



## Extinction risks forced by climatic change and intraspecific variation in the thermal physiology of a tropical lizard

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### ARTICLE INFO

#### Keywords:

Amazon

Cerrado

Ecophysiology

Global warming

Preferred temperature

Performance

### ABSTRACT

Temperature increases can impact biodiversity and predicting their effects is one of the main challenges facing global climate-change research. Ectotherms are sensitive to temperature change and, although predictions indicate that tropical species are highly vulnerable to global warming, they remain one of the least studied groups with respect to the extent of physiological variation and local extinction risks. We model the extinction risks for a tropical heliothermic teiid lizard (*Kentropyx calcarata*) integrating previously obtained information on intraspecific phylogeographic structure, eco-physiological traits and contemporary species distributions in the Amazon rainforest and its ecotone to the Cerrado savannah. We also investigated how thermal-biology traits vary throughout the species' geographic range and the consequences of such variation for lineage vulnerability. We show substantial variation in thermal tolerance of individuals among thermally distinct sites. Thermal critical limits were highly correlated with operative environmental temperatures. Our physiological/climatic model predicted relative extinction risks for local populations within clades of *K. calcarata* for 2050 ranging between 26.1% and 70.8%, while for 2070, extinction risks ranged from 52.8% to 92.8%. Our results support the hypothesis that tropical-lizard taxa are at high risk of local extinction caused by increasing temperatures. However, the thermo-physiological differences found across the species' distribution suggest that local adaptation may allow persistence of this tropical ectotherm in global warming scenarios. These results will serve as basis to further research to investigate the strength of local adaptation to climate change. Persistence of *Kentropyx calcarata* also depends on forest preservation, but the Amazon rainforest is currently under high deforestation rates. We argue that higher conservation priority is necessary so the Amazon rainforest can fulfill its capacity to absorb the impacts of temperature increase on tropical ectotherms during climate change.

### 1. Introduction

Climate change is occurring at different spatial scales (IPCC, 2014), and responses to rising temperatures have included changes in phenology, shifts in elevation and local extinction of populations (Colwell et al., 2008; Sinervo et al., 2010; Winter et al., 2016). Several studies suggest that tropical ectotherms are more vulnerable to warming temperatures than species farther from the equator (e.g., Deutsch et al.,

2008; Sinervo et al., 2010; Huey et al., 2012). Thus, climate change in tropical regions is expected to induce a decrease in biodiversity, because these regions tend to be the most affected by rapid increases in temperature as well as they support the highest numbers of species (Dillon et al., 2010).

Current species distribution models (SDMs) mainly describe the environmental niches of focal species through correlations between climatic factors and distributional data, without incorporating species-

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specific ecophysiological predictors (e.g., body and operative environmental temperatures) in the modeling (Kearney and Porter, 2009). However, such SDMs may be limited because they are not based on traits measured directly from the physiology of species. In contrast, mechanistic SDMs might overcome these drawbacks by using physiological data to represent the complexity of interrelationships between organismal physiology, microclimate, behavior, and distribution (Gunderson and Leal, 2012).

Climate change has been shown to have global impacts on reptile diversity (Winter et al., 2016) and to cause local declines in lizard diversity (Sinervo et al., 2010). Such climatic disturbances likely caused changes in thermal niches by reducing hours of activity ( $H_a$ ) and increasing hours of restriction (i.e.,  $H_r$  or the total number of daylight hours in which environmental temperatures are above or below critical limits that restrict lizard activity). Models estimating extinction risks on a global scale indicate that by 2070, > 39% of local populations of ~20% of lizard species could become extinct (Sinervo et al., 2010). These predictions are especially alarming for large and biodiverse areas in the Neotropics, such as the Amazon rainforest, the Cerrado savanna and their ecotone in South America, which are expected to become high extinction-risk regions for lizards (Sinervo et al., 2010).

Until recently, SDMs based on ecophysiology were mainly premised on extinction risk across lizard families. As they were not based on individual species, they need further refinement and validation with field data obtained in situ (Sinervo et al., 2010). Recent studies on lizards have refined estimates of extinction-risk at local scales, considering the interrelations between physiology and microclimate (e.g., Ceia-Hasse et al., 2014; Lara-Resendiz et al., 2015; Kubisch et al., 2015). However, few such analyses exist for lizard species inhabiting tropical rainforests (Sinervo et al., 2010).

Individuals of the same species at distinct locations are subject to variation in local environmental conditions, which is particularly true for species with broad geographic distributions (Hoffman and Sgro, 2011). Geographic variation in physiological traits between and within populations, which could result from plasticity or adaptation, indicates that climate sensitivity of tropical species may not be as rigid as previously believed (Huey et al., 2009; Llewelyn et al., 2016) and reveal the spatial structure of adaptive potential throughout species distributions (Moritz et al., 2012). For example, differences in physiological traits and sensitivity to temperature can confer differences in vulnerability of populations according to local habitat structure (Piantoni et al., 2016).

Differences in physiological traits have been revealed between species from temperate and tropical regions. For example, differences in locomotor performance at different body temperatures between different populations within a clade were detected in skinks in Australia (Moritz et al., 2012) and *Anolis sagrei* populations in the Bahamas (Logan et al., 2014). However, differences in performance were not found in *A. cristatellus* on Puerto Rico, which is a close relative of *A. sagrei* (Gunderson and Leal, 2012). Body temperature can vary with sex, age, and season, as well as among individuals at different locations (e.g., Van Damme et al., 1986; Gunderson and Leal, 2012). Clusella-Trullas et al. (2011) suggested that differences in preferred temperature might be more related to other climatic variables, such as variation in environmental temperature and precipitation, than to mean temperature.

Critical thermal limits ( $CT_{min}$  and  $CT_{max}$ ) are considered key predictors of ectotherm physiological sensitivity, and differences in both traits have been used to investigate macrophysiological patterns relative to temperature and precipitation (e.g., Clusella-Trullas et al., 2011), latitude (e.g., Addo-Bediako et al., 2000; Araujo et al., 2013), and habitat (e.g., terrestrial versus marine; Sunday et al., 2011). Likewise, critical thermal limits may vary intraspecifically (e.g., Moritz et al., 2012; Logan et al., 2014), but the extent of their variation remains underexplored (Losos, 2008). Uncovering intraspecific



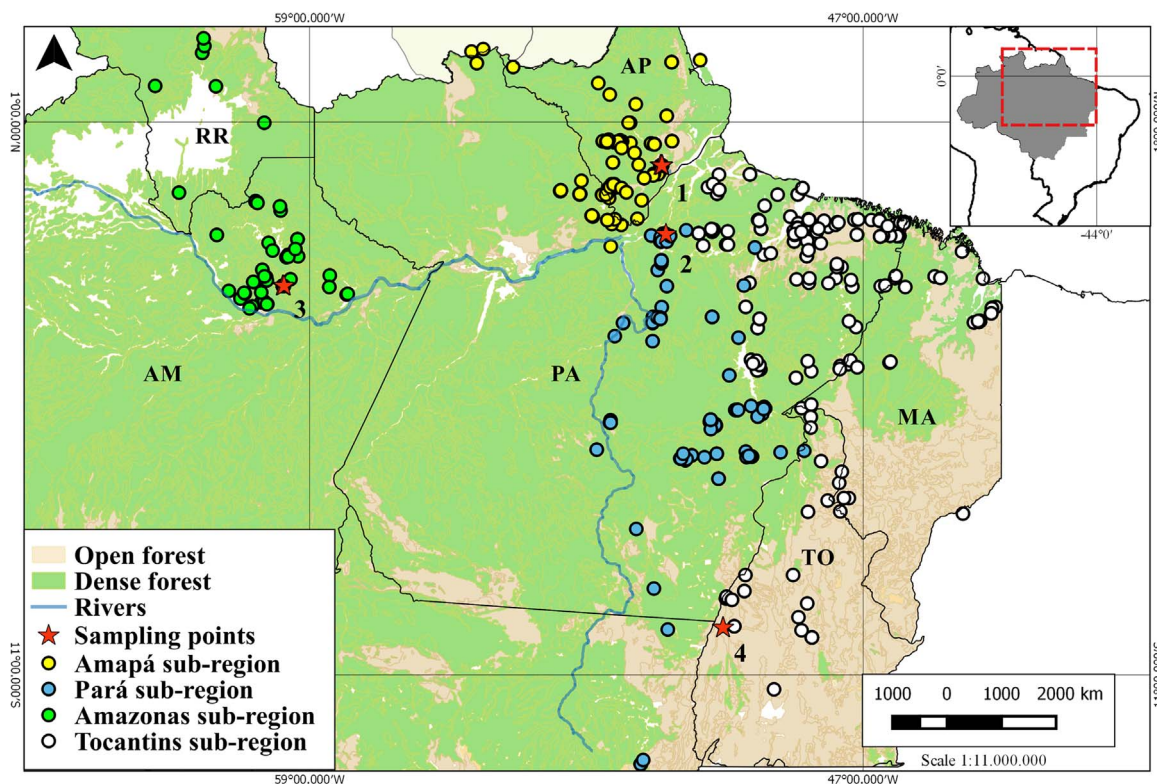
Fig. 1. *Kentropyx calcarata* photographed in Ribeirão Cascalheira, Mato Grosso, Brazil by Guarino R. Colli.

differences may help clarify whether critical thermal limits are evolutionarily conserved or labile (Pearman et al., 2008). The upper critical temperature is known to vary little and tends to be conserved among populations (Moritz et al., 2012), and among ectotherm species (Addo-Bediako et al., 2000; Araujo et al., 2013). Thus, it is assumed that temperature increases will impose selective pressure over a wide range of species and populations (Sunday et al., 2011).

Regional diversification of some tropical lineages has been described and can be integrated with evolutionary-physiological studies (Moritz et al., 2012). Here, we use this approach using the lizard species *Kentropyx calcarata* (Spix 1825; Squamata: Teiidae) as a model system (Fig. 1). The species has a broad distribution in the northeastern part of South America including Brazil and the Guianas. Individuals are found in forest environments near streams, natural clearings or forest edges (Vitt, 1991). The species has a relatively well-known phylogeographic history with at least ten main intraspecific lineages previously identified (Werneck et al., 2009; Avila-Pires et al., 2012; Cronemberger, 2015). Given this species distribution and the assumed conservative nature of thermal traits typical of tropical ectotherms (Deutsch et al., 2008; Huey et al., 2009), *K. calcarata* is predicted to be sensitive to climate change. Most open-habitat heliotherms occupy heterogeneous thermal environments and are thermal generalists, which may explain their wide geographic distributions (Bonino et al., 2015). *Kentropyx calcarata* thermal-microhabitat use contrasts with those of heliotherms of open areas and forest nonheliotherms (Vitt et al., 1991; Vitt et al., 1997). Thus, *K. calcarata* belongs to an under-explored group in relation to its thermal biology.

Most teiid lizards are heliotherms that maintain body temperatures above air or ambient temperatures during activity and avoid heat stress by shuttling between sun and shade (e.g., Sartorius et al., 1999). Behavioral thermoregulation may buffer lizards from warming impacts. However, estimates of mean field or preferred temperature may not be suitable to estimate restriction in activity times for heliothermic lizards (Kearney and Porter, 2009). For heliothermic lizards, the voluntary thermal maximum (VTM) for activity is probably a better threshold to indicate activity limitation (Vitt et al., 1997).

Here, we estimate relative extinction risks for *K. calcarata* in large sub-regions within the Brazilian Amazon and the Cerrado-Amazon ecotone occupied by four of the main intraspecific lineages recognized by Cronemberger (2015), based on the calculation of thermally imposed hours of restriction, alone and in interaction with rainfall and vegetation cover. We also investigate how the thermal biology of *K. calcarata* is affected by different thermal environments to predict how differences in thermal biology might affect vulnerability of populations from different regions.



**Fig. 2.** Study area and locality records of *Kentropyx calcarata* used in extinction-risk modeling across four sub-regions in Dense forests (Amazon areas) and Open forests (Cerrado savannas areas). The geographic limits used to sub-select sampling points were based on the distribution of *Kentropyx calcarata* intraspecific lineages inferred from Cronemberger (2015) as follows: green circles refer to the Amazonas sub-region that corresponds to Amazonas State (AM) north of the Amazon river extending to Roraima (RR); yellow circles refer to the Amapá sub-region that corresponds to the Amapá State (AP); white circles refer to the Tocantins sub-region that corresponds to sampling points in Tocantins State (TO) and extends to Maranhão (MA) and eastern Pará (PA); blue circles refer to the Pará sub-region that corresponds to sampling points in Pará State, interfluvium of Xingu River (west), Araguaia River (east) and Amazon River (north). Red stars depict the sampling points for *K. calcarata* ecophysiological data, as follows: 1 – Floresta Nacional do Amapá, Amapá; 2 – Floresta Nacional de Caxiuanã, Pará; 3 – Reserva Florestal Adolpho Ducke, Amazonas; and 4 – Centro de Pesquisas Canguçu, Tocantins. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

## 2. Methods

### 2.1. Study area and sample method

Specimen sampling and experiments were carried in the Brazilian Amazon in September 2014 and between July and October 2015. These months correspond to the dry season in all sampling and experimental locations. The four sampling localities were chosen based on the regions occupied by four different intraspecific clades recovered by recent phylogeographic studies (Avila-Pires et al., 2012; Cronemberger, 2015): 1 – Floresta Nacional (FLONA) de Caxiuanã (1°44' S, 51°27' W; elevation 28 m), Melgaço/Portel, Pará State (PA); 2 – Reserva Florestal Adolpho Ducke (2°57' S, 59°55' W; elevation 68 m), Manaus, Amazonas State (AM); 3 – Floresta Nacional (FLONA) do Amapá (0°55' N, 51°36' W; elevation 14.4 m), Amapá State (AP); and the Cerrado-Amazon ecotone: 4 – Centro de Pesquisas Canguçu (9°56' S, 49°47' W; elevation 150 m), Pium, Tocantins State (TO) (Fig. 2).

The three Amazon sampling localities are covered by rainforests, have high mean annual precipitation (2200 mm, 2272 mm and 2300 mm for AM, PA and AP, respectively), but similar mean annual temperatures and small annual temperature range (approx. 2 °C difference between the coldest and hottest months). The Cerrado-Amazon ecotone sampling locality is characterized by transitional forest, where many trees are seasonally deciduous (Marimon et al., 2006), with mean annual rainfall of 1760 mm and a more variable climate (INMET, 2016).

At each sampling site, individuals of *K. calcarata* were captured by active search, nooses, pitfall traps and funnel traps. Each lizard was

housed individually in containers with base area of 22.5 cm × 15.0 cm and height of 8.0 cm at room temperature (approximately 25–30 °C) and provided with water ad libitum until the experiments were performed on the same day of collection in a nearby field lab.

Collected individuals and tissue samples were deposited at institutions listed in Appendix II. All protocols involving live animals were approved by the Comissão de Ética no Uso de Animais na Pesquisa do Instituto Nacional de Pesquisas da Amazônia (INPA) (Code: 029/2014).

We restricted extinction-risk modeling (see below) to distribution records from areas adjacent to where physiological data were collected and within areas occupied by the four *K. calcarata* intraspecific clades (Cronemberger, 2015). For the **Tocantins sub-region**, we considered geographic points east of the Araguaia River, except for disjunct records on the east coast; for the **Amazonas sub-region** we considered geographic records in the area around Manaus extending to Boa Vista (Roraima) and south of Pantepui; for the **Pará sub-region** we considered geographic records in the interfluvium of the Xingu River (west), Araguaia (east) and Amazonas (north) Rivers; and for the **sub-region of Amapá** we considered geographic records from the entire Amapá State (Fig. 2).

Geographic (SDM-input) data were assembled that included a total of 2013 points distributed between sub-regions as follows: Pará (416), Amazonas (82), Amapá (449) and Tocantins (1066). When points were separated by 1 km or less from the next point (defined as one locality; Sinervo et al., 2010), we randomly selected one to be used in modeling. Geographic records were available in online databases of Scientific Collections and Museums records (Appendix I).

## 2.2. Operative temperatures, occupancy index and extinction risks

To explore the natural thermal environment (i.e., microclimate) of *K. calcarata* individuals in each location, we recorded local operative ( $T_e$ ) and air ( $T_{air}$ ) temperatures. Laboratory data were collected simultaneously with operative-temperature recording in all locations. For this purpose, we used temperature data-loggers (HOBO™ Onset Pro V2, approximately 25 by sampling locality) connected to PVC tubes matching adult *K. calcarata* in size and shape (3 cm in diameter, 14 cm long). The  $T_e$  estimates using PVC pipe models has been validated against body temperatures for small ectotherms (Adolph, 1990; Sinervo et al., 2010; Lara-Reséndiz et al., 2014; Lara-Reséndiz et al., 2015, Kubisch et al., 2015; Belasen et al., 2016; Kirchoff et al., 2017). We also used data-loggers unconnected to models to record air temperatures (30 by sampling locality). Each temperature data-logger had two probes, each inserted in a model tube. We placed temperature recorders in microhabitats most likely occupied by lizards (natural clearings, edges of the forest and stream borders) at heights up to two meters (approximated natural height used by *K. calcarata*), always with a probe shaded by leaves and the other placed in a position exposed to sunlight (Sinervo et al., 2010). Temperature was recorded every two minutes throughout the field period. We considered the temperature recorded by the probes to represent the operative temperature ( $T_e$ ) of *K. calcarata* in each sampling locality. We selected only temperature records within the time period when the species is active, based on the time of the first and the last sighting in the field (07:00 h and 16:00 h).

We fit a species-distribution model, using hours of activity, hours of restriction to activity, precipitation and vegetation surfaces, and interactions between those surfaces, as predictors for the distribution of *Kentropyx calcarata* across each sub-region. The hours of activity surfaces were compound from two previous surfaces, each one with different assumptions about the animals' physiology (heliotherm and nonheliotherm). Those surfaces were combined using a vegetation surface as a spatial criterion. This was a simple binary surface indicating where forests or open areas are predominant. Data from the nonheliotherm surface were assigned to forest areas and data from the heliotherm surface were assigned to open areas, creating the compound variable hours of activity surface. The same was done for hours of restriction.

We define hours of activity as the number of hours in a day in which the animal's body temperature was above lower thermal limit ( $T_{lower}$ ), and hours of restriction as the number of hours above the upper thermal limit ( $T_{upper}$ ), following Sinervo et al. (2010). Other than the choice of thermal threshold ( $T_{lower}$  or  $T_{upper}$ ), all the calculations are identical for both surfaces. We calculated the extinction risks based on highest body temperatures recorded in the field instead of preferred body temperatures estimated in laboratory.  $T_{lower}$  is defined as the lowest air temperature at which the species was found active (26.9 °C) and  $T_{upper}$  as highest body temperature at which the species was found active

(40.2 °C).

For the nonheliotherm surfaces, we simulated daily temperature variation across the projected area (See Fig. 2), by constructing a sinusoidal curve ranging from minimum to maximum temperature values for each cell, extracted respectively from WorldClim minimum and maximum temperature surfaces (Hijmans et al., 2005, 2.5 min resolution). Then we counted how many hours at each cell fell outside the established thermal threshold ( $T_{lower}$  or  $T_{upper}$ ). Those values would then be used to create the nonheliotherm hours of activity or restriction surfaces, for each month of the year.

For the heliotherm surfaces, we used operative temperature data ( $T_e$ ) collected from microhabitats used by the species at locations where the species was sampled. This data gave us actual measures of daily temperature variation, which we then used to count the number of hours outside thermal thresholds ( $T_{lower}$  or  $T_{upper}$ ) on each day and in each microhabitat. However, since this data was representative only of the locations where *K. calcarata* was collected, we used a model to extrapolate those values across the projected area. This was done by fitting a generalized logistic model (Richard's curve) of hours above threshold to maximum air temperature, collected for the same location and days where the operative temperature measures were taken. This model was then applied to a WorldClim maximum temperature surface in order to predict hours of restriction and hours of activity across the projected area.

The heliotherm and nonheliotherm surfaces were then compounded using a forest surface, as described above, creating the final hours of activity and restriction surfaces for each month of the year. Each monthly variable (hours of restriction, activity and precipitation) was also averaged across the animal's breeding season and also across the months outside the breeding season. All the calculations were done in R. Those surfaces, together with the forest surface and the interactions between surfaces were used as predictors in species-distribution models assembled from GLM, GAM and MaxEnt algorithms, using 1000 pseudo absence points per run, done with the package biomod2 (Thuiller et al., 2005) in program R. Then, model predictions were extrapolated to the years 2050 and 2070, using WorldClim climate projections under the RCP 4.5 scenario of the global climate model MPI-GSM-LR.

From each future projection, we extracted the occurrence probability or Occupancy Index of *Kentropyx calcarata* at each known current point of occurrence. We then subtracted those probabilities from the occurrence probabilities in the present for the same points, which gives us a probability of the species being extant at that point at that year, complementary to the probability of being extinct. Further details on the modeling and rationale can be found on Appendix III.

## 2.3. Thermal-trait estimates

We measured the thermal traits of the individuals of *K. calcarata* through the following experiments, with their respective sample sizes of

**Table 1**  
Thermal physiology (preferred temperatures -  $T_{pref}$  and critical thermal limits -  $CT_{min}$  and  $CT_{max}$ ) and operative temperatures ( $T_e$ ) of *Kentropyx calcarata* at four sampling locations. We present the sample sizes (n), mean estimates / variance (var), and the minimum and maximum values for each location in parentheses.

	$T_{pref}$ (°C)	$CT_{min}$ (°C)	$CT_{max}$ (°C)	$T_e$ (°C)
Amapá	n = 17 29.9 / var = 5.2 (24.5–33.1)	n = 5 7.8 / var = 27.9 (3.5–16.5)	n = 5 39.8 / var = 12.8 (34.9–43.7)	24.6 ± 1.1 (21.7 – 29.5)
Amazonas	n = 22 31.3 / var = 8.1 (23.3–35.4)	n = 7 11.1 / var = 9.3 (9.4–18.0)	n = 7 40.7 / var = 37.8 (31.8–49.2)	26.8 ± 1.0 (23.5 – 31.3)
Pará	n = 8 30.4 / var = 7.2 (24.8–33.7)	n = 7 14.0 / var = 41.6 (5.1–23.0)	n = 7 41.4 / var = 11.0 (37.9–47.1)	31.1 ± 5.9 (22.7 – 46.3)
Tocantins	n = 61 37.1 / var = 28.7 (30.8–40.2)	n = 10 12.8 / var = 1.1 (10.6–14.3)	n = 10 43.18 / var = 0.8 (41.3–44.4)	32.1 °C ± 7.8 (15.4 – 64.3)

**Table 2**

Significance of coefficients obtained with Generalized Linear Models (GLM) for each model parameter: cumulative hours of restriction (Total\_hr), hours of activity (Total\_ha), and precipitation (Total\_prec), and the quadratic terms: precipitation (Sqprec: Total\_prec<sup>2</sup>), hours of restriction (Sqhr: Total\_hr<sup>2</sup>), hours of activity (Sqha: Total\_ha<sup>2</sup>) and the interactions between precipitation and hours of restriction (Prechr: Total\_prec x Total\_hr), precipitation and hours of activity (Precha: Total\_prec x Total\_ha) and vegetation cover (Forest Layer). Degrees of Freedom: 1.094 (total).

	Estimate	Std. Error	z value	p value
Intercept	- 1.796e + 02	3.765e + 01	- 4.771	< 0.0001
Total_hr	4.195e + 01	5.830e + 00	7.195	< 0.0001
Total_ha	1.076e + 01	1.364e + 00	7.891	< 0.0001
Total_prec	1.233e - 02	1.745e - 03	7.068	< 0.0001
Sqprec	1.461e - 06	5.859e - 07	2.494	0.012639
Sqhr	1.864e - 03	4.567e - 04	4.082	< 0.0001
Sqha	1.909e - 03	5.927e - 04	3.221	0.001278
Prechr	- 9.328e - 08	1.913e - 08	4.877	< 0.0001
Precha	2.287e - 07	2.346e - 08	9.747	< 0.0001
Forest Layer	1.676e + 02	3.735e + 01	4.488	< 0.0001

the all sub-regions: Preferred temperature -  $T_{pref}$  (108), Critical thermal minimum -  $CT_{min}$  (29), Critical thermal maximum -  $CT_{max}$  (29) and Sensitivity of thermal performance (33). We used different individuals for the different experiments, except for  $CT_{min}$  and  $CT_{max}$ . The specific sample sizes of each region are detailed on Tables 1, 3.

#### 2.4. Preferred temperature

The preferred temperature of individual lizards was estimated in a thermal gradient with base area of 2.0 m × 0.3 m and 0.65 m high. The gradient was divided into lanes, where individuals were placed. A heat lamp was placed at one end of the lane to maintain a substrate temperature of 48 °C, simulating direct daylight, while gel ice packs and a fan kept the other end of the lane at an approximate temperature of 21 °C, which simulated a natural shaded microenvironment.

We measured the initial body temperature of individuals before placing them in the lane for the experiment. Body temperature was measured with an infrared laser thermometer (Digitech QM7221) at a distance of < 0.25 m or through an automated data logger with thermocouple probes attached to the belly of each individual. Both estimate instruments were calibrated against each other. We validated the use of body temperatures based on infrared thermometers with data on *Zootoca vivipara*, with high correlation between core and surface temperatures (0.85; n = 34, P < 0.001). This species is in similar size to most of the lizards in our data set. Before each round of estimates, lizards were left undisturbed in the thermal gradient for 10–15 min of acclimation. Regardless of the method,  $T_{pref}$  estimation lasted 2 h with body temperatures measured every 5 min, resulting in 25 estimates per individual. We used the mean temperature of the 25 estimates to represent each individual's preferred temperature,  $T_{pref}$ .

#### 2.5. Sensitivity of thermal performance

We measured individual ability to sustain physical activity to simulate natural activity conditions where mobility is required for

territorial defense, escape from predators or foraging (Robson and Miles, 2000). Lizards were stimulated to run in a circular track by gentle touches and only using data when lizards did not run to the sides (Clobert et al., 2000). The track was made of aluminum, with a diameter of the inner wall of 60 cm and of the outer wall of 150 cm. Lizards ran in a track with a width of 25 cm. We used the soil from each collection site as a substrate to mimic natural conditions.

We measured the endurance elapsed until exhaustion and motor-response loss of individual locomotion. We estimated the endurance to account for effects of size and temperature on speed (distance) (Clobert et al., 2000).

Each individual was acclimated to run at seven different body temperatures (15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 40 °C and 43 °C) in a randomized sequence. The test started when the individual's body temperature was within 0.5 °C of the target temperature. Lizards had a rest interval of 4 h between each race at a new temperature. Within individuals, running temperatures were given in a random sequence. The substrate temperature of the circular track was adjusted to each running body temperature through intercalation of the track between shadow and sun. The optimum performance temperature was estimated based on the body temperature at which each individual had the best performance (Huey and Stevenson, 1979).

#### 2.6. Thermal tolerance: $CT_{min}$ and $CT_{max}$

The thermal-tolerance range represents the physiological performance interval in which the activities of an organism are possible and is limited by the critical thermal minimum ( $CT_{min}$ ) and maximum ( $CT_{max}$ ) temperature (Huey and Stevenson, 1979). We estimated  $CT_{min}$  and  $CT_{max}$  of *K. calcarata* individuals at each study site using cold and heat sources to manipulate body temperature to a critical limit while monitoring at approximately 2-min intervals the loss of righting response, and individual body temperature was taken with a quick-reading cloacal thermometer (Miller and Weber 4K8893) when the individual lost motor function.

To estimate  $CT_{min}$ , lizards were isolated in Styrofoam boxes with gel ice packs. To estimate  $CT_{max}$ , lizards were directly exposed to sunlight or to bottles containing heated water placed in styrofoam boxes. To represent the critical thermal limit, we used the body temperature at which the individual did not move after being turned belly up (Clobert et al., 2000). We used the difference between  $CT_{max}$  and  $CT_{min}$  to represent the thermal-tolerance range of each individual ( $Amp_{tol}$ ).

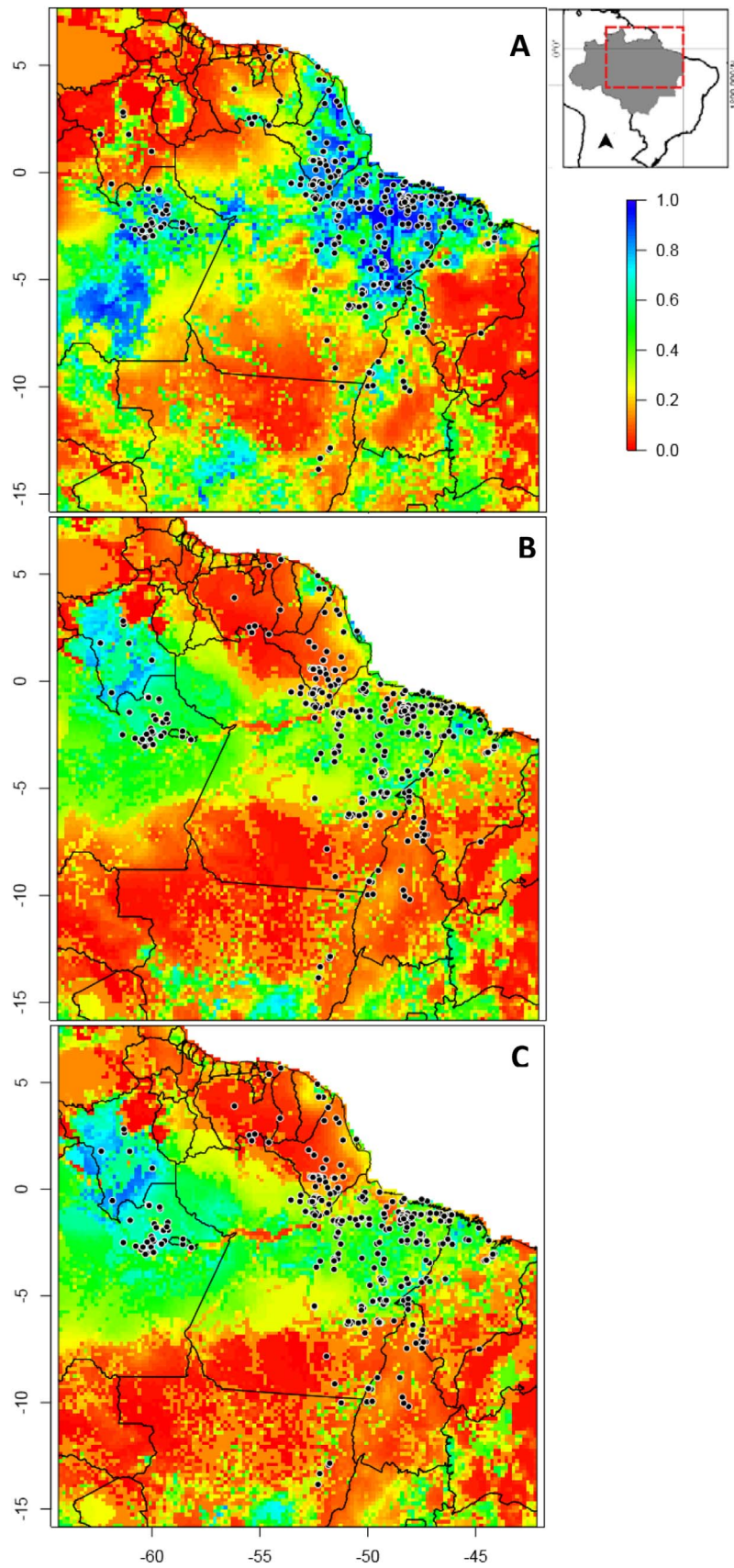
#### 2.7. Data analysis

We compared the thermal biology among the four sampling localities using either an analysis of variance (ANOVA) or Kruskal-Wallis test. When significant (P < 0.05) differences were found in mean trait values among sites, we used the Tukey *post hoc* test to identify pairwise differences. The shapes of thermal performance curves (TPC's) of individuals from each site were estimated and plotted using generalized additive mixed models (GAMM), using the function "gamm" in the package mgcv (Woods, 2006) and ggplot2 (Wickham, 2009). Because size also may influence locomotor performance, we included snout-to-

**Table 3**

Results of generalized additive mixed models describing thermal performance curves for *Kentropyx calcarata* at the four sampling locations. Statistics are given for the effect of temperature and SVL on performance (temperature - Temp and snout-to-vent length - SVL, respectively), R<sup>2</sup> value, the optimum temperature for the performance estimates ( $T_{opt}$ ), and the temperature range in which at least 80% of the maximum performance of the individual is possible.

	Temp	SVL	R <sup>2</sup>	$T_{opt}$ (°C)	$B_{80}$ (°C)
Amazonas (n = 10)	F = 31.6; P = .0001	P = .2	0.5	37.2	34.3–39.4
Pará (n = 5)	F = 8.8; P = .0002	P = .7	0.2	29.1	24.2–33.9
Amapá (n = 8)	F = 5.2; P = .0003	P = .4	- 0.08	25.8	21.6–29.4
Tocantins (n = 10)	F = 34.2; P = .0001	P = .0001	0.5	35.9	33.3–38.1



**Fig. 3.** *Kentropyx calcarata* occupancy index for the studied sub-regions, based on the habitat suitability for the present (A), 2050 (B) and 2070 (C). The color scale indicates a gradient of favorable areas for species occurrence, ranging from blue (1.0 - suitable areas) to red (0.0 - unsuitable areas). Black dots refer to the current *K. calcarata* occurrence records in the four sub-regions used in partitioned modeling (models were estimated separately for each sub-region but plotted simultaneously). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

vent length (SVL) as a covariate. Sex of individuals not was used as a covariate. Gravid females and immature individuals were not used in the tests. To compute the curve,  $CT_{max}$  and  $CT_{min}$  mean values of individuals from each location were given a performance value of 0. The breadth of optimum performance was estimated as the range of body temperatures at which lizards attained 80% ( $B_{80}$ ) of maximum performance, by multiplying the maximum performance speed at the best temperature ( $T_{opt}$ ) by 0.8 (Huey and Stevenson, 1979). Statistical analyses were performed with R (R Development Core Team, 2014). Results are shown using mean  $\pm$  standard deviation and sample sizes ( $n$ ).

### 3. Results

#### 3.1. Operative temperatures, occupancy index and extinction risks

The estimated operative temperatures for *K. calcarata* differed significantly between sampling sites ( $F_{3,13} = 91$ ,  $P = 0.0001$ ). At the Tocantins site, models had higher mean  $T_e$  and an amplitude 93% higher than in other locations (Table 1). At Amapá and Amazonas sites, models had the lowest  $T_e$  (7.5 °C lower than TO) and were more thermally homogeneous.  $T_e$  at Pará site was higher and also had greater variability than in other Amazonian sites (Table 1).

The traits  $H_r$  and  $H_a$ , as well as precipitation and historical Amazon forest cover, were significant explanatory variables in our SDM, along with most of their interaction effects and quadratic terms (Table 2).

The occupancy index in the present (Fig. 3a) for the Tocantins sub-region was of 83.8%, 70.9% for the Amapá sub-region, 61.8% for the Amazonas sub-region and 74.3% for the Pará sub-region. The average extinction risk of local populations for 2050 (Fig. 3b) in the Pará sub-region was 57.8%, 60.2% for the Tocantins sub-region, 26.1% for the Amazonas sub-region and 70.8% for the Amapá sub-region. The mean extinction risk of populations for 2070 (Fig. 3c) in the Pará sub-region was of 85.2%, 86.6% for the Tocantins sub-region, 52.8% for the Amazonas sub-region and 92.8% for the Amapá sub-region.

#### 3.2. Preferred temperatures and thermal tolerances

Individuals of *K. calcarata* from different locations exhibited significant differences in  $T_{pref}$  ( $F_{3,94} = 123$ ,  $P < 0.0001$ ; Appendix IV). Tocantins individuals selected temperatures 23% higher ( $37.1 \pm 5.3$  °C) than the mean  $T_{pref}$  of other locations (AM:  $31.3 \pm 2.8$  °C; PA:  $30.4 \pm 2.6$  °C and AP:  $29.9 \pm 2.2$  °C). Lizards from the remaining three locations exhibited no significant difference in  $T_{pref}$  (Table 1).

No significant differences were found in mean thermal-tolerance range among the sampling locations, although there was a trend for differences in minimum critical temperature ( $CT_{min}$ ) among locations

( $CT_{min}$ ,  $P = 0.08$ ;  $CT_{max}$ ,  $P = 0.3$ ;  $Amp_{tol}$ ,  $P = 0.5$ ). Amapá individuals had the lowest  $CT_{min}$  (6.2 °C difference from PA individuals, which had the highest  $CT_{min}$ ). In contrast, variances of minimum and maximum critical limits were significantly different between locations (Levene test,  $CT_{min}$ ,  $F_{3,26} = 3.2$ ,  $P = 0.03$ ;  $CT_{max}$ ,  $F_{3,27} = 3.6$ ,  $P = 0.02$ ). Individuals from Pará ( $CT_{min}$ ,  $14.0 \pm 6.4$  °C;  $CT_{max}$ ,  $41.4 \pm 3.3$  °C), Amazonas ( $CT_{min}$ ,  $11.1 \pm 3.0$  °C;  $CT_{max}$ ,  $40.7 \pm 6.1$  °C), and Amapá ( $CT_{min}$ ,  $8 \pm 5.2$  °C;  $CT_{max}$ ,  $39.8 \pm 3.5$  °C) were similar to each other, showing greater variability in thermal-tolerance estimates than Tocantins individuals ( $CT_{min}$ ,  $12.8 \pm 1.1$  °C;  $CT_{max}$ ,  $43.1 \pm 0.8$  °C) (Table 1).

There was no significant relationship between mean operative temperature or maximum operative temperature ( $T_{e,max}$ ) and  $T_{pref}$ ,  $T_{opt}$  or the upper temperature limit for 80% of maximum performance measured in the laboratory ( $P \geq 0.3$  in all cases). However, there was a strong positive relationship between maximum operative temperatures ( $T_{e,max}$ ) and critical thermal maximum ( $CT_{max}$ ) measured in the laboratory ( $CT_{max} = 37 \pm 0.1 * T_{e,max}$ ,  $F_{(3,45)} = 37.1$ ,  $P = 0.02$ ; Fig. 4).

#### 3.3. Thermal sensitivity of locomotor performance

Body temperatures significantly affected individual performance at all sampling locations (AM:  $F_{(3,38)} = 31.6$ ,  $P < 0.0001$ ,  $R^2 = 0.5$ ; PA:  $F_{(3,46)} = 8.8$ ,  $P = 0.0002$ ,  $R^2 = 0.2$ ; AP:  $F_{(4,27)} = 5.2$ ,  $P = 0.0003$ ,  $R^2 = 0.08$  and TO:  $F_{(5,91)} = 34.2$ ,  $P = 0.0001$ ,  $R^2 = 0.5$ ), with reduced performance at higher temperatures. We detected a significant effect ( $P < 0.0001$ ) of SVL on locomotor performance only among Tocantins individuals (Table 3), with smaller individuals exhibiting lower locomotor performance than larger ones, generating a bimodal performance curve (Appendix V). We tested males and females from each location separately to evaluate possible sex effects on performance before calculating performance curves, but there were no statistically significant sexual differences in any sampling location ( $P > 0.05$ , in all cases) and, therefore, analyses were conducted without regard to sex.

The optimum-performance temperature was highest at the Amazonas site (37.2 °C) and lowest for Amapá individuals (25.8 °C), while Tocantins (35.9 °C) and Pará (29.1 °C) individuals exhibited better performance at intermediate temperatures (Table 3). Individuals from PA, AP and TO selected higher body temperatures than their optimum-performance temperatures, whereas the estimated  $T_{pref}$  for AM individuals was lower than the optimum-performance temperature (Table 1).

The optimum-performance temperature was higher at sampling sites where the 80% performance breadth ( $B_{80}$ ) was narrower (TO and AM), while in places where  $AT_{80}$  was wider (PA and AM), individuals showed better locomotor performance under a lower  $T_{opt}$  (Table 3), reflecting differences in the curve shapes (Appendix V).

### 4. Discussion

Here we present the first study with a tropical forest heliothermic lizard integrating previously obtained information on intraspecific phylogeographic structure with thermal biology traits and geographic distribution data to predict extinction risks due to climate change in a mega-diverse region. We also present an updated model of Sinervo et al. (2010) to calculate extinction risks based on highest body temperatures recorded in the field instead of preferred body temperatures estimated in laboratory, which can serve as a baseline for future studies with forest heliotherms.

While Sinervo et al. (2010) predicted an extinction risk for teiids of 13.6% by 2050 and 21% by 2080, our geographically more refined physiological models that included more habitat variables in the SDM for *K. calcarata* predicted higher relative extinction risks, with a mean across lineages of 53.7% for 2050 and 79.4% for 2070. A possible reason for the difference between the studies is that 30 out of the 36 heliothermic species Sinervo et al. (2010) considered for their

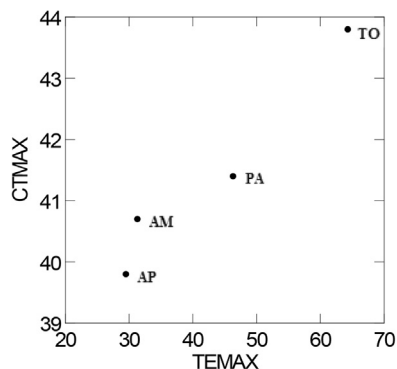


Fig. 4. Relationship between maximum operative temperature ( $T_{e,max}$ ) and *K. calcarata* critical thermal maximum ( $CT_{max}$ ) measured in the laboratory for the four studied locations.

predictions inhabit open vegetation areas and deserts, and experience higher activity temperatures (mean body activity temperatures = 38.6 °C, data from Sinervo et al., 2010) than the few inhabiting forest or aquatic environments (mean body activity temperatures = 34.8 °C, data from Sinervo et al., 2010). However, even using other species, the model of Sinervo et al. (2010) should have had a greater relative extinction rate because they used a narrower thermal tolerance. Our modeling suggests that heliothermic lineages of *Kentropyx calcarata* living in densely forested environments (PA, AM, AP) have lower thermal preferences than the lineage living in the Amazon-Cerrado ecotone (Tocantins sub-region), and this elevates the risk of extinction. However, our interpretation of the differences between forested versus ecotone regions may also be because we included the effects of habitat change and not only thermal biology on extinction risk calculations.

In a similar approach, Kubisch et al. (2015) calculated the restriction hours for a Patagonian gecko thermoconformer (*Homonota darwini*) and predicted that 20% of populations could become extinct by 2080, while Sinervo et al. (2010) predicted that the family as a whole would not suffer impacts due to climate change. As in our study, extinction-risk modeling by Kubisch et al. (2015) was conducted at the species level on a high latitude species of Phyllodactylid gecko, and thus, may represent a finer scale risk assessment.

Our SDM results suggest that ecophysiological traits, such as the thermal breadth for activity,  $H_r$ ,  $H_a$ , and forest cover provide a useful explanation of the occurrence of *K. calcarata* in certain locations of the Amazon. In addition, the significant effect of precipitation (alone and in interaction with hours of restriction) suggests that a study of egg development and/or adult water loss (Muth, 1980), and the potential for climate change induced drought (Westphal et al., 2016), might prove to be a fruitful avenue of research at a deeper mechanistic level than we have done with our study of thermal ecophysiological traits across the four lineages of *K. calcarata*.

There was little relationship between mean or maximum operative temperatures and most of the thermal traits we measured. This might be expected, because the cost associated with different environments is mainly related to behavioral modifications that might not strongly impact fitness. However, passing the critical thermal maximum has a very strong immediate fitness cost (death) (Angilletta, 2009), because the lizard cannot escape from the immobilizing conditions, so it is not surprising that it is related to the maximum environmental temperature of the site.

Thermal traits appear to be, at least in part, determined by the environmental temperatures at each site, as evidenced by the strong relationship between critical thermal maximum and maximum operative temperatures. This indicates selection for different thermal traits at different sites (Gunderson and Leal, 2012; Moritz et al., 2012; Piantoni et al., 2016; Llewelyn et al., 2016). The variance in laboratory measures of thermal critical limits was greater in some sites than others, especially those sites that had low mean preferred temperatures. Therefore, adaptation to forested environments with lower environmental temperatures does not appear to have resulted in narrow amplitudes of thermal traits and loss of adaptive potential to climate changes. Huey et al. (2012) and Deutsch et al. (2008) predicted that presumed narrow amplitudes of thermal traits of tropical ectotherms would increase their vulnerability to climate change, but we show that individuals of *Kentropyx calcarata*, although living in thermally homogeneous environments, exhibit high variability in critical limits, which may favor individuals with increased tolerance to changes in temperature under a global-warming scenario.

When populations are polymorphic, variability tends to increase the niche amplitude in a way that thermal traits with greater variability may be favored by natural selection caused by climate change (Logan et al., 2014). Thus, we suggest that the potential adaptive amplitude will be greater in populations or areas whose individuals exhibit greater variation in thermal tolerance. Populations of *K. calcarata* in the Amazonian sites clearly show high flexibility in thermal critical limits

and, therefore, may have higher potential adaptive amplitude. In contrast, critical limits were less variable in the population at the ecotone. This could be a result of higher selection pressure at the ecotone, resulting in reduced variance in this trait. Despite the lower variability, under a scenario of climate warming, this could favor these populations as  $T_{pref}$  was higher at the ecotone. Further studies investigating the geographic and adaptive basis of genetic diversity variation and signals of selective pressure at the genomic level of *K. calcarata* are needed to test these hypotheses.

Most thermal biology studies consider the mean values of traits to evaluate differences between species, populations or locations (Hoffman and Sgro, 2011). We found geographic differences in thermal traits mean values, such as critical limits, but also in variability values. We suggest that future studies should pay closer attention to the variability of thermal traits. Variability in responses to environmental conditions can be considered a proxy for adaptive potentials of natural populations (e.g., Logan et al., 2014; Llewelyn et al., 2016) and, therefore, of persistence in response to climate change (Hoffman and Sgro, 2011).

Large areas of tropical forest have been predicted as potential refuges for biodiversity with increasing temperatures (Kearney et al., 2009; Clusella-Trullas et al., 2011). Our estimates are of increased extinction risks in most regions, mainly with the reduction of favorable areas for occurrence in lower-altitude Amazon forest regions that could contribute to local declines. However, even if the species goes extinct in some areas, colonization processes facilitated by large land connections that exist in the Amazon region may lead to species persistence in the long term.

As a forest heliotherm, persistence of *Kentropyx calcarata* also depends on forest preservation. Nonetheless, the Amazon rainforest is currently under high deforestation rates and some sub-regions are particularly impacted, such southern Pará state, which has the second-highest absolute deforestation rate of the Amazon (Fonseca et al., 2015). In addition to extinction risks estimated for the Tocantins sub-region, the Cerrado-Amazon ecotone largely coincides with the “Arc of Deforestation” of the Brazilian Amazon and is under intense anthropogenic change characterized by intense substitution of natural landscapes for cattle ranching and agriculture (Costa and Pires, 2010). Combined effects of temperature increases on a global scale (Sinervo et al., 2010) and on a local scale (Lara-Resendiz et al., 2015) as a result of landscape conversion are factors that may further increase extinction risks of *K. calcarata* in these regions.

Our results show that *Kentropyx calcarata* thermal physiology varies geographically and thermal maxima appear to be conditional on the thermal environment of each surveyed locality. We used a novel approach to model extinctions risks for intra-specific lineages of a tropical heliothermic lizard that takes into account activity body temperatures measured in the field, vegetation cover and precipitation as environmental variables. Our results support the hypothesis that tropical lizard taxa are at higher risk of local extinction and this factor is geographically structured, with some regions within the species range predicted to become more or less favorable as the climatic conditions change over this century. Also, our laboratory experiments indicate significant differences in thermal traits between individuals from the same or distinct populations. We propose that this variability in thermal traits is the basis of local adaptation and might buffer some populations from extinction as the climate warms. Large areas of dense forest vegetation, thermal-tolerance variability and natural selection for heat tolerance are factors with considerable capacity to absorb the impacts of temperature increase on tropical ectotherms during climate change, and they should be included in future models of extinction risk.

## Acknowledgements

We would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) - FPW - 475559/2013-4; GRC -



456926/2014-3, Fundação de Amparo à Pesquisas do Estado do Amazonas-FAPEAM - FPW - 062.00665/2015 and 062.01110/2017 and Partnerships for Enhanced Engagement in Research from the US National Academy of Sciences and US Agency for International Development (PEER NAS/USAID PGA-200005316) for funding this research. We thank Dra. Teresa Avila-Pires for support in data collection in Pará, the staff of INPA reserves for support in data collection in AM (Valdecira, Valdir and Rubenildo), and to all people who gave direct assistance for data collection in all locations: AM = G. Lima, L. Chrisley, J. Buckner, K. Bajer, O. R. Molnar, Sr. Compensa and Jesus; AP = A. Missassi, A. Nunes, J. Cosenza, and Sr. Maranhão; PA = C. Sette, A. Paula, Sr. Mór and Dorinha; and TO = C. Morais, S. Balbino, D. Tucker, M. Kucinick and S. Bee. FPW thanks financial support from L'Oréal-UNESCO-ABC Para Mulheres na Ciência - Brazil Werneck 2016 and L'Oréal-UNESCO For Women In Science awards - IRT Werneck 2017. GRC thanks financial support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - GRC - 88881.068430-2014-01 and Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF) - Guarino Colli - 193.000.576/2009. B.S. and D. B. M. were supported by NSF EF-1241848. JCS thanks Jack W. Sites, Jr. (BYU) for his support as a postdoctoral fellow under NSF EF-1241885 and SJU for the start-up funds a new faculty. WEM was supported by the PPBio and CENBAM, and both contributed to the availability of a data repository. The authors have no conflict of interest to declare.

#### Authors' contributions

FPW, BS, DBM, WEM and GRC conceived the ideas and designed methodology; EPS, GHC, GRC, LMDV, JF and JCS collected the data; EPS, GHC, WEM and JCS analyzed the data; EPS led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

#### Data accessibility

Data deposited in the KNB Data Repository. Identifier knb.1111.4 (<https://knb.ecoinformatics.org/#view/knb.1111.4>)

#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.jtherbio.2018.01.013>.

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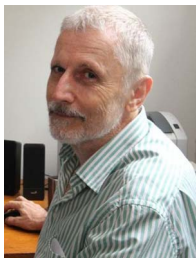
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