterion corrected for small sample size (AICc, Burnham & Anderson, 2002; Butler & King, 2004). Preferred model presents lowest AICc with differences larger than 2.

Results

Relations between morphological variables and microhabitat use

The phylogenetic principal component analysis performed on morphometric traits retained two components. Component 1 of the morphological data set for 64 species (PC1 64) explained 57.75% of the total variance and was related to a positive association between measurements taken from hindlimb (femur, tibiofibula, tarsus and foot), humerus and hand, which are the largest contributors, besides the radioulna. Thus, in PC1 64, all variables are represented and can be interpreted as hindlimb plus forelimb distal elements. The second component (PC2 64) explained 18.78% of total variance and was mainly related to forelimb measurements (radioulna – Table 1).

The results of maximum-likelihood estimation, as shown in Table 2, suggest that OUM is the preferred evolutionary model among the four candidates for PC1 64 (morphological component related to overall limb lengths), implying that there are multiple optima of limb lengths associated with different microhabitats. The evolutionary optima estimated for this model reveal a trend of increasing hindlimbs in torrent, arboreal and semi-aquatic species, whereas fossorial and terrestrial species evolve towards optima with shorter hindlimbs (Table 2). For PC2 64 (humerus and radioulna), the preferred model is Brownian motion with different variance rates across microhabitats (BMS), implying that interspecific variation in this structure is not associated with selection related with microhabitat use. Moreover, some microhabitats, such as fossorial and terrestrial, allow higher morphological variance in this morphological component than others (Table 3). Regarding body size, the OU1 models described stabilizing selection towards a single global optimum for all species ($\theta = 1.5461$) that is not associated with selective regimes of microhabitat use.

Relationship among morphological variables, microhabitat use and locomotor performance

Descriptive statistic of morphological variables from each species are represented in Table S2. The phylogenetic principal component analysis performed on morphometric traits resulted in two components. Component 1 of the morphological data set for 19

Table 1 Component loadings from the phylogenetic principal component analysis (pPCA) performed on morphological variables for 64 anuran species from Brazil and Australia.

Component	1	2
Eigenvalues (λ)	4.04	1.31
Total variance explained (%)	57.75	18.78
Cumulative variance (%)	57.75	76.53
Humerus length	–0.580	0.547
Radioulnar length	–0.503	0.755
Hand length	–0.736	0.346
Femur length	–0.878	–0.223
Tibiofibular length	–0.890	–0.352
Tarsus length	–0.863	–0.231
Foot length	–0.776	–0.313

Table 2 AIC scores from models of morphological evolution. AICc = Modified Akaike information criterion; BM1 = fits a single morphological variation rate across species; BMS = fits a model with different morphological variation rates for each microhabitat; OU1 = fits a single optimum for all species; and OUM = fits different optimum for each microhabitat. PC1 64 and PC2 64 = principal component 1 and principal component 2, respectively, of the morphological data set for 64 species. Model fit statistics for PC1 64 (hindlimb: femur, tibiofibular, tarsus, foot; and forelimb: hand), PC2 64 (radioulnar) and body size (SVL 64). The preferred models are highlighted in bold.

Evolutionary models	PC1 64 AICc	PC2 64 AICc	SVL 64 AICc
BM1	23.0733	6.86908	2.83938
BMS	26.1423	**0**	7.85394
OU1	17.9985	8.55756	**0**
OUM	**0**	16.0904	8.48335

Table 3 Parameter estimates for the preferred model PC1 64 (hindlimb: femur, tibiofibular, tarsus, foot; and forelimb: hand) and PC2 64. The estimated optimal values (θ) and morphological diversification rate (σ) for each microhabitat.

Microhabitat use	PC1 64 (OUM) θ	PC2 64 (BMS) Σ
Arboreal	−23.0346	0.5439
Fossorial	3.1506	2.3177
Terrestrial	−11.2799	1.3568
semi-aquatic	−20.9494	0.9386
Torrent	−29.5412	0.1352

species (PC1 19) explained 47.77% of the total variance and was related to a positive association between measurements taken from hindlimb (femur, tibiofibula, tarsus and foot) and hand. The second component (PC2 19) explained 23.87% of total variance and was mostly related to a positive association between forelimb elements (humerus and radioulna) (Table 4).

Table 4 Component loadings from the phylogenetic principal component analysis (pPCA) performed on morphological variables for 19 anuran species.

Component	1	2
Eigenvalues (λ)	3.34	1.67
Total variance explained (%)	47.77	23.87
Cumulative variance (%)	47.77	71.64
Humerus length	−0.425	0.768
Radioulnar length	0.201	0.877
Hand length	−0.515	0.312
Femur length	−0.891	−0.034
Tibiofibular length	−0.948	−0.103
Tarsus length	−0.808	−0.403
Foot length	−0.715	0.201

Table 5 Results for preferred models testing whether locomotor performance is related to morphological variation and microhabitat use. PC1 19 and PC2 19 = principal component 1 and principal component 2, respectively, of the morphological data set for 19 species; HAB = microhabitat use (arboreal, fossorial, terrestrial, torrent, semi-aquatic); AICc = modified Akaike information criterion; dAICc = delta Akaike information criterion; df = number of parameters in the model. The preferred model is highlighted in bold.

Dependent variation	Models	AICc	dAICc	df	Weight
Jumping distance	PC1 19*HAB + PC2 19*HAB	48.8	42.5	8	< 0.001
	PC1 19*HAB + PC2 19	32.4	26.1	7	< 0.001
	PC1 19 + PC2 19 + HAB	16.6	10.3	6	0.0058
	PC1 19 + PC2 19	6.3	**0**	5	**0.9941**

Results from preferred models showed that interspecific differences in locomotor performance relate to both morphological components (Table 5). Species with higher jumping performance were characterized by lower scores from the morphological component 1 [PC1 19] (longer hindlimbs and hands). Thus, arboreal, semi-aquatic and torrent species were characterized by higher jumping performance and longer hindlimbs, than fossorial and terrestrial species (Fig. 1a). Species with higher jumping performance were also characterized by higher scores from the morphological component 2 [PC2 19] (longer humerus and radioulnar lengths), with a less clear association with differences in habits. However, it is noteworthy that torrent species are characterized by short forelimbs for their high locomotor performance (Fig. 1b).

Discussion

Our comparative analysis supports the hypothesis of adaptive morphological diversification within anurans, associated with microhabitat use. Additionally, we show that interspecific morphological diversity is associated with differences in locomotor performance, corroborating functional morphological analyses from previous studies (Emerson, 1985; Nauwelaerts *et al.*, 2007; Gomes *et al.*, 2009). In general, arboreal, semi-aquatic and torrent species are characterized by longer hindlimbs and higher jumping ability than terrestrial and, mainly, fossorial ones. These results emphasize that the inclusion of locomotor performance measurements is highly informative for comparative studies that seek to understand the evolutionary relationship between ecology (e.g. microhabitat use) and morphology (Losos, 1990a,b; Wainwright, 1991; Herrel *et al.*, 2004). Moreover, anurans from two different geographic regions (Brazil and Australia) were included in the morphological analyses,

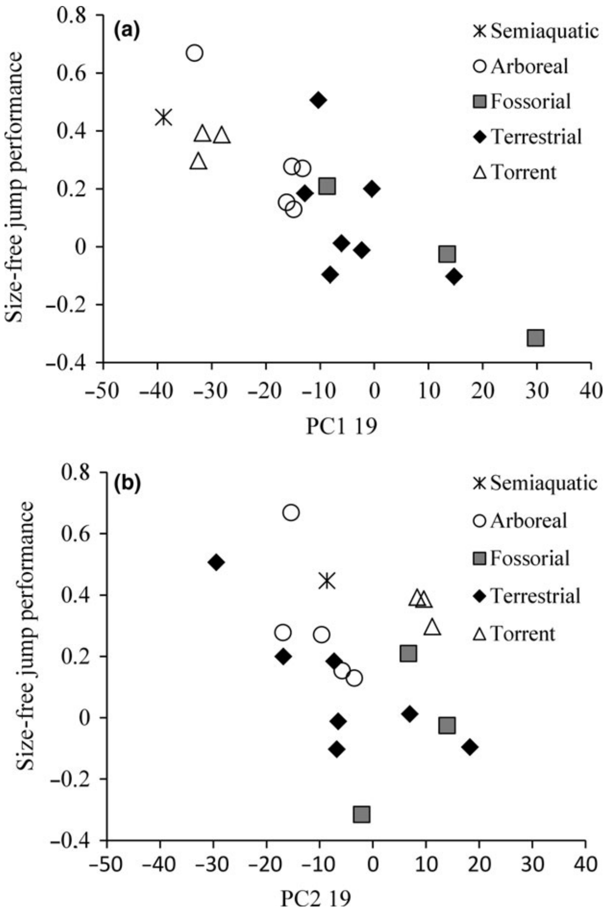

Fig. 1 Scatterplots of morphological components and size-free locomotor performance: (a) describe here better of PC1 19 (hindlimb: femur, tibiofibular, tarsus and foot) and size-free jump performance; (b) describe here better of PC2 19 (humerus and radioulnar) and size-free jump performance.

maximizing transitions between microhabitat uses across the anuran phylogenetic history.

Our analyses suggest that the evolutionary trajectories of morphological traits have changed in a predictable manner associated with changes in microhabitat use. The evolution of morphological components related to relative overall limb length was best explained by OUM. It indicates that the adaptive optima differed between microhabitat types. Biomechanical studies predict distinct adaptive optima associated with the gradient of microhabitat use (Zug, 1972, 1978; Emerson, 1978, 1985; Gomes *et al.*, 2009; Moen *et al.*, 2013). Although torrent and arboreal habits are associated with evolutionary trajectories towards longer relative overall limbs, fossorial habits lead to opposed trajectories. The long limbs that characterized riparian and stream breeder anurans might represent the selective outcome associated with improved ability to swim under high-flow rates (Dennis & Mahony, 1994; Lewis & Rohweder, 2005; Hoskin, 2010; Vidal-García *et al.*, 2014). *Heleophryne hewitti* (Heleophrynidae), for example, is an African anuran that inhabits fast-flowing mountain streams and has disproportionately long and muscular legs, which may have evolved in association with improved swimming ability against high water resistance (Laurent, 1964). Longer hindlimbs also increase the time of muscular force application against the substrate and thus the distance jumped. Interestingly, our results highlight an interspecific variation in jumped distance associated with the microhabitat use and corroborate previous studies showing that hindlimbs evolve in association with higher jumping performance in anurans (Zug, 1972; Emerson, 1985; Gomes *et al.*, 2009).

Our results confirm those from previous studies, showing that fossorial species possess tibiofibulae particularly shortened in comparison with jumping anurans (Enriquez-Urzelai *et al.*, 2015). In opposition to the longer hindlimbs, shorter tibiofibula function as outlevers (Hildebrand, 1974). In this way, shorter hindlimbs increase the force generated during scooping (Emerson, 1976), improving burrowing performance (Duellman & Trueb, 1986; Wells, 2007; Vidal-García *et al.*, 2014). Reduced hindlimb lengths can be found in species that burrow either forwards or backwards (Vidal-García *et al.*, 2014), and shorter legs are also found in species that walk rather than hop as demonstrated in two species of *Arenophryne* (Tyler *et al.*, 1980; Vidal-García *et al.*, 2014). Moreover, previous studies have associated the presence of skin toxins of more terrestrial and active forager anurans with limited ability to flee from predators (Bennett & Licht, 1973, 1974; Toft, 1981). In our study, for example, two terrestrial species (*Leptodactylus labyrinthicus* and *Rhinella schneideri*) were the weakest jumpers and frequently remained motionless when stimulated to jump in locomotor performance tests. Toledo *et al.* (2005) also observed that *Leptodactylus labyrinthicus,* a species characterized by toxic and distasteful skin secretions, commonly relied on immobility when exposed to simulated predation events. Additionally, toads (including genus *Rhinella*) evolved specialized groups of large secretory glands on their shoulders, the parotid glands, which provide a mixture of substances with powerful effects against predators (Jared *et al.*, 2011).

Forelimbs, in contrast to the hindlimbs, are generally considered to be conserved among anurans (Manzano *et al.*, 2008), and they are associated with absorbing impact forces during landing and providing body support during sitting or walking (Emerson, 1983; Nauwelaerts *et al.*, 2007). Moreover, frogs often use their forelimbs to capture and transport prey (Gray *et al.*, 1997). Our results show that fossorial species indeed exhibit higher variation in radioulnar length, which may explain their higher morphological forelimb disparity than the hindlimbs. The preferred evolutionary model for morphological components related to forelimbs (mainly radioulnar length) in our study was the BM process with different rates of evolution among selective regimes. Vidal-García *et al.* (2014) observed that Australian forward burrowers (*Arenophryne, Myobatrachus*) had relatively longer arms. Otherwise, their sister taxon *Metacrinia* (Read *et al.*, 2001), which does not burrow (Tyler & Doughty, 2009), had relatively shorter arms, suggesting that longer arms are important in burrowing forward (Vidal-García *et al.*, 2014). Within the species included in the present study, information on digging mode is available for the Brazilian *Dermatonotus muelleri*, which is described as a forward burrowing frog by Nomura *et al.* (2009). It is worth noting that *D. muelleri* is one of the species characterized by longer forelimbs in our data set. However, precise information of burrowing behaviour is lacking for most of the species included in our data set, limiting our ability to detect patterns of association between forelimb lengths and digging mode. Otherwise, we show that species associated with fast-flowing streams and waterfalls exhibit lower variation in radioulnar length and short forelimbs for their high locomotor performance. Additional studies on biomechanics of locomotion in riparian anurans are necessary to understand the functional implications of this morphological pattern.

Regarding body size, we found just one adaptive optimum among all microhabitat use (OU1), corroborating previous results from an interspecific comparison of Western Mediterranean anurans varying in locomotory mode (Enriquez-Urzelai *et al.*, 2015). In fact, interspecific variation in body size is larger within than between categories of microhabitat use in our data set, with SVL ranging from 18.01 to 94.33 mm, 17.07 to 149.33 mm, 35.12 to 51.70 mm, 24.70 to 82.87 mm and 17.48 to 91.49 mm, respectively for fossorial, terrestrial, semi-aquatic, torrent and arboreal anurans. In this way, our analyses suggest that variation in anuran body size is not particularly associated with diversification in microhabitat use.

Anuran locomotor modes comprise burrowing, swimming, walking, hopping, jumping, climbing and gliding (Jorgensen & Reilly, 2013), and anuran interspecific variation in locomotor mode has been frequently associated with morphological diversity (Rand, 1952; Zug, 1972, 1978; Emerson, 1979, 1988; Gomes *et al.*, 2009; Jorgensen & Reilly, 2013; Moen *et al.*, 2013 Vidal-García *et al.*, 2014). Our analyses suggest that hindlimb morphological traits have changed in association with jumping performance, and diversification in microhabitat use is an important selective pressure moulding these evolutionary trajectories. According to our results, torrent and arboreal anurans are characterized by longer hindlimbs and higher jumping ability. Among the torrent anurans from the Atlantic Forest (Brazil), males from the genus *Hylodes* are territorial and call from rocks along the streams to attract females and to keep conspecific males at distance. These frogs are wary, generally escaping through one long jump to the water when disturbed (Pavan *et al.*, 2001). Calling behaviour in this genus is also complemented by leg stretching and foot flagging (Narvaes, 1997), a behavioural pattern associated with frogs that inhabit noisy streams, facilitating the transmission of information about location of the signaller (Hödl & Amézquita, 2001). However, a possible cost of this type of behaviour would be greater exposure to predators, and this disadvantage is possibly circumvented by the rapid escape through diving into the water (Narvaes, 1997). In this way, longer hindlimbs and higher jumping ability might have evolved in the context of elaborated courtship and escape behaviour in torrent anurans. We recognize that a limitation of the present study is that only Brazilian torrent species were included in the comparative analyses. The inclusion of torrent anurans from different phylogenetic groups and zoogeographical areas might provide stronger evidence of convergent evolution of post-cranial skeleton in association with torrent microhabitat use.

Our results corroborate previous studies suggesting that hindlimb length evolved in association with a higher jumping performance in anurans (Zug, 1972; Emerson, 1985; Gomes *et al.*, 2009). The origin and diversity of adaptations for jumping performance in anurans have been associated with different selective pressures, including predation (Gans & Parsons, 1966; Zug, 1972; Emerson, 1978; Gomes *et al.*, 2009). Predation is intense during metamorphosis and early juvenile life of amphibians (Arnold & Wassersug, 1978), and the origin of saltatory locomotion in anurans has been suggested to be an adaptation to escape predation (Gans & Parsons, 1966). However, it is important to consider that although anurans show a clear pattern of coevolution of hindlimb length and saltatory performance, the proportion of the maximum jumping performance elicited in response to predators is affected by several factors, including microhabitat structure (Gomes *et al.*, 2002; Martín *et al.*, 2005). Rapid adaptive increase in anuran hindlimb length has been also observed in alternative ecological contexts, such as the coevolution with increased rates of movement and dispersal during the invasion of *Rhinella marina* in Australia (Phillips *et al.*, 2006).

In conclusion, our results show that microhabitat use is an important selective pressure associated with jumping performance and post-cranial morphological diversification in anurans. Particularly, arboreal, semi-aquatic and torrent anurans are characterized by overall limb lengths and higher jumping performance than terrestrial and, particularly, fossorial species. In this way, these results corroborate the hypothesis that evolutionary modifications of overall limb morphology have been important in the diversification of locomotor performance across the anuran phylogeny and that such evolutionary changes converged in different phylogenetic groups adapted to similar microhabitat use in different zoogeographical regions. The discussion presented here emphasizes that different or even conflicting selective pressures, such as jumping ability and burrowing efficiency, might act on the evolution of complex structures such as vertebrate hindlimbs in association with diversification in microhabitat use. Further studies investigating possible associations between pelvic morphology and habit, together with data on different patterns of antipredator behaviour, would enable a better understanding of the functional implications of morphological divergence in this phylogenetic group.

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References

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

Arnold, S.J. & Wassersug, R.J. 1978. Differential predation on metamorphic anurans by garter snakes (*Thamnophis*): social behaviour as a possible defense. *Ecology* **59**: 1014–1022.

Ascarrunz, E., Rage, J.-C., Legreneur, P. & Laurin, M. 2016. *Triadobatrachus massinoti*, the earliest known lissamphibian (Vertebrata: Tetrapoda) re-examined by μCT-Scan, and the evolution of trunk length in batrachians. *Contrib. Zool.* **85**: 201–234.

Baldissera, F.A.B. Jr, Caramaschi, U. & Haddad, C.F.B. 2004. Review of the *Bufo crucifer* species group, with descriptions of two new related species (Amphibia, Anura, Bufonidae). *Arq. Mus. Nac.* **62**: 255–282.

Beaulieu, J.M., Jhwueng, D.C., Boettiger, C. & O'Meara, B.C. 2012. Modeling stabilizing selection: expanding the Ornstein-Uhlenbeck model of adaptive evolution. *Evolution* **66**: 2369–2383.

Bennett, A.F. & Licht, P. 1973. Relative contributions of anaerobic and aerobic energy production during activity in Amphibia. *J. Comp. Physiol.* **87**: 351–360.

Bennett, A.F. & Licht, P. 1974. Anaerobic metabolism during activity in amphibians. *Comp. Biochem. Physiol.* **48A**: 319–327.

Bertoluci, J. & Rodrigues, M.T. 2002. Utilização de hábitats reprodutivos e microhábitats de vocalização em uma taxocenose de anuros (Amphibia) da Mata Atlântica do sudeste do Brasil. *Pap. Avul. Zool.* **42**: 287–297.

Burnham, K.P. & Anderson, D.R. 2002. *Model selection and multi-model inference: a practical information-theoretic approach*. Springer, New York, NY.

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

Christian, K.A. & Tracy, R. 1981. The effect of the thermal environment on the ability of hatchling Galapagos land iguanas to avoid predation during dispersal. *Oecologia* **49**: 218–223.

Crompton, R.H. 1984. Foraging, habitat structure, and locomotion in two species of *Galago*. In: *Adaptations for Foraging in Non-human Primates* (P.S. Rodman, J.G.H. Cant, eds), pp. 73–111. Columbia University Press, New York, NY.

Dennis, A. & Mahony, M. 1994. *Experimental translocation of the endangered sharp-snouted day frog Taudactylus acutirostris* and observations of the cause of declines among montane riparian frogs. Unpublished report prepared for Wet Tropics Management Authority, Cairns.

Duellman, W.E. & Trueb, L. 1986. *Biology of amphibians*. McGraw-Hill, New York, NY.

Emerson, S.B. 1976. Burrowing in frogs. *J. Morphol.* **149**: 437–458.

Emerson, S.B. 1978. Allometry and jumping in frogs: helping the twain to meet. *Evolution* **32**: 551–564.

Emerson, S.B. 1979. The ilio-sacral articulation in frogs: form and function. *Biol. J. Linn. Soc.* **11**: 153–168.

Emerson, S.B. 1983. Functional analysis of frog pectoral girdles. The epicoracoid cartilages. *J. Zool.* **201**: 293–308.

Emerson, S.B. 1985. Jumping and leaping. In: *Functional Vertebrate Morphology* (M.E. Hildebrand, D. Bramble, K. Laim & D. Wake, eds), pp. 58–76. Harvard University Press, Cambridge, MA.

Emerson, S.B. 1988. Convergence and morphological constraint in frogs: variation in postcranial morphology. *Fieldiana Zool.* **43**: 1–19.

Emerson, S.B. 1991. The ecomorphology of Bornean tree frogs (family Rhacophoridae). *Zool. J. Linn. Soc.* **101**: 337–357.

Engqvist, L. 2005. The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Anim. Behav.* **70**: 967–971.

Enriquez-Urzelai, U., Montori, A., Llorente, G.A. & Kaliontzopoulou, A. 2015. Locomotor mode and the evolution of the hindlimb in western Mediterranean anurans. *Evol. Biol.* **42**: 199–209.

Faivovich, J. 2002. A cladistic analysis of *Scinax* (Anura: Hylidae). *Cladistics* **18**: 367–393.

Frost, D.R. 2015. *Amphibian Species of the World: An Online Reference*. Version 6.0. American Museum of Natural History, New York. Eletronic Database accessible at http://research.amnh.org/herpetology/amphibia/index.html (accessed on 18 March 2015).

Gans, C. & Parsons, T.S. 1966. On the origin of the jumping mechanism in frog. *Evolution* **20**: 92–99.

Garland, T.J. 1999. Laboratory endurance capacity predicts variation in field locomotor behaviour among lizard species. *Anim. Behav.* **58**: 77–83.

Gomes, F.R., Bevier, C.R. & Navas, C.A. 2002. Environmental and physiological factors influence antipredator behavior in *Scinax hiemalis* (Anura: Hylidae). *Copeia* **2002**: 994–1005.

Gomes, F.R., Rezende, E.L., Grizante, M.R. & Navas, C.A. 2009. The evolution of jumping performance in anurans: morphological correlates and ecological implications. *J. Evol. Biol.* **22**: 1088–1097.

Gray, L., O'Reilly, J.C. & Nishikawa, K.C. 1997. Evolution of forelimb movement patterns for prey manipulation in anurans. *J. Exp. Zool.* **277**: 417–424.

Haddad, C.F.B., Toledo, L.F. & Prado, C.P.A. 2008. *Anfíbios da Mata Atlântica: Guia dos anfíbios anuros da Mata Atlântica*. Editora Neotropica, São Paulo. 244p.

Hansen, T.F. 1997. Stabilizing selection and the comparative analysis of adaptation. *Evolution* **51**: 1341–1351.

Harrison, A.S., Revell, L.J. & Losos, J.B. 2015. Correlated evolution of microhabitat, morphology, and behavior in West Indian *Anolis* lizards: a test of the habitat matrix model. *Behaviour* **152**: 1187–1207.

Herrel, A., Vanhooydonck, B., Joachim, R. & Irschick, D.J. 2004. Frugivory in polychrotid lizards: effects of body size. *Oecologia* **140**: 160–168.

Herrel, A., Moureaux, C., Laurin, M., Daghfous, G., Crandell, K., Tolley, K.A. *et al.* 2016. Frog origins: inferences based on ancestral reconstructions of locomotor performance and anatomy. *Fossil Imprint* **72**: 108–116.

Hildebrand, M. 1974. *Analysis of Vertebrate Structure*. John Wiley and Sons, New York, NY.

Hödl, W. & Amézquita, A. 2001. Visual signaling in anuran amphibians. In: *Anuran Communication* (M.J. Ryan, ed.), pp. 121–141. Smithsonian Institution Press, Washington, DC.

Hoskin, C. 2010. Breeding behaviour of the Barred Frog *Mixophyes coggeri*. *Mem. Queensl. Mus.* **55**: 1–7.

Husak, J.F., Fox, S.F., Loven, M.B. & Van Den Bussche, R.A. 2006. Faster lizards sire more offspring: sexual selection on whole-animal performance. *Evolution* **60**: 2122–2130.

Irschick, D.J. & Losos, J.B. 1999. Do lizards avoid habitats in which performance is submaximal? The relationship between sprinting capabilities and structural habitat use in Caribbean anoles. *Am. Nat.* **154**: 293–305.

Jared, C., Antoniazzi, M.M., Verdade, V.K., Toledo, L.F. & Rodrigues, M.T. 2011. The Amazonian toad *Rhaebo guttatus* is able to voluntarily squirt poison from the paratoid macroglands. *Amphibia-Reptilia* **32**: 546–549.

Jorgensen, M.E. & Reilly, S.M. 2013. Phylogenetic patterns of skeletal morphometrics and pelvic traits in relation to locomotor mode in frogs. *J. Evol. Biol.* **26**: 929–943.

Laurent, R. 1964. Adaptive modifications in frogs of an isolated highland fauna in central Africa. *Evolution* **18**: 458–467.

Lewis, B. & Rohweder, D. 2005. Distribution, habitat, and conservation status of the Giant Barred Frog, *Mixophyes iteratus* in the Bungawalbin catchment, northeastern New South Wales. *Pac. Conserv. Biol.* **11**: 189–197.

Losos, J.B. 1990a. Concordant evolution of locomotor behavior, display rate, and morphology in West Indian *Anolis* lizards. *Anim. Behav.* **39**: 879–890.

Losos, J.B. 1990b. The evolution of form and function: morphology and locomotor performance ability in West Indian Anolis lizards. *Evolution* **44**: 1189–1203.

Maddison, W.P. & Maddison, D.R. 2011. Mesquite: a modular system for evolutionary analysis. Version 2.75. http://mesquiteproject.org (Accessed 20 April 2015).

Manzano, A.S., Abdala, V. & Herrel, A. 2008. Morphology and function of the forelimb in arboreal frogs: specializations for grasping ability? *J. Anat.* **213**: 296–307.

Marsh, R.L. 1994. Jumping ability of anurans. In: *Comparative Vertebrate Exercise Physiology* (J.H. Jones, ed.), pp 51–111. Academic Press, San Diego, CA.

Martín, J., Luque-Lurena, J.J. & López, P. 2005. Factors affecting escape behavior of Iberian green frogs (*Rana perezi*). *Can. J. Zool.* **83**: 1189–1194.

Martins, E.P. & Hansen, T.F. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am. Nat.* **149**: 646–667.

Martins, M., Araújo, M.S., Sawaya, R.J. & Nunes, R. 2001. Diversity and evolution of macrohabitat use, body size and morphology in a monophyletic group of Neotropical pitvipers (*Bothrops*). *J. Zool.* **254**: 529–538.

Meylan, S. & Clobert, J. 2004. Maternal effects on offspring locomotion: influence of density and corticosterone elevation in the lizard *Lacerta vivipara*. *Physiol. Biochem. Zool.* **77**: 450–458.

Moen, D.S., Irschick, D.J. & Wiens, J.J. 2013. Evolutionary conservatism and convergence both lead to striking similarity in ecology, morphology and performance across continents in frogs. *Proc. Biol. Sci.* **280**: 20132156.

Moreno, E. & Carrascal, L.M. 1993. Leg morphology and feeding postures in four *Parus* species: an experimental ecomorphological approach. *Ecology* **74**: 2037–2044.

Narvaes, P. 1997. Comportamento territorial e reprodutivo de uma nova espécie de *Hylode*s (Amphibia, Anura, Leptodactylidae) da Mata Atlântica do Sudeste do Brasil. [Dissertation]. University of Sao Paulo. 104p. www.teses.usp.br/teses/disponiveis/41/41133/tde-22032004.../Hylodes.pdf

Nauwelaerts, S., Ramsay, J. & Aerts, P. 2007. Morphological correlates of aquatic and terrestrial locomotion in a semi-aquatic frog, *Rana esculenta*: no evidence for a design conflict. *J. Anat.* **210**: 304–317.

Nomura, F., Rossa-Feres, D. & Langeani, F. 2009. The burrowing behavior of *Dermatonotus muelleri* (Anura, Microhylidae) with reference to the origin of the burrowing behavior. *J. Ethol.* **27**: 195–201.

Pavan, D., Narvaes, P. & Rodrigues, M.T. 2001. A new species of leptodactylid frog from the Atlantic forest of southeastern Brazil with notes on the status and the speciation of the *Hylodes* species groups. *Pap. Avulsos Zool.*, **41**: 407–425.

Phillips, B., Brown, G.P., Webb, J.K. & Shine, R. 2006. Invasion and the evolution of speed in toads. *Nature* **439**: 803.

Pombal, J.P. Jr & Izecksohn, E. 2011. Uma nova espécie de Brachycephalus (Anura, Brachycephalidae) do estado do Rio de Janeiro. *Pap. Avulsos Zool.* **51**: 443–451.

Purvis, A., Gittleman, J.L., Cowlishaw, G. & Mace, G.M. 2000. Predicting extinction risk in declining species. *Proc. Biol. Sci.* **267**: 1947–1952.

Pyron, R. 2014. Biogeographic analysis reveals ancient continental vicariance and recent oceanic dispersal in amphibians. *Syst. Biol.* **63**: 779–797.

Pyron, R. & Wiens, J. 2011. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Mol. Phylogenet. Evol.* **61**: 543–583.

R Core Team. 2010. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Rand, A.S. 1952. Jumping ability of certain anurans, with notes on endurance. *Copeia* **1952**: 15–20.

Read, K., Keogh, J.S., Scott, I., Roberts, J. & Doughty, P. 2001. Molecular phylogeny of the australian frog genera *Crinia, Geocrinia*, and allied taxa (Anura, Myobatrachidae). *Mol. Phylogenet. Evol.* **21**: 294–308.

Revell, L.J. 2009. Size-correction and principal components for interspecific comparative studies. *Evolution* **63**: 3258–3268.

Shubin, N.H. & Jenkins, F.A. 1995. An early Jurassic jumping frog. *Nature* **377**: 49–52.

Stuart-Fox, D.M. & Ord, T.J. 2004. Sexual selection, natural selection and the evolution of dimorphic coloration and ornamentation in agamid lizards. *Proc. Biol. Sci* **271**: 2249–2255.

Toft, C.A. 1981. Feeding ecology of Panamanian litter anurans: patterns in diet and foraging mode. *J. Herpetol.* **15**: 139–144.

Toledo, L.F., Tozetti, A. & Zina, J. 2005. *Leptodactylus labyrinthicus* (Pepper Frog): defensive repertoire. *Herpetol. Bull.* **90**: 29–31.

Tyler, M.J. & Doughty, P. 2009. *Field Guide to Frogs of Western Australia*. Western Australia Museum, Perth, WA.

Tyler, M.J. & Walker, S.J. 2011. *Frogs of South Australia*, 3rd edn. Michael Tyler and Associates, Belair SA.100 p.

Tyler, M.J., Roberts, J. & Davies, M. 1980. Field observations on *Arenophryne rotunda* Tyler, a leptodactylid frog of coastal sandhills. *Aust. Wild. Res.* **7**: 295–304.

Vidal-García, M., Byrne, P.G., Roberts, J.D. & Keogh, J.S. 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, P.C. 1991. Ecological morphology: experimental functional anatomy for ecological problems. *Am. Zool.* **31**: 680–693.

Wells, K. 2007. *The Ecology and Behavior of Amphibians*. The University of Chicago Press, Chicago, IL.

Zug, G.R. 1972. Anuran locomotion: structure and function. 1. Preliminary observations on the relation between jumping and osteometrics of appendicular and postaxial skeleton. *Copeia* **1972**: 613–624.

Zug, G.R. 1978. Anuran locomotion: structure and function, 2: jumping performance of semiaquatic, terrestrial, and arboreal frogs. *Smithson. Contrib. Zool.* **276**: 1–3.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1 Composite phylogenetic tree for the 64 anuran species from Brazil and Australia included in the present morphological study, with topology and divergence times estimates (Mya) based on the literature.

Figure S2 Composite phylogenetic tree for the 19 anuran species from Brazil and Australia included in the present locomotor performance study, with topology and divergence times estimates (Mya) based on the literature.

Table S1 Morphological traits (mean ± standard deviation) of 44 Brazilian species and 20 Australian species of anurans that differ in habits.

Table S2 Morphological traits (mean ± standard deviation) of 15 Brazilian species and 4 Australian species of anurans that differ in habits.

Table S3 Species, localities and collect dates.

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