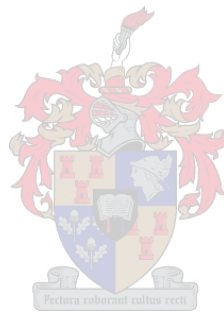


**Investigating the morphology, locomotory performance  
and macroecology of a sub-Saharan African frog radiation  
(Anura: Pyxicephalidae)**

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Thesis presented in partial fulfillment of the requirements for the degree  
Master of Science in Zoology at Stellenbosch University

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March 2017

## **Declaration**

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## Abstract

The phenotypic diversity among closely related species is often attributed to the process of natural selection. This process retains heritable traits within a population, increasing effectiveness of movement within the environment they occupy to maximise their fitness. Morphological traits can be selected to modify aspects of locomotion to better suit certain requirements. Such traits could also have an effect on distributional patterns, and could drive large-scale macroecological patterns. Understanding how interspecific differences in morphology relate to functional and distribution patterns can provide clues to the evolutionary and macroecological processes that drive them.

In this study I compare interspecific differences in morphology and locomotor performance of the Pyxicephalidae. I hypothesise that morphology will affect locomotor performance and that these differences are best explained by the habitat and ecology of the species. Additionally I investigate whether morphological and reproductive traits can explain interspecific differences in geographic range size; and use modelling to determine their affect on colonisation ability and niche breadth.

Morphology and locomotion was assessed for 25 wildcaught pyxicephalid species. Swimming and jumping performance was filmed at a high-frame rate, endurance was assessed by chasing frogs around a circular track and adhesive performance by rotating frogs on a non-stick surface. Specimens were measured and dissected from museums for reproductive and additional morphological data. Range size was calculated using a minimum convex polygon from distributional data. MaxEnt was used to model habitat suitability with Worldclim and topographic predictors. Colonisation Index was derived from habitat suitability to quantify the ability of a species to occupy nearby suitable habitats and niche breadth was calculated with the Outlier Mean Index (OMI) analysis, using the same predictor variables, but constraining the geographic extent to South Africa and species therein.

Species morphology had a significant influence on the measured locomotive traits, which confirmed similar functional relationships found for other frog clades. Furthermore, I find support that separate selective optima for morphology between burrowing, terrestrial and semi-aquatic ecotypes, but not for locomotor performance. However, specific tests between traits showed that semi-aquatic ecotypes had support for a separate performance selective optimum. Species geographic range size was positively correlated with body size and relative clutch size, but not relative head width or hindlimb length. The Colonisation Index was not robust for comparing species from different environments and range extents. Species niche breadth was not explained by either body size or relative clutch size, but by relative hindlimb length, suggesting that these former traits do not affect range size by increasing species ability to colonise and occupy a broader range of environmental conditions.

In summary, species body size and reproductive output are indirectly linked to range size patterns, but these patterns appear to be the result of an indirect association with abundant habitats or the ability to disperse and colonise within suitable habitat. The morphological diversity of the Pyxicephalidae has functional significance for locomotor performance, and some of these traits do represent ecotype adaptations. However, the limited evidence presented in this study does not support the Pyxicephalidae as an adaptive radiation.

### **Keywords**

Africa; Pyxicephalidae; Anura; locomotion; morphology; ecomorphology; macroecology; distribution; adaptation; radiation; dispersal; colonisation

## Opsomming

Die fenotipiese diversiteit tussen nouverwante spesies word dikwels toegeskryf aan die proses van natuurlike seleksie. Hierdie proses behou oorerflike eienskappe binne 'n doeltreffende beweging wat die oorlewing verhoog binne in hulle omgewing. Seleksie van morfologiese eienskappe kan lei tot aanpassings wat aspekte van beweging beïnvloed om hul behoeftes beter te pas. Hierdie eienskappe kan 'n uitwerking op verspreidingspatrone, en grootskaalse makro-ekologiese patrone te hê. Hoe die interspesifieke verskille in die morfologie verband hou met die funksionele en verspreidingspatrone, kan aandui hoe die evolusionere en makro-ekologiese prosesse die patrone beïnvloed.

In hierdie studie vergelyk ek interspesifieke verskille in morfologie en lokomotoriese prestasie van die Pyxicephalidae. My hipotese is dat morfologie die lokomotoriese prestasie sal beïnvloed en dat hierdie verskille verander met habitat en ekologie. Verder het ek kyk of die morfologiese en reprodktiewe kenmerke die grootte van die geografiese verskeidenheid kan verduidelik en gebruik modelleer die kolonisasie vermoë en nisbreedte.

Morfologie en voortbeweging was vir 25 pyxicephalid spesies beoordeel. Swem en spring prestasie is getoets deur stadige aksie verfilming; uithouvermoë was bepaal deur die paddas te jaag om 'n sirkelvormige baan; en adhesie is gemeet op 'n kleefvrye oppervlak wat gedraai was. Paddas van museums was gemeet en ontleed om morfologiese en reprodktiewe kenmerke te kry. Verspreidings grootte was bereken met behulp van 'n minimum konvekse veelhoek vir verspreiding data. MaxEnt is gebruik om habitat geskiktheid met Worldclim en topografiese voorspellers te modelleer. Kolonisasie indeks is afgelei van habitat geskiktheid om die vermoë van 'n spesie om nabygeleë geskikte habitate te gebruik te kwantifiseer. Breedte bereken met die ontleding Uitskieter Gemiddelde Indeks (OMI), met hulp van dieselfde voorspeller veranderlikes, maar met die beperking van die geografiese mate tot Suid-Afrikaanse en spesies daarin.

Die morfologie van spesies het 'n beduidende invloed op lokomotoriese prestasie wat gemeet was. Verder vind ek ondersteunende bewyse dat verskillende selektiewe optima vir morfologie tussen grawende, land- en semi-akwatiese eko-tipes, maar nie vir lokomotoriese prestasie nie. Spesifieke toetse wat gedoen was tussen eienskappe wys dat semi-akwatiese paddas 'n verskillende selektiewe optimale vir lokomotoriese prestasie het in vergelyking met die ander eko-tipe hê. Die geografiese verskeidenheid in grootte van die paddas is positief gekorreleer met liggaamsgrootte en relatiewe broeiselgrootte, maar is nie gekorreleer met relatiewe kop breedte of agterbeen lengte nie. Die nisbreedte van spesies kan nie verduidelik word deur liggaamsgrootte of relatiewe broeiselgrootte nie, maar kan verduidelik word deur relatiewe agterbeenlengte, wat daarop dui dat

hierdie voormalige eienskappe beïnvloed nie die verskeidenheid grootte deur die verhoging van spesies vermoë om te koloniseer nie.

Om op te som, spesies liggaamsgrootte en reprodktiewe uitset is indirek gekoppel aan die gebiedserspreidings patrone maar dit lyk asof hierdie patrone verduidelik kan word deur indirekte assosiasie tussen die beskikbaarheid van habitate en die vermoë van 'n spesies om te versprei en te koloniseer in beskikbare habitate. Die morfologiese diversiteit van die Pyxicephalidae het funksionele betekenis vir lokomotoriese prestasie, en 'n paar van hierdie eienskappe verteenwoordig eko-tipe-aanpassings. Die beperkte bewyse van hierdie studie ondersteun nie die Pyxicephalidae as 'n aanpasbare radius.

**Trefwoorde**

Afrika; Pyxicephalidae; Anura; voortbeweging; morfologie; ecomorphology; makro-; verspreiding; aanpassing; bestraling; verspreiding; kolonisasie

## **Acknowledgements**

My supervisor John Measey has pushed me hard over the last two years, but this enabled me to accomplish more than I would have previously thought possible. I am grateful for his hard work, his sense of humour and for the opportunity to work on an ambitious and enjoyable project with him. I would also like to thank the members of the Measey Lab, whom have made my stay in Stellenbosch more enjoyable.

For their hospitality, I am indebted to David and Gill Weaver, Mike Cherry, Nico and Lyzette van der Linde, Werner and Christa Conradie and my parents for hosting me during fieldwork. I would like to thank Atherton de Villiers, Andrew Turner, Michael Cunningham, Jeanne Tarrant, Louis du Preeze, Graham Alexander, James Harvey, James Vonesh, Mike McCoy, Nick Evans and Raquel Garcia for their useful advice during my MSc. For fieldwork assistance I am grateful to Michael Cunningham, Werner Conradie, Giovanni Vimercati, James Morton, Corey Thorpe, Mohlamatsane Mokhatla and Savel Daniels. For logistical and lab support I was luckily enough to have Christy Momberg, Mathilda van der Vyver, Erika Nortje and Suzaan Kritzinger-Klopper for assistance. A special thanks to Liza Carne who measured many of the museum specimens that contributed to my study and Erin Jooste who helped analyse many hours of video.

For assisting with access to specimen collections I would like to thank Alan Channing, Andrew Turner, Werner Conradie and Eli Greenbaum. I would also like to thank the following institutions for allowing access to their specimen collections: Port Elizabeth Museum, Iziko Museum, Ditsong Museum, California Academy of Sciences, University of Texas, National Museum of Bloemfontein and South African Institute for Aquatic Biodiversity. I am grateful for the access to species distribution data that was provided by the Animal Demography Unit, South African National Biodiversity Institute, iSpot, Endangered Wildlife Trust, Port Elizabeth Museum, Iziko Museum and Ditsong Museum.

I would like to thank Stefan Richerts (SanParks), Clive (Crowned Eagle Conservancy), Paul Painter (Alphenvale Retirement Village) and CapeNature for enabling me to collect frogs on their properties.

I would like to thank the Centre for Invasion Biology (CIB), Stellenbosch University (merit bursary) and the National Research Foundation (NRF) for funding.

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## Chapter 1: General introduction

The diversity of form and function observed around the world is attributed to the process of natural selection that has been modifying heritable traits since life began (Darwin 1859). Adaptive traits directly affect an organism's survival and reproductive success and are selected to maximise these outcomes. Strong directional selection can result in great phenotypic differences between lineages (Rieseberg et al. 2002), even between closely related species (Glor et al. 2003). This can arise following novel ecological opportunities that create multiple adaptive peaks, enabling lineages to climb new adaptive peaks (Losos & Malher 2010). Morphology can functionally constrain an organism, due to the physical properties of biological structures (Koehl 1996). Morphology can thus affect the survival of an organism through its influence on locomotory performance, and become adapted for a specific function within a lineage. Differences in external morphological features are often distinguishable between species and can be useful to test for a functional link with performance traits.

Locomotion is ecologically relevant for most animals, being integral for foraging/prey capture, predator escape and reproduction (Garland & Losos 1994; Dickinson et al. 2000; Sinervo et al. 2000). Locomotion encompasses a suite of functional traits which are likely to be under strong selection, making it an ideal candidate to investigate adaptations. Lineages have evolved adaptive traits in the context of their surrounding environment (Losos & Malher 2010). Organisms that find themselves in novel environments can experience an adaptive mismatch, where their physiology/behaviour is not suited to the prevailing conditions, which decreases the chance of survival (Hayes & Barry 2008). For example, Karpestam et al. (2012) found that dark-coloured grasshoppers had a higher mortality when relocated to environments with more solar radiation. However, mismatches between adaptive traits and the environment have the potential to rekindle adaptation by removing the barriers of stabilising selection. Furthermore, ecological opportunities change the adaptive landscape, facilitating the emergence of different adaptive traits and lineage divergence, as is famously demonstrated in the Galapagos finches (Lack 1947; Dobzhansky 1948). Ecological opportunities can arise from the colonisation of novel environments (Bilton et al. 2002), presence of dominant or competing species and through evolution of key innovations (Gavrilets & Losos 2009; Losos & Malher 2010). Lineages that experience multiple speciation events with associated diversification of adaptive traits are termed adaptive radiations (Glor 2010), and these provide a unique opportunity to investigate the functional significance of traits and ecological processes driving selection (Losos 2009).

The adaptive nature of a trait can be investigated by testing its functional role in the performance of an organism and then measuring the difference in fitness between environments (Koehl 1996), where evolutionary fitness represents the ability of an organism to survive and produce offspring. However, with advances in genomics, we are now able to use new phylogenetic methods to test evolutionary hypotheses without a measure of fitness (Butler & King 2004). Environmental variables can be categorised into microhabitat uses, such as aquatic, terrestrial, burrowing or arboreal, in order to test whether different selective pressures have altered the morphological or performance traits measured in contemporary species. Different functional roles and their associated morphology have been demonstrated to support separate selective forces between these microhabitat uses. For example, Moen et al. (2013) show that arboreal frogs have enlarged finger tips for clinging, semi-aquatic species have more pedal webbing for swimming and that burrowing species have larger meta-tarsal tubercles and shorter limbs for digging. However, morphological traits are not always relevant for microhabitat use, as has been demonstrated in an adaptive radiation of plethodontid salamanders, where morphology is largely uncoupled from microhabitat use (Blankers et al. 2012). It is therefore important to test for evidence of selection on a trait between environments to be able to infer its adaptive significance.

Locomotion and morphology also have other ecological ramifications, such as influencing distribution patterns of a species. However, the influence of such traits on macroecological patterns remains poorly understood, despite the availability of distribution data and the relevance to invasion and conservation biology. In theory, locomotion determines the kernel that a species can disperse and colonise suitable habitats. Furthermore, locomotion affects migration between populations, which can enable populations on marginal habitat suitability to persist even if there is a local decline in population growth.

Dispersal of a species is proportional to both dispersal distance and the number of individuals dispersing over time. More individuals allow rapid colonisation of new environments, increase the chances that individuals will disperse large distances, and supply more individuals to bolster declining populations. The number of individuals dispersing can be approximated by the natural density of individuals within the environment and the annual reproductive output. The latter is only relevant if a proportion of the offspring survive to the dispersal phase. However, empirical studies rarely find evidence for an effect of dispersal on range size (Lester et al. 2007). This has been attributed to the presence of other dispersal-independent factors that can influence distribution dynamics (Dispersal might not be the limiting factor of range size). Habitat fragmentation determines the importance of dispersal, where continuous suitable habitat or impassable barriers result in the same species range extent regardless of species' dispersal ability. Species are confined to accessible suitable

habitat, thus the range size should also be proportional to the abundance of accessible suitable habitat. However, species with broader environmental tolerances or generalist habitat requirements should be able to occupy a broader range of habitat types. Therefore, a species range size should represent the abundance of accessible suitable habitat, as determined by its dispersal and life-history traits.

In this thesis, I focus on the family Pyxicephalidae, an ecologically diverse anuran lineage consisting of 77 species within 12 genera (Frost 2016). It is claimed that this family has undergone an adaptive radiation across its geographic extent in sub-Saharan Africa (van der Meijden et al. 2005), but evidence is needed to define a radiation as adaptive (Losos & Malher 2010). The Pyxicephalidae offers an opportunity to study functional, evolutionary and macroecological patterns in a family that has been largely omitted from such studies in the past. It is a particularly useful clade to investigate these topics because all its taxa share a common ancestor (van der Meijden et al. 2005), which allows powerful phylogenetic comparisons; they occupy an overlapping geographic extent and they exhibit a diversity of life-history strategies and phenotypic differences (Bittencourt-Silva et al. 2016; van der Meijden et al. 2005; van der Meijden et al. 2011). The pyxicephalid common ancestor is estimated to have originated in the late Mesozoic (70 Mya) and is thought to have been a medium to large frog that occupied the widespread savanna and lowland forest habitats, utilising water for breeding (van der Meijden et al. 2005; Bittencourt-Silva et al. 2016). This ancestor is hypothesised to have diverged into multiple lineages that subsequently adapted to novel environments and gave rise to a number of ecologically and phenotypically distinct clades within sub-Saharan Africa (van der Meijden et al. 2005; van der Meijden et al. 2011).

The most striking difference between pyxicephalid species is the difference in body size, which ranges from some of the smallest frogs in the region (*Cacosternum* & *Arthroleptella*: snout-vent length (SVL) 10-20 mm) through intermediate sizes to the largest (*Pyxicephalus adspersus*: SVL 150-250 mm; Figure 1.1; van der Meijden et al. 2011). The stubby short legs of *Pyxicephalus* and *Tomopterna* are contrasted with the long elegant legs of *Strongylopus* and *Natalobatrachus* (Figure 1.2) and their impressive ability to cover great distances within a single jump (Minter et al. 2004). The extent of webbing between the toes extends up to the 5th phalange in the river-associated *Amietia vertebralis*, but varies between species and is absent in some taxa, such as *Arthroleptella* that avoid open water (Lambiris 1989; Channing & Baptista 2013; Minter et al. 2004). The head of *Pyxicephalus* and *Amietia vertebralis* are remarkably wide (Tarrant et al. 2008; Minter et al. 2004), possibly related to dietary requirements, while many *Strongylopus* species have narrow heads and long-toes that match their ability to jump through and on top of dense Restionaceae and sedges (Rose 1926; Measey pers. comm.). The monotypic *Natalobatrachus bonebergi* is a particularly

intriguing pyxicephalid species due to its greatly expanded finger and toe tips (Lambiris 1989), which it presumably uses to adhere to slippery plant leaves and rocks it lives upon (Minter et al. 2004).

The ecological diversity of this family is just as remarkable (Figure 1.3; Addendum A.7). Some taxa, such as *Pyxicephalus*, *Tomopterna* and *Cacosternum capense*, dig their own burrows and remain buried underground for extended periods (Rose 1929; de Villiers 1931; Withers & Loveridge 1981). Terrestrial species often utilise crevices, debris and vegetation as refugia and spend most of their time foraging on the ground, such as *Strongylopus* and *Cacosternum* (Minter et al. 2004). Pyxicephalids have also entered the aquatic environment, *Amietia* are associated with the edges of waterbodies that they dive into at any sign of danger (Rose 1926), while *Aubria* and *Amietia vertebralis* and *Amietia hymenopus* spend more time within the water itself (Burger, pers. comm.; pers. obs.). Some species only occupy permanently moist seeps, in the mountains of the Cape Fold Belt (*Arthroleptella* & *Poyntonia*) (Channing & Boycott 1989; Turner 2010), Eastern Arc Mountains (Bittencourt-Silva et al. 2016), alongside streams in Afromontane forests leaf litter (*Anhydrophryne hewitti* & *A. ratrayi*), and in grassland seeps (*A. ngongoniensis*) (Dawood & Stam 2006; Minter et al. 2004). Finally, *Natalobatrachus bonebergi* occurs alongside forested streams where it lives upon low shrubs and rocky ledges (Minter et al. 2004; van der Meijden et al. 2005).

Pyxicephalid species vary in both the reproductive output and the oviposition site of their eggs. *Tomopterna* and *Pyxicephalus* are considered to be ‘explosive breeders’ that produce thousands of eggs per female per breeding event around temporary pans following rainfall (Balinsky & Balinsky 1954; van der Meijden et al. 2011). *Cacosternum* breed in similar temporary wetlands but produce a smaller clutch size (Rose 1926), possibly due to limitation by their small body size (Kuramoto 1978). *Amietia* breed over a longer period within permanent waterbodies, producing large clutches, while *Strongylopus* specialise by laying their eggs on the ground at the edge of the water during rain, which then hatch when the water-level rises and floods them (Rose 1929, pers. obs.). *Natalobatrachus* utilise their arboreal advantage by ovipositing their moderately-sized clutch on vegetation overhanging a waterbody (Minter et al. 2004); such strategies have been suggested to reduce ovule predation from aquatic predators (Goin & Goin 1962). Some pyxicephalids take it to the next extreme: they oviposit their small clutches in moist areas where they develop into tadpoles that live in the shallow seepage waters (*Poyntonia*) or develop directly into froglets (*Arthroleptella* & *Anhydrophryne*) (van der Meijden et al. 2011).

In the following chapters, I will explore how interspecific differences in morphological traits influence ecologically relevant locomotory traits, whether there are differences consistent

with adaptation to specific microhabitats and finally, whether common macroecological patterns can be observed and understood using phenotypic and reproductive traits within the Pyxicephalidae.

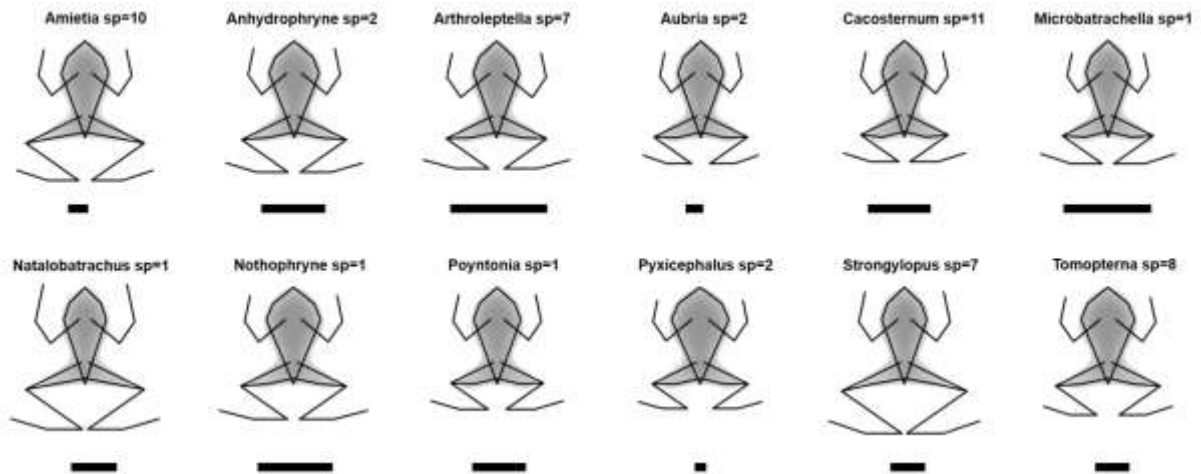


Figure 1.1. Graphical representations of relative body proportions for averaged over species for each pyxicephalid genus. The number of species averaged within each plot is given by the “sp.”, the scale bar beneath each plot represents 10 mm. Measurements used to plot these genera include snout-vent length, head width, humerus length, radius length, longest finger length, femur length, tibiofibular length, metarsus length and longest toe.



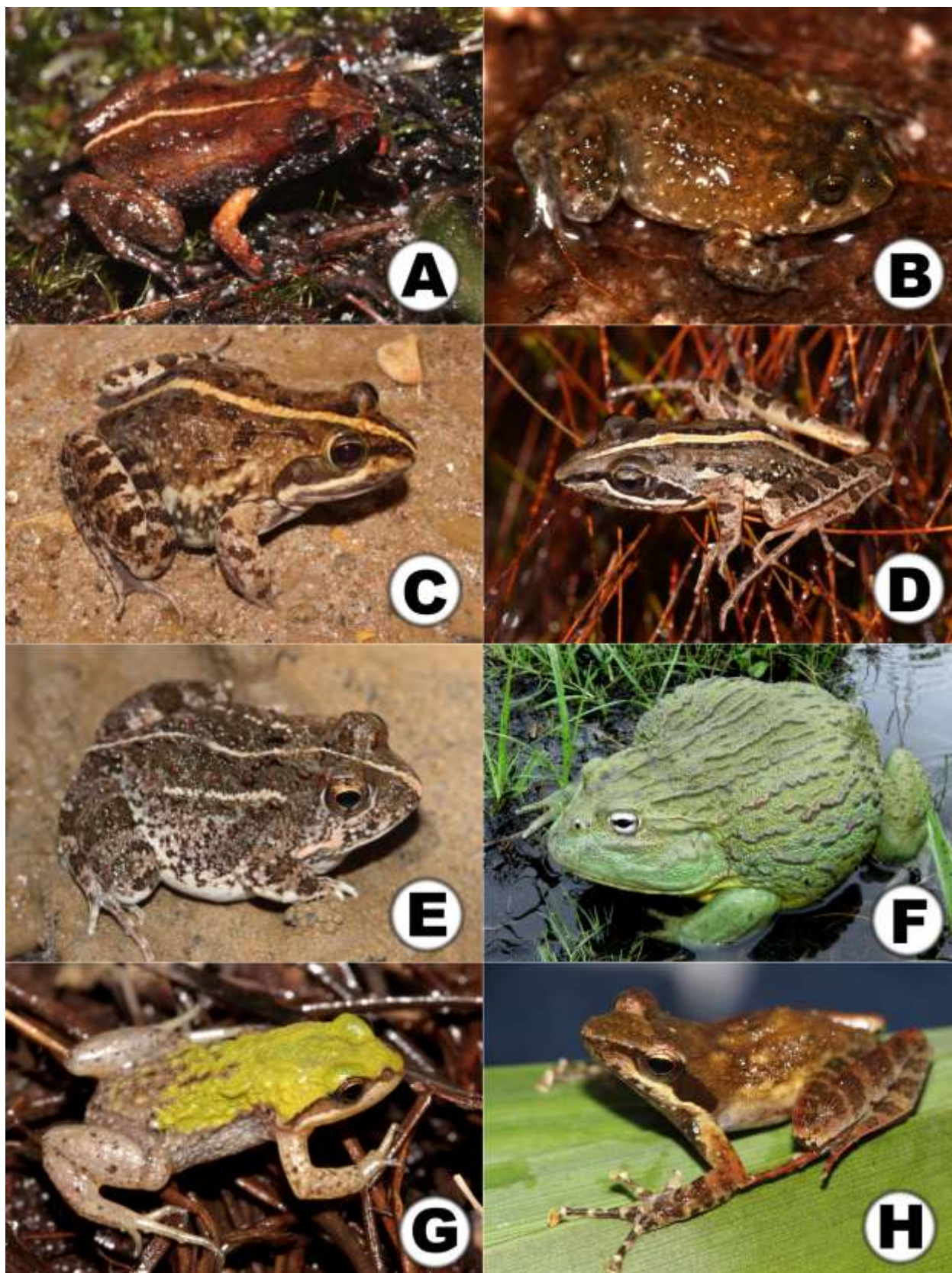


Figure 1.2. Photographs representing most of the phenotypic diversity within the Pyxicephalidae. A: *Arthroleptella lightfooti*; B: *Poyntononia paludicola*; C: *Amietia fuscigula*; D: *Strongylopus bonaespei*; E: *Tomopterna cryptotis*; F: *Pyxicephalus adspersus*; G: *Cacosternum thorini*; H: *Natalobatrachus bonebergi*.

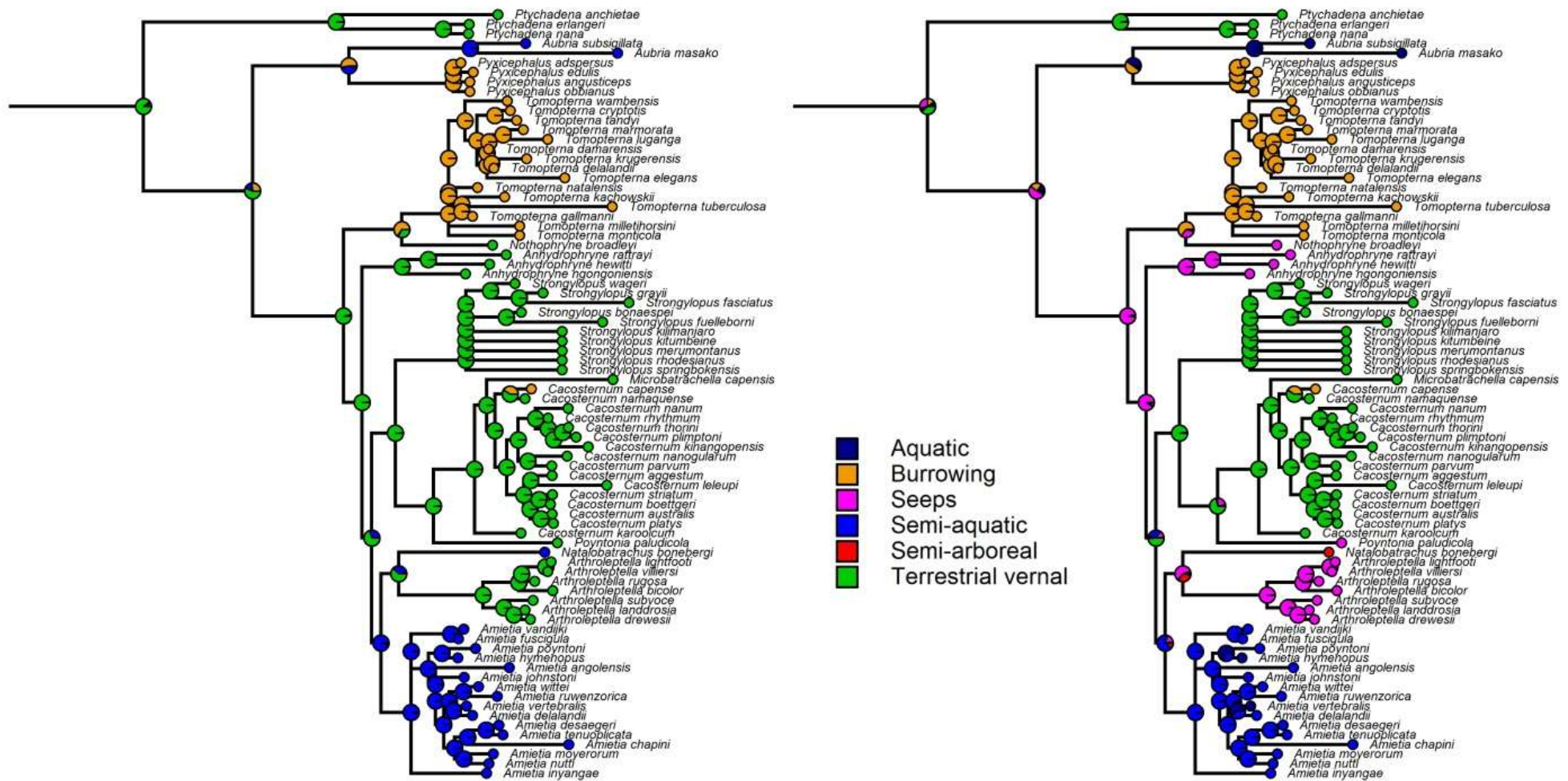


Figure 1.3. The remarkable ecological diversity of the Pyxicephalidae categorised into different coloured ecotypes and plotted on a ML phylogeny constructed from concatenated 16S, 12S and Tyrosinase genes (Addendum A.5). The ancestral ecotype states were reconstructed using the function ‘ace’ with ML and marginal reconstruction in the R package ape (Paradis et al. 2004). The broad ecotype scheme is shown on the left and the narrow on the right; note that seeps are considered as the terrestrial ecotype in the broad scheme.

## **Chapter 2: Locomotor performance constrained by morphology but not habitat: ecomorphology and adaptive insights from an African frog radiation (Anura: Pyxicephalidae)**

### **2.1 Introduction**

Differences in heritable traits between species can arise through several evolutionary processes, but natural selection is thought to have generated the majority of trait diversity (Funk 1998). Natural selection can act rapidly on a lineage, sometimes resulting in an adaptive radiation where multiple lineages diverge and undergo unique adaptations for resources in a variety of environments (Schluter 1996). The adaptive advantage of certain traits can be investigated by testing their effect on ecologically relevant performance traits (Koehl 1996). For example, Losos & Sinervo (1989) found that shorter limbs in *Anolis* lizards increased stability while moving along thin perches, granting an adaptive advantage for the arboreal specialists. Locomotion is a vital component to many aspects of vertebrate life, such as foraging ability, reproductive success and predator escape (Dickinson et al. 2000; Sinervo et al. 2000; Garland & Losos 1994), and should therefore be under strong selection. These traits can be compared between species to contrast their fitness optima and test ecological hypotheses.

Morphological traits have a strong impact on the whole-body locomotor performance between anuran species (Zug 1972; Zug 1978). Basic principles of physics predict that the velocity of a body will increase as the amount of force per unit time and the duration of the applied force increases. This has been demonstrated in anurans, where thicker thigh muscles (Choi et al. 2003; Choi & Park 1996) and longer legs (Rand 1952; Howell 1944 in Gans & Parsons 1966; Zug 1972; Zug 1978; Emerson 1978; Emerson 1986; Gomes et al. 2009; Herrel et al. 2014) enable frogs to jump with increased velocity and cover a greater distance. Maximum jump distance and acceleration could mean the difference between life and death during a predation attempt (Gans & Parsons 1966; Choi & Park 1996). However, not all frog species are excellent jumpers (Zug 1972).

Multiple morphological traits can contribute to the locomotor performance of an individual (Gould & Lewontin 1979; Koehl 1996; Scales & Butler 2016). For example, body size can also influence multiple aspects of locomotion. Hill (1950) predicted that larger animals will jump at the same velocity and cover the same distance as smaller animals. However, Emerson (1978) found that larger frog species jump farther. Gomes et al. (2009) also found

that jump distance increases with body size, but increases with diminishing returns for increasing body size. Body size was also hypothesised to affect endurance: Hill (1950) predicted that fatigue resistance should increase with the body size of the organism. In contrast, James et al. (2007) hypothesised that smaller species that can utilise elastic energy in jumps will be more energetically efficient than larger species that rely purely on muscle action.

Different aspects of locomotion can have conflicting demands on morphological traits. For example, burst performance has been shown to compromise endurance capacity in human athletes (Van Damme et al. 2002) and among lizard species (Huey et al. 1984; Vanhooydonck et al. 2001; Scales & Butler 2016). A population comparison of Cane Toads revealed that locomotor endurance was higher at the invasion edge, but that this was unrelated to morphology and did not support a trade-off with movement velocity (Llewelyn et al. 2010). However, interspecific comparisons of endurance ability between closely related anurans are needed to test this adequately. Endurance capacity can be beneficial for species that utilise seasonally available or spatially diffuse resources (Alerstam et al. 2003). Some anurans migrate considerable distances to their breeding sites during the rainy season (Spieler & Linsenmair 1998) and others move frequently while actively foraging for food (Wells 2007).

The habitat of an organism dictates the most appropriate aspect of performance that maximises the fitness within that environment (Koehl 1977; Garland & Losos 1994). Studies on anurans have revealed that different habitat types can explain some interspecific variation of morphology and locomotor performance traits (Moen et al. 2013; Vidal-García et al. 2014). Effective swimming ability is important for aquatic prey capture, escape from predatory fish, and to overcome water currents (Richards 2008). Swimming ability can be enhanced with more extensive pedal webbing (Stamhuis & Nauwelaerts 2005), larger thigh muscles (Moen et al. 2013) and adjusting proportions of hindlimb element ratios (Richards & Clemente 2013). Arboreal species require exceptional adhesion in order to move across slippery and vertical surfaces such as leaves and bark. Specialised toe and finger pads, which are expanded at the tip, have a specialised micro-surface that greatly enhances adhesive ability (Emerson & Diehl 1980; Blackburn et al. 2013; Chakraborti et al. 2014). Alternatively, some traits are also linked to other important ecological roles and can result in conflicting demands. For example, burrowing frogs require powerful movements for digging, which limits their hindlimb length (Hill 1950; Enriquez-urzelai et al. 2015) and thus they tend to be poor jumpers (Gomes et al. 2009). However, not all traits are expected to match simple

habitat categorisation, for example frogs of different body sizes are often found within the same habitat (Enriquez-urzelai et al. 2015).

Adaptions that have evolved in the context of a specific habitat are often not beneficial in different environments, and could even be maladaptive (Ward-Fear et al. 2009). Therefore, it is expected, and has been demonstrated in previous anuran studies that some locomotor and morphological traits represent ecotype adaptations (Gomes et al. 2009; Moen et al. 2013; Moen et al. 2016). In this study, I investigate the effect of morphology on locomotor performance and test for evidence of adaptation in the Pyxicephalidae, which has undergone a potentially adaptive radiation (van der Meijden et al. 2005). This sub-Saharan African frog family consists of 77 species within twelve genera (Frost 2016) that inhabit a variety of ecotypes and display a remarkable diversity of morphological, locomotor and reproductive traits (van der Meijden et al. 2005; van der Meijden et al. 2011; Bittencourt-Silva et al. 2016). Pyxicephalidae encompasses semi-aquatic (*Amietia*, *Aubria*), terrestrial (*Strongylopus*, *Arthroleptella*, *Anhydrophryne*, *Nothophryne*, *Poyntonia*, *Microbatrachella*, *Cacosternum*), burrowing (*Pyxicephalus*, *Tomopterna*) and semi-arboreal (*Natalobatrachus*) ecotypes. In this study I predict that hindlimb length and muscle mass positively correlate with both the burst and endurance aspects of terrestrial and aquatic locomotion (Gray 1968; Choi & Park 1996; Choi et al. 2003; James et al. 2007; James & Wilson 2008; Gomes et al. 2009; Herrel & Bonneaud 2012; Jorgensen & Reilly 2013; Moen et al. 2013; Herrel et al. 2014), with pedal webbing extent positively correlating with swim locomotion (Moen et al. 2013). Furthermore, body size and fingertip diameter is expected to affect adhesive performance by increasing the downwards force and surface area for adhesion respectively (Moen et al. 2013). Finally, I test whether aspects of both morphological and locomotor traits have evolved according to separate selective optima between ecotypes, as opposed to a nonadaptive model.

## 2.2 Methods

Twenty-five pyxicephalid species were selected to measure morphometrics and test locomotor performance. These were specifically chosen to provide a good representation of the morphology, phylogenetic relatedness and ecotype preference within the family. Representatives for 10 of the genera were tested, including *Amietia*, *Arthroleptella*, *Anhydrophryne*, *Cacosternum*, *Natalobatrachus*, *Poyntonia*, *Pyxicephalus*, *Strongylopus* and *Tomopterna*. The remaining two genera, *Aubria* and *Nothophryne*, were not tested due to logistical constraints. Ethical clearance was obtained from Stellenbosch University's REC: ACU (Protocol #: SU-ACUD15-00101). Collections were conducted under permits for Cape Nature (0056-AAA043-00009), DETEA Free State (S45C-515111613151), DEDEAT Eastern Cape (CRO 204/15CR), Ezemvelo KZN (OP 3825/2015) and GDARD (ToPS 0-09534).

### Specimen capture

Species-specific searches targeted known localities (Addendum A.1) during periods of breeding activity, for ease of detection. Frogs were located by visual or auditory cues and captured by hand or hand net. Up to 10 adult male specimens were collected for each species; individuals with injuries or deformations were not captured. Female frogs were excluded from this study because their eggs can affect locomotor performance (Zug 1978; Herrel et al. 2014), and these could bias functional relationships due to interspecific breeding strategies as well as increasing the variability of performance if females were collected after oviposition. However, collecting 10 adult males was not possible for all species due to their scarcity or difficulty to determine sex. Specimens were transported in individual sealable plastic bags, pre-moistened and maintained at a temperature below 25 °C.

### Testing environment

Locomotor performance traits were tested within the Department of Botany and Zoology research facilities at Stellenbosch University when possible, or else at temporary accommodation facilities. Any surfaces and equipment exposed to test subjects were disinfected with a 10% bleach solution and thoroughly rinsed before testing individuals from different localities. Tapwater used in swimming components was left overnight to dechlorinate and reach room temperature. Frogs were kept at room temperature on the night prior to, and during the performance testing. Because temperature is known to have a confounding effect on jump performance, performance tests were confined to temperatures between 18 and 23° C where possible and was recorded for each performance trial (Addendum A.2).

### Procedure

Performance testing extended over two days for each group of specimens, which comprised of six performance aspects: jump, sprint, swim, adhesion, terrestrial and aquatic endurance (Addendum A.3). These traits were selected to address a range of known locomotor functions with potential for adaptation (Moen et al. 2013). On the first day, jump, sprint and terrestrial endurance performance were tested, in that order. Swim, followed by aquatic endurance performance was tested on the second day. Each frog was rested for at least an hour after jumping, sprinting and swimming before continuing with subsequent trials. Adhesion was measured directly after terrestrial endurance to minimise the frog activity during the trial. Performance trials were scheduled to end following terrestrial endurance on the first day and aquatic endurance on the second, maximising the time available for recovery after this strenuous activity. Endurance trials were tested at the end of each day to

reduce the effects of fatigue on tests requiring sudden, maximum exertion performance. Frogs were released at the capture site on the second or third day of testing.

### Jumping

Frogs were placed on flat cork tiles against a perpendicular background. A known distance was marked on the background to be used as calibration during video analysis. A camera (Canon powershot G16) was positioned on a tripod to face the background and the full trajectory of the jumps was filmed at 240 frames per second (fps). Frogs were induced to jump by lightly touching the legs or blowing air on them from behind. Frogs were filmed until at least three successful jumps were made, defined as being parallel with the background and with maximal exertion, although some frogs cleared the distance in a single jump. This method incorporates the ability of the frog to recover from the landing of the previous jump and could be relevant for predicting escaping from a pursuing predator. Frogs that showed visible declines in performance before these jumps could be filmed were rested for 1-2 minutes before retrying.

### Sprinting

The velocity of frogs for multiple consecutive jumps was measured within a rectangular track, with a flat bottom length of 1 m and width 0.3 m, with vertical walls of 0.3 m. The camera was placed on a tripod above the track to face down and view the length of the track. A known distance was marked on the track to be used as calibration during video analysis. Frogs were induced to move down the track, while being filmed at 120 fps, by touching/blowing the frog in rapid succession without letting the frog rest during one length. This was continued until each frog completed at least three lengths of the track. Smaller frog species were only required to move a fraction of the track's length. Initially the frogs were placed on the polyester track surface, but this was replaced at later stage in the study with cork tiles due to the lack of traction provided by the polyester (Addendum A.1).

### Swimming

The same track and setup described in the sprint test was filled with water to a depth that ensured that a frog could not kick off the floor of the track. Frogs were filmed at 120 fps until at least three sequences of at least 5 consecutive kicks were obtained, or until the performance decreased noticeably.

### Endurance

The ability for frogs to resist fatigue during locomotion was tested using a circular track. The track consisted of an inner and outer vertical wall to constrain frog movement in a circular

motion. The circumferences of the inner and outer walls were 2.6 and 4.5 m, respectively. Frogs were induced to move in a single direction until they either became exhausted or twenty minutes had passed (only 15 min for the aquatic trial). Exhaustion was declared if the frog refused to move for more than 15 consecutive seconds. Both terrestrial and aquatic trials were tested once for each individual. The bottom of the track was filled with water for the aquatic trial and frogs induced to swim in a similar manner to the terrestrial trial. Laps and half laps were timed for the duration of the trial and the lap number was counted. The distance of a single lap was measured as the circumference at the midpoint between the inner and outer walls (3.5 m).

### Adhesion

Frog adhesive ability was measured by placing frogs on a non-stick (tetrafluoroethane) surface and rotating the surface at 20 degrees per second until the frog was dislodged. This surface was chosen because it has been used previously to simulate the surface of waxy leaves or rocks (Moen et al. 2013). The degree of the surface at the point of dislodgement was recorded for each rotation. Frogs were orientated to face both upwards and downwards for three repetitions each. Trials where frogs jumped off the rotating surface before losing adhesion were repeated.

### Trait measurements

The morphometrics of the live specimens were measured at the end of performance trials (Addendum A.4). Initially electronic callipers were used with the aid of a dissecting microscope. This was later replaced by measuring photographs using ImageJ ver. 1.49 (Rasband 1997), as this method had more consistent measurements to the nearest 0.1 mm. Frogs were positioned and photographed flush against 10 mm<sup>2</sup> gridded paper with both a dorsal and ventral view. These morphological measurements included snout-vent length (SVL; snout tip to end of ischium), snout-urostyle length (SUL; snout tip to end of urostyle), body width (BW; maximum lateral width of body), head width (HW; maximum width of head), mid-femur width (MFW, relaxed width of middle of thigh), femur length (FM; mid-pelvic girdle to knee), tibiofibula length (TB; length of bone), tarsus length (MT; heel to proximal metatarsus), foot length (FTL; proximal metatarsus to end of longest toe), mid toe width (TW; width at the middle of the second last phalange of the longest toe), terminal toe disk diameter (TDD; maximum width of last phalange on longest toe), mid radio-ulna width (MTW), humerus length (HM; from one third of pelvic width to tip of elbow), radio-ulna length (RD; tip of elbow to proximal metacarpal), hand length (HNDL; proximal metacarpal to tip of longest finger), mid finger width (FW; width at the middle of the second last phalange of the longest finger), terminal finger disk diameter (FDD; maximum width of last phalange on



longest finger). Pedal webbing was scored for each species according to method outlined in Zimkus et al. (2012). Frogs were then weighed to the nearest 0.01 g on a top pan balance (Radwag WTB 2000).

#### Video-data extraction

All videos were examined and clipped into smaller files to create individual instances of the performance activity. Three clips were visually selected for each filmed activity per individual and used in further data extraction and analysis. These clips were selected to represent the maximal performance of an individual in terms of jump distance, sprint/swim velocity and continuous movement (in sprints/swimming).

Two data extraction methods were used. The first extracted the distance and velocity of frog jumps by tracking the trajectory of the frog across individual frames using video editing software (Blender 3D). These coordinates were calibrated using a known distance from the video and exported with the reference frame number. The distance and time were calculated by correcting the pixel distance by the known calibration distance marked on the track and dividing the frame number by the fps of the recording, respectively. A second order polynomial function was fitted to the jump trajectory in order to derive the total horizontal distance for jumps that exited the video frame before landing. The instantaneous velocities along the jump trajectory were calculated in EXCEL (Microsoft Corp.) and filtered using a fourth order Butterworth filter with a cut-off frequency of 20 Hz (VBA for EXCEL; Van Wassenbergh 2007). Only the propulsion (take-off) phase of the trajectory was used to derive the maximum smoothed velocity as the second half of the trajectory was occasionally interrupted or went out of frame.

The second method calculated the average velocity over multiple frames by extracting the coordinates and frame number from sprinting and swimming videos using the Blender system. A predetermined calibration distance was also measured, as before, to provide a scaling factor. The average velocity was calculated from the total distance travelled and time between the start and end points.

#### Endurance data

In addition to total distance and time to exhaustion, I created a new metric to better capture the ability for frogs to resist fatigue. Fatigue is difficult to extract from distance and time if many taxa are able to continue moving up to and beyond the twenty-minute cut-off time, while the distance moved is related to the baseline velocity of the species. A new metric, forthwith called 'exhaustion index' (EI) was thus created. The EI was calculated by dividing

the average velocity for the first lap of the endurance trial by that of the last lap. Individuals that became exhausted by the end of the endurance trial were given an EI value of zero. For example, individuals that maintain the same velocity from start to finish will have an EI of one, while those that slow down over the duration of the trial will tend towards a lower EI value. This index appears to be effective at discriminating some degree of fatigue resistance, as species within the same genus show similar EI values and standard deviations are low for some species (Addendum A.3).

### Phylogeny estimation

A maximum likelihood (ML) phylogeny was estimated from available sequences of mitochondrial 16S and 12S, and nuclear Tyrosine for all pyxicephalid species available on Genbank (Addendum A.5; Benson et al. 2005). These genes had the best species coverage for which morphology and performance data were collected, while providing species-level, in addition to higher, phylogenetic resolution. Sequences used in Bittencourt-Silva et al. (2016) were preferentially chosen, but were replaced if other sequences with better gene coverage for a single specimen could be found, or if a BLAST search for that sequence did not match closely with other conspecific sequences of that gene on Genbank. All sequences of a gene were aligned using MUSCLE in MEGA 6 (Tamura et al. 2013). The two hyper-variable regions of 16S [55 and 23 bp, respectively] were removed from the dataset. Genes were concatenated if they were sampled from the same specimen, or else entered on a new line with blank-value genes missing from that specimen. The concatenated sequences were analysed with RAxML-HPC BlackBox v.8.2.8 (Miller et al. 2010; Stamatakis 2006) using a partition file and default settings (GTR+GAMMA) to create the ML tree and a GTRCAT model to produce non-parametric bootstrapping replicates (100 replicates).

The phylogeny was scrutinised in relation to the ML phylogeny of Bittencourt-Silva et al. (2016) and the gene with the best corresponding topology was selected for species with different genes from multiple specimens. However, in Bittencourt-Silva et al. (2016), *Strongylopus grayii* was nested within the *Amietia* clade, which is clearly an error and was noted by the authors. Thus instead of their 16S sequence, I selected a 12S sequence that placed it within the *Strongylopus* clade. I manually inserted all other missing pyxicephalid species using the function 'bind.tree' in the R package 'ape'. These species were inserted at the base of their respective genus with a branch length calculated as the average branch within that genus. Each manually inserted species was given a dichotomous branching structure with an small branch length of 0.000001 to enable estimation of ancestral character states.

### Data analyses

All analyses were performed in R (R Core Team 2015). All traits were first logged and then normalised, using the R function 'scale'. Preliminary correcting of traits for body size without taking phylogenetic relatedness into account can increase estimator variance and type 1 error (Revell 2009). To avoid this I used the function 'phyl.resid' in the R package 'phytools' (Revell 2012) to correct allometric scaling traits with the snout-vent length (SVL). The phylogenetic generalised least squares regression (PGLS) analysis was used with simultaneous estimation of Pagel's  $\lambda$ , as recommended in Revell (2010), using the R package 'caper' (Orme 2013). Only biologically sensible traits or those confirmed in previous literature were included in the regression model for any particular performance trait. Best-fit models were identified using AICc values to determine which morphological predictors were correlated to the locomotor performance trait.

Categories of ecotype were selected for testing evolutionary hypotheses using the Ornstein-Uhlenbeck (OU) process. Ecotype is here defined as the functional habitat that the species utilises during its non-breeding period. I divided this trait into two subsets, one to test broad differences between species and another for finer derivation of ecotype. Broad ecotypes were divided into four categories, namely semi-aquatic, terrestrial, burrowing and arboreal, as recognised in other publications (Zug 1978; Gomes et al. 2009; Moen et al. 2013; Moen et al. 2016). For narrow ecotypes I further divided this into aquatic, semi-aquatic, terrestrial opportunist, montane seeps, arboreal and burrowing. Ecotype states were assigned to species using literary accounts as well as personal field observations (Addendum A.6).

The OU process is used to simulate the stochastic variation of a trait with a tendency to converge over time on an optimal value (Butler & King 2004). This method can test for multiple selective optima within trait data given a selective regime across a phylogeny. However, this process requires classification of the ancestral nodes of the phylogeny according to the selective regime being tested. These discrete ancestral states were estimated using maximum likelihood with a Brownian motion model and marginal estimation in the function 'ace' from the R package 'ape' (Paradis 2012). The full phylogeny, including all pyxicephalid species, was used in the ancestral character estimation. The out-group taxa, *Ptychadena anchietae*, *P. erlanderi*, *P. mascareniensis* and *P. nana*, were selected due to their close relationship with pyxicephalids, within the basal Ranoidea (van der Meijden et al. 2005). The state with the greatest support was chosen for each node state, with the exception of seep ecotypes, which were replaced with terrestrial ecotypes because they are thought to have derived multiple times independently (van der Meijden et al. 2011), especially given the large geographic isolation between clades.

The adaptive influence of ecotype on morphological and performance traits of the Pyxicephalidae were tested using OU models of adaptive evolution (Butler & King 2004; Hansen 1997). This method is particularly suitable as it is able to distinguish between clade conservatism, single and multiple selective optima. I used a pruned version of the phylogeny, only including species for which both morphology and performance traits were measured. The modelling was done in the R package 'ouch' (King & Butler 2009). The pruned phylogeny was converted into an ouchtree object and used in the functions 'brown' and 'hansen' to model different selective regimes. I used eight different scenarios that differed according to the categorisation of ecotypes and number of selective optima. These included a null Brownian motion model with no selective optimum, a single optimum OU model, an OU model with selective optima for each genus (clade history), and finally 5 OU models with different categorisations schemes for ecotypes (Table 2.1).

Phylogenetic principal components analyses were conducted separately on morphological and locomotor performance trait variation using the function 'phyl.pca' in the R package 'phytools' (Revell 2012). Principal component scores for all axes were used to test the OU models, following the methods by Moen et al. (2016). In addition, separate sets of OU models were run for a selection of individual morphological and locomotor performance traits, specifically testing for an adaptation within a single ecotype compared with all other ecotypes. Only the relevant ecotype was classified within these models and all other ecotypes were grouped into an 'unassigned' ecotype. These traits have been demonstrated to be ecologically important in other anuran groups (Moen et al. 2013) and included: relative fingertip diameter, relative length of longest finger and clinging ability for the semi-arboreal ecotype, extent of pedal webbing and swimming velocity for the semi-aquatic ecotype and relative hindlimb length and jump velocity for the burrowing ecotype. Best-fit models were identified and compared using AICc values.

## 2.3 Results

### Morphology & Locomotor performance

Most locomotor performance traits were correlated with one morphological trait or a combination thereof (Tables 2.1-2.6). I found that body size was included in the best model and positively correlated for: jump distance ( $R^2_{adj}=0.92$ ;  $P<0.001$ ), jump take-off velocity ( $R^2_{adj}=0.89$ ;  $P<0.001$ ), sprint velocity ( $R^2_{adj}=0.88$ ;  $P<0.001$ ), swim velocity ( $R^2_{adj}=0.71$ ;  $P<0.001$ ), terrestrial endurance distance ( $R^2_{adj}=0.11$ ;  $P=0.08$ ), terrestrial endurance velocity ( $R^2_{adj}=0.48$ ;  $P<0.001$ ), aquatic endurance distance ( $R^2_{adj}=0.19$ ;  $P=0.027$ ) and aquatic

endurance velocity ( $R^2_{\text{adj}}=0.52$ ;  $P<0.001$ ). In addition, body size was negatively correlated for both upward ( $R^2_{\text{adj}}=0.85$ ;  $P<0.001$ ) and downward adhesive ability ( $R^2_{\text{adj}}=0.75$ ;  $P<0.001$ ).

Relative hindlimb length (sum of femur, tibio-fibula, calcaneum and longest toe) affected the most performance measures after body size. Relative hindlimb length was included in the best models for jump distance ( $R^2_{\text{adj}}=0.92$ ;  $P<0.001$ ), jump take-off velocity ( $R^2_{\text{adj}}=0.89$ ;  $P<0.001$ ), sprint velocity ( $R^2_{\text{adj}}=0.88$ ;  $P<0.001$ ) and terrestrial endurance velocity ( $R^2_{\text{adj}}=0.48$ ;  $P<0.001$ ), in which it was positively correlated. In addition, relative hindlimb length was included in the best models for terrestrial endurance time to exhaustion ( $R^2_{\text{adj}}=0.39$ ;  $P<0.001$ ), terrestrial endurance index ( $R^2_{\text{adj}}=0.51$ ;  $P<0.001$ ) and aquatic endurance index ( $R^2_{\text{adj}}=0.19$ ;  $P=0.045$ ), but with a negative correlation. Therefore, species with relatively long legs could reach greater take-off velocities, jump farther and move faster over a set distance, while shorter-legged species were more resistant to fatigue.

Relative mid-femur width was included in the best models for jump distance ( $R^2_{\text{adj}}=0.92$ ;  $P=0.002$ ), jump take-off velocity ( $R^2_{\text{adj}}=0.89$ ;  $P<0.001$ ), sprint velocity ( $R^2_{\text{adj}}=0.8849$ ;  $P=0.001$ ), terrestrial endurance velocity ( $R^2_{\text{adj}}=0.48$ ;  $P=0.081$ ) and aquatic endurance time ( $R^2_{\text{adj}}=0.16$ ;  $P=0.002$ ), in which it was positively correlated. Extent of foot webbing was included in the best models for swim velocity ( $R^2_{\text{adj}}=0.71$ ;  $P<0.001$ ) and aquatic endurance time ( $R^2_{\text{adj}}=0.10$ ;  $P=0.031$ ), in which it was also positively correlated.

Finger disk diameter relative to toe diameter was included in the best models for both upward ( $R^2_{\text{adj}}=0.85$ ;  $P=0.002$ ) and downward adhesive ability ( $R^2_{\text{adj}}=0.75$ ;  $P=0.004$ ), in which it was positively correlated. Relative finger length was included in the best models for both upward ( $R^2_{\text{adj}}=0.85$ ;  $P=0.08$ ) and downward orientated adhesive ability ( $R^2_{\text{adj}}=0.75$ ;  $P=0.14$ ) in which it was positively, but not significantly, correlated. Relative body mass was not included in the best models for any performance traits.

### Overall ecotype selection

The evolution of frog performance traits was best explained by a simple Brownian motion model, which outperformed models that incorporated separate optima for different ecotype types (Table 2.8). On the other hand, morphological traits were better explained by a multiple ecotype optima model, which included separate optima for burrowing, semi-aquatic and terrestrial species. The greatest signal strength for the optimal morphological model was for principal component 2, an axis related to multiple morphological traits, such as positively with leg length and negatively with head width, body size and forelimb length (once most variation in body mass and body size was accounted for in PC1; Addendum A.7).

### Specific trait ecotype selection

To separate the selection signal of supposed habitat-specific traits from other traits, I modelled these select performance and morphological traits individually (Tables 2.9 & 2.10 respectively). These habitat specific traits were tested in the following scheme: jump velocity and relative hindlimb with burrowing, swimming velocity and pedal webbing score with semi-aquatic, and relative finger disk diameter, relative hand length and adhesive performance with semi-arboreal ecotypes. I found that jump velocity, was best explained by a Brownian motion model of evolution, while relative hindlimb length was best explained by clade history. However, both swimming velocity and pedal webbing score were best explained by a separate selective optimum for semi-aquatic ecotypes. Finally, adhesive performance was best explained by Brownian motion, while relative finger disk diameters combined with relative hand length were best explained by a separate selective optima including the semi-arboreal ecotype.

Table 2.1. Ecotype combinations and coding strategies for the five scenarios used to test for separate selective optima between pyxicephalid ecotypes using the Ornstein-Uhlenbeck process. Ecotype abbreviations are: Ar (semi-arboreal), A (aquatic), B (burrower), S (seepage), SA (semi-aquatic), and T (terrestrial).

Scenario	Ecotypes	Description
1	B, SA, T	Broad: main ecotype habitat types, aquatic and arboreal with semi-aquatic, seepage with terrestrial
2	Ar, A, B, S, SA, T	Narrow: all ecotypes
3	Ar, A, S, SA, T,	same as 2, but burrowers with terrestrial
4	SA, T	same as 1, but burrowers with terrestrial
5	Ar, B, S, SA, T	same as 2, but aquatic with semi-aquatic

Table 2.2. Results for morphological correlates of jump performance within the Pyxicephalidae, using phylogenetic generalised least squares with simultaneous estimation of  $\lambda$ . Predictor variable abbreviations include SVL (snout-vent length), MASS (size-corrected body mass), HLEG (size-corrected hind-leg length), and MFW (size-corrected mid-femur width). Column names include log-likelihood (lnL), second-order Akaike Information Criterion (AICc), weight of evidence ( $w_i$ ) and number of model parameters ( $K$ ). The AICc best-fit models are denoted in bold.

Model predictors	lnL	AICc	$\Delta$ AICcs	$w_i$	$K$
<b>Jump Distance</b>					
SVL+HLEG+MFW	28.67	-47.34	0.00	<b>0.81</b>	4
SVL+HLEG+MFW+MASS	28.73	-44.31	3.04	0.18	5
SVL+HLEG	22.87	-38.59	8.75	0.01	3
HLEG+MFW	19.98	-32.81	14.53	0.00	3
HLEG	14.49	-24.44	22.90	0.00	2
HLEG+MASS	14.76	-22.39	24.96	0.00	3
SVL+MASS	5.60	-4.06	43.29	0.00	3
MFW	3.83	-3.12	44.22	0.00	2
SVL+MFW	4.34	-1.54	45.81	0.00	3
MFW+MASS	4.20	-1.26	46.09	0.00	3
SVL	1.01	2.52	49.87	0.00	2
null	-0.40	2.98	50.32	0.00	1
MASS	0.29	3.97	51.31	0.00	2
<b>Jump Velocity</b>					
SVL+HLEG+MFW	2.62	4.75	0.00	<b>0.66</b>	4
SVL+HLEG+MFW+MASS	3.22	6.71	1.96	0.25	5
SVL+HLEG	-0.85	8.84	4.09	0.09	3
HLEG+MFW	-8.10	23.35	18.60	0.00	3
HLEG	-10.63	25.81	21.06	0.00	2
HLEG+MASS	-10.53	28.20	23.45	0.00	3
SVL+MASS	-21.00	49.15	44.40	0.00	3
MFW	-23.61	51.77	47.02	0.00	2
SVL+MFW	-22.33	51.81	47.06	0.00	3
MFW+MASS	-22.72	52.59	47.83	0.00	3
SVL	-24.94	54.43	49.68	0.00	2
null	-26.44	55.06	50.30	0.00	1
MASS	-25.96	56.46	51.71	0.00	2

Table 2.3. Results for morphological correlates of sprint velocity within the Pyxicephalidae, using phylogenetic generalised least squares with simultaneous estimation of  $\lambda$ . Predictor variable abbreviations include SVL (snout-vent length), MASS (size-corrected body mass), HLEG (size-corrected hind-leg length), and MFW (size-corrected mid-femur width). Column names include log-likelihood (lnL), second-order Akaike Information Criterion (AICc), weight of evidence ( $w_i$ ) and number of model parameters (K). The AICc best-fit model is denoted in bold.

Model predictors	lnL	AICc	$\Delta$ AICcs	$w_i$	K
Sprint Velocity					
null	21.14	-32.28	0.00	<b>0.77</b>	1
SVL+HLEG+MFW+MASS	21.49	-29.82	2.46	0.22	5
SVL+HLEG+MFW	14.94	-22.73	9.55	0.01	4
SVL+HLEG	4.59	-4.63	27.64	0.00	3
SVL+MFW	5.37	-3.60	28.68	0.00	3
SVL+MASS	5.05	-2.95	29.33	0.00	3
HLEG+MFW	4.07	-1.01	31.27	0.00	3
HLEG+MASS	3.83	-0.52	31.75	0.00	3
MFW+MASS	0.03	4.49	36.77	0.00	3
SVL	-1.76	8.07	40.35	0.00	2
HLEG	-1.76	10.66	42.93	0.00	2
MFW	-4.37	10.91	43.19	0.00	2
MASS	-4.10	12.75	45.02	0.00	2

Table 2.4. Results for morphological correlates of swim velocity within the Pyxicephalidae, using phylogenetic generalised least squares with simultaneous estimation of  $\lambda$ . Predictor variable abbreviations include SVL (snout-vent length), TWS (pedal webbing score), HLEG (size-corrected hind-leg length), and MFW (size-corrected mid-femur width). Column names include log-likelihood (lnL), second-order Akaike Information Criterion (AICc), weight of evidence ( $w_i$ ) and number of model parameters (K). The AICc best-fit model is denoted in bold.

Model predictors	lnL	AICc	$\Delta$ AICcs	$w_i$	K
SVL+TWS	30.52	-53.89	0.00	<b>0.52</b>	3
SVL+HLEG+TWS	30.71	-51.42	2.47	0.15	4
SVL+HLEG+MFW+TWS	32.18	-51.20	2.68	0.13	5
HLEG+TWS	28.67	-50.19	3.70	0.08	3
SVL+MFW+TWS	30.04	-50.07	3.81	0.08	4
HLEG+MFW+TWS	29.00	-48.01	5.88	0.03	4
TWS	24.94	-45.34	8.55	0.01	2
MFW+TWS	25.46	-43.78	10.11	0.00	3
SVL	23.16	-41.77	12.12	0.00	2
SVL+MFW	24.31	-41.49	12.40	0.00	3
SVL+HLEG	23.74	-40.35	13.54	0.00	3
SVL+HLEG+MFW	24.04	-38.08	15.80	0.00	4
HLEG	17.33	-30.11	23.77	0.00	2
null	15.95	-29.72	24.17	0.00	1
MFW	16.48	-28.42	25.47	0.00	2
HLEG+MFW	17.63	-28.12	25.77	0.00	3



Table 2.5. Results for morphological correlates of adhesive performance within the Pyxicephalidae, using phylogenetic generalised least squares with simultaneous estimation of  $\lambda$ . Predictor variable abbreviations include SVL (snout-vent length), MASS (size-corrected body mass), FD (size-corrected fingertip diameter), and HNDL (size-corrected longest finger length). Column names include log-likelihood (lnL), second-order Akaike Information Criterion (AICc), weight of evidence ( $w_i$ ) and number of model parameters (K). The AICc best-fit models are denoted in bold.

Model predictors	lnL	AICc	$\Delta$ AICcs	$w_i$	K
Upwards Adhesive Angle					
SVL+FD+HNDL	-110.31	230.61	0.00	<b>0.49</b>	4
SVL+FD	-112.12	231.38	0.77	0.33	3
SVL+MASS+FD+HNDL	-109.97	233.10	2.49	0.14	5
SVL	-116.24	237.03	6.41	0.02	2
SVL+MASS	-115.90	238.95	8.33	0.01	3
SVL+HNDL	-116.16	239.47	8.85	0.01	3
null	-129.93	262.03	31.42	0.00	1
FD	-129.08	262.72	32.10	0.00	2
MASS	-129.34	263.23	32.62	0.00	2
HNDL	-129.94	264.42	33.80	0.00	2
Downwards Adhesive Angle					
SVL+FD+HNDL	-116.61	243.22	0.00	<b>0.49</b>	4
SVL+FD	-118.90	244.95	1.72	0.21	3
SVL	-120.81	246.16	2.94	0.11	2
SVL+MASS+FD+HNDL	-116.51	246.17	2.95	0.11	5
SVL+MASS	-120.51	248.16	4.93	0.04	3
SVL+HNDL	-120.80	248.75	5.52	0.03	3
FD	-126.32	257.18	13.96	0.00	2
null	-127.61	257.40	14.17	0.00	1
HNDL	-127.63	259.80	16.58	0.00	2
MASS	-127.69	259.93	16.70	0.00	2

Table 2.6. Results for morphological correlates of terrestrial endurance within the Pyxicephalidae, using phylogenetic generalised least squares with simultaneous estimation of  $\lambda$ . Predictor variable abbreviations include SVL (snout-vent length), MASS (size-corrected body mass), HLEG (size-corrected hind-leg length), and MFW (size-corrected mid-femur width). Column names include log-likelihood (lnL), second-order Akaike Information Criterion (AICc), weight of evidence ( $w_i$ ) and number of model parameters ( $K$ ). The AICc best-fit models are denoted in bold.

Model predictors	lnL	AICc	$\Delta$ AICcs	$w_i$	$K$
<b>Terrestrial Endurance Distance</b>					
SVL	-97.80	200.15	0.00	0.17	2
SVL+MASS	-96.53	200.21	0.06	0.17	3
SVL+HLEG	-96.60	200.35	0.20	0.15	3
HLEG	-98.29	201.13	0.98	0.10	2
null	-99.74	201.66	1.51	0.08	1
HLEG+MASS	-97.62	202.39	2.24	0.06	3
SVL+MFW	-97.73	202.60	2.46	0.05	3
MASS	-99.11	202.76	2.61	0.05	2
MFW+MASS	-97.96	203.06	2.92	0.04	3
SVL+HLEG+MFW+MASS	-94.98	203.12	2.97	0.04	5
SVL+HLEG+MFW	-96.61	203.22	3.07	0.04	4
MFW	-99.54	203.62	3.48	0.03	2
HLEG+MFW	-98.27	203.68	3.54	0.03	3
<b>Terrestrial Endurance Time</b>					
HLEG	-174.62	353.78	0.00	<b>0.35</b>	2
SVL+HLEG	-173.88	354.90	1.11	0.20	3
HLEG+MFW	-173.91	354.97	1.19	0.19	3
SVL+HLEG+MFW	-172.88	355.77	1.98	0.13	4
HLEG+MASS	-174.62	356.37	2.59	0.09	3
SVL+HLEG+MFW+MASS	-172.63	358.43	4.64	0.03	5
MFW	-179.92	364.39	10.61	0.00	2
null	-181.27	364.70	10.92	0.00	1
MFW+MASS	-179.09	365.33	11.54	0.00	3
SVL+MFW	-179.51	366.16	12.38	0.00	3
SVL	-181.11	366.76	12.98	0.00	2
MASS	-181.18	366.90	13.12	0.00	2
SVL+MASS	-180.94	369.02	15.24	0.00	3
<b>Terrestrial Endurance Velocity</b>					
SVL+HLEG+MFW	63.25	-116.51	0.00	<b>0.37</b>	4
SVL+HLEG	61.40	-115.66	0.85	0.24	3
SVL+HLEG+MFW+MASS	64.17	-115.19	1.32	0.19	5
SVL+MFW	60.36	-113.57	2.94	0.08	3
HLEG+MASS	59.77	-112.41	4.10	0.05	3
SVL	58.13	-111.71	4.80	0.03	2
HLEG	57.57	-110.60	5.91	0.02	2
SVL+MASS	58.34	-109.54	6.97	0.01	3
HLEG+MFW	57.76	-108.38	8.13	0.01	3

null	53.50	-104.82	11.69	0.00	1
MFW	54.49	-104.43	12.08	0.00	2
MASS	53.58	-102.61	13.90	0.00	2
MFW+MASS	54.49	-101.83	14.68	0.00	3
Terrestrial Endurance Index (EI)					
HLEG	13.98	-23.41	0.00	<b>0.40</b>	2
HLEG+MFW	14.51	-21.88	1.53	0.19	3
SVL+HLEG	14.37	-21.61	1.81	0.16	3
HLEG+MASS	14.25	-21.37	2.05	0.15	3
SVL+HLEG+MFW	14.89	-19.78	3.63	0.07	4
SVL+HLEG+MFW+MASS	15.08	-16.99	6.42	0.02	5
null	8.16	-14.15	9.26	0.00	1
MFW	9.01	-13.48	9.93	0.00	2
SVL	9.00	-13.45	9.96	0.00	2
SVL+MFW	9.63	-12.12	11.30	0.00	3
MASS	8.16	-11.77	11.64	0.00	2
MFW+MASS	9.43	-11.73	11.68	0.00	3
SVL+MASS	9.00	-10.85	12.56	0.00	3

Table 2.7. Results for morphological correlates of aquatic endurance within the Pyxicephalidae, using phylogenetic generalised least squares with simultaneous estimation of  $\lambda$ . Predictor variable abbreviations include SVL (snout-vent length), TWS (pedal webbing score), HLEG (size-corrected hind-leg length), and MFW (size-corrected mid-femur width). Column names include log-likelihood (lnL), second-order Akaike Information Criterion (AICc), weight of evidence ( $w_i$ ) and number of model parameters ( $K$ ). The AICc best-fit models are denoted in bold.

Model predictors	lnL	AICc	$\Delta$ AICcs	$w_i$	$K$
Aquatic Endurance Distance					
SVL	-95.41	195.36	0.00	<b>0.43</b>	2
SVL+MFW	-95.18	197.51	2.15	0.15	3
SVL+HLEG	-95.42	197.99	2.63	0.12	3
SVL+HLEG+TWS	-94.61	199.21	3.85	0.06	4
NULL	-98.52	199.21	3.86	0.06	1
SVL+MFW+TWS	-94.63	199.25	3.89	0.06	4
SVL+HLEG+MFW	-95.18	200.35	4.99	0.04	4
HLEG	-98.53	201.61	6.25	0.02	2
MFW	-98.58	201.71	6.35	0.02	2
TWS	-98.71	201.97	6.61	0.02	2
SVL+HLEG+MFW+TWS	-94.61	202.37	7.01	0.01	5
HLEG+MFW	-98.55	204.24	8.88	0.01	3
HLEG+TWS	-98.64	204.42	9.06	0.00	3
MFW+TWS	-98.71	204.56	9.21	0.00	3
HLEG+MFW+TWS	-98.64	207.27	11.91	0.00	4
Aquatic Endurance Time					
TWS	-164.47	333.49	0.00	0.21	2
MFW	-164.63	333.81	0.32	0.18	2

MFW+TWS	-163.73	334.60	1.11	0.12	3
NULL	-166.28	334.73	1.24	0.11	1
HLEG+TWS	-164.21	335.55	2.06	0.07	3
SVL+MFW	-164.26	335.65	2.16	0.07	3
HLEG+MFW	-164.65	336.45	2.96	0.05	3
SVL	-166.12	336.78	3.29	0.04	2
HLEG	-166.24	337.03	3.53	0.04	2
HLEG+MFW+TWS	-163.57	337.14	3.65	0.03	4
SVL+MFW+TWS	-163.61	337.23	3.74	0.03	4
SVL+HLEG+TWS	-164.19	338.39	4.90	0.02	4
SVL+HLEG+MFW	-164.29	338.57	5.08	0.02	4
SVL+HLEG	-166.23	339.60	6.11	0.01	3
SVL+HLEG+MFW+TWS	-163.41	339.98	6.49	0.01	5
Aquatic Endurance Velocity					
SVL	73.52	-142.50	0.00	<b>0.59</b>	2
SVL+HLEG	73.48	-139.81	2.69	0.15	3
SVL+MFW	73.37	-139.60	2.90	0.14	3
SVL+HLEG+TWS	73.49	-136.97	5.53	0.04	4
SVL+HLEG+MFW	73.41	-136.82	5.68	0.03	4
SVL+MFW+TWS	73.34	-136.69	5.81	0.03	4
SVL+HLEG+MFW+TWS	73.40	-133.63	8.87	0.01	5
NULL	63.90	-125.63	16.87	0.00	1
TWS	64.99	-125.42	17.08	0.00	2
HLEG	64.11	-123.68	18.83	0.00	2
MFW	64.11	-123.67	18.84	0.00	2
HLEG+TWS	65.16	-123.17	19.33	0.00	3
MFW+TWS	65.01	-122.87	19.63	0.00	3
HLEG+MFW	64.39	-121.65	20.86	0.00	3
HLEG+MFW+TWS	65.22	-120.45	22.06	0.00	4
Aquatic Endurance Index (EI)					
HLEG	3.06	-1.57	0.00	<b>0.29</b>	2
SVL+HLEG	3.84	-0.54	1.04	0.17	3
HLEG+MFW	3.70	-0.26	1.31	0.15	3
SVL+HLEG+MFW	4.47	1.06	2.63	0.08	4
HLEG+TWS	3.00	1.15	2.72	0.07	3
NULL	0.01	2.16	3.73	0.04	1
SVL+HLEG+TWS	3.88	2.24	3.81	0.04	4
HLEG+MFW+TWS	3.76	2.48	4.05	0.04	4
MFW	0.96	2.63	4.21	0.03	2
SVL+HLEG+MFW+TWS	4.84	3.48	5.05	0.02	5
SVL	0.50	3.55	5.13	0.02	2
SVL+MFW	1.34	4.46	6.04	0.01	3
TWS	-0.13	4.80	6.38	0.01	2
MFW+TWS	1.07	5.01	6.58	0.01	3
SVL+MFW+TWS	1.76	6.48	8.05	0.01	4

Table 2.8. Comparison of Ornstein-Uhlenbeck model criteria between different ecotype scenarios for all pyxicephalid morphological and performance principal components. Ecotype abbreviations are: Ar (semi-arboreal), A (aquatic), B (burrower), S (seepage), SA (semi-aquatic), T (terrestrial). The AICc best-fit models are denoted in bold. Column names include log-likelihood (lnL), second-order Akaike Information Criterion (AICc), weight of evidence ( $w_i$ ) and number of model parameters ( $K$ ). The AICc best-fit models are denoted in bold.

Class	Model predictors	lnL	AICc	$\Delta$ AICcs	$w_i$	$K$
<b>Performance</b>						
	Brownian motion	83.87	-126.82	0.00	<b>0.62</b>	2
	Single optimum OU	94.97	-125.66	1.16	<b>0.35</b>	3
	SA, T	105.29	-120.58	6.25	0.03	4
	B, SA, T	115.59	-112.75	14.07	0.00	5
	Ar, A, T, S, SA	128.52	-107.04	19.78	0.00	6
	Ar, B, T, S, SA	145.07	-104.85	21.97	0.00	7
	Ar, A, B, T, S, SA	152.34	-79.67	47.15	0.00	8
	Only clades	236.18	157.64	284.47	0.00	14
<b>Morphology</b>						
	B, SA, T	107.59	-149.39	0.00	<b>0.71</b>	5
	SA, T	98.31	-146.61	2.78	0.18	4
	Ar, B, T, S, SA	123.80	-144.66	4.73	0.07	7
	Ar, A, B, T, S, SA	134.33	-143.66	5.73	0.04	8
	Brownian motion	81.23	-139.72	9.67	0.01	2
	Ar, A, T, S, SA	111.43	-139.54	9.86	0.01	6
	Single optimum OU	80.51	-125.31	24.09	0.00	3
	Only clades	237.20	-124.40	24.99	0.00	14

Table 2.9. Comparison of Ornstein-Uhlenbeck model criteria between different ecotype scenarios for specific habitat relevant performance traits, jump take-off velocity, sprint velocity and upward orientated adhesive performance of the Pyxicephalidae. Ecotype abbreviations are: Ar (semi-arboreal), A (aquatic), B (burrower), S (seepage), SA (semi-aquatic), T (terrestrial), U (unassigned). Column names include log-likelihood (lnL), second-order Akaike Information Criterion (AICc), weight of evidence ( $w_i$ ) and number of model parameters ( $K$ ). The AICc best-fit models are denoted in bold.

Class	Model predictors	lnL	AICc	$\Delta$ AICcs	$w_i$	$K$
Jump velocity						
	Brownian motion	75.15	-145.75	0.00	<b>0.66</b>	2
	Single optimum OU	75.51	-143.88	1.87	0.26	3
	B, U	75.83	-141.66	4.09	0.08	4
	Only clades	101.11	-132.22	13.54	0.00	14
Swim velocity						
	SA, U	26.48	-42.96	0.00	<b>1.00</b>	4
	Single optimum OU	17.06	-26.98	15.97	0.00	3
	Brownian motion	12.33	-20.11	22.84	0.00	2
	Only clades	16.90	36.20	79.15	0.00	14
Grip (forward)						
	Brownian motion	-130.22	264.98	0.00	<b>0.89</b>	2
	Single optimum OU	-131.36	269.85	4.87	0.08	3
	Ar, U	-130.80	271.59	6.61	0.03	4
	Only clades	-114.68	299.37	34.38	0.00	14

Table 2.10. Comparison of Ornstein-Uhlenbeck model criteria between different ecotype scenarios for specific habitat relevant morphological traits of the Pyxicephalidae. Morphology abbreviations are: HLEG (size corrected hindlimb length), TWS (toe webbing score), FD (finger disk diameter corrected by finger width), HNDL (size corrected hand length). Ecotype abbreviations are: Ar (semi-arboreal), B (burrower), SA (semi-aquatic), U (unassigned). Column names include log-likelihood (lnL), second-order Akaike Information Criterion (AICc), weight of evidence ( $w_i$ ) and number of model parameters ( $K$ ). The AICc best-fit models are denoted in bold.

Class	Model predictors	lnL	AICc	$\Delta$ AICcs	$w_i$	$K$
HLEG						
	Only clades	-58.75	187.50	0.00	<b>0.79</b>	14
	Brownian motion	-92.99	190.52	3.02	0.17	2
	B, U	-92.03	194.06	6.57	0.03	4
	Single optimum OU	-95.29	197.71	10.22	0.00	3
TWS						
	SA, U	-29.69	69.37	0.00	<b>0.98</b>	4
	Single optimum OU	-35.02	77.18	7.81	0.02	3
	Brownian motion	-38.90	82.35	12.98	0.00	2
	Only clades	-15.04	100.08	30.70	0.00	14
FD+HNDL						
	Ar, U	-0.48	20.95	0.00	<b>1.00</b>	4
	Brownian motion	-26.09	61.27	40.32	0.00	2
	Only clades	35.27	69.47	48.52	0.00	14
	Single optimum OU	-29.11	72.50	51.55	0.00	3

## 2.4 Discussion

I find strong support for the influence of morphology on locomotor performance and demonstrate that the recent African pyxicephalid radiation is governed by similar mechanical constraints found in other anuran clades already studied (Gomes et al. 2009; Moen et al. 2013). Overall, the best fitted model of evolution for the morphological traits includes separate selective optima for burrowing, semi-aquatic and terrestrial ecotypes. On the other hand, performance traits are equally well explained by a Brownian motion model of evolution and a single selective optimum, with no support for separate ecotype scenarios. However, some habitat-specific morphological and locomotor traits were found to differ between ecotypes. For example, the semi-aquatic ecotype of *Amietia* supports the evolution of more extensive pedal webbing as well as the associated increase in swimming speed. Therefore, ecotype appears to have influenced specific functional morphological adaptations.

I find that jump distance and velocity, as well as sprint velocity, all increase with relative hindlimb length, consistent with biomechanical predictions (Gray 1968) and findings of other anuran studies (Choi et al. 2003; James et al. 2007; James & Wilson 2008; Gomes et al. 2009; Jorgensen & Reilly 2013; Herrel et al. 2014). This suggests that species that are under strong selection pressure to escape active predators might evolve relatively long limbs; these taxa included the genera *Amietia*, *Strongylopus* and *Natalobatrachus*, which were also the best jumpers. While the *Cacosternum* and *Arthroleptella* clades appear to have independently lost their jumping proficiency and have become miniaturized, which is possibly associated with a habitat transition (Zimkus et al. 2012), allowing them to hide from predators within grass tufts and mossy beds, respectively.

Surprisingly, the time to exhaustion during terrestrial endurance was negatively related to hindlimb length, contrary to intraspecific studies on *Xenopus* (Herrel & Bonneaud 2012; Herrel et al. 2014). For distance moved during the endurance trial, I predicted that species with longer legs would be able to move faster and thus reach a greater distance within the trial, assuming fatigue was equal between species. However, species with longer legs tired faster and could not sustain movement for as long shorter-legged species, as suggested by Putnam & Bennett (1981). This hypothesis is reinforced by the negative correlation hindlimb length on the endurance index for both terrestrial and aquatic endurance trials. Therefore, long-legged species also showed a greater reduction in velocity between the start and end of the trial. Indeed, I observed that species with longer legs often struggled to bring their legs back into jump position from a previous landing when fatigued (such as *Strongylopus* spp.). These antagonistic outcomes could be interpreted as a trade-off between speed and endurance, as found in lacertid lizards (Vanhooydonck et al. 2001), because longer

hindlimbs are optimal for burst performance which creates a morphology-function conflict (Van Damme et al. 2002). However, the mechanism driving this trade-off is unclear: proportions of muscle fibre type can determine the maximum power and fatigue properties of a muscle (James & Wilson, 2004). It is likely that performance requirements have influenced relative proportions of these fibres between species such that longer-legged species also have high burst, but easily fatigued fibres, as seen in some lizards (Bonine et al. 2001; Scales et al. 2009). Selection pressure should favour burst performance in species that forage in exposed habitats or areas with abundant and active predators, whereas species required to migrate long distance could benefit more energy efficient locomotion. However, I can only speculate whether this is the artefact of predatory avoidance or longer limbs actually limit persistent movement in standard dispersal type locomotion, due to the predator escape nature of the trial.

Contrary to Hill's (1950) prediction, I found that body size was positively correlated with most movement performance traits, namely jump distance, jump velocity, sprint velocity, swim velocity, as well as both terrestrial and aquatic endurance distance and velocity. Hill predicted that organisms should jump the same distance regardless of body size, due to allometric scaling and the increased acceleration with smaller body size. However, Emerson (1978) demonstrated that larger frogs could jump greater absolute distances than small frogs, because they were able to accelerate at the same rate as smaller frogs possibly due to compensation by larger muscles or a higher density of muscle fibres. It is speculative to assume that body size would represent an adaptation specifically for locomotion, given the role it plays in so many other aspects of an organism's life-history (Farrell & Macmahon 1969; Peters 1986; Shine 1988). The only negative performance correlation with body size was with adhesive performance. This was consistent with biomechanical predictions that adhesion is proportional to the ratio of surface contact to body mass, which decreases with increasing body size (Emerson 1991; Moen et al. 2013).

Previous studies have shown that hindlimb muscle mass is positively correlated with jump velocity (Choi & Park 1996), which was corroborated by my findings despite using thigh muscle width as an approximation of muscle mass. I found a positive correlation with jump distance, jump velocity, sprint velocity and terrestrial endurance velocity, suggesting that muscle mass contributes to burst performance together with hindlimb length. Frogs with relatively thick thighs should have a greater cross-sectional area of muscles that can be engaged to produce force during a jump (Emerson 1978) and can thus accelerate their limbs faster and jump greater distances. Much like hindlimb length, thigh muscle mass should be beneficial for species requiring quick bursts of speed, but is unnecessary for slow persistent



movement. No significant negative correlation between mid-femur width and endurance was found, which reinforces the link between fatigue and the ability for a long-legged frog to manoeuvre its legs back into jump position.

Relative mid-femur width did not correlate with swim velocity, contrary to predictions that larger muscles would enhance propulsion, as found by Moen et al. (2013). However, Richards & Clemente (2013) found power output is optimised by both the gear ratio of the hindlimb elements and the extent of toe webbing. Therefore, accounting for these gear ratios might be required to properly test the effect of thigh muscle width on swimming performance. Furthermore, swim velocity was not correlated with relative hindlimb length. An additional explanation is that shorter-legged species perform a series of kicks that can rival the average velocity of the long glides from the single powerful kicks of long-legged species. The only morphological trait that was related to swim velocity, apart from body size, was the extent of pedal webbing, which positively correlated with velocity, as predicted (Moen et al. 2013).

I found that relative finger disk diameter was positively correlated with adhesive ability, as demonstrated for relative finger and toe-tip area by Moen et al. (2013), which supports the hypothesis that these tips are responsible for generating adhesive forces (Emerson & Diehl 1980; Blackburn et al. 2013; Chakraborti et al. 2014). However, I did not find a significant correlation between relative hand length and adhesive ability, where the greater reach of longer fingers could be associated with arboreality. It appears that this method was not ideal to test the true functional advantage of this trait in an arboreal environment. Many of the species were small enough to maintain adhesion through belly contact without the aid of specialised finger/toe pads. Instead it would have been more ecologically relevant to test the adhesive ability of frogs moving across a surface of varying angles.

In summary for functional morphology in the Pyxicephalidae, it is evident that some morphological traits are correlated with locomotor performance in accordance to predictions based on previous work as well as biomechanical predictions. These correlations suggest that the morphological traits measured could have played an adaptive role in ecotype specialisation among pyxicephalid species. This is particularly notable in the Pyxicephalidae due to their divergence from a common ancestor (ca. 70 Mya; van der Meijden et al. 2005; Bittencourt-Silva et al. 2016), indicating that these differences are more likely to be associated with the transition to novel ecotypes, rather than arising through chance and phylogenetic conservatism. To test this I used the Ornstein-Uhlenbeck process to model

morphological and locomotor performance traits and determine the best-fit model of evolution.

I predicted that selective optima would differ between pyxicephalid ecotypes and would thus result in distinct groups between measured morphological and performance traits. The support of a Brownian motion evolutionary model suggests that the performance traits did not show separate selection pressures between the different ecotypes. Zug (1978) also compared jump performance between different ecotypes and found them to highly overlap in performance, but he only considered a small sample of closely related species. Interestingly, morphological traits were best explained by a model with separate selective optima for burrowing, semi-aquatic and terrestrial ecotypes, suggesting that morphology was distinct between these species occupying these ecotypes. Burrowing species had relatively shorter hindlimbs, while semi-aquatic species had more extensive pedal webbing. But hindlimb length did not show differences between ecotypes. However, Nauwelaerts & Aerts (2006) showed that short, but fast, jumps can prevent predators from predicting the landing position (Royan et al. 2010), where success depends on the ability to recover rapidly after landing. This could suggest a many-to-one mapping (Alfaro et al. 2005) of performance to ecology, where different locomotor abilities can be employed with similar success when combined with specific behaviours. Scales & Butler (2016) found that differences in ecotype could not explain the adaptive significance of performance traits in lizards. They explain that multiple performance-related strategies might be compatible with a single ecotype, which is certainly possible within the terrestrial species of pyxicephalids, due to the variety of environments that terrestrial species could adapt within.

The fact that morphology is more associated with ecotype than performance traits, despite morphology influencing performance, could be due to a number of reasons. Firstly, morphology could be associated with other ecological functions or performance traits that are important for specific ecotypes, but which were not included in this study, such as traits associated with breeding, feeding or physiological tolerance. Secondly, performance traits could have adapted according to the selective optima of different ecotypes, and my ecotype categorisation was inadequate. Thirdly, the method in which the performance traits were measured might not have captured the nuances that are relevant for ecotype, such as concurrent mobility and adhesion. Fourthly, performance traits could be harder to quantify than morphological traits, resulting in more variable estimates between species that obscure true differences between species. Finally, some of the traits included might not be relevant, and thus obscure evidence for selective optima.

To resolve whether adaptive traits are being obscured by irrelevant traits, I individually tested three of the most obviously ecologically relevant performance traits and their associated morphological correlates using the OU process. As predicted by (Moen et al. 2013), semi-aquatic species had a distinct optimum from all other ecotypes, with more extensive webbing and a greater maximum velocity while swimming. Webbing should increase both acceleration and manoeuvrability within an aquatic environment, being beneficial for semi-aquatic species which need to swim to underwater cover as soon as possible to avoid pursuing terrestrial predators. Alternatively, and possibly complimentary, is the vulnerability of pedal webbing to abrasive terrestrial surfaces, that overland movement is likely to damage the webbing, and that webbing could hamper locomotion by getting snagged.

Burrowing frog species generally have short hindlimbs (Emerson 1976) and their jump performance is thus constrained by the reduced period that the feet are in contact with the ground (Gomes et al. 2009). I found that the preferred model of evolution for leg length in pyxicephalid species was clade history, suggesting that hindlimb length was highly conserved within genera. Jump velocity was best explained by Brownian motion, closely followed by a single optimum. Although burrowing species appear to have relatively short hindlimbs, a subset of terrestrial species occurring in montane and forest seeps also had relatively short hindlimbs. The habitat of these small species provides dense cover from most predators, and thus might not require long hindlimbs to escape predators. Furthermore, because these species are small, their absolute hindlimb length is also smaller than the larger burrowing species. Long and delicate hindlimbs are likely to be unwieldy within confined spaces and easily damaged in the process of burrowing, but these patterns may not be evident because non-burrowing species are not necessarily all under selection for enhanced jump performance.

Arboreal frogs have enlarged toe and finger tips that have been shown to enhance the ability to cling on smooth surfaces (Moen et al. 2013). I found non-arboreal frogs had strikingly smaller fingertip diameter relative to finger width. However, the upwards orientated adhesive ability was better explained by the null model, suggesting that non-arboreal species did not differ in adhesive ability. This is not surprising, as body size has been found to be a major influence on adhesive performance for this method (Moen et al. 2013), thus many of the small terrestrial species, with a large surface-area to mass ratio, could still adhere without expanded fingertips. Furthermore, only one species of the family was semi-arboreal, *Natalobatrachus bonebergi* (which has finger disks of a diameter far greater than other species relative to finger width), which makes it difficult to obtain statistical support. Another possible contributing factor is the amount of time for *Natalobatrachus* to evolve optimal

arboreal traits, where the finger disk area to snout-urostyle ratio is 0.05, while similarly sized arboreal *Dendropsophus* species are around 0.18 (Moen et al. 2013). The adhesive performance tested was passive, but this is not necessarily useful for arboreal species which need to move around, relying on hands and feet for purchase. Designing a new test for active adhesive performance may provide results of greater relevance to arboreal lifestyle and would likely emphasise the importance of toe and fingertip morphology. Therefore, more research could be needed to understand the functional relevance at traits at a finer ecological scale.

My findings support the hypothesis that morphological traits have diversified according to different ecotypes, but locomotor performance does not show consistent differences between ecotypes, despite the correlation between many morphological and performance traits. Testing individual traits and their proposed ecotype scenario reveals that two of the three morphological traits and one locomotor performance trait show support for their respective ecotype optimum. However, adhesive performance is very likely to show a selective optimum for semi-arboreal ecotypes, as results were biased by body size and an inadequate adhesive technique (discussed above). Therefore, these individual traits support an adaptive role of functional morphology for semi-aquatic and semi-arboreal ecotypes in comparison to pyxicephalids from other ecotypes. Other studies that found separate selective optima for burrowing frog morphology also considered the size of the metatarsal tubercle, which was larger in burrowing frogs (Moen et al. 2013; Moen et al. 2016). Unfortunately these structures were too difficult to measure on live animals. Likewise, burrowing performance is difficult to test and thus was not measured. Furthermore, endurance distance and time to exhaustion were poorly described by morphological traits, possibly due to the inadequate duration of the trials. Improving performance testing methods and including other additional morphological and functional traits could have provided greater support for separate selective optima.

The ancestral reconstruction of these ecotypes suggests that burrowing behaviour evolved two to three times independently within the Pyxicephalidae, between *Pyxicephalus* and *Cacosternum*, and possibly between *Pyxicephalus* and *Tomopterna*. Semi-aquatic ecotypes arose independently within *Aubria* and *Amietia*, while the association of the closely related *Natalobatrachus* and *Arthroleptella* with streams could suggest that they originated from a semi-aquatic ancestor. *Natalobatrachus* is the only species that has adapted to a semi-arboreal ecotype, with expanded toe-pads unique within the family and an ability to climb on foliage. Terrestrial species appear to be the ancestral form, suggesting that burrowing, semi-aquatic and semi-arboreal species have diverged from this ecotype. The number of ecotypes

that the Pyxicephalidae have occupied is remarkable in comparison to other southern African frog families, which rarely occupy more than two ecotypes (Minter et al. 2004). However, some ecotypes remain unconquered. For example, torrent specialists that live in the strong headwaters of montane streams are only represented by a single family in southern Africa, the Heleophrynidae or ghost frogs. Interestingly, *Amietia* are often found alongside heleophrynids (pers. obs.), which suggests that they are generalist enough to persist within these environments. *Heleophryne* is placed basally as the sister clade to all other neobatrachians (Alexander Pyron & Wiens 2011), indicating that Pyxicephalidae have only recently arrived and are likely to have missed the ecological opportunity for torrent ecotypes. Likewise, pipids are also a basal group that occupy aquatic environments to a greater degree than either *Amietia* or *Aubria*, which could mean that competition has also prevented further specialisation into this ecotype. This raises further interesting questions, such as why the Pyxicephalidae were able to occupy these other ecotypes? Are these ecotypes experiencing more frequent environmental perturbations that eliminate competitive species?

Losos & Malher (2010) suggest that adaptive radiations should refer to clades that show exceptional differences in adaptive phenotype as well as ecology, regardless of species richness. In this study I demonstrate the functional significance of interspecific phenotypic differences, and argue that that some of these traits are consistent with separate evolutionary trajectories between specific ecotypes. However, the ecotype transitions between pyxicephalid clades are severely limited and occur across a broad time range (ca. 25 to 60 Mya; Bittencourt-Silva et al. 2016), with high niche conservatism at the genus level (figure 2.3). Therefore, I propose that although the Pyxicephalidae show specific adaptations to different ecotypes, my results cannot support the 'exceptional' accumulation of adaptive diversity as described in adaptive radiations (Losos & Malher 2010), as found in *Anolis* lizards (Losos et al. 2006). It does, however, show some remarkable adaptive traits between closely related species, and certainly represents a non-adaptive radiation, given the species richness within genera. This radiation did not occur over a short burst, suggesting that multiple independent events were likely to have caused this family to steadily gain phenotypic and ecological diversity.

## Conclusion

The diversity of morphological traits between pyxicephalid species was found to have important functional implications with regards to locomotor performance. In general, these relationships followed the biomechanical predictions that have been demonstrated in other anuran groups at a global scale and between multiple families (Zug 1972; Gomes et al. 2009; Moen et al. 2013). Additionally, I find that a potential trade-off between burst performance and fatigue resistance, due to their positive and negative correlation with relative hindlimb length respectively. Although this trade-off has been noted in small sample sizes of distantly related anurans (Rand 1952; Putnam & Bennett 1981), it has been challenged by Zug (1978) and has not been tested within a closely related frog clade while accounting for phylogenetic relatedness. Pyxicephalidae have speciated relatively recently (van der Meijden et al. 2005; Bittencourt-Silva et al. 2016), which suggests that interspecific differences have arisen from shared opportunities given the shared geographic and ecological proximity, reinforcing the evidence for adaptive processes (Glor 2010).

Overall interspecific morphological variation supported separate selective optima among semi-aquatic, terrestrial and burrowing ecotypes. However, overall locomotor performance was better explained by a Brownian motion model of evolution, rather than separate ecotype optima. Testing individual morphological and locomotor traits revealed that pedal webbing score and swimming velocity was associated with a semi-aquatic selective optimum and fingertip diameter with a semi-arboreal optimum, as found by Moen et al. (2013). Therefore, although most of the locomotor and some of the morphological differences between species are not predicted by separate ecotype optima, there is evidence for functional morphological adaptations with regards to different environmental conditions. Van der Meijden et al. (2005) referred to the Pyxicephalidae as an adaptive radiation, but I find little support for exceptional adaptive diversity (Losos & Malher 2010), especially given the high niche conservatism within the family. However, these results raise new questions about the Pyxicephalidae and methods for testing adaptations. Why are morphological traits better explained by ecotypes than locomotor traits which should be directly influencing fitness? Are some locomotor performance traits independent of the environment, or is the coding for the ecotype categorisation insufficient to quantify important environmental factors (Blankers et al. 2012)?

## **Chapter 3: Macroecology of the Pyxicephalidae: can morphological or reproductive traits explain range size and ability to colonise suitable habitat?**

### **3.1 Introduction**

The distributional patterns of many species are becoming increasingly well documented, yet it remains poorly understood what determines the limits of these ranges and thus why some species are distributed more widely than others (Lester et al. 2007). The simplest explanation is that a species' range is directly determined by environmental conditions, and thus limited to the abundance of habitat that is suited to a species' phenotype. However, it has become evident that certain species traits are linked to range size (Gaston & Blackburn 1996c). This is indicative of a complexity in macroecological processes that requires a biologically informed perspective to unravel the underlying mechanisms.

A prominent pattern is the positive relationship between body size and range size, which has been demonstrated in freshwater fish, birds, primates, amphibians, reptiles and some terrestrial mammals (Gaston & Blackburn 1996c; Murray & Dickman 2000; Brown et al. 2015). Furthermore, this relationship generally appears to have a triangular form when range size is plotted against body size (Addendum B1), where small-bodied species can occupy both small and large ranges, but large species tend to only occupy large ranges (Hanski 1982; Gaston & Blackburn 1996a; Gaston et al. 2000). Potential explanations for these patterns include: colonisation ability, time since speciation for dispersal, spatial requirements for minimum viable population size, incidental correlation with latitude (Gaston & Blackburn 1996a), niche breadth (Gaston & Blackburn 1996c) and speciation patterns (Pabijan et al. 2012). Conversely, one mechanism, known as rates of increase, predicts that smaller-bodied species should have larger ranges due to their rapid ability to recover population numbers (Gaston & Blackburn 1996c). This pattern of body size has inspired a number of plausible hypotheses that could be influencing range size, but other traits could provide further insight into the specific mechanisms.

Dispersal ability is commonly assumed to have an important role in macroecological patterns, despite a lack of empirical support (Lester et al. 2007). Efficient dispersal allows some species to occupy more marginal and stochastic habitats, because they can utilise spatially diffuse or seasonally available resources (Fahrig & Merriam 1994; Gaston & Blackburn 1996b; Alerstam et al. 2003), re-establish extinct populations, or maintain

population densities in less than adequate environments (Haldane 1956; Gaston & Blackburn 2000; Bielby et al. 2008). In terms of evolutionary processes, according to the intermediate dispersal model, vagile species are less likely to become spatially isolated and undergo cladogenesis (Hansen 1980; Claramunt et al. 2012), preventing the range from being subdivided (Lester et al. 2007; Price & Kirkpatrick 2009; Pabijan et al. 2012). On the other hand, vagile species might experience gene swamping, hampering local adaptation and occupation of marginal habitats (Haldane 1956; Kirkpatrick & Barton 1997; Case & Taper 2000).

Theoretically, endurance and speed of locomotion should determine the upper limit on how far an individual can disperse, while the rate of reproduction determines the number of dispersing individuals and the strength of intraspecific competition. Dispersal ability has been quantified using species traits such as wing length in stoneflies (McCulloch et al. 2016) and pelagic larval duration in marine organisms (Lester et al. 2007), as well as the more sophisticated methods of population genetics (Lester et al. 2007; Pabijan et al. 2012). However, the dispersal ability of a species is only relevant with regards to fragmented or discontinuous habitat (Wiens 2011), which might explain why dispersal ability often fails to predict range size, in which case other mechanisms, such as niche breadth, could play a role (Lester et al. 2007).

Unsuitable environments negatively affect the survival or reproduction of individuals, undermining the long-term persistence of a species within that habitat. Species with a greater niche breadth should be able to persist within more habitats and thus occupy a larger range (Brown 1984; Gaston 1996). In contrast, species that depend on specific environmental conditions or habitat features are likely to occupy narrower ranges, such as diet specialists that are also constrained by the distribution of their prey. For example, host-specific moths tend to have smaller range sizes than generalist feeders (Loder et al. 1998). However, there are a number of potential factors influencing the habitat suitability for a single species, as well as potential interactions between these, which might explain why no single factor has been found acting across different groups of organisms (Gaston & Blackburn 1996c; Gaston et al. 2000; Lester et al. 2007). It is therefore sensible to select study species that are likely to be constrained by similar processes to one another. Amphibians share a hydric physiology (Thorson 1955; Rittenhouse et al. 2008) and are generally dispersal limited (Blaustein et al. 1994; but see Smith & Green 2005).

Biogeographic studies of anurans that have investigated potential mechanisms influencing range size have found strong and consistent relationships for several traits. Murray et al.



(1998) showed that Australian frogs displayed the positive triangular pattern, where smaller bodied species occupy both small and large ranges and large bodied species only occupy large ranges, but further investigation by Murray & Hose (2005) revealed that range size could also be explained by species abundance and egg size. They argue that species with smaller eggs and higher density of individuals can colonise larger areas, especially because smaller eggs are able to develop faster (Komoroski et al. 1998). Diniz-Filho et al. (2004) also found the same triangular pattern when correlating body size and range size for Central Brazilian anurans, and that species at the lowest ratio of body size to range size occurred at lower densities and had greater population fluctuations. Global studies on anurans have also found positive correlations between clutch size and range size (Cooper et al. 2008), in addition to body size and range size (Tingley et al. 2010).

Body size in frogs is important for physiological tolerance, such as thermoregulation and desiccation tolerance (Farrell & Macmahon 1969; Nevo 1973; Tracy et al. 2010) and could thus impact the niche breadth of a species. However, it is also correlated with many other traits, such as locomotory ability (Choi et al. 2000; Nauwelaerts et al. 2005; Gomes et al. 2009), clutch size (Cooper et al. 2008; Liedtke et al. 2014) and gape size. This makes it difficult to disentangle correlative factors from the causative factors that might be influencing range size (Gaston & Blackburn 1996a). Vignoli & Luiselli (2012) demonstrated that amphibians with large gape sizes have a broader range of potential prey. Clutch size is highly variable between anuran species, ranging from tens to thousands of eggs being produced by a female at a single breeding event (Kuramoto 1978), with direct developing species that tend to have small clutch sizes with large eggs (Salthe & Duellman 1973; Pupin et al. 2010; Liedtke et al. 2014). The combination of many dispersing offspring and high intraspecific competition is likely to increase the movement of individuals of a species across unfavourable habitats (Pittman et al. 2014). While interspecific locomotory endurance could contribute to dispersal, it is poorly understood in anurans, however, I demonstrated that hindlimb length is negatively correlated with endurance ability within the Pyxicephalidae (Chapter 1). Despite the correlation of these traits with body size, the residual variation could still be informative if one of these mechanisms is influencing range size.

In this study I investigate macroecological patterns of the Pyxicephalidae, an ecologically diverse sub-Saharan frog family. This group is appropriate for studying macroecological patterns, because its members occur within the same geographic region, have a variety of range sizes and share a common ancestor from the late Mesozoic era (ca. 70 Mya)(van der Meijden et al. 2005; Bittencourt-Silva et al. 2016). Furthermore, amphibian population genetics are often highly spatially structured (Bonin et al. 1995; Avise 2000). Therefore, it is

likely that distribution of the Pyxicephalidae, as found for other anurans, will be constrained by geographical features (Nielson et al. 2001; Tolley et al. 2010; Evans et al. 2011; Rodríguez et al. 2015), and that climatic conditions will determine the suitability and connectivity of habitats according to species behaviour and breeding requirements.

One shortcoming of macroecological studies is that it is difficult to measure the amount of suitable habitat. Perhaps a species simply has an extensive range just because the suitable habitat is common and certain traits are adaptations to this environment and in reality they provide no advantages for colonisation and range expansion. Range size is inadequate to tease apart these subtleties, and more sophisticated methods should also be used that can account for differences in abundance of suitable habitats between species. The wide range of morphological and reproductive traits within the Pyxicephalidae has created a natural experiment to test patterns of colonisation and population persistence across evolutionary time. In this study I test the macroecological correlations for four traits: body size, relative clutch size, relative hindlimb length and relative head width while taking phylogenetic relatedness into account. Firstly, I hypothesise that pyxicephalid species' range sizes show the same positive correlation with body size and clutch size as found for other frog groups. Additionally, I test whether relative hindlimb length and head width positively correlate with range size. Secondly, using the Outlying Mean Index (OMI), I test for a correlation between species niche breadth and range size, and whether the species traits are related to niche breadth. Thirdly, I hypothesise that colonisation ability is positively correlated with the species traits, independent of the available suitable habitat differences among species by using habitat suitability predictions from species distribution modelling suitable habitat.

## **3.2 Methods**

### Spatial data

Species distribution data were obtained from regional conservation authorities, museums and citizen science initiatives (Addendum A). These data were separated to species rank and checked for obvious identification or locality errors by comparing them with current distribution range maps (IUCN 2015), consulting experts and looking for contradictions between locality and coordinates. Records with errors were removed from the dataset if they could not be verified by the observer or by an expert. Certain morphologically similar species can only be reliably distinguished based on call or by genetic sampling, such as *Amietia poyntoni*, *A. fuscigula* and *A. delalandii*; *Tomopterna delalandii*, *T. cryptotis* and *T. tandyi*; and *Cacosternum aggestum*, *C. australis* and *C. platys*. The distribution records for each of these groups were pooled and then assigned a new identification to match the known distribution range based on confirmed genetic samples and/or expert opinion.

Species distribution data were imported into the R environment (R Core Team 2015) and transformed into spatial points using the African Albers-Equal projection with the R package 'sp' (Pebesma & Bivand 2005). A minimum convex polygon (MCP) and a 5km and 30km buffered polygon were created from the distribution data of each species using the R function 'chull' and the package 'rgeos' (Bivand & Rundel 2013), respectively. These two buffered polygons represent the current realised distribution range and the total range within proximity of colonisation. The smaller polygon with a radius increase of 5km was chosen to represent the realised niche, to effectively cover the patchy and incomplete sampling of the distribution data. The anuran dispersal distance for standard metapopulation genetic differentiation is generalised to 11-13km by Smith & Green (2005), and also follows a power law that enables colonisation at greater distances over long periods. Therefore, I propose that a distance of 30km should represent a reasonable upper estimate for the distance within which habitats are accessible for colonisation by a standard anuran species over many generations.

#### Climate and Topographic predictors

Ten bioclim variables were selected from WorldClim (Hijmans et al. 2016) at the 30 arc-second resolution as climatic predictors, based on their biological relevance for amphibians and included bio3, bio7, bio8, bio9, bio10, bio11, bio13, bio15, bio17 and bio19 (Mokhatla et al. 2015). In addition, I included elevation, slope and aspect layers derived from the 90m SRTM (Jarvis et al. 2008) using the function 'terrain', which was then resampled at the same extent and resolution of the bioclim variables using the R package 'raster' (Hijmans & van Etten 2012). The final set of included MaxEnt predictors used for modelling each species included elevation, aspect and slope, in addition to the bioclim variables. Additionally, the latitudinal coordinates were extracted for the MCP centroids of each pyxicephalid species.

#### MaxEnt modelling

The extent of a species' distribution cannot discriminate between areas that are unsuitable and those that are suitable but inaccessible. Species-specific habitat suitability was estimated using maximum entropy models in MaxEnt version 3.3.3k (Phillips et al. 2006). A sample of 1000 random background points was limited to within a radius of 250km around the distribution points for each species. The distribution data were highly biased towards populated areas and within the boundaries of South Africa due to national level sampling (Botts et al. 2015). This was controlled in MaxEnt by constructing a bias file from combining the distribution data of all anuran species recorded in sub-Saharan Africa (from the same databases), converting into a 30 arc-second resolution raster and smoothing it using the

function 'kde2d' with an h value of 0.5 in the R package 'MASS' (Venables & Ripley 2002). This bias file was included in MaxEnt runs, but all other parameters were left as default. Model performance was assessed using the average Area Under the Receiver Operating Characteristic Curve (AUC), for 100 cross-validated replicate runs. The average standard MaxEnt logistic output (Phillips & Dudík 2008) for each raster cell was used in further analysis.

### Colonisation Index

The proportion of suitable habitat (obtained from the MaxEnt logistic output) between the 5km and 30km buffer was used to create an index, hereby referred to as the Colonisation Index (CI). This was calculated by averaging the habitat suitability (0-1) values of raster cells within this zone for each species. The presence data of a species used in the MaxEnt modelling enables prediction of the habitat suitability in the surrounding areas. Under the assumption that species can occupy habitat as suitability increases, I propose that the suitability of nearby habitat that remains unoccupied by a species can be used to estimate its ability to colonise and establish. Effective colonisers should be able to occupy all suitable habitat in the nearby vicinity until all suitable habitat is used up and only very unsuitable habitat remains. Whereas a poor coloniser should have unoccupied habitats of high suitability nearby and thus have a large CI. This index is independent of range size as it only considers the proportion of habitat suitability within and outside a range of species.

### Traits

Morphological traits were obtained by measuring both live and museum specimens using electronic callipers or from photographs using ImageJ (Rasband 1997) to the nearest 0.1 mm (Addendum B.3). Measurements included the snout-vent length, head width, femur length, tibia length, calcaneum length and foot length. These latter four measurements were summed to give the hindlimb length. The clutch size was obtained by dissecting adult female museum specimens and estimating the total the number of eggs from a counted subset. However, not all species were available for dissection and the clutch size of some species was estimated from the mean clutch size of the genus (Addendum B.4). Ethical clearance was obtained from Stellenbosch University's REC: ACU (Protocol #: SU-ACUD15-00101). Collections were conducted under permits for Cape Nature (0056-AAA043-00009), DETEA Free State (S45C-515111613151), DEDEAT Eastern Cape (CRO 204/15CR), Ezemvelo KZN (OP 3825/2015) and GDARD (ToPS 0-09534).

### Niche breadth

The Outlying Mean Index analysis (OMI; Dolédec et al. 2000) was used to determine the niche breadth of pyxicephalid species within the study region. Niche breadth represents the variability in the measured conditions where a species is present. The climatic and topographical variables from the MaxEnt modelling were used for this analysis at a resolution of 30 arc-seconds. Due to both computational constraints and poor distribution-data coverage outside of South Africa, the study region was confined to South Africa and only species contained within this region were included in this analysis (Addendum B.5). The niche breadth was calculated using the function 'niche' in the package 'ade4' (Dray & Dufour 2007).

### Data analysis

The phylogeny constructed in Chapter 2 was pruned to include all species for which morphological and clutch size data were available, and was used in subsequent analyses. All continuous variables were log transformed and scaled using the R function 'scale'. The head width, hind-leg length and clutch size were corrected by snout-vent length assuming a Brownian motion model of evolution with the function 'phyl.resid' in the R package 'phytools' (Revell 2012). I used phylogenetic generalised least squares regression with simultaneous estimation of Pagel's lambda, as suggested by Revell (2010), in the package 'caper' (Orme 2013), to test three macroecological variables: species range size, CI, and species niche breadth. Explanatory variables included snout-vent length, relative head width, relative hind-leg length and relative clutch size. The best-fit models were determined using the second-order Akaike Information Criterion (AICc).

## **3.3 Results**

### Trait correlation

Prior to size correction, all predictor traits were significantly and positively correlated with species snout-vent length: clutch size ( $R_{2adj}^2=0.54$ ,  $P<0.001$ ), hindlimb length ( $R_{2adj}^2=0.92$ ,  $P<0.001$ ) and head width ( $R_{2adj}^2=0.96$ ,  $P<0.001$ ). These strong correlations with body size indicate the importance of correcting for body size. Clutch size had the largest residual variance, compared to head width and hindlimb length.

### MCP range size and position

Species range size, calculated from a minimum convex polygon, displayed a triangular relationship with body size, where smaller species had both narrow and broad ranges, while larger species had mostly broad ranges (Figure 3.1). Species range size was best explained by the combination of body size ( $R_{adj}^2=0.52$ ;  $P<0.001$ ) and corrected clutch size ( $R_{adj}^2=0.52$ ;

$P < 0.001$ ), with both having a positive correlation (Table 3.1). Neither relative hindlimb length nor relative head width explained much variance of species range size, even when body size or corrected clutch size was included in the model. The central latitude position of a species range was positively correlated with both species range size ( $R^2_{\text{adj}} = 0.35$ ,  $P < 0.001$ ) and body size ( $R^2_{\text{adj}} = 0.34$ ,  $P < 0.001$ ).

#### Colonisation Index

The Colonisation Index (CI), defined as the climatic/topographic suitability at the species range boundary, was best explained by the corrected clutch size alone, but the combination of body size and corrected clutch size performed similarly (Table 3.2). Colonisation Index was positively correlated with corrected clutch size ( $R^2_{\text{adj}} = 0.26$ ,  $P < 0.002$ ) and body size ( $R^2_{\text{adj}} = 0.26$ ,  $P = 0.267$ ), although body size was not significant. As with range size, hindlimb length and head width did not contribute to explaining variance in the CI. The CI for models with an AUC value higher than 0.75 did not have an obvious best-fit model, and the null model performed similar to the best fit model (Table 3.3). However, CI was found to positively correlate with corrected clutch size ( $R^2_{\text{adj}} = 0.22$ ,  $P = 0.004$ ).

#### Niche breadth

The niche breadth, obtained from the Outlying mean index (OMI) analysis, was significantly positively correlated with species range size ( $R^2_{\text{adj}} = 0.14$ ,  $P = 0.008$ ), where species occupying extensive ranges occurred in a greater variety of conditions. In terms of species' traits, relative hindlimb length was positively correlated with niche breadth ( $R^2_{\text{adj}} = 0.23$ ,  $P < 0.001$ ), while body size, corrected clutch size and relative head width did not contribute towards niche breadth (Table 3.4).

Table 3.1. Results for morphological correlates of species range size within the Pyxicephalidae, using phylogenetic generalised least squares with simultaneous estimation of  $\lambda$ . SVL=body size, CS=corrected clutch size, HL=relative hindlimb length, HW=relative head width. Column names include log-likelihood (lnL), second-order Akaike Information Criterion (AICc), weight of evidence ( $w_i$ ) and number of model parameters (K). The AICc best-fit model is denoted in bold.

Model predictors	lnL	AICc	$\Delta$ AICcs	$w_i$	K
SVL+CS	-123.10	252.69	0.00	<b>0.55</b>	3
SVL+CS+HLEG	-122.66	254.18	1.49	0.26	4
SVL+CS+HLEG+HW	-122.48	256.26	3.57	0.09	5
SVL	-126.76	257.76	5.07	0.04	2
SVL+HLEG	-126.31	259.11	6.42	0.02	3
SVL+HW	-126.60	259.69	7.00	0.02	3
CS	-128.62	261.49	8.80	0.01	2
HLEG	-129.61	263.47	10.78	0.00	2
HW	-131.39	267.02	14.33	0.00	2
null	-133.87	269.82	17.13	0.00	1

Table 3.2. Results for morphological correlates of Colonisation Index (all species distributions models) within the Pyxicephalidae, using phylogenetic generalised least squares with simultaneous estimation of  $\lambda$ . SVL=body size, CS=corrected clutch size, HL=relative hindlimb length, HW=relative head width. Column names include log-likelihood (lnL), second-order Akaike Information Criterion (AICc), weight of evidence ( $w_i$ ) and number of model parameters (K). The AICc best-fit model is denoted in bold.

Model predictors	lnL	AICc	$\Delta$ AICcs	$w_i$	K
CS	26.51	-50.94	0.00	<b>0.44</b>	1
SVL+CS	32.54	-53.77	0.65	0.32	5
SVL+CS+HLEG	32.43	-56.00	2.87	0.10	4
SVL+CS+HLEG+HW	32.36	-58.22	5.10	0.03	3
HLEG	29.56	-52.63	5.88	0.02	3
SVL	28.99	-51.49	6.00	0.02	3
HW	28.56	-52.87	6.17	0.02	2
SVL+HLEG	31.56	-58.87	6.25	0.02	2
SVL+HW	28.62	-52.99	7.38	0.01	2
null	28.47	-52.70	7.93	0.01	2

Table 3.3. Results for morphological correlates of Colonisation Index (species distributions models with an AUC>0.75) within the Pyxicephalidae, using phylogenetic generalised least squares with simultaneous estimation of  $\lambda$ . SVL=body size, CS=corrected clutch size, HL=relative hindlimb length, HW=relative head width. Column names include log-likelihood (lnL), second-order Akaike Information Criterion (AICc), weight of evidence ( $w_i$ ) and number of model parameters (K). The AICc best-fit model is denoted in bold.

Model predictors	lnL	AICc	$\Delta$ AICcs	$w_i$	K
CS	18.20	-32.00	0.00	0.21	2
null	16.91	-31.69	0.31	0.18	1
SVL	17.95	-31.50	0.50	0.16	2
SVL+CS	18.90	-30.97	1.03	0.13	3
HLEG	17.36	-30.33	1.67	0.09	2
HW	17.00	-29.59	2.41	0.06	2
SVL+HW	18.12	-29.40	2.60	0.06	3
SVL+HLEG	17.95	-29.08	2.92	0.05	3
SVL+CS+HLEG	19.16	-28.89	3.10	0.04	4
SVL+CS+HLEG+HW	19.25	-26.27	5.73	0.01	5

Table 3.4. Results for morphological correlates of niche breadth within the Pyxicephalidae, using phylogenetic generalised least squares with simultaneous estimation of  $\lambda$ . SVL=body size, CS=corrected clutch size, HL=relative hindlimb length, HW=relative head width. Column names include log-likelihood (lnL), second-order Akaike Information Criterion (AICc), weight of evidence ( $w_i$ ) and number of model parameters (K). The AICc best-fit model is denoted in bold.

Model predictors	lnL	AICc	$\Delta$ AICcs	$w_i$	K
HLEG	-73.27	150.84	0.00	<b>0.64</b>	2
SVL+HLEG	-73.23	153.10	2.26	0.21	3
SVL+CS+HLEG	-73.03	155.14	4.30	0.07	4
HW	-76.33	156.97	6.14	0.03	2
SVL+CS+HLEG+HW	-72.79	157.25	6.42	0.03	5
SVL+HW	-76.09	158.82	7.98	0.01	3
CS	-77.97	160.25	9.41	0.01	2
null	-79.39	160.89	10.05	0.00	1
SVL	-78.48	161.26	10.42	0.00	2
SVL+CS	-77.39	161.41	10.57	0.00	3



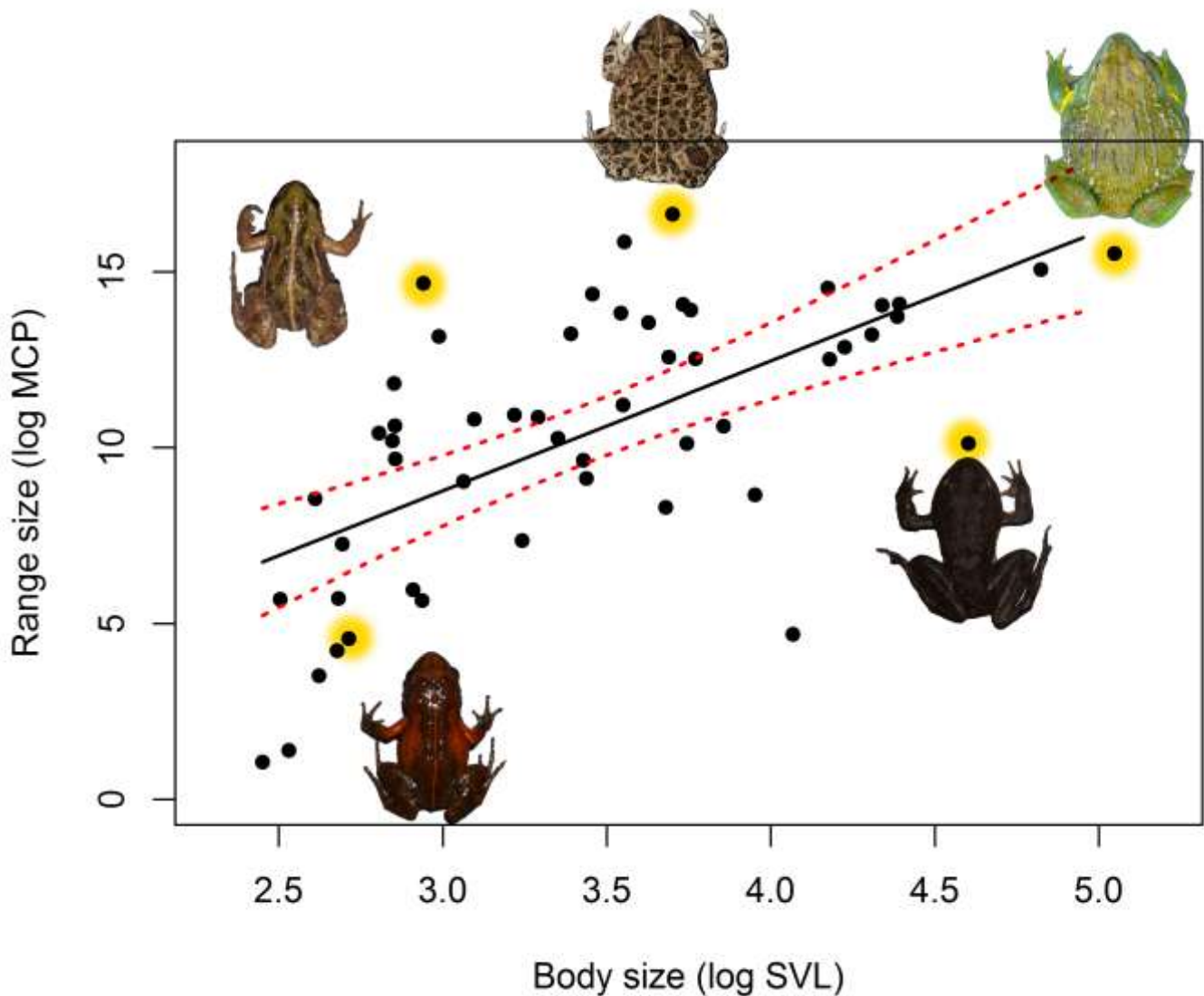


Figure 3.1. The relationship between species geographic range size (minimum convex polygon) and body size (snout-vent length) for Pyxicephalidae, showing predictions (black line) and 95% confidence intervals (red dashed line) for a simple linear regression. Species names of photographs (top left clockwise) *Cacosternum boettgeri*, *Tomopterna cryptotis*, *Pyxicephalus adspersus*, *Amietia vertebralis*, *Arthroleptella bicolor*. Photographs are not shown to scale.

### 3.4 Discussion

Pyxicephalid species range size was found to positively correlate with a combination of body size and corrected clutch size. However, the results for Colonisation Index were counter-intuitive, showing that a species' ability to colonise the surrounding suitable habitat is negatively correlated with its corrected clutch size. Remarkably, despite a positive relationship between niche breadth and range size, niche breadth was not correlated with either body size or corrected clutch size, but instead with relative hindlimb length. These results suggest that large-bodied species with relatively large clutch sizes have more extensive distribution ranges, but do not facilitate the colonisation of a broader range of environmental conditions. The Colonisation Index, however, is inadequate to investigate colonisation ability due to bias of model output with range size (see below).

Studies investigating the full extent of species ranges show a positive correlation between range size and body size for a wide range of taxa (Gaston & Blackburn 1996c), including anurans (Murray et al. 1998; Wollenberg et al. 2011; Brown et al. 2015). Body size is frequently studied and is often associated with a wide range of life-history traits (Blackburn & Gaston 1994), such as metabolism, respiration, predation, locomotion, home range, abundance and population turnover (Calder 1984; Peters 1986). In the African Pyxicephalidae, I found that the best-fitting model included both body size and corrected clutch size as positive correlates of species range size. Some of the mechanisms suggested to explain the positive body size relationship include the area required to support a minimum viable population, dispersal ability, niche breadth and latitudinal correlations (Gaston & Blackburn 1996c). Alternatively, a negative correlation is predicted in terms of generation time (Gaston & Blackburn 1996c), but clutch size is a better measure of reproductive output than body size in anurans. One of these mechanisms can be easily addressed: increasing latitude results in a decrease in both pyxicephalid body size and range size. This pattern is opposite to that of Bergmann and Rapoport (Gaston & Blackburn 1996c) and lacks a convincing mechanism, except perhaps the presence of the Cape Fold Belt in the South that is associated with high levels of endemism, particularly in the flora (Linder 2003), and that the geographic area decreases towards the South.

It is important to note that clutch size increases with body size in pyxicephalids, a trend that is common in other anurans, even across different reproductive strategies (Prado & Haddad 2005). Absolute clutch size has been found to be positively correlated with anuran range size in Australian frogs (Cooper et al. 2008). Therefore, it is possible that the reason body size positively correlates with range size related entirely to clutch size, given that corrected clutch size and body size approximate the absolute clutch size of a species. However,

Pabijan et al. (2012) found that genetic divergence between frog populations decreased with body size, and suggest that dispersal ability of small bodied species constrains their expansion and connectivity. Therefore, it is difficult to rule out other factors explained by body size. Species that produce more offspring should be better at persisting within harsh or temporally unstable environments because more individuals are dispersing into these areas (Murray & Hose 2005).

Other indices for dispersal ability have been used to predict range size, such as morphological or behavioural traits, both successfully (McCulloch et al. 2016) and unsuccessfully (Lester et al. 2007). I found that interspecific locomotory endurance is negatively associated with relative hindlimb length in the Pyxicephalidae (see chapter 2). Therefore, I hypothesised that relative hindlimb length should also be negatively correlated with range size. However, I found no relationship between hindlimb length and range size. This result is surprising, given that many phylogeographic studies have revealed that anurans have high genetic structuring over short spatial distances, indicating that their dispersal is limiting (Bonin et al. 1995; Nielson et al. 2001; Zancolli et al. 2014). Although dispersal is necessary for a population to occupy any space over time, it might be of little concern if distances between suitable habitats far exceed dispersal abilities or if other factors prevent dispersal irrespective of endurance ability (Lester et al. 2007), such as desiccation. Another possibility is that environmental fluctuations could create corridors between habitats, making all suitable habitats temporarily accessible and eliminating the advantage of dispersal ability. Alternatively, the number of dispersing individuals makes up for poor dispersal ability, which could be determined by reproductive output and animal density (Gaston & Blackburn 2003). Perhaps hindlimb length is not a reliable proxy for dispersal ability, because although longer-legged frogs fatigue faster under duress, a more conservative form of locomotion may be utilised for long-distance movements. Finally, a reduction in hindlimb length is also associated with burrowing species (Gomes et al. 2009; Vidal-García et al. 2014; Vidal-García & Keogh 2015; Chapter 2), which are better at surviving seasonally variable conditions (Rittenhouse et al. 2008), and thus may occupy a greater extent than non-burrowers. However, this prediction would also require a negative correlation between range size and relative hindlimb length, which I did not find. Furthermore, in Chapter 2 I did not find support for short-legged species being exclusively burrowers.

Larger gapes are known to enable frogs to ingest a broader diet (Emerson 1985; Vignoli & Luiselli 2012). Species with relatively larger heads could consume a greater availability of prey items during resource depressions or in marginal habitats. I therefore hypothesised that

species with relatively larger heads would also have larger range sizes. I did not find any support for this hypothesis in pyxicephalids, which could suggest that dietary specialisation does not predispose populations to extinction, for example, competition for resources with other species limits all prey sizes equally, prey abundance is thus not a limiting factor (invertebrates are often abundant) and/or during low abundance of prey other factors become more important, such as desiccation (Rittenhouse et al. 2008). It is also possible that relatively head width cannot predict dietary requirements as well as absolute head width.

In Pyxicephalidae there are a number of species that fall far outside the linear positive trend between body size and range size. Species that are small bodied but have relatively extensive ranges are all within the genus *Cacosternum*, while some medium sized *Tomopterna* are also relatively extensive. Both these genera produce larger clutches than other similar sized pyxicephalids, on average 262 and 2344 eggs, respectively (Addendum B.4). *Tomopterna* are considered as explosive breeders (Vences et al. 2000). If *Cacosternum* and *Tomopterna* are indeed better colonisers than other species of similar body size, they should exhibit relatively low genetic structure across the same geographic distance. Channing et al. (2013) shows that *C. boettgeri*, the most widespread species in the genus, has a 16S uncorrected p-distance of 0-0.5% across the extent of its range. The widespread *Strongylopus grayii* shows similar levels of phylogeographic structuring (0.6%; Tolley et al. 2010), while *Amietia wittei* and *A. angolensis* showed much lower structuring, 0.083% and 0.002% respectively, but over a much smaller area (Zancolli et al. 2014). South African *Amietia* species differ in genetic distance from 1.3-10% (Channing et al. 2016), which is much higher than within *C. boettgeri*. In comparison to the small bodied *Arthroleptella* that show intraspecific genetic distance of 2-4% within their highly restricted range extents (Turner 2010). This suggests that *Cacosternum boettgeri* (and possibly other members of the genus), have considerable gene-flow across their ranges. The phylogeography of *Tomopterna* has yet to be studied in detail. The extensive distribution of these species could be due to a combination of the continuous nature of its breeding habitat-typically temporary wetlands (Channing et al. 2013) in addition to the large number of dispersing individuals.

At the bottom right corner of the body size-range size plot (Figure 3.1), outlier species have large bodies and small ranges and include specific *Amietia* species. These represent range-restricted *Amietia*, which produce clutch sizes comparable to their more widespread conspecifics, but are only associated with montane streams and rivers. For example, *A. johnstoni* occurs only on Mount Mulanje and *A. hymenopus* and *A. vertebralis* in the Lesotho Highlands. Yet widespread *Amietia* species occur in the adjacent low-lying areas, suggesting

that differences in habitat preferences are preventing expansion, possibly due to larval adaptations to cool, fast flowing water (Measey pers. comm.). Thus range size is limited by the amount of available habitat. The persistence of these large species in small ranges indicates that the minimum viable population mechanism (Gaston & Blackburn 1996a) only becomes relevant at much smaller distribution ranges, possibly due to the high population abundance and commensality of anurans in general. Another possibility is that some species have smaller ranges because they have recently diverged and have yet to occupy their full distribution potential (Paul et al. 2009). However, this seems unlikely for small-range pyxicephalids, because many are often limited by montane environments or have already speciated across the range, indicating that these species have already reached a distribution in equilibrium with the environment. For example, *Arthroleptella* have mostly allopatric ranges coinciding with topographic features in the Cape Fold Mountains (Turner 2010), suggesting that dispersal ability, rather than time since divergence, is limiting the realised range size. However, competition and hybridisation between species can prevent some species from occupying that same range (Tingley et al. 2014), which results in a smaller range extent.

#### Colonisation ability

Species distribution modelling was used to create an index for colonisation ability by calculating the average suitability of environmental conditions within a buffer zone around known species presences. Species that have colonised all available suitable habitats should have low suitability values outside their range, indicating their superior colonisation ability. Furthermore, this index avoids the 'extent of suitable habitat' conundrum, because the average suitability of the buffer zone is independent from the total area of suitable habitat. However, a strong positive correlation between species range size and Colonisation Index was found for all species ( $R^2_{adj}=0.51$ ), as well as those with SDMs scoring an AUC  $>0.75$  ( $R^2_{adj}=0.63$ ). This indicates that species with larger ranges had more suitable habitat in the surrounding buffer zone than species with small ranges. This might be understood by narrow-ranging species living in highly specialised environmental conditions, but this could also be an artefact of SDMs (discussed below).

I hypothesised that species with a larger body size, relative clutch size and relative head width, but shorter relative hindlimbs would be able to colonise more of the nearby suitable habitat and thus have a lower Colonisation Index. Surprisingly, I found that corrected clutch size and body size, combined in a single model, were positively associated with Colonisation Index. This is opposite to expectations, given that larger bodied species with relatively large clutch sizes occupy larger ranges. One interpretation of this is that narrow-ranging species

are associated with topographic features (like mountains), and because these features are important for climate and input data into SDMs, these ranges are better defined than widespread species that occur in the lowlands or a range of elevations. This would result in SDMs predicting low suitability in lowland areas for highland narrow ranged species and moderate suitability in areas surrounding widespread species. Furthermore, CI could be biased according to extent of distribution simply due to differences in the abundance of presence records and a wider range of environmental conditions. Finally, the AUC value is not suitable for comparing model accuracy at different spatial extents (Lobo et al. 2008), and thus narrow ranging species may still have a high AUC even if the model is inaccurate. It does not make sense to interpret these results as species large ranges being poor colonisers given the theoretical advantages of their large bodies and high reproductive output.

What evidence is there for bias in SDM outputs for species with different range sizes? From the outputs I noticed that habitat suitability for wide-ranging species was generally lower than narrow-ranged species, even for areas well within the current range of the species. On the other hand, species with small ranges had better predictions that matched their native range, with low suitability values in the surrounding areas. One interpretation of this is that wide-ranging species are less constrained by climatic variables, and thus the SDMs cannot distinguish suitable habitats from unsuitable habitats. Another explanation, possibly complementary, is that species with small ranges are over-fitted, because there is a greater chance that random bioclimatic patterns will coincide with a smaller area. This was apparent in *Arthroleptella*, which have speciated within the Cape Fold Mountains, coinciding with breaks in suitable edaphic and geological features (Turner 2010), whereas SDMs predicted that neighbouring mountains (occupied by congeners) would be unsuitable habitat based on bioclimatic variables. Therefore, species that have a small distribution range will be predicted to have a lower suitability in surrounding areas than a wide-ranging species, even if their niche breadths are equivalent. Finally, the probability to detect a species could limit the amount of data to construct accurate distribution models. Habitat type and range location in relation to human infrastructure is known to bias the number of presence points collected (Botts et al. 2015), in addition to other factors body size, behaviour and abundance of species that further limit detection probability. These biases could affect the differential performance of models, rendering the comparison of model outputs meaningless. Therefore, I am convinced that the method was flawed for the task to which it was applied, and provides limited interpretation for colonisation ability. More robust methods for modelling habitat suitability need to be devised. Additionally, it is not ideal to test mechanisms that have multiple inputs from different traits (such as dispersal), by using a single trait. Instead,

nucleotide divergence can be interpreted as a measure of historic geneflow between populations (Pabijan et al. 2012; Rodríguez et al. 2015) and simulations of range dynamics with tweaks in actual mechanisms (Higgins & Richardson 1999) could directly test these mechanisms without relying on potentially inadequate surrogate traits.

### Niche breadth

There was a positive correlation between the Outlying Mean Index niche breadth and species range size in the Pyxicephalidae. This finding is consistent with the hypothesis that species able to survive within a wider range of habitats should have a more extensive distribution range. Biases between niche breadth and range size were not investigated in this study, but should not be discounted lightly. Traits that correlated with the niche breadth of a species did not include body size and corrected clutch size, which were previously found to be positively correlated with range size. Therefore, species with larger bodies and clutch sizes have more extensive ranges, but do not occupy a broader range of habitats. This suggests that either these species are able to colonise suitable habitat better, or that their suitable habitats tend to be more abundant and/or continuous. However, niche breadth was found to positively correlate with relative hindlimb length. In the context of this study, hindlimb length is relevant for dispersal ability and predator escape. However, neither of these mechanisms seem important for survival across a wide range of environmental conditions. One possible incidental reason is that many long-legged *Amietia* and *Strongylopus* species are generalist breeders (Minter et al. 2004), whereas many of the short-legged species require more specialised breeding sites that are dependent on climate and topography (Zimkus et al. 2012).

### Conclusion

The Pyxicephalidae have diversified within southern Africa with recognisable macroecological patterns arising from differences in habitat requirements and life history traits between species. I find the distinctive triangular relationship between body size and range size found across other groups (Hanski 1982; Gaston & Blackburn 1996a; Gaston et al. 2000). Furthermore I find that relative clutch size explains more variation in range size in combination with body size, than body size alone, consistent with previous studies on anurans (Murray et al. 1998; Bielby et al. 2008; Cooper et al. 2008) Furthermore, I find that niche breadth cannot explain why species with larger bodies and relative clutch sizes have more extensive ranges. Therefore, these traits either enable species to colonise a greater proportion of the suitable habitat or must be indirectly associated with the abundance of suitable habitat. More research is required to isolate the process that is creating this correlation with range size, and to determine whether the abundance of suitable habitat can

single-handedly explain correlations between species traits and macroecology within the Pyxicephalidae.



## Chapter 4: Overall conclusion

Morphological traits measured for 5 to 10 individuals for a total of 25 pyxicephalid species were shown to correlate with almost all the locomotory traits that were tested. These included the filming of jumping, sprinting and swimming to obtain measures of velocity, adhesive performance on a non-stick surface and both terrestrial and aquatic endurance by chasing frogs around a circular track. These relationships were consistent with biomechanical predictions, indicating that morphology played a functional role within this clade. In Chapter 2, I confirm the trade-off between burst locomotion and endurance within the confines of a single anuran family, supporting the findings that more proficient jumpers also fatigue faster, demonstrated both between species (Rand 1952) and within males of a single species (Herrel & Bonneaud 2012). Furthermore, I find a negative correlation between relative hindlimb length and endurance, but experimental evidence is needed to determine whether the underlying mechanisms is attributable to hindlimb length or muscle fibre type composition (Bonine et al. 2001). Morphological traits of Pyxicephalidae are consistent with a model of evolution with separate selective regimes for three different ecotypes: terrestrial, semi-aquatic and burrowing. However, only a select few locomotory traits show evidence for separate ecotype selective optima. This suggests that morphology may be linked to ecotype by factors other than locomotion, and that single ecotypes could support multiple strategies for most aspects of locomotory performance (Blankers et al. 2012). These morphological patterns support the speculation of van der Meijden et al. (2005) that the phenotypic diversity of the Pyxicephalidae is adaptive, but only in the early stages of diversification where novel ecotypes were colonised, before reaching a state of niche conservatism and simple vicariance (see Kozak et al. 2006).

The geographic range sizes of the Pyxicephalidae show a positive correlation with body size, which produces the typical triangular pattern seen in some other groups (Gaston & Blackburn 1996). Size-corrected clutch size also explains range size differences between species, as found for Australian frogs (Murray et al. 1998; Murray & Hose 2005). Since clutch size is positively correlated with body size (Kuramoto 1978), it is possible that clutch size alone could explain range size, but more research is needed to separate other processes correlated with body size (Gaston & Blackburn 2003), such as predator prey interactions, minimum viable population and environmental tolerance linked to substrate. Species with larger clutch sizes produce more offspring, which increases the number of dispersing individuals (Gaston & Blackburn 2003), especially since the majority of dispersal occurs at the juvenile stage in amphibians (Gill 1978; Berven & Grudzien 1990), which might

be exacerbated by high levels of intraspecific competition at the natal pond (Waser 1985). However, species with large body sizes and relatively large clutch sizes do not have a greater habitat tolerance, suggesting that these traits are either linked to range size via speciation mechanism or due to the abundance of suitable habitat.

My study focuses on a single frog family distributed across the African continent, the Pyxicephalidae. Despite this, the ecomorphological findings are consistent with Anura studied at a global scale (Gomes et al. 2009; Moen et al. 2013). This indicates that the effect of morphology on locomotory performance could be relevant to other frog clades around the world. It is important to note that morphological traits do not always have a strong effect on a performance trait. This could be a form of many-to-one mapping (Wainwright et al. 2005), where multiple morphological traits can produce similar performance outputs, or the result of conflicting selective forces on the morphology for other functions or constraints that are not measured (Blankers et al. 2012). This warrants caution for interpretation when assuming a direct and clear relationship between morphology and function. The effects of traits on macroecology are also complex, due to the combination of multiple factors and their interactions. It remains a challenge to distinguish between causation and correlation (Gaston 2009), but mechanisms that contradict macroecological patterns are more likely to be identified by comparing multiple independent macroecological patterns. Due to differences in life history and environmental constraints, the processes affecting macroecological patterns of the anurans are unlikely to be informative for other animal groups. However, the methods and reasoning used to justify macroecological mechanisms can be applied to different patterns in other groups. Much remains to be understood regarding interspecific distribution patterns, with relevance for conservation and invasion biology in specific groups of organisms (Cooper et al. 2008; Tingley et al. 2010).

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## Addendum A

**A.1** The collection details for pyxicephalid species captured for use in performance testing, showing the date of capture, locality and decimal degree coordinates. N represents the number of specimens caught and tested from each site. Coordinates are in decimal degrees.

Species	n	Date found	locality	Latitude (dec)	Longitude (dec)
<i>Amietia delalandii</i>	4	11/12/2015	Bergwoning, Golden Gates	-28.5213	28.5595
<i>Amietia fuscigula</i>	4	8/16/2016	Alphenvale Retirement Village, Constantia	-34.0172	18.4409
<i>Amietia hymenopus</i>	10	12/19/2015	Drakensberg, before Tugela Falls	-28.7509	28.8855
<i>Amietia poyntoni</i>	4	12/28/2015	Retiefklip, canal	-28.5528	29.1283
<i>Amietia vertebralis</i>	9	12/18/2015	Drakensberg, first site: Khubedu East	-28.7761	28.9000
<i>Anhydrophryne hewitti</i>	10	12/23/2015	Royal Natal, Tugela gorge	-28.7472	28.9126
<i>Anhydrophryne rattrayi</i>	10	6/4/2016	Isidenge harvested area	-32.6887	27.2780
<i>Arthroleptella bicolor</i>	9	13/9/2016	Bainskloof pass	-33.5972	19.1183
<i>Arthroleptella villiersi</i>	10	5/8/2015	lower Franschhoek pass	-33.9563	19.1743
<i>Cacosternum aggestum</i>	5	6/15/2016	Klipheuwel	-33.6921	18.7350
<i>Cacosternum australis</i>	10	1/10/2015	next to Donut vlei, Rooisand, Kleinmond	-34.3345	19.0880
<i>Cacosternum boettgeri</i>	10	12/25/2015	Harrismith vlei	-28.2825	29.1121
<i>Cacosternum capense</i>	10	6/16/2016	Klipheuwel site 2	-33.6992	18.7068
<i>Cacosternum nanum</i>	5	12/1/2016	Mount Moreland, eastern wetland	-29.6391	31.0969
<i>Cacosternum nanum</i>	1	3/31/2016	Fort Fordyce, west bushpig trail	-32.6709	26.4904
<i>Cacosternum nanum</i>	1	6/4/2016	northern Isidenge area, Patchwood	-32.4031	27.4485
<i>Cacosternum thorini</i>	5	12/4/2016	Type locality, Hogsback	-32.5800	26.9430
<i>Microbatrachella capensis</i>	11	9/22/2015	Donut vlei, Rooisand, Kleinmond	-34.3340	19.0880
<i>Natalobatrachus bonebergi</i>	10	5/1/2016	Crowned Eagle Park	-29.7975	30.8018
<i>Poyntonia paludicola</i>	8	5/8/2015	lower Franschhoek pass	-33.9556	19.1742
<i>Pyxicephalus adspersus</i>	2	4/13/2016	<b>Captive</b> , Bayworld	na	na
<i>Strongylopus bonaespei</i>	1	6/23/2015	lower Franschhoek pass	-33.9566	19.1743
<i>Strongylopus bonaespei</i>	9	9/9/2015	Peninsula dam, Kogelberg	-34.1722	18.9708
<i>Strongylopus fasciatus</i>	5	3/31/2016	Fort Fordyce, nearby vlei to West	-32.6785	26.4883
<i>Strongylopus fasciatus</i>	5	6/4/2016	Sandiles Rest	-32.6639	27.2992
<i>Strongylopus grayii</i>	10	6/16/2015	Jonkershoek fishery	-33.9636	18.9259
<i>Tomopterna cryptotis</i>	10	12/30/2015	Harrismith vlei	-28.2825	29.1121
<i>Tomopterna delalandi</i>	11	10/20/2015	Rooisands, Kleinmond	-34.3447	19.0834
<i>Tomopterna natalensis</i>	9	12/28/2015	Retiefklip, canal	-28.5528	29.1283

**A.2** Average temperatures for different performance tests and the presence of cork substrate during the sprint trial for species tested. D.End= dry endurance, A.End= Aquatic endurance. Question marks denote temperatures that were not measured due to a lack of apparatus, and not due to temperature extremes.

Species	n	Date found	locality	Latitude (dec)	Longitude (dec)
<i>Amietia delalandii</i>	4	11/12/2015	Bergwoning, Golden Gates	-28.5213	28.5595
<i>Amietia fuscigula</i>	4	8/16/2016	Alphenvale Retirement Village, Constantia	-34.0172	18.4409
<i>Amietia hymenopus</i>	10	12/19/2015	Drakensberg, before Tugela Falls	-28.7509	28.8855
<i>Amietia poyntoni</i>	4	12/28/2015	Retiefklip, canal	-28.5528	29.1283
<i>Amietia vertebralis</i>	9	12/18/2015	Drakensberg, first site: Khubedu East	-28.7761	28.9000
<i>Anhydrophryne hewitti</i>	10	12/23/2015	Royal Natal, Tugela gorge	-28.7472	28.9126
<i>Anhydrophryne rattrayi</i>	10	6/4/2016	Isidenge harvested area	-32.6887	27.2780
<i>Arthroleptella bicolor</i>	9	13/9/2016	Bainskloof pass	-33.5972	19.1183
<i>Arthroleptella villiersi</i>	10	5/8/2015	lower Franschhoek pass	-33.9563	19.1743
<i>Cacosternum aggestum</i>	5	6/15/2016	Klipheuwel	-33.6921	18.7350
<i>Cacosternum australis</i>	10	1/10/2015	next to Donut vlei, Rooisand, Kleinmond	-34.3345	19.0880
<i>Cacosternum boettgeri</i>	10	12/25/2015	Harrismith vlei	-28.2825	29.1121
<i>Cacosternum capense</i>	10	6/16/2016	Klipheuwel site 2	-33.6992	18.7068
<i>Cacosternum nanum</i>	5	12/1/2016	Mount Moreland, eastern wetland	-29.6391	31.0969
<i>Cacosternum nanum</i>	1	3/31/2016	Fort Fordyce, west bushpig trail	-32.6709	26.4904
<i>Cacosternum nanum</i>	1	6/4/2016	northern Isidenge area, Patchwood	-32.4031	27.4485
<i>Cacosternum thorini</i>	5	12/4/2016	Type locality, Hogsback	-32.5800	26.9430
<i>Microbatrachella capensis</i>	11	9/22/2015	Donut vlei, Rooisand, Kleinmond	-34.3340	19.0880
<i>Natalobatrachus bonebergi</i>	10	5/1/2016	Crowned Eagle Park	-29.7975	30.8018
<i>Poyntonia paludicola</i>	8	5/8/2015	lower Franschhoek pass	-33.9556	19.1742
<i>Pyxicephalus adspersus</i>	2	4/13/2016	<b>Captive</b> , Bayworld	na	na
<i>Strongylopus bonaespei</i>	1	6/23/2015	lower Franschhoek pass	-33.9566	19.1743
<i>Strongylopus bonaespei</i>	9	9/9/2015	Peninsula dam, Kogelberg	-34.1722	18.9708
<i>Strongylopus fasciatus</i>	5	3/31/2016	Fort Fordyce, nearby vlei to West	-32.6785	26.4883
<i>Strongylopus fasciatus</i>	5	6/4/2016	Sandiles Rest	-32.6639	27.2992
<i>Strongylopus grayii</i>	10	6/16/2015	Jonkershoek fishery	-33.9636	18.9259
<i>Tomopterna cryptotis</i>	10	12/30/2015	Harrismith vlei	-28.2825	29.1121
<i>Tomopterna delalandi</i>	11	10/20/2015	Rooisands, Kleinmond	-34.3447	19.0834
<i>Tomopterna natalensis</i>	9	12/28/2015	Retiefklip, canal	-28.5528	29.1283

**A.3** Locomotory performance for all pyxicephalid species tested (females removed), showing mean values and SD in brackets. Abbreviated performance traits are: J\_dist (jump distance), J\_vel (jump take-off velocity), SP\_vel (sprint velocity), SW\_vel (swim velocity), G\_UP (upward orientated adhesive), G\_UD (downward orientated adhesive), Tdist (terrestrial endurance distance), T\_time (terrestrial endurance time to exhaustion), T\_EI (terrestrial endurance index), A\_dist (aquatic endurance distance), A\_time (aquatic endurance time to exhaustion), and A\_EI (aquatic endurance index). See A.4 for sample # per species.

Species	J_dist (mm)	J_vel (m/s)	SP_vel (m/s)	SW_vel (m/s)	G_UP (°)	G_UD (°)	Tdist (m)	T_time (sec)	T_EI	A_dist (m)	A_time (sec)	A_EI
<i>Amietia delalandii</i>	1110 (205.3)	3.82 (0.54)	1.08 (0.2)	0.43 (0.07)	47.5 (6.74)	45.8 (7.26)	13.61 (0.83)	216 (16.8)	0 (0)	23.93 (1.65)	647.8 (60.3)	0.28 (0.18)
<i>Amietia fuscigula</i>	715 (58.7)	2.94 (0.16)	0.81 (0.26)	0.43 (0.12)	56.3 (5.51)	56.3 (3.44)	46.2 (15.48)	894 (342.7)	0.12 (0.12)	45.38 (9.09)	889.5 (21.6)	0.43 (0.11)
<i>Amietia hymenopus</i>	454 (71.4)	2.49 (0.41)	0.43 (0.08)	0.49 (0.04)	100.2 (47.16)	58 (13.01)	15.68 (7.83)	628 (225.2)	0.01 (0.03)	24.02 (8.13)	432.4 (114.4)	0.22 (0.17)
<i>Amietia poyntoni</i>	916 (230.5)	3.82 (0.35)	1.29 (0.37)	0.48 (0.04)	41.3 (2.1)	40 (1.92)	20.63 (5.64)	157 (36.4)	0 (0)	37.95 (8.73)	409 (73.7)	0.12 (0.14)
<i>Amietia vertebralis</i>	800 (139.9)	3.28 (0.59)	0.92 (0.34)	0.77 (0.18)	43.7 (7.21)	39.3 (5.66)	13.2 (2.61)	316 (108.4)	0 (0)	21.27 (4.24)	322.3 (121.8)	0.15 (0.06)
<i>Anhydrophryne hewitti</i>	507 (83.3)	2.46 (0.27)	0.48 (0.11)	0.19 (0.04)	180 (0)	178 (6.32)	11.88 (3.19)	349 (117.5)	0.06 (0.03)	8 (3.73)	321.3 (123.1)	0 (0)
<i>Anhydrophryne rattrayi</i>	301 (35.2)	1.79 (0.15)	0.27 (0.03)	0.18 (0.03)	153.7 (20.09)	173.2 (21.61)	7.76 (1.57)	242 (91.3)	0 (0)	4.29 (0.85)	249.7 (64)	0.13 (0.05)
<i>Arthroleptella bicolor</i>	162 (23.4)	1.21 (0.15)	0.15 (0.03)	0.14 (0.02)	180 (0)	173.9 (9.53)	12.38 (1.73)	1231 (108.1)	0.19 (0.1)	5.5 (1.7)	752.3 (222.9)	0.14 (0.16)
<i>Arthroleptella villiersi</i>	211 (54.8)	1.61 (0.39)	0.16 (0.04)	0.16 (0.05)	180 (0)	157.7 (24.47)	17.66 (9.93)	1011 (401.7)	0.12 (0.1)	7.34 (9.35)	508 (450.5)	0.06 (0.08)
<i>Cacosternum aggestum</i>	171 (30.7)	1.32 (0.11)	0.27 (0.11)	0.19 (0.03)	155.3 (14.45)	98.3 (16.41)	30.03 (7.77)	1200 (18.9)	0.65 (0.21)	29.04 (5.3)	892.4 (8.3)	0.7 (0.18)
<i>Cacosternum australis</i>	164 (25)	1.3 (0.16)	0.25 (0.07)	0.21 (0.04)	162.3 (16.85)	118.2 (38.03)	24.59 (4.15)	1176 (21.7)	0.54 (0.17)	28.88 (4.42)	835.2 (86.7)	0.79 (0.2)
<i>Cacosternum boettgeri</i>	186 (27.1)	1.48 (0.2)	0.21 (0.09)	0.16 (0.03)	171.7 (180 (0))	22.28 (18)	980 (8.98)	980 (127.1)	0.33 (0.14)	26.9 (3.12)	823.5 (45.5)	0.75 (0.3)
<i>Cacosternum capense</i>	126 (7.9)	1.1 (0.06)	0.26 (0.06)	0.16 (0.02)	72 (11.73)	63.2 (9.64)	31.35 (7.82)	1219 (55.9)	0.69 (0.39)	25.58 (4.81)	909.7 (14.5)	0.67 (0.2)
<i>Cacosternum nanum</i>	228	1.77	0.33	0.18	180 (0)	180 (0)	29.23	1167	0.27	28.52	892.3	0.55

	(49.3)	(0.25)	(0.14)	(0.02)			(10.51)	(56.9)	(0.13)	(3.65)	(21.8)	(0.15)
	145	1.23	0.16	0.15			14.85	1134	0.12	17.49	873.6	0.42
<i>Cacosternum thorini</i>	(14.8)	(0.11)	(0.02)	(0.04)	180 (0)	180 (0)	(4.81)	(52)	(0.03)	(2.5)	(29.8)	(0.19)
<i>Microbatrachella capensis</i>	201	1.51	0.2	0.18	165.2	150.8	19.5	1185	0.12	24.75	851.1	0.63
	(27.9)	(0.17)	(0.03)	(0.06)	(18.45)	(27.99)	(2.93)	(24.9)	(0.05)	(4.78)	(34.2)	(0.22)
<i>Natalobatrachus bonebergi</i>	670	2.99	0.76	0.37			7.1	70		4.62	211.4	
	(123.9)	(0.26)	(0.17)	(0.07)	180 (0)	180 (0)	(1.11)	(26.3)	0 (0)	(1.52)	(71.5)	0 (0)
	236	1.62	0.24	0.19	85.8	65.1	17.21	919	0.07	15.68	848.5	0.17
<i>Poyntonia paludicola</i>	(57)	(0.37)	(0.04)	(0.03)	(24.29)	(13.21)	(10.9)	(460.1)	(0.07)	(11.55)	(449.3)	(0.16)
<i>Pyxicephalus adspersus</i>	307	2.23	0.36	0.18	27.8	31.7	57.75	1195	0.36	57.75	879.5	1.18
	(13)	(0.04)	(0.06)	(0.02)	(12.49)	(9.43)	(16.33)	(70.7)	(0.11)	(7)	(7.8)	(0.07)
<i>Strongylopus bonaespei</i>	900	3.91	0.91	0.31	100.5	79.8	13.53	235		22.77	547.4	0.13
	(235.4)	(0.98)	(0.1)	(0.08)	(16.16)	(14.09)	(4.32)	(121.1)	0 (0.01)	(8.04)	(88.3)	(0.09)
	729	3.26	0.81	0.35	104	93.2	14.19	205		46.04	816.7	0.45
<i>Strongylopus fasciatus</i>	(104.2)	(0.58)	(0.26)	(0.05)	(32.8)	(28.5)	(4.81)	(98.8)	0 (0)	(9.28)	(91)	(0.29)
	554	2.67	0.63	0.22	76.8	61.9	29.54	999	0.07	29.54	628.7	0.75
<i>Strongylopus grayii</i>	(100.7)	(0.41)	(0.11)	(0.04)	(7.26)	(9.23)	(4.43)	(206.1)	(0.07)	(4)	(79.9)	(0.32)
	381	2.04	0.52	0.25	98	89.8	63.69	1189	0.37	50.49	880.5	0.48
<i>Tomopterna cryptotis</i>	(54.4)	(0.13)	(0.09)	(0.05)	(9.62)	(13.18)	(9.08)	(23.5)	(0.15)	(6.42)	(14.2)	(0.1)
	380	1.99	0.46	0.34	51.6	52.6	74.4	1187	0.34	65.7	915.7	0.81
<i>Tomopterna delalandii</i>	(57.3)	(0.25)	(0.04)	(0.04)	(7.7)	(4.7)	(14.34)	(20.3)	(0.24)	(6.34)	(11.6)	(0.34)
	510	2.89	0.34	0.25	126.9	90.9	53.53	1208	0.18	46.93	891	0.43
<i>Tomopterna natalensis</i>	(130.8)	(0.59)	(0.11)	(0.03)	(17.25)	(5.78)	(6.12)	(17.2)	(0.05)	(3.61)	(19.6)	(0.1)

A 4. Morphological traits for all pyxicephalid species measured (live captured, females removed), showing mean values and SD in brackets. Abbreviated morphological traits are: MASS (body mass), SVL (snout-vent length), MFW (thigh muscle width), HLEG (hindlimb length (including longest toe), HNDL (longest finger length), FW (mid-finger width), FDD (fingertip width), and TWS (pedal webbing score). N represents the number of specimens measured.

Species	n	MASS (g)	SVL (mm)	MFW (mm)	HLEG (mm)	HNDL (mm)	FW (mm)	FDD (mm)	TWS
<i>Amietia delalandii</i>	4	21.65 (8.56)	62.68 (2.74)	13.51 (1.53)	115.39 (13.23)	12.85 (0.96)	0.9 (0.09)	0.94 (0.05)	3
<i>Amietia fuscigula</i>	4	18.09 (2.47)	58.44 (3.27)	13.03 (0.21)	97.85 (4.4)	13.56 (1.3)	0.87 (0.06)	0.83 (0.05)	3
<i>Amietia hymenopus</i>	10	4.32 (1.05)	39.46 (8.77)	6.69 (0.91)	68.04 (4.69)	8.81 (0.56)	0.58 (0.1)	0.51 (0.12)	5
<i>Amietia poyntoni</i>	4	50.25 (15.48)	79.87 (12.41)	18.56 (2.81)	142.3 (18.67)	18.05 (2.42)	1.46 (0.22)	1.18 (0.02)	3
<i>Amietia vertebralis</i>	9	68.09 (22.99)	97.29 (1.72)	19.12 (1.56)	160.53 (19.33)	20.39 (3.17)	1.49 (0.36)	1.34 (0.3)	5
<i>Anhydrophryne hewitti</i>	10	0.72 (0.14)	21.15 (1.08)	3.81 (0.45)	37.48 (3.02)	5.67 (0.59)	0.38 (0.06)	0.36 (0.04)	1
<i>Anhydrophryne rattrayi</i>	10	0.91 (0.15)	21.27 (0.63)	4.42 (0.26)	30.58 (1.8)	4.66 (0.42)	0.42 (0.04)	0.4 (0.04)	1
<i>Arthroleptella bicolor</i>	6	0.19 (0.02)	13.78 (0.72)	2.55 (0.16)	19.36 (0.71)	3.07 (0.07)	0.26 (0.03)	0.25 (0.02)	1
<i>Arthroleptella villiersi</i>	10	0.19 (0.03)	13.29 (0.94)	1.99 (0.19)	21.05 (0.96)	3.53 (0.24)	0.29 (0.02)	0.31 (0.02)	1
<i>Cacosternum aggestum</i>	5	0.83 (0.07)	21.82 (0.79)	4.1 (0.36)	29.45 (1)	4.96 (0.17)	0.47 (0.05)	0.35 (0.06)	1
<i>Cacosternum australis</i>	10	0.37 (0.06)	17.08 (0.96)	2.87 (0.35)	24.88 (1.95)	3.55 (0.36)	0.33 (0.03)	0.27 (0.04)	1
<i>Cacosternum boettgeri</i>	10	0.39 (0.05)	31.93 (2.29)	3.34 (0.28)	24.95 (1.48)	3.78 (0.28)	0.35 (0.06)	0.25 (0.04)	1
<i>Cacosternum capense</i>	10	2.89 (0.74)	18.2 (0.88)	5.61 (0.68)	40.17 (2.16)	7.66 (0.56)	0.83 (0.08)	0.58 (0.09)	1
<i>Cacosternum nanum</i>	7	0.48 (0.07)	14.82 (0.75)	3.55 (0.31)	27.13 (1.9)	4.23 (0.24)	0.36 (0.02)	0.31 (0.04)	1
<i>Cacosternum thorini</i>	5	0.27 (0.04)	14.37 (1.01)	2.91 (0.29)	21.51 (0.31)	3.51 (0.21)	0.26 (0.06)	0.2 (0.01)	1
<i>Microbatrachella capensis</i>	11	0.27 (0.04)	28.3 (1.06)	2.59 (0.16)	23.25 (1.92)	3.22 (0.3)	0.27 (0.04)	0.23 (0.02)	3
<i>Natalobatrachus bonebergi</i>	10	1.46 (0.11)	23.56 (2.9)	5.05 (0.33)	52.38 (1.07)	10.08 (0.23)	0.5 (0.04)	1.61 (0.1)	3
<i>Poyntonia paludicola</i>	8	1.3 (0.44)	23.56 (9.62)	4.47 (1.01)	37.06 (5.32)	5.9 (0.78)	0.5 (0.05)	0.38 (0.04)	3
<i>Pyxicephalus adspersus</i>	2	610.8 (89.94)	162.5 (4.05)	25.94 (0.62)	177.19 (1.32)	29.01 (0.62)	4.3 (0.1)	3.73 (0.37)	2
<i>Strongylopus bonaespei</i>	10	2.47 (1.1)	33.59 (2.11)	5.83 (0.76)	74.6 (9.99)	9.09 (0.84)	0.54 (0.07)	0.5 (0.09)	1
<i>Strongylopus fasciatus</i>	10	3.5 (0.64)	36.45 (1.55)	6.73 (0.29)	77.82 (4.63)	10.15 (0.66)	0.57 (0.05)	0.59 (0.05)	1
<i>Strongylopus grayii</i>	10	2.1 (0.38)	31.12 (1.02)	4.99 (0.47)	58.32 (4.77)	8.14 (0.47)	0.47 (0.03)	0.51 (0.06)	2
<i>Tomopterna cryptotis</i>	10	6.14 (0.64)	38.32 (1.65)	7.09 (0.44)	56.16 (1.06)	8.65 (0.35)	0.84 (0.06)	0.61 (0.11)	2
<i>Tomopterna delalandii</i>	11	6.32 (1.17)	38.85 (1.76)	7.12 (0.73)	57.66 (3.34)	8.95 (0.48)	0.78 (0.09)	0.59 (0.07)	2
<i>Tomopterna natalensis</i>	9	2.08 (0.39)	28.6 (0)	5.71 (0.44)	46.53 (2.57)	6.73 (0.51)	0.55 (0.06)	0.49 (0.07)	2



A.5 Voucher specimen numbers and accession numbers for the three genes deposited on GenBank used to construct the phylogeny of the Pyxicephalidae (Figure 1.3). NS denotes genes with no record of a voucher specimen number.

Species	specimen number	16S	12S	Tyrosinase
<i>Amietia angolensis</i>	AC3016	KC756291	KU693707	KU694141
<i>Amietia chapini</i>	ZMB81748	KU693869	KU693762	KU694190
<i>Amietia delalandii</i>	AACRG797	KU693851	KU693733	KU694167
<i>Amietia desaegeri</i>	MD576	KU693871	KU693764	KU694192
<i>Amietia fuscigula</i>	AC3181	KC756314	KU693756	KU694185
<i>Amietia hymenopus</i>	ZMB83110	KU693805	KU693657	KU694103
<i>Amietia johnstoni</i>	PEM A7853	KU693787	KU693618	KU694067
<i>Amietia moyeromum</i>	AC2225	KU693828	KU693684	KU694124
<i>Amietia nutti</i>	MD625	KU693876	KU693768	KU694195
<i>Amietia poyntoni</i>	ZMB78569	KU693822	KU693677	KU694119
<i>Amietia ruwenzorica</i>	SL456	KU693818	KU693672	KU694115
<i>Amietia vandijki</i>	ZMB83106	KC756305	KU693591	KU694041
<i>Amietia vertebralis</i>	AACRG2791	KU693840	KU693711	KU694145
<i>Amietia wittei</i>	MP4807	KU693867	KU693757	KU694186
<i>Amietia tenuoplicata</i>	ZMB81817	KU693821	KU693676	KU694118
<i>Anhydrophryne hewitti</i>	AH2	AY838890		
<i>Anhydrophryne rattrayi</i>	AC1206		DQ022319	
<i>Anhydrophryne rattrayi</i>	NS			HQ014446
<i>Anhydrophryne rattrayi</i>	16S121Anhydro	AF215504		
<i>Anhydrophryne ngongoniensis</i>	TM83892	AY838888		
<i>Arthroleptella bicolor</i>	AMNH A144967		DQ283070	DQ282910
<i>Arthroleptella drewesii</i>	CNCH6752	AY454341	AY453276	
<i>Arthroleptella landdrosia</i>	NS		AF330244	
<i>Arthroleptella landdrosia</i>	AC1204	AY205276		
<i>Arthroleptella lightfooti</i>	NS		AF330242	
<i>Arthroleptella lightfooti</i>	AC968	AY205282		
<i>Arthroleptella rugosa</i>	ZR 52097	EU840262		
<i>Arthroleptella subvoce</i>	CNCH6745	AY205284	AY205266	
<i>Arthroleptella villiersi</i>	isolate 1219	DQ347344	DQ347062	DQ347195
<i>Aubria masako</i>	UTEP:21202	KU560021	KU559938	
<i>Aubria subsigillata</i>	ZMB:79260	KF991276		
<i>Aubria subsigillata</i>	DPL 4936 (UTA)			DQ282975
<i>Cacosternum aggestum</i>	MHNG 2690.25	KF144411		KF144571
<i>Cacosternum australis</i>	MHNG 2699.39	KF144417		KF144572
<i>Cacosternum boettgeri</i>	isolate 0948	DQ347299	DQ347007	DQ347141
<i>Cacosternum capense</i>	TMSA84242	DQ022354	DQ022323	
<i>Cacosternum capense</i>	isolate 629			KF144548
<i>Cacosternum karooicum</i>	MHNG 2709.67	KF144470		KF144574
<i>Cacosternum kinangopensis</i>	E126.9	EU978471		
<i>Cacosternum leleupi</i>	MHNG 2740.69	KF144472		KF144531
<i>Cacosternum namaquense</i>	MHNG 2699.44	KF144475		KF144576
<i>Cacosternum nanogularum</i>	MHNG 2740.83	KF144483		KF144549
<i>Cacosternum nanum</i>	MHNG 2741.1	KF144501		KF144556

<i>Cacosternum parvum</i>	MHNG 2741.3	KF144503		KF144557
<i>Cacosternum platys</i>	MHNG 2699.38	KF144512		KF144563
<i>Cacosternum plimptoni</i>	E151.22	EU978472		
<i>Cacosternum rhythmum</i>	isolate 645	KF144515		KF144545
<i>Cacosternum striatum</i>	MHNG 2741.21	KF144524		KF144533
<i>Cacosternum thorini</i>	PEMA10094	KJ461737		
<i>Microbatrachella capensis</i>	CNC6698	DQ022357		
<i>Microbatrachella capensis</i>	TMSA84315		DQ022329	
<i>Natalobatrachus bonebergi</i>	isolate 0952	DQ347302	DQ347011	DQ347144
<i>Nothophryne broadleyi</i>	QQ 0710	KU761283	KU761275	
<i>Poyntonia paludicola</i>	isolate 1066	DQ347341	DQ347058	DQ347191
<i>Ptychadena anchietae</i>	ZNHM (AM-032)	JX464879	JX465088	
<i>Ptychadena erlangeri</i>	ZNHM (AK-2029)	JX464871	JX465080	
<i>Ptychadena mascareniensis</i>	AMNH A167415		DQ283031	DQ282899
<i>Ptychadena nana</i>	isolate XF-934	KF380455		KF380278
<i>Pyxicephalus adspersus</i>	JPB 6584	AF206472	AF206091	
<i>Pyxicephalus edulis</i>	AMNH A168412	DQ283157	DQ283157	DQ282941
<i>Strongylopus bonaespei</i>	isolate 1221	DQ347345	DQ347063	DQ347196
<i>Strongylopus fasciatus</i>	NS		DQ019594	
<i>Strongylopus fasciatus</i>	16SStrongylopus_fasciatus	AF215412		
<i>Strongylopus fuelleborni</i>	RdS 994			KC180300
<i>Strongylopus grayii</i>	TMSA84854	DQ022367		
<i>Strongylopus wageri</i>	J3J_Sw	FJ411442		
<i>Tomopterna cryptotis</i>	AC1100	AY255090	AF371199	
<i>Tomopterna damarensis</i>	AC1668	AY255091		
<i>Tomopterna delalandii</i>	AC942	AY255086	AF371153	
<i>Tomopterna elegans</i>	MVZ:Herp:242739		HQ700692	
<i>Tomopterna gallmanni</i>	NMK:A/5159	JX088645		
<i>Tomopterna kachowskii</i>	MVZ:Herp:241323	HQ700691		
<i>Tomopterna krugerensis</i>	isolate AC1508	AY255098	AF371209	
<i>Tomopterna luganga</i>	AD310	DQ017056		
<i>Tomopterna marmorata</i>	AC1534	AY255084	AF371204	
<i>Tomopterna natalensis</i>	TM84342	AY205286	AY205274	
<i>Tomopterna tandyi</i>	AC1567	AF436073	AF371190	
<i>Tomopterna tuberculosa</i>	RdS 880	KC179967		KC180298
<i>Tomopterna wambensis</i>	NMK:A/4323	JX088656		

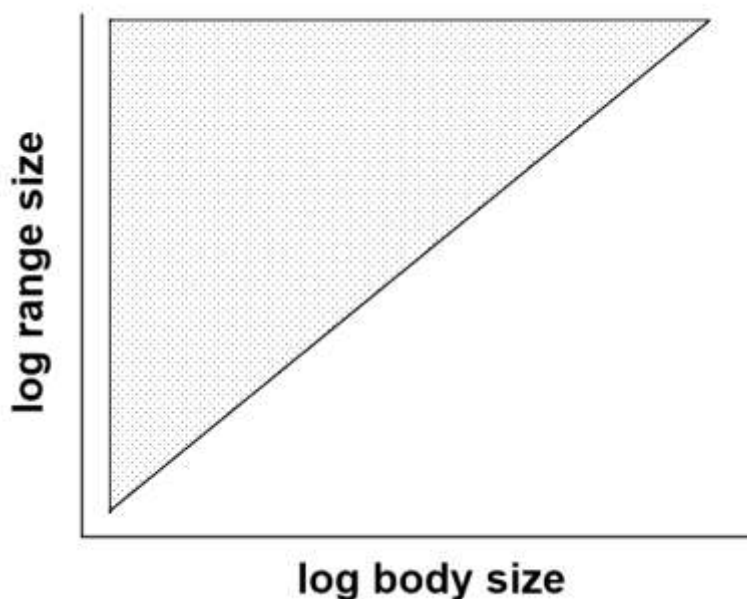
## A.6 Justification for ecotype categorisation of pyxicephalid taxa from field notes and expert opinion.

Taxon	Narrow category	Ecotype use justification	Broad category	Ecotype use justification
<i>Pyxicephalus</i>	Burrowing	digs own burrow and spends dry season underground (Withers & Loveridge, 1981)	Burrowing	"
<i>Aubria</i>	Aquatic	more aquatic than most Amietia species, spending more time in water (pers. comm. Burger, M.)	Semi-aquatic	aquatic is sub-category of semi-aquatic
<i>Nothophryne</i>	Seep	lives in montane seeps among moss (Bittencourt et al 2016)	Terrestrial	seep is sub-category of terrestrial
<i>Tomopterna</i>	Burrowing	burrows underground during the day, digs own burrow (Rose 1926, Dawood et al. 2002; pers. obs.)	Burrowing	"
<i>Strongylopus</i>	Terrestrial	lives under debri (pers. obs.), or at the base of vegetation and grassy wetlands and in deep earth cracks (Rose 1926)	Terrestrial	"
<i>Poyntonia</i>	Seep	montane shallow seepages, shallow rocky streams and marshy areas (Channing & Boycott, 1989) where water forms a shallow film upon substrate (pers. obs.)	Terrestrial	seep is sub-category of terrestrial
<i>Cacosternum</i>	Terrestrial	lives in mud cracks, base veg or under debris (pers. obs. & De Villiers pers. comm.)	Terrestrial	"
<i>Cacosternum capensis</i>	Burrowing	underground during the dry season, digs own burrow (Rose 1926) unlike other <i>Cacosternum</i> species	Burrowing	"
<i>Microbatrachella</i>	Terrestrial	edges of temporary vleis, found burrowed at base of vegetation during the dry season (Rose 1926), near edges of temporary vleis (Rose 1929)	Terrestrial	"
<i>Amietia</i>	Semi-aquatic	sits partially submerged in water or on the edge of rivers or pools (Rose 1926), readily jumps in and swims downward when approached (pers. obs.)	Semi-aquatic	"
<i>Amietia vertebralis</i> & <i>hymenopus</i>	Aquatic	spends more time in water, rather than sitting on the edge of water, vertebralis swims underwater occasionally when undisturbed (pers. obs.)	Semi-aquatic	aquatic is sub-category of semi-aquatic
<i>Anhydrophryne</i>	Seep	lives in Afromontane leaf litter/mossy seeps near streams, and grassland seeps (Dawood & Stam 2006; Minter et al. 2004)	Terrestrial	seep is sub-category of terrestrial
<i>Arthroleptella</i>	Seep	permanently moist montane seeps and moss beds along streams (Rose 1926; Turner, 2009)	Terrestrial	seep is sub-category of terrestrial
<i>Natalobatrachus</i>	Semi-arboreal	forested streams and pools, utilise overhanging vegetation (Minter et al. 2004; van der Meijden et al. 2005)	Semi-aquatic	dives into water when approached (Minter et al. 2004; pers. obs.)

A.7 Principal component loadings for the first four PC axes for all morphological traits measured in captured pyxicephalid species. This shows the different contributions of morphological traits that are captured in each principal component axis. Abbreviation of morphological traits are: MASS (body mass), SVL (snout-vent length), SUL (snout-urostyle length), BW (mid-body width), HW (head width), MFW (thigh muscle width), ILL (inter-limb length), FM (femur length), TB (tibia length), MT (calcaneum length), FTL (foot length including longest toe), TW (mid-toe width), TDD (toe-tip width), MRW (mid radioulnar width), HM (humerus length), RD (radioulnar length), HNDL (longest finger length), FW (mid-finger width), FDD (fingertip width), and TWS (pedal webbing score).

PC comp.	MASS	SVL	SUL	BW	HW	MFW	ILL	FM	TB	MT
PC1	0.98	0.88	0.87	0.96	0.85	0.78	0.91	0.78	0.73	0.76
PC2	0.18	-0.47	-0.50	-0.17	-0.49	-0.59	-0.37	-0.61	-0.65	-0.60
PC3	0.00	0.02	0.01	0.02	0.14	-0.13	0.18	-0.11	-0.20	-0.20
PC4	-0.01	0.00	0.00	0.20	-0.07	0.09	0.01	-0.01	-0.02	-0.06
PC comp.	FTL	TW	TDD	MRW	HM	RD	HNDL	FW	FDD	TWS
PC1	0.72	0.96	0.95	0.88	0.79	0.82	0.81	0.95	0.90	-0.11
PC2	-0.67	-0.24	-0.15	-0.44	-0.58	-0.55	-0.57	-0.25	-0.18	0.18
PC3	-0.14	0.05	-0.09	0.15	0.14	0.10	0.01	0.02	-0.05	-0.51
PC4	-0.04	0.02	0.03	0.00	-0.09	-0.08	0.01	0.12	0.03	0.50

## Addendum B



B.1. The triangular relationship formed between species range size and body size, adapted from Gaston & Blackburn (1996). The shaded area represents the region where the majority of species are plotted, indicating that large species tend only to have large distribution ranges.

B.2. List of institutions that provided distribution data on pyxicephalid species used to calculate range size and for species distribution modelling purposes. Many of these institutions also provided locality data for other anuran species for use in MaxEnt as the bias file.

Organisation or Institution	Acronym	Distribution records
Animal Demography Unit	ADU	46064
Cape Nature	CN	17684
Endangered Wildlife Trust	EWT	13607
Ditsong Museum	TM	12521
Bayworld Museum	PEM	8287
KwaZulu Natal Museum	KZNM	6590
iSpot southern Africa		4614
Iziko South African Museum	ZR	3644
South African Institute for Aquatic Biodiversity	SAIAB	1772
South African National Biodiversity Institute	SANBI	989
Vertnet		969
Mpumalanga Tourism and Parks Agency	MTPA	670
Survey of Cederberg Amphibians and Reptiles for Conservation and Ecotourism	SCARCE	97

B.3. Morphological traits for all pyxicephalid species (measured from both live and museum specimens), showing mean values and SD in brackets. Abbreviated morphological traits are: SVL (snout-vent length), MASS (body mass), HW (head width), FM (femur length), TB (tibia length), MT (calcaneum length), and FTL (foot length including longest toe). N represents the number of specimens measured.

Species	n	SVL	HW	FM	TB	MT	FTL
<i>Amietia angolensis</i>	14	67.74 (7.96)	22.85 (2.57)	35.15 (3.73)	41.48 (4.28)	20.86 (2.3)	40.29 (3.94)
<i>Amietia delalandii</i>	18	64.79 (6.87)	21.81 (2.6)	33.68 (3.71)	37.98 (4.58)	17.67 (2.89)	36.15 (4.25)
<i>Amietia fuscigula</i>	19	67.21 (13.87)	24.82 (4.85)	32.76 (6.09)	34.17 (6.12)	16.04 (4.15)	34.64 (5.66)
<i>Amietia hymenopus</i>	12	38.55 (3.68)	14.64 (1.44)	18.4 (1.74)	19.17 (1.46)	8.56 (0.51)	20.57 (1.65)
<i>Amietia johnstoni</i>	6	58.44 (5.63)	21.56 (2.2)	28.37 (1.88)	30.69 (3.96)	15.77 (1.71)	32.19 (3.69)
<i>Amietia lubrica</i>	2	76.72 (3.51)	27.42 (3.37)	38.28 (4.17)	41 (1.99)	16.03 (0.27)	44.09 (4.57)
<i>Amietia poyntoni</i>	5	80.86 (7.91)	30.55 (4.17)	41.33 (4.6)	42.21 (4.9)	18.1 (2.52)	41.82 (4.54)
<i>Amietia poyntoni?</i>	2	51.34 (3.05)	20.61 (1.6)	25.05 (0.12)	25.5 (1.2)	11.86 (0.93)	26.82 (1.53)
<i>Amietia sp.nov.</i>	2	76.98 (4.03)	28.78 (1.35)	43.19 (0.93)	48.65 (1.27)	19.38 (2.77)	45.31 (1.39)
<i>Amietia vandijki</i>	1	52.09 (NA)	17.78 (NA)	24.75 (NA)	25.66 (NA)	11.3 (NA)	24.49 (NA)
<i>Amietia vertebralis</i>	17	99.84 (13.17)	43.01 (9.36)	47.44 (5.44)	48.61 (5.92)	22.03 (3.97)	49.55 (6.6)
<i>Anhydrophryne hewitti</i>	32	22.13 (2.94)	8.46 (1.14)	10.01 (1.24)	11.41 (1.57)	5.82 (0.9)	12.06 (1.61)
<i>Anhydrophryne rattrayi</i>	29	18.46 (2.46)	7.49 (1.01)	7.8 (0.93)	7.8 (0.96)	4.11 (0.49)	8.24 (0.96)
<i>Arthroleptella bicolor</i>	19	14.75 (2.13)	5.22 (0.58)	6.01 (0.74)	6.04 (0.77)	3.36 (0.69)	6.33 (0.9)
<i>Arthroleptella drewesii</i>	3	13.77 (1.87)	5.16 (0.8)	6.33 (0.86)	6.6 (1.1)	3.85 (0.13)	7.02 (1.66)
<i>Arthroleptella landdrosia</i>	5	12.24 (0.5)	4.62 (0.27)	5.36 (0.77)	5.63 (0.47)	3.34 (0.42)	5.82 (0.5)
<i>Arthroleptella lightfooti</i>	15	14.62 (2.6)	4.87 (0.55)	5.5 (0.43)	5.65 (0.77)	3.58 (0.54)	6.47 (0.87)
<i>Arthroleptella rugosa</i>	4	11.6 (0.88)	4.4 (0.28)	4.89 (0.29)	5.42 (0.13)	3.18 (0.15)	5.2 (0.48)
<i>Arthroleptella subvoce</i>	5	12.57 (0.96)	4.52 (0.46)	5.37 (0.8)	5.39 (0.67)	3.21 (0.29)	5.69 (0.42)
<i>Arthroleptella villiersi</i>	21	13.51 (1.42)	4.77 (0.6)	5.55 (0.52)	5.63 (0.65)	3.54 (0.54)	6.13 (0.44)
<i>Aubria masako</i>	3	74.33 (9.16)	26.55 (2.94)	29.96 (5.35)	28.76 (2.79)	12.82 (0.59)	33.01 (3.59)
<i>Aubria subsigillata</i>	6	80.34 (9.47)	28.74 (2.31)	30.35 (2.71)	30.97 (3.05)	16.69 (1.79)	36.54 (2.12)
<i>Cacosternum aggestum</i>	5	21.82 (0.79)	7.81 (0.31)	7.51 (0.4)	8.2 (0.22)	4 (0.35)	9.74 (0.35)
<i>Cacosternum australis</i>	12	17.45 (1.29)	6.19 (0.43)	6.33 (0.52)	6.74 (0.65)	3.97 (0.47)	8.52 (0.94)
<i>Cacosternum boettgeri</i>	26	18.09 (1.74)	6.27 (0.54)	6.6 (0.64)	7.26 (0.76)	4.27 (0.78)	8.64 (0.84)
<i>Cacosternum capense</i>	42	29.76 (4.2)	11.66 (1.51)	10.46 (1.35)	10.63 (1.29)	5.63 (0.69)	11.78 (1.38)
<i>Cacosternum karrooicum</i>	7	26.86 (2.54)	9.11 (1.25)	10.1 (0.44)	10.8 (0.56)	5.8 (0.43)	12.7 (0.82)
<i>Cacosternum namaquense</i>	15	25 (2.38)	8.84 (1.22)	8.56 (0.96)	9.18 (0.79)	5.56 (0.69)	10.86 (0.8)
<i>Cacosternum nanum</i>	20	19.86 (1.98)	6.9 (0.55)	7.67 (0.93)	8.32 (0.81)	4.57 (1.01)	9.77 (0.89)
<i>Cacosternum parvum</i>	2	17.32 (4.91)	5.39 (0.99)	6.66 (0.88)	7.45 (0.21)	3.9 (0.28)	8.56 (0.51)
<i>Cacosternum platys</i>	5	18.9 (1.54)	6.51 (0.48)	6.84 (0.64)	7.69 (0.4)	4.78 (0.14)	9.84 (0.37)
<i>Cacosternum striatum</i>	8	16.53 (1.2)	5.2 (0.46)	5.53 (0.53)	6.45 (0.76)	3.91 (0.7)	8.75 (1.18)
<i>Cacosternum thorini</i>	11	15.1 (1.3)	5.27 (0.31)	5.42 (0.33)	5.93 (0.33)	3.36 (0.35)	7.42 (0.69)
<i>Microbatrachella capensis</i>	39	14.79 (1.35)	5.13 (0.47)	5.56 (0.46)	6 (0.83)	3.59 (0.46)	7.78 (0.88)
<i>Natalobatrachus bonebergi</i>	24	28.53 (2.75)	9.51 (0.85)	14.33 (1.17)	16.51 (1.42)	8.03 (1.09)	15.8 (1.26)
<i>Nothophryne broadleyi</i>	8	17.24 (1.29)	7.35 (0.42)	7.65 (0.98)	8.25 (0.49)	4.63 (0.55)	8.05 (0.61)
<i>Poyntonia paludicola</i>	27	24.29 (3.59)	8.95 (1.06)	9.32 (1.29)	9.27 (1.26)	5.48 (0.74)	11.01 (1.52)
<i>Pyxicephalus adspersus</i>	24	149.3 (20.35)	68.87 (11.04)	61.65 (10.7)	54.75 (9.12)	28.32 (4.94)	53.78 (8.14)
<i>Pyxicephalus edulis</i>	14	100.92 (19.15)	43.84 (10.55)	38.43 (9.5)	35.34 (6.95)	19.85 (4.63)	36.92 (6.51)
<i>Strongylopus bonaespei</i>	27	34.79 (5.03)	11.07 (1.49)	18.61 (2.51)	22.96 (3.7)	11.12 (1.6)	25.61 (4.29)
<i>Strongylopus fasciatus</i>	20	37.56 (4.1)	12.35 (1.68)	21.36 (2.52)	24.77 (3.35)	11.16 (2.52)	27.6 (3.3)

<i>Strongylopus fuelleborni</i>	6	43.45 (5.47)	14.61 (2.05)	22.7 (3.82)	27.04 (5.12)	12.79 (2.05)	30.2 (5.62)
<i>Strongylopus grayii</i>	22	34.3 (6.02)	12.32 (2.51)	17.4 (3.6)	20.02 (3.99)	9.27 (1.74)	20 (4.23)
<i>Strongylopus kitumbeine</i>	2	44.22 (7.16)	16.16 (2.74)	22.9 (6.2)	26.6 (7.06)	11.57 (2.26)	29.21 (6.07)
<i>Strongylopus rhodesianus</i>	1	31.13 (NA)	11.22 (NA)	18.74 (NA)	21.21 (NA)	10.05 (NA)	23.46 (NA)
<i>Strongylopus springbokensis</i>	7	42.28 (10.01)	16.61 (4.09)	20.96 (4.95)	22.54 (5.6)	11.01 (2.91)	22.74 (5.31)
<i>Tomopterna cryptotis</i>	23	39.87 (4.63)	17.25 (2.18)	17.85 (2.48)	16.99 (2.01)	8.58 (1.83)	18.62 (3.03)
<i>Tomopterna delalandii</i>	22	40.25 (3.01)	17.53 (1.41)	17.16 (1.26)	15.98 (1.47)	7.84 (1.5)	19.52 (1.56)
<i>Tomopterna kachowskii</i>	1	47.27 (NA)	20.23 (NA)	21.28 (NA)	20.81 (NA)	11.07 (NA)	22.91 (NA)
<i>Tomopterna krugerensis</i>	12	42.78 (5.35)	17.58 (2.12)	18.18 (2.89)	17.18 (2.14)	8.84 (1.75)	17.68 (2.49)
<i>Tomopterna marmorata</i>	9	41.82 (6.57)	16.95 (1.7)	17.84 (2.83)	16.7 (2.06)	7.59 (1.2)	17.11 (2.4)
<i>Tomopterna natalensis</i>	23	29.69 (2.24)	12.29 (0.93)	14.27 (1.44)	14.76 (1.46)	6.8 (1.11)	15.44 (1.72)
<i>Tomopterna tandyi</i>	2	35.35 (4.67)	14.77 (1.68)	14.95 (2.3)	14.16 (1.99)	7.68 (0.68)	16.26 (2.57)
<i>Tomopterna tuberculosa</i>	10	32.28 (3.06)	13.64 (1.58)	14.28 (1.67)	13.72 (1.02)	6.47 (1.19)	13.92 (1.02)

B.4. The clutch size for all pyxicephalid species that had both morphological data (A.3) and distribution data, showing mean values and SD in brackets. N represents the number of specimens measured and species marked under the column 'genus average' were given a clutch size estimated from the average of other members of their genus.

Species	n	Clutch size	genus average
<i>Amietia angolensis</i>	7	1356 (575)	0
<i>Amietia delalandii</i>	8	1912 (1020)	0
<i>Amietia fuscigula</i>	4	2048 (590)	0
<i>Amietia hymenopus</i>	1	600 (na)	0
<i>Amietia inyangae</i>	0	2194 (na)	1
<i>Amietia johnstoni</i>	1	684 (na)	0
<i>Amietia nutti</i>	0	2194 (na)	1
<i>Amietia poyntoni</i>	0	2194 (na)	1
<i>Amietia sp.nov.</i>	0	2194 (na)	1
<i>Amietia vandijki</i>	0	2194 (na)	1
<i>Amietia vertebralis</i>	6	6561 (5505)	0
<i>Anhydrophryne hewitti</i>	4	29 (16)	0
<i>Anhydrophryne ratrayi</i>	18	66 (76)	0
<i>Arthroleptella bicolor</i>	4	14 (4)	0
<i>Arthroleptella drewesii</i>	0	14 (na)	1
<i>Arthroleptella landdrosia</i>	0	14 (na)	1
<i>Arthroleptella lightfooti</i>	3	23 (10)	0
<i>Arthroleptella rugosa</i>	0	14 (na)	1
<i>Arthroleptella subvoce</i>	0	14 (na)	1
<i>Arthroleptella villiersi</i>	1	6 (na)	0
<i>Aubria masako</i>	0	4785 (na)	1

<i>Aubria subsigillata</i>	2	4785 (3051)	0
<i>Cacosternum aggestum</i>	0	262 (na)	1
<i>Cacosternum australis</i>	0	262 (na)	1
<i>Cacosternum boettgeri</i>	14	288 (312)	0
<i>Cacosternum capense</i>	6	225 (81)	0
<i>Cacosternum karrooicum</i>	0	262 (na)	1
<i>Cacosternum namaquense</i>	8	222 (69)	0
<i>Cacosternum nanum</i>	6	530 (710)	0
<i>Cacosternum parvum</i>	0	262 (na)	1
<i>Cacosternum platys</i>	0	262 (na)	1
<i>Cacosternum rhythmum</i>	0	262 (na)	1
<i>Cacosternum striatum</i>	6	233 (141)	0
<i>Cacosternum thorini</i>	2	72 (24)	0
<i>Microbatrachella capensis</i>	5	74 (16)	0
<i>Natalobatrachus bonebergi</i>	9	94 (40)	0
<i>Nothophryne broadleyi</i>	1	304 (na)	0
<i>Poyntonia paludicola</i>	10	119 (154)	0
<i>Pyxicephalus adspersus</i>	0	3346 (na)	1
<i>Pyxicephalus edulis</i>	4	3346 (772)	0
<i>Strongylopus bonaespei</i>	3	177 (110)	0
<i>Strongylopus fasciatus</i>	6	637 (200)	0
<i>Strongylopus fuelleborni</i>	1	224 (na)	0
<i>Strongylopus grayii</i>	6	554 (259)	0
<i>Strongylopus kitumbeine</i>	0	449 (na)	1
<i>Strongylopus rhodesianus</i>	0	449 (na)	1
<i>Strongylopus springbokensis</i>	2	656 (608)	0
<i>Tomopterna cryptotis</i>	7	2849 (1890)	0
<i>Tomopterna delalandii</i>	5	1360 (654)	0
<i>Tomopterna kachowskii</i>	1	4210 (na)	0
<i>Tomopterna krugerensis</i>	2	2156 (1058)	0
<i>Tomopterna marmorata</i>	4	2194 (924)	0
<i>Tomopterna natalensis</i>	5	1297 (758)	0
<i>Tomopterna tandyi</i>	0	2344 (na)	1
<i>Tomopterna tuberculosa</i>	0	2344 (na)	1

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B.5. Species distribution and Outlier Mean Index (OMI) outputs for all pyxicephalid species that had both morphological data (A.3) and distribution data. Tol represents the niche breadth of species with regards to the climatic and topographical input predictors, see Dolédec et al. (2000) for more parameter definitions. CA represents the Colonisation Index, calculated as the proportion of suitable habitat in a zone surrounding the distribution range of a species. The distribution range was calculated from a minimum convex polygon (mcp) as shown in the table. Note that only South African pyxicephalids were included for the OMI analysis.

Species	inertia	OMI	Tol	Rtol	omi	tol	rtol	CA	mcp (km <sup>2</sup> )
<i>Amietia delalandii</i>	17.67	6.05	2.71	8.92	34.2	15.3	50.5	0.49	2.1E+06
<i>Amietia fuscigula</i>	53.09	32.73	6.39	13.96	61.7	12	26.3	0.44	2.7E+05
<i>Amietia hymenopus</i>	82.62	81.57	0.41	0.64	98.7	0.5	0.8	0.12	4.0E+03
<i>Amietia poyntoni</i>	12.15	3.34	1.9	6.91	27.5	15.7	56.8	0.45	1.3E+06
<i>Amietia vandijki</i>	41.83	36.15	1.41	4.27	86.4	3.4	10.2	0.20	5.8E+03
<i>Amietia vertebralis</i>	57.49	52.82	2.85	1.82	91.9	5	3.2	0.35	2.5E+04
<i>Anhydrophryne hewitti</i>	23.3	14.04	1.47	7.8	60.2	6.3	33.5	0.35	4.9E+04
<i>Anhydrophryne ratrayi</i>	29.15	26.76	0.62	1.77	91.8	2.1	6.1	0.24	3.8E+02
<i>Arthroleptella bicolor</i>	116.42	114.58	0.27	1.57	98.4	0.2	1.3	0.08	6.8E+01
<i>Arthroleptella drewesii</i>	72.12	70.48	0.6	1.04	97.7	0.8	1.4	0.04	3.4E+01
<i>Arthroleptella landdrosia</i>	126.8	123.25	0.35	3.2	97.2	0.3	2.5	0.08	2.9E+02
<i>Arthroleptella lightfooti</i>	111.73	105.61	2.49	3.63	94.5	2.2	3.3	0.01	3.0E+02
<i>Arthroleptella rugosa</i>	64.65	63.27	0.22	1.15	97.9	0.3	1.8	0.09	2.9E+00
<i>Arthroleptella subvoce</i>	68.74	67.22	0.36	1.16	97.8	0.5	1.7	0.17	4.0E+00
<i>Arthroleptella villiersi</i>	94.43	86.02	3.65	4.76	91.1	3.9	5	0.17	5.1E+03
<i>Cacosternum aggestum</i>	44.24	39.38	1.86	3	89	4.2	6.8	0.25	8.3E+03
<i>Cacosternum australis</i>	37.8	32.06	0.78	4.96	84.8	2.1	13.1	0.27	1.6E+04
<i>Cacosternum boettgeri</i>	11.72	1.67	1.95	8.1	14.2	16.6	69.1	0.44	2.3E+06
<i>Cacosternum capense</i>	40.84	35.25	2.07	3.52	86.3	5.1	8.6	0.31	1.5E+04
<i>Cacosternum karoicum</i>	19.35	10.07	2.53	6.75	52	13.1	34.9	0.26	5.2E+04
<i>Cacosternum namaquense</i>	17.16	13.54	0.22	3.4	78.9	1.3	19.8	0.45	5.5E+04
<i>Cacosternum nanum</i>	26.07	13.99	3.92	8.16	53.7	15	31.3	0.42	5.2E+05
<i>Cacosternum parvum</i>	27.11	19.24	1.28	6.59	71	4.7	24.3	0.46	1.4E+05
<i>Cacosternum platys</i>	77.45	74.19	0.75	2.51	95.8	1	3.2	0.06	2.8E+02
<i>Cacosternum rhythmum</i>	15.93	10.06	0.91	4.97	63.1	5.7	31.2	0.41	4.1E+04
<i>Cacosternum striatum</i>	21.62	11.07	1.31	9.24	51.2	6.1	42.7	0.39	3.3E+04
<i>Cacosternum thorini</i>	37.69	37.05	0.04	0.6	98.3	0.1	1.6	0.12	9.6E+01
<i>Microbatrachella capensis</i>	53.01	48.9	0.94	3.17	92.2	1.8	6	0.05	1.4E+03
<i>Natalobatrachus bonebergi</i>	25.66	23.09	1.08	1.5	90	4.2	5.8	0.26	2.9E+04
<i>Poyntonia paludicola</i>	96.28	91.12	1.98	3.18	94.6	2.1	3.3	0.16	1.6E+03
<i>Pyxicephalus adspersus</i>	8.36	0.77	0.88	6.7	9.2	10.6	80.2	0.44	5.5E+06
<i>Pyxicephalus edulis</i>	17.76	9.92	2.73	5.1	55.9	15.4	28.7	0.26	3.5E+06
<i>Strongylopus bonaespei</i>	74.09	58.62	4.49	10.97	79.1	6.1	14.8	0.34	7.4E+04
<i>Strongylopus fasciatus</i>	28.16	12.23	4.2	11.74	43.4	14.9	41.7	0.44	7.7E+05
<i>Strongylopus grayii</i>	41.84	21.27	6.17	14.4	50.8	14.7	34.4	0.43	1.0E+06
<i>Strongylopus springbokensis</i>	19.62	14.32	1.25	4.06	72.9	6.3	20.7	0.37	2.5E+04
<i>Tomopterna cryptotis</i>	8.03	0.82	0.36	6.84	10.3	4.5	85.2	0.39	1.7E+07
<i>Tomopterna delalandii</i>	35.37	20.99	4.59	9.79	59.3	13	27.7	0.45	2.9E+05
<i>Tomopterna krugerensis</i>	16.69	10.79	3.18	2.72	64.7	19	16.3	0.41	1.1E+06

<i>Tomopterna marmorata</i>	10.56	7.57	0.51	2.48	71.7	4.8	23.5	0.48	1.3E+06
<i>Tomopterna natalensis</i>	13.88	5.83	1.61	6.44	42	11.6	46.4	0.45	5.6E+05
<i>Tomopterna tandyi</i>	10.33	2.59	1.26	6.48	25	12.2	62.7	0.23	7.7E+06
<i>Amietia angolensis</i>								0.17	3.8E+05
<i>Amietia johnstoni</i>								0.01	1.1E+02
<i>Amietia nutti</i>								0.41	1.3E+06
<i>Aubria masako</i>								0.52	5.4E+05
<i>Aubria subsigillata</i>								0.14	9.1E+05
<i>Nothophryne broadleyi</i>								0.47	2.7E+04
<i>Strongylopus fuelleborni</i>								0.57	2.7E+05
<i>Strongylopus rhodesianus</i>								0.45	9.1E+03
<i>Tomopterna kachowskii</i>								0.45	4.0E+04
<i>Tomopterna tuberculosa</i>								0.40	1.7E+06

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