Morphology, locomotor performance and habitat use in southern African agamids

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Understanding the relationships between form and function can help us to understand the evolution of phenotypic diversity in different ecological contexts. Locomotor traits are ecologically relevant as they reflect the ability of an organism to escape from predators, to catch prey or to defend territories. As such, locomotion provides a good model to investigate how environmental constraints may influence an organism's performance. Here, we investigate the ecomorphological relationships between limb morphology, locomotor performance (sprint speed and endurance) and habitat use in six southern African agamid species. The investigated agamid species showed differences in hind limb and toe lengths. Both of these traits were further correlated with endurance capacity. This association was supported by stepwise multiple regression analyses. However, we demonstrate trade-offs in locomotor performance traits, suggesting that specialization towards speed comes at the detriment of endurance capacity. Overall, the single arboreal species studied had longer hind limbs, a higher exertion capacity and a higher mean speed. However, for a given hind limb length, the arboreal species was slower than the other habitat specialists. This study provides insights into the evolutionary mechanisms that have driven the morphological and functional evolution in southern African agamid lizards.

ADDITIONAL KEYWORDS: *Agama* – habitat – lizards – locomotion – morphometrics – performance.

INTRODUCTION

Natural selection can drive evolutionary changes in animal phenotypes (Darwin, 1859). Whole-organism traits such as performance and behaviour are particularly relevant in this context as they are the direct target of selection (Huey *et al.*, 2003). Selection often acts on multiple traits (Arnold, 1983) to maximize the ability of an organism to perform ecologically and socially relevant functions (Irschick *et al.*, 2008). To understand the potential evolutionary significance of morphological variation, it is essential to evaluate how variation in morphology translates into variation in ecologically relevant performance traits (Arnold, 1983). Only by understanding the complex relationship between phenotypic traits and performance can we understand the effect of variation in morphology on fitness among individuals in a given environment (Arnold, 1983). Therefore, it is essential to examine whether differences in morphological traits among species occupying different habitats translate into differences in performance capabilities in order to shed light on the underlying evolutionary drivers (Vanhooydonck & Van Damme, 2003).

One of the most common performance traits examined in this context is locomotion. Locomotor performance, for example running, jumping, climbing and swimming, can improve survival (e.g. foraging or hunting, escape from predation) and reproductive success (e.g. finding mates, and defending territories) in many animals. This is particularly well documented in lizards (Sinervo & Losos, 1991; Garland & Losos, 1994;

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Vanhooydonck & Van Damme, 2003; Edwards *et al.*, 2016). Evolution towards higher sprint speed in many taxa has gone hand in hand with the evolution of body size (Losos, 1990). Therefore, larger individuals should be sprinting faster due to allometric relationships resulting in longer limbs. Irrespective of variation in size, longer legged individuals should also show higher sprint speeds (Sinervo & Losos, 1991; Vanhooydonck & Van Damme, 2001). Yet, improving performance in one task may result in trade-offs with other traits. For example, fast sprinters typically have longer hind limbs relative to their forelimbs (Vanhooydonck & Van Damme, 2001). However, arboreal species typically have fore- and hind limbs of equal length as the forelimbs take up an important role in providing propulsion during climbing (Vanhooydonck *et al.*, 2000). As such, a trade-off between climbing and running may be present. Another locomotor trade-off that has been documented in several taxa is between sprint speed and endurance capacity. For example, a negative correlation between sprinting and sustained locomotion has been demonstrated in lacertid lizards due to the involvement of different structural features (muscle fibre types) involved in the two locomotor traits (Vanhooydonck *et al.*, 2001, 2014).

Despite the many studies focusing on locomotion in lizards, relatively little is known about locomotion in agamids. Yet, many agamids appear specialized for high-speed locomotion and some even use bipedal locomotion to escape from predators (e.g. *Ctenophorus cristatus*, *Ctenophorus caudicinctus*, *Chlamydosaurus kingii* and occasionally *Stellagama stellio*) (Snyder, 1962; Clemente *et al.*, 2008). When disturbed, agamas often scurry at top speed, then stop abruptly, further relying on camouflage to escape detection (Branch, 1998). These quick bursts are not only necessary for predator escape but also for catching prey. African agamids (hereafter agamas) are of interest as they are a group of ecologically diverse species, making them ideal for conducting evolutionary and ecological studies (Leaché *et al.*, 2014). The group is thought to have diverged about 23 Mya and radiated into multiple clades about 10 Mya (Pyron *et al.*, 2013; Leaché *et al.*, 2014), suggesting relatively rapid speciation. The radiation of agamas throughout the African continent coincided with the period of massive contraction in rainforest cover, linked to the increase in aridity in the late Miocene (Kissling *et al.*, 2016). Furthermore, the highest diversification occurred in topographically rich areas, especially southern Africa.

Agamas are widespread and fairly common across southern Africa. Their radiation is represented by three monophyletic groups (Leaché *et al.*, 2014; although the taxonomic status of *Agama aculeata distanti* remains unclear). Interestingly, three different habitat specializations can be observed in these species: rupicolous or rock dwelling, psammophilous or sand dwelling, and arboreal or tree dwelling. *Agama atra*, *A. anchietae* and *A. aculeata distanti* are dependent on rocky outcrops whereas both *A. aculeata aculeata* and *A. armata* appear to be associated with sandy areas (sandveld and savannahs) (Branch, 1998; Bates *et al.*, 2014). *Acanthocercus atricollis* is the only agama species in southern Africa that does not belong to the genus *Agama*, and is the only arboreal agama in our study group. All of these agamas are diurnal and oviparous occurring over large areas of southern Africa. Although occupying different habitat types, *A. a. distanti* and *A. a. aculeata* are currently considered subspecies of *A. aculeata*. The structural features of the microhabitats occupied by these agamas are probably sufficiently different to impose different selective pressures on locomotor capacity. As such, variation in morphology and performance can be expected for species occupying different microhabitats.

Despite the differences in habitat use, these agamas look strikingly similar, so much so that even identifying species by morphology alone has met with poor success (Jacobsen, 1992). Yet, biomechanical considerations predict that conflicting body designs should be selected for in their respective environments. Here, we examined the limb morphology and two types of locomotor performance of six agamas from southern Africa with different habitat preferences to investigate the association between morphology and performance across species and determine the potential relevance of habitat use to such an association. We first investigated (1) whether these agamas are morphologically different? We asked what morphological traits best predicted variation in performance. Based on previous studies, individuals with longer hind limbs should be better runners because long hind limbs increase stride length (Garland & Losos, 1994). We also explored (2) whether functional trade-offs exist between different locomotor traits. We predicted that sprint speed should trade-off with endurance capacity as has been observed for other lizards (Vanhooydonck *et al.*, 2001, 2014). Finally, we (3) explored whether microhabitat use has been a selective factor in driving the evolution of morphology and/or performance capabilities. We predicted that climbers should have fore- and hind limbs of similar length (Sinervo & Losos, 1991) and a dorso-ventrally flattened body (Miles, 1994). By contrast, lizards living in open habitat should be good runners with relatively long hind limbs (Garland & Losos, 1994), short forelimbs (Losos, 1990) and a laterally compressed body (Snyder, 1962).

MATERIAL AND METHODS

Field sampling was conducted in South Africa. Lizards were caught by hand or by noose. A total of

155 individuals were included in the study. *Agama atra* samples (*N* = 41) were collected mainly from the Muizenberg mountains (34°05′S, 18°26′E) and the Grootwinterhoek reserve (33°09′S, 19°05′E) and other parts of the Western Cape in March 2008 and January 2011. Both *A. anchietae* (*N* = 11) and *A. aculeata aculeata* (*N* = 10) were sampled in Tswalu game reserve (27°17′S, 22°23′E), Northern Cape, in January 2010, with the exception of three *A. anchietae* from Gobabis (22°26′S, 18°57′E) and Swakopmund (22°15′S, 15°4′E), Namibia, and one *A. a. aculeata* from Zwartskraal farm (33°10′S, 22°34′E), Western Cape. *Agama aculeata distanti* (*N* = 39) were sampled in Welgevonden Reserve (24°12′S, 27°54′E) and Kruger National Park (23°58′S, 31°31′E), Limpopo province, in November 2011 and March 2017. *Agama armata* (*N* = 14) were collected at Alicedale Farms (22°38′S, 30°08′E) and Greater Kuduland Safaris (22°32′S, 30°40′E) in Limpopo in January 2010 and February 2017. Finally, *Acanthocercus atricollis* (*N* = 40) were caught in the suburban area of Mtunzini (28°57′S, 31°44′E) and Zululand Nurseries, Eshowe (28°52′S, 31°28′E), KwaZulu-Natal, in February 2017. All lizards caught were marked with a temporary non-toxic marker (to avoid recapturing and repeated measurements of the same individual) and returned to their exact site of capture after being measured and tested for performance. We did not preferentially sample an age or sex group. the data presented consist of adults and juveniles [we consider individuals with snout–vent length (SVL) < 100 mm for *Acanthocercus atricollis* and SVL < 70 mm for *Agama* species as juveniles] as we found them in the field. Male agamas were distinguished from females by the bulging of the hemipenes at the base of their tails. A lack of a particular sex or age group represents an absence of these individuals at the sampling sites.

MORPHOMETRICS

All individuals (see Supporting Information, Table S1 for sample sizes) were measured using digital callipers (Mitutoyo; precision 0.01 mm). The following morphological traits were measured following Edwards *et al.* (2012) (Fig. 1): SVL, body width (BW) and height (BH), tail length (TL), inter-limb length (ILL), femur length (FmL), tibia length (TbL), metatarsus length (MtL), length of longest hind toe (LtoeH), humerus length (HmL), radius length (RL), metacarpus length (McL) and length of longest front toe (LtoeF). All measurements were taken on the left side of the lizard. Hind limb length (HLL) was calculated by adding the values for FmL, TbL, MtL and LtoeH. The mass of each lizard was measured using a Pesola spring balance.

RUNNING PERFORMANCE

Sprint speed (see Supporting Information, Table S1 for sample sizes) was measured by allowing lizards to run along a flat 1.5-m-long race track with 25-cm markers (Vanhooydonck *et al.*, 2015). Each lizard was chased three times across the track. We recorded the runs using a digital camera (Canon PowerShot G16) at 240 frames per second. Sprint speed was computed as follows:

$$\text{Velocity } \left(\text{cms}^{-1}\right) \; = \frac{25cm}{\frac{1}{240} \times \; no. \; of \; frames}$$

Velocity was calculated each time the lizard completed a 'valid' run by crossing any 25-cm interval without stopping. The highest velocity over 25 cm for each lizard was considered as the maximal speed for that animal.

Endurance capacity was measured by chasing the lizard around a circular track (circumference of 4.1 m) until exhaustion, identified by a lack of a response from the lizard when being tapped ten times at the base of the tail. The righting response normally used in studies of lizards (Vanhooydonck *et al.*, 2015) was not used in this study as these agamas tend to right themselves even when exhausted. The time and number of laps were recorded. Three or four trials were performed and the longest time to exhaustion (stamina in seconds), the longest distance run (exertion in centimetres), and highest mean speed per lap (exertion/stamina) were taken as indicators of maximal endurance capacity.

All trials were performed at the ambient temperature during which the animals are active in the field. Sprint and endurance tests were conducted with a 1-h rest period between every trial to allow the lizards to rest, and sprint and endurance were tested on different days. Between trials animals were kept in cloth bags and put in half sun half shade allowing them to thermoregulate. *Agama a. aculeata* was excluded from the performance analyses as endurance data were not available for this subspecies.

STATISTICAL ANALYSES

Morphological and performance variation

All continuous data were $\log_{10}$-transformed before further analyses to fulfil conditions of normality and homoscedascity. Multivariate analyses of variance (MANOVAs) were used to test for differences among the six agamas. Potential sex-effects were not tested because our sample size was low for each sex per species. To explore how species differed in terms of morphology, we ran principal component analyses (PCAs) on the raw log-transformed data. A varimax rotation was used to reduce the number of variables with high loadings on each factor. As
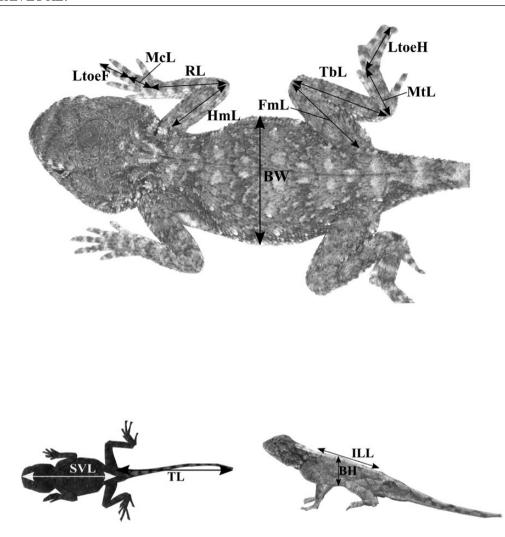
Figure 1. Limb and body dimensions recorded for each lizard. SVL, snout–vent length; BW, body width; BH, body height; TL, tail length; ILL, inter-limb length; FmL, femur length; TbL, tibia length; MtL, metatarsus length; LtoeH, length of longest hind toe; HmL, humerus length; RL, radius length; McL, metacarpus length; LtoeF, length of longest front toe.

nearly 90% of the variation in body measurements was explained by the first principal component (PC) being a measure of size, all morphological and performance variables were regressed against SVL, and the residuals were extracted. These residuals were then entered into a new PCA to assess the variation in body shape. We ran a separate PCA on the performance traits and saved the first two PCs. We next ran a Pearson correlation on the shape and performance PC axes to test for ecomorphological relationships.

Drivers of variation in performance and performance trade-offs

To explore the morphological variables that best explained the variation in performance, stepwise multiple regression analyses were performed. To test for

trade-offs between performance traits we ran Pearson correlations on the residual performance traits.

Impact of habitat use on morphology and performance

We grouped the species into three main microhabitat categories or habitat specialists: rupicolous (*A. atra*, *A. anchietae*, *A. a. distanti*), psammophilous (*A. armata*, *A. a. aculeata*) and arboreal (*Acanthocercus atricollis*) (Bates *et al.*, 2014). However, note that habitat categories may only apply to these particular populations sampled. We next performed an ANOVA to test for differences in SVL among these habitat specialists. We also performed paired *t*-tests to investigate whether there are differences between sexes in limb morphology and performance. Next, we used MANOVA and ANOVA comparisons on each

morphological and performance trait, with habitat specialization as factor. Subsequently, we performed post-hoc tests [Tukey's honest significant difference (HSD)] to test for differences between pairs of habitat specialists. If the habitat specialists were significantly different in SVL, multivariate analysis of covariance (MANCOVA) and ANCOVA comparisons with SVL as covariate were performed on the same morphological parameters. Lastly, to test for differences in performance, we ran MANOVAs on all locomotor performance traits (maximal sprint, stamina, exertion and mean speed). Subsequent MANCOVAs with hind limb length as covariate were also performed. Hind limb length was used here as it has been suggested to determine locomotor performance in other lizard species (Sinervo & Losos, 1991; Vanhooydonck & Van Damme, 2001). All analyses were performed in IBM-SPSS v24 (SPSS Inc., Chicago, IL, USA).

RESULTS

MORPHOLOGICAL AND PERFORMANCE VARIATION

Species differed significantly in multivariate morphological space (MANOVA: Wilk's $\lambda = 0.011$, $F_{75,650.85} = 13.51$, $P < 0.01$). A PCA on the 14 size-adjusted morphological variables retained three PCs. Together, they explained 62% of the total shape variation. The first axis, which explained 25.4% of the variance, was positively correlated with residual metatarsus, humerus and metacarpus length and body width (Table 1; Fig. 2). Residual hind limb length, residual femur and tibia, and residual radius loaded strongly on the second PC, which explained a further 22.2% of the variation. Residual front and hind toe lengths, on the other hand, were strongly correlated with the third PC (explaining 14.4% of the total variance).

A PCA on the residual performance traits yielded two PCs jointly accounting for 79% of the total variance (PC1: 45%; PC2: 34%). Residual stamina and exertion loaded strongly on the first PC whereas residual maximal sprint speed and mean speed were strongly correlated to the second PC (Table 1). Pearson correlations indicated significant associations between the first performance PC and the second ($r = 0.339$; $P < 0.01$) and third ($r = 0.238$; $P = 0.011$) morphological PCs (see Fig. 3).

DRIVERS OF VARIATION IN PERFORMANCE

Inter-limb length ($\beta = 0.73$) and body height ($\beta = -0.30$) were significant predictors of maximal sprint speed ($R^2 = 0.23$; $P < 0.01$) (Supporting Information,

Table 1. Loadings on the first principal components for agama species using size-free (residual) data

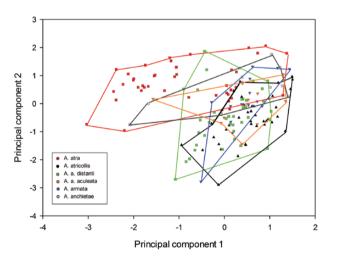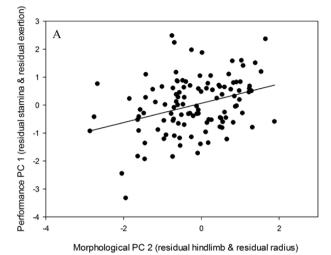|	PC1	PC2	PC3
Sprint speed	−0.084	0.69	
Morphological variable			
Tail length	0.063	0.134	0.442
Femur	−0.138	**0.852**	−0.028
Tibia	−0.266	**0.862**	−0.161
Metatarsus	**0.861**	−0.061	0.134
Longest hind toe	−0.119	0.046	**0.792**
Hindlimb	0.087	**0.891**	0.366
Humerus	**0.810**	0.116	0.084
Radius	0.282	**0.710**	0.163
Metacarpus	**0.741**	0.083	0.125
Longest front toe	−0.003	0.005	**0.855**
Body width	**0.823**	−0.189	−0.300
Body height	0.531	−0.224	0.040
Interlimb length	−0.367	0.325	−0.519
Mass	0.455	0.308	−0.076
Eigenvalue	3.56	3.11	2.02
% Variance	25.44	22.19	14.44
Performance variable			
Stamina	**0.83**	−0.47	
Exertion	**0.95**	0.211	
Average speed	0.042	**0.90**	
Eigenvalue	1.79	1.37	
% Variance	44.73	34.20	

Figure 2. The positions of the six agama species in the morphological space described by the first two principal components using size-adjusted data. Although there appears to be a considerable overlap between the all agama species at higher PC1 values, these overlaps are associated with the juvenile individuals in our dataset. Symbols refer to habitat use: ▲, arboreal; ■, rupicolous; ▼, psammophilous.
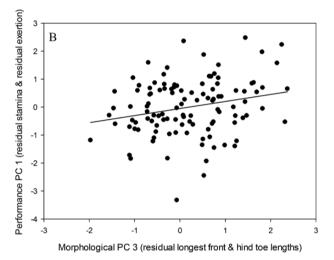
Figure 3. Correlations between morphology and performance in the southern African agamas. A, a clear positive correlation is apparent between hind limb (+radius) and stamina + exertion across all species in the dataset. B, similarly, a positive correlation is noticeable between toe lengths (front and hind) and stamina + exertion across all species.

Table S2). Similarly, a significant model ($R^2 = 0.17$; $P < 0.01$) with femur ($\beta = 0.65$) and body width ($\beta = -0.38$) as predictors was retained for stamina. Hind limb length ($\beta = 1.11$) and body height ($\beta = -0.25$) were significant predictors of exertion capacity ($R^2 = 0.78$; $P < 0.01$). For mean speed we obtained significant models ($R^2 = 0.72$; $P < 0.01$) with mass ($\beta = 0.53$) and metatarsal length ($\beta = 0.34$) as predictors.

TRADE-OFFS

Pearson correlations showed a significant positive correlation between residual stamina and residual exertion capacity ($r = 0.63$, $P < 0.01$, Fig. 4A). However, residual stamina was negatively correlated with residual maximal sprint speed ($r = -0.23$, $P = 0.01$, Fig. 4B) and residual mean speed ($r = -0.45$, $P < 0.01$, Fig. 4C). Exertion capacity was, however, positively correlated with mean speed ($r = 0.25$, $P < 0.01$). There was also a significant positive correlation between maximal sprint speed and mean speed ($r = 0.30$, $P < 0.01$, Fig. 4D).

IMPACT OF HABITAT USE ON MORPHOLOGY AND PERFORMANCE

There was a significant difference in SVL between habitat specialists ($F_{2,154} = 0.88$, $P < 0.01$). Subsequent post-hoc tests confirmed that the arboreal species were significantly larger than other groups. Paired *t*-tests also indicated some significant differences between sexes (Supporting Information, Table S3).

Significant differences between habitat specialists were detected for limb and body measurements (MANOVA, Wilks' $\lambda = 0.10$, $F_{28,280} = 21.89$, $P < 0.01$). Subsequent univariate ANOVAs also detected significant differences in all limb and body measurements (all $P < 0.01$, Supporting Information, Table S4). Post-hoc tests revealed that the arboreal species had longer limbs and trunks than psammophilous and rupicolous species, while psammophilous species had a shorter femur and longer front toe than rupicolous species (Table 2). A MANCOVA, with SVL as covariate, also showed significant differences in limb and body dimensions between habitat specialists (Wilks' $\lambda = 0.12$, $F_{28,278} = 19.14$, $P < 0.01$). Univariate ANCOVAs further demonstrated significant differences in most of the measurements (Table S5). Pairwise comparisons indicated that rupicolous species had a relatively longer femur but relatively shorter metatarsal and metacarpal compared to other habitat specialists. The arboreal species, on the other hand, had relatively shorter tibiae and a relatively shorter inter-limb length, but a relatively longer digit length (followed by rupicolous species) and relatively longer hind limbs and taller bodies than the other groups.

A MANOVA testing for difference in running performance between arboreal, rupicolous and psammophilous species was significant (Wilks' $\lambda = 0.53$, $F_{8,220} = 10.37$, $P < 0.01$). Univariate ANOVAs revealed that only exertion capacity and mean speed differed between habitat specialists (Supporting Information, Table S6). Post-hoc tests revealed that the arboreal species had a higher exertion capacity and mean speed than the other groups (Table 2). Sex did not have a significant effect on locomotor performance except for stamina and mean speed in the arboreal species (Table S3). Arboreal males sprinted faster and for longer than

Table 2. Morphology and performance traits for the three habitat specialists

	Psammophilous			Rupicolous			Arboreal		
	Males	Females	Juveniles	Males	Females	Juveniles	Males	Females	Juveniles
Morphology									
N	8	7	9	37	26	28	9	20	11
SVL (mm)	86.63 ± 11.53	85.90 ± 9.56	32.02 ± 4.20	85.6 ± 12.01	78.77 ± 7.23	40.00 ± 9.52	126.67 ± 7.93	112.88 ± 11.3	58.91 ± 23.64
Tail (mm)	114.37 ± 26.09	108.32 ± 21.46	35.65 ± 7.52	103.71 ± 25.22	90.96 ± 16.77	47.42 ± 14.99	170.64 ± 10.70	143.01 ± 29.04	81.00 ± 32.92
Femur (mm)	19.68 ± 2.88	19.07 ± 1.65	7.99 ± 1.56	21.76 ± 3.60	20.15 ± 2.42	9.85 ± 3.25	27.33 ± 1.74	24.53 ± 2.55	13.8 ± 4.96
Tibia (mm)	20.23 ± 2.15	19.96 ± 1.55	7.57 ± 1.62	20.77 ± 3.23	19.22 ± 1.89	9.48 ± 3.17	26.57 ± 1.51	23.32 ± 1.83	12.52 ± 5.21
Metatarsus (mm)	10.33 ± 1.80	9.53 ± 1.47	4.11 ± 0.60	8.01 ± 1.71	7.38 ± 1.58	5.01 ± 1.52	15.18 ± 1.48	12.41 ± 1.69	7.11 ± 2.58
Longest hind toe (mm)	9.66 ± 1.52	9.68 ± 1.97	4.43 ± 0.60	9.69 ± 2.15	9.26 ± 1.32	5.36 ± 1.50	16.74 ± 1.81	14.83 ± 1.45	9.88 ± 3.18
Hind limb length (mm)	59.90 ± 7.21	58.24 ± 4.74	24.1 ± 4.20	60.24 ± 8.18	56.01 ± 4.96	29.70 ± 9.30	85.83 ± 4.01	75.10 ± 5.86	43.30 ± 15.70
Humerus (mm)	17.89 ± 1.60	17.22 ± 0.85	8.01 ± 1.46	16.50 ± 2.97	15.62 ± 2.43	9.36 ± 2.36	26.06 ± 1.89	22.76 ± 2.08	12.94 ± 4.64
Radius (mm)	16.02 ± 1.98	15.46 ± 0.71	5.57 ± 1.27	15.11 ± 2.22	14.16 ± 1.22	7.08 ± 2.31	22.66 ± 1.13	19.88 ± 1.61	10.45 ± 4.00
Metacarpus (mm)	5.80 ± 0.74	5.64 ± 0.56	2.76 ± 0.52	5.41 ± 2.29	4.74 ± 1.03	2.98 ± 0.93	8.72 ± 0.78	7.33 ± 0.73	4.67 ± 1.71
Longest front toe (mm)	6.85 ± 0.99	6.35 ± 0.91	3.09 ± 0.52	7.28 ± 1.14	7.13 ± 1.04	3.86 ± 1.08	13.73 ± 1.22	11.84 ± 1.12	7.46 ± 2.38
Body width (mm)	27.03 ± 7.21	23.89 ± 7.48	10.26 ± 2.00	20.4 ± 6.04	19.41 ± 6.17	12.47 ± 3.32	33.28 ± 4.34	29.29 ± 2.59	14.65 ± 6.20
Body height (mm)	16.00 ± 2.55	11.99 ± 3.75	5.32 ± 1.53	13.02 ± 3.20	12.65 ± 3.21	6.91 ± 1.81	24.83 ± 3.34	21.08 ± 3.09	9.39 ± 5.22
Interlimb length (mm)	49.21 ± 8.46	47.47 ± 5.63	16.00 ± 2.57	47.75 ± 8.11	44.23 ± 4.11	21.95 ± 7.06	64.32 ± 3.98	56.95 ± 5.67	29.31 ± 12.31
Mass (g)	28.26 ± 12.63	22.95 ± 8.36	1.60 ± 0.70	22.66 ± 7.5	18.33 ± 5.59	3.12 ± 2.88	82.11 ± 16.10	50.00 ± 9.94	10.86 ± 12.34
Performance									
N	3	6	8	23	20	23	9	20	10
Sprint speed (cm/s)	222.73 ± 58.17, N = 8	252.97 ± 48.35	141.53 ± 48.47	206.4 ± 64.07, N = 37	190.70 ± 52.14, N = 26	157.21 ± 44.51, N = 24	207.53 ± 32.46	178.55 ± 33.3	126.56 ± 37.98
Stamina (s)	43.26 ± 8.83	–	46.18 ± 8.51	55.28 ± 24.99	55.71 ± 17.54	38.42 ± 13.29	51.84 ± 10.28	54.2 ± 13.52	58.56 ± 21.63
Exertion (cm)	1287.4 ± 405.11	–	563.75 ± 121.59	1571.35 ± 589.98	1492.44 ± 471.60	511.04 ± 226.41	2391.53 ± 576.19	2030.36 ± 464.28	1349.31 ± 541.06
Average speed (cm/s)	31.43 ± 5.68	–	14.57 ± 1.55	31.85 ± 10.14	29.84 ± 9.17	16.92 ± 4.72	51.75 ± 10.27	42.01 ± 5.99	26.89 ± 10.54

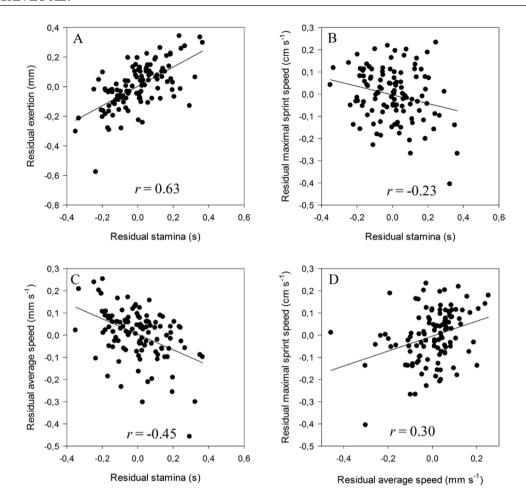Table entries are means ± SD; N = sample size.

Figure 4. Correlation between different performance traits for the southern African agamas. Residual performance traits are plotted, and the correlation coefficient (*r*) is shown.

females. Differences in running performance remained after correcting for hind limb length (MANCOVA, Wilks' $\lambda = 0.68$, $F_{8,216} = 5.87$, $P < 0.01$). Univariate ANCOVAs showed that only maximal sprint speed was different ($F_{2,111} = 10.47$, $P < 0.01$) with the arboreal species sprinting slower than the rupicolous and psammophilous species in a pairwise comparison test.

DISCUSSION

We have investigated the relationship between morphology and locomotor performance in agamas from different microhabitats (psammophilous, rupicolous and arboreal species) to understand whether the different habitats exert different selective pressures on morphology and performance. Our results revealed ecomorphological variation, trade-offs in locomotor performance and potential traits which may have been involved in promoting segregation between habitat specialists across different ecological niches.

Despite the striking similarity in appearance among these agamas, there are interspecific morphological differences among them. It has been shown that differences in body size are a strong structuring agent between agama species (Leaché *et al.*, 2014). Our analyses further show that these agamas are also distinguishable to some degree in body shape (Fig. 2). Our results provide the first quantitative analysis of this, and provide evidence for a significant differentiation between southern African agamas in terms of limb shape. When examining size-independent variables, agama species can be separated by the lengths of their fore- and hind limbs. As expected, limb size is largely responsible for differences among species in their performance capability at ecologically relevant tasks (Losos, 1990). However, it appears that these agamas have not reached the degree of morphological differentiation observed in, for example, *Anolis* lizards (Velasco & Herrel, 2007). Much of the variation in morphology is driven principally by the tree agama (*Acanthocercus atricollis*) and the rupicolous agama

(*A. atra*). They appear to be diverging from the other species. Interestingly, although overlapping to some degree due to the abundance of juveniles in our dataset, there is some divergence among adults of *A. a. distanti* and its relatives, *A. a. aculeata* and *A. armata*. A reduction in both fore- and hind limb size in *A. a. distanti* (see Fig. 2) could be advantageous in climbing and increase stability on inclined surfaces such as rocks (e.g. Sinervo & Losos, 1991). However, given the relationship among these agamas (Pyron *et al.*, 2013), differences between groups seem to be greatest between phylogenetically distant groups. Clearly, additional studies of agama species are needed to test the principal drivers of divergence among African agamas.

Pearson correlation analyses indicated a close association between morphology and performance capabilities (Fig. 3). This morphology–performance comparison revealed that long-legged and long-toed lizards can run for longer and farther. Our multiple regression analyses further showed that femur length is the best predictor of stamina (although explaining only 13% of its variation) while hind limb length explains 76% of the variation in exertion. However, hind limb length was not a good determinant of sprint speed. This is in contrast to simple biomechanical models, which suggest that longer legged individuals should have higher sprint speeds, all else being equal (Losos, 1990). However, agamas with longer hind limbs perform better in endurance, which could be an adaptation for predatory escape (Vanhooydonck & Van Damme, 2003). Mean speed was best predicted by relative mass (mass predicted 70% of its variation) and metatarsal length. This finding was rather unsurprising as most studies report an effect of body mass on speed (Pérez-Tris *et al.*, 2004; Zamora-Camacho *et al.*, 2014). Heavier lizards are impaired in sprinting abilities or, in this case, long-distance sprinting due to a trade-off between fat storage and escape efficiency. When under predation risk, lizards have been shown to lose weight to improve their ability to flee from predators (Pérez-Tris *et al.*, 2004).

TRADE-OFFS
We documented a trade-off between sprint speed and stamina. This was observed across all individuals in our study and illustrates the constraints operating on the evolution of locomotor capacity (see Vanhooydonck *et al.*, 2001; Fig. 4B). Higher muscle masses help lizards to achieve fast sprints but at the expense of higher costs of locomotion, and therefore possibly coming at the cost of a decreased exertion capacity. Moreover, a trade-off in muscle fibre types exists, with sprinters having fast fibres and endurance specialists having a larger proportion of slow fibres in their muscles

(Vanhooydonck *et al.*, 2014). For example, in humans, world class sprinters and marathon runners have different fibre type distributions in their leg muscles (Abe *et al.*, 2000). Observations on muscle physiology indicate that sprint athletes have fast-twitch muscles with high contraction rate but that fatigue fast, whereas endurance athletes have slow-twitch muscles that are better capable of resisting fatigue (Rivero *et al.*, 1993).

HABITAT USE
Species from different microhabitats differed from each other in size. Our data revealed that the arboreal species, *Acanthocercus atricollis*, is the largest and has longer limbs. It is important to note that we have data for only one species representing the arboreal habitat in this study. Moreover, it is the only tree-climbing agama in southern Africa (Bates *et al.*, 2014). Body size is often an important driver of variation in morphology and locomotor performance (Losos, 1990; James *et al.*, 2015). Indeed, the arboreal species excels with regard to exertion and mean speed compared to species from other habitats. Lizards with larger body size are expected to have higher muscle power output to compensate for a relative increase in body mass but at the expense of increased muscle stress (James *et al.*, 2015). A previous study, which accounted for phylogeny, demonstrated a maintenance of performance between species of different body size due to the changes in mechanical properties of muscles (James *et al.*, 2015). As a result, differences in body size may, at least in part, drive habitat partitioning among species (Losos 1994). Our results further showed functional relationships in limb and body shape corresponding to habitat use in these lizards when size is removed. The arboreal species, for example, had longer digits on the hands, longer hind limbs and taller bodies than the other two habitat specialists. This is quite unexpected as climbing ability is normally associated with short hind limbs (Sinervo & Losos, 1991; Losos *et al.*, 1993) and a dorso-ventrally flattened body (Miles, 1994). However, in cases where species climb broad steep surfaces, long limbs may be advantageous (Vanhooydonck & Van Damme, 2001). Indeed, long hind limbs may be extended laterally to grasp available points of support (Kramer, 1951), thus facilitating climbing in an arboreal environment. Longer digits on each forelimb may additionally help when gripping large vertical surfaces while the hind legs push off.

For all performance traits, we detected significant differences between habitat specialists, with or without correcting for hind limb length. This indicates significant differences in intrinsic muscle properties, muscle size or skeletal architecture between habitat specialists. Variation in fibre type and limb morphology

can explain species variation in locomotor performance as has been shown for lacertid lizards (Vanhooydonck *et al.*, 2014; James *et al.*, 2015). The arboreal species appeared to be a poor sprinter for a given hind limb length when compared to other habitat specialists. Species living in open microhabitats, such as the sandy or rocky sandveld in southern Africa, may be under selection for sprint speed as they are visible to predators in these open habitats (Vanhooydonck & Van Damme, 2003). This has been demonstrated for lacertid lizards from these same areas (Edwards *et al.*, 2012, 2016). This observation also revealed two general patterns. First, despite having a slower sprint speed, the arboreal species seemed to outperform lizards from the other habitat groups in terms of exertion and mean speed during the endurance trials. This could be the result of the trade-off between endurance and sprint speed. Previous studies have also suggested a negative correlation between climbing capacity and sprint speed in arboreal lizards, such as *Anolis* (Sinervo & Losos, 1991) and chameleons (Losos *et al.*, 1993). Second, having a better grip or 'surefootedness' and relatively high sprint speed on arboreal surfaces may not be possible without relatively shorter legs, in turn reducing speed on the ground (Herrel *et al.*, 2013). A paper on locomotor efficiency on inclined surfaces by Huey & Hertz (1984) reported a sharp decrease in speed on steep slopes in *Stellagama stellio*. It has been suggested that although arboreal lizards may run well on a flat surface, their muscle configuration is not tuned to do so, resulting in uncomfortable performance due to high stresses, muscular fatigue and energetic costs (Aerts *et al.*, 2000). Further research needs to be done on climbing in agamas in order to better understand the underlying trade-offs.

CAVEATS

We did not test for potential sexual differences within species due to the low sample sizes in our study. However, males of the arboreal species typically had a larger body and longer limbs, allowing them to run faster than the females. This association was not apparent in the rupicolous and psammophilous species. Males and females of rupicolous species did appear to differ in shape: males had relatively longer hind- and forelimbs and a higher body mass, yet these morphological differences did not translate into differential performance. Possibly in these rupicolous species other factors such as adaptation to crevice use or the evolution of sexually selected traits may play a bigger role in driving intersexual differences in morphology (Leaché *et al.*, 2014).

Another possible drawback of our study is that body temperature was not controlled for during the performance measurements. Variation in

body temperature affects locomotor performance in ectotherms (Autumn *et al.*, 1999). Although environmental conditions such as air temperatures and humidity could not be controlled in this study due to the lack of facilities in the field, we ensured that all animals were run at the temperatures at which they were active in the field (25–35 °C). Furthermore, the optimal temperature for sprinting is often similar to field active body temperatures (Huey, 1983; Perry *et al.*, 2004), suggesting that any bias introduced in our study is probably minor.

CONCLUSIONS

Our data show that the southern African agamas examined here are different morphologically and their body size and limb shape are tightly linked to locomotor performance. Our data further suggest that southern African agamas may be habitat specialists with morphological traits that allow them to perform better in their respective habitats. However, being adapted to a certain habitat may come at a cost as trade-offs between different locomotor traits were detected. Future studies should examine a greater range of species in a phylogenetically comparative context to test the generality of these results in addition to quantifying the microhabitats used by these species.

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SUPPORTING INFORMATION

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

Table S1. Morphology and performance traits for the six agama species studied.
Table S2. Best predictors of running performances based on stepwise multiple regression analyses with performance as the dependent variable and all morphological traits as independent variables.
Table S3. Paired samples *t*-test on the potential sexual differences in limb measurements and performance between habitat specialists.
Table S4. ANOVAs on limb and body measurements between the three habitat specialists.
Table S5. ANCOVAs on limb and body measurements between the three habitat specialists.
Table S6. ANOVAs on locomotor performance traits between the three habitat specialists.