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Multiple paths to aquatic specialisation in four species of Central American *Anolis* lizards

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Aquatic anoles present an interesting ecomorphological puzzle. On the one hand, the link between habitat use and morphology is well established as convergent within the Caribbean anole radiation. On the other hand, aquatic anoles do not appear to form an ecomorphological group – rather, it appears that there may be several ways to adapt to aquatic habitats. We explore this issue by examining the ecology, morphology and performance of four species of Central American aquatic anoles belonging to two different lineages. Overall, we find that aquatic anoles overlap in multiple ecological and morphological dimensions. However, we do find some differences in substrate use, claw and limb morphology, and bite force that distinguish *Anolis aquaticus* from the other three species (*A. lionotus*, *A. oxylophus* and *A. poecilopus*). Our results suggest that *A. aquaticus* is adapted to climb on boulders, whereas the other species utilise vegetation in streamside habitats.

Keywords: adaptation; ecomorph; functional morphology; aquatic lizard; *Anolis*

Introduction

The specialisation for life in aquatic environments has evolved in at least 11 lizard families and, as a group, aquatic lizards exhibit significant ecological, morphological and behavioural diversity (Pianka and Vitt 2003; Bauer and Jackman 2008). Aquatic specialisation can take many forms: some species are found only near slow-moving water, whereas others are found near rapidly flowing streams. Some aquatics are only sometimes observed near water, whereas others spend nearly all their time in aquatic habitats. Aquatic specialisation has, in many cases, involved the evolution of novel behaviours (e.g. sprinting on water, as seen in *Basiliscus* and *Uranoscodon superciliosus*) and the evolution of specialised morphologies (e.g. laterally compressed tails in *Sphenomorphus cryptotis* and *Varanus indicus*; discussed in Bauer and Jackman 2008).

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Aquatic specialisation in the genus *Anolis* is particularly intriguing, as anoles are a classic example of ecomorphological specialisation (reviewed in Losos 2009), but it remains uncertain whether aquatic anoles, particularly those from Central America, can be considered a single ecomorph (Leal et al. 2002). Of the nearly 400 species of anoles, only 11 are known to have adopted the aquatic habitat. There are nine species of aquatics found in the Latin American mainland. Only one species, *Anolis barkeri* (Schmidt 1939), is known from México. Costa Rica has two species, *A. aquaticus* Taylor, 1956 and *A. oxylophus* Cope, 1876. *Anolis lionotus* Cope, 1861 is the sister taxon to *A. oxylophus* and is distributed parapatrically in Panamá. The aquatic *A. poecilopus* Cope, 1862 is also found in Panamá, though it is also found in Colombia. Also known from Colombia are *A. macrolepis* Boulenger, 1911, *A. maculigula* Williams, 1984 and *A. rivalis* Williams, 1984. *Anolis lynchi* Miyata, 1985 is also found in Colombia, though it can also be found in Ecuador. The two Caribbean species, *A. eugenegrahami* Schwartz, 1978 and *A. vermiculatus* Cocteau in Duméril and Bibron, 1837, are found on the islands of Hispaniola and Cuba, respectively.

Based on an examination of seven species, Leal et al. (2002) found that aquatic anoles form three morphologically disparate groups: the two Caribbean species, *A. eugenegrahami* and *A. vermiculatus*, are divergent from each other and from the five mainland species examined (*A. aquaticus*, *A. barkeri*, *A. lionotus*, *A. oxylophus* and *A. poecilopus*). Further, the mainland aquatics were found to overlap greatly in morphological dimensions, leading to the tentative conclusion that these species may represent a single ecomorphological group. They also found that the mainland aquatics were morphologically quite similar to Caribbean ‘trunk–ground’ anoles, suggesting that these lizards may represent ‘trunk–ground’ anoles that utilise stream-side environments (Leal et al. 2002). However, detailed information about ecology, behaviour and performance characteristics were then lacking to make a more complete assessment of how similar mainland aquatic anoles are.

In this study, we address the potential ecomorphological overlap in four of the Central American aquatics in greater detail by examining their ecology (perch characteristics) and performance (bite force, sprint speed) alongside their morphology, including a new analysis of toe characteristics. We perform these analyses in four species of Central American aquatic anoles studied in Leal et al. (2002): *Anolis aquaticus*, *A. lionotus*, *A. oxylophus* and *A. poecilopus*, which all belong to the Norops clade of anoles (Nicholson et al. 2005). These four species represent two independent derivations of aquatic specialisation: *Anolis lionotus*, *A. oxylophus* and *A. poecilopus* are closely related; in fact *A. lionotus* and *A. oxylophus* have been treated as synonyms (Köhler 2008; but see Williams 1984; Köhler 2014). *Anolis aquaticus* is much more distantly related to the other three species, and independently evolved aquatic specialisation (Poe 2004; Nicholson et al. 2005).

Methods and materials

Species and habitat sampling

We sampled *Anolis aquaticus* and *A. oxylophus* in Costa Rica in July 2008 and the *A. lionotus* and *A. poecilopus* in Panamá in July 2009. Each habitat had a range of

available substrates that aquatic anoles are known to utilise. *Anolis aquaticus* was studied in a rocky stream at the Playa Piro Biological Station on the Osa Peninsula (8°23'24.00"N, 83°19'12.00"W) and *A. oxylophus* from slow-moving streams at the La Selva Biological Station in Heredia province (10°25'48.14"N, 83°58'46.34"W). In Panamá, we sampled *A. lionotus* at a small rocky stream and *A. poecilopus* at a slow-moving stream near Gamboa (9°07'12.00"N, 79°42'00.00"W). We searched for lizards during daytime hours (0700–1800) in each habitat by walking along rivers and streams; when an individual was sighted, we recorded the following habitat features: substrate type (e.g., log, trunk, boulder), perch height and perch diameter. We recorded perch diameter for flat surfaces such as boulders as 35.6 cm. This value was chosen as a conservative estimate for how broad a surface would need to be for our largest lizard (hindlimb length = 59.5 mm) to experience a flat surface (Spezzano and Jayne 2004). We captured lizards using a noose made from fishing line attached to an extendible panfish pole (Cabela's) and transported them to the field station to measure and record morphological and performance data. All individuals were returned to their original point of capture within 36 hours.

Morphological measurements

We collected the following morphometric measurements in millimetres using digital calipers (Mitutoyo): snout-vent length (SVL), forelimb segment lengths (humerus, radius, metatarsus, longest digit), hindlimb segment lengths (femur, tibia, metatarsus and fourth toe), head dimensions (head length, height, width, distance from the coronoid to the tip of the jaw, distance from the quadrate to the tip of the lower jaw, and lower jaw length), and body dimensions (height, width and inter-limb length). We measured head length as the distance from the back of the occipital to the tip of the snout; head width at the widest part of the head, typically just behind the eyes; and head height at the deepest part of the head, often at the level of the fronto-parietal junction. We measured body height as the deepest part of the chest at the level of the forelimbs, body width as the widest point of the body between the two limb pairs and inter-limb length as the distance from the shoulder to the hip. We calculated two additional measurements – the jaw opening inlever and closing inlever – that reflect the biomechanics of jaw movement and are related to bite force (Herrel et al. 2006, 2008). The opening inlever was calculated by subtracting the distance from the quadrate to the tip of the lower jaw from the total lower jaw length, whereas the inlever for jaw closing was estimated by subtracting the distance from the coronoid, estimated by the back of the jugal bone, to the tip of the jaw from the jaw outlever (= the distance from the anterior tip of the quadrate to the tip of the lower jaw, or the posteriormost point of the retroarticular process).

Toe pad and claw characteristics were measured from the fourth hind toe of preserved specimens of adult males from all four species (Table S1). Toe pad images were generated using a flatbed scanner and were quantified using ImageJ (1.40 g, Rasband). We measured aspects of claw morphology relevant to performance (Zani 2000; Dai et al. 2002): claw height, length, tip angle and curvature (Figure S1). The toe pad characters measured were toe pad area, lamella number and toe pad width.

Maximum running velocity

Sprint speeds were calculated by measuring the time to run 25 cm on a dowel (diameter 1.5 cm) placed at a 45° angle. Pairs of photocells were set at 25-cm intervals and connected to a portable computer, and we recorded the times at which the lizard passed the cells. Lizards were encouraged to run by tapping the base of the tail. Three trials were conducted for each individual at hourly intervals, and the highest speed recorded over a 25-cm interval was taken as that individual's maximum sprint speed ability. Performance in ectotherms such as lizards is tightly linked to temperature, and the optimal range can vary among species (Huey and Stevenson 1979; Huey 1982). Sprint trials were conducted at ambient temperature, which ranged between 24 and 28°C. Previous work has shown that this temperature range encompasses the mean temperatures measured for field-active *A. lionotus* and *A. poecilopus* (Campbell 1973), as well as the optimal performance range for *A. lionotus* (Van Berkum 1986, 1988). All trials were scored as good (i.e. trials where lizards ran continuously and without stopping along the entire track) or bad, and only trials that were scored as being 'good' were retained for analysis.

Bite force

Bite force capacity was measured in the field using an isometric Kistler force transducer (type 9203) mounted on a purpose-built holder and connected to a portable charge amplifier (type 5995; for details of the setup, see Herrel et al. 1999). Lizards were induced to bite the transducer five times, and the highest bite force recorded was used as an estimate of an individual's maximal bite performance.

Statistical analyses

To compare substrate use among species, we binned perch types into different categories. We binned branches, twigs, leaves and vines into a 'leafy vegetation' category, and binned tree trunks, roots, logs and posts into a 'woody vegetation' category. Because we were interested in the relationship between perch use and morphology, we excluded observations of lizards on the ground (3.8% of total observations) from our analyses. We used Fisher's exact test (two-tailed) to test for differences in substrate use (woody vegetation, leafy vegetation and boulders) among different species.

We log-transformed all continuous traits (perch height, perch diameter, morphological traits [except for claw curvature and tip angle], bite force and sprint speed) for adult lizards for analysis in SPSS (version 17.0). We recovered no significant differences between males and females in perch height (analysis of variance, ANOVA: $F_{1,123} = 1.27$; $p = 0.263$) or perch diameter (ANOVA: $F_{1,122} = 0.19$; $p = 0.663$). Thus, we pooled habitat data for males and females for subsequent analyses. However, even after accounting for body size (SVL), multivariate analysis (multivariate analysis of covariance, MANCOVA: sex = fixed factor; morphological traits = dependent variables, SVL = covariate) revealed that morphology differed between males and females (Wilks' $\lambda = 0.27$, $F_{2,21} = 6.90$, $p < 0.001$), and so the sexes were analysed separately for each species. We pooled individuals and reduced the number of morphological variables through principal component analysis (PCA; varimax

rotation) performed using the correlation matrix of the residuals of size-corrected morphological variables. To compare morphology among species, we conducted one-way ANOVAs on all principal components (PCs) with eigenvalues greater than 1.0, with species as the explanatory factor.

We found that tip angle, claw curvature and lamella number are not correlated with body size, which is consistent with previous research finding that lamella number is fixed at hatching (Hecht 1952). We reduced the number of toe pad characters through PCA (as described above) on the residuals of claw height, claw length and toe pad area, and raw values for tip angle, claw curvature and lamella number. We conducted one-way ANOVAs on all PCs with eigenvalues greater than 1, with species as the explanatory factor.

Similarly, we recovered no sex differences in performance (bite force and sprint speed) using a multivariate test (MANCOVA: sex = fixed factor, performance traits = dependent variables, SVL = covariate), and so we pooled data for males and females for subsequent analyses (Wilks' $\lambda = 0.97$, $F_{2,63} = 0.85$, $p = 0.43$). We compared bite force and sprint speed among species using one-way analysis of covariance (ANCOVA; performance = dependent variable, SVL = covariate).

Results

Habitat use and perching behaviour

The four aquatic anoles were found perching on the following substrates: boulders, leafy vegetation (branches, leaves, twigs, vines) and woody vegetation (tree trunks, roots, logs, posts) (Table 1). *Anolis aquaticus* was most frequently observed on boulders (56.7% of observations) and less frequently on logs (23.3%). *Anolis lionotus* and *A. poecilopus* were most often observed perching on woody vegetation, namely roots and trunks (77.1% and 59.5%, respectively), whereas *A. oxylophus* was most often observed on a variety of woody and leafy vegetation, including leaves, posts and tree trunks (60%). We generally did not observe lizards on narrow perches, such as twigs or vines. We found that *A. aquaticus* utilised rocks more than any of the other species (Fisher's exact test: comparison between use of rocks vs. woody vegetation: $p < 0.001$ in all comparisons), but after correction for multiple tests, none of the other comparisons in perch use were significant. Species were most often observed perching within a metre of the ground, and perch height did not differ among species (ANOVA: $F_{3,139} = 1.88$, $p = 0.136$). Mean perch diameter was not significantly different among species either (ANOVA: $F_{3,136} = 2.48$, $p = 0.064$).

Morphology and performance

In the factor analysis for males, the first six PC axes explained 79% of the morphological variation among individuals (Table 2; Table S2), whereas in the analysis for females, the first five PC axes explained 76% of the variation among species (Table 3). In males, the first factor loaded most strongly for head length and width characters, the second with body height and body width, the third with hindlimb dimensions, the fourth with forelimb dimensions, the fifth with the forelimb toe length and the sixth with inter-limb length. In females, the first factor loaded heavily with fore- and hindlimb length dimensions, the second with head length dimensions, the third with

Table 1. Summary of perching behaviour for the four species sampled. The number of observations per species is given in parentheses. The percentage of observations for each species on different substrates is given, as are mean perch height and perch diameter. Perch height is given in metres and diameter in centimetres (mean \pm 1 standard error of the mean).

	Ground	Boulders	Woody	Post	Root	Trunk	Vegetation			Vine	Perch	Perch
			Vegetation				Leafy	Leaf	Twig		height	diameter
			log				branch					
<i>A. aquaticus</i> (30)	3.3	56.7	23.3	0	3.3	0	6.7	6.7	0	0	0.60 \pm 0.07	25.8 \pm 2.3
<i>A. litoralis</i> (36)	0	11.4	2.9	0	60	17.1	5.7	0	2.9	0	0.61 \pm 0.07	20.8 \pm 4.6
<i>A. oxylophus</i> (26)	8.0	0	4.0	16.0	8.0	24.0	8.0	20.0	12.0	0	0.60 \pm 0.12	22.2 \pm 5.7
<i>A. poecilopus</i> (42)	4.7	14.3	7.1	0	35.7	23.8	7.1	0	2.4	4.7	0.57 \pm 0.08	24.1 \pm 7.0

Table 2. Loadings, eigenvalues (≥ 1) and percentage of variance explained from the principal component (PC) analysis conducted on the residuals for 21 morphological variables for male aquatics.

Variable	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Head length	0.873	0.099	0.073	-0.175	0.220	-0.058
Lower jaw length	0.941	0.120	0.000	-0.058	0.237	0.091
Quadrate – tip	0.963	0.045	0.077	-0.003	0.056	-0.037
Coronoid – tip	0.892	0.150	0.007	-0.147	-0.166	-0.122
Body height	0.185	0.864	0.103	0.088	-0.002	0.230
Body width	0.033	0.792	0.012	-0.065	0.264	-0.255
Tibia	0.356	0.308	0.711	0.084	-0.126	-0.034
Metatarsus	-0.155	-0.054	0.801	0.012	0.121	0.085
Hindlimb length	0.165	-0.075	0.922	0.187	0.114	-0.038
Humerus	-0.121	-0.051	0.553	0.592	0.057	-0.003
Metacarpus	-0.156	-0.108	-0.063	0.879	-0.015	-0.008
Forelimb length	-0.063	0.196	0.353	0.873	0.208	-0.034
Interlimb length	-0.051	-0.099	0.039	-0.009	0.095	0.912
Longest toe, forelimb	0.111	0.337	0.297	0.213	0.686	-0.098
Head width	0.681	0.344	0.162	0.152	-0.346	0.311
Head height	0.413	0.606	-0.202	-0.088	-0.111	-0.095
Femur	0.006	-0.683	0.513	0.257	-0.062	0.056
Longest toe, hindlimb	0.167	0.553	0.462	0.004	0.423	-0.199
Radius	0.259	0.687	0.514	0.214	0.071	0.064
Open	0.074	0.224	-0.199	-0.130	0.514	0.338
Close	0.126	-0.252	0.173	0.352	0.531	0.187
Eigenvalue	5.82	4.22	2.59	1.46	1.34	1.18
% variance explained	27.7	20.1	12.3	6.9	6.4	5.6

head height and inter-limb length and the fourth with jaw lever arms. Multivariate analysis (multivariate analysis of variance, MANOVA: species = fixed factor; PC scores = dependent variables) revealed that morphological dimensions differed significantly among species in males (Wilks' $\lambda = 0.07$, $F = 8.50$, $p < 0.001$) and in females (MANOVA: Wilks' $\lambda = 0.11$, $F = 6.09$, $p < 0.001$). Male *A. aquaticus* have taller and wider bodies than other species, and *A. poecilopus* have relatively longer forelimbs (Figure 1; Table S3). Female *A. aquaticus* have relatively long fore- and hindlimbs, greater head height and greater inter-limb lengths (Figure 1; Table S3).

In the factor analysis of toe characteristics, the first two PC axes explained 65.9% of the variation (Table 4). The first factor (44.2% variance explained) most strongly loaded with claw height, claw length and lamella number, whereas the second factor (21.7% variance explained) loads with toe pad area. Species were marginally different in PC 1 (ANOVA: $F_{3,26} = 3.06$, $p = 0.049$), a difference driven primarily by relatively shorter claw height and length and fewer lamellae in *A. aquaticus*, particularly with respect to *A. oxylophus* (Tukey honest significant difference post hoc: mean diff. = -1.46, $p = 0.042$; Table S4). In contrast, PC 2 did not significantly differ among species (ANOVA: $F_{3,26} = 0.65$, $p = 0.590$).

Species differed considerably in sprint speed and bite force (Table S5). Univariate analyses (ANCOVA: species = fixed factor, SVL = covariate) reveal that species

Table 3. Loadings, eigenvalues (≥ 1) and percentage of variance explained from the principal component (PC) analysis conducted on the residuals for 21 morphological variables for female aquatics.

Variable	PC 1	PC 2	PC 3	PC 4	PC 5
Forelimb length	0.889	0.241	0.079	-0.179	0.000
Hindlimb length	0.870	0.307	0.055	0.057	0.238
Tibia	0.856	0.217	0.061	0.083	0.067
Humerus	0.805	0.034	-0.105	-0.223	-0.287
Metatarsus	0.759	0.047	0.178	-0.035	-0.047
Femur	0.699	0.141	-0.045	0.210	0.323
Quadrate – tip	0.229	0.937	0.106	0.064	0.075
Coronoid – tip	0.207	0.910	0.002	0.173	-0.254
Lower jaw length	0.190	0.903	0.173	0.285	0.041
Head length	0.247	0.896	0.148	-0.144	0.056
Head height	0.056	0.249	0.844	0.201	-0.047
Interlimb length	-0.031	0.018	-0.830	0.089	-0.188
Open	-0.096	0.077	0.251	0.784	-0.124
Close	0.045	0.005	0.241	-0.271	0.735
Radius	0.692	0.328	0.449	0.025	0.031
Longest toe, forelimb	0.677	0.294	0.017	-0.197	0.149
Head width	0.471	0.423	0.450	0.240	0.060
Longest toe, hindlimb	0.547	0.565	0.060	-0.160	0.272
Body width	-0.045	0.163	-0.343	0.654	-0.303
Body height	0.282	0.378	0.546	-0.237	-0.357
Metacarpus	0.328	0.042	-0.269	-0.161	0.456
Eigenvalue	8.37	2.96	2.10	1.49	1.05
% variance explained	39.8	14.1	10.0	7.1	5.0

differ in sprint speed ($F_{3,68} = 12.76$, $p < 0.001$) and bite force ($F_{3,71} = 16.22$, $p < 0.001$) among aquatics. Specifically, *A. poecilopus* is a faster sprinter than *A. aquaticus* ($F_{1,38} = 30.86$, $p < 0.001$) and *A. lionotus* ($F_{1,43} = 29.11$, $p < 0.001$; Table 5). *Anolis aquaticus* has a much stronger bite than the other three species (Table 5), and *A. lionotus* has a stronger bite than *A. poecilopus* ($F_{1,28} = 12.29$, $p = 0.002$).

Discussion

Aquatic specialisation has evolved multiple times in 11 families (Pianka and Vitt 2003; Bauer and Jackman 2008) – such specialisation has evolved at least five times in *Anolis* lizards (Nicholson et al. 2005). In a study of five Central American aquatic anoles, Leal et al. (2002) found that they substantially overlapped in morphology. Our results largely agree with Leal et al. (2002) – we found considerable overlap in habitat use, morphology and performance among four Central American species. Despite this overlap, however, we observed several notable differences in substrate use and performance that indicate there may be more than one way for anoles to utilise streamside environments.

Lizards were observed using a wide range of woody, leafy and rocky substrates. Consistent with previous observations (Vitt et al. 1995; Eifler and Eifler 2010), all

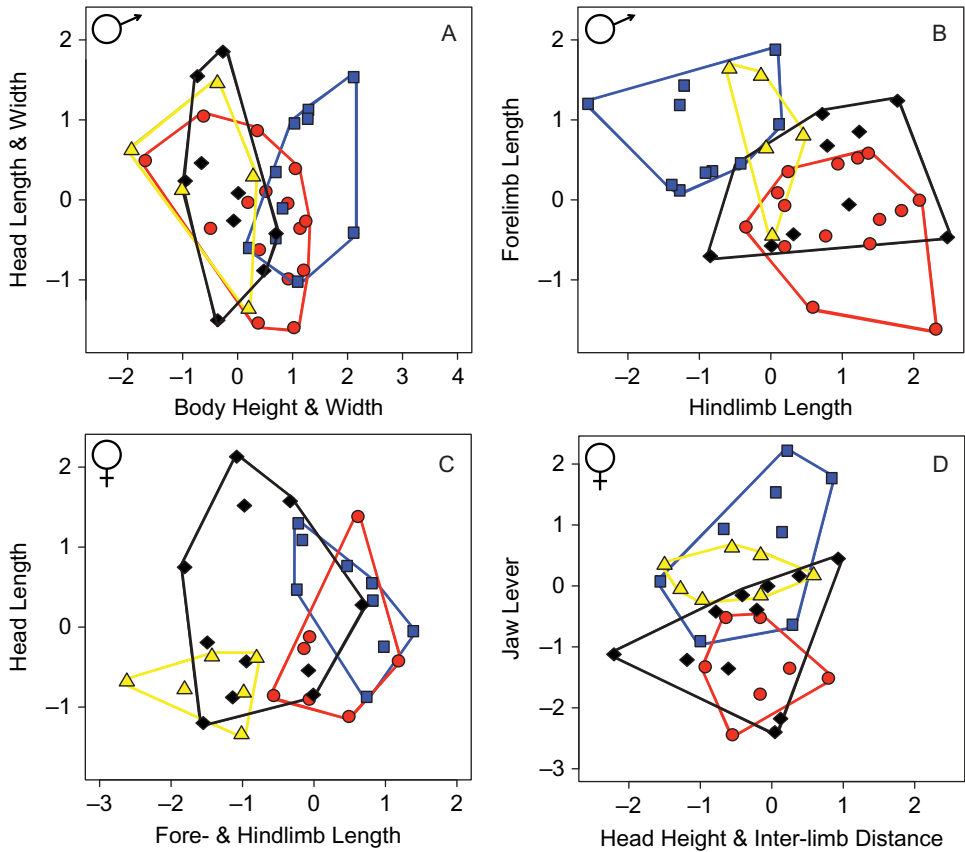


Figure 1. Plots of the four principal component (PC) axes for males (A + B) and females (C + D). Species denoted as follows: *Anolis aquaticus* (blue squares), *A. lionotus* (black diamonds), *A. oxylophus* (yellow triangles) and *A. poecilopus* (red circles). (A) PC 1 (head length and width) plotted against PC 2 (body height/body width) for males. (B) PC 3 (hindlimb length) plotted against PC 4 (forelimb length) for males. (C) PC 1 (fore- and hindlimb length) plotted against PC 2 (head length) for females. (D) PC 3 (head height and inter-limb length) plotted against PC 4 (jaw lever) for females.

Table 4. Loadings, eigenvalues (≥ 1) and percentage of variance explained from the principal components (PC) analysis conducted on the residuals for 6 toe characteristics. See Methods for a description of the traits.

Variable	PC 1	PC2
Tip angle	-0.596	0.647
Curvature	0.199	0.549
Claw height	0.856	0.131
Claw length	0.796	0.267
Lamella number	0.686	0.533
Toepad area	0.246	0.754
Eigenvalue	2.65	1.30
% variance explained	44.2	21.7

Table 5. Post-hoc test (Tukey) for ANCOVA on sprinting performance (left) and maximum bite force (right). Significance is denoted as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Comparison	Sprint speed		Max bite force	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
<i>A. aquaticus</i> – <i>A. lionotus</i>	1.18	0.286	14.23	0.001**
<i>A. aquaticus</i> – <i>A. oxylophus</i>	3.67	0.068	30.28	< 0.001***
<i>A. aquaticus</i> – <i>A. poecilopus</i>	30.86	< 0.001***	33.52	< 0.001***
<i>A. lionotus</i> – <i>A. oxylophus</i>	4.16	0.051	12.29	0.002**
<i>A. lionotus</i> – <i>A. poecilopus</i>	29.11	< 0.001***	3.71	0.061
<i>A. oxylophus</i> – <i>A. poecilopus</i>	6.15	0.019*	5.45	0.026*

species generally perch within a metre of the ground. In their analysis, Leal et al. (2002) found that the Central American aquatic species were morphologically more similar to ‘trunk–ground’ anoles than to any other Caribbean ecomorph. Our ecological data are consistent with this finding – trunk–ground anoles utilise almost any substrate, particularly trunks, logs and rocks, within a metre of the ground (reviewed in Williams 1983; Losos 2009).

Nonetheless, differences among species in substrate use indicate that *A. aquaticus* has adopted a different aquatic lifestyle than the other three species (*A. lionotus*, *A. oxylophus* and *A. poecilopus*). Unique among the species examined, *A. aquaticus* was more often observed on boulders than on other perches. The observed variation in boulder use could represent different substrate preferences between *A. aquaticus* and the other species, differences in substrate availability among sites, or both. Whereas previous studies have shown that *A. aquaticus* frequently uses rocky perches (e.g., Savage 2002; Eifler and Eifler 2010), others find that *A. oxylophus* does not tend to utilise boulders (<8% observations; Vitt et al. 1995). Thus, our observations potentially represent differences among species in substrate preferences.

The observation that *A. aquaticus* is frequently found on boulders, however, is not associated with morphological adaptations for crevice dwelling or with running on flat surfaces. Boulder- and cliff-dwelling lizards tend to be dorsoventrally flattened, with smaller bodies and longer hindlimbs, likely due to functional constraints for hiding in crevices and/or for maintaining balance on vertical surfaces (Vitt et al. 1997; Revell et al. 2007; Goodman et al. 2008; Collar et al. 2011). In contrast, *A. aquaticus* has a taller head and body than the other species. Both male and female *A. aquaticus* have proportionately short hindlimbs and, consequently, slower sprint speeds than the other species, especially with respect to *A. poecilopus*. What, then, is the link, if any, between perch use and morphology in this species?

Our analyses of limb and claw morphology strongly suggest that *A. aquaticus* is adapted to climb, rather than to sprint, on boulders and cliff walls. *Anolis aquaticus* has relatively short hindlimbs and long forelimbs; limb length parity is a feature strongly associated with specialised climbers (Cartmill 1985; Autumn et al. 1998; Vanhooydonck and Van Damme 2001). Further, *A. aquaticus* has shorter claws with fewer lamellae than the other species, especially with respect to *A. oxylophus*. Anoles that are less arboreal tend to exhibit reduced toe pads and fewer lamellae (Glor

et al. 2003; Macrini et al. 2003; Pinto et al. 2008), suggesting that claws are more important for clinging to boulder faces or cliff walls in scansorial anoles. Further, higher claws appear to perform better on rocky substrates (Zani 2000). Thus, claw height and length may work concurrently to provide additional access to rocky surfaces while also improving overall climbing performance. The limb and claw traits suggest that this species can cling to and climb on vertical rock surfaces – indeed, this species was commonly seen on bare rock walls, sometimes even clinging upside down (A. Herrel, pers. obs.). These differences in claw morphology may also reflect adaptations to different sleeping tactics – *Anolis aquaticus* is frequently observed clinging to boulder faces in the splash zone at night (Mason Ryan, pers. comm.), whereas *A. oxylophus* are most often observed sleeping on leafy vegetation (Vitt et al. 1995). These observations emphasise that our work focused on the connection between morphology and diurnal perching behaviour. Nocturnal perching behaviour may represent an equally important, though less explored, dimension of ecomorphological variation in aquatic anoles (Singhal et al. 2007).

Bite force, which is known to correlate with differences in diet and foraging style (Herrel et al. 2001), varied considerably among species. The diet of Central American aquatics is composed mostly of nonaquatic invertebrates (Leal et al. 2002). *Anolis aquaticus*, however, has a significantly stronger bite than the other species, suggesting that it may incorporate harder aquatic prey into its diet. If so, this would further link the lifestyle of *A. aquaticus* to the Caribbean aquatic species, which are known to incorporate harder prey, such as shrimp and fish, into their diets (Leal et al. 2002). While differences in prey choice may differ and be linked to variation in head shape and bite force between species, this remains to be tested.

It is not surprising that *A. lionotus*, *A. oxylophus* and *A. poecilopus* strongly overlap in ecomorphology as they are closely related and almost certainly represent a single derivation of the aquatic lifestyle (Poe 2004; Nicholson et al. 2005). In fact, *A. lionotus* and *A. oxylophus* may even be synonymous (Köhler 2008). However, we did find that *A. lionotus* has a stronger bite than *A. oxylophus*, suggesting that there may be some important ecological differences in feeding behaviour between these taxa, though further study is needed to assess this possibility. The evolution of aquatic specialisation in *A. aquaticus*, however, is clearly different from that of the other three species. Adaptation to boulders and rock walls appears to exert different selective pressures on morphology, which has produced strong differences in skull shape, claw morphology and performance capacities in this species.

Anolis aquaticus is not the only aquatic anole to be frequently observed on boulders and cliff walls – the Cuban species, *A. vermiculatus*, exhibits a strong preference for boulders (Rodriguez-Schettino et al. 2010, 71% of observations in females), as does *A. eugenegrahami* from Haiti (Schwartz 1978). Both of these species have notably longer hindlimbs, consistent with adaptation to running on flat surfaces such as rocks (Leal et al. 2002; Losos et al. 2002; Revell et al. 2007). These Caribbean species were strongly divergent in morphology from the Central American aquatics, as well as from each other (Leal et al. 2002). Thus, aquatic anoles appear to be adapted to perching on leafy or woody vegetation (*A. lionotus*, *A. oxylophus* and *A. poecilopus*) or on boulders; and if the latter, either to run (*A. eugenegrahami* and *A. vermiculatus*) or to climb (*A. aquaticus*).

Here we assessed only four of the nine recognised species of aquatic specialists from the Latin American mainland. Future study is required to assess how

specialisation has occurred in the other species, but, based on our results, we can predict that differences due to adaptation to boulders versus adaptation to vegetation in streamside habitats will also manifest. *Anolis maculigula* (Dactyloa clade) appears to exhibit a strong preference for rocky substrates, and has been observed perching vertically on boulders (Williams 1984); thus, its ecomorphology may be expected to align more closely with *A. aquaticus* than with the other Norops clade aquatics. In contrast, it is likely that most of the mainland aquatics from the Norops clade (*A. lynchi*, *A. macrolepis*, *A. macrolepis* and *A. rivalis*) are more likely to share ecomorphological affinities with *A. lionotus* (and related species) than with *A. aquaticus*. For example, Williams (1984) noted that *A. rivalis* tended to avoid the big boulders preferred by *A. maculigula* (though it did use smaller rocks along streambeds). One possible exception is *A. barkeri*, which has been recorded to more often utilise boulders than vegetation (Robinson 1962; Kennedy 1965; Meyer 1968; Birt et al. 2001; but see Brandon et al. 1966), though further study is required to assess the perching affinities of this, and other, aquatics from mainland Latin America.

In summary, as previously demonstrated (Leal et al. 2002), we find that the Central American aquatic anoles share some similarities in habitat use, morphology and performance. However, differences in substrate choice, morphology (skull shape, limb dimensions and claw shape), sprinting performance and bite force suggest that *A. aquaticus* is specialised to life on boulders or cliffs, whereas the other three species may represent, as Leal et al. (2002) postulated, ‘trunk–ground’ anoles restricted to streamside environments. The two derivations of aquatic specialisation examined here indicate that there are likely at least two different ways to be an aquatic anole.

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Supplemental material

Supplemental Figure and Tables can be found online: <http://dx.doi.org/10.1080/00222933.2015.1005714>

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