

# Basking behavior predicts the evolution of heat tolerance in Australian rainforest lizards

Martha M. Muñoz,<sup>1,2</sup> Gary M. Langham,<sup>3</sup> Matthew C. Brandley,<sup>4</sup> Dan F. Rosauer,<sup>5,6</sup> Stephen E. Williams,<sup>7</sup> and Craig Moritz<sup>5,6</sup>

<sup>1</sup>*Department of Biology, Duke University, Durham, North Carolina 27708*

<sup>2</sup>*E-mail: mmm109@duke.edu*

<sup>3</sup>*National Audubon Society, Washington, District of Columbia*

<sup>4</sup>*School of Life and Environmental Sciences, University of Sydney, Sydney, New South Wales, Australia*

<sup>5</sup>*Centre for Biodiversity Analysis, Australian National University, Canberra, Australian Capital Territory, Australia*

<sup>6</sup>*Research School of Biology, Australian National University, Canberra, Australian Capital Territory, Australia*

<sup>7</sup>*Centre for Tropical Biodiversity and Climate Change, James Cook University, Townsville, Queensland, Australia*

Received August 31, 2015

Accepted August 31, 2016

There is pressing urgency to understand how tropical ectotherms can behaviorally and physiologically respond to climate warming. We examine how basking behavior and thermal environment interact to influence evolutionary variation in thermal physiology of multiple species of lygosomine rainforest skinks from the Wet Tropics of northeastern Queensland, Australia (AWT). These tropical lizards are behaviorally specialized to exploit canopy or sun, and are distributed across marked thermal clines in the AWT. Using phylogenetic analyses, we demonstrate that physiological parameters are either associated with changes in local thermal habitat or to basking behavior, but not both. Cold tolerance, the optimal sprint speed, and performance breadth are primarily influenced by local thermal environment. Specifically, montane lizards are more cool tolerant, have broader performance breadths, and higher optimum sprinting temperatures than their lowland counterparts. Heat tolerance, in contrast, is strongly affected by basking behavior: there are two evolutionary optima, with basking species having considerably higher heat tolerance than shade skinks, with no effect of elevation. These distinct responses among traits indicate the multiple selective pressures and constraints that shape the evolution of thermal performance. We discuss how behavior and physiology interact to shape organisms' vulnerability and potential resilience to climate change.

**KEY WORDS:** Australian Wet Tropics, behavioral thermoregulation, climate change, physiological evolution, skinks, thermal physiology.

Anthropogenic climate warming presents an unprecedented threat to global biodiversity (Thomas et al. 2004; Parmesan 2006; Barnosky et al. 2011), and its impacts are predicted to be particularly pernicious for tropical ectotherms such as lizards, which already function near their upper physiological limits (Huey et al. 2009; Sinervo et al. 2010; Buckley and Huey 2016). Whether organisms can buffer rising temperatures with behavior

or physiology, or adapt genetically, is thus a central question (Deutsch et al. 2008; Hoffman and Sgrò 2011; Huey et al. 2012; Hoffmann et al. 2013). Evolutionary studies of physiology in lizards have yielded mixed results. Case studies in Caribbean *Anolis* lizards reported adaptive shifts in cold tolerance (Leal and Gunderson 2012) and the optimal sprinting temperature (Logan et al. 2014), indicating that some physiological traits



can respond to changes in the thermal environment. In contrast, other studies found that heat tolerance is evolutionarily inert in various lizard clades (Labra et al. 2009; Bonino et al. 2011; Muñoz et al. 2014a, but see van Berkum 1988). Evolutionary stability in heat tolerance implies a limited capacity for this trait to respond rapidly enough to meet the pace of environmental warming.

Theory suggests that behavior impacts patterns of physiological divergence (Angilletta 2009). By determining how organisms interact with their thermal habitats, behavior can modulate the pace and magnitude of physiological evolution across environments (Huey et al. 2003). For example, by preferentially selecting cooler microsites in a warm habitat, behavior can limit divergence in upper physiological limits (e.g.,  $T_{opt}$  and  $CT_{max}$ ) (Huey et al. 2003). In contrast, behavior should be considerably less effective at shielding lower physiological limits from selection (i.e.,  $CT_{min}$ ), as there is limited thermal variation in colder habitats, particularly at night (Sarmiento 1986; Ghalambor et al. 2006). Studies simultaneously comparing thermal habitat preference and physiology in an evolutionary framework are sparse.

In this study, we compare patterns of behavioral and physiological divergence in seven species of forest floor skinks from low to high elevations in the tropical rainforests of Australia. The Australian Wet Tropics (AWT) has been experiencing dramatic changes in climate over the past several decades (Hughes 2003) and has been highlighted as a highly endemic system that is vulnerable to future climate change (Williams et al. 2003; Shoo et al. 2005). Further, rainforests provide highly heterogeneous thermal environments, with marked temperature differences between closed canopy and gap environments—this heterogeneity provides opportunity for marked behavioral partitioning of the thermal environment (Kearney et al. 2009). Lizards in this region are spread across a wide altitudinal distribution, thus experiencing—and enabling physiological adaptation to—a broad range of thermal conditions. Moreover, the latitudinal range of the AWT encompasses deep phylogeographic splits with species, allowing us to examine divergence patterns across climatic gradients both within and among species (Moritz et al. 2009, 2012). We first compare thermal microhabitat characteristics and basking tendencies among species and demonstrate that they partition the thermal niche in two key ways—whereas some species preferentially bask in relatively warm microenvironments, others are nearly always observed in cool, canopied habitats. We then employ a phylogenetically informed approach to test whether ecologically important physiological traits—cold tolerance ( $CT_{min}$ ), the optimal sprinting temperature ( $T_{opt}$ ), the optimal performance range ( $B95$ ), and heat tolerance ( $CT_{max}$ )—shift across altitude, and ask how basking behavior—strong differences among species in the use of sun and shade—influences these relationships. Finally, we

explore how our results can inform assessments of vulnerability to future warming in this system.

## Methods

### STUDY SPECIES AND SAMPLING

We studied seven widespread species of lygosomine skinks from four genera found in the Wet Tropics World Heritage rainforests in northeast Queensland, Australia (Table 1; Fig. 1). The species examined were as follows: *Carlia rubrigularis*, *Gnypetoscincus queenslandiae*, *Lampropholis coggeri*, *L. robertsi*, *Saproscincus basiliscus*, *S. czechurae*, and *S. tetradactyla*. Of these, *L. robertsi* and *S. czechurae* are montane taxa (mostly > 1000 m), whereas the other species have broad elevation ranges. We sampled all species across their elevational ranges (Table 1). Where possible, we sampled populations from multiple mountain ranges, including the Paluma, Carbine, Herberton, and Atherton Uplands (Fig. 1). Populations from the northern AWT (Carbine Uplands) are phylogenetically distinct from those in the southern ranges (Paluma, Herberton, and Atherton Uplands; Moritz et al. 2009). Hence, our sampling design captures both shallow and deep phylogeographic splits within species, allowing for examination of independent lineages within and among species across thermal boundaries.

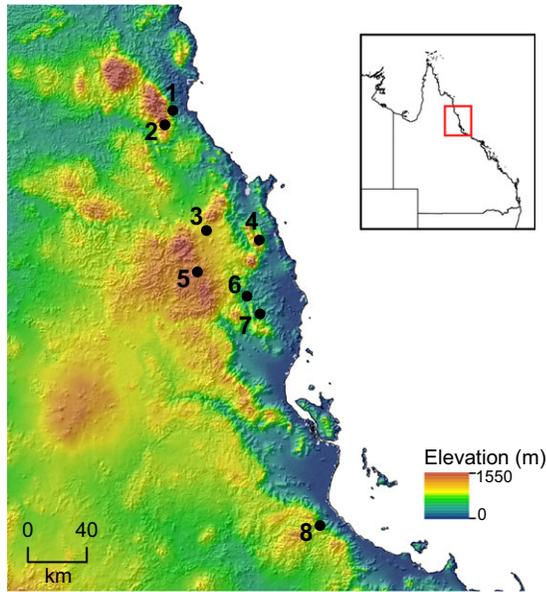
### BEHAVIORAL THERMAL MICROHABITAT USE

We obtained estimates of thermal habitat use from the target species throughout the AWT using the long-term ecology survey data from Williams et al. (2010). The sites where we sampled organisms for physiological experiments are the same as those used in these ecological surveys, affording us comparable estimates of ecology and physiology. Briefly, sampling surveys were conducted by walking along a 1 km transect during a set 30 min period. Lizards were spotted visually on the surface or found by flipping logs and boulders. For each observation, the temperature of the perch surface ( $T_{surf}$ ) was taken using an infrared thermometer placed ~1 cm above where the lizard was observed. The basking behavior (i.e., whether the lizard was perching in the sun or in the shade) was also recorded. Based on these observations of substrate temperature and basking behavior (see Results), we classified each species into one of two thermal behavior groups. The basking species included *C. rubrigularis* ( $n = 571$ ), *L. coggeri* ( $n = 540$ ), and *L. robertsi* ( $n = 30$ ). The shade dwellers included *G. queenslandiae* ( $n = 186$ ), *S. basiliscus* ( $n = 43$ ), and *S. czechurae* ( $n = 12$ ). Sufficient data were lacking for *S. tetradactyla*, but this species is known to behave similarly to its congeners (C. Moritz and S. E. Williams, pers. Obs.), *S. basiliscus* and *S. czechurae*, and so we also assigned it to the “shade-dwelling” category. We used basking behavior as a fixed

**Table 1.** Summary data are given for each physiological trait—critical thermal minimum ( $CT_{min}$ ), optimal sprinting temperature ( $T_{opt}$ ), optimal sprinting temperature range (B95), and critical thermal maximum ( $CT_{max}$ ) for each locality and species.

Species	Locality	$CT_{min}$	$T_{opt}$	B95	$CT_{max}$	$T_{surf}$ (Prop. Sun)
<i>Carlia rubigularis</i> (bask)	CU 100	15.58 ± 0.91 (4)	26.30 (1)	4.60 (1)	—	25.55 (0.833)
	CU 1000	13.6 ± 0.52 (6)	31.26 ± 3.12 (5)	5.80 ± 0.41 (5)	43.29 ± 0.52 (4)	22.49 (0.742)
	AU 100	14.03 ± 0.86 (6)	26.70 ± 1.76 (7)	6.09 ± 0.40 (7)	42.94 ± 0.47 (9)	24.67 (0.661)
	AU 1000	11.73 ± 1.21 (4)	28.50 ± 1.63 (6)	5.95 ± 0.41 (6)	43.31 ± 0.58 (8)	28.06 (0.740)
	HR 1000	15.7 ± 0.50 (2)	—	—	—	25.48 (0.917)
PA 750	10.76 ± 0.32 (4)	29.19 ± 1.74 (7)	6.96 ± 0.34 (7)	43.44 ± 0.25 (5)	30.62 (0.818)	
<i>Gnypetoscincus queenslandiae</i> (shade)	CU 1000	14.27 ± 0.47 (7)	29.75 ± 1.91 (7)	6.16 ± 0.23 (7)	36.85 ± 0.86 (6)	16.81 (0.000)
	AU 400	12.23 ± 0.65 (8)	27.82 ± 1.01 (6)	4.98 ± 0.26 (6)	36.89 ± 1.02 (4)	19.03 (0.000)
	AU 1000	11.29 ± 1.22 (7)	24.7 ± 0.58 (5)	5.28 ± 0.39 (5)	36.32 ± 0.27 (3)	17.43 (0.000)
<i>Lampropholis coggeri</i> (bask)	CU 100	14.4 ± 0.3 (2)	24.9 (1)	4.4 (1)	—	26.25 (0.769)
	AU 100	14.62 ± 0.87 (6)	29.28 ± 1.73 (4)	5.43 ± 0.80 (4)	41.78 ± 0.38 (9)	28.15 (0.746)
	AU 1000	13.48 ± 0.37 (12)	28.80 ± 2.07 (8)	6.61 ± 0.81 (8)	42.57 ± 0.42 (6)	26.63 (0.811)
	HR 1000	12.57 ± 0.71 (6)	28.20 ± 1.92 (3)	5.57 ± 0.87 (3)	42.05 ± 0.58 (5)	25.34 (0.888)
	AU 1000	12.97 ± 0.75 (3)	31.90 (1)	6.10 (1)	43.05 ± 0.29 (3)	30.86 (0.800)
<i>Lampropholis robertsi</i> (bask)	AU 1600	9.96 ± 0.55 (7)	31.43 ± 0.29 (7)	6.83 ± 0.22 (7)	43.42 ± 0.35 (5)	30.83 (0.667)
	HR 1000	10.1 (1)	36.10 (1)	8.50 (1)	—	—
	AU 100	14.3 ± 0.47 (8)	29.50 ± 3.19 (3)	5.33 ± 0.19 (3)	39.00 ± 0.36 (5)	23.06 (0.188)
<i>Saproscincus basiliscus</i> (shade)	AU 1000	11.77 ± 0.65 (9)	27.88 ± 1.56 (8)	5.44 ± 0.33 (8)	38.04 ± 0.65 (5)	21.36 (0.250)
	HR 1000	9.4 (1)	—	—	—	19.68 (0.417)
	PA 750	13.48 ± 0.54 (4)	32.25 ± 0.81 (4)	5.63 ± 0.70 (4)	37.96 ± 0.69 (4)	21.6 (0.000)
<i>Saproscincus czechurae</i> (shade)	AU 1000	11.77 ± 1.27 (4)	29.88 ± 2.40 (5)	5.36 ± 0.57 (5)	37.75 ± 1.37 (4)	20.04 (0.200)
	AU 1600	9.33 ± 0.12 (3)	31.00 ± 2.19 (3)	6.50 ± 0.85 (3)	36.97 ± 1.20 (3)	—
<i>Saproscincus tetradactylus</i> (shade)	AU 100	16.7 ± 0.96 (5)	30.35 ± 2.31 (4)	4.83 ± 0.39 (4)	35.52 (1)	19.88 (—)
	PA 750	13.3 (1)	—	6.1 (1)	—	—

For each trait, the mean in °C ± 1 SEM is given, with sample size provided in parentheses. The mean surface temperature in °C and proportion of basking observations for each locality based on Williams et al. (2010) is also given. Locality abbreviations refer to the specific mountain range (+ approximate elevation) from which species were sampled as follows: AU = Atherton Uplands, CU = Carbine Uplands, HR = Herberton Range, and PA = Paluma Range.



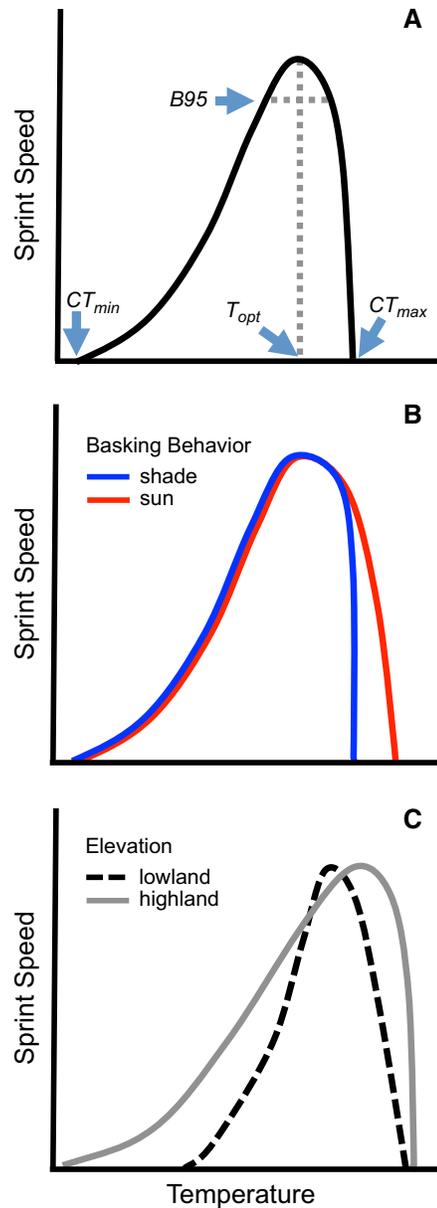
**Figure 1.** Map showing the altitudinal variation in the Australian Wet Tropics (AWT) region and localities for each species sampled in this study. The map inset shows the location of the AWT within northeastern Queensland. Color indicates elevation and ranges from sea level (blue) to 1550 m. a. s. l. (red). Localities are denoted as follows: (1) CU 100; (2) CU 1000; (3) AU 1000; (4) AU 1600; (5) HR 1000; (6) AU 400; (7) AU 100; and (8) PA 750. Locality details are given in Table 1.

effect in subsequent analyses (see “Phylogenetic Analyses of Physiology and Behavior” next), which we considered as discrete (species were designated as either “baskers” or “shade-dwellers”) or continuous (basking behavior was the arcsine square root transformed proportion of total basking observations).

**PHYSIOLOGICAL TRAIT MEASUREMENT**

Thermal performance curves (TPCs) describe the relationship between an organism’s core body temperature and its performance (Huey and Stevenson 1979). In ectotherms such as lizards the ability to perform a task such as sprinting is contingent on core temperature (Fig. 2A). Performance is maximized at a particular temperature (the thermal optimum,  $T_{opt}$ ), remains high over a range of temperatures (the performance breadth,  $B_{95}$ ), and decreases at higher or lower temperatures until the animal is immobilized (the critical thermal minimum [ $CT_{min}$ ] and maximum [ $CT_{max}$ ]).

Between February and September 2001, we collected lizards from eight localities within the AWT that, together, span the altitudinal distribution of the seven species (Fig. 1; Table 1). From the field sites, we transferred captured individuals to plastic containers (14 × 8 × 12 cm) with leaf litter, which were kept in a portable cooler to maintain temperatures near 24°C during transport. Once back at the laboratory, each lizard was measured and weighed and then returned to the container. Air conditioning in



**Figure 2.** (A) Hypothetical performance curve showing the thermal dependence of sprint speed. Sprinting ability is maximized at  $T_{opt}$ , and functions at 95% of maximum capacity in the  $B_{95}$  temperature range (or “performance breadth”). Performance declines at temperatures above and below  $T_{opt}$  until the critical thermal maximum ( $CT_{max}$ ) and critical thermal minimum ( $CT_{min}$ ), respectively, when the organism is immobilized. Hypothetical curve based on Huey and Stevenson (1979). (B and C) Display schematic representations of thermal performance curves based on results described in Figure 4. (B) Within a given locality, basking species (red) and shade dwellers (blue) exhibited similar  $CT_{min}$ ,  $T_{opt}$ , and  $B_{95}$ , but differed markedly in  $CT_{max}$ . (C) Lizards at low elevation tended to have higher  $CT_{min}$ , lower  $T_{opt}$ , and a narrower performance breadth ( $B_{95}$ ) relative to lizards at high elevation.  $CT_{max}$ , however, remained constant independent of elevation. These altitudinal patterns manifested in both basking and shade-dwelling skinks.

the lizard facility was used to maintain an ambient temperature of  $\sim 24^{\circ}\text{C}$  and humidity near 40%. Lizards were maintained on a 12-h light:12-h dark schedule and provided with water and mealworms daily. Containers were stored on a rack with a heating element running along the back end and heated daily from 0900 to 1500 h. Lizards were all tested within a few days of capture.

We measured  $CT_{min}$  and  $CT_{max}$  with standard righting tests (Spellerberg 1972). Briefly, to conduct  $CT_{min}$  experiments, we placed lizards in a plastic container sitting in an ice bath, causing core temperature to drop at  $\sim 1^{\circ}\text{C}/\text{min}$ . We checked for sluggish behavior, and then performed righting tests. The righting test consisted of flipping the lizard onto its back and stimulating it to right itself by squeezing its thighs and the base of its tail with blunt tweezers. As soon as a lizard could no longer right itself, we measured body temperature,  $T_b$ , from the cloaca with a fine-gauge, quick-read thermocouple (TC-1000 Sable Systems<sup>®</sup>). To test for  $CT_{max}$ , we placed skinks in a plastic container with high walls and placed a 245 W bulb above the container. We observed each skink closely and then performed righting tests and temperature readings as described above. We immediately transferred the container to a cool water bath following the experiment. No animals died during either test.

Following tolerance experiments, we tested the thermal sensitivity of sprint speed. Each skink was run across a range of temperatures that correspond with field site temperatures ( $15\text{--}40^{\circ}\text{C}$ ). Five groups of 10–30 skinks were run in a series of randomized temperatures: group 1 ( $28^{\circ}\text{C}$ ,  $24^{\circ}\text{C}$ ,  $19.2^{\circ}\text{C}$ ,  $15.7^{\circ}\text{C}$ ,  $31.6^{\circ}\text{C}$ ,  $26^{\circ}\text{C}$ , and  $30^{\circ}\text{C}$ ); group 2 ( $15.7^{\circ}\text{C}$ ,  $19.2^{\circ}\text{C}$ ,  $36.2^{\circ}\text{C}$ ,  $31.6^{\circ}\text{C}$ ,  $28^{\circ}\text{C}$ ,  $24^{\circ}\text{C}$ , and  $40^{\circ}\text{C}$ ); group 3 ( $19.2^{\circ}\text{C}$ ,  $36.2^{\circ}\text{C}$ ,  $15.7^{\circ}\text{C}$ ,  $31.6^{\circ}\text{C}$ ,  $28^{\circ}\text{C}$ , and  $24^{\circ}\text{C}$ ); group 4 ( $36.2^{\circ}\text{C}$ ,  $19.2^{\circ}\text{C}$ ,  $24^{\circ}\text{C}$ ,  $31.6^{\circ}\text{C}$ ,  $15.7^{\circ}\text{C}$ , and  $28^{\circ}\text{C}$ ); and group 5 ( $28^{\circ}\text{C}$ ,  $15.7^{\circ}\text{C}$ ,  $31.6^{\circ}\text{C}$ ,  $19.2^{\circ}\text{C}$ ,  $24^{\circ}\text{C}$ , and  $36.2^{\circ}\text{C}$ ). Skinks were run during normal field activity times on a track ( $60 \times 95 \times 700$  cm) that consisted of white acrylic walls and a wood base with 180-grain sandpaper for traction, with a darkened shelter at the end of the track. A video camera (Sony<sup>®</sup> TCV 500) mounted on a tripod 1 m above the track filmed trials. To run sprint trials, all skinks were initially placed into a large incubator at the appropriate temperature. When lizards reached the sprinting temperature, they were transferred to the base of the track and encouraged to run by gently touching the tail with a paintbrush. Skinks ran at each temperature five times in a given day, and were given an hour rest between trials.

#### FITTING TPCs

Video frames from sprinting trials were manually scored to find the fewest number of frames (30/sec) along any 10 cm segment of the track for a given temperature and individual. Then, we used this fastest segment to calculate maximum velocity (m/sec) at each temperature for each individual. These speeds were then

plotted against temperature along with  $CT_{min}$  and  $CT_{max}$  for each individual, and curves were fit with an Splus<sup>®</sup> script (R. Huey, pers. comm.). The script fit a Gaussian (left side)  $\times$  Gompertz (right side) curve, using the nonlinear regression package, to approximate the known shape of lizard performance curves. Curves were then assayed for peak speed ( $V_{max}$ ) and peak sprinting temperature ( $T_{opt}$ ). To calculate optimal temperature range at 95% of maximal performance (i.e.,  $B95$ ), we multiplied  $V_{max}$  by 0.95 and recorded the intersection with the fitted curve. The intersections represent the upper and lower bounds of  $B95$ , where sprint speeds are 95% of maximum potential speed (e.g., Hertz et al. 1983). As predicted (see Huey and Pianka 2007), physiological traits ( $CT_{min}$ ,  $T_{opt}$ ,  $B95$ , and  $CT_{max}$ ) did not differ between sexes (multivariate analysis of variance [MANOVA]: Wilk's  $\lambda = 0.934$ ,  $F = 1.04$ ,  $P = 0.393$ ); hence, we combined data for males and females for subsequent analyses. Further, neither body mass nor body length (i.e., snout-vent length, SVL) influenced physiological estimates (all  $P > 0.05$ ); hence, we used uncorrected variables in subsequent analyses.

#### ENVIRONMENTAL LAYERS AND CLIMATIC PROJECTIONS

For each site we obtained daily maximum and minimum temperatures by downscaling existing weather station data against spatial topography and vegetation layers at a grid resolution of  $250\text{ m}^2$  (Storlie et al. 2013). These fine-scale layers more accurately depict the thermal landscape, providing more realistic estimates of environmental temperatures as experienced by these forest floor skinks (Storlie et al. 2014). Lizard physiology is a combination of both genetic and environmentally induced variation (i.e., acclimation). For estimates of thermal environment to be most ecologically relevant, mean temperature on a temporal scale appropriate to the conditions influencing physiology at time of capture should be used. Given that most physiological acclimation in lizards occurs within four weeks (Kolbe et al. 2012; Pintor et al. 2016), we estimated environmental temperature as the mean of the maximum daily temperature for the month preceding capture for  $T_{opt}$  and  $CT_{max}$ . For  $CT_{min}$  and  $B95$ , we used the mean of the minimum daily temperature for the month preceding capture (see “Phylogenetic Analyses” next for explanation). Nonetheless, results were robust to different estimates of mean environmental temperature, ranging from the week prior to capture to several months preceding capture (Table S1).

#### INFERRING PHYLOGENETIC RELATIONSHIPS

We obtained tissue samples (ethanol-preserved tail tips) from one individual per species per sampling locality, and extracted DNA using Qiagen DNeasy<sup>™</sup> columns. We sequenced fragments of four independently evolving loci, including the mitochondrial gene ND1 and flanking tRNA<sup>LEU</sup>, tRNA<sup>ILE</sup>, and tRNA<sup>GLN</sup> genes

(1206 bp), and the three nuclear genes BDNF (670 bp), R35 (634 bp), and RAG1 (2000 bp). Polymerase chain reaction (PCR) amplification and Sanger sequencing procedures were identical to Brandley et al. (2011). tRNAs were aligned using Muscle version 3.8.31 (Edgar 2004) under default settings, and we excluded from subsequent phylogenetic analysis 23 tRNA characters that we subjectively determined to be unalignable.

We identified 13 data partitions a priori including the separate codon positions of ND1, BDNF, R35, and RAG1 and a single partition for the combined mtDNA tRNAs. Using Partition Finder version 1.1.1 (Lanfear et al. 2012), we then estimated the optimal partitioning scheme by combining a priori partitions that may be explained by the same model of DNA substitution under the Bayesian information criterion (BIC). Partition Finder identified seven total partitions that were used in subsequent phylogenetic analyses (Table S2).

To infer the phylogenetic relationships among populations and species, we performed Bayesian phylogenetic analyses using MrBayes version 3.2.1 (Ronquist et al. 2012). For each MrBayes analysis, we conducted two runs of four Markov Chain Monte Carlo (MCMC) chains and enforced the optimal DNA substitution models and partitioning scheme estimated by Partition Finder. We did not specify a limit on the number of MCMC generations, but rather stopped the analyses when the average SD of split frequencies between the two runs fell below 0.5% (estimated every  $10^6$  generations); at this point, we considered the two runs to have converged on the same posterior distribution. We also ensured that the effective sample sizes (ESS) of the model parameters were  $\geq 200$  using Tracer version 1.5 (Rambaut and Drummond 2007). We repeated these analyses four times for a total of eight independent runs. We deleted the first 20% of all trees in the posterior distribution as burn-in and then calculated at consensus tree from all post-burn-in trees from the eight runs, and used the maximum clade credibility (MCC) tree in subsequent analyses. Estimated clade posterior probabilities (PP)  $\geq 0.95$  are considered strongly supported (Huelsenbeck and Rannala 2004).

## PHYLOGENETIC ANALYSES OF PHYSIOLOGY AND BEHAVIOR

Following Revell (2010), we performed phylogenetic generalized least squares (PGLS) analyses in which the maximum likelihood estimate of phylogenetic signal (Pagel's  $\lambda$  [Pagel 1999]) was simultaneously calculated with the regression model. This method recovers the performance of the best model under a wide range of conditions (Revell 2010). We also performed PGLS analyses using the random walk (Brownian motion [BM]) model and an Ornstein–Uhlenbeck (OU) model with a single optimum (Martins and Hansen 1997; Blomberg et al. 2003). To perform PGLS, we used the *gls* function in the nlme package (Pinheiro et al. 2016) in R (R Core Development Team 2014). We compared

model fits using  $\Delta AIC_C$ . Following Burnham and Anderson (2002), we considered models with  $\Delta AIC \geq 2$  from the lowest score to be better supported. We found that the model using the  $\lambda$  branch transformation was either best supported or equally good (i.e.,  $\Delta AIC \leq 2$  as compared to best model) (Table S3); hence, we present those results. We hypothesized that, due to cooler temperatures at high elevation, montane lizards should be more cold tolerant (lower  $CT_{min}$ ), which should also lead to a wider performance breadth ( $B95$ ). Correspondingly, we found a strong correlation between the independent contrasts of  $CT_{min}$  and  $B95$  using regression through the origin with the *lmargin* function in APE (Paradis 2006). Hence, we analyzed these two traits together using PGLS. None of the other estimated correlations among traits were significant and we thus analyzed  $CT_{max}$  and  $T_{opt}$  separately (Table S4).

We expected thermal traits to correlate with local thermal environment. Hence, we first regressed each trait (or combination of traits) against environmental temperature. We then compared whether the model fit was significantly improved by adding basking behavior as an explanatory factor to test the hypothesis that basking behavior influences the relationship between physiology and environment. As described above (see “Behavioral Thermal Microhabitat Use” above), separate analyses were run treating basking behavior as either a continuous (arcsine square root transformed proportion of basking observation) or discrete (“basking” vs. “shade-dwelling”) variable. Both approaches yielded similar results. For all models, we report the Akaike information criterion corrected for small sample sizes ( $AIC_C$ ; Sugihara 1978) as a heuristic indicator of model support. Following Burnham and Anderson (2002), we considered the model with the lowest  $AIC_C$  score to be the best model, and models within 2 units of the lowest score to have substantial support.

Given our finding that  $CT_{max}$  differs substantially between “baskers” and “shade-dwellers” (see Results), we further tested whether heat tolerance evolves toward distinct evolutionary optima by fitting three models of trait evolution in the OUCH package (King and Butler 2009) in R. The three models were BM, a random walk such that trait divergence is proportional to branch length; single peak OU, a random walk in which characters tend to a single optimum, and two peak OU, in which the OU model is pulled toward to two different adaptive peaks, one for each baskers and shade dwellers (Butler and King 2004). Testing the multipeak model requires assigning a binary behavioral type to each of the tree's nodes. We reconstructed ancestral states for each of the behavioral types by estimating the marginal likelihood for each node. Although ancestral state reconstructions introduce uncertainty, most of the marginal likelihoods for node assignment were extremely high (0.99) (Table S3). Only two nodes, in particular the root node (marginal likelihood  $\sim 0.5$ ), gave uncertain assignments (Table S5). Hence, we ran the two peak OU models

using all four possible reconstructions of these nodes; we found that our results were robust to the different reconstructions (Table S6), and present model results for the best reconstruction (based on marginal likelihood) in the Results. As above, we used  $AIC_C$  to compare models.

## Results

### ANALYSIS OF PHYLOGENETIC RELATIONSHIPS

All Bayesian phylogenetic analyses converged by  $10^6$  generations and the consensus of 6400 post-burn-in trees revealed extremely high support for most phylogenetic relationships (Fig. 3). All clades are supported by a  $PP \geq 0.95$  except for the relationships between *Carlia* and *Saproscincus* ( $PP = 0.68$ ). Relationships among these genera were also unresolved in another analysis of these species based on much larger taxon and locus sampling (415 loci; Brandley et al. 2015), but uncertainty at this level is unlikely to influence our comparative analyses, as these are based on relative branch lengths. We do not discuss the phylogenetic results further other than to emphasize that the phylogenetic framework underlying all subsequent statistical comparative analyses is reasonably robust.

### DIFFERENCES IN THERMAL MICRO- AND MACROHABITAT

The sampling localities for this study spanned an altitudinal range of  $\sim 1500$  m and, correspondingly, thermal macrohabitat (i.e., among-site variation) varied substantially. Mean minimum temperatures (representing lowest overnight temperatures) ranged from  $11.6^\circ\text{C}$  at high elevation (AU—1600 m) to  $22.7^\circ\text{C}$  near sea level (CU—100 m). Mean daytime maximum temperatures varied more than overnight minima, and ranged across localities from  $14.5^\circ\text{C}$  (AU—1600 m) to  $28.5^\circ\text{C}$  (AU 400).

In addition to marked variation in thermal macrohabitat in the AWT, we also found clear differences in basking tendencies and thermal microhabitat use by skinks within sites (Table 1). Although *C. rubrigularis*, *L. coggeri*, and *L. robertsi* almost always used sunlit perches, regardless of elevation, *G. queenslandiae*, *S. basiliscus*, and *S. czechurae* were more often observed in shaded habitat (Table 1). These results are exceedingly robust given that ecological surveys were conducted under various weather conditions and across different years—regardless of ambient conditions, the differences in microhabitat use persisted among taxa. This difference in sun use translated into basking species occupying warmer thermal microhabitats: Perch temperature ( $T_{surf}$ ) was significantly lower (phylogenetic analysis of variance [ANOVA]:  $F = 45.118$ , degrees of freedom [df] = 1,19,  $P = 0.031$ ) in the shade-dwelling species, on average  $7.2^\circ\text{C}$  cooler than those of the basking species (Table 1). Given the relationships among these

taxa (Fig. 3), we can be confident there was at least one evolutionary shift in basking behavior in these lizards.

### PHYSIOLOGICAL DIVERGENCE ACROSS THERMAL CLINES

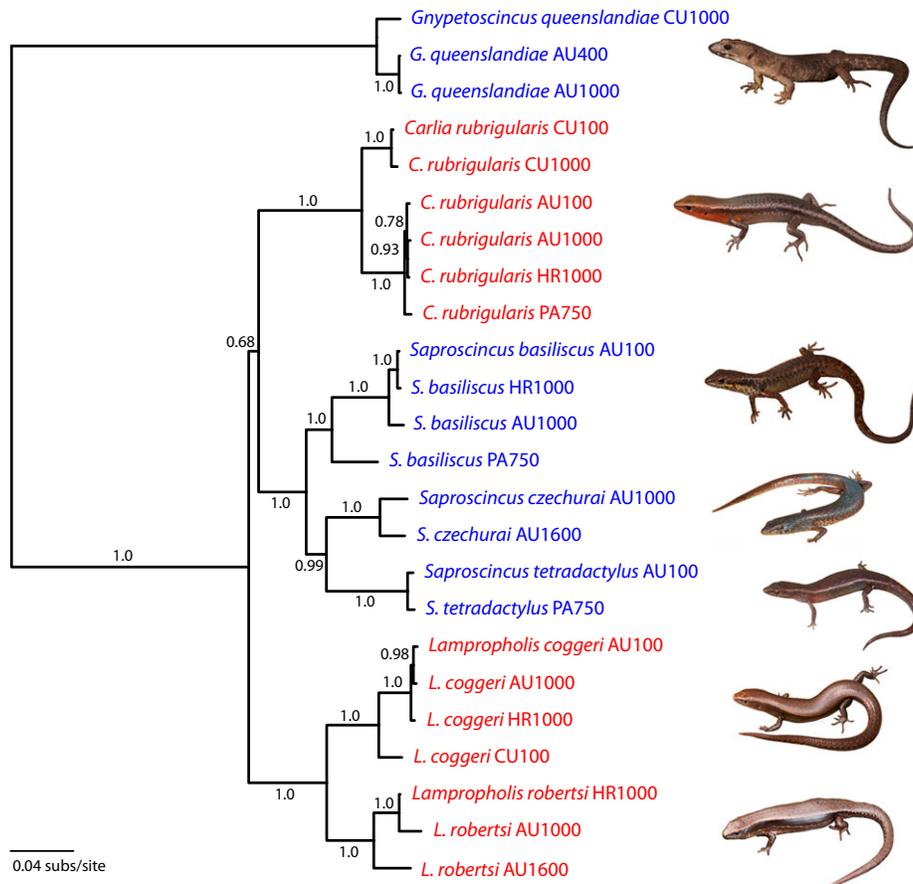
Cold tolerance ( $CT_{min}$ ) and performance breadth ( $B95$ ) each correlated strongly with minimum environmental temperatures, though in different directions. As predicted, lizards from higher elevations were more cold tolerant and also had a wider performance breadth (Table 2; Fig. 4). The differences were substantial: values at 100 m were up to  $11^\circ\text{C}$  higher than at 1000 m for  $CT_{min}$ , and  $8^\circ\text{C}$  lower for  $B95$ . Unexpectedly, the optimal sprinting temperature ( $T_{opt}$ ) was inversely correlated with mean maximum environmental temperature, indicating that lizards from warmer habitats are specialized to sprint at cooler temperatures than their montane counterparts (Table 2; Fig. 4). By contrast, heat tolerance,  $CT_{max}$ , did not vary significantly with elevation (Table 2; Fig. 4).

Although  $CT_{max}$  did not shift across elevation, it did vary substantially between basking and shade-dwelling species, whether basking behavior was treated as a discrete or continuous trait (Table 2; Fig. 4). Specifically, the basking taxa, *Carlia* and *Lampropholis*, were considerably more heat tolerant (range among population means =  $41.8$ – $43.4^\circ\text{C}$ ) than the shade skinks, *Gnypetoscincus* and *Saproscincus* (range =  $36.3$ – $39.0^\circ\text{C}$ ). Hence, basking behavior greatly influenced the magnitude of heat tolerance, whereas thermal differences among sites exerted little to no effect at all. Furthermore, the evolution of basking behavior (treated as a binary trait) was best represented as a multi-peak OU model in which baskers and shade-dwellers evolved toward distinct heat tolerance optima ( $37^\circ\text{C}$  for shade skinks and  $43^\circ\text{C}$  for basking species; Table 3). Unlike heat tolerance, basking behavior had no effect on the relationships between  $T_{opt}$ ,  $B95$ , and  $CT_{min}$  and environmental temperature (basking behavior term  $P > 0.05$ ).

## Discussion

### PATTERNS AND MECHANISMS OF PHYSIOLOGICAL EVOLUTION

Evolutionary patterns of physiology are important for understanding organisms' potential response to the constraints imposed by climate change (Chown et al. 2010). However, exposure to climate-related selection is altered by behavior, so that both behavioral and physiological phenotypes should be considered jointly (Helmuth et al. 2005; Huey et al. 2012; Muñoz and Moritz 2016). By synthesizing information on basking behavior, environment, physiology, and genetics in seven skink species across steep altitudinal gradients in the AWT, we confirm that physiological traits respond to different parameters. Although  $CT_{min}$ ,  $T_{opt}$ , and  $B95$  shifted with elevation (thermal macrohabitat) (Fig. 2C), variation



**Figure 3.** Phylogenetic relationships of taxa in this study based on Bayesian analysis of four loci. Site localities are described in Table 1. Basking species are shown in red and shade dwellers in blue. Numbers above or below branches are clade posterior probabilities. Photos were taken by Stephen Zozaya.

**Table 2.**  $CT_{min}$ ,  $T_{opt}$ , and  $B95$  are best predicted by thermal environment, whereas  $CT_{max}$  is best predicted by basking behavior.

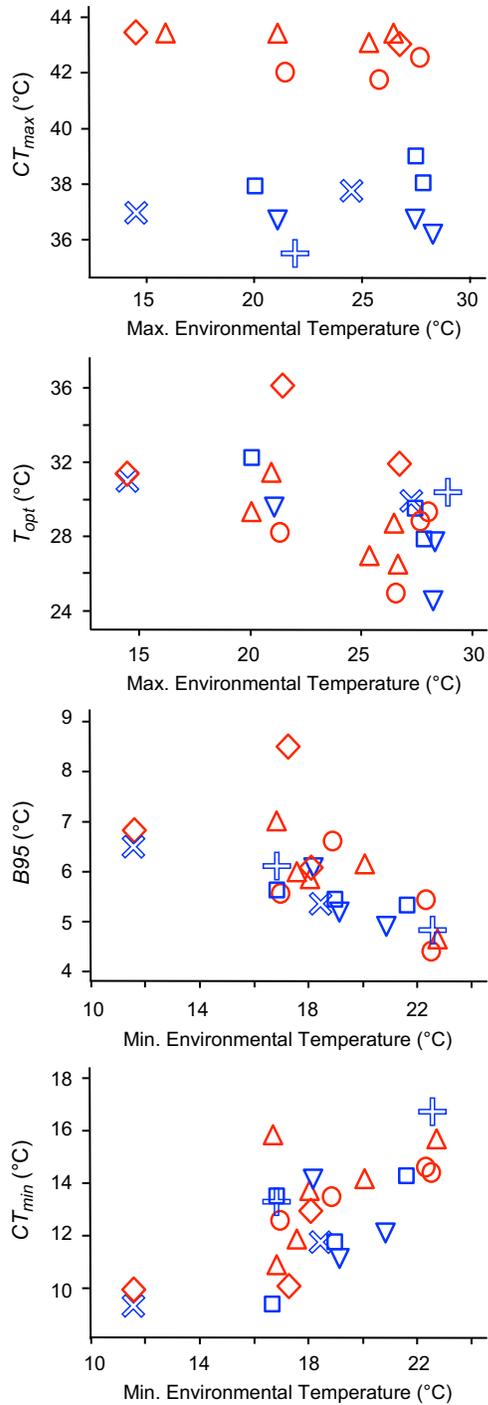
Dependent Variable(s)	Temperature			Temperature + Behavior (Continuous)			Temperature + Behavior (Discrete)		
	Coefficient ± std. error	AIC <sub>C</sub>	P	Coefficient ± std. error	AIC <sub>C</sub>	P	Coefficient ± std. error	AIC <sub>C</sub>	P
<i>CT<sub>min</sub> + B95</i>	<i>0.549 ± 0.077</i>	<i>52.899</i>	<i>&lt;0.001</i>	0.251 ± 0.522	55.536	0.636	0.187 ± 0.488	55.637	0.706
<i>T<sub>opt</sub></i>	<i>-0.337 ± 0.122</i>	<i>76.411</i>	<i>0.014</i>	-0.629 ± 1.010	78.865	0.543	-0.739 ± 0.943	78.603	0.446
<i>CT<sub>max</sub></i>	-0.007 ± 0.053	54.572	0.901	5.395 ± 0.568	50.179	<0.001	<i>4.992 ± 0.509</i>	<i>34.434</i>	<i>&lt;0.001</i>

PGLS models were fit comparing physiological traits to environmental temperature (left) and environmental temperature + basking behavior (center and right panels). For analyses including behavior, the coefficients for the additional behavioral term are given. As described in the Methods, when treated as a continuous variable, basking behavior is represented as the arcsine square-root transformed proportion of basking observations during ecological surveys. Based on the hypotheses described in the text,  $CT_{min}$  and  $B95$  were regressed against the mean minimum environmental temperature of the month prior to capture, whereas  $T_{opt}$  and  $CT_{max}$  were regressed against the mean maximum environmental temperature of the month prior to capture. The best-supported models (based on AIC<sub>C</sub> score) are shown in italics.

in  $CT_{max}$  was driven by differences in basking behavior (thermal microhabitat), with little to no effects due to elevation (Fig. 2B).

One possibility is that the physiological variation among lizards that we observed was due to environment-induced plasticity, rather than genetic differences. However, the range of variation observed for these traits was much greater than that typically induced through experimental acclimation. Targeted ecological

studies within lizard species (e.g., Kolbe et al. 2012; Muñoz et al. 2014a; Phillips et al. 2016) and broader meta-analyses of acclimation (Gunderson and Stillman 2015) all find that physiological traits typically only shift by 1–2°C in response to acute or sustained environmental shifts (but see meta-analysis of  $CT_{min}$  acclimation in Pintor et al. 2016), whereas our measurements often differed by up to 12°C (Fig. 4). This indicates that, while plasticity



**Figure 4.** Population means for critical thermal minimum ( $CT_{min}$ ), the optimal performance range ( $B95$ ), the optimal sprinting temperature ( $T_{opt}$ ), and the critical thermal maximum ( $CT_{max}$ ). The x-axis denotes mean maximum environmental temperature for  $CT_{max}$  and  $T_{opt}$  and mean minimum environmental temperature for  $CT_{min}$  and  $B95$ . Species are denoted in different colors and shapes as follows: *Carlia rubrigularis*, circle; *Gnypetoscincus queenslandiae*, inverse triangle; *Lampropholis coggeri*, triangle, *Lampropholis robertsi*, diamond; *Saproscincus basiliscus*, square; *S. czechurae*, x mark; *S. tetradactyla*, cross. Basking species are shown in red and shade dwellers in blue.

can be expected to contribute, there is likely a genetic basis for much of the variation observed among lizards.

Previous studies on closely related ectotherms generally report stable heat tolerances (Hoffmann 2010; Sunday et al. 2011; Kellermann et al. 2012; but see Gilchrist and Huey 1999). However, among the lizard taxa we sampled,  $CT_{max}$  varied considerably, ranging from 35.1°C (*S. czechurae*—AU 1000) to 45.2°C (*C. rubrigularis*—AU 1000). This range—more than 10°C—is particularly remarkable given that those lizards were from the same locality. The differences in heat tolerance that we observe between sympatric lizards are usually observed over substantially greater geographic scales or across biomes (e.g., between temperate and tropical taxa; Huey et al. 2009).

Heat tolerance evolved toward two distinct optima, with edge habitat species (*Carlia* and *Lampropholis*) exhibiting considerably higher heat tolerance ( $\sim 6^\circ\text{C}$ ) than the shade specialists (*Gnypetoscincus* and *Saproscincus*). Nonetheless, we found no additional effects of local thermal environment on  $CT_{max}$ , indicating that heat tolerance evolution largely occurred when species behaviorally specialized to a specific microhabitat—forest interior or canopy gaps/forest edges. The evolutionary differences in heat tolerance between shade skinks and baskers emphasize that tropical landscapes, though more thermally stable than temperate habitats, provide sufficient within-site thermal heterogeneity for marked physiological trait specialization.

Although basking behavior exerted a strong influence on  $CT_{max}$ , it had no clear effects on any of the other traits. Rather, thermal macroenvironment was the primary driver of variation in  $CT_{min}$ ,  $T_{opt}$ , and  $B95$ . All lizards, regardless of basking behavior, were more cold tolerant in cooler (higher elevation) habitats. This finding is broadly concordant with a variety of geographic studies on ectotherms. Interspecific studies, for example, have demonstrated that  $CT_{min}$  exhibits considerably more geographic variation than  $CT_{max}$  (Sunday et al. 2011; Araújo et al. 2013), lower phylogenetic signal (Kellermann et al. 2012), and faster rates of evolution than other physiological traits (Muñoz et al. 2014a). Basking species and shade dwellers alike are confronted with cool, thermally stable conditions at night, which greatly limit their ability to thermoregulate efficiently (Ghalambor et al. 2006; Muñoz et al. 2014a). In the absence of behavioral refuges from the cold, lizards have no option but to adjust their physiology. As organisms become more cold tolerant (while also remaining equally heat tolerant), the performance curve becomes progressively more left-skewed, explaining why performance breadth is considerably greater in montane habitats and why  $CT_{min}$  and  $B95$  were strongly correlated.

In addition to more constricted performance breadths, lizards in warm environments also had lower optimal sprinting temperatures ( $T_{opt}$ ). This pattern, which we observe in a broad interspecific study, is supported by a detailed interpopulational study in *L.*

**Table 3.** Model comparisons for heat tolerance ( $CT_{max}$ ) evolution.

	AIC <sub>C</sub>	$\theta$	$\sigma^2$	Log likelihood
BM	65.90529	NA	15.912	-30.553
OU single peak	69.54214	39.138	19.621	-30.914
OU multi peak	<b>50.12621</b>	43.006, 37.146	18.659	-19.525

For each model, the AIC<sub>C</sub> score is given, along with  $\theta$  (optimal trait values),  $\sigma^2$  (Brownian motion rate parameter), and the log-likelihood. Bold indicates the best-supported model.

*coggeri* (Llewelyn et al. 2016). One possibility is that counter-gradient selection is occurring, such that lizards in cooler habitats exhibit greater behavioral sensitivity to temperature (e.g., Schultz et al. 1996; Laugen et al. 2003). The inverse relationship between  $T_{opt}$  and environmental temperature may thus be a by-product of reduced surface activity in hotter environments. At low elevation, lizards are at much greater risk of overheating than freezing and should be expected to alter their activity patterns to avoid heat stress (Kearney et al. 2009). Recent empirical work on Honduran *Anolis* lizards by Logan et al. (2015) supports this idea—whereas lizards became less active when temperatures exceeded  $T_{opt}$ , activity patterns were unrelated to temperatures when the habitat was cooler than  $T_{opt}$ . Similarly, Vickers et al. (2011) found that *Carlia* skinks thermoregulated most effectively during summer months, when environmental temperatures were highest (and, therefore, the risk of overheating was greatest). In a recent meta-analysis of various lizard species, Huey et al. (2012) also detected an inverse (though nonsignificant) correlation between maximum summer temperature and optimal sprinting speed. Fine-scale studies of thermoregulatory behavior across thermal gradients would help resolve the mechanism(s) underlying this physiological pattern.

The sampling strategy employed here maximized the number of populations sampled, and focused on capturing physiological variation across phylogenetic splits and geographic clines. Despite relatively modest sample sizes within populations, we feel that our sampling strategy accurately captured the range of physiological trait variation, particularly because of the high trait variation observed (Table 1; Fig. 4), and because we focused on sampling across the altitudinal ranges of species (Bonino et al. 2011; Muñoz et al. 2014a). Nonetheless, more detailed intraspecific studies would be useful to further explore the relationships described here. For example, in a detailed study of *L. coggeri*, Llewelyn et al. (2016) found extensive among-population variation in  $CT_{min}$  and considerably less among-population variation for  $CT_{max}$ , a pattern concordant with the results presented here.

## BEHAVIOR AND PHYSIOLOGY INFLUENCE VULNERABILITY TO CLIMATE CHANGE

By examining the physiological traits of ectotherms from an evolutionary perspective, we can predict their potential for adaptive response to rising temperatures, and hence vulnerability to future climate change (Williams et al. 2008; Huey et al. 2012). Our empirical data underscore the especially high vulnerability of the shade-specialist lizards, *Gnypetoscincus* and *Saproscincus*, due to their low heat tolerance as compared to edge habitat species. These results indicate that even in tropical rainforests, which tend to be relatively thermally stable habitats, there is enough environmental heterogeneity for even closely related species to differ substantially in their vulnerability to climate change (discussed in Huey et al. 2009).

The shifts we observed in all species for  $CT_{min}$ ,  $T_{opt}$ , and  $B95$  across elevation suggest high evolutionary lability in these traits. It is important to note, however, that an accelerated evolutionary rate—a process often inferred from such relationships—may in fact reflect a number of different possible evolutionary processes (Revell et al. 2008). Whatever the cause, it is clear that these traits shift strongly across thermal gradients, due to genetic changes, plastic shifts, or a combination of both, whereas  $CT_{max}$  does not.

The fact that  $CT_{max}$  does not shift with elevation may suggest that this trait is unable to respond adaptively, or to do so fast enough to meet the predicted pace of environmental warming. Stability in  $CT_{max}$  is a general pattern observed across a variety of ectotherms (Araújo et al. 2013), and is alarming because the imminent challenge facing such organisms is to avoid overheating. In the case of these rainforest skinks, once lizards adapt to a given thermal microhabitat, heat tolerance remains stable. Although rigidity in  $CT_{max}$  suggests a limited capacity for lizards to adaptively respond to warming, plasticity in thermal traits is predicted to confer greater resilience in the face of rising temperatures (Seebacher et al. 2015). Heat hardening, or a rapid, transient increase in  $CT_{max}$  in response to heat shock, may provide physiological buffering against greater and more intense heat waves. Indeed, Phillips et al. (2016) found that one of our target species, *L. coggeri*, exhibits a marked heat hardening response. However, they also found that organisms in environments that are already approaching thermal limits have a reduced capacity to shift heat tolerance, which has also been observed in flies (Kristensen et al. 2015) and crabs (Stillman 2003). Hence, either through a limited ability to shift heat tolerance, heat hardening, or both, ectotherms are likely to be confronted with increasingly hostile thermal environments.

Examining intraspecific variation in physiology across altitudinal clines revealed patterns that would not have been evident by averaging species' means alone (Muñoz et al. 2014b). Our finding that  $T_{opt}$  is higher in montane populations was not predicted a priori (although there is substantial theoretical

support), and provides an unexpected source of adaptive diversity and promising new options for mitigation strategies, such as assisted migration (Aitken and Whitlock 2013). Because the relationship between  $T_{opt}$  and thermal environment is known to vary in many ways among species (reviewed in Angilletta 2009), it is only through targeted intraspecific studies that we can discover how adaptive diversity in this trait is distributed across species' ranges.

Consistent with what other studies have posited in various geographic regions (Kearney et al. 2009; Sinervo et al. 2010; Sunday et al. 2014), simple extrapolations to future climate scenarios for the Wet Tropics indicate that maximum temperatures could exceed  $T_{opt}$  of these lizards in lowland environments (e.g., Table S7), and so challenge the persistence of these populations. The shade skinks are mostly active early mornings and evenings (C. Moritz and S. E. Williams pers. obs.), and in the warmer months basking species such as *Carlia* also become more crepuscular (Vickers et al. 2011). Whether these species will shift their diurnal rhythms and structural habitat use remains to be explored, and requires microclimatic measurements to capture the full effects of behavior on energy budgets (Kearney et al. 2009). We further point out that rising temperatures represent only one dimension of climate warming—other key factors, such as shifts in humidity and cloud cover, could also stress ectotherms, particularly those from tropical environments (e.g., Pounds et al. 1999; Vickers 2014).

By examining variation both within and among species, we were able to reveal patterns in basking behavior and their effect on heat tolerance evolution. Intraspecific analyses revealed clinal variation in  $CT_{min}$ ,  $T_{opt}$ , and  $B95$  that would be absent at the species level. Conversely, interspecific analyses revealed marked differences in  $CT_{max}$  due to basking behavior that would not have been apparent by examining clinal variation within species. Such patterns reveal how ecologically relevant phenotypic diversity is distributed across species' ranges. Hence, understanding the potential impacts of climate change on reptiles requires a more detailed consideration of how behavioral and physiological phenotypes interact, and is best accomplished by studies integrating information both within and among species.

#### ACKNOWLEDGMENTS

We thank C. Storlie, and B. Phillips for assistance with analyses. R. Huey, D. Miles, M. Sears, A. Gunderson, P. Cooper, and G. Bakken provided useful comments and feedback on this manuscript. Funding was provided by a National Science Foundation postdoctoral fellowship (GML) and the Australian Research Council (CM). Fieldwork support was provided by Earthwatch Institute Australia and the Tropical Ecosystems National Environmental Research Program. Intersect Australia Ltd. provided high performance computing resources. Use of animals in this study was approved by the University of California, Berkeley IACUC (Moritz\_278) and the Australian National University (A2013-08). Fieldwork and physiological experiments in the Wet Tropics were conducted under a permit

from the Queensland Department of Environment and Resource Management to SEW (WITK05468508). The authors declare no conflict of interest.

#### LITERATURE CITED

- Aitken, S. N., and M. C. Whitlock. 2013. Assisted gene flow to facilitate local adaptation to climate change. *Ann. Rev. Ecol. Evol. Syst.* 44:367–388.
- Angilletta, M. J. Jr. 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford Univ. Press, Oxford, U.K.
- Araújo, M. B., F. Ferri-Yáñez, F. Bozinovic, P. A. Marquet, F. Valladares, and S. L. Chown. 2013. Heat freezes niche evolution. *Ecol. Lett.* 16:1206–1219.
- Barnosky, A. D., N. Matzke, S. Tomiya, W. O. U. Wogan, B. Swartz, T. B. Quental, C. Marshall, J. L. McGuire, E. L. Linsley, K. C. Maguire, et al. 2011. Has the Earth's sixth mass extinction already arrived? *Nature* 471:51–57.
- Blomberg, S. P., T. Garland, and A. R. Ives. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57:717–745.
- Bonino, M. F., D. L. Moreno Azócar, M. J. Tulli, C. S. Abdala, M. G. Perotti, and F. B. Cruz. 2011. Running in cold weather: morphology, thermal biology, and performance in the southernmost lizard clad in the world (*Liolaemus lineomaculatus* section: Liolaemini: Iguania). *J. Exp. Zool.* 315:495–503.
- Brandley, M. C., Y. Wang, X. Guo, A. Nieto Montes de Oca, M. Fería Ortíz, T. Hikida, and H. Ota. 2011. Accommodating locus-specific heterogeneity in molecular dating methods: an example using inter-continental dispersal of *Plestiodon* (*Eumeces*) lizards. *Syst. Biol.* 60:3–15.
- Brandley, M. C., J. G. Bragg, D. G. Chapple, C. K. Jennings, A. R. Lemmon, E. M. Lemmon, S. Singhal, M. B. Thompson, and C. Moritz. 2015. Evaluating the performance of anchored hybrid enrichment at the tips of the tree of life: a phylogenetic analysis of Australian *Eugongylus* group scincid lizards. *BMC Evol. Biol.* 15:62. doi:10.1186/s12862-015-0318-0.
- Buckley, L. B., and R. B. Huey. 2016. Temperature extremes: geographic patterns, recent changes, and implications for organismal vulnerabilities. *Global Change Biol.* doi:10.1111/gcb.13313.
- Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. 2nd ed. Springer Verlag, New York.
- Butler, M. A., and A. A. King. 2004. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. *Am. Nat.* 164:683–695.
- Chown, S. L., A. A. Hoffmann, T. N. Kristensen, M. J. Angilletta Jr., N. C. Stenseth, and C. Pertoldi. 2010. Adapting to climate change: a perspective from evolutionary physiology. *Clim. Res.* 43:3–15.
- Deutsch, C. A., J. J. Tewksbury, R. B. Huey, K. S. Sheldon, C. K. Ghalambor, D. C. Haak, and P. R. Martin. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. USA* 105:6668–6672.
- Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and throughput. *Nucleic Acids Res.* 32:1792–1797.
- Ghalambor, C. K., R. B. Huey, P. R. Martin, J. J. Tewksbury, and G. Wang. 2006. Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integr. Comp. Biol.* 46:5–17.
- Gilchrist, G. W., and R. B. Huey. 1999. The direct response of *Drosophila melanogaster* to selection on knock-down temperature. *Heredity* 83:15–29.
- Gunderson, A. R., and J. H. Stillman. 2015. Plasticity in thermal tolerances has limited potential to buffer ectotherms from global warming. *Proc. R. Soc. B* 282:20150401.

- Helmuth, B., J. G. Kingsolver, and E. Carrington. 2005. Biophysics, physiological ecology, and climate change: does mechanisms matter? *Annu. Rev. Physiol.* 67:177–201.
- Hertz, P. E., R. B. Huey, and E. Nevo. 1983. Homage to Santa Anita: thermal sensitivity of sprint speed in agamid lizards. *Evolution* 37:1075–1084.
- Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* 25:1965–1978.
- Hoffmann, A. A. 2010. Physiological climatic limits in *Drosophila*: patterns and implications. *J. Exp. Biol.* 213:870–880.
- Hoffmann, A. A., and C. M. Sgrò. 2011. Climate change and evolutionary adaptation. *Nature* 470:479–485.
- Hoffmann, A. A., S. L. Chown, and S. Clusella-Trullas. 2013. Upper thermal limits in terrestrial ectotherms: how constrained are they? *Funct. Ecol.* 27:934–949.
- Huelsenbeck, J. P., and B. Rannala. 2004. Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. *Syst. Biol.* 53:904–913.
- Huey, R. B., and E. R. Pianka. 2007. Lizard thermal biology: do genders differ? *Am. Nat.* 170:473–478.
- Huey, R. B., and R. D. Stevenson. 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *Integr. Comp. Biol.* 19:357–366.
- Huey, R. B., P. E. Hertz, and B. Sinervo. 2003. Behavioral drive versus behavioral inertia in evolution: a null model approach. *Am. Nat.* 161:357–366.
- Huey, R. B., C. A. Deutsch, J. J. Tewksbury, L. J. Vitt, P. E. Hertz, H. J. Alvarez Perez, and T. Garland Jr. 2009. Why tropical forest lizards are vulnerable to climate warming. *Proc. R. Soc. B* 276:1939–1948.
- Huey, R. B., M. R. Kearney, A. Krockenberger, J. A. M. Holtum, M. Jess, and S. E. Williams. 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Phil. Trans. R. Soc. B* 367:1665–1679.
- Hughes, L. 2003. Climate change and Australia: trends, projections and impacts. *Aus. Ecol.* 28:423–443.
- Kearney, M., R. Shine, and W. P. Porter. 2009. The potential for behavioral thermoregulation to buffer “cold-blooded” animals against climate warming. *Proc. Natl. Acad. Sci. USA* 106: 3835–3840.
- Kellermann, V., V. Loeschke, A. A. Hoffmann, T. N. Kirstensen, C. Fløjgaard, J. R. David, J.-C. Svenning, and J. Overgaard. 2012. Phylogenetic constraints in key functional traits behind species’ climate niches: patterns of desiccation and cold resistance across 95 *Drosophila* species. *Evolution* 66:3377–3389.
- King, A. A., and M. A. Butler. 2009. Ouch: Ornstein–Uhlenbeck models for phylogenetic comparative hypotheses (R package). Available at <http://ouch.r-forge.r-project.org>.
- Kolbe, J. J., P. S. VanMiddlesworth, N. Losin, N. Dappen, and J. B. Losos. 2012. Climatic niche shift predicts thermal trait response in one but not both introductions of the Puerto Rican lizard *Anolis cristatellus* to Miami, Florida, USA. *Ecol. Evol.* 2:1503–1516.
- Kristensen, T. N., J. Overgaard, J. Lassen, A. A. Hoffmann, and C. Sgrò. 2015. Low evolutionary potential for egg-to-adult viability in *Drosophila melanogaster* at high temperatures. *Evolution* 69:803–814.
- Labra, A., J. Pienaar, and T. F. Hansen. 2009. Evolution of thermal physiology in *Liolaemus* lizards: adaptation, phylogenetic inertia, and niche tracking. *Am. Nat.* 174:204–220.
- Lanfear, R., B. Calcott, S. Y. W. Ho, and S. Guindon. 2012. Partition finder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol. Biol. Evol.* 29:1695–1701.
- Laugen, A. T., A. Laurila, K. Rasanen, and J. Merila. 2003. Latitudinal counter-gradient variation in the common frog (*Rana temporaria*) development rates—evidence for local adaptation. *J. Evol. Biol.* 16:996–1005.
- Leal, M., and A. R. Gunderson. 2012. Rapid change in the thermal tolerance of a tropical lizard. *Am. Nat.* 180:815–822.
- Llewelyn, J., S. Macdonald, A. Hatcher, C. Moritz, and B. L. Phillips. 2016. Intraspecific variation in climate-relevant traits in a tropical rainforest lizard. *Divers. Distrib.* 22:1000–1012. doi:10.1111/ddi.12466.
- Logan, M. L., R. M. Cox, and R. Calsbeek. 2014. Natural selection on thermal performance in a novel thermal environment. *Proc. Natl. Acad. Sci. USA* 111:14165–14169.
- Logan, M. L., S. G. Fernandez, and R. Calsbeek. 2015. Abiotic constraints on the activity of tropical lizards. *Funct. Ecol.* 29:694–700.
- Martins, E. P., and T. F. Hansen. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am. Nat.* 149:646–667.
- Matsui, T., K. Matsumoto, Y. Hijioka, T. Kinoshita, T. Nozawa, S. Ishiwatari, E. Kato, P. R. Shukla, Y. Yamagata, and M. Kainuma. 2011. An emission pathway for stabilization at 6 Wm<sup>-2</sup> radiative forcing. *Climatic Change* 109:59–76.
- Moritz, C., C. Hoskin, J. MacKenzie, B. Phillips, M. Tonione, N. Silva, J. VanDerWal, S. Williams, and C. Graham. 2009. Identification and dynamics of a cryptic suture zone in tropical rainforest. *Proc. R. Soc. B* 276:1235–1244.
- Moritz, C., G. Langham, M. Kearney, A. Krockenberger, J. VanDerWal, and S. E. Williams. 2012. Integrating phylogeography and physiology reveals divergence of thermal traits between central and peripheral lineages of tropical rainforest lizards. *Phil. Trans. R. Soc. B* 367:1680–1687.
- Muñoz, M. M., and C. Moritz. 2016. Adaptation to a changing world: evolutionary resilience to climate change. *In: J. B. Losos, and R. E. Lenski, eds. How evolution shapes our lives: essays on biology and society.* Princeton Univ. Press, Princeton, NJ.
- Muñoz, M. M., M. A. Stimola, A. C. Algar, A. E. Conover, A. Rodriguez, M. A. Landestoy, G. S. Bakken, and J. B. Losos. 2014a. Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. *Proc. R. Soc. B* 281:20132433.
- Muñoz, M. M., J. E. Wegener, and A. C. Algar. 2014b. Untangling intra- and interspecific effects on body size clines reveals divergent processes structuring convergent patterns. *Am. Nat.* 184:636–646.
- Pagel, M. D. 1999. Inferring the historical patterns of biological evolution. *Nature* 401:877–884.
- Paradis, E. 2006. *Analysis of phylogenetics and evolution with R.* Springer, New York, NY.
- Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Syst.* 37:637–669.
- Phillips, B. L., M. M. Muñoz, A. Hatcher, S. L. Macdonald, J. Llewelyn, V. Lucy, and C. Moritz. 2016. Heat hardening in a tropical lizard: geographic variation explained by the predictability and variance in environmental temperatures. *Funct. Ecol.* 30:1161–1168.
- Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2016. nlme: linear and nonlinear mixed effects models. R package version 3.1–128.
- Pintor, A. F. V., L. Schwarzkopf, and A. K. Krockenberger. 2016. Extensive acclimation in ectotherms conceals interspecific variation in thermal tolerance limits. *PLoS ONE* 11:e0150408.
- Pounds, J. A., M. P. L. Fogden, and J. H. Campbell. 1999. Biological response to climate change on a tropical mountain. *Nature* 398:611–615.
- R Development Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rambaut, A., and A. J. Drummond. 2007. Tracer v1.4. Available at: <http://beast.bio.ed.ac.uk/Tracer>: 0.004472.
- Revell, L. J. 2010. Phylogenetic signal and linear regression on species data. *Methods Ecol. Evol.* 1:319–329.
- Revell, L. J., L. J. Harmon, and D. C. Collar. 2008. Phylogenetic signal, evolutionary process, and rate. *Syst. Biol.* 57:591–601.

- Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61:539–542.
- Sarmiento, G. 1986. Ecological features of climate in high tropical mountains. Pp. 11–46 in F. Vuilleumier, and M. Monasterio, eds. *High altitude tropical biogeography*. Oxford Univ. Press, New York, NY.
- Schultz, E. T., K. E. Reynolds, and D. O. Conover. 1996. Countergradient variation in growth among newly hatched *Fundulus heteroclitus*: geographic differences revealed by common-environment experiments. *Funct. Ecol.* 10:366–374.
- Seebacher, F., C. R. White, and C. E. Franklin. 2015. Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim. Chang.* 5:61–66.
- Shoo, L. P., S. E. Williams, and J.-M. Hero. 2005. Climate warming and the rainforest birds of the Australian Wet Tropics: using abundance data as a sensitive predictor of change in total population size. *Biol. Cons.* 125:335–343.
- Sinervo, B., F. Méndez-De-La-Cruz, D. B. Miles, B. Heulin, E. Bastiaans, M. V.-S. Cruz, R. Lara-Resendiz, N. Martínez-Méndez, M. L. Calderón-Espinosa, R. N. Meza-Lázaro et al. 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328:894–899.
- Spellerberg, I. F. 1972. Temperature tolerances of southeast Australian reptiles examined in relation to reptile thermoregulatory behavior and distribution. *Oecologia* 9:23–46.
- Stillman, J. H. 2003. Acclimation capacity underlies susceptibility to climate change. *Science* 301:65.
- Storlie, C., A. Merino-Viteri, B. Phillips, J. VanDerWal, J. Welbergen, and S. Williams. 2014. Stepping inside the niche: microclimate data are critical for accurate assessment of species' vulnerability to climate change. *Biol. Lett.* 10:20140576.
- Storlie, C. J., B. L. Phillips, J. J. VanDerWal, and S. E. Williams. 2013. Improved spatial estimates of climate predict patchier species distributions. *Divers. Distrib.* 19:1106–1113.
- Sugihara, N. 1978. Further analysis of the data by Akaike's information criterion and the finite corrections. *Commun. Stat. Theory Methods A* 7:13–26.
- Sunday, J. M., A. E. Bates, and N. K. Dulvy. 2011. Global analysis of thermal tolerance and latitude in ectotherms. *Proc. R. Soc. B* 278:1823–1830.
- Sunday, J. M., A. E. Bates, M. R. Kearney, R. K. Colwell, N. K. Dulvy, J. T. Longino, and R. B. Huey. 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proc. Natl. Acad. Sci. USA* 111:5610–5615.
- Thomas, C. D., A. Cameron, R. E. Green, M. Bakkenes, L. J. Beaumont, Y. C. Collingham, B. F. N. Erasmus, M. Ferreira de Siqueira, A. Grainger, L. Hannah, et al. 2004. Extinction risk from climate change. *Nature* 427:145–148.
- Van Berkum, F. H. 1988. Latitudinal patterns of the thermal sensitivity of sprint speed in lizards. *Am. Nat.* 132:327–343.
- Vickers, M. 2014. Thermoregulation in tropical lizards. PhD dissertation. School of Marine and Tropical Biology, James Cook University, Townsville, Australia.
- Vickers, M., C. Manicom, and L. Schwarzkopf. 2011. Extending the cost-benefit model of thermoregulation: high-temperature environments. *Am. Nat.* 177:452–461.
- Williams, S. E., E. E. Bolitho, and S. Fox. 2003. Climate change in Australian tropical rainforests: an impending environmental catastrophe. *Proc. R. Soc. B* 270:1887–1892.
- Williams, S. E., L. P. Shoo, J. L. Isaac, A. A. Hoffmann, and G. Langham. 2008. Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biol.* 6:2621–2626.
- Williams, S. E., J. VanDerWal, J. Isaac, L. P. Shoo, C. Storlie, S. Fox, E. E. Bolitho, C. Moritz, C. J. Hoskin, and Y. M. Williams. 2010. Distributions, life-history specialization, and phylogeny of the rain forest vertebrates in the Australian Wet Tropics. *Ecology* 91:2489.

Associate Editor: E. Derryberry  
Handling Editor: R. Shaw

## Supporting Information

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

- Table S1.** PGLS models comparing physiological traits to environmental temperature (left) and environmental temperature + basking behavior (right).
- Table S2.** Partitions for the molecular dataset used in the Bayesian phylogenetic analyses.
- Table S3.** Comparison of model likelihoods for PGLS analyses when different branch transformations are employed.
- Table S4.** Correlations among traits determined using regression of independent contrasts through the origin.
- Table S5.** Marginal likelihood values for ancestral state reconstruction of basking behavior for each node in the phylogeny.
- Table S6.** Extended model comparisons for heat tolerance ( $CT_{max}$ ) evolution.
- Table S7.** As a heuristic indicator of temperature change due to environmental warming, we determined the predicted increase in average daily maximum temperature for each site, using gridded climate data sourced from WorldClim (worldclim.org) at 30 arcsec (~900 m) resolution (Hijmans et al. 2005).