The role of phylogeny and ecology in shaping morphology in 21 genera and 127 species of Australo-Papuan myobatrachid frogs

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Abstract

Body shape is predicted to differ among species for functional reasons and in relation to environmental niche and phylogenetic history. We quantified morphological differences in shape and size among 98.5% of the 129 species and all 21 genera of the Australo-Papuan endemic myobatrachid frogs to test the hypothesis that habitat type predicts body shape in this radiation. We tested this hypothesis in a phylogenetic context at two taxonomic levels: across the entire radiation and within the four largest genera. Thirty-four external measurements were taken on 623 museum specimens representing 127 species. Data for seven key environmental variables relevant to anurans were assembled for all Australian-distributed species based on species' distributions and 131,306 locality records. The Australo-Papuan myobatrachid radiation showed high diversity in adult body size, ranging from minute (15 mm snout–vent length) to very large species (92 mm), and shape, particularly sin relative limb length. Five main morphological and environmental summary variables displayed strong phylogenetic signal. There was no clear relationship between body size and environmental niche, and this result persisted following phylogenetic correction. For most species, there was a better match between environment/habitat and body shape, but this relationship did not persist following phylogenetic correction. At a broad level, species fell into three broad groups based on environmental niche and body shape: 1) species in wet habitats with relatively long limbs, 2) species in arid environments with relatively short limbs (many of which are forward or backward burrowers) and 3) habitat generalist species with a conservative body shape. However, these patterns were not repeated within the four largest genera – *Crinia*, *Limnodynastes*, *Pseudophryne* and *Uperoleia*. Each of these genera displayed a highly conservative anuran body shape, yet individual species were distributed across the full spectrum of Australian environments. Our results suggest that phylogenetic legacy is important in the evolution of body size and shape in Australian anurans, but also that the conservative body plan of many frogs works well in a wide variety of habitats.

Introduction

Morphological differences between populations or species evolve for a wide variety of reasons. For example,

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they can reflect adaptive responses to competition among individuals for mates (sexual selection), specific physiological requirements (Lewis, 1970; Feder & Burggren, 1992) and local habitats (Duellman & Trueb, 1986; Bossuyt & Milinkovitch, 2000; Wells, 2007). Body size and shape, in particular, can vary enormously among even closely related species, and understanding the cause of this variation has been a long-standing focus of evolutionary research (Hutchinson & MacArthur, 1959; LaBarbera, 1989; Blackburn & Gaston, 1994). Some body-shape

patterns are more easily produced than others (Foote, 1994; Beldade *et al.*, 2002). The evolution of different morphologies could be a consequence of environmental, developmental and/or evolutionary constraints (Losos & Miles, 1994). For example, at a broad scale, in mammals, Bergmann's rule posits that species tend to be larger in cooler environments due to the need to minimize heat loss (Brown & Lee, 1969; James, 1970). At a finer scale, in *Anolis* lizards, body size, shape, and in particular, limb proportions are demonstrated to be the result of intensive selection on habitat partitioning following colonization (Losos, 1990; Harmon *et al.*, 2005). Consequently, if we compare morphology of lineages of organisms that have radiated across continents with high levels of variation in climate, topography and vegetation structure, we might expect to see predictable patterns in the evolution of morphotypes reflected across an entire radiation and to have these patterns repeated at lower taxonomic levels within species-rich groups.

Anuran amphibians (frogs and toads) are a morphologically homogeneous group relative to other tetrapod radiations, but they still display a great deal of specialization across the 54 families and 6328 species (AmphibiaWeb., 2013), with recurrent patterns in morphotype (Duellman & Trueb, 1986; Bossuyt & Milinkovitch, 2000; Wells, 2007; van Buskirk, 2009). For example, arboreal species have evolved independently in multiple lineages (rhacophorids, ranids, hylids), and in each case, 'suckers' on the toes and relatively elongate limbs are strongly correlated with this habit type (Duellman & Trueb, 1986; Wells, 2007). Similarly, many burrowing frogs have relatively short limbs, a rotund, squat body shape and well-developed metatarsal tubercles (Duellman & Trueb, 1986; Wells, 2007). The rotund body shape has been interpreted as one that minimizes surface area-to-volume ratios and therefore minimizes evaporative water loss (Tyler, 1989), whereas the metatarsal tubercle is thought to improve burrowing performance. However, there are multiple solutions to both these problems. Forward burrowing has evolved several times in frogs with no metatarsal tubercle (e.g. *Arenophryne*: Davies, 1984; *Hemisus*: Nomura *et al.*, 2009), and water loss can be reduced by forming impermeable cocoons by incomplete sloughing and keratinization of the skin in burrowing species (Lee & Mercer, 1967; Flanigan *et al.*, 1993; Withers & Richards, 1995; Christian & Parry, 1997) or by changing resistance to water loss through the skin, for example, by covering the skin with wax (Shoemaker *et al.*, 1987; Tracy *et al.*, 2010). Laurent (1964) suggested that frogs from very wet environments have relatively long limbs, but this may not be an adaptation to wet habitats *per se*. For instance, many 'wet' habitat species might live in high-flow stream systems where longer limbs give better propulsion when swimming (Emerson, 1978). Furthermore, many of these species climb trees and make use of the canopy layer in rainforest systems, so relatively long limbs may improve arboreal agility (Emerson, 1978, 1985, 1991; Zug, 1978).

Testing between mechanical (all burrowers have a common shape) and physiological requirements (all arid-zone frogs are rotund to reduce surface area-to-volume ratios) requires comparisons of frogs across habitats and repeated measures across lineages to assess generality of pattern. The Australian myobatrachid frogs represent two major lineages: the subfamilies Limnodynastinae and the Myobatrachidae (Pyron & Wiens, 2011). Both groups occupy a wide range of habitats and climate types across the whole Australian continent, including: rainforest (tropical to temperate), wood and grasslands in the wet–dry tropics, extreme arid deserts in the continental interior and seasonally arid Mediterranean climates across Southern Australia (such as forests, woodland, shrub and grasslands) (Tyler, 1989; Littlejohn *et al.*, 1993; Roberts, 1993). Within both subfamilies, there are speciose genera (*Crinia*, *Limnodynastes*, *Pseudophryne*, *Uperoleia*), each with species that specialize in a broad range of habitats, from arid deserts with unpredictable rainfall to predictably wet rainforest. Some myobatrachid species also can burrow (forwards or backwards, Cogger, 1979; Emerson, 1976; Davies, 1984; Tyler & Doughty, 2009) and can spend extended periods underground with and without specific mechanisms for preventing water loss (Blaylock *et al.*, 1976; McClanahan *et al.*, 1978; Withers *et al.*, 1984; McClanahan *et al.*, 1994; Cartledge *et al.*, 2006). Thus, they present an ideal group for looking at broad patterns in adaptive morphology, testing for repeated evolution of similar patterns within species-rich genera and investigating environmental correlates and phylogenetic constraints.

We inferred the environmental niche and examined body size and shape variation displayed by all species and genera of myobatrachid frogs to test whether environmental factors determine their morphology. We did this by testing two simple hypotheses: 1) is the rotund, short-limbed morphology of burrowing frog species an adaptation to aridity and 2) are frog species from wet environments more likely to have longer legs? Based on these hypotheses, we predicted that: 1) the species occurring in arid habitats (such as *Arenophryne*, *Myobatrachus* and *Notaden*) would display more squat bodies and short limbs, 2) species from wet habitats (such as *Mixophyes*, *Taudactylus* and *Philoria*) would display stream-lined bodies with long legs and 3) species occurring in intermediate habitats would display intermediate or conservative anuran body shapes. We test these predictions with detailed morphological and environmental data sets for all species, and in the context of their phylogenetic history, to assess whether the different morphological patterns are constrained by phylogeny, restricting directional selection. This was evaluated in both a broad-scale analysis across all myobatrachid frog species and at a finer scale in the four largest genera: *Crinia*, *Limnodynastes*, *Pseudophryne* and *Uperoleia*.

We also provide alternative explanations for the evolution of some of these general anuran body forms.

Materials and methods

We examined 127 species of the Australian myobatrachid radiation from the following genera: *Adelotus, Arenophryne, Assa, Crinia, Geocrinia, Heleioporus, Lechriodus, Limnodynastes, Metacrinia, Mixophyes, Neobatrachus, Notaden, Paracrinia, Philoria, Platyplectrum, Pseudophryne, Rheobatrachus, Spicospina, Taudactylus* and *Uperoleia*. We did not include myobatrachid species from New Guinea as appropriate environmental data were not available, but New Guinea only has four species, all of which are members of included genera. We measured five adult specimens in 123/127 species, but for the remaining four species, we measured three or four frogs due to limited availability. This sample size was suitable for our purposes as our primary interests were interspecific and intergeneric differences, not individual variation. Specimens were provided by Australian Museum (AM), South Australian Museum (SAM), Museum Victoria (VM), Western Australian Museum (WAM), Queensland Museum (QM), Tasmanian Museum (TM). Details are provided in Appendix S1.

Morphological traits

We preferentially measured female frogs to avoid the confounding issue of sexually selected traits in males and sexual size dimorphism. When choosing specimens, we looked at external cues to determine sex as our loan conditions prevented dissection of all individuals, but in a small number of species, sex was confirmed through dissection, with permission. We identified female specimens by external cues: for example, absence of vocal sacs in *Adelotus* and *Mixophyes*, lack of hip pouches in *Assa darlingtoni*, absence of spines on fingers in *Heleioporus*, lack of black throats in *Crinia*, cloacal fimbriation, lack of black throats in *Uperoleia*, lack of nuptial pads in *Limnodynastes* and *Platyplectrum* species or eggs visible through the ventral skin. In the absence of external cues, specimens were dissected and females were selected based on the presence of ovaries. We took 33 external morphological measurements (nearest 0.1 mm) with digital callipers to obtain a thorough picture of head, body and limb morphology (Fig. S1 and Table S1). To ensure consistency, all measurements were taken by M. Vidal-García. We obtained data on forward versus backward burrowing behaviour from Cogger (1979) and Tyler and Knight (2009).

Environmental variables

Data on distribution of individual frog species, as well as numerous environmental variables (see below), were obtained from the Atlas of Living Australia (ALA –

Atlas of Living Australia, 2011) online database. This resource amalgamates Australian Museum specimen records and allowed us to plot the nearly complete geographic distribution of each species in Australia. Records from the Queensland Museum (not included in the Atlas of Living Australia) were added manually to complete coverage of species distributions. In total, we assembled 21 environmental variables for 10×10 km blocks centred on the location of each museum record. We collected this information for every Australian myobatrachid species, resulting in 131,306 records. For further analyses, we then chose seven of these variables that represent the full spectrum of habitat variables and that are of relevance to frogs: annual mean evaporation, precipitation in the warmest quarter (Bio18: Bioclim-Global Climate Data, 2013), temperature in the warmest period (Bio05: Bioclim-Global Climate Data, 2013), soil nutrient status, annual mean moisture index (Bio28: Bioclim-Global Climate Data, 2013), topographic slope and mean net primary productivity. To avoid obtaining a biased characterization of the habitat occupied by each species, we scored each species as present or absent in each 10×10 km block irrespective of the total number of records in any one grid square. For each species, we then calculated the mean values for the seven environmental variables based on the whole geographic distribution of each species.

Statistical analysis

We first carefully examined the data for all 33 morphological characters in order to determine whether any of the variables were prone to measurement errors. Shoulder width and forearm width proved difficult to measure accurately and were discarded from further analysis. Width of the tympanic membrane could not be measured in many species because tympana were absent (e.g. *Pseudophryne* and some *Crinia* species) or covered by skin, so it also was discarded. The length of individual fingers and toes on a given individual was highly correlated. To avoid over-representing finger and toe lengths in multivariate analyses, we only included I and IV finger length, and, I and V toe length - fingers and toes that vary the most between species.

The remaining 25 morphological raw variables were first evaluated using principal component analysis (PCA). For analyses, we calculated standard principal components with imputation of missing values so that all animals could be included in the PCA (few values were missing). Our primary interest was in the quantification of body size and shape differences between species and genera. The first principal component represented variation in body size, and the second principal component summarized shape differences among the species. We also used PCA to summarize the seven environmental variables for each species, and PC 1

from this analysis was used as an overall measure of environmental niche. We then tested for any predictive relationship between body size (morphology PC 1) and body shape (morphology PC 2) and the environment occupied by each species (environment PC 1) with linear regression. We also examined the distribution of burrowing behaviour (forward borrowing, backward burrowing, no borrowing) as it relates to body size/shape and habitat. All PCA analyses and nonphylogenetically corrected regression analyses were conducted using JMP® 8.0 software (SAS-Institute., 2009)

Phylogenetic history also could strongly impact the distribution of body size and shape and habitat preferences; therefore, we also performed tests of phylogenetic signal on individual variables and phylogenetic generalized least squares (PGLS) regression analyses. For all the analyses, we used a multilocus maximum likelihood molecular phylogeny with branch lengths that we generated for most (92%) of the taxa for which we had morphological and environmental data (J.S. Keogh, P.G. Byrne & J.D. Roberts, unpublished data). The data comprised 3658 base pairs from two mtDNA genes (ND2 and 12S) and two nDNA loci (Rag1 and Rhodopsin) and are part of ongoing phylogenetic work on the group. Multiple specimens of each species were used to confirm the monophyly of the species, with a single specimen of each species then used in the final tree for phylogenetic comparative analyses. We used the package *ape* (Paradis *et al.*, 2004) to produce an ultrametric tree with branch lengths approximating proportions of the total age and then performed the phylogenetic signal tests and PGLS tests in the R statistical environment (Ihaka & Gentleman, 1996). We used the package *geiger* (Harmon *et al.*, 2009) to calculate Pagel's lambda (λ) (Pagel, 1999), as a measure of phylogenetic signal for six relevant variables: morphology PC 1 (body size), morphology PC 2 (shape), relative arm (arm length/snout–vent length ratio), relative leg (leg length/snout–vent length ratio), environmental PC 1 (environmental niche), environmental PC 2 (environmental niche). Pagel's λ can take a value from 0, which means that phylogeny has no impact on the distribution of the trait and the values can be treated as independent, to a value of 1 which means that phylogeny predicts the distribution of the trait. Pagel's λ can also be estimated by maximum likelihood given the data and a phylogeny. For each set of variables, we calculated the log-likelihood of a model when $\lambda = 1$ (strong phylogenetic signal), $\lambda = 0$ (no phylogenetic signal) and when λ was estimated from the data. We then performed likelihood ratio tests to test for statistically significant differences between the likelihood based on the estimated λ and the likelihood based on $\lambda = 1$ or 0. We then repeated our regression analyses of body size (morphology PC 1) and body shape (morphology PC 2) against environmental niche (environmental PC 1) with PGLS in BayesTraits (Pagel & Meade, 2007) and

also performed the same tests in the R package *caper* (Orme *et al.*, 2012) as a cross-check.

Results

The first two principal components on the morphological data set accounted for 95% of the total morphometric variation. PC 1 explained 90.829% of the variance and PC 2 an additional 4.357% (Table S2). PC 1 was highly correlated with body size (SVL, r = 0.976, $P < 0.001$) justifying our expectation that it was best treated as an estimate of absolute body size. PC 2 represented body shape. Absolute body size (PC 1) and body shape (PC 2) differed greatly between species and genera (Fig. 1). Variation in absolute body size (SVL) was large with individual frogs ranging from 15 mm to nearly 100 mm, with the biggest species averaging 91.7 ± 6.8 mm (*Mixophyes iteratus*) and the smallest 15.2 ± 0.8 mm (*Crinia deserticola*) (Fig. 1). Body shape differed conspicuously between genera, with the genus *Heleioporus* displaying the most extreme negative loadings in PC 2 (short-limbed, round body) and *Mixophyes* displaying the most extreme positive values (long-limbed, elongated body) (Fig. 1). All other genera displayed intermediate body sizes and shapes. Of all the morphological measurements, limb length, particularly hindlimb length, explained most of the morphological variance among genera. The bivariate plot of relative arm length (humerus length + forearm length + hand length) and relative leg length (femur length + tibial length + foot length) illustrates the large differences among genera (Fig. 2). *Metacrinia* has the shortest limbs, whereas *Mixophyes* and *Lechriodus* have extremely long limbs. The forward burrowing *Arenophryne* and *Myobatrachus* have relatively long arms in comparison with the legs, despite an otherwise rotund morphology (Figs 1 and 2). Three of the tested variables (body size, body shape and relative arm length) displayed strong phylogenetic signal with estimated λ values equivalent to 1 and the other three (relative leg length and environmental PC1 and PC 2) displayed intermediate levels of phylogenetic signal (Table S4).

For the environmental data set, the first principle component (PC 1) explained 63% of the environmental variation, and the second component explained an additional 20% (Table S3). The ecological niche of each species was defined according to its position on the first principal component axis (PC 1) - arid-distributed species had negative loadings on PC 1, and the species from wetter habitats had positive loadings (Fig. 3). There was no clear relationship between body size and environmental niche when phylogeny was not taken into account ($r^2 = 0.02$, $P < 0.1119$), and there was a very weak relationship following phylogenetic correction with PGLS ($r^2 = 0.04$, $P < 0.0001$). There was a stronger relationship between body shape and environmental niche when phylogeny was not taken into
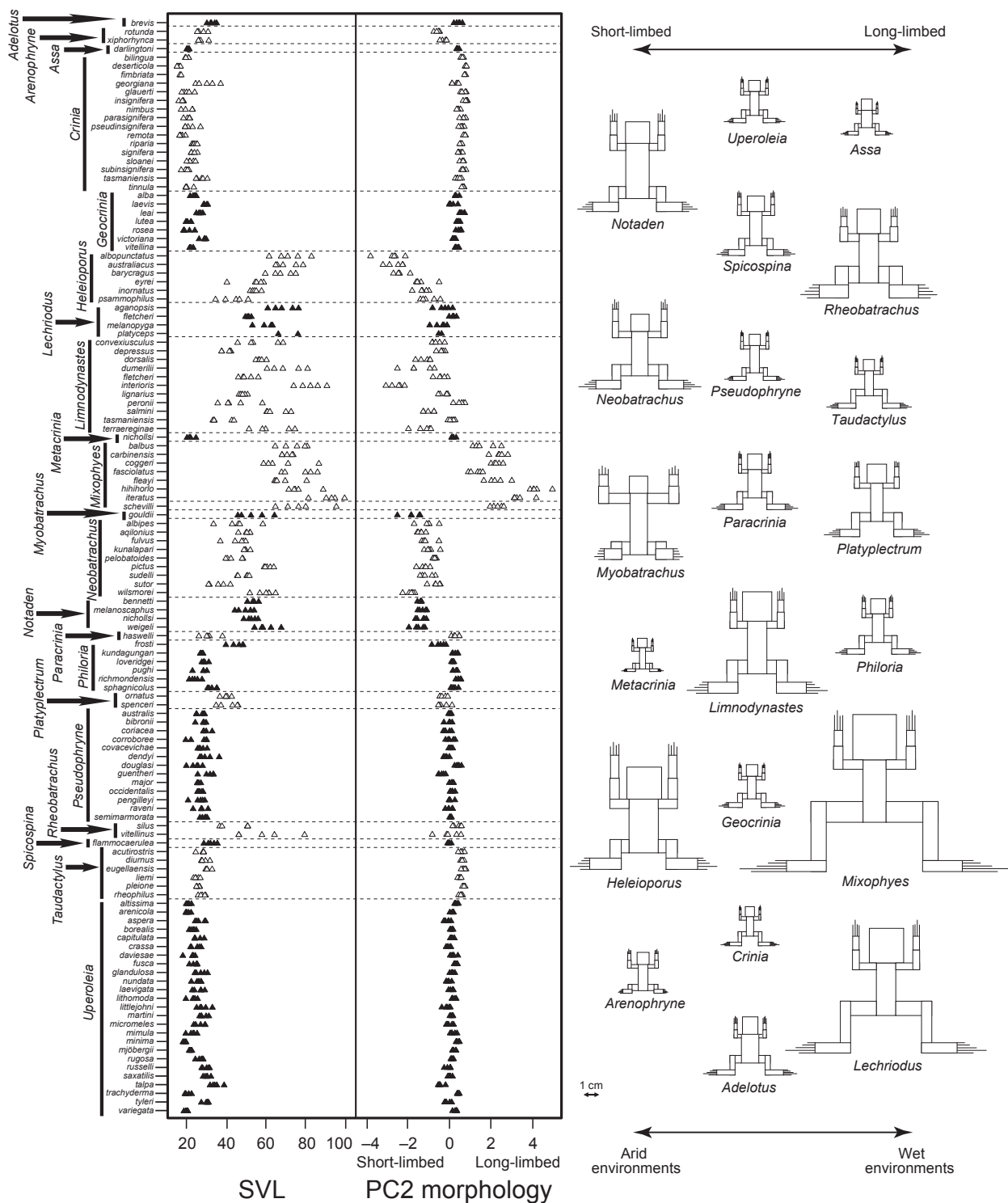
Fig. 1 Scatterplot by species of snout–vent length (SVL) and PC 2 (representing shape) from the morphological data set. The representative drawings summarize mean measurements and body shape patterns displayed by each genus and are arranged according to the kind of environment they occupy (from wet to arid habitats).
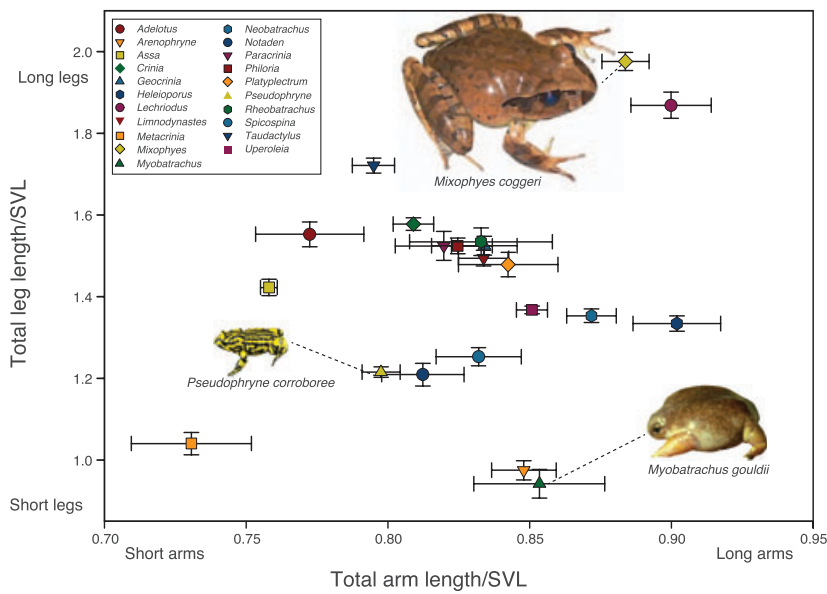
Fig. 2 Scatterplot of the ratio of relative arm length (arm length/SVL) to relative leg length (leg length/SVL). The means ± SD are displayed for each genus. Pictures show *Myobatrachus gouldii*, *Mixophyes coggeri* and *Pseudophryne corroboree*, representative morphologies according to relative limb length.
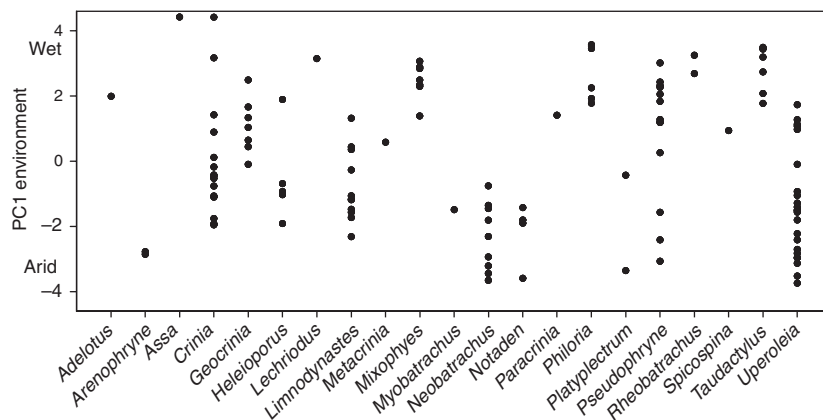

Fig. 3 Summary of mean PC 1 values of the environmental data set for each species showing the environmental breadth displayed in each genus.

account (r^2 = 0.14, P < 0.0001), but this relationship did not persist following phylogenetic correction with PGLS (r^2 = 0.01, P = 0.2075). Because our goal was to estimate the correlation between the two variables, we estimated Lambda at the same time as estimating the correlation, as recommended by Pagel & Meade (2007) In both cases, the estimated Lambda approached 1 (0.996 and 0.986, respectively), reaffirming that phylogeny is important.

There are three broad groups that can be identified (Fig. 4a, b): a) wet environments - mostly long-limbed species, but with a few exceptions; b) short-limbed species from arid environments; c) ubiquitous species that are more typically 'frog-shaped'. Environmental niche breadths were greater in the most speciose genera (*Crinia*, *Limnodynastes*, *Pseudophryne* and *Uperoleia*), yet there was little variation in the morphological PC 2 among species in those genera (Figs 3 and 4b). Both

forward burrowing species (*Arenophryne* and *Myobatrachus*) and backward burrowing species (*Notaden*, *Neobatrachus*, *Heleioporus* and some *Limnodynastes*) share a common short-limbed body shape pattern, and usually occur in drier environments, with one exception, *Heleioporus australiacus* (Fig. 4c).

Discussion

This study investigated whether variance in myobatrachid frog body form correlates with habitat variation throughout the Australian continent. Specifically, we were interested in examining whether the rotund, short-limbed morphology of burrowing frogs may be an adaptation to aridity and, by contrast, whether more slender body forms might be adapted to wetter environments. Myobatrachid frog genera can be broadly categorized on overall body size and relative limb length.
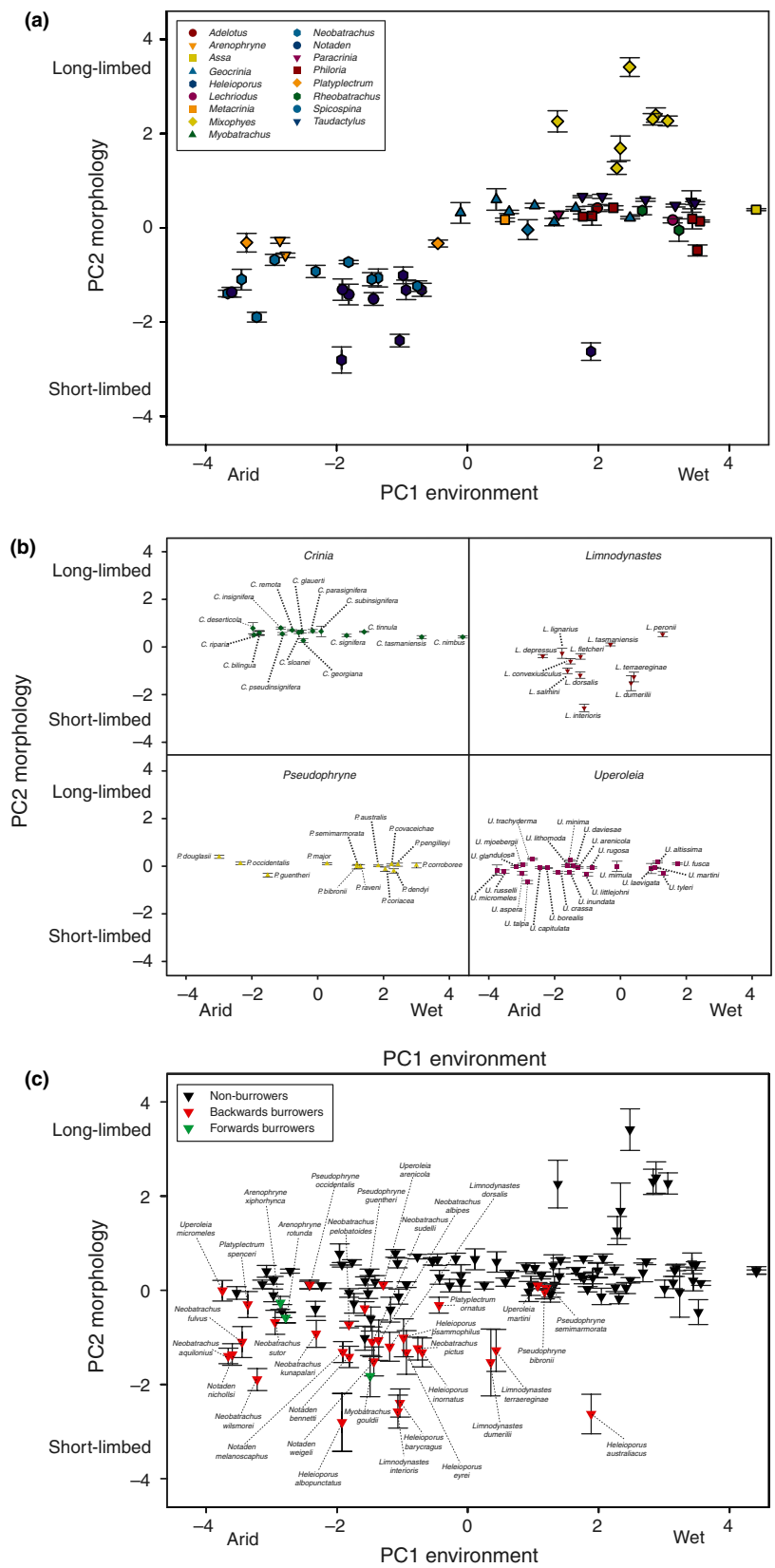
Fig. 4 (a) Summary of mean values ± SD for PC 1 of the environmental data set and PC 2 of the morphological data set by genus (except *Crinia, Pseudophryne* and *Uperoleia*). (b) Summary of mean values ± SD for PC 1 of the environmental data set and PC 2 of the morphological data set for *Crinia, Pseudophryne* and *Uperoleia* species. (c) Summary of mean values ± SD for PC 1 of the environmental data set and PC 2 of the morphological data set for each myobatrachid species with burrowing behaviour noted. Species names are shown for all backward and forward burrowers.

Small body size is characteristic of most myobatrachid frogs, such as *Adelotus*, *Arenophryne*, *Crinia*, *Geocrinia*, *Metacrinia*, *Paracrinia*, *Philoria*, *Pseudophryne*, *Spicospina*, *Taudactylus* and *Uperoleia*. Conversely, large body size characterizes two groups (*Lechriodus* and *Mixophyes)*, whereas five groups (*Heleioporus*, *Limnodynastes*, *Neobatrachus*, *Notaden* and *Rheobatrachus)* include species with markedly differing body sizes.

Despite the differences among and within genera, we found no obvious climatic correlation with body size, except that the genera (*Mixophyes*), with uniformly large frogs, typically occur in very wet habitats. Other genera with some large species, such as *Limnodynastes* (e.g. *L. interioris*) and *Heleioporus* (e.g. *H. australiacus*, *H. barycragus and H. albopunctatus*) are found in temperate to relatively arid habitats, and some very small species (e.g. many *Uperoleia* spp. and some *Crinia* spp.) occur in the driest climate zones. Burrowing species, irrespective of climate zone, showed reduced limb length, and this applied to species that burrow either forwards or backwards. Forward burrowers (*Arenophryne*, *Myobatrachus*) had relatively longer arms, but 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. Previous claims that rotund, short-limbed forms reduce surface area and therefore evaporative water loss in dry habitats (Tyler, 1989; Thompson *et al.*, 2005), sound intuitively correct, but are not supported by the different geographic occurrence of certain body forms in our data (Fig. 4a, b). It is well established that frogs possess an array of physiological techniques for reducing water loss, such as cocoon formation (Loveridge & Withers, 1981; Withers & Richards, 1995; Cartledge *et al.*, 2006), 'wax' coatings (Blaylock *et al.*, 1976; McClanahan *et al.*, 1978; Shoemaker *et al.*, 1987; Tracy *et al.*, 2010), skin morphology (Prates & Navas, 2009), changes in metabolic rates (Gomez *et al.*, 2006) and plastic or permanent increases in the resistance of epidermal layers to water loss (Withers *et al.*, 1984; Navas *et al.*, 2004). Critically, there is also good evidence that these techniques are effective irrespective of body shape (Tracy *et al.*, 2010). Burrowing may contribute to reductions in water loss or allow water uptake from the soil. For example, *Notaden*, which has no specific waterproofing potential with its skin, can persist in deserts by burrowing into deep, moist sands (Thompson *et al.*, 2005; Cartledge *et al.*, 2006). The specific morphology of burrowing frogs will be a trade-off between the mechanics of osmotic balance and other physiological demands, such as locomotion during foraging or breeding. However, the duration of these demands may be short because many desert species (e.g. *Notaden*, *Neobatrachus*) are explosive breeders that are only active for short periods after rain (Littlejohn *et al.*, 1993; Roberts, 1993). Thus, the rotund and short-limbed body shape pattern that typically occurs in arid environments is also tightly associated with the burrowing behaviour.

Reduction in limb length also is related to mode of locomotion (Emerson, 1978), irrespective of climate. Shorter legs are found in species that walk rather than hop as demonstrated in our data for two species of *Arenophryne* (Tyler *et al.*, 1980), and its sister taxa *Myobatrachus* and *Metacrinia* that occur in successively wetter climate zones (Tyler & Doughty, 2009). It is not clear whether limb length presents a pathway of change based on habitat shifts, and if so, what advantage might accrue from relatively long limbs in some genera (e.g. *Lechriodus*, *Mixophyes* and *Taudactylus)* inhabiting very wet environments. One possibility is that long limbs provide locomotory benefits in more aquatic habitats. None of the species in these genera are arboreal, but the latter two are either riparian or stream breeders (Dennis & Mahony, 1994; Lewis & Rohweder, 2005; Hoskin, 2010), and longer legs may be critical in resisting high flow rates. This notion has been put forward to explain long limbs in stream breeding anurans from other parts of the world. For example, Hewitt's Ghost frog (*Heleophryne hewitti*) inhabits fast-flowing mountain streams in Africa and has disproportionately long muscular legs, which may have evolved to enhance swimming ability (Laurent, 1964). Equally, as frogs with elongated hind limbs and short fore limbs should be able to jump further (Emerson, 1985; Choi *et al.*, 2003; James & Wilson, 2008), longer legs might be of some advantage in escaping predators (Gans & Parsons, 1966) or negotiating complex vegetation structure (Emerson, 1991). Furthermore, it could also be the case that frogs in wet habitats have longer limbs because they do not face the same physiological and morphological constraints as species inhabiting arid environments.

Contrary to the differences among genera, at a lower taxonomic level, body size and shape differences are much more subtle. This is especially observed in the species-rich and widely distributed genera *Crinia*, *Limnodynastes*, *Pseudophryne* and *Uperoleia*, which are each morphologically highly conservative despite being distributed across the full spectrum of Australian habitats. Similarly, the genus *Heleioporus* is comprised primarily of rotund frogs distributed in semi-arid environments to forests. Why has there been a limited degree of evolutionary change in morphology in some myobatrachid anurans? One possibility is that the duration of isolation between groups has not been sufficient to permit divergence. However, this explanation seems implausible given that the divergence times within these genera are up to 30 million years old (Read *et al.*, 2001; Morgan *et al.*, 2007; Catullo *et al.*, 2011). Additionally, rapid morphological changes have been observed in other anurans (e.g. in *Rhinella marina*, Phillips & Shine, 2005) and in other taxa (Keogh *et al.*, 2005). An alternative explanation is that evolutionary constraints have restricted directional selection. If mutational changes at genes involved in patterning the limbs or body plan have negative pleiotropic effects upon other body parts,

these genes will not be selectively favoured. For example, mutations that alter the number of digits present on a hand or a foot in tetrapods often are associated with serious abnormalities that reduce fitness (Castilla *et al.*, 1998; Lewis *et al.*, 1999; Muranjan & Bharucha, 2000). However, Galis *et al.* (2001) argued that the potential for such pleiotropic effects is relatively low in amphibians because limb development occurs late in ontogeny, effectively decoupling limb development from the interactivity of the phylotypic stage. This notion is supported by the observation that amphibians display extreme variation in limb patterning, such as the presence and size of phalanges, carpal and tarsal elements (Lynch, 1971; Gollman, 1991; Rienesl & Wagner, 1992; Shubin *et al.*, 1995), a finding consistent with our data set for the *Arenophryne*, *Myobatrachus* and *Metacrinia*. Therefore, perhaps the simplest explanation for the general conservation of limb and body morphology observed across *Crinia, Limnodynastes, Pseudophryne* and *Uperoleia* species, is that, despite ecological diversification early in the radiation history of myobatrachid frogs, these groups have evolved various behavioural and physiological adaptations that have permitted them to persist across a range of mesic and xeric environments (Warburg *et al.*, 2000).

Our data suggest that the major morphological changes occurred rapidly and very early in the myobatrachid's diversification history, and this was followed by minimal morphological changes, despite recent ecological diversification. Speciation rates and the rate of phenotypic evolution are often correlated (Harmon *et al.*, 2003; Mahler *et al.*, 2010; Rabosky & Adams, 2012), and several models of adaptive radiation are characterized by rapid trait changes, followed by a relative diversification stasis as ecological opportunities decrease (McPeek, 2008; Rabosky & Lovette, 2008; Gavrilets & Losos, 2009; Harmon *et al.*, 2010; Sallan & Friedman, 2012). This pattern is observed in other old adaptive vertebrate radiations such as Hawaiian honeycreepers (Lovette *et al.*, 2002), acanthomorph teleosts (Friedman, 2010) and Silurian gnathostomes (Anderson *et al.*, 2011), in contrast to the rapid phenotypical diversification observed in more recent radiations, such as Darwin's finches (Abzhanov & Tabin, 2004), the Caribbean Anolis lizards (Losos *et al.*, 1998), parrotfish (Streelman *et al.*, 2002) seedeaters (Campagna *et al.*, 2011) or African cichlids (Albertson *et al.*, 1999). Furthermore, the morphological disparity observed between, but not within, genera also could be due to an early saturation of the morphological space (Foote, 1994).

Anurans have had a highly conserved body shape pattern since at least the early Jurassic (Shubin & Jenkins, 1995; Jenkins & Shubin, 1998). At a broader level, there were predictable differences in body size and shape related to environmental variation in our data set. However, our data also show that the conservative anuran body size and shape that is displayed by many species is one that can work under a wide variety of environments.

Acknowledgments

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Codes of the specimens per species.
Table S1 Description of the 33 external morphological measurements taken from each specimen.
Table S2 PCA loadings for the morphological data set.
Table S3 PCA loadings for the environmental data set.
Table S4 Summary of phylogenetic signal tests.
Figure S1 Illustration of the 33 external morphological measurements taken from each specimen (see Table 1 for descriptions).