

Locomotor performance constrained by morphology and habitat in a diverse clade of African frogs (Anura: Pyxicephalidae)

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Understanding how morphology relates to function and how the two change across species in different environments and regions can provide clues to the drivers of speciation and diversity within a clade. Locomotion is a vital trait for most animals to function, and locomotor adaptations should involve morphological changes that maximize fitness in specific environments. We focus on the Pyxicephalidae, a speciose sub-Saharan anuran family with remarkable morphological and ecological diversity, and hypothesize that morphological differences among these species affect locomotor performance and that these represent adaptations to their surrounding environment. We tested 215 individuals across 25 species for various aspects of locomotor performance by filming frogs jumping and swimming, inducing sustained movement around a circular track and rotating a non-stick surface until adhesion failure. We then used Ornstein–Uhlenbeck models to determine whether species showed trait selection between different environments. We found that some morphological traits were significantly correlated with aspects of locomotor performance between species. Microhabitats showed separate selective optima for both morphology and locomotor performance, specifically between combinations of semi-aquatic, fossorial, semi-arboreal and terrestrial species. Our study shows how ecological conditions coincide with morphological and locomotor differences within a continental arena, generating a remarkably diverse African frog family.

ADDITIONAL KEYWORDS: adaptive radiation – Africa – Anura – locomotion – microhabitats – morphology – performance – Pyxicephalidae.

INTRODUCTION

Adaptive radiations provide opportunities to showcase evolutionary processes over a diverse array of life forms (Schluter, 2000; Losos & Malher, 2010). Although the process is considered to be ubiquitous, classical examples refer to adaptive radiations in isolated communities (e.g. islands, lakes; Schluter, 1995; Rainey & Travisano, 1998; Grant & Grant, 2002), with few examples that test adaptation on continental clades (Schluter, 1988; Hughes & Eastwood, 2006). The paucity of continental adaptive diversifications might reflect a genuine change in the evolutionary process or simply the increased effort required to sample their diversity. This uncertainty will be resolved only by objective assessments of more continental groups.

The comparative method enables exactly such robust substantiation of traits as adaptations (Felsenstein, 1985). With the comparative method, we can determine whether groups with remarkable diversity are adaptive and gain better insight into the ecological drivers of their diversification.

The fitness of a species can sometimes be approximated by a morphological trait that is easier to quantify than a direct measure of the performance or fitness, but this ignores the complexity of the relationship between traits and their function (Wainwright *et al.*, 2005). Instead, Arnold (1983) suggests measuring a performance trait directly, which gives greater insight for ecological adaptations. Here, we focus on locomotor performance because it is well studied within animals and has a direct link to evolutionary fitness through its importance for escape from predators, foraging and successful reproduction (Garland & Losos, 1994; Miles *et al.*, 2000; Smith &

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Green, 2006). By comparing locomotor performance of species from different habitats and linking morphology to locomotion, we can infer possible ecological adaptations and which morphological changes were made to improve fitness.

Anurans are well known for their characteristic and conserved 'bauplan' that has facilitated the occupation of both terrestrial and aquatic habitats (Gans & Parsons, 1966; Handrigan & Wassersug, 2007; Reilly & Jorgensen, 2011). Despite this, anurans occupy a broad range of environments, from lakes and forests to desert floodplains, and have remarkably different modes of locomotion (Emerson, 1979), suggesting that superficial differences in morphology can be of functional significance. Most anurans can hop or jump, propelling themselves into the air using their hindlimbs and creating a movement that is analogous to simple ballistics (Marsh, 1994). Some frog species are particularly adept at jumping and can escape predators by quick, successive and unpredictable hops (Royan *et al.*, 2010) or by a single strong leap to safety (Citadini *et al.*, 2018). Others move either by walking or with short hops, which may be better suited for long-distance movements, such as migrations to breeding sites (Smith & Green, 2006). The ecological and locomotor diversity among anurans, in combination with their constrained bauplan, enables us to use the comparative method to explore adaptation in anurans.

Studies on anuran locomotion show that jump performance is correlated with body size, relative limb length or relative muscle mass, both within and between species (Rand, 1952; Gomes *et al.*, 2009; Moen *et al.*, 2013; Enriquez-Urzelai *et al.*, 2015; Astley, 2016; Citadini *et al.*, 2018). Indeed, the areas of cross-sectional muscles and their attachment sites are correlated in *Leptodactylus* frogs, and these were larger in jumping species than in hopping species (Ponssa *et al.*, 2018). Morphology can also be correlated with swimming and adhesive performance, increasing with the extent of pedal webbing and the width of finger/toe pads, respectively (Emerson & Diehl, 1980; Moen *et al.*, 2013). However, other aspects of locomotion, such as endurance, remain poorly understood in anurans and have been thoroughly addressed only with intraspecific studies (Herrel & Bonneaud, 2012; Herrel *et al.*, 2014) or studies between a few species (Rand, 1952; Putnam & Bennett, 1981). Although Zug (1978) investigated fatigue within multiple species, he did not note any morphological correlates apart from body size. Selective trade-offs between speed and sustained movement caused by conflicting locomotor requirements, as shown with isolated muscles (Wilson & James, 2004), might encourage niche separation and adaptation within anurans. Specific aspects of locomotor performance might have a greater fitness benefit in some types of habitats, where predation risk

is high or where species migrate to breed, and thus promote performance-enhancing morphological traits.

Some aspects of anuran morphology and locomotion are shown to be related to the type of habitat that a species occupies, with the most prevalent finding that fossorial species generally have relatively short hindlimbs (Zug, 1978; Moen *et al.*, 2013; Vidal-García *et al.*, 2014); these may enable powerful excavation movements, but constrain jumping performance (Zug, 1978; Gomes *et al.*, 2009; Citadini *et al.*, 2018). In addition, arboreal frogs have specialized pads on their fingers and toes that increase the surface area in contact with the substrate and enhance adhesion to slippery surfaces (Emerson & Diehl, 1980). Aquatic species have more webbing than terrestrial species, which strengthens swimming ability (Moen *et al.*, 2013), in addition to differences in hip rotation and limb velocity while swimming (Robovska-Havelkova *et al.*, 2014). In addition, semi-aquatic species that sit at the edge of water have a sharp decline in jump performance after successive jumps, whereas fossorial species are able to sustain movement for longer periods (Zug, 1978). There are clearly links between habitat, morphology and locomotion in some clades of anurans, but do these predictions hold for anurans in general?

Although many studies have investigated the ecological context of interspecific differences in anuran locomotion, relatively few have specifically tested locomotor performance and examined multiple taxa within a phylogenetic framework. Furthermore, these studies have mostly overlooked African anurans, addressing taxa from the Americas, Europe, Australasia and Asia (Rand, 1952; Zug, 1978; Moen *et al.*, 2013; Enriquez-Urzelai *et al.*, 2015; Astley, 2016; Citadini *et al.*, 2018). In addition, most of these studies have compared distantly related taxa (with an exception of Hylidae; Moen *et al.*, 2013). Distantly related taxa have had more time to accrue non-locomotor adaptations since vicariance, and these changes might affect morphology or locomotion. By restricting comparisons to more closely related species, we can reduce the effects that might obscure locomotor adaptations. In this study, we investigate whether there is evidence for microhabitat adaptation within a diverse frog clade from sub-Saharan Africa.

The Pyxicephalidae comprises 84 species within 12 genera (Frost, 2018) and is the largest and most ecologically diverse frog family in southern Africa, with species from semi-aquatic, terrestrial, burrowing and semi-arboreal microhabitats. The family encompasses a remarkable morphological diversity that rivals any other frog family in the region (van der Meijden *et al.*, 2011) and is therefore suitable for testing predictions of functional morphology and adaptation in an African context. In this study, we hypothesize that locomotor performance can be explained by morphology and

that species from different microhabitats have consistent changes in morphological and locomotor traits (separate selective optima). Here, we explore the potential for ecological adaptation by comparing both morphological and locomotor traits between microhabitats.

MATERIAL AND METHODS

SPECIMEN CAPTURE AND HANDLING

The morphological and locomotor performance traits were measured for 25 wild-caught pyxicephalid species, including ten out of the 12 genera (Fig. 1). We prioritized the testing of species with unique morphology and/or

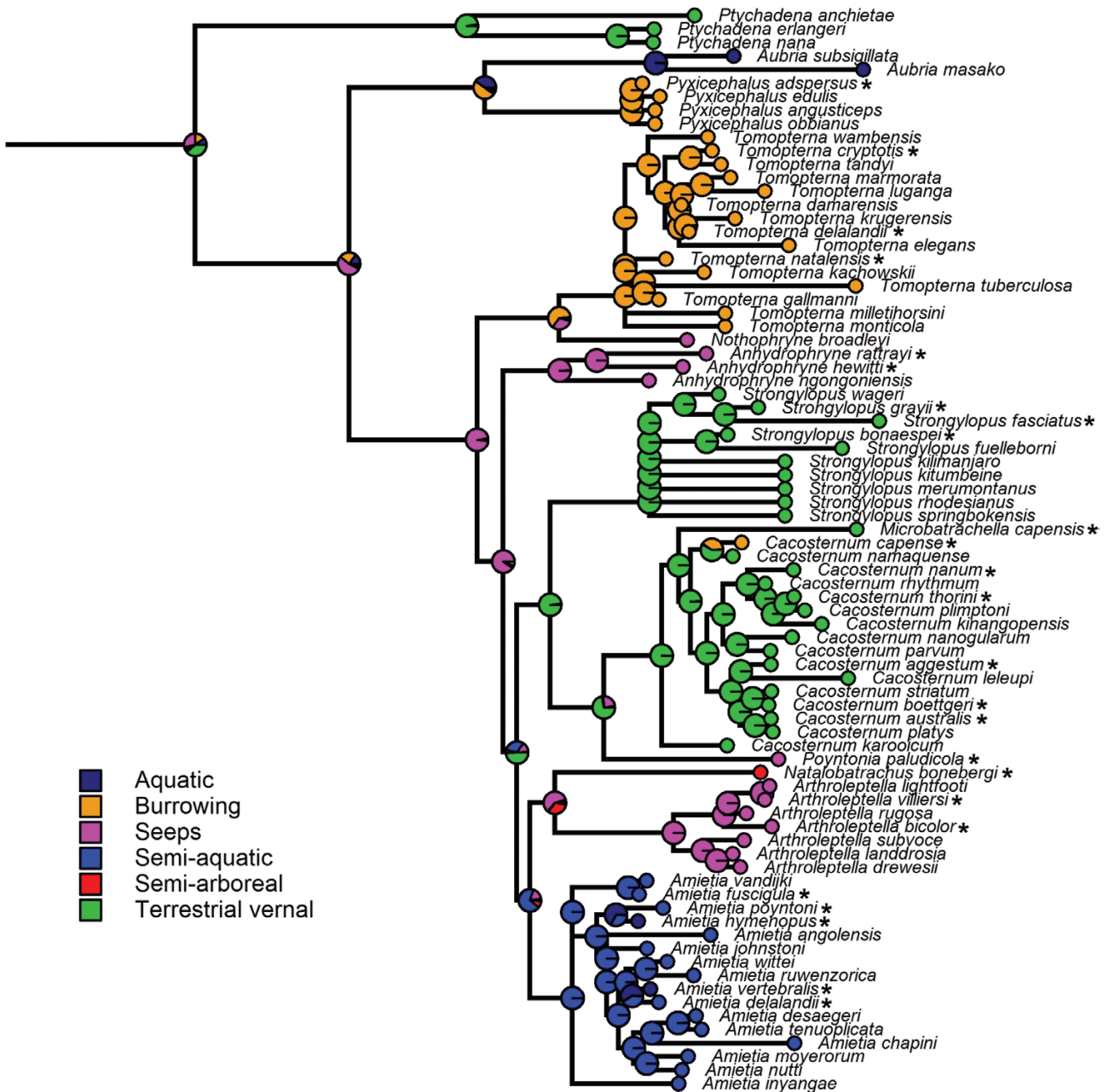


Figure 1. Maximum likelihood tree for the Pyxicephalidae using 12S and 16S RNA and Tyr genes. *Ptychadena anchietae*, *Ptychadena erlangeri* and *Ptychadena nana* were used as outgroups. The ancestral state reconstructions of microhabitats are shown as pie charts at the nodes, which indicate the proportional likelihood of that ancestor occupying one of six microhabitats. Microhabitats are as follows: A, aquatic; Ar, arboreal; F, fossorial; S, seep; SA, semi-aquatic; and T, terrestrial. *Species included in this study.

ecology, such as the slow, rotund *Poyntonia paludicola*, the lithe, semi-arboreal *Natalobatrachus bonebergi* and the massive *Pyxicephalus adspersus*. We collected and tested up to ten adult males per species, avoiding specimens with abnormalities. Captured individuals were transported to an experimental facility and acclimated for ≥ 10 h before starting performance trials. Temperatures within the experimental arena were controlled at ~ 20 °C. Tested individuals were released within 48 h of capture.

PERFORMANCE TRAITS

Frogs were tested for six locomotor performance activities: jumping, sprinting, swimming, terrestrial endurance, aquatic endurance and adhesion (see Supporting Information, Fig. S1). In anurans, a jump is generally defined as a distance greater than eight lengths of the body (Reilly & Jorgensen, 2011), but for the sake of simplicity we use the term ‘jump’ to describe both hops and jumps regardless of the distance-to-body length ratio. We used the term ‘sprint’ to define sustained movement over a short distance (≤ 1.5 m), usually composed of a series of consecutive jumps. Below, we provide a summary of the procedure used to test performance (see Supporting Information for more details).

Three of the locomotor activities (jumping, sprinting and swimming) were filmed to measure the speed of frogs during these respective exercises. Jumping was filmed side-on to capture both the vertical and horizontal components, whereas sprinting and swimming were filmed from above to capture movements along a horizontal plane. For jumping, frogs were individually placed on a flat, cork tile substrate parallel to a vertical scaled background and were induced to jump by lightly touching the legs or blowing air on them from behind. Sprinting was tested by placing individual frogs in a rectangular track (dimensions: 1.5 m \times 0.3 m and vertical walls of 0.3 m) lined with cork tiles, and once again frogs were induced to move, but this time in rapid succession without any rest until the other side of the track was reached. In this activity, the camera was placed above the track so that the full length of the track filled the frame. This same set-up was used for the swimming activity, except that the track was filled with water to a depth such that the frog being tested could not contact the bottom while being induced to swim a distance along the surface. The distance and velocity of frogs were extracted from these videos by tracking movement between frames.

The endurance performance was tested using a circular track with an inner and outer wall of circumference 2.6 and 3.5 m, respectively. Individual frogs were placed inside the track and induced to

move in a single direction until they either became exhausted or a time limit was reached. Each lap time was recorded, and the distance moved was calculated from the lap number. We tested both terrestrial and aquatic endurance in the same manner, the latter requiring the bottom of the track to be filled with water. Each endurance activity was tested once for an individual frog, and when the frog refused to move for a continuous period > 15 s it was declared exhausted. From these distance and time measurements, we calculated the total distance moved, time to exhaustion, average velocity and exhaustion index (EI), which was defined as the proportion of the average final velocity to the average initial velocity.

To test frog adhesive ability, we used a non-stick (tetrafluoroethane) tray that was hinged at one side, such that we could lift one side smoothly at a constant rate of 20° s^{-1} and create a pivoting motion in the horizontal plane. Each frog was placed on the surface and rotated until the displacement of the frog, marking the angle of adhesion failure. We controlled for frog orientation by placing the frogs facing up and down the inclining surface and made three repeat measurements for each orientation per frog.

Finally, individuals were weighed and photographed on scaled grid paper. From the images, we measured key morphological traits using ImageJ (Rasband, 1997), which included snout–vent length, mid-femur width, hindlimb length, hand length and terminal finger disc diameter (for more details, see Supporting Information). The extent of pedal webbing was estimated by counting the number of phalanges free of webbing on the longest digit (see summary morphological and performance traits in Supporting Information, Tables S16, S17).

DATA ANALYSES

Mensural morphological traits were first logged, normalized and then phylogenetically corrected for body size using the function ‘*phyl.resid*’ in ‘*phytools*’ (Revell, 2012). The effect of morphology on each locomotor performance trait was tested using the phylogenetic generalized least squares regression (PGLS) analysis with simultaneous estimation of Pagel’s λ in ‘*caper*’ (Orme, 2013). Owing to our small sample size, only morphological traits relevant to locomotor performance were included as model fixed effects (see Supporting Information, Tables S1–S6). We used multiple models covering all combinations of the included morphological traits and used the corrected Akaike information criterion (AICc) to compare model fit. All analyses were done in R v.3.0.2 (R Core Team, 2015). Trait phylogenetic signal was calculated using λ (Pagel, 1999) and K (Blomberg *et al.*, 2003) with the function ‘*phylosig*’ in ‘*phytools*’.

The Ornstein–Uhlenbeck (OU) process models both the intensity of a stochastic component and the strength of selection towards an optimal value for a given trait across a phylogeny (Butler & King, 2004). We used the OU process to model whether microhabitats have different selective optima for morphology or locomotor traits. First, we categorized species into microhabitats according to literary accounts and personal field observations. These microhabitats included aquatic, semi-aquatic, terrestrial opportunist, montane seeps, semi-arboreal and fossorial categories (see Supporting Information, Table S7), most of which have been used in previous anuran analyses (Gomes *et al.*, 2009; Moen *et al.*, 2013; Vidal-García *et al.*, 2014; Citadini *et al.*, 2018). Second, we created seven different microhabitat scenarios, with different combinations and classifications of microhabitats to model separate selective optima, including a Brownian motion (no optimum) and single optimum scenario (see Supporting Information, Table S8). Third, we assigned ancestral nodes to specific microhabitats using maximum likelihood (ML) with marginal estimation in the function ‘ace’ from the R package ‘ape’ (Paradis, 2012), with an exception for seep microhabitats because the clades are geographically isolated and it is unlikely that conservatism or dispersal is responsible. Fourth, we conducted phylogenetic principal component (PC) analyses, using ‘phytools’ (Revell, 2012), for morphology and performance separately (see Supporting Information, Tables S9 and S10). Fifth, the principal component scores for each PC axis were used as input for the OU process, in the R package ‘ouch’ (King & Butler, 2009), using a pruned version of the ML best tree. Sixth, the sums of the AICc values over all PC axes were used to select the best scenario for morphological and locomotor performance traits, as done by Moen *et al.* (2016).

PHYLOGENY ESTIMATION

We required a phylogeny to remove the effects of shared ancestry in our analyses. We used available GenBank DNA sequences of mitochondrial 12S and 16S and nuclear *Tyr* to estimate a maximum likelihood phylogeny for 67 pyxicephalid species (see Supporting Information, Table S11). These genes had the best species coverage and provided a genetic resolution at both the species level and deeper taxonomic units. Sequences used by Bittencourt-Silva *et al.* (2016) were chosen preferentially, but were replaced if all genes had not been acquired from a single specimen or if a BLAST search did not match the sequence with that of conspecifics in GenBank. All sequences of a gene were aligned using MUSCLE in MEGA v.6 (Tamura *et al.*, 2013). Genes were concatenated only if they were sampled from the same specimen. We

partitioned the two mitochondrial genes separately and the nuclear gene by codon (for more details, see Supporting Information). The concatenated sequences were analysed with RAxML-HPC BlackBox v.8.2.8 (Stamatakis, 2006; Miller *et al.*, 2010) using the partition file and default settings (GTR+ Γ) to estimate the ML tree. The topology of our ML tree generally agreed with that of other published phylogenies (for details, see Supporting Information and Fig. S2).

RESULTS

PHYLOGENETIC SIGNAL

Morphological traits were found to have large and significant estimates of phylogenetic signal, between 1.6 and 2.6 for Blomberg’s K and close to one for Pagel’s λ (see Supporting Information, Table S12). Although locomotor performance traits had smaller estimates than morphological traits, the values were nonetheless large and significant, with most values being close to one for Blomberg’s K and Pagel’s λ .

MORPHOLOGICAL CORRELATES OF LOCOMOTOR PERFORMANCE

The 25 pyxicephalid species tested in this study showed strong support for many of the established morphological correlations with locomotor performance traits (see Supporting Information, Tables S1–S6). Morphological traits were corrected for body size and are referred from here on in a relativistic sense. Hindlimb length and snout–vent length had the most numerous correlations with locomotor performance traits. The jump distance, take-off velocity and sprint velocity all included snout–vent length, hindlimb length and femur muscle width as predictors in the best models ($w_i = 0.67$, PGLS $R^2_{\text{adj}} = 0.89$; $w_i = 0.78$, PGLS $R^2_{\text{adj}} = 0.86$; and $w_i = 0.63$, PGLS $R^2_{\text{adj}} = 0.88$, respectively; see Supporting Information, Tables S1 and S2). Swimming velocity included snout–vent length and extent of toe webbing in the model with the lowest AICc ($w_i = 0.50$, PGLS $R^2_{\text{adj}} = 0.68$; see Supporting Information, Table S3), although another model that included hindlimb length in addition to snout–vent length and extent of toe webbing performed similarly ($w_i = 0.24$, PGLS $R^2_{\text{adj}} = 0.68$), suggesting that hindlimb length is inconclusive with regard to swim velocity.

We measured four different performance traits for both the terrestrial (see Supporting Information, Table S4) and aquatic endurance activities (see Supporting Information, Table S5). The best model for total terrestrial distance moved included snout–vent length and hindlimb length ($w_i = 0.34$, PGLS $R^2_{\text{adj}} = 0.28$), but the model including only snout–vent length performed

similarly ($w_i = 0.21$, PGLS $R^2_{adj} = 20$), suggesting that the effect of hindlimb length is inconclusive. The best model for time taken to exhaustion included hindlimb length and femur muscle width ($w_i = 0.30$, PGLS $R^2_{adj} = 0.4$), but the model including only hindlimb length performed similarly ($w_i = 0.27$, PGLS $R^2_{adj} = 0.36$), thus we consider the effect of thigh muscle width inconclusive. There were no clear best models for the average terrestrial velocity during the endurance trials; the model with the lowest AICc (see Supporting Information, Table S4) included snout–vent length, hindlimb length and thigh muscle width ($w_i = 0.32$, PGLS $R^2_{adj} = 0.53$), although snout vent length was present as a predictor in all the models with the lowest AICc scores. This was also the case for terrestrial endurance index, which had the same morphological predictors in the ‘best’ model ($w_i = 0.36$, PGLS $R^2_{adj} = 0.49$), except that hindlimb length was present in most models with the lowest AICc scores.

The best model for total distance swum during the aquatic endurance trials included only snout–vent length as a predictor ($w_i = 0.40$, PGLS $R^2_{adj} = 0.17$). Time to exhaustion in aquatic trials performed similarly with different predictors and was thus generally inconclusive. The best model for average velocity during

aquatic trials included snout–vent length as the sole predictor ($w_i = 0.54$, PGLS $R^2_{adj} = 0.59$). Finally, the best model for aquatic endurance index included hindlimb length ($w_i = 0.41$, PGLS $R^2_{adj} = 0.18$), although another model including both hindlimb length and thigh muscle width performed similarly ($w_i = 0.2$, PGLS $R^2_{adj} = 0.18$), thus the effect of thigh muscle width is regarded as inconclusive. Two aspects of adhesive performance were measured, namely upward and downward facing adhesive ability. Both upward and downward adhesive ability shared a best model that included the snout–vent length and the finger-tip diameter ($w_i = 0.50$, PGLS $R^2_{adj} = 0.83$; and $w_i = 0.56$, PGLS $R^2_{adj} = 0.72$, respectively; see Supporting Information, Table S6), and both featured models including hand length in addition to snout–vent length and finger-tip diameter as performing similarly ($w_i = 0.32$, PGLS $R^2_{adj} = 0.84$; and $w_i = 0.29$, PGLS $R^2_{adj} = 0.73$, respectively), thus we consider the effect of hand length on adhesive ability to be inconclusive.

MICROHABITAT SELECTIVE OPTIMA

The best model for morphology included separate morphological adaptive optima for semi-arboreal,

Table 1. Ornstein–Uhlenbeck OU) model outputs with different models of evolution for both morphological and locomotor performance traits

Class	Model description	lnL	AICc	ΔAICc	w_i	K
Morphology	Null					
	Brownian motion	305.26	−574.17	5.56	0.060	2
	Single optimum OU	312.69	−568.24	11.49	0.000	3
History only	Only clades	449.16	−338.32	241.41	0.000	14
Microhabitat	Ar, A, F, T, S, SA	373.90	−547.81	31.92	0.000	8
	Ar, F, T, S, SA	364.68	−564.66	15.07	0.000	7
	Ar, A, T, S, SA	349.23	−565.13	14.60	0.000	6
	F, SA, T	331.42	−557.58	22.15	0.000	5
	Ar, SA, T	342.50	−579.73	0.00	0.900*	5
	SA, T	326.79	−573.59	6.14	0.040	4
	F, T	318.36	−556.72	23.01	0.000	4
Performance	Null					
	Brownian motion	62.71	−84.50	8.91	0.005	2
	Single optimum OU	77.28	−90.27	3.14	0.088	3
History only	Only clades	215.70	198.61	292.02	0.000	14
Microhabitat	Ar, A, F, T, S, SA	142.78	−60.56	32.85	0.000	8
	Ar, F, T, S, SA	128.33	−71.36	22.05	0.000	7
	Ar, A, T, S, SA	108.34	−66.68	26.73	0.000	6
	F, SA, T	105.04	−91.67	1.74	0.177*	5
	Ar, SA, T	98.38	−78.34	15.07	0.000	5
	SA, T	91.39	−92.77	0.64	0.307*	4
	F, T	91.71	−93.41	0.00	0.423*	4

Different selective optima scenarios include the following microhabitats: A, aquatic; Ar, arboreal; F, fossorial; S, seep; SA, semi-aquatic; and T, terrestrial. The table includes the log-likelihood (lnL), corrected Akaike information criterion (AICc), weight of evidence (w_i) and number of model parameters (K) for each model. *Model with low AICc value.

semi-aquatic and terrestrial species (Table 1), whereas the other candidate models fitted the data poorly ($\Delta\text{AICc} > 5$). The greatest differences for morphological selective optima were explained by PC axes 2 and 3 (see Supporting Information, Table S13). These PC axes predominantly accounted for variation in the extent of pedal webbing and finger disc diameter, respectively (see Supporting Information, Table S9), with more extensive webbing in semi-aquatic species and wider fingertips in semi-arboreal species (Fig. 2A).

For locomotor performance, two of the best models had equivalent AICc scores ($\Delta\text{AICc} = 0.64$); the first had separate selective optima for fossorial and terrestrial species, and the second for semi-aquatic and terrestrial species (Table 1). A third model also

performed relatively well ($\Delta\text{AICc} = 1.74$), which included separate selective optima for fossorial, semi-aquatic in addition to terrestrial species. The difference in locomotor selective optima between fossorial and non-fossorial species was greatest for PC axis 1, but PC axes 2 and 7 also showed some differences (see Supporting Information, Table S14). Principal component 1 predominantly explained adhesive ability and endurance ability, whereas PC 2 represented sprint velocity, jump distance and jump velocity, and PC 7 explained variation in endurance, specifically where an increase in terrestrial distance moved was associated with a decline in aquatic distance moved (see Supporting Information, Table S10). However, the difference in PC 7 between fossorial and terrestrial species appears to be caused solely by the species

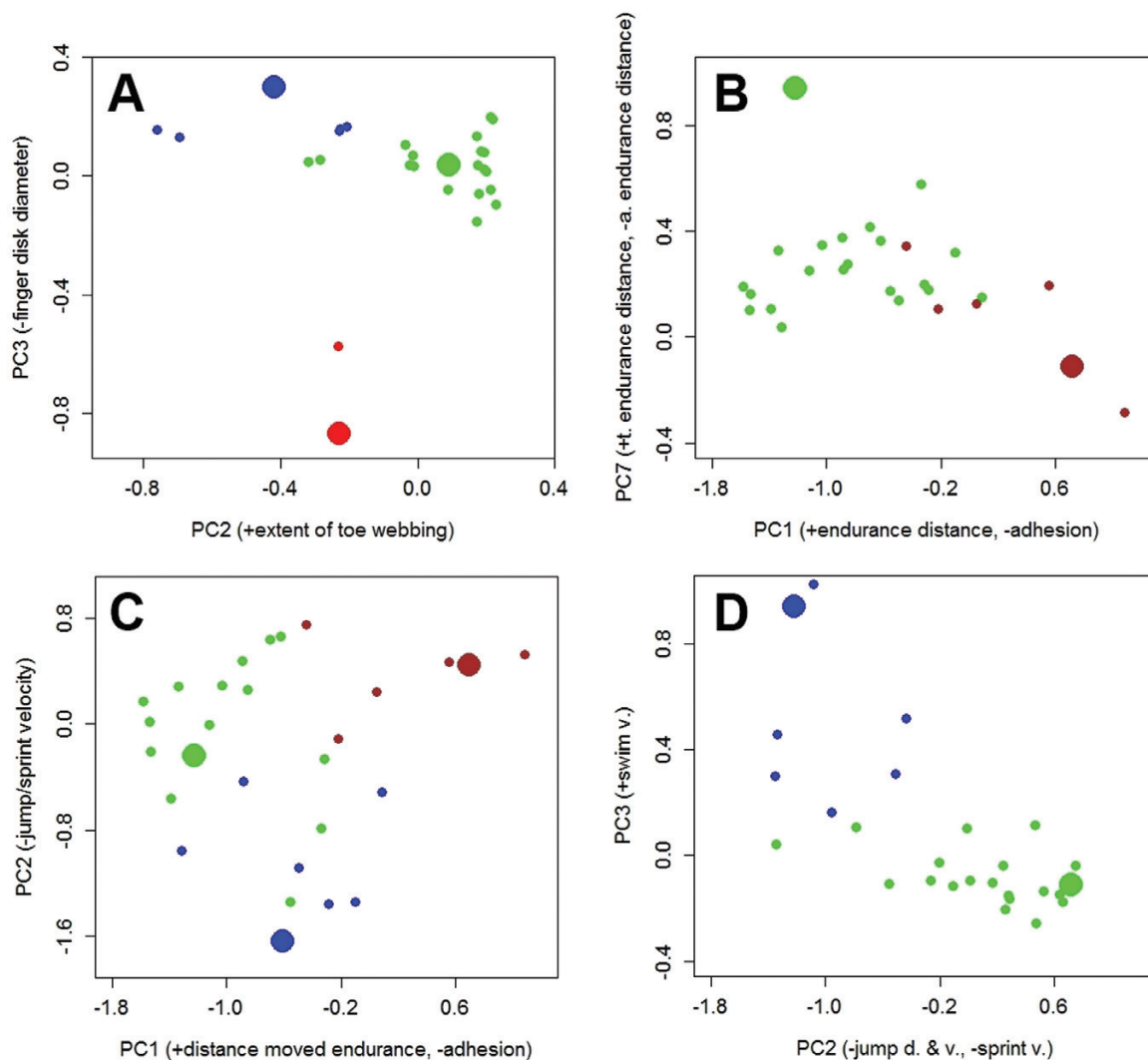


Figure 2. The phylogenetic principal component (PC) scores for morphological traits (A) and locomotor performance traits (B–D) showing semi-aquatic (blue), semi-arboreal (red), terrestrial (green) and fossorial (brown) species. The large circles show Ornstein–Uhlenbeck selective optima using microhabitat scenarios MH4 (A), MH7 (B), MH4 (C) and MH6 (D).

Pyxicephalus adspersus. Therefore, fossorial species had weaker jumps, moved greater total distances during the endurance trial and had poor adhesive ability in comparison to non-fossorial species (Figs 2B, 2C). The greatest difference between semi-aquatic and terrestrial species was for PC axes 1, 2 and 3 (see Supporting Information, Table S15), although data visualization of PC 1 shows that variation far exceeds the differences in selective optima (Fig. 2C). Principal component 2 predominantly explained distance and burst velocity of jumping, and PC 3 represented the average swim velocity (see Supporting Information, Table S10). Therefore, semi-aquatic species had stronger jumps than terrestrial (and especially fossorial) species (Fig. 2C) and swam faster (Fig. 2D).

DISCUSSION

We found that locomotor performance was correlated with aspects of morphology and that specific morphological and locomotor traits had separate selective optima for some microhabitats in the Pyxicephalidae, a large family of sub-Saharan frogs. For example, jump distance, take-off velocity and sprint velocity increased with body size, relative hindlimb length and relative femur width. Our data show that a single clade of African anurans shares similar mechanical constraints with anurans from other continents (Moen *et al.*, 2013; Enriquez-Urzelai *et al.*, 2015; Citadini *et al.*, 2018). Furthermore, from our results we infer that specific microhabitats have altered the selective gradient for African pyxicephalids to favour specific aspects of locomotor performance and their morphological counterparts. Our finding corroborates the general theme that inherited phenotypic traits that cause a change in performance and thereby provide a fitness benefit to an organism become fixed within a lineage as a morphological adaptation (Arnold, 1983). Our results add to a growing body of literature comparing functional traits across different lineages on islands (Losos, 1990; Grant & Grant, 2002) or isolated habitats, such as lakes (Liem, 1973). But here, we show the importance of ecologically mediated selection producing a radiation of morphological forms in a continental arena.

FUNCTIONAL MORPHOLOGY

Our study fills a large geographical gap in the literature documenting morphology on locomotor performance in anurans and testing these questions on a diverse African frog clade. We found that jump distance, jump velocity and sprint velocity all increase with relative hindlimb length. This has been found for other anurans (Choi *et al.*, 2003; James *et al.*, 2007;

James & Wilson, 2008; Gomes *et al.*, 2009; Jorgensen & Reilly, 2013; Herrel *et al.*, 2014) and is expected for a body that is under acceleration for an extended time (Gray, 1968). In addition, thigh width was positively correlated with jump distance, jump velocity, sprint velocity and terrestrial endurance velocity, possibly because proportionally larger muscles can exert a greater force. Cross-sectional muscle area has been shown to correspond to frog locomotion mode (Ponssa *et al.*, 2018), and muscle mass has been shown to be positively correlated with jump velocity in other frogs (Choi & Park, 1996). However, we found that neither hindlimb length nor thigh width was correlated with swimming velocity in pyxicephalids, whereas the latter was found to be correlated by Moen *et al.* (2013). Instead, we found that the extent of pedal webbing was positively correlated with swim velocity, as found in other frogs (Moen *et al.*, 2013), and this is expected because it enables a greater volume of water to be displaced. We found that body size was positively correlated with almost all performance traits, with the exception of adhesive ability, which was negatively correlated; this is not surprising given that the adhesive force required is proportional to the ratio of surface contact to body mass, which decreases with increasing body size (Emerson, 1991; Moen *et al.*, 2013). In addition, we found that adhesive ability was positively correlated with relative finger disc diameter, as shown by Moen *et al.* (2013), suggesting that expanded finger tips enhance adhesive forces (Emerson & Diehl, 1980; Blackburn *et al.*, 2013; Chakraborti *et al.*, 2014). Therefore, we have confirmed many of the correlations between morphological and locomotory traits for African pyxicephalids that have been demonstrated in anurans from other regions.

Our trials on endurance, a frequently overlooked performance trait, suggest that the species that slowed or stopped owing to exhaustion during the terrestrial endurance activity also tended to have relatively long hindlimbs, a finding that is novel for anuran locomotion. Augmenting this, we found that species with relatively long hindlimbs (that jumped swifter and further) fatigued faster, which might be indicative of a more general functional trade-off between burst speed and endurance in anurans. Astley (2016) hypothesized that the contrast in hindlimb length between jumping and walking anurans could be attributable to a conflict between long hindlimbs and walking, or simply a case of optimizing growth to locomotor requirements; our results tend to support the former hypothesis. Long hindlimbs might encumber recovery in between jumps, whereas short hindlimbs facilitate a cyclic bound (Reilly *et al.*, 2015), as seen for *Pyxicephalus adspersus* (A.D.R., pers. obs.), and reduce anaerobic metabolic activity (Taigen *et al.*, 1982). However, interspecific differences in muscle fibre composition might also

play a role (Esbjörnsson *et al.*, 1993; Wilson & James, 2004). Indeed, faster muscle fibre contractions shown for anuran species with strong jumps were also found to result in rapid muscle fatigue (Astley, 2016). It is likely that species undergoing selection for enhanced jump performance will maximize both muscle fibre performance and leg length simultaneously, and the exact effect of these traits on fatigue will need to be tested in a carefully designed and isolated *in vitro* experiment. Regardless of the exact mechanical trade-off, the ability to jump faster and further is extremely valuable for evading predators (Herrel *et al.*, 2016). It is therefore interesting that some species are weak jumpers, because this suggests that predation pressure is not a strong selective force for them. A good example of this are the toxic dendrobatid frogs, which have no need to escape predators actively (Reilly & Jorgensen, 2011). We can speculate that some species are less affected by predation because they spend most of their time underground (such as *Tomopterna* and *Pyxicephalus*) or that others remain hidden within vegetation or mud (such as *Arthroleptella*, *Anhydrophryne* and *Poyntonia*). However, it is also possible that the ability to escape from predators has been traded off against endurance or burrowing ability, such that selection might override the advantages of being a strong jumper in favour of powerful legs for excavation (Zug, 1978; Moen *et al.*, 2013; Vidal-García *et al.*, 2014) or endurance to forage or to reach a breeding site (Smith & Green, 2006).

ECOMORPHOLOGICAL ADAPTATION

The evolutionary constraints and opportunity imposed by the environment on a lineage can facilitate speciation and diversification (Schluter, 2009). Microhabitats have been associated with anuran morphology (Vidal-García *et al.*, 2014; Enriquez-Urzelai *et al.*, 2015; Moen *et al.*, 2016), in addition to locomotion for many large clades (Zug, 1978; Gomes *et al.*, 2009; Moen *et al.*, 2013; Robovska-Havelkova *et al.*, 2014). We tested this in a family of African anurans and found that both morphology and locomotor performance have been influenced by selective pressures from microhabitats. Specifically for morphology, we found that semi-aquatic species have more extensive webbing and semi-arboreal species have dramatically wider fingertips, as found for anurans on other continents (Emerson & Diehl, 1980; Moen *et al.*, 2013). Like Moen *et al.* (2013), we also found that morphology was more distinctive between microhabitats than locomotor performance, in addition to having larger estimates of phylogenetic signal. This could mean that locomotion is less conserved than morphology, that performance experiments have additional sources of error, or it might even be indicative that we measured

performance traits that are not the most relevant to the fitness of these anurans.

LOCOMOTOR ADAPTATION

The greatest support for different locomotor selective optima between microhabitats was for fossorial species, which were weaker jumpers with poor adhesive ability but were able to move further during endurance trials than non-fossorial species. Fossorial species are known to have short hindlimbs (Zug, 1978; Gomes *et al.*, 2009; Moen *et al.*, 2013; Vidal-García *et al.*, 2014), which reduces the time that the foot is able to generate force during take-off (Demes *et al.*, 1996; Choi *et al.*, 2003) and therefore impedes jump performance (Zug, 1978; Gomes *et al.*, 2009; Citadini *et al.*, 2018). The robust bodies of fossorial species should constrain acceleration during a jump, because more mass needs to be accelerated, and the ratio of mass to area in contact with the substrate should reduce adhesive ability (Emerson & Diehl, 1980). However, we demonstrated that relatively short hindlimbs might be beneficial for sustained movement, which might be a strong selective force for species that migrate to breed (Smith & Green, 2006). Interestingly, Zug (1978) also found that fossorial species took longer to fatigue, but the lack of endurance experiments between multiple anuran species has limited further insight on the subject.

Semi-aquatic species were found to have considerably stronger jumps than other groups, as previous findings suggest (Rand, 1952; Citadini *et al.*, 2018). The refuge that water provides from terrestrial predators might have driven this association, where frogs that can reach the water in a single jump are less likely to be eaten. Surprisingly, adhesive ability did not differ drastically for semi-arboreal species, despite considerable differences in finger disc diameter and contrary to results in previous studies (Moen *et al.*, 2013). In hindsight, this might have been because some terrestrial species were minute in size (< 30 mm), which enabled static adhesion by surface friction between the ventral surface and the substrate. We expect that the semi-arboreal species would have outperformed other species while actively climbing with only the surfaces of their hands and feet in contact with the substrate.

We were not surprised to find high estimates of phylogenetic signal in both morphology and locomotion, given the similarities between species from the same genera and their shared microhabitat preferences. The large Blomberg's *K* values (greater than one) for morphology suggest that these traits are being conserved and that the locomotor traits are not phylogenetically independent. This reinforces the importance of using a phylogeny to prevent

pseudoreplication. We postulate that the phylogenetic signal represents evidence for convergent selection for clades that have adapted successfully to their respective niches and that these niches are partly encompassed by our interpretation of species microhabitat.

However, despite being phylogenetically conserved, not all morphological traits contribute to differences in selective optima between microhabitats, such as body size, hindlimb length and thigh muscle width. Body size was also found to show no difference between microhabitats for anurans in other studies (Moen *et al.*, 2013; Vidal-García *et al.*, 2014). It is possible that these morphological traits have no functional effect (Blankers *et al.*, 2012) but were nonetheless correlated with locomotor performance. Interestingly, although jumping ability had a separate optimum for semi-aquatic microhabitats, relative hindlimb length did not, despite being correlated with jump ability. Hindlimb length cannot account fully for all variation in jumping ability, and this might explain why it was not different for species from semi-aquatic microhabitats. This emphasizes the importance of selecting and measuring a trait that is relevant to performance, rather than simply using a morphological trait that is expected to affect fitness in a specific manner (Arnold, 1983). Furthermore, it suggests that some traits are not linked to microhabitat, such as body size. Whether this is because our microhabitat classification is flawed or because other factors are influencing suitability of habitats, such as larval requirements, whose developmental constraints might not yet be appreciated fully in adults, remains to be seen. Another explanation is that responses to predators differ between species, where some might rely on crypsis or small but unpredictable jumps to avoid predators, and these might be linked to subtle differences in microhabitats, such as the substrate. Finally, we acknowledge the small sample size ($N = 25$) for conducting phylogenetic analysis (Garamszegi, 2014), and these results should be interpreted with caution.

IMPLICATIONS FOR CONTINENTAL EVOLUTION IN ANURANS

Although most interspecific evolutionary studies use clades from islands or between continents, we chose one that is constrained to a single continent. Despite the obvious lack of land boundaries for lineage isolation, we find that the Pyxicephalidae has managed to colonize and adapt to a wide range of habitats in sub-Saharan Africa. This suggests that ecological opportunity, as seen in island systems (Grant & Grant, 2002), also facilitates diversification within continents. Although this was always assumed (Schluter, 2000), continental examples have remained absent for most taxa. The

diversification of the Pyxicephalidae occurred roughly in concert with other African anuran genera (Bossuyt *et al.*, 2006; Feng *et al.*, 2017), which is surprising because niche competition should have limited the ecological opportunity (Schluter, 1988). However, it is possible that environmental changes in southern Africa, such as rainfall patterns/seasonality, unlocked ecological opportunity and caused isolation events (Tolley *et al.*, 2014), analogous to radiations on island systems. It has been suggested that diversification of the remarkable ecological radiation of Australian Hylidae (Moen *et al.*, 2013) resulted from aridification over the last 15 Myr (Vidal-García & Keogh, 2015). In fact, the major diversification of anurans in general appears to coincide with the late Cretaceous–early Tertiary, which might relate to the ecological opportunity from the rise of Angiosperms in this period (Roelants *et al.*, 2007). It appears that speciation after colonization and isolation in montane environments has occurred in the pyxicephalid genera *Arthroleptella* (Turner & Channing, 2017) and *Nothophryne* (Bittencourt-Silva *et al.*, 2016), and for a couple species of *Amietia* (Larson *et al.*, 2016) and *Strongylopus*. However, the mechanism or event driving speciation in other members of the family is more difficult to assess given their wide and often sympatric distribution. Here, we show that anuran adaptive diversification is not limited to separate landmasses or islands and that pyxicephalids were still colonizing new environments alongside their, possibly more specialized, anuran relatives.

CONCLUSION

We conclude that pyxicephalid frogs have adapted, in both morphology and locomotion, to environments on the African continent. However, much of the variation in morphology and performance cannot be explained by microhabitat. The question remains: can other aspects of life history account for these species' differences? Some morphological traits (body size, relative hindlimb length and relative thigh width) and microhabitats (seeps and aquatic) show no association with adaptation through locomotory performance. In this study, we have reinforced the link between morphology and anuran locomotion to include African anurans and, for the first time between anuran species, supported a clear trade-off between burst speed and endurance. We emphasize that, although island systems are useful models for adaptive radiation, adaptive diversification can still occur on single landmasses.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Results for morphological correlates of jump performance within the Pyxicephalidae, using phylogenetic generalized least squares with simultaneous estimation of λ . Predictor variable abbreviations include: HLEG, size-corrected hind-leg length; MASS, size-corrected body mass; MFW, size-corrected mid-femur width; and SVL, snout–vent length. Column names include: AICc, second-order Akaike information criterion; lnL, log-likelihood; K, number of model parameters; and w_i , weight of evidence. The AICc best models are denoted in bold.

Table S2. Results for morphological correlates of sprint velocity within the Pyxicephalidae, using phylogenetic generalized least squares with simultaneous estimation of λ . Predictor variable abbreviations include: HLEG, size-corrected hind-leg length; MASS, size-corrected body mass; MFW, size-corrected mid-femur width; and SVL, snout–vent length. Column names include: AICc, second-order Akaike information criterion; lnL, log-likelihood; K, number of model parameters; and w_i , weight of evidence. The AICc best model is denoted in bold.

Table S3. Results for morphological correlates of swim velocity within the Pyxicephalidae, using phylogenetic generalized least squares with simultaneous estimation of λ . Predictor variable abbreviations include: HLEG, size-corrected hind-leg length; MFW, size-corrected mid-femur width; SVL, snout–vent length; and TWS, pedal webbing score. Column names include: AICc, second-order Akaike information criterion; lnL, log-likelihood; K, number of model parameters; and w_i , weight of evidence. The AICc best model is denoted in bold.

Table S4. Results for morphological correlates of terrestrial endurance within the Pyxicephalidae, using phylogenetic generalized least squares with simultaneous estimation of λ . The AICc best models are denoted in bold.

Table S5. Results for morphological correlates of aquatic endurance within the Pyxicephalidae, using phylogenetic generalized least squares with simultaneous estimation of λ . Predictor variable abbreviations include: HLEG, size-corrected hind-leg length; MFW, size-corrected mid-femur width; SVL, snout–vent length; and TWS, pedal webbing score. Column names include: AICc, second-order Akaike information criterion; lnL, log-likelihood; K, number of model parameters; and w_i , weight of evidence. The AICc best models are denoted in bold.

Table S6. Results for morphological correlates of adhesive performance within the Pyxicephalidae, using phylogenetic generalized least squares with simultaneous estimation of λ . Predictor variable abbreviations include: FD, size-corrected fingertip diameter; HNDL, size-corrected longest finger length; MASS, size-corrected body mass; and SVL, snout–vent length. Column names include: AICc, second-order Akaike information criterion;

$\ln L$, log-likelihood; K , number of model parameters; and w_i , weight of evidence. The AICc best models are denoted in bold.

Table S7. The criteria used to categorize pyxicephalid species into different microhabitats.

Table S8. Microhabitat combinations and coding strategies for the seven scenarios used to test for separate selective optima using the Ornstein–Uhlenbeck process. Abbreviations include: A, aquatic; Ar, semi-arboreal; F, fossorial; MH, microhabitat; S, seepage; SA, semi-aquatic; and T, terrestrial.

Table S9. The loadings of morphological traits for phylogenetic principal component (PC) axes. Abbreviations: FDD, finger-tip diameter; FM, femur length; FTL, foot length; MASS, body mass; MFW, mid-femur width; MT, tarsus length; SVL, snout–vent length; TB, tibia length; TWS, extent of pedal webbing.

Table S10. The loadings of locomotor performance traits for phylogenetic principal component (PC) axes. Abbreviations: ADH, adhesive ability; DDIST, terrestrial endurance distance; DEI, terrestrial endurance index; J_DIST, jump distance; J-VEL, jump take-off velocity; SPR_VEL, sprint velocity; SW_VEL, swim velocity; WEI, aquatic endurance index; WDIST, aquatic endurance distance.

Table S11. Voucher specimen numbers and accession numbers for the three genes deposited on GenBank used to construct the phylogeny of the Pyxicephalidae. NS denotes genes with no record of a voucher specimen number.

Table S12. The phylogenetic signal estimates for morphological and locomotor performance traits of 25 species of pyxicephalid frogs.

Table S13. The optimal morphological traits for microhabitat in MH5 estimated from an Ornstein–Uhlenbeck (OU) model using maximum likelihood. The principal component (PC) axes and their strength of selection (α), rate of drift (σ^2) and the selective optima for semi-arboreal (Ar), semi-aquatic (SA) and terrestrial (T) species are shown. Note that in this scenario terrestrial species represent non-aquatic species and that PC1, accounting for body size, was omitted from the OU modelling.

Table S14. The optimal locomotor traits for microhabitat in MH7 estimated from an Ornstein–Uhlenbeck (OU) model using maximum likelihood. The principal component (PC) axes and their strength of selection (α), rate of drift (σ^2) and the selective optima for fossorial (F) and terrestrial (T) species are shown. Note that in this scenario terrestrial species represent all non-fossorial species.

Table S15. The optimal locomotor traits for microhabitat in MH6 estimated from an Ornstein–Uhlenbeck (OU) model using maximum likelihood. The principal component (PC) axes and their strength of selection (α), rate of drift (σ^2) and the selective optima for semi-aquatic (SA) and terrestrial (T) species are shown. Note that in this scenario terrestrial species represent all non-aquatic species.

Table S16. Summary of morphological traits for all adult frogs included in the analyses, showing the mean values on the left and the standard deviation on the right. Abbreviations: FDD, longest finger disc diameter; FW, longest finger width; HLEGL, hindlimb length; HM, humerus length; HNDL, hand length; MFW, mid-femur width; N , number of specimens; RD, radio-ulna length; SVL, snout–vent length; TWS, number of phalanges free of webbing.

Table S17. Summary of locomotor performance traits for all adult frogs included in the analyses, showing the mean values on the left and the standard deviation on the right. Abbreviations: A_{down}, adhesive ability facing downwards; A_{up}, adhesive ability facing upwards; Dist_AE, distance moved during aquatic endurance; Dist_TE, distance moved during terrestrial endurance; EI_AE, aquatic endurance index; EI_TE, terrestrial endurance index; JDist, jump distance; JVel, jump velocity; N , number of specimens; SpVel, sprint velocity; SwVel, swim velocity; Time_AE, time spent moving during aquatic endurance; Time_TE, time spent moving during terrestrial endurance.

Figure S1. Apparatus used for performance experiments: A, jumping; B, sprinting/swimming; C, endurance; and D, adhesive ability.

Figure S2. Maximum likelihood tree for the Pyxicephalidae using 12S and 16S RNA and *Tyr* genes. Maximum likelihood bootstrap support values are shown at the nodes. *Ptychadena anchietae*, *Ptychadena erlangeri* and *Ptychadena nana* were used as outgroups. *Species included in this study.