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Research

Thermophysiology, microclimates, and species distributions of lizards in the mountains of the Brazilian Atlantic Forest

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Thermophysiological traits, particularly thermal tolerances and sensitivity, are key to understanding how organisms are affected by environmental conditions. In the face of ongoing climate change, determining how physiological traits structure species' ranges is especially important in tropical montane systems. In this study, we ask whether thermal sensitivity in physiological performance restricts montane lizards to high elevations and excludes them from the warmer environments reported at low elevations. For three montane lizard species in the Brazilian Atlantic Forest, we collect thermophysiological data from lizards in the highest elevation site of each species' distribution, and ask how well the individuals exhibiting those traits would perform across the Atlantic Forest. We use microclimatic and organism-specific models to directly relate environmental conditions to an organism's body temperature and physiological traits, and estimate measures of thermophysiological performance. Our findings demonstrate that thermophysiological constraints do not restrict montane lizards to high elevations in this system, and thus likely do not determine the warm boundaries of these montane species' distributions. Results also suggest that competition may be important in limiting the warm boundaries of the species' ranges for two of the focal species. These experimental results suggest that caution should be used when claiming that physiology drives patterns of diversity and endemism within montane environments. They also highlight the importance of interdisciplinary experimental studies that bridge the fields of evolution and ecology to improve predictions of biological responses to future environmental shifts.

Keywords: species distributions, thermophysiology, montane lizards

Introduction

Understanding how physiology defines species distributions and responses to climate change is central to evolutionary ecology and conservation (Kearney and Porter 2009). In montane systems, and especially in the tropics, physiology becomes a particularly relevant topic. Tropical mountains play a central role in the generation and maintenance



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of biodiversity over evolutionary time by isolating and harboring montane populations over long periods (McCain and Colwell 2011, Graham et al. 2014). Because the limited seasonality of lowland tropical regions is expected to result in narrow thermal tolerances for species in these areas, tropical mountains also act as important thermal barriers to dispersal, and thus agents of isolation and diversification, for lowland species (Janzen 1967). Still, present-day communities at higher elevations are regarded as highly threatened by anthropogenic climate shifts: population isolation and further contraction due to climate warming may lead to local extinction (La Sorte and Jetz 2010, McCain and Colwell 2011), and the distributional shifts upslope of species previously restricted to lower elevation environments is expected to impose additional biotic pressures on montane communities (Colwell et al. 2008, Gilman et al. 2010, Sinervo et al. 2010, Urban and Zarnetske 2013).

Despite high endemism in montane environments globally (Kier et al. 2009), we still have a poor understanding of what factors drive habitat restriction in montane species. Are tropical montane species presently trapped in mountaintop, cooler environments due to narrow thermal tolerances and an inability to function at warmer, lowland temperatures? While this question has implications for understanding patterns of species' distributions in the past (Graham et al. 2010), present (McCann et al. 2014), and into the future (Moritz and Agudo 2013), few studies have incorporated physiological data to directly assess potential drivers of species' distributions (Kearney and Porter 2009, Buckley et al. 2010, Tingley et al. 2014).

The topographically complex Atlantic forests of Brazil are one case in point. In this system, phylogeographic and population genetic analyses have suggested that the ability of species to respond to climate change explains current and former species ranges, and contribute to patterns of genetic diversity and population dynamics (Carnaval et al. 2009, 2014). Specifically, genetic signatures of range and population contraction for lowland species during the Last Glacial Maximum (LGM), and subsequent expansion (Carnaval et al. 2009, de Mello Martins 2011), suggest that these species track warm environments. In contrast, data from montane species indicate population and range expansions during the LGM and post-LGM contractions (Amaro et al. 2012, Carnaval et al. 2014, Leite et al. 2016), suggesting that relatively high-elevation communities are instead restricted to cooler environments presumably because their thermophysiological traits are not well suited to the warm temperatures found at lower elevations.

In this study, we investigated how thermophysiological traits determine range limits in tropical montane taxa through an integrative physiological and ecological study of three lizard species that inhabit the Brazilian Atlantic Forest mountains. Lizards are particularly vulnerable to warming temperatures associated with global climate change and hence constitute an appropriate system to study (Sinervo et al. 2010). As ectotherms, they are directly affected by ambient

temperatures (Tewksbury et al. 2008), and as low-dispersal organisms, these species also have limited capability to escape unsuitable temperatures (Huey et al. 2009).

We collected thermophysiological data from specimens living at the highest elevation point of each one of our focal species. Then, we examined how well the individuals exhibiting those traits would perform across the Atlantic Forest, based on descriptors of the many microclimates available within the domain – and including areas occupied by their closest relatives. If the evidence suggests that the thermophysiological traits observed at high elevations allow organisms to perform well in the cold environments in which they are found, but not in warmer environments found at lower elevations, then we can conclude that our study is consistent with the view that an inability to cope with warmer conditions may limit the range of these taxa. In contrast, if the traits measured in high elevation populations confer high performance under other microclimates of the Atlantic Forest, in which the species is nonetheless not found, then we can conclude that thermophysiology does not exclude these organisms from warmer regions, and thus does not define the warm boundaries of these species' distributions. Under the latter scenario, we further examined the possibility that competition with related species excludes montane species from thermally suitable regions. In this case, we expect that thermally suitable areas in which the focal species is absent will be occupied by its close relatives.

While we fully acknowledge that physiological data from a single site may not represent the traits of the species as a whole (Mimura and Aitken 2010, Keller et al. 2013, Valladares et al. 2014), we expect that traits from high elevation extremes will show the most cold-adapted traits of the species and likely the highest trade-off in terms of tolerance to heat (Angilletta et al. 2006, Labra et al. 2009). Performance metrics based on these traits will therefore provide the most extreme test of the hypothesis that the traits of montane species exclude them from lower elevations.

Methods

Overview

We focused on high-elevation populations of three lizard species in the Brazilian Atlantic Forest with different range sizes, and generated thermophysiological data through experimentation. To evaluate how physiological traits would enable or hinder the occupation of the many climatic spaces available throughout the forest, we chose to derive microclimatic, substrate temperatures for each 1 km² grid cell throughout the Atlantic Forest based on thermodynamic first principles and the biophysical properties of each cell. Because lizards are able to behaviorally thermoregulate to alter their body temperatures, we experimentally collected thermal preference data from each species and compared them to the distribution of microclimatic temperatures accessible in each grid cell

across the forest to estimate what body temperatures would be exhibited, throughout the forest, by lizards with the observed physiological traits. Finally, we evaluated the performance of these traits across the forest using four thermophysiological performance metrics: hours within optimal temperatures, hours within critical temperatures, hours within preferred temperatures, and temperature dependent sprint score. For each focal species, we mapped these metrics across space and compared values within occupied and unoccupied areas, as well as those areas occupied by closely related species.

Study system

Our three focal species were *Caparaonia itaquara* and *Colobodactylus dalcyanus* (Gymnophthalmidae), and *Mabuya dorsivittata* (Scincidae). *Caparaonia itaquara* is restricted to Parque Nacional do Caparaó, a ~200 km² region, in which it is found in open areas with high elevation rocky outcrops (Fig. 1 red squares, Rodrigues et al. 2009a). Its close relative, *C. dalcyanus*, has a slightly broader distribution, yet occupies similar environments (Fig. 1 blue triangles, Bernardo et al. 2011). *Mabuya dorsivittata*, a geographically widespread South American species, is found in open areas with grasslands and rocky outcrops, including those on cool mountaintops (Vrcibradic et al. 2004, Fig. 1 orange circles).

Thermophysiology

Physiological data were collected from lizards captured at the highest elevation sites within each species' range: the Parque Nacional de Itatiaia at 2100–2400 m a.s.l. (for individuals of *C. dalcyanus*), and the Parque Nacional do Caparaó at

2100–2600 m a.s.l. (for *C. itaquara* and *M. dorsivittata*, Fig. 1, indicated with arrows). Lizards were captured by hand between 10 am and 5 pm.

Within four days of capture, we measured critical thermal maxima (CT_{max}), critical thermal minima (CT_{min}), and thermal preferences (T_{pref}) for 27 individuals of *Caparaonia itaquara*, 9 *Colobodactylus dalcyanus*, and 13 *Mabuya dorsivittata*. We also collected individual sprint speed data at four different temperatures from 16 individuals of *C. itaquara*, eight *C. dalcyanus*, and seven *M. dorsivittata*. Using sprint speed as a measure of physiological performance, we estimated thermal performance curves for each species.

We chose to assess critical thermal maxima (CT_{max}) and critical thermal minima (CT_{min}) because the critical thermal limits of ectotherms are frequently used to mark temperatures unsuitable for the organisms (Angilletta 2009). For that, we monitored righting response while raising or lowering body temperature at a rate of 0.5°C min⁻¹ (Lutterschmidt and Hutchison 1997, Ribeiro et al. 2012). We measured thermal preference (T_{pref}), the temperature voluntarily selected by the organism when there is no cost to thermoregulation, by calculating the mean body temperature achieved by each individual during a period of two hours spent in a thermal gradient spanning 12–48°C, where temperatures were recorded via a thermocouple every 30 s (Angilletta 2009, Moritz et al. 2012). Experiments were always conducted in the same order: T_{pref}, CT_{min}, and CT_{max}. Only one experiment was conducted per day per animal.

We evaluated the thermal dependence of performance by collecting data on sprint speed for each individual at four temperatures. The thermal sensitivity of sprint speed is a commonly used measure of thermal performance (Angilletta

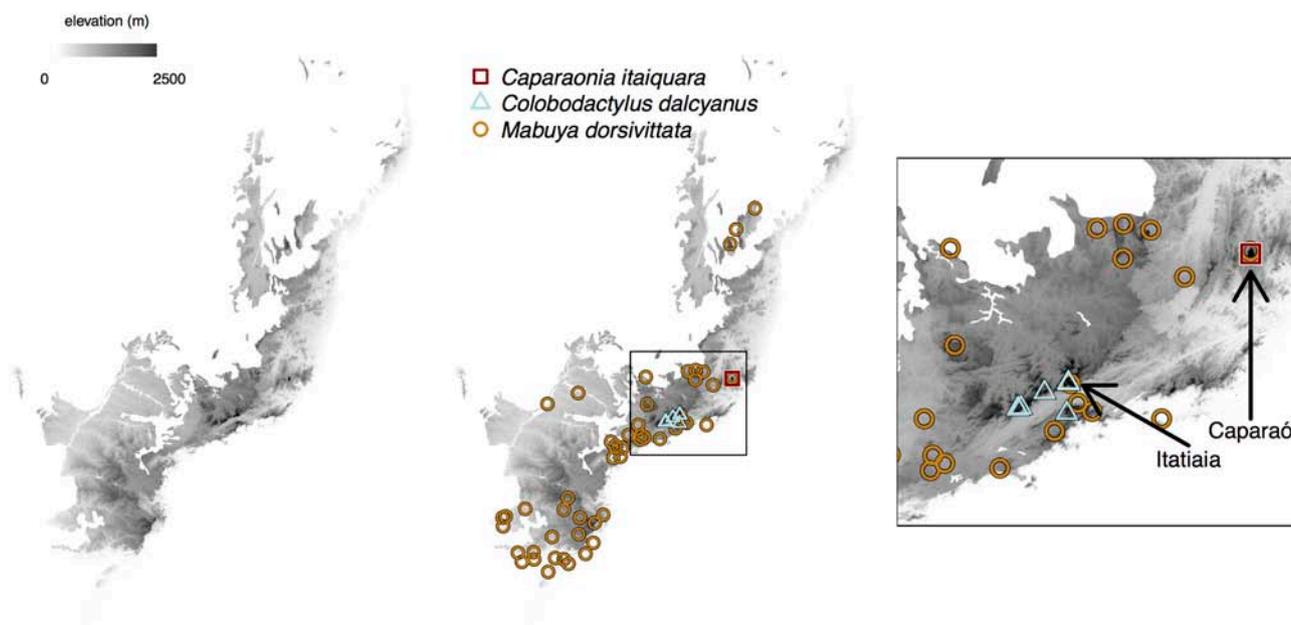


Figure 1. Elevation map (left) with known occurrence records within the Atlantic Forest for the three target species (center), shown with inset of the central Atlantic Forest (right). Collection sites, Parque Nacional do Caparaó (for *Caparaonia itaquara* and *Mabuya dorsivittata*) and Parque Nacional de Itatiaia (for *Colobodactylus dalcyanus*), are indicated with arrows.

2009, but see also Sinclair et al. 2016), and tends to correlate highly with many activities essential to survival and reproduction (Jayne and Bennett 1990, Angilletta et al. 2002, Miles 2004, Sinclair et al. 2016, Gilbert and Miles 2017). Sprint speeds were measured in the same randomly selected sequence of four body temperatures (19°C, 14°C, 24°C, 29°C), with only one temperature tested each day (Angilletta et al. 2002, Zajitschek et al. 2012; see Supplementary material Appendix 1 for additional details).

For each species, we estimated the shape of the thermal performance curve at the experimentation site with generalized additive mixed-models (GAMMs) in the R package 'mgcv' (Wood 2011). GAMMs use non-parametric smoothing functions to model non-linear relationships. To model sprint performance across temperatures for each species, we pooled CT_{min}, CT_{max}, and sprint speed at four temperatures from all individuals and applied a cubic spline smoothing function. We also corrected for the effect of individual identity, body weight, and sex on the shape of the curve (Zajitschek et al. 2012, Beal et al. 2014, see Supplementary material Appendix 1 for additional details).

Microhabitat and body temperatures

Relative to the commonly used interpolated weather-station data (Hijmans et al. 2005), microhabitat climate characterizations are expected to more accurately reflect the thermal regimes experienced by small organisms (Potter et al. 2013, Scheffers et al. 2013, Storlie et al. 2014, Woods et al. 2014). To derive the full range of microhabitat temperatures available to these lizards throughout the entire Atlantic Forest, we used the microclimate model in the R package 'NicheMapR' ver. 1.0.0 (Kearney and Porter 2017). Specifically, we used this approach to model hourly substrate temperatures at three substrate depths (surface, -2.5 cm and -5 cm) and two shade levels (90% shade and the average shade coverage for each site, see below), for one day per month. This totaled 12 d per 1 km² grid cell, each with 24 h measurements at each of three substrate depths, at both average and maximum shade coverage available at the site. For each 1 km² grid cell within the Atlantic Forest domain, we used 'NicheMapR' to obtain macroclimatic data (diurnal temperature range, relative humidity, sunshine duration, wind speed, etc., at the coarse resolution of 15 km²; New et al. 2002), and, with more fine-scale data on biophysical conditions associated with the habitat type within the 1 km² area (soil elevation, and vegetation cover), derive microclimatic temperature and humidity measures. These values represent the average conditions found in each grid cell. In this process, elevation layers at 1 km² resolution were obtained from <www.worldclim.com>, and an adiabatic lapse rate of 5.6°C km⁻¹ (Bush and Silman 2004) was used to refine temperature values from the New et al. 2002 dataset. Soil maps (1 km² resolution; <www.soilgrids.org>) were used to categorize soil type as sand, soil, or rock, following Campbell and Norman (1998). The average available shade coverage for each site was derived from remote sensing data via the MODIS product Vegetation Continuous

Fields (1 km² resolution; <www.glcf.umd.edu/data/vcf/index.shtml>). Soil moisture estimates and their effects on substrate temperatures were included in the microclimate model, as per Kearney et al. (2014).

Because lizards move across substrates and shade levels to achieve their preferred temperatures and avoid unsuitable temperatures, species with different physiological traits may experience different body temperatures in the same localities, or vice versa (Dzialowski 2005). To estimate the body temperatures that lizards with the observed physiological traits would have throughout the region, we used the ectotherm model in 'NicheMapR'. This model incorporates the full range of microclimatic temperatures available to lizards in each locality at every hour (derived from 'NicheMapR's microclimate model), and combines them with information on the organism's thermophysiology, body mass, shape, and behavior, to estimate hourly body temperatures.

Metrics of thermophysiological performance

To estimate how individuals with the physiological traits observed at high elevations would perform in the many microclimatic thermal environments available across the Atlantic Forest, we generated spatial layers depicting four thermo-physiological performance metrics per species. They are: 1) the average daily number of hours within critical temperatures; 2) the average daily number of hours within optimal temperatures; 3) the average daily number of hours within preferred temperatures; and 4) the average daily sprint score.

The first three metrics evaluate the prevalence of body temperatures suitable for activity and survival. We first summarized the critical thermal minimum and maximum of each species at the high elevation site by taking the mean value for all individuals of the species. For each day of the year, we calculated the number of hours available within these physiological limits in each grid cell daily over a full year; those hours outside the limits were classified into hours below and above the critical limits. Then, we calculated the number of hours within optimal temperatures by identifying how often those per-grid-cell temperatures allow organisms to sprint at 80% of their maximum capacity or higher (Angilletta 2009, Moritz et al. 2012, Logan et al. 2013), as determined from each species' sprint performance curve. This threshold is thought to promote activities essential to survival, such as foraging and reproduction (Grant and Dunham 1988, Sinervo et al. 2010). Because all three species are diurnal, this analysis was restricted to daytime hours. Similarly, we calculated the number of hours within preferred temperatures by identifying how often temperatures allow organisms to achieve body temperatures within the interquartile range of temperatures selected during thermal preference experiments. While methodologically similar to hours within optimal temperatures, hours within preferred temperatures can be used in the absence of sprint speed data.

Lastly, we evaluated the potential for activity across the Atlantic Forest domain by calculating sprint scores, a relative

metric of the sprint capacity enabled by each thermal environment. For each daytime hour in which temperatures that are within the lizard's optimal thermal range (the temperatures at which they are able to sprint at 80% or higher of their maximum capacity) are available, we used the sprint performance curve to determine the sprint capacity of the organism relative to its own maximum. For example, if the available temperature during one hour could allow it to sprint at maximum speed, the relative sprint capacity for that hour is 1. If temperatures during the next hour allow it to sprint at 80% of its maximum speed, this hour receives a score of 0.8. We then summed these measures over the entire period in which temperatures are within the optimal thermal range to generate a sprint score – a relative metric of the sprinting capacity that a given thermal environment allows. The sprint score can vary between 0 and 12, with a score of 0 indicating that daytime temperatures are never suitable for activity, and 12 indicating that temperatures at every daytime hour allows the organisms to sprint at maximum capacity. The sprint score is hence influenced by both the duration of temperatures within the optimal thermal range and the capacity for sprinting that these temperatures allow. This is similar to the comprehensive fitness function developed by Martin and Huey (2008).

Ranges of closely related species

To evaluate physiological performance at the sites occupied by each focal species, as well as those inhabited by their closely related species, we first compiled a species occurrence dataset for each taxon. For each species, we compiled and vetted occurrence records from our own fieldwork, literature searches, and verified online databases (Supplementary material Appendix 1 Table A6). To reduce spatial autocorrelation in this dataset, we used the R package 'spThin' (Aiello-Lammens et al. 2015) to remove localities within 15 km of each other. To represent the range of close relatives, we included locality data for all species sharing a common ancestor with the three focal species within the last 12 million yr (my). Although arbitrary, this period corresponds to the estimated time of origin of a monophyletic clade within the *Mabuya* genus (Miralles and Carranza 2010), and a phylogenetic break splitting the clade containing *C. itaiquara*, *C. dalcyanus* and four closely related species from the remaining gymnophthalmid lizards (Strangas unpubl.). This procedure resulted in the selection of a phylogenetic clade comprising six described species for comparison with *C. dalcyanus* and *C. itaiquara*, and 16 described species for *M. dorsivittata* (Supplementary material Appendix 1 Table A6).

Data deposition

Data and R scripts available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.8t6184v>> (Strangas et al. 2018).

Results

Thermophysiology

The lizards from the three focal species differed in their thermal tolerances, with individuals from the small-ranged *C. itaiquara* and *C. dalcyanus* showing narrower thermal tolerances relative to the more widespread and phylogenetically distant *M. dorsivittata* (Fig. 2). Mean thermal tolerance, plus/minus one standard deviation, spanned from $8.1 \pm 1.5^\circ\text{C}$ to $36.4 \pm 1.5^\circ\text{C}$ for *C. itaiquara*, $7 \pm 2.4^\circ\text{C}$ to $36.3 \pm 1.1^\circ\text{C}$ for *C. dalcyanus*, and $6.9 \pm 2.1^\circ\text{C}$ to $44.4 \pm 3.8^\circ\text{C}$ for *M. dorsivittata*. The interquartile range of preferred temperatures was $22.03\text{--}25.01^\circ\text{C}$ with a mean of $23.35 \pm 2.67^\circ\text{C}$ in *C. itaiquara*, $24.30\text{--}28.02^\circ\text{C}$ with a mean of $26.75 \pm 1.33^\circ\text{C}$ in *C. dalcyanus*, and $22.39\text{--}27.03^\circ\text{C}$ with a mean of $24.72 \pm 6.85^\circ\text{C}$ for *M. dorsivittata*. Models of sprint performance revealed that the temperatures that allow organisms to perform at 80% or higher of their maximum (the B_{80} range) ranged from $18.9 \pm 0.1^\circ\text{C}$ to $28.9 \pm 0.2^\circ\text{C}$ in *C. itaiquara*, $19.8 \pm 0.45^\circ\text{C}$ to $30.3 \pm 0.5^\circ\text{C}$ in *C. dalcyanus*, and $18.1 \pm 0.6^\circ\text{C}$ to $30.6 \pm 0.9^\circ\text{C}$ in *M. dorsivittata*. Optimal temperatures for sprinting (T_{opt}) were 24.51°C for *C. itaiquara*, 25.81°C for *C. dalcyanus*, and 24.47°C for *M. dorsivittata*. The thermal safety margin, or difference between CT_{max} and T_{opt} , was 13.45°C for *C. itaiquara*, 9.73°C for *C. dalcyanus*, and 16.29°C for *M. dorsivittata*.

Thermophysiological performance across geography

The metrics of thermophysiological performance show similar patterns across all three species. Hours within optimal temperatures, hours within preferred temperatures and sprint score closely follow elevation gradients within the Atlantic Forest, with lower performance at high elevations. For all

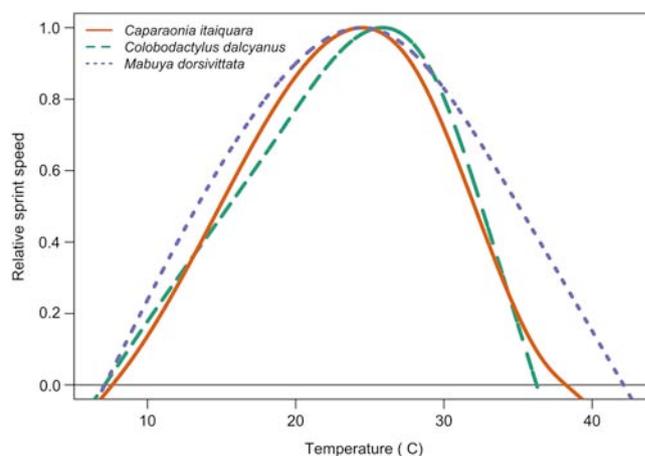


Figure 2. Sprint performance curves from the three target species at their collection sites. These curves show broader thermal tolerances and performance for the geographically widespread *Mabuya dorsivittata* (purple dotted line), and narrow tolerances for *Colobodactylus dalcyanus* (green dashed line) and *Caparaonia itaiquara* (red solid line), which have more restricted ranges.

three species, these metrics were lowest in the high elevation areas where the thermophysiological traits were collected, and highest at mid and low elevations (Fig. 3). Mid elevation areas in the north-west regions of the forest domain also showed relatively low performance – yet not as low as was seen at high elevations. Because these metrics showed very similar patterns, we only show maps of hours within optimal temperatures here (Fig. 3). Maps of hours within preferred temperatures and sprint score can be found in Supplementary material Appendix 1 Fig. A2, A3.

Hours within critical temperatures were also lowest at high elevations, though differences across the forest were minimal. While most areas provided 24 h d⁻¹ of tolerable temperatures, our analyses show that the values were slightly lower at high elevation areas. For *C. itaiquara* and *C. dalcyanus*, these areas included the sites where the traits were collected (Supplementary material Appendix 1 Fig. A4). In all cases, the low measures of hours between critical temperatures at the occurrence sites of the animals tested were driven entirely by the number of hours where temperatures reached below the critical thermal minimum, as modeled body temperatures never exceeded the lizards' critical thermal maxima (Supplementary material Appendix 1 Fig. A4).

All traits measured at high elevation sites indicate that those individual lizards would achieve high performance in regions of the forest that are not occupied by them presently. For all three species, hours within optimal temperatures, hours within preferred temperatures, and sprint score were higher in sites occupied by their closely related species relative to the site of experimentation (Table 1). For *M. dorsivittata*, these metrics were also higher in sites occupied by other members of the species relative to the site of experimentation (Table 1). Hours within critical temperatures were not significantly different across sites.

Discussion

Our findings suggest that thermophysiological constraints, assessed through critical thermal limits and sprint speed, do not restrict tropical montane lizards to high elevations. In all three montane Atlantic Forest lizard species targeted in this study, traits observed at high elevation sites were predicted to confer relatively low thermophysiological performance to lizards in those high elevation environments. However, for all three species, the traits seen in high elevations were shown to confer high thermophysiological performance in mid- and low-elevation regions of the Atlantic Forest (Fig. 3, Supplementary material Appendix 1 Fig. A2–A4). These findings suggest that temperatures in other areas of the forest are compatible with the physiological traits of these cold-environment individuals.

These results suggest that warm temperatures at lower elevations allow for higher thermophysiological performance without an accompanying threat of lethal temperatures. Because estimates of hours of optimal temperatures, hours of preferred temperatures and sprint score incorporated the

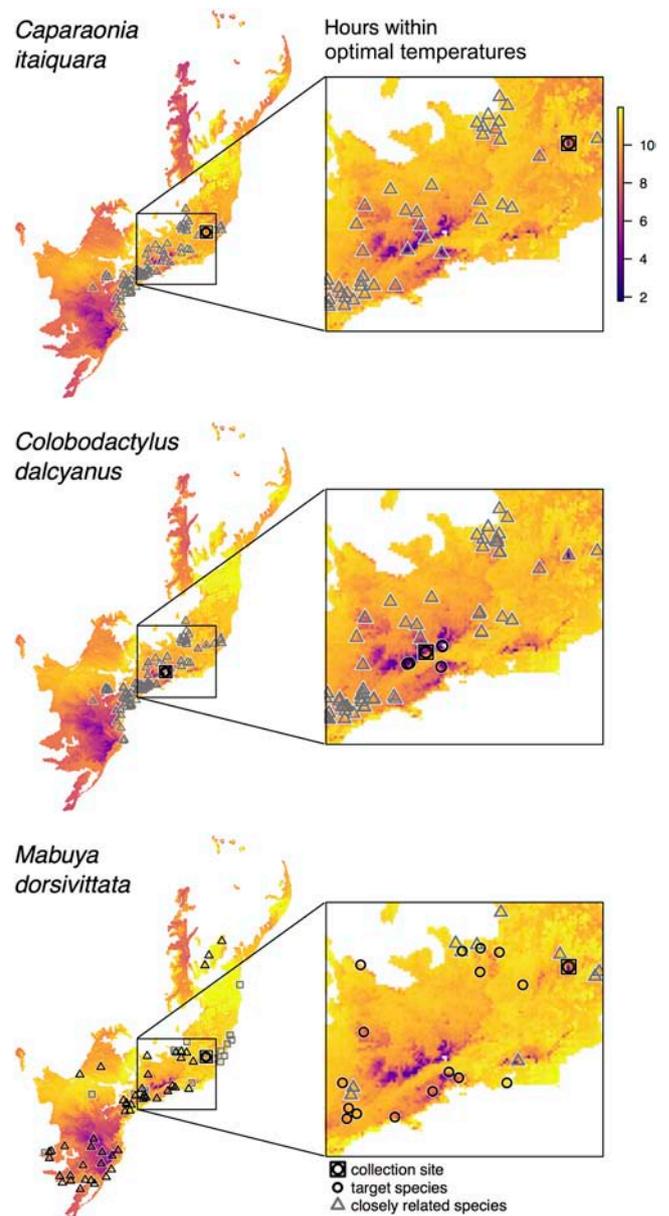


Figure 3. Maps of estimated thermophysiological performance across the Atlantic Forest with inset of the forest, showing the average daily number of hours within optimal temperatures for each species based on physiological data from a high elevation population. Maps for *Caparaonia itaiquara* (top), *Colobodactylus dalcyanus* (center), and *Mabuya dorsivittata* (bottom) all show fewer hours within optimal temperatures in the sites where these traits were collected, and, in the case of *C. itaiquara* and *C. dalcyanus*, in all sites where the species occur. For these species, close relatives occupy areas that offer higher thermophysiological performance. In the case of *M. dorsivittata*, many members of the target species as well as closely related species occupy areas with high estimated performance. Color scale indicates the daily average hours within optimal temperatures, with yellow indicating more hours, and dark blue indicating fewer.

distribution of hourly temperatures throughout the year, we avoid mischaracterization of the threat of extreme temperatures that is associated with using mean temperatures

Table 1. Mean daily values for the suitability metrics at the collection point, in sites occupied by other populations of the focal species, and in sites occupied by their closely related species. HrOT=hours within optimal temperatures; HrPT=hours within preferred temperatures; HrCT=hours within critical temperatures; SpScore=relative sprint score. Different letters denote groups with statistically significant differences as per Wilcoxon signed-rank tests, $p < 0.05$.

| | Metric | Collection point | Target species | Close relatives |
|---------------------------------|---------|------------------|----------------|-----------------|
| <i>Caparaonia itaiquara</i> | HrOT | 1.79a | – | 9.73b |
| | HrPT | 1.28a | – | 9.04b |
| | HrCT | 23.80a | – | 23.97b |
| | SpScore | 1.71a | – | 9.47b |
| <i>Colobodactylus dalcyanus</i> | HrOT | 2.12a | 3.21b | 9.58c |
| | HrPT | 0.95a | 1.82b | 7.75c |
| | HrCT | 24.00a | 24.00a | 24.00a |
| | SpScore | 2.01a | 3.08b | 9.32c |
| <i>Mabuya dorsivittata</i> | HrOT | 1.81a | 9.33b | 10.81c |
| | HrPT | 1.19a | 9.81b | 13.17c |
| | HrCT | 24.00a | 24.00b | 24.00a |
| | SpScore | 1.76a | 9.12b | 10.51c |

(Martin and Huey 2008). In addition, we found that low elevation sites occupied by close relatives were not exposed to temperatures above the lizards' heat tolerance limits or, for *C. itaiquara* and *C. dalcyanus*, their optimal thermal ranges more often than the high elevation sites where they do occur (Supplementary material Appendix 1 Table A5, Fig. A5). This suggests that the warmer areas, in which hours of optimal temperatures, hours of preferred temperatures, and sprint score are predicted to be higher, are unlikely to be dangerous due to extreme heat (Martin and Huey 2008). The cold environments at high elevations do appear to be thermally suboptimal given the thermophysiological traits of the lizards – as shown in the reduction of estimated hours of optimal and optimal temperatures driven by cold temperatures (a higher number of hours below Critical Temperature Minima and below the optimal thermal range) at these occupied, montane sites.

Thermophysiological performance and species' ranges

Beyond the performance of individuals, the results also imply that thermophysiological constraints likely do not determine the warm boundaries of montane species' distributions. For the two narrowly endemic species, *Caparaonia itaiquara* and *Colobodactylus dalcyanus*, the observed thermophysiological traits were predicted to only perform well in areas where the species does not occur, while low performance was predicted in areas occupied by the species. While the distribution of the more widespread *Mabuya dorsivittata* falls within regions of high predicted performance, many areas of the forest that allowed for high thermophysiological performance are not occupied, despite being geographically accessible. Thermophysiological performance as measured in this study is therefore not a strong predictor of range limits for any of the three focal species.

Our conclusion that thermophysiological performance is not driving warm-boundary range limits is robust to the potential for local adaptation across these species' ranges. Despite our finding that the traits of montane populations

are better suited to the low-elevation environments in which they do not occur, the populations studied here may nonetheless be better adapted to cold environments than are their low-elevation relatives. It is widely recognized that local adaptation may generate variation in thermophysiological traits among populations within species, thus making it difficult to infer species' range dynamics with data from a single population (Mimura and Aitken 2010, Keller et al. 2013, Valladares et al. 2014). However, if local adaptation in these traits did occur in our study system, we would expect the traits seen in the highest elevation, most thermally extreme populations, to be the most suited to cold, and perhaps unsuited to warm temperatures if trade-offs are present (Angilletta et al. 2006, Labra et al. 2009). Still, we found that the traits of lizards expected to be most suited to cold environments confer higher performance in warmer rather than colder environments; if populations from lower elevations are locally adapted to their environments, they will likely also perform well, if not better, in these warm environments.

Other possible determinants of range limits

Biotic interactions may play a role in determining the warm boundaries of the species' ranges for the two montane endemics, *Caparaonia itaiquara* and *Colobodactylus dalcyanus*. While areas with high predicted performance are unoccupied by these focal species, many such areas are inhabited by closely related species (Fig. 3). Our physiological results from *C. itaiquara* and *C. dalcyanus* are consistent with the hypothesis that the presence of closely related species may exclude these species from mid and low-elevation areas, despite the apparent thermal suitability. Little is known about interactions between these close relatives, yet many have approximately the same body size (with the exception of *Heterodactylus imbricatus*; Camacho et al. 2015), and tend to occupy similar microhabitats (Rodrigues et al. 2009a, b). While no experiments have been conducted to directly evaluate the effect of competition between these species, it is possible that they could compete for similar territories

or food sources. The presence of predators at lower elevations may have also driven these species upslope. While we do not have widespread data on the identity or distribution of these species' predators, anecdotal evidence is compatible with this hypothesis. For example, the only snake obtained during our intensive fieldwork at the high altitudes of Parque Nacional do Caparaó where *M. dorsivittata* and *C. itaquara* are prevalent was an unidentified species of the genus *Thamnodynastes*, which primarily feeds on frogs, not lizards (Dorigo et al. 2014). Biotic factors have been previously suggested as important in limiting species distributions at warm range boundaries. While this phenomenon was first proposed Darwin (Darwin 1859), it has also been supported by several recent macroecological studies (Sunday et al. 2012, Louthan et al. 2015, Cunningham et al. 2016), and is consistent with our findings.

Competition with close relatives may be less important in determining range limits for the widely distributed species, *Mabuya dorsivittata*. Unlike the other two focal species, *M. dorsivittata* is not restricted to high elevations where thermal performance is low. Populations of the species occur throughout areas predicted to allow high performance, as well as some lowland areas predicted to allow poor performance. Closely related species do occur within regions unoccupied by *M. dorsivittata* yet they also exist in sympatry (Fig. 3), suggesting that competition with these species is not a key factor in restricting the range of *M. dorsivittata*. The prevailing factors limiting the species distribution for *M. dorsivittata* are yet unclear. Precipitation patterns and vegetation type, as well as biotic interactions with other potential competitors, predators, and prey items, may play important roles (Guisan and Thuiller 2005, Wisz et al. 2013).

Potential mechanisms shaping thermophysiology of montane lizards

Why do montane lizard populations and species show traits poorly suited to their environments? The mechanisms shaping thermophysiology may differ across the species we studied. In the narrowly endemic *C. itaquara* and *C. dalcyanus*, which have limited environmental heterogeneity throughout their ranges, phylogenetic niche conservatism in thermal traits may play a role in explaining thermophysiological response. If these species retreated to high elevations due to competition with close relatives, as we have speculated here, they may have retained warm-adapted traits inherited from lowland ancestors. Phylogenetic conservatism of upper thermal limits has been consistently observed in ectotherms (Araújo et al. 2013, Sunday et al. 2014). Low standing genetic variation may explain the apparent retention of ancestral traits (Kellermann et al. 2009, 2012, Blackburn et al. 2014), and upper thermal limits emerge as a byproduct of genetic and physiological architecture, rather than in response to direct selective pressure (Blows and Hoffmann 2005). While further research is necessary to evaluate the prevalence of trait conservatism in these species groups, our work suggests that there is great need to learn more about the underlying genetic

architecture of these traits, and to explicitly consider evolutionary history when examining relationships between traits and environment.

In the widespread *M. dorsivittata*, however, it may be possible that gene flow among populations in heterogeneous environments influence the traits observed in cold environment. Gene flow within a species range can potentially limit evolution in environmentally and geographically marginal populations (Haldane 1956, Kirkpatrick and Barton 1997, Bridle and Vines 2007, Hoffmann and Sgrò 2012). This has been observed in tadpoles from many frog species, for instance, for which thermal traits better reflect the conditions experienced at the core of the species range rather than at its margins (Gouveia et al. 2013). For *M. dorsivittata*, it remains to be tested whether gene flow from warmer environments to the montane population sampled here impedes adaptation to colder temperatures.

Implications for montane lizards and the Atlantic Forest

The importance of factors beyond thermophysiology complicate predictions of species' distributions into future climate scenarios. If thermophysiological traits in tropical montane lizards remain stable, rising temperatures may, in the short term, provide conditions conducive to improved performance for montane populations, as has been predicted for other open-habitat tropical lizards (Logan et al. 2013) and several temperate insects (Deutsch et al. 2008). However, the challenges that may be posed by accompanying changes in the biotic community are unclear and have not been tested here.

Importantly, our experimental results suggest that caution should be used when claiming that physiology, when tied to current environmental conditions, holds the key to understanding patterns of diversity and endemism within Atlantic Forest lineages (Amaro et al. 2012, Carnaval et al. 2014). Although this study has not addressed whether physiological performance can preclude lowland Atlantic Forest species from occupying higher elevations, our results suggest that physiological trade-offs, habitat availability, or species interactions may be acting to determine the warmer edge of the ranges of montane species. This has important implications for the interpretation of models of species distribution based on climate: although climate is tightly correlated with species distributions – both lowland and montane – the underlying cause of this correlation is likely multifaceted, rather than based solely on thermophysiology.

Conclusion

In this study, we collected data on three poorly studied species in an understudied hotspot of biodiversity and applied novel microclimatic resources and methods to assess the geographical patterns of thermophysiological performance for montane species of different range sizes. Our results suggest that species differ in the factors that structure their ranges, and that this difference may be tied to the overall breadth

of their ranges. We found that thermophysiological traits in cold environments predict low thermophysiological performance in those same environments, while predicting high performance in areas not occupied by the species. This suggests that such performance is a poor predictor of occurrence for these montane species.

We implemented an integrative framework that relies on physiological assays of a single population per study species. While studies of physiological variation throughout species' ranges and across large phylogenies shed important light on the exact mechanisms shaping these traits (Damasceno et al. 2014), the framework presented here can be particularly efficient in the face of limited or hard-to-collect interspecific physiological data. Our results point to the importance of incorporating data on mechanisms other than thermophysiology, and cannot discard the hypothesis that species interactions may well hold part of the key to understanding ranges (Ricklefs 2010) – at least in montane tropical species. Our incipient knowledge of the interplay between thermophysiology and biological interactions, given a backdrop of environmental conditions, presently limits our understanding of the mechanisms that drive species responses to climate change. As a corollary, it poses a challenge to predictive studies under future climatic shifts. Emphasis on interdisciplinary field studies that bridge the realms of evolution and ecology will improve predictions of biological responses to future environments.

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Supplementary material (Appendix ECOG-03330 at <www.ecogeography.org/appendix/ecog-03330>). Appendix 1.