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# Thermal physiology of Amazonian lizards (Reptilia: Squamata)

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# Abstract

We summarize thermal-biology data of 69 species of Amazonian lizards, including mode of thermoregulation and field-active body temperatures (T<sub>b</sub>). We also provide new data on preferred temperatures ( $T_{pref}$ ), voluntary and thermal-tolerance ranges, and thermal-performance curves (TPC's) for 27 species from nine sites in the Brazilian Amazonia. We tested for phylogenetic signal and pairwise correlations among thermal traits. We found that species generally categorized as thermoregulators have the highest mean values for all thermal traits, and broader ranges for T<sub>b</sub>, critical thermal maximum (CT<sub>max</sub>) and optimal (T<sub>opt</sub>) temperatures. Species generally categorized as thermoconformers have large ranges for T<sub>pref</sub>, critical thermal minimum (CT<sub>min</sub>), and minimum voluntary (VT<sub>min</sub>) temperatures for performance. Despite these differences, our results show that all thermal characteristics overlap between both groups and suggest that Amazonian lizards do not fit into discrete thermoregulatory categories. The traits are all correlated, with the exceptions of (1) T<sub>opt</sub>, which does not correlate with  $CT_{max}$ , and (2)  $CT_{min}$ , and correlates only with  $T_{opt}$ . Weak phylogenetic signals for T<sub>b</sub>, T<sub>pref</sub> and VT<sub>min</sub> indicate that these characters may be shaped by local environmental conditions and influenced by phylogeny. We found that open-habitat species perform well under present environmental conditions, without experiencing detectable thermal stress from high environmental temperatures induced in lab experiments. For forest-dwelling lizards, we expect warming trends in Amazonia to induce thermal stress, as temperatures surpass the thermal tolerances for these species.



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# Introduction

Body temperature  $(T_b)$  in ectotherms influences all physiological and behavioral processes [1]. Consequently, maintenance of  $T_b$  within suitable limits is essential for ectotherms survival [2]. Thermoregulators actively maintain  $T_b$  within a restricted range of temperatures by heliothermy, i.e., by basking in the sun, or by thigmothermy, i.e., by contact with warm surfaces [3]. Thermoconformers do not actively thermoregulate, so their  $T_b$  parallels fluctuations in the environmental temperature [1, 4]. However, no lizard species has been shown to be a complete thermoconformer; all will move to avoid unfavorable extreme temperatures. This category is often used for species that select areas with relatively uniform temperatures, such as shaded forest, where active thermoregulation is not needed to maintain relatively stable body temperatures. Using a strictly thermoconforming strategy requires that species have broad thermal tolerances [1], and experience high variation in  $T_b$  throughout the day, season and geographic range.

In the field, lizards are usually active at a restricted range of  $T_b$ . It is commonly assumed that these temperatures represent their actual thermal preferences [5]. However, laboratory experiments show that the variance in  $T_b$  range observed in nature for tropical lizards exceeds both the preferred  $T_b$  and the voluntary  $T_b$  range observed when the animals are subjected to thermal gradients [6–7]. Consequently, tropical lizards may already be experiencing  $T_b$ 's at or above their physiological optima [8], putting them dangerously close to their upper thermal thresholds. These upper thermal limits are likely to be exceeded in the next few decades as a consequence of climate change [9]. An alternative interpretation is that preferred  $T_b$  and voluntary  $T_b$  reflect not only physiological limits, but are also tailored to specific activities, such as digestion, reproduction and foraging for different types of prey [1], and that laboratory studies do not fully reflect the range of motivational states. Field activity temperatures may vary seasonally, independent of variation in environmental temperatures (e.g., [10]).

The influence of ambient temperature on key physiological traits is described by thermal performance curves (TPC) [2]. A species' thermal sensitivity can be visualized and quantified through TPCs, which reveal several important thermal properties of ectotherms. These include the optimal temperature  $(T_{opt})$ , for maximal animal performance; the breadth of temperatures that results in a species performing at  $\geq 80\%$  of its optimal capacity (B<sub>80</sub>); and the thermal tolerance range, which is the difference between the critical thermal minimum and maximum temperatures (CT<sub>min</sub> and CT<sub>max</sub>), i.e., the extreme temperatures that an individual can maintain locomotor function [11]. T<sub>opt</sub> can vary within and among species and varies among physiological traits, according to the hypothesis of multiple physiological optima [1, 12]. Locomotor performance is one of the best-studied traits in thermal physiology, because it is related to Darwinian fitness and presumably reflects the ability to escape from predators, capture prey, and reproduce [13]. TPCs are also useful in assessing extinction risk of ectotherms. Because global warming may alter the spatial distribution of preferred microclimates [14], animals that rely on behavioral thermoregulation may experience a reduction in the time available for activity during periods when preferred microclimates become too rare to locate without overheating [15]. Restriction in activity time can result in extirpation or extinction if the remaining time is insufficient to perform all the necessary functions for successful breeding and recruitment [16].

Amazonia is a biogeographic region predicted to be strongly affected by climate change [17–18]. It covers about eight million square kilometers spread over nine South American countries [19]. Current estimates suggest that at least 210 species of lizards occur in the Amazon, although the actual diversity is poorly known [20–21]. Observed trends in the region's climate include an overall reduction in precipitation and increased duration and intensity of

droughts, especially in southern Amazonia [22], where climate change interactions with landuse change are stronger [23–24]. Recent studies indicate a long-term decreasing trend of carbon accumulation in Amazonia due to increased tree turnover and mortality rates [25]. Moreover, increased dryness may result in large-scale reductions in biomass, carbon uptake and net primary productivity [26]. Some models suggest that these changes may induce biome shifts in Amazonia, with the forest being replaced by drier vegetation associations, such as seasonal forests and savannas [27]. Therefore, recent and projected climate trends in Amazonia will likely result in a more open canopy and increased ambient temperature for forest-dwelling lizards. Despite the vastness and complexity of Amazonian habitats, thermal-physiology data for Amazonian lizards are limited, with most studies scattered among the major groups of Squamata. Most data are focused on reports of field-active  $T_b$  and there have been few controlled experiments on preferred or optimal  $T_b$ .

We aim to provide the first comprehensive summary of thermal physiology characteristics of Amazonian lizards, which is essential to enhance our understanding of the effects of global warming on current and future lizard diversity in this megadiverse region. We first characterize patterns of variation in  $T_{bs}$  of Amazonian lizards (including some species that occur peripherally, at the ecotone between the Amazonian rainforest and the savanna-like Cerrado, an ecophysiological tension interface). We also provide new data on the thermal biology of some of these species and summarize the information on lizards' modes of thermoregulation. Moreover, we next analyze evolutionary trends among thermal and physiological traits by examining the consistency of trait variation with phylogeny (i.e., phylogenetic signal) and the correlations among traits after controlling for the influence of phylogeny.

## Material and methods

#### Literature review

We carried out a literature survey for data on seven physiological traits of Amazonian lizards: field-active  $T_b$ , preferred temperature  $(T_{pref})$ , minimum and maximum voluntary temperatures ( $VT_{min}$  and  $VT_{max}$ ), critical thermal minimum and maximum ( $CT_{min}$ ,  $CT_{max}$ ) and the optimal temperature for locomotor performance ( $T_{opt}$ ). Only data on  $T_b$  were available. Because some species have distributions extending beyond Amazonia into other biomes, our review extended beyond Amazonia, and included species from the Atlantic Rainforest, Caatinga, and Cerrado regions of Brazil, as well as the Lavrado, a savanna enclave in northeastern Roraima, Brazil. We also included data from lizards occurring in tropical forests of Central America that have similar ecophysiological traits. Species were classified as thermoregulators or thermoconformers based on whether the studies indicated they were heliotherms (thermoregulators) or non-heliotherms (defined here as thermoconformers). We also reviewed the literature to search for substrate ( $T_{sub}$ ) and air-temperature ( $T_{air}$ ) data associated with  $T_b$ , and obtained 45 studies from the last 50 years, with reported  $T_b$ 's from 62 species occurring in Amazonia.

#### Field data

We completed our dataset with data collected by the authors throughout the year. First, we included T<sub>b</sub> data on eight species collected from 1993 to 1999 in seven localities in Amazonia: Estación Biológica de la Pontifícia Universidad Católica (Quito), within the Reserva de Producción Faunística Cuyabeno (Sucumbíos Province, Ecuador, 0°0'N, 76°10'W); Juruá River Basin, ca. 5km north of Porto Walter (Acre, Brazil, 8°15'S, 72°46'W); Ituxi River (Amazonas, Brazil, 8°20'S, 65°43'W); 30km NW of Caracaraí (Roraima, Brazil, 2°50'N, 60°40'W); Parque Estadual Guajará–Mirim, on the Formoso River (Rondônia, Brazil, 10°19'S, 72°47'W); SE of



**Fig 1. Thermal traits' sampling localities**. Body-temperature data were collected between 1993 and 1999 and thermal preference and performance data were collected between 2014 and 2016. Lizards are illustrative of the eleven families found in the field. Numbers are representative of the localities, as follows: 1) 30km NW of Caracaraí (Roraima, Brazil, 2°50'N, 60°40'W); 2) Floresta Nacional do Amapá (Amapá, Brazil, 0°55'N, 51°36'W); 3) Estación Biológica de la Pontifícia Universidad Católica (Sucumbíos Province, Quito, Ecuador, 0°0'N, 76°10'W); 4) Floresta Nacional de Caxiuanã (Pará, Brazil, 1°44'S, 51°27'W); 5) Yasuni National Park (Ecuador, 1°5'S, 75°55'W); 6) Reserva Florestal Adolpho Ducke (Manaus, Brazil, 2°57'S, 59°55'W); 7) Agropecuária Treviso (Pará, Brazil, 3° 9' S, 54° 50'W); 8) SE of Manaus (Amazonas, Brazil, 3°20'S, 59°4'W); 9) Juruá River Basin (Acre, Brazil, 8°15'S, 72° 46'W); 10) Ituxi River (Amazonas, Brazil, 8°20'S, 65°43'W); 11) Centro de Pesquisas Canguçu (Pium, Tocantins, Brazil, 9°56'S, 49° 47'W); 12) Parque Estadual Guajará–Mirim (Rondônia, Brazil, 10°19'S, 72°47'W); and 13) Los Amigos Biological Station (Peru, 12° 34'S, 70°6'W).

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Manaus, on the margin of the Amazon River (Amazonas, Brazil, 3°20'S, 59°4'W); and Agropecuária Treviso, 101 km S and 18 km E of Santarém, close to Curuá-Una River (Pará, Brazil, 3° 9' S, 54° 50'W) (Fig 1). For the species from these localities, cloacal temperatures were measured from adult individuals with Miller & Weber quick reading cloacal thermometers (resolution of 0.2°C). Literature and empirical data provide information on the general thermal characteristics of each species, and we do not address within-species variation due to factors such as reproduction, digestion and infection.

Ecophysiological data were collected in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Comissão de Ética no Uso de Animais (CEUA)–INPA (Permit Number: 029/2014), CEUA–MPEG (Permit Number: 01/2015), and CEUA UnB (33716/

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2016). All collecting in Brazil was done under pertinent IBAMA (073/94-DIFAS) and SISBIO (13324–1, 49241, 50381, 44832–1) permits. All efforts were made to minimize discomfort to research animals.

We collected thermal-preference and performance data on 27 species between 2014 and 2016 in six localities, five in Amazonia—Floresta Nacional do Amapá (Amapá, Brazil, 0°55'N, 51°36'W), Floresta Nacional de Caxiuanã (Pará, Brazil, 1°44'S, 51°27'W), Reserva Florestal Adolpho Ducke (Manaus, Brazil, 2°57'S, 59°55'W); Los Amigos Biological Station (Peru, 12°34'S, 70°6'W), and Yasuni National Park (Ecuador, 1°5'S, 75°55'W), and one in the Amazonia-Cerrado ecotone—Centro de Pesquisas Canguçu (Pium, Tocantins, Brazil, 9°56'S, 49° 47'W) (Fig 1). Specimens were captured by active search or with pitfall traps checked twice daily. Lizards were kept in captivity for a period of no more than three days, and were released at their site of capture after a recovery time of at least four hours after the last trial. While in captivity, animals were housed individually in plastic containers with air holes and a damp cloth for moisture, without access to food. We measured Tpref, CTmin, CTmax, and thermal performance curves on captive lizards. Table 1 shows the number of individuals used in each test by species. We measured snout-vent length (SVL) to 0.1mm with a Vernier caliper. A few voucher specimens of each species were euthanized with a lethal dose of Tiopental anesthetics, fixed in 10% formalin, and permanently stored in 70% ethanol. Voucher specimens were deposited in the Herpetological Collections of Museu Paraense Emílio Goeldi (MPEG), Pará, Brazil; Instituto Nacional de Pesquisas da Amazônia (INPA), Amazonas, Brazil; Universidade de Brasília, Distrito Federal (CHUNB), Brazil; Monte L. Bean Life Science Museum, Utah, USA; and Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos, Lima, Peru.

We characterized the thermal biology of captured lizards with the following protocol. We measured the lizards' T<sub>b</sub> by using infrared thermometers, focusing the laser on the mid-portion of the animal's ventral side, with approximately 15cm between animal and thermometer. We validated the use of body temperatures based on infrared thermometers with data on Zoo*toca vivipara*, with high correlation between core and surface temperatures (0.85; n = 34, P<0.001). This species is of similar size to most of the lizards in our data set. Smaller species should present even higher correlations between core and surface temperatures, and we did not include any large species in the laboratory tests. Tpref and voluntary upper (VTmax) and lower (VT<sub>min</sub>) temperatures were measured using a thermal gradient. Lizards were placed for 2 hours in plywood tracks 1m in length and 40cm wide, with a photothermal gradient of 15-40°C generated across each track using ice at one end and a heating lamp ~100W full spectrum at the other (sensu [28]). T<sub>b</sub> was measured every 3-5 minutes, and T<sub>pref</sub> was estimated as the mean of all T<sub>b</sub> values recorded. The first measurement was made after five minutes of the animals' positioning inside the track, to allow lizards to get acclimated to the track and reach their preference. VT<sub>max</sub> and VT<sub>min</sub> for each individual during this interval were estimated by the interquartile range of T<sub>pref</sub> [29]. We measured T<sub>pref</sub> for all individuals captured. Diurnal lizards were tested during the day, while nocturnal lizards were tested after sunset. Afterwards, lizards were arbitrarily chosen to undergo either the thermal tolerance or performance tests.

Critical temperatures were measured on 485 individuals of 26 species. An individual's body temperature was decreased or increased in a chamber cooled by ice packs or heated by hot water until the animal lost its righting response. Each animal was tested for both  $CT_{min}$  and  $CT_{max}$ , and heated/cooled to their  $T_{pref}$  immediately after the tests. We always measured  $CT_{min}$  before  $CT_{max}$ , since the last may get the animals most impaired and thus needs that the animals have a longer recovery time. To calculate  $T_{opt}$ , we measured locomotor-capacity experiments on 254 individuals. We stimulated each individual to run once at 2–7 randomly-assigned temperatures (15°, 20°, 25°, 30°, 35°, 40° and 43°C). Species that only occur in shady

#### Table 1. Thermal traits of Amazonian lizards.

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Species	ActP	EAR LAR	Ть	T <sub>sub</sub>	T <sub>air</sub>	T <sub>pref</sub>	VT <sub>min</sub>	VT <sub>max</sub>	CT <sub>min</sub>	CT <sub>max</sub>	T <sub>opt</sub>	SVL	SR
GEKKONIDAE	-												
Hemidactylus mahouia * [75]	N		27.4 (116)	25.6	24.9	27.4 (10)	26.5 (10)	28.7 (10)	10.6 (9)	36.1.(9)	_	50.3 (10)	AI
			20.6-29.6		21.9	18.9_33.8	20.5 (10)	20.7 (10)	10.0 (5)	50.1 (5)		50.5 (10)	11, 1
Hamidactulus palaichthus [76, 77]	N	18:00h	26.7 (76)	25.4	25.2	10.9-33.0						18 8 (8)	ΔΙ
Themiauciyius palatentinus [76-77]	IN IN	22.001	20.7 (70)	23.4	23.2	-	-	-	-	-	-	40.0 (0)	A, L
	_	22:00h	24.2-33.2										
			a.a. (a.a)									ao = (a=a)	
Gymnodactylus amaralı [78–79]	N	-	30.2 (28)	26.8	26.5	-	-	-	-	-	-	39.5 (370)	Ce
	_		26.2-34.1										
Pnyuopezus politcaris [80–81]	C	13:00h	28.9 (10)	29.5	28.6	-	-	-	-	-	-	-	Ca, Ce
		24:00h	27.8-36.6										
<i>Thecadactylus rapicauda</i> * [ <u>75</u> , <u>82</u> – <u>83</u> ]	N	20:00h	26.9 (80)	26.2	26	28.0 (7)	26.9 (7)	29.4 (7)	3.1 (2)	38.4 (3)	-	110.0 (7)	A
		23:00h	24.2-28.6			21.8-33.8							
SPHAERODACTYLIDAE													
Chatogekko amazonicus * [65]	D	09:00h	27.5 (36)	27.5	27.9	23.8 (117)	22.08 (117)	25.3 (117)	9.4 (37)	38.6 (32)	25.8 (24)	20.4 (112)	A
		17:30h	24.6-30.2			16.1-39.5							
Coleodactylus septentrionalis [65]	D	09:00h	27.4 (50)	-	27.4	-	-	-	-	-	-	26.9 (1)	A
		15:00h	-	]									
<i>Gonatodes annularis</i> * [20]	D	12:30h	-	-	-	31.8 (1)	31.0 (1)	32.5 (1)	16.5 (1)	-	-	33 (1)	A
		15:30h				31-32.5							
Gonatodes concinnatus [83]	D	09:30h	27.0 (156)	25.5	25.9	-	-	-	-	-	-	43.3 (1)	A
		15:00h	25.2-30.3										
Gonatodes hasemani [84]	D	07.00h	30.6 (22)	27	26.9	-	-	-	-	-	_	399(1)	A
		19.00h	28.2_33.2		20.5							0,	
Gonatodes humeralis *[20, 84–86]	D	07:00h	20.2 33.2	27.3	27.3	26.0 (212)	24.8 (212)	27.2 (212)	87(68)	40.9 (63)	26.0 (66)	36.7 (186)	Δ
		107.00h	29.2 (110)	- 27.5	27.5	15 2 33 0	24.0 (212)	27.2 (212)	0.7 (00)	40.9 (03)	20.0 (00)	50.7 (180)	
		19.0011	24.0-50.4			15.2-55.9							
		10.001	20.2 (22)	20.1	20	27.2 (4)	22.0 (4)	20.7 (4)	0.0 (2)	20 ( (2)		77.0 (5)	
Dactyloa punctata [87]		10:00h	29.2 (32)	28.1	28	27.2 (4)	25.8 (4)	30.7 (4)	8.0 (2)	39.6 (2)	-	//.9(5)	A
		17:00h	25.8-32			24.5-29.6						= = = = = = = = = = = = = = = = = = = =	<u> </u>
Dactyloa transversalis * [87]	D	08:00h	29.0 (12)	26	26.3	24.1 (1)	24.0 (1)	24.3 (1)	-	-	-	76.5 (2)	A
	_	16:00h	25.4-29.7			23.9-24.3							<u> </u>
Norops auratus [76]	D	09:00h	33.9 (36)	29.9	29.2	-	-	-	-	-	-	43.9 (123)	A
	_	17:30h	30.2-37.2	<u> </u>									
Norops brasiliensis [88]	D	08:00h	30.6 (46)	30.2	31	-	-	-	-	-	-	65.3 (36)	Ce
		17:00h	26.5-34.6										
Norops chrysolepis *	D	09:30h	-	-	-	29.15 (26)	28.1 (26)	30.16(26)	9.4 (19)	39.7 (19)	-	46.1 (13)	A
		16:00h				27.2-33.4							
Norops fuscoauratus * [89]	D	08:30h	28.6 (86)	27	27	27.02 (105)	25.75 (105)	28.23 (105)	8.4 (48)	39.8 (46)	27.8 (30)	43.6 (122)	A
		17:30h	25.7-33.8			19.2-33.1							
Norops ortonii *	D	08:30h	30.3 (7)	28	27.5	27.8 (14)	26.9 (14)	28.8 (14)	9.7 (4)	42.3 (3)	-	44.0 (11)	A
		16:00h	27.5-31.2	]		22.9-33.5							
Norops planiceps * [90–91]	D	-	28.3 (19)	26.1	26.2	29.1 (16)	27.5 (16)	29.3 (16)	9.6 (11)	40.3 (11)	-	55.7 (13)	A
			26.3-30.8	1		23.1-33							
Norops scypheus [20, 92–93]	D	-	27.3 (36)	26.6	26.3	-	-	-	-	-	-	-	A
			24.8-28.8	1									
Norops tandai [94]	D	08.00h	277(33)	27	26.9	-	-	-	-	-	_	_	Се
		17.00b	25 2-31 2										
Norops trachyderma [82 02]		09.001	27.8 (31)	26.0	26.0							53.1.(1)	Δ
1010ps tructyuermu [03, 23]		16.001	27.0 (31)	20.9	20.9				-	-	-	33.1 (1)	
HODIOCEDCIDAE	_	10:000	23.0-29.8										
		00.001	25.6.(6)	25.2	25.5							114.0 (1)	
Enyalioides laticeps [88]	0	09:00h	25.6 (6)	25.3	25.7	-	-	-	-	-	-	114.0 (1)	A
		15:00h	25-26.1	1									

(Continued)

#### Table 1. (Continued)

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Species	ActP	EAR	Ть	T <sub>sub</sub>	Tair	T <sub>pref</sub>	VT <sub>min</sub>	VT <sub>max</sub>	CT <sub>min</sub>	CTmax	Topt	SVL	SR
		LAR											
IGUANIDAE													
Iguana iguana [95–96]	D	-	35.3 (6)	28.5	28.5	-	-	-	-	-	-	387.5 (1)	С
			26.7-42.4										
POLYCHROTIDAE													
Polychrus acutirostris [81]	D	09:00h	35.0 (8)	32.6	30.7	-	-	-	-	-	-	125.1 (1)	Ca
		15:00h	34.2-36.4	1									
Polychrus marmoratus *	D	-	29.0 (1)	26.1	26.2	-	-	-	-	-	-	127.5 (1)	A
TROPIDURIDAE													
<i>Plica plica</i> * [ <u>97–99</u> ]	D	08:00h	29.1 (56)	27.8	27.4	26.2 (23)	25.2 (23)	27.4 (23)	9.3 (17)	41.5 (17)	27.4 (10)	109.1 (21)	A
		18:00h	25.6-33.8			18.4-33							
<i>Plica umbra</i> * [83, 100]	D	09:30h	28.7 (38)	27.6	27.6	27.2 (15)	25.9 (15)	28.3 (15)	9.9 (10)	39.7 (10)	-	85.0 (19)	A
		14:00h	24.8-32.0			16.2-31.3							
Stenocercus roseiventris *	D	09:00h	28.2 (3)	27.6	28	-	-	-	-	-	-	85.0 (1)	A
		14:30h	26.2-32.0										
Tropidurus hispidus * [99, 101]	D	10:30h	34.2 (130)	33.1	30.3	29.1 (2)	28.8 (2)	30.1 (2)	13.2 (2)	43.1 (2)	-	96.8 (82)	A, Ce
		17:00h	30.6-39.6			27.8-30.3							
Tropidurus insulanus [79, 97]	D	-	34.5 (51)	30	28.1	-	-	-	-	-	-	75.2 (-)	Ce
			-										
Tropidurus oreadicus [102]	D	08:30h	32.9 (159)	30.4	28.7	-	-	-	-	-	-	-	Ce
		18:00h	32.0-38.1										
Uracentron flaviceps [103]	D	08:30h	31.2 (22)	27.9	27.6	-	-	-	-	-	-	107.3 (11)	A
		17:30h	25-36.7										
Uranoscodon superciliosus *	D	11:00h	27.8 (24)	27.3	27.1	28.3 (7)	27.0 (7)	29.5 (7)	11.3 (5)	39.5 (5)	-	108.9 (7)	A
		16:00h	24.8-30.1			26.8-33.6							
SCINCIDAE													
Copeoglossum nigropunctatum * [100]	D	10:00h	33.2 (121)	29.9	28.7	29.1 (23)	28.0 (23)	30.3 (23)	10.4 (19)	44.3 (19)	27.3 (11)	92.5 (24)	A
		16:00h	28.0-37.4			22.2-33.5							
Notomabuya frenata [98, <u>104</u> ]	D	07:00h	31.8 (145)	26.2	26.4	-	-	-	-	-	-	56.7 (56)	AF
		18:00h	21.7-37										
Varzea bistriata [105]	D	08:00h	32.9 (11)	-	-	-	-	-	-	-	-	87.2 (24)	A
		16:00h	27.6-36.8										
GYMNOPHTHALMIDAE													
Alopoglossus angulatus * [106]	D	10:00h	27.3 (10)	25.1	25.6	23.8 (3)	19.9 (3)	25.6 (3)	9.0 (2)	37.2 (2)	-	49.0 (3)	A
		17:00h	25.4-33.0			20.3-27.5							
Alopoglossus atriventris [20,106–108]	D	09:00h	28.2 (12)	25.9	26.4	-	-	-	-	-	-	53 (1)	A
		18:00h	24.9-34.0										
Arthrosaura kockii * [20]	D	10:00h	-	-	-	26.5 (43)	25.3 (43)	27.4 (43)	10.1 (29)	43.4 (28)	25.2 (12)	30.3 (42)	A
		15:00h				20.0-30.1							
Arthrosaura reticulata* [20]	D	09:00h	27.0 (34)	25.9	26.1	24.4 (39)	23.4 (39)	25.6 (39)	8.6 (19)	36.1 (19)	25.6 (8)	50.7 (39)	A
		16:30h	23.8-28.2			15.4-27.7							
Cercosaura argulus * [109]	D	09:00h	29.0 (13)	27.2	27.3	25.8 (1)	25.6 (1)	25.8 (1)	-	-	-	34.5 (1)	A
		16:00h	26.2-30.8			25.3-28.0							
Cercosaura eigenmanni * [20, 91]	D	09:30h	29.7 (20)	27.7	27.4	25.3 (3)	24.9 (3)	25.4 (3)	-	-	-	45.5 (1)	A
		15:30h	27.6-31.9			25.0-25.7							
Cercosaura manicata [109]	D	-	29.7 (2)	28	28	-	-	-	-	-	-	-	A
Cercosaura ocellata * [20, 91]	D	09:30h	28.1 (13)	-	-	28.4(3)	27.4 (3)	29.6 (3)	-	-	-	52.2 (2)	A
		15:00h	24-30.2			26.6-29.8							
Cercosaura oshaughnessyi [20, 83, 91]	D	09:00h	29.5 (13)	26.7	26.7	-	-	-	-	-	-	37.4 (1)	A
		15:30h	26.2-30.8										
Iphisa elegans <sup>*</sup> [20, <u>109</u> ]	D	09:00h	28.2(1)	30.2	29.5	25.4 (2)	24.6 (2)	26.1 (2)	3.1 (1)	38.4 (1)	-	46.7 (3)	A
		17:00h				21.3-29.9							

(Continued)

#### Table 1. (Continued)

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Species	ActP	EAR	Ть	T <sub>sub</sub>	T <sub>air</sub>	Tpref	VT <sub>min</sub>	VT <sub>max</sub>	CT <sub>min</sub>	CT <sub>max</sub>	Topt	SVL	SR
		LAR											
Leposoma guianense * [20]	D	09:00h	-	-	-	25.6 (26)	23.6 (26)	28.0 (26)	10.4 (15)	37.3 (11)	-	28.5 (25)	Α
		17:00h	1			20.8-28.6							
Leposoma osvaldoi * [20]	D	09:30h	-	-	-	24.3 (11)	23.1 (11)	25.8 (11)	9.9 (9)	36.4 (8)	-	29.6 (11)	Α
1		15:30h				20.5-32.4							
Leposoma percarinatum * [20]	D	08:00h	29.7 (8)	26.7	26.6	24.1 (49)	22.3 (49)	25.8 (49)	9.0 (30)	38.7 (30)	28.8 (17)	32.3 (32)	А
		17:00h	28.2-31.8			13.4-31.9							
Micrablepharus maximiliani [95, 98, 102]	D	10:00h	29.1 (4)	-	-	-	-	-	-	-	-	36 (1)	Ce
		18:00h											
Potamites ecpleopus [20, 110]	D	08:30h	27.0 (63)	25.5	26	-	-	-	-	-	-	61.8 (1)	A
		18:00h	23.8-31.8										
Potamites juruazensis [110]	D	08:30h	26.4 (8)	26.1	26	-	-	-	-	-	-	41.6 (1)	А
		16:30h	25.4-27.8										
Tretioscincus agilis * [20]	D	09:00h	-	-	-	27.8 (10)	26.8 (10)	29.4 (10)	9.3 (7)	40.2 (7)	-	52.2 (15)	A
o		14:00h				23.5-33.0							
TEIIDAE													A
<b>Ameiva ameiva</b> * [111–112]	D	10:00h	37.4 (283)	32.2	30.3	29.2 (68)	27.7 (67)	30.4 (67)	11.0 (47)	46.1 (45)	34.5 (20)	127.7 (68)	A
		16:00h	26.2-41.7			18.5-38.3							
Ameiva parecis [113]	D	09:00h	38.2 (54)	34.8	30.9	-	-	-	-	-	-	64.5 (1)	A
		13:00h	31.0-42.0										
Cnemidophorus cryptus* [113]	D	09·30h	39.4 (11)	37.4	32.3	27.6 (40)	267(40)	28 4 (40)	84(20)	50 1 (20)	30.5 (20)	65 4 (40)	А
		16:00h	34.6-44.4			14.5-32							
Cnemidophorus gramivagus [79, 113]	D	09:00h	37.6 (42)	-	-		-	-	-	-	-	56 (1)	А
		14.00h	30.4-40.0										
Cnemidophorus lemniscatus [76]	D	09.00h	37.6 (96)	37.2	31.6	-	-		-	-	-	642(1)	А
F		16:00h	29.1-40.7										
Crocodilurus amazonicus [114]	D	11:00h	31.2 (30)	30.4	27.6		_	-		-	-	220.0 (2)	А
		16:00h	27 4-35 0		27.0							22010 (2)	
Dracaena guianensis [114]	D	-	32.2 (1)	29	29	-	-	-	-	-	-	330(1)	А
Kentropyx altamazonica [77]	D	09·30h	36.0 (66)	30.9	29.4		_	-		-	-	85 (1)	A
		15:30h	28-41.2										
Kentropyx calcarata * [47]	D	10:00h	34.7 (99)	30.6	29	34.2 (97)	32.9 (97)	35.6 (97)	11.9 (31)	41.7 (30)	-	100.8 (145)	A
		16:00h	28.7-41.0		-	23.3-39.1							
Kentropyx pelviceps [83, 115]	D	10:00h	35.1 (143)	29.9	28.6	-	-	-	-	-	-	104.3 (32)	A
		16:00h	26-40.5										
Kentropyx striata [76,116]	D	09.00h	357(111)	30.3	29.5		_	-		-	-	91.6 (110)	L
		17:00h	28.8-41.0		2,							,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	2
Salvator merianae [81]	D	09.00h	35.0 (8)	32.6	31.2	_	_	-	_	-	-	-	Ca
		15:00h	34.2-36.4										54
Tupinambis longilineus *	D	-	35.5 (3)	29.8	30	-	-	-	-	-	-	196.0 (1)	A
			33.5-37.2										
Tupinambis quadrilineatus*	D	-	37.2 (1)	-	-	-	_	-	-	_	-	-	A
Tupinambis teguixin [76, 117]	D	09:30h	33.2 (11)	30.4	28.9	-	-	-	-	_	-	362,1 (8)	I.
	-	15:30h	26.1-37.2										_
	1			1	1		1	1	1		1	1	

Number of analyzed specimens in parenthesis after mean values. Species in bold are consider heliotherms in literature. Numbers in brackets are the references for the data obtained from literature, and asterisks (\*) represents newly data provided in this study. ActP = Activity period (D = Diurnal, N = Nocturnal, C = Cathemeral); EAR = Earliest Activity Record; LAR = Latest Activity Record; T<sub>b</sub> = body temperature (mean and range); T<sub>sub</sub> = substrate temperature (mean); T<sub>pref</sub> = preferred temperature (mean and range); VT<sub>min</sub> = minimum voluntary temperature; VT<sub>max</sub> = maximum voluntary temperature; CT<sub>min</sub> = critical thermal minimum; T<sub>opt</sub> = optimal temperature; SVL = snout-vent length; SR = study region (A = Amazonian rainforest; AF = Brazilian Atlantic Forest; C = Central America; Ca = Brazilian Caatinga; Ce = Brazilian Cerrado; L = Brazilian Lavrado).

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environments may suffer at extreme temperatures, and such species were run at 20°, 25°, 30° and 35°C. Lizards were allowed to recover at least four hours between trials. During the recovery period, we monitored their  $T_b$  and activity inside their containers at least once every hour, in order to assess their health and well-being after the stress tests. We only released the animals after we assessed that they had recovered their normal activity pattern. No animal died prior to the end of the experiments.

To measure performance, the experimenter manually stimulated lizards to run around a circular track [30]. A track with a 4m circumference was used for lizards with SVL $\geq$ 50mm, and a track with 1m circumference was used for lizards SVL<50mm. Each trial ended when the lizard reached exhaustion and was unable to right itself when placed in a supine position. Animal performance was calculated as the voluntary distance traveled (number of times around the track x track circumference). T<sub>opt</sub> was the body temperature that yielded the highest value of locomotor performance. We determined T<sub>opt</sub> from the thermal performance curves.

## Analysis

We used the statistical software environment R 3.3.3 [31] for all calculations. Dependence between thermal physiology parameters of a priori classification of thermoregulation modes, SVL, families and species were analyzed by simple stepwise regression and one-way analysis of variance. Shapiro and Levene's tests were used, respectively, to test assumptions of normality and homogeneity of variance for parametric variables. We used the Pearson correlation coefficient to determine the correlation between T<sub>b</sub>, T<sub>sub</sub> and T<sub>air</sub>. For comparative analyses, we used the chronogram for Squamata estimated by Zheng & Wiens [32], which included all of the species for which we were able to assemble thermophysiological traits. Phylogenetic signal was calculated based on Blomberg's K [33], which is an evolutionary model-based metric of phylogenetic-signal strength. A K-value of one indicates that the distribution of trait values follows the expectation of Brownian motion model of evolution along the tree [33]. This indicates that trait variance among species accumulates in direct proportion to their divergence time, as measured by the branch lengths separating them in a phylogenetic tree [34-35]. Values of K<1 indicate that traits are less conserved than expected, an indication of adaptive evolution, whereas values of K>1 indicate that trait values are more conserved than expected by Brownian motion evolution. We used phytools [36] to calculate Blomberg's K and to measure the phylogenetic pairwise correlations between all thermal traits.

TPC's were generated for each species using the packages ggplot2 [37], grid [31], mgcv [38] and proc [39] to do a Generalized Additive Mixed Modeling (GAMM) [40]. These models use additive nonparametric functions to model covariate effects while accounting for overdispersion and correlation, by adding random effects to the additive predictor [41]. Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC) were used to select the best correlation structure prior to estimating the TPC. AIC measures the quality of fit of the model, penalized by model complexity, and BIC additionally considers the number of observations included in the model [42]. Lizard performance at different temperatures was the response and individual was included as a random effect. The extremes of the curve were fixed at the average  $CT_{min}$  and  $CT_{max}$  values for that species. We tested several correlation structures to select the best fit including: temporal correlation structures (autoregressive process [corAR1], continuous autoregressive process [corCAR1], and autoregressive moving average process [corARMA]) and spatial residual correlation structures (Gaussian spatial correlation [cor-Gaus], exponential spatial correlation structure [corExp], rational quadratics spatial

	No. specimens	No. species	No. families
T <sub>b</sub>	80	8	5
T <sub>pref</sub> / VR	1010	27	9
Physiological Performance	254	10	6
Thermal Tolerance Range (CT <sub>min</sub> , CT <sub>max</sub> )	485	26	8

#### Table 2. Number of specimens and taxa used as source of data reported here for the first time.

 $T_b$  = body temperature;  $T_{pref}$  = preferred temperature; VR = voluntary range

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correlation [corRatio] and spherical spatial correlation [corSpher]). We chose the correlation structure that yielded the lowest AIC and BIC values [42].

#### Results

We obtained thermal data for 69 lizard species from eleven families (Table 1), including new data on field-active  $T_b$ ,  $T_{pref}$ , thermal performance and tolerance from 27 species (Table 2). Among all species with physiological data, 64 are diurnal, one is cathemeral, and four nocturnal. Based on the literature, 38 species are classified as thermoconformers, while 31 are thermoregulators.

Lizards' body temperature was positively correlated with environmental temperature (T<sub>b</sub> and  $T_{sub}$ : r = 0.80, P<0.01;  $T_b$  and  $T_{air}$ : r = 0.67, P<0.05). Seven species generally classified as thermoconformers had T<sub>sub</sub>/T<sub>air</sub> higher than T<sub>b</sub>, suggesting that these species do not gain additional heat from the environment, but may be thermoregulating by selecting lower temperatures or using evaporative cooling. A one-way ANOVA revealed significant differences in all physiological traits in relation to a priori classification of thermoregulation mode, SVL, family, and species. Fig 2 shows the range of temperatures for each evaluated trait for each thermoregulation mode. Species generally classified as thermoregulators had higher mean values for all thermal traits than those generally classified as thermoconformers, as well as larger variation in T<sub>b</sub>, CT<sub>max</sub> and T<sub>opt</sub>. Variation in T<sub>pref</sub>, VT<sub>min</sub> and CT<sub>min</sub> was lower in species classified as thermoregulators and greater in species classified as thermoconformers, though mean values were relatively similar (Fig 3). In spite of these differences, our results show an overlap in most thermal traits between species classified as thermoregulators and those classified as thermoconformers, with some lizards considered thermoregulators having ranges of temperatures similar to others identified as thermoconformers. Thus, a dichotomous classification of thermoregulation mode may not be satisfactory.

We also found phylogenetic pairwise correlations between all thermal traits evaluated (T<sub>b</sub>, T<sub>pref</sub> VT<sub>min</sub>, VT<sub>max</sub>, CT<sub>min</sub>, CT<sub>max</sub>, and T<sub>opt</sub>), except between (1) T<sub>opt</sub> and CT<sub>max</sub>, and (2) CT<sub>min</sub>, which only correlates with T<sub>opt</sub> (Table 3). Thus, selection on one thermal characteristic affects the evolution of all those considered here, except, possibly, in the two cases mentioned above. We detected significant departures from Brownian motion evolution for T<sub>b</sub> (K = 0.64, P = 0.0001), T<sub>pref</sub> (K = 0.49, P = 0.04), and VT<sub>min</sub> (K = 0.5, P = 0.01), but not for the other thermal traits (VT<sub>max</sub>, K = 0.39, P = 0.21; CT<sub>min</sub>, K = 0.49, P = 0.12; CT<sub>max</sub>, K = 0.50, P = 0.079; T<sub>opt</sub>, K = 0.74, P = 0.17). Lizards in the family Teiidae are comprised only of species classified as thermoregulators, and had the highest values for all three thermal traits. The lowest T<sub>b</sub> was found in one species of Hoplocercidae (forest-dwelling lizards), and the lowest VT<sub>min</sub> was observed in Gekkonidae, both families containing only species classified as thermoconformers. Gekkonidae and Dactyloidae presented the lowest T<sub>pref</sub>. Although Dactyloidae is a mixed family, the only dactyloid species classified as thermoregulator in this study has no T<sub>pref</sub> data available. Thus, all T<sub>pref</sub> measurements for this family are from species classified as





**Fig 2. Temperature frequency distribution by thermal regulation mode for each thermal trait evaluated.** Species were classified a priori as thermoregulators or thermoconformers. Values of temperature (x-axis) correspond to the mean value for each species.

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thermoconformers (six out of ten analyzed species), which may explain the low value found. Families with both thermoregulation modes tended to have intermediate mean values for all thermal parameters (Table 4). Tropiduridae had five species classified as thermoregulators with mean T<sub>b</sub> 32.2°C. Of those, data on T<sub>pref</sub> and VT<sub>min</sub> value (29.1° and 28.8°C, respectively) were available only for *Tropidurus hispidus*. Among the three species of tropidurids classified as thermoconformers, the mean values of T<sub>b</sub>, T<sub>pref</sub> and VT<sub>min</sub> were 28.5°, 27.5° and 26.3°C, respectively. Four gymnophthalmids were classified as thermoregulators and they had mean values of T<sub>b</sub>, T<sub>pref</sub> and VT<sub>min</sub> of 29.4°, 27.5° and 25.8°C, respectively, while the other 13 species that are considered thermoconformers had mean values of T<sub>b</sub>, T<sub>pref</sub> and VT<sub>min</sub> of 28.2°, 25.1° and 23.8°C, respectively. These results reflect the among-family pattern where families with species considered to be thermoregulatory had higher mean values of T<sub>b</sub>, T<sub>pref</sub> and VT<sub>min</sub> than families which only have species classified as thermoconformers.

We measured the thermal dependence of locomotor performance for ten species of Amazonian lizards (Fig 4). The best correlation structure and estimated TPC parameters for each of these ten species are presented in Table 5. The  $CT_{max}$  among all species classified as thermoregulators varied between 45–50°C. We observed small variation in the shape of the TPCs,

Tuble 51 Correlation	in between thermal trui					
	CT <sub>min</sub>	Ть	T <sub>opt</sub>	T <sub>pref</sub>	VT <sub>max</sub>	VT <sub>min</sub>
CT <sub>max</sub>	0.19, 0.65	0.84, 0	0.25, 0.241	0.57, 0.002	0.57, 0.002	0.57, 0.002
CT <sub>min</sub>	-	0.19, 0.21	0.78, 0.005	0.19, 0.72	-0.14, 0.49	-0.20, 0.84
T <sub>b</sub>	-	-	0.62, 0.05	0.60, 0.002	0.59, 0.002	060, 0.0014
T <sub>opt</sub>	-	-	-	0.68, 0.018	0.68, 0.02	0.58, 0.05
T <sub>pref</sub>	-	-	-	-	0.87, 0	0.77, 0
VT <sub>max</sub>	-	-	-	-	-	0.85, 0

Table 3.	Correlation	between	thermal	traits	( <b>r</b> , <b>P</b> ).
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As hypotheses were independent, no correction was made for multiple tests.

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Family	T <sub>b</sub>	Tpref	VT <sub>min</sub>	TrM
Hoplocercidae (1)	25.6	-	-	TC
Gekkonidae (2)	27	27.4	23.7	TC
Sphaerodactylidae (6)	28.3	27.8	25.8	TC
Phyllodactylidae (3)	28.7	28	27.9	TC
Gymnophthalmidae (17)	28.4	25.6	24.2	М
Dactyloidae (11)	29.2	27.4	25	М
Tropiduridae (8)	30.8	27.9	26.9	М
Polychrotidae (2)	32	-	-	TR
Scincidae (3)	32.6	29.4	28.2	TR
Iguanidae (1)	35.3	-	-	TR
Teiidae (15)	35.7	31.1	29.8	TR

Table 4.	Mean values of body	v temperature (T <sub>b</sub>	), preferred tem	perature (T <sub>mas</sub> ) an	d minimum voluntar	v temperature (	(VT) h	ov familv
I GOIC II	incult fulles of bou	, compensato (1)	, preferred tem	perature ( i pref) all	a minimum vorantai	, componacate ,	$- m_{\rm m}/c$	<i>, , , , , , , , , ,</i>

The number of species we obtained data for each family is in parentheses. TrM = a priori classification of thermoregulation mode (TR = thermoregulator; M = mixed; TC = thermoconformer).

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with exception of Copeoglossum nigropunctautm and Cnemidophorus cryptus, which had almost linear curves and a high confidence interval. Gonatodes humeralis and Norops fuscoauratus are forest shade species that were classified a priori as thermoconformers whose TPC's have broad plateaus, with CT<sub>max</sub> reaching 40°C. In G. humeralis, T<sub>opt</sub> is closer to T<sub>pref</sub>, while in N. fuscoauratus Topt is closer to VTmax. The TPC for Arthrosaura kockii had a plateau, which was slightly inclined at lower temperatures. Plica plica had a pattern similar to that of A. kockii, even though the former is considered a thermoconformer and the latter a thermoregulator. Plica plica had a Topt closer to VTmax, while in A. kockii Topt was closer to VTmin. For Ameiva ameiva there was a steep performance increase with higher body temperature, with a Topt greater than the mean values for VT and T<sub>pref</sub>, and closer to T<sub>b</sub>. In Cnemidophorus cryptus, T<sub>opt</sub> was greater than VT's and T<sub>pref</sub>, which is similar to A. ameiva, but below its T<sub>b</sub>. Chatogekko amazonicus, Arthrosaura reticulata, and Leposoma percarinatum are found in the leaf litter and all three species have CT<sub>max</sub> values near 35°C. T<sub>opt</sub> for Chatogekko amazonicus and A. reticulata's was close to their  $VT_{max}$ , while for L. percarinatum's  $T_{opt}$  is nearer its  $T_b$ .  $T_{opt}$  values for both species classified as thermoregulators and thermoconformers partially overlap, similar to values for the other thermal physiological parameters analyzed.

### Discussion

We observed a non-significant phylogenetic correlation between  $T_{opt}$  and  $CT_{max}$ , and considering that  $T_{opt}$  is correlated with all other thermal traits, this is in agreement with the argument that tolerance limits have less relevance to thermoregulation than  $T_{opt}$  [43–44]. On the other hand, this is incongruent with the results of Huey and Kingsolver [12] and Huey *et al.* [45], who found that  $T_{opt}$  and  $CT_{max}$  are correlated and tend to coevolve. Possibly this is because these studies included many shade-loving species in which the highest obtainable temperatures are well below those likely to cause physiological stress. Conversely, the correlation between  $CT_{min}$  and  $T_{opt}$  suggests that directional selection on  $CT_{min}$  will have a direct effect on locomotor performance, raising or lowering  $T_{opt}$  and the mid-level performance temperature range. This is also in accordance with the hypothesis that species that restrict their activities to deeply-shaded areas may be more limited by low rather than high temperatures [46–47].

Blomberg *et al.* [33] found relatively low values of K indicative of low phylogenetic signal for physiological traits in Australian skinks (e.g.,  $T_{pref}$  and  $T_{opt}$ ), as we report here for  $T_b$ ,  $T_{pref}$ 



**Fig 4. Thermal-performance curves and mean values of thermal traits.** A) *Chatogekko amazonicus*; B) *Gonatodes humeralis*; C) *Copeoglossum nigropunctatum*; D) *Norops fuscoauratus*; E) *Plica plica*; F) *Arthrosaura kockii*; G) *Arthrosaura reticulata*; H) *Leposoma percarinatum*; I) *Ameiva ameiva*; and J) *Cnemidophorus cryptus*. Gray shaded region shows the 95% confidence interval. Black points represent the results of individual tests at different body temperatures: 15°, 20°, 25°, 30°, 35°, 40° and 43° for species classified as thermoregulators and 20°C, 25°C, 30°C and

35°C for shade-associated species classified as thermoconformers. Short vertical black lines indicate the number of trials at each temperature. Black lines at the curves' extremes are the critical thermal minimum ( $CT_{min}$ ) and critical thermal maximum ( $CT_{max}$ ).  $T_{opt}$  = optimal temperature;  $T_b$  = body temperature;  $T_{pref}$  = preferred temperature;  $VT_{min}$  = minimum voluntary temperature;  $VT_{max}$  = maximum voluntary temperature.

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and  $VT_{min}$ . Such a result was expected considering the importance of environment on the thermal characters of ectotherms, which in turn influences nearly all their physiological processes. These traits are apparently influenced by factors other than phylogeny, such as phenotypic plasticity, which is the ability of an organism to express different phenotypes depending on its biotic and abiotic environments [48]. This factor influences not only the thermal physiology of lizards, but also their life histories [49–51]. The low signal could also be due to local adaptation, since it indicates that these traits have not been conserved evolutionarily [52].

For the thermal parameters  $VT_{max}$ ,  $CT_{min}$ ,  $CT_{max}$  and  $T_{opt}$ , we found no departure from Brownian motion evolution. Nevertheless, some of these traits may be limited by physiological constraints common to all lizards, hindering the chances of evolutionary changes that would be reflected in the phylogeny. According to Huey & Kingslover [12], if the population lacks the underlying genetic variation to shift its thermal sensitivity adequately in response to selection, we might expect that this population will not be able to adapt and evolve rapidly enough to track changes in environmental temperatures, such as those caused by the climate warming. For example, broad variation in  $T_{pref}$  can be too low [53] or additive genetic variation can be overwhelmed by maternal effects [54] to allow rapid adaptation to climate warming measured on decadal time scales [16]. Alternatively, our sampling was insufficient to detect local adaptation since we did not designed our study for that. The ages of these species are probably all in the range of millions of years, indicating that all have passed through many climatic fluctuations that affected all parts of Amazonia to some extent (e.g., [55–58]), which may have led to genetic variation within and between populations.

Species	CS	BIC (R <sup>2</sup> )	B <sub>80</sub> (°C)	T <sub>opt</sub> (°C)	BP (m)	CT <sub>min</sub> (°C)	CT <sub>max</sub> (°C)
SPHAERODACTYLIDAE				-			
Chatogekko amazonicus	CorGaus	0.65	24.9-33.5	25.8	7.6	11.6	43.8
Gonatodes humeralis	CorAr1/CorARMA	0.72	11.9-35.2	26.0	5.6	8.4	41.0
SCINCIDAE							
Copeoglossum nigropunctatum	CorARMA	0.1	15.0-43.0	27.3	26.2	9.3	49.3
DACTYLOIDAE							
Norops fuscoauratus	CorAr1	0.43	20.1-35.4	27.8	2.98	8.5	44.2
TROPIDURIDAE							
Plica plica	CorSpher	0.73	25.0-35.3	27.4	6.5	7.8	44.7
GYMNOPHTHALMIDAE							
Arthrosaura kockii	CorRatio	0.65	10.1-30.1	25.2	17.6	9.0	49.6
Arthrosaura reticulata	CorAr1	0.48	19.6-35.1	25.6	19.1	8.6	42.3
Leposoma percarinatum	CorARMA	0.45	19.8-35.0	28.8	7.2	8.9	36.5
TEIIDAE							
Ameiva ameiva	CorRatio	0.60	24.5-42.9	34.5	67.5	9.9	46.1
Cnemidophorus cryptus	CorARMA	0.17	14.8-43	30.5	29.3	10.6	49.1

Table 5. Selected correlation structures (CS) used in GAMM fitting of thermal performance curves (TPC) of Amazonian lizards.

Values indicate Bayesian Information Criterion (BIC) and performance (range of temperatures that are  $\geq$  80% of optimal capacity = B<sub>80</sub>; optimal temperature = T<sub>opt</sub>; best performance = BP; critical thermal minimum = CT<sub>min</sub>; and critical thermal maximum = CT<sub>max</sub>). Thermoregulatory species are shown in bold.

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Thermal conditions within lowland tropical forest are likely insufficient to permit Tbs below the level of thermoregulation found [6]. The ability to attain  $T_{pref}$  by basking in rainforest microenvironments may be limited due to lower heterogeneity in the thermal environment. All species with performance and T<sub>b</sub> data available had T<sub>opt</sub> < T<sub>b</sub>, although their T<sub>b</sub>'s are within the broad plateau of their TPC. This is in disagreement with Bennett [59], who suggested that T<sub>opt</sub> is always higher than T<sub>pref</sub> and T<sub>b</sub>. T<sub>opt</sub> in all species is between 7.7-24.4°C below CT<sub>max</sub>. By comparing T<sub>b</sub> with CT<sub>max</sub> in 19 species with available data, CT<sub>max</sub> was between 6-12°C above the mean values of T<sub>b</sub>, consistent with Ji et al.'s [60] study on Sphenomorphus indicus and Du et al.'s study [7] on Eumeces elegans males. When we consider the highest values of T<sub>b</sub> measured, the difference drops to 4-11°C for most species, except Kentropyx calcarata and Tropidurus hispidus, where the difference is ~0.5°C and 3.5°C, respectively. Overall, our results partially support Hoffman and Sgró's claim [9] that tropical lizards have their thermal traits close to their upper thermal thresholds, which are likely to be exceeded in the next few decades. Nevertheless, we measured Topt only for running speed. It may be that  $T_{opt}$  for other activities is closer to that observed for  $T_b$ . Within the forest, it is less likely that availability of temperatures lower than T<sub>opt</sub> will limit any of the species in the near future, especially in the case of heliotherms, which are probably presently more limited by low than high temperatures.

Although we found statistical differences between the thermoregulatory modes generally attributed to the species for all physiological traits, most species appear to show substantial overlap in their thermal physiology regardless of their a priori classification into thermoregulatory modes. Thus, the tropical lizard species here studied do not form discrete categories, as suggested by Pough and Gans [3]. We tested the a priori categories for thermoregulatory mode because these continue to be used to describe lizard thermoregulation, especially in relation to the predicted effects of climate change. However, the relationship between body and environmental temperatures in lizards shows a cline rather than discrete categories [3, 61] and future studies would gain by abandoning them and using continuous variables when more detailed information is available [62].

Geographic variation in climate can lead to differences in thermal physiology among species [63], so the physiological data obtained for some species outside Amazonia must be seen as an approximation of the thermal traits for those species in this region. Among all species included in this study, smaller animals had the lowest temperatures for all traits, probably due to their relatively low thermal inertia [64–65]. *Chatogekko amazonicus, A. ameiva* and *C. cryptus* had T<sub>opt</sub> close to their T<sub>b</sub>'s, so these species can achieve their highest locomotor performance under current environmental conditions. In contrast, *P. plica* and *A. reticulata* achieve their T<sub>opt</sub> closer to T<sub>pref</sub>, and *G. humeralis, C. nigropunctatum* and *A. kockii* achieve T<sub>opt</sub> closer to VT<sub>max</sub>; in both cases, T<sub>opt</sub> was lower than T<sub>b</sub> obtained from field measurements. *Norops fuscoauratus* and *Leposoma percarinatum* had T<sub>opt</sub> considerably above T<sub>b</sub>, T<sub>pref</sub>, and VT<sub>max</sub>, closer to CT<sub>max</sub>. For these species, T<sub>opt</sub> might reflect the thermal optimum of other physiological processes, or an intermediate thermal optimum for different processes [64]. Among the species with T<sub>b</sub>>T<sub>opt</sub>, the high T<sub>b</sub> likely reflects the thermal optima for other physiological process, such as digestion [11, 66–68].

We hypothesize that many tropical rainforest lizards may be affected by high environmental temperatures, considering that their locomotor performance is better at lower temperatures than they are already experiencing in field. Our inferences are in agreement with other studies with fewer species and at higher latitudes, which predict that many tropical lizards are at an imminent risk of extinction due to human induced global warming [8, 16, 69]. Additional factors are affecting large parts of Amazonia such as forest loss, degradation, and fragmentation [70]. These factors can cause rapid microclimate changes towards hotter and drier conditions

that climate models are unable to predict with accuracy [71]. Most of the species in this study had some margin for an increase in average  $T_b$  with low cost to performance, since their  $T_b$ s are still within the broad plateau of their TPC. However, selection is weaker on species with broad TPCs than narrow TPCs, which have a lower capacity to evolve to track changes in climate [12].

Sinervo *et al.* [16] provided the first model of the potential effects of a warming climate on species distributions based on thermal physiology. Integrative models such as those that incorporate phenotypic plasticity and genetic variability will allow projections of adaptive radiation occurring under warmer environments [72–73]. Both phenotypic plasticity and genetic variability are directly dependent on environmental conditions that allow lizards to gain and lose heat [16, 60, 74]. We expect that, with more temperature data on a higher diversity of Amazonian lizards' species, we can better understand the effects of climate change on these animals. Also, investigations taking into account the phylogeographic history of Amazonian lizard species, many of which are known to exhibit cryptic diversity and high population structure, will be important to refine and help detect geographic divergence of thermal traits and extinction risks.

# Conclusions

This study represents the first effort to compile and provide novel thermal-biology data obtained across wide geographic ranges and taxonomic diversity of Amazonian lizards. We integrated field and literature data with phylogenetic inferences to better understand how updated ecophysiological traits can serve as a baseline to inform predictions of global warming effects on the future of rainforest lizards.

Although lizards generally classified as thermoregulators and thermoconformers show significant differences in their thermophysiological characters, our results indicate that these groups do not form discrete categories, since most species are intercalated in their thermal physiology regardless of their thermoregulation modes. Most species considered to be thermoconformers in Amazonia prefer warmer microhabitats to gain additional heat from the environment and thus cannot be considered thermoconformers in the strict sense, corroborating the idea that thermoconformity is one extreme of a continuum with thermoregulation in the other extreme [3].

Our results suggest that selection on  $CT_{min}$  will affect locomotor performance directly by amplifying or reducing  $T_{opt}$  and the range of temperatures of mid-level performance. We found no phylogenetic signal for  $CT_{max}$ ,  $CT_{min}$ ,  $T_{opt}$  and  $VT_{max}$ . In turn,  $T_b$ ,  $T_{pref}$  and  $VT_{min}$  are less conserved than expected, so they appear to be influenced by factors other than phylogeny, such as strong selection or phenotypic plasticity.

Lizards are excellent models for investigating the biological effects of climate change. Although Amazonian lizards have an apparent margin for an increase in  $T_b$  with low cost to performance, suggesting they may show some resilience to warming, their broad TPC's may not allow rapid evolutionary response to a quickly warming climate. More studies on the thermal physiology of Amazonian lizards are needed to obtain data representative of the high species diversity in the region so we can better understand the effects of climate change on their distribution and densities.

# **Supporting information**

S1 File. Preferred, voluntary and critical temperatures from all individuals tested in ecophysiological experiments.  $T_{pref}$  = preferred temperature;  $VT_{min}$  = Minimum voluntary

temperature;  $VT_{max}$  = Maximum voluntary temperature;  $CT_{min}$  = Minimum critical temperature;  $CT_{max}$  = Maximum critical temperature. Available on doi: 10.6084/m9.figshare. 5293756. (XLSX)

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