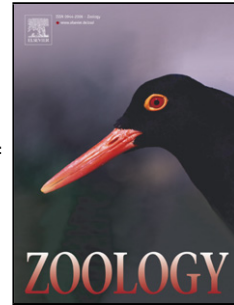


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**How and when melanic coloration is an advantage for lizards: the case of three closely-related species of *Liolaemus*.**

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Highlights

- *Liolaemus gununakuna*, *L. elongatus*, and *L. shitan* lizards differ in colouration.
- Sprint speed performance curves are similar for the three species.
- The darkest species (*L. shitan*) gains heat and runs faster than the lighter-coloured ones.
- Support was found for the thermoregulatory function of melanism in lizards.
- Behaviour plays a key role in less-melanic species.

## ABSTRACT

Body temperature affects various aspects of ectotherm biology. Reptiles, as ectotherms, gain and control their temperature mainly through behavioural adjustments, although some body traits may also be advantageous. According to the thermal melanism hypothesis (TMH) dark colour may be thermally advantageous in cold environments. Additionally, differences in thermoregulatory capacity may also affect performance. We analysed the role of melanism in the thermoregulation and sprint speed performance of three species of *Liolaemus* lizards from Argentinean Patagonia. *Liolaemus shitan*, *L. elongatus* and *L. gununakuna* are phylogenetically close, with similar body sizes and life history traits, but differ in their melanic colouration, *L. shitan* being the darkest and *L. gununakuna* the lightest species. We estimated sprint speed performance curves and heating rates, and recorded final body temperature and sprint speed achieved after a fixed heating time, from two different initial body temperatures, and with and without movement restriction. Performance curves were similar for all the species, but for *L. gununakuna* the curve was more flattened. Darker species showed faster heating rates, ran faster after fixed heating trials at the lowest temperature, and reached higher body temperatures than *L. gununakuna*, but this was compensated for by behavioural adjustments of the lighter lizards. Similarity of sprint speed performance may be due to the conservative nature of this character in these species, while variation in heating ability, particularly when starting from low temperatures, may reflect plasticity in this trait. The latter provides support for the TMH in these lizards, as melanism helps them increase their body temperature. This may be especially advantageous at the beginning of the day or on cloudy days, when temperatures are lower.

Keywords: Cold climate, Patagonia, reflectance, sprint speed performance, thermal melanism hypothesis.

## 1. Introduction

Body temperature is a variable of major influence in biological processes of ectotherms (Angilletta et al., 2006). As it affects different aspects such as digestion, muscle performance, and developmental time (Angilletta, 2001), a significant deviation from their optimal body temperatures may compromise performance in these organisms (Angilletta et al., 2002). Body temperature in ectotherms depends both on the magnitude of thermal environment variation and on the organism's ability to regulate heat exchange (Belliure and Carrascal, 2002). This thermoregulation ability is possible due to a set of physiological and behavioural strategies, plus

different morphological traits that evolved according to their combined influence on fitness (Angilletta, 2009). These attributes, together with the life history of a species, may be subjected individually or collectively to environmental selective forces, or they may respond to evolutionary restrictions (Andrews, 1998; Angilletta, 2009). Therefore, the evolution of behavioural and/or morphological characters does not occur in isolation, but may rather be associated with the evolution of physiological aspects of the organisms, particularly in ectotherms (e.g. Forsman et al., 2002; Angilletta et al., 2004; Tulli et al., 2011; Vanhooydonck et al., 2014; Zamora-Camacho et al., 2015). At the same time, certain morphological attributes may directly or indirectly affect different performance characters, and may in turn influence fitness (Arnold, 1983).

As ectothermic animals, reptiles gain and control heat mainly through behavioural adjustments (Bartholomew, 1982; Huey, 1982; Stevenson, 1985). Certain body traits may also be adaptive for thermal regulation; for example, a melanistic (dark coloured) individual with low reflectance should gain heat faster than one with high reflectance (light colouration) at the same body size and environmental conditions (Watt, 1969). Thus, melanism is expected to be advantageous in cold climates, as proposed by the thermal melanism hypothesis (TMH; Watt, 1968; Clusella-Trullas, et al., 2008). However, previous studies regarding the influence of melanism on heating rates of reptiles reported different, sometimes contradictory, results for the TMH (Tanaka, 2005, 2007; Clusella-Trullas et al., 2009; Geen and Johnston, 2014; Moreno Azócar et al., 2016). From these studies we can hypothesize that if melanism does have a thermoregulatory function, it may be more likely at lower temperatures. As light and dark animals are able to adjust thermoregulation via behaviour, such a difference would vanish once body temperature reached the thermal preferendum, and after this, lizards start shuttling between different thermal sources to thermoregulate.

Combining behavioural and morphological adjustments for thermoregulation may result in a better performance of the individual. An ecologically and socially-relevant performance function is locomotion, essential for escaping from predators, feeding, mating and territorial defence (Domenici, 2001; Blumstein et al., 2004; Husak et al., 2006; Husak, 2006; Husak et al., 2008; Mowles et al., 2010; Pruitt, 2010; Irschick and Higham, 2016). Multiple studies analysed variation in locomotor capacities between species in relation to ecological, environmental, or morphological traits (e.g. Angilletta et al., 2002; Bonino et al., 2011; Zamora-Camacho et al., 2015; Irschick and Highman, 2016). Comparison of sprint speed performance curves, or speed achieved after an equal amount of heat exposure, may reflect the effect of behaviour and/or morphology on the individuals.

In the present study we analysed the effects of melanism on the thermoregulation and sprint speed performance of three *Liolaemus* lizard species from Patagonia, Argentina. *Liolaemus shitan*, *L. elongatus* and *L. gununakuna* are phylogenetically close (they belong to the *L. elongatus* group; Lobo et al., 2010). These species show similar body size and shape (see Supplementary Material, Tables S1 and S2), ecological and life history traits (the three species are viviparous and insectivorous), but they differ in their melanic colouration. While *Liolaemus shitan* is completely black, *L. gununakuna* has a striped pattern with an iridescent green background colour, and *L. elongatus* is brownish and darker to the naked eye than *L. gununakuna*. Our data on these species come from rocky areas located at similar latitudes in Northern Patagonia (Fig. 1). Regardless of the colour observed by the naked eye, the differences in skin reflectance must be verified using spectrophotometry. It is important to evaluate whether species' heating rates differ under experimental conditions, and determine the basal information from performance curves of sprint speed, because they will later be related to melanism. Taking these performance curves as a reference, we studied which species was able to draw closest to their optimum temperature after a fixed heating time, starting at equal initial temperatures. According to our prediction, if melanism accelerates heat gain, the darkest species (*L. shitan*) will show faster heating rates (Clusella Trullas et al., 2007); additionally, this species will show higher body temperature and a speed closer to its maximum after a fixed heating time than the other two paler species (Zhang et al., 2008). These results may be considered evidence for the influence of melanism on species performance (Grigg et al., 1979), and as a consequence, demonstrate the advantage gained when inhabiting cold climates.

## 2. Material and methods

### 2.1. Study species, collection sites and specimen housing

We studied three lizard species of the *Liolaemus* genus. *Liolaemus shitan* (Abdala et al., 2010, Fig. 1A), has a uniform, deep black dorsal colour, and ventral colour is grey. Scapular or paravertebral spots are absent, as are dorsolateral and vertebral lines. Average snout-vent length (SVL) is  $88.1 \text{ mm} \pm 6.7$ , (Abdala et al., 2010). The collection site is located in El Cuy department, Río Negro province, at Estancia Piedras Blancas ( $39^\circ 55' 38'' \text{ S}$ ;  $68^\circ 20' 43'' \text{ W}$ ). *Liolaemus elongatus* (Cei, 1974, Fig. 1B) ground colour is greyish or pale brownish with three black, irregular, longitudinal stripes, one vertebral and two lateral, interconnected by irregular transverse dark bars; its belly is greyish and unspotted (Cei, 1974). SVL range is 85-90 mm (Scolaro, 2005). Specimens were collected 20 km west of Comallo town, beside National highway 23, in Río Negro Province ( $41^\circ 02' 00'' \text{ S}$ ;  $70^\circ 16' 00'' \text{ W}$ ). Finally, *L. gununakuna* (Ávila et al., 2004, Fig. 1C) ground dorsal colour is iridescent yellow-green, with a series of transversal, irregular, partially fused dark bars along the vertebral line (“tigroid pattern”, Ávila

et al., 2004). Average SVL is 93 mm (Scolaro, 2005). This species was collected near Piedra Grande, Zapala Department, Neuquén Province ( $38^{\circ} 54' 10''$  S;  $70^{\circ} 03' 54''$  W, Fig. 2). All three species are saxicolous, insectivorous and viviparous (*L. gununakuna*: Ávila et al., 2004, *L. elongatus*: Scolaro, 2005, *L. shitan*: Abdala et al., 2010; reproductive mode: Moreno Azócar unpublished data).

Specimen collection was carried out on consecutive days during the active season (November-March). Only adult males and non-pregnant adult females were collected, to avoid ontogenetic and reproductive differences in thermal biology and locomotor performance (Fernández et al., 2017). Lizards were carried to the laboratory in cloth bags; they were then fed periodically with *Tenebrio molitor* larvae and water was provided *ad libitum*.

## 2.2. Reflectance measurements

In order to verify differences in species colouration observed by the naked eye, we measured spectral reflectance for at least three individuals per species. These measurements were taken on 9 different patches of the dorsal body of the specimens (Fig. 3) using a JAZ EL200-XR1 spectrophotometer (Ocean Optics, Inc.). Although the spectrophotometer gives reliable reflectance from 240 to 850 nm, for our purpose we analysed only the visible spectra (from 400 to 700 nm) at 0.42 nm intervals, which is related to melanism coloration and the supposedly higher solar radiation absorption. A bifurcated fibre optic cable was used with illumination provided by a PX-2 pulsed xenon light source, collecting data at  $45^{\circ}$  with respect to the body surface. The probe was covered with a black rubber tube so as to maintain a distance of 5 mm between the probe and the skin, and avoid the incidence of ambient light. As the end of the tube is cut at an angle of  $45^{\circ}$ , the resulting patch is elliptical, covering an area of  $10 \text{ mm}^2$ .

Reflectance measures were obtained by setting 10 averages and Boxcar width 5, using Ocean View program (Corbalán et al., 2018). Calibration was carried out with a WS-1 diffuse reflectance standard, and both white and black measurements were taken every 20 minutes. Dorsal patch measurements were head, nape, inter-scapular region, mid-dorsum, posterior region (between the hind legs), and tail proximal portion. Dorsolateral measurements were taken of the flank, cheek, and shoulder (anti-humeral spot, Fig. 3).

We analysed the spectral curves, taking the average of all the patches (dorsal and dorsolateral) measured for each individual. We quantified colour using the segment classification method proposed by (Endler, 1990). Segments correspond to the blue (400-475 nm), green (475 -550 nm), yellow (550-625 nm) and red (626-700 nm) part of the visible spectrum. The shape and height of reflectance spectra are described by three variables: brightness (the total area under the curve), chroma (or saturation, the slope of the curve as it approaches peak reflectance) and hue

(the location of the wavelength with the greatest reflectance value). All these variables were calculated following the Smith protocol (2014). Typically, melanic individuals have lower skin reflectance than non-melanic individuals (Clusella Trullas et al., 2009; Geen and Johnston, 2014). In our study then, the variable of major interest is brightness, since the area under the curve determines the difference between dark animals (less reflective curves) and light animals (more reflective curves).

### 2.3. Heating rates

Heating rates between body temperatures of 15 °C and 35 °C were measured for a minimum of six individuals per species, in a walk-in chamber with controlled temperature and luminosity. Individuals were immobilized by fixing their limbs and the base of the tail to a cork plate, using 3M Micropore® tape. We then placed the plate, with the animal, in a bucket of 30 cm diameter and 35 cm height in order to prevent escape, and an incandescent 100 W daylight lightbulb (Philips Inc.) was placed 45 cm above this. Body temperatures were registered from 15 °C to 35 °C by taking thermographic images with a Testo 735-1 thermal image camera (resolution 160 by 120 pixels, Testo, Germany) located 40 cm above the specimen and at a 45 ° angle; images were taken every 30 seconds, and timing was controlled with a digital chronometer (Modena MS302, precision 1/100s). Although we observed that the lizards did not try to move or to escape within this temperature range, which could have been considered a sign of stress, immobilization may induce stress and therefore alter body temperature. Every time we observed a lizard trying to escape from the immobilization device or moving continuously, we removed it from the sample. We determined external body temperature at the same spots used to evaluate skin reflectance, using the software Testo IRSofT (Testo, 2016), which allows us to set different points and to transfer them from one image to the next, in order to determine temperature over time always in the same spot for the same individual. These measurements allowed us to determine heating rates for each animal as the average of all the measured spots.

Heating rates were calculated as thermal time constants ( $\tau$ ; Bell, 1980; Cossins and Bowler, 1987), following Labra et al. (2009). We estimated the constants for individuals as  $\tau = -0.4343/b$ , where  $b$  is the slope of  $\ln(T_i - T_a)$  against time;  $T_i$  is the body temperature of the experimental animal recorded at different time points and  $T_a$  is ambient (air 1cm above the substrate) temperature, which was held constantly at 35 °C.  $\tau$  values vary from 0 to infinite; the smaller the value, the faster the heat exchange.

### 2.4. Thermal sensitivity of sprint speed and thermal performance curves (TPC)



To determine the thermal tolerance range, we also measured both critical minimum (CT<sub>min</sub>) and critical maximum temperatures (CT<sub>max</sub>) for each species. The critical temperature is defined as the temperature at which an organism loses motor coordination, failing to get back to its normal posture after being set in a supine position (Carothers et al., 1997; Cruz et al., 2005). We determined the thermal tolerance range following protocols proposed by Cruz et al. (2005) and Bonino et al. (2011). These critical thermal measurements later constituted the edges of the TPC.

We estimated the thermal sensitivity of sprint speed by conducting races for each lizard on a horizontal racetrack 1.2 m in length, with 10 cm-high side panels to prevent escape. It was equipped with seven LED sensors arranged every 15.5 cm, connected to a timer circuit and to a data collector (personal computer). The base of the racetrack was covered with a cork sheet to provide grip and traction. For each run a lizard was placed at one end (start) of the track and then released; gentle taps on the sacral region were used as stimuli until the entire track was completed (Angilletta et al., 2002; Aguilar and Cruz, 2010; Bonino et al., 2015). Speed was measured between consecutive sensors (15.5 cm). Lizards' runs were performed at five discrete body temperatures within the species thermal tolerance ranges (22 °C, 26 °C, 30 °C, 34 °C and 38 °C). Lizards were heated to the desired test body temperatures in an incubation chamber (Semedic FT 290; Semedic, Buenos Aires, Argentina). Runs were conducted on consecutive days, and temperatures were assigned randomly. When a lizard refused to run or escaped before the end of the run the race was considered a failure and discarded from the analyses. Each specimen completed six runs on the track for each of the five temperatures, following Losos et al. (2002); thus, each lizard ran 30 times within a period of 5 days. No more than six runs per day were made per individual, and they were divided into three series of two races, with an interval of at least 2 h between them for the lizards to rest. Body temperature of the specimens was recorded (prior to each race) with an ultra-thin K-type thermocouple connected to a digital thermometer (Extech 421502; Extech Instruments, Waltham, MA, USA). From the six runs for each discrete temperature we retained the highest speed value recorded between two of the seven consecutive LED sensors (15.5 cm intervals), which was considered the maximum speed (m/s) at each temperature. This method has been considered valid when sprint speed is measured in relation to escape behaviour (Gomes et al., 2017), although the same authors pointed out the advantages of instantaneous speed measurements in terms of precision and repeatability, as maximum speed measurements may be influenced by strategies which differ between species (e.g., short bursts vs. long runs), racetrack length, and by the stimuli used (food search vs. escape from predators). In our study we used maximum speed following Cruz et al. (2005), Tulli et al. (2011), Bonino et al. (2011, 2015) to make data comparable with other *Liolaemus*.



To estimate the thermal sensitivity of sprint speed we used the series of speed/temperature points obtained for the individuals of each species. We also included those obtained after fixed time heating runs (see below), as these were obtained with the same protocol. From all of these, we constructed the TPCs with the species'  $CT_{min}$  and  $CT_{max}$  as extreme values (with null speed), and by using TableCurve 2D Demo (© SYSTAT Software Inc., 2002). While Angilletta (2006, 2009) proposed that the simple Gaussian function provides a more acceptable description than other more complex models under the Akaike criterion, we based model selection on the Bayesian approach. Under these criteria we used a set of the asymmetric functions with left skewness and biological significance, among them the Gaussian model from Angilletta (2006). The best fit was selected from AIC values, and then used to estimate performance parameters. From the curves we obtained for each lizard a maximum sprint speed ( $V_{max}$ ) and optimum temperature ( $T_o$ ), defined as the temperature at which the individual reaches its maximum speed. We then standardized the performance for each of the three lizard species, obtaining the relative velocity at each temperature (instantaneous velocity at each temperature/maximum speed reached). Following this, for each species we calculated the optimal temperature range and thermal performance breadth, defined as the body temperature range over which the lizard can run at 95 % of the optimal temperature (B95) or 80 % (B80) of the  $V_{max}$ , respectively (Hertz et al., 1983).

## 2.5. Body temperature and sprint speed after fixed heating time

We aimed to evaluate the capacity of the species to run after heating under similar conditions. We selected two initial temperatures: to simulate a situation where the environment has low thermal quality (as occurs at the beginning of the day, and/ or during cloudy days) the initial body temperature of lizards was set to 15 °C; to simulate a more favourable situation (a temperature at which lizards may already be active in the day), the initial body temperature of the animals was set to 25 °C. These body temperatures were achieved by placing the lizards individually in cloth bags, in an incubator chamber (Semedic FT 290; Semedic, Buenos Aires, Argentina), set 0.5 °C above the target temperature. Once lizards achieved the target temperature, they were placed in a bucket (20 cm diameter, 20 cm high), with the bottom covered by a thin layer of sand in order to prevent overheating. Heat was provided by an incandescent light bulb (Philips daylight 100 W) placed 40 cm above the base of the bucket, for a fixed time of 10 min. We also tested for behavioural adjustments by setting the individuals with restricted movement at an initial temperature of 15 °C. We decided not to test heating with restricted movement at an initial temperature of  $T_i = 25$  °C, as we considered it could imply a high overheating risk. After this time, lizards were removed and final body temperature was immediately measured in their flanks with a thermometer connected to an ultrathin

thermocouple (Moreno Azócar et al., 2013). The lizard then ran three races on the same racetrack, following the protocol explained above for TPC. Every lizard repeated the sequence twice at each initial body temperature (15 °C and 25 °C), but no more than one series of each temperature per day. On completing a trial, each lizard was transferred to a terrarium and allowed to recover, and water was provided *ad libitum*. From each series we obtained the maximum sprint speed, calculated as explained before.

## 2.6. Statistical analyses

We measured 81 individuals of the three *Liolaemus* species (*L. shitan*, *L. elongatus*, and *L. gununakuna*). Reflectance was measured for 19 individuals, heating rates for 26 individuals, and sprint speed for 59 individuals; 34 were heated for a fixed time (10 min) from both initial temperatures (15 °C and 25 °C) without restriction of movement, while 26 were heated from 15 °C initial body temperature with movement restriction. Some specimens were used in more than one experiment, because of collection permit restrictions on sample size. Before running the statistical analyses we examined the data and discarded all the outliers observed, according to the Tukey method, which identifies values outside the IQR 1.5 (Interquartile range approach), the IQR being the difference between Q75 and Q25.

To analyse species colour brightness, we first searched for differences between sexes within each species, using the T-test, then compared brightness between species with ANOVAs using the ‘aov’ function from the ‘stats’ package in R (R Core team, 2019). Significant differences were then analysed using a Tukey *post-hoc* comparison (function ‘TukeyHSD’ from the ‘stats’ package as well).

Prior to any statistical analysis, we tested each variable for homoscedasticity of variances through Levene’s tests. To analyse differences between species in each of the response variables considered (heating rates, TPC variables, final body temperature and sprint speed after fixed heating time), we used ‘gls’ function in ‘nlme’ package (Pinheiro et al., 2019) to perform generalized least squares models from an inferential perspective (Garibaldi et al., 2017), and to take into account the heterogeneity of variance plus covariables. We compared models with homogeneous and heterogeneous variance, including all models derived from the two full models:  $\text{gls}(\text{Vardep} \sim \text{Species} + \text{SVL}, \text{weights} = \text{varIdent}(\text{form} = \sim 1|\text{Species}))$ , and  $\text{gls}(\text{Vardep} \sim \text{Species} + \text{BM}, \text{weights} = \text{varIdent}(\text{form} = \sim 1|\text{Species}))$ , where weights denote estimation of different variances between groups. We then chose the best fitted model according to AICc values for each group of models; that is, either including SVL or BM (Burnham and Anderson, 2004). We analysed intergroup differences by using ‘glht’ function from the ‘multcomp’ package (Hothorn et al., 2008), using Tukey’s all pairs comparison.

### 3. RESULTS

#### 3.1. Reflectance

In agreement with our visual observation, we detected that the three studied species differed in colour and brightness (Fig. 4). The mean brightness values were 361.71 for *Liolaemus shitan*, 671.76 for *L. elongatus* and 1271.15 for *L. gununakuna*, which was significantly lighter (ANOVA Brightness:  $F_{(2, 17)} = 60.5$ ,  $p < 0.001$ ; Chroma:  $F_{(2, 17)} = 21.85$ ,  $p < 0.001$ ; Hue:  $F_{(2, 17)} = 73.88$ ,  $p < 0.001$ ). All levels of the ‘species’ factor were homogeneous and significantly different from each other according to TukeyHSD *posthoc* analysis (Fig. 4, supplementary material Table S3). Because we were not able to test all the individuals collected we did not use reflectance or any of the component variables as continuous; we used this information to ratify the differences in colour between species observed with the naked eye, and therefore used species as a group factor in further analyses.

#### 3.2. Heating rates

Homoscedasticity fails for TAU values between species (Supplementary Material, table S4). We observed no influence of body length on heating rates within the species (lineal model SVL \* Species:  $R^2 = 0.1854$ ,  $F_{(5, 22)} = 1.002$ ,  $p = 0.4399$ ), but we did find a significant effect of body mass (lineal model BM \* Species:  $R^2 = 0.4949$ ,  $F_{(5, 20)} = 3.919$ ,  $p = 0.01223$ , Fig.5A), mainly for *L. gununakuna*, which showed faster heating rates at larger body mass.

The best gls model to describe differences in heating rates between species was the one modelling TAU ( $\tau$ ) with respect to species, including the heterogeneous variance between species factor (Table 1). *Post hoc* analysis showed that *L. shitan* had lower values of TAU (faster heating rates) and smaller variance, and it differed significantly from *L. gununakuna* (Table 1, Fig. 5B). Mean thermal time constants for the species were 6.353 ( $\pm 1.087$ ) for *L. shitan*; 8.302 ( $\pm 1.480$ ) for *L. elongatus*, and 10.231 ( $\pm 3.462$ ) for *L. gununakuna* (Fig. 5B).

#### 3.3. Thermal sensitivity of sprint speed.

Before estimating TPC for sprint speed, we obtained thermal critical minimum and maximum values for *L. gununakuna* and *L. shitan*, to be used as the extremes of the thermal sensitivity curves. CTmin and CTmax for each species were: *L. gununakuna*, 7.42 °C ( $\pm 1.57$ ) and 43.49 °C ( $\pm 0.46$ ); *L. shitan*, 8.12 °C ( $\pm 2.24$ ) and 43.66 °C ( $\pm 0.50$ ); *L. elongatus*, 7.42 °C ( $\pm 1.98$ ) and 44.32 °C ( $\pm 0.63$ ) (data obtained from Bonino et al., 2015). After adjusting the sprint speed registered for all individuals of the three species, the best model to adjust a thermal sensitivity

curve to the data after AIC selection, for all species, was the Asymmetric Logistic (#8043) function.

Among TPC variables, only  $V_{max}$  lacks homogeneity of variance (Supplementary material table S1). The TPC of the lizards showed no significant differences between species other than in  $V_{max}$ , as *Liolaemus elongatus* runs significantly faster than *L. gununakuna*, while *L. shitan* runs similarly to both species (Fig. 6, Table 2).

In most of the variables analysed, the best models were those including the interaction of ‘species’ and ‘SVL’, with or without heterogeneous variance (Table 2). BM was not significant for any model. GLS and ANOVAs indicated differences between species only for  $V_{max}$  (*posthoc*: *L. gununakuna*-*L. elongatus*  $z$  value = -2.503;  $p$  = 0.032), and for B80L (Low B80 value in the curve), but the latter was not recovered by the *posthoc* analysis (Fig. 6, Table 2).

### 3.4. Fixed-time heating experiments

In this experiment we aimed to test whether species reached different body temperatures and/or responded differently in their sprint speed after exposure to the same amount of time under a heating source, using SVL and BM as co-variables, due to the possible influence they may have on heat gain and speed.

We did not find differences in variance between groups for the variables analysed here (Supplementary material, Table S4). According to the AICc values, the best model analysing final body temperatures included species as a factor, and homogeneous variance (Table 3). This was true in all three cases studied ( $T_i$  = 15 °C with unrestricted movement,  $T_i$  = 15 °C with restricted movement, and  $T_i$  = 25 °C with unrestricted movement). With unrestricted movement inside the bucket, *L. elongatus* reached significantly higher body temperatures than the other two species, independently of the initial body temperature (Table 3, Fig. 7A and 7C). In contrast, when lizards were unable to adjust their body postures (that is with movement restricted), *L. gununakuna* final body temperatures were significantly lower than *L. shitan* body temperatures, while *L. elongatus* showed intermediate values (Table 3, Fig. 7B).

Regarding sprint speed, at  $T_i$  = 15 °C, the best model considered heterogeneous variance and species as factors, excluding covariables, while at  $T_i$  = 25 °C the best model included homogeneous variance between species levels, also excluding covariables (Table 3). We observed similar responses for the two temperature settings studied: *L. gununakuna* was the slowest species, whereas *L. elongatus* ran at an intermediate speed and *L. shitan* was the fastest (Table 3, Fig. 8A). However, although at 15°C the lighter species was significantly different from the other two, at 25°C it differed significantly only from *L. shitan* (Table 3, Fig. 8B). We

were unable to test sprint speed after the restricted movement trial, as the manipulation needed to release the lizards greatly affected their body temperature.

#### 4. DISCUSSION

Up to now, evidence for the thermal melanism hypothesis in ectothermic animals has been contradictory, reporting either a clear relationship between colour and heat flux or a lack of influence (Harris et al., 2013; Geen and Johnston, 2014; Yin et al., 2015; Zamora-Camacho et al., 2015; Moreno Azócar et al., 2016; Köhler et al., 2017; De Souza et al., 2017; Kuyuku et al., 2018; among others). We aimed to clarify the relevance of this hypothesis by comparing a study system that would allow us to reduce the noise produced by differences in traits such as body size, diet, and reproductive mode (for further details see Moreno Azócar et al., 2016), as well as colour polymorphism. Here we studied three clearly different species in terms of colouration and reflectance, which allowed us to test thermal melanism. The three lizard species inhabit similar latitudes, share several ecological and biological characteristics but clearly differ in colour. The body shape of the species is similar, mainly cylindrical, without significant differences in external measurements related to the locomotor system or thermoregulation (see supplementary material Tables S1 and S2). First, spectrometry confirmed the differences in colouration between the species as we had visually observed; as expected, higher brightness indicated the highest reflectance in *L. gununakuna* (the lightest coloured species) and the lowest reflectance in *L. shitan* (the darkest one). These differences, in accordance with our predictions, agree with the observed heating rates, where the darkest species gained heat significantly faster than the lightest (paler) one. In view of this, we found support for the prediction that suggests melanism helps ectothermic animals to gain heat faster, and probably enables them to maintain their optimal body temperatures for a longer time (Clusella-Trullas et al., 2008; Harris et al., 2012; De Keyser et al., 2015; Mathews et al., 2016).

Previous studies failed to find differences in heat gain between dark and pale morphs of the lizard *Podarcis dugesii* (Crisp et al., 1979), the snake *Thamnophis sirtalis* (Bittner et al., 2002), and the *Cordylus* genus (Clusella Trullas et al., 2009). However, other studies found support for the TMH in vipers, lizards, snakes and frogs (Gibson and Falls, 1979; Forsman, 1995a, 1995b; Vences et al., 2002; Tanaka, 2005; Geen and Johnston, 2014), while others observed mixed results when studying the distribution of several *Cordylus* species to test the association of melanistic species with cold climates (Janse van Rensburg et al., 2009). Therefore, the nature of dark colouration cannot be linked universally to thermoregulation in cold climates, nor can it be considered in isolation. Despite our promising results, there is still a number of other aspects in which dark colouration may play a role; such as sexual communication (Protas and Patel, 2008),

immunology (Getting, 2006), camouflage, intraspecific communication, and UV protection (Porter, 1967; Gunn, 1998; Böhm et al., 2005).

In a series of studies, Tanaka (2005, 2007, 2009) observed differences in heating of the rat snake (*Elaphe quadrivirgata*) between laboratory and wild experiments, probably associated with behavioural adjustments. In the case of *Liolaemus* species, a previous study in the *L. goetschi* group also reported mixed results; for example, live animals did not show differences in heat gain due to melanism, but inanimate ones showed a positive relationship between melanic colour and heating rates (Moreno Azócar et al., 2016). It is therefore possible that behavioural or physiological aspects compensate for colour differences, suggesting the importance of behaviour during experimental trials. Additionally, the species of *Liolaemus* previously studied by Moreno Azócar et al. (2016) may correspond to polymorphic species with regard to melanism (e.g. *Liolaemus xanthoviridis*; Escudero, et al., 2016). Therefore, the search for good study systems is fundamental in order to clarify the role of melanism-based colouration in reptile thermoregulation. Comparing natural morphs within a single species (Geen and Johnston, 2014), or between closely-related species with clear differences in colour but similarities in size or other life history traits (present study) helps to control for variability caused by biological or ecological traits, as well as genetic variation. While studies within the same species imply gene flux between different morphs and therefore genuine adaptive responses, inter-specific genetic isolation may obscure these adaptive responses; however, studying different species (although morphologically similar and closely related) under similar environmental conditions may constitute a good study system to help us analyse the adaptive role of a trait, in this case melanism.

As we proposed, it is expected that if darker individuals heat up faster than lighter ones, and this represents an advantage for lizards living in cold climates, such an advantage should be reflected in their performance and ultimately, their fitness (Arnold, 1983). We found evidence to support this prediction when behavioural adjustments were prevented during heating rates (in the case of impaired mobility). In these trials, *L. shitan* showed a consistently faster heating rate, reaching a higher final body temperature than the other two species. This species also shows the most homogeneous colouration of the studied species, which could also explain the lower variation observed. However, in the fixed-time heating experiments where the lizards were able to move inside the bucket and change posture, we found different results; although *L. shitan* did show a higher mean final temperature than *L. gununakuna* when heating started at 15 °C, *L. elongatus* showed even higher final body temperatures. Furthermore, *L. shitan* showed the lowest final temperatures when heating started at 25 °C (Fig. 6C). These findings show the importance of melanism for these lizards in terms of heat gain, but also how relevant



behavioural adjustments are, evidenced by movements and postures that allowed the lighter species to reach similar temperatures to the darkest one; however, this result may also show that *L. shitan* controls its body temperature above a certain threshold, to avoid overheating. Thus, dark colour may help to increase temperature, but behaviour is key to controlling the rate of heating and prevent undesirable consequences.

Our results from the sprint speed performance tests to evaluate the potential adaptive advantages of melanism show that the darker species (*L. shitan* and *L. elongatus*) perform better after heating at a cold initial temperature (15 °C) than the lighter one (*L. gununakuna*). These results suggest an improved response when animals need to flee from predators or capture prey in the early hours of the day, when activity begins. However, we observed increased dispersion of the data at higher temperatures, suggesting that the temperature achieved when heating began at 25 °C was warm enough to allow all lizards to perform close to their optimal temperatures. This data dispersion may also be due to the action of different factors (such as physiological status, body wellness, stress, behavioural adjustments, etc.) plus the effect of melanism on sprint speed performance. Few studies have considered the influence of melanism on fitness (the final step of Arnolds, 1983 paradigm) in ectothermic vertebrates, or provided support for the TMH. Castella et al. (2013) found that the body condition of two colour morphs (patterned light and melanistic individuals) of *Vipera aspis* differed between sexes and sites, and observed increased predation on males of the melanistic morph; thus resulting in enhancement of body condition associated with melanism only for females. Similar results were reported by Forsman (1995a, 1995b) for the adder *Vipera berus*. For this species, Capula and Luiselli (1994) reported advantages of possessing melanism that were positively related to higher litter frequency and survivorship in darker females. Our study, although using a common performance measurement (sprint speed), does not enable us to make generalizations about the influence of melanism. However, our results allow us to infer that melanism is in fact advantageous in cold weather, enhancing speed in lizards that may be advantageous for escaping from predators or capturing prey, particularly at the beginning of daily activity, when temperatures are lower.

According to Arnold's Paradigm (Arnold, 1983), which states a correlation between the phenotype, performance and fitness of an organism, we expected to observe that differences in heat gain after the same amount of time under a heat source would translate into enhanced locomotor performance for the darkest (in terms of morphology) species (*L. shitan*) compared to the lighter coloured ones, as a result of faster heating rates (better performance). However, we found only partial support for this prediction. What we actually found was that the lightest coloured species (*L. gununakuna*) was slower than the darker ones, while *L. shitan* was as fast as *L. elongatus*; therefore, our results show the disadvantage of light colouration in cold



climates when lizards are exposed to a heat source for a limited time, but not a clear advantage for the darkest (or even totally black) species. Other aspects, such as behaviour and physiological flexibility, may be involved in the final success of a species.

## 5. CONCLUSIONS

The present study provides evidence to support the thermal function of dorsal melanistic colouration and its influence in reaching near-optimal temperatures from a starting point of 15 °C (a low temperature, typical of early morning in spring-summer in Patagonia), thus favouring the thermal melanism hypothesis. Our results also indicate that paler species reach reasonable body temperatures, at which they can sustain activity either by behavioural or physiological strategies or by choosing short distances from shelter, as is the case of *L. gununakuna* (Moreno Azócar, unpublished data). For these reasons, it remains unclear if melanism is crucial for lizards inhabiting cold habitats. In addition to the thermal advantages for dark lizards (or the disadvantage of being too pale in cold regions), other functions of melanism such as sexual selection, camouflage, UV protection, and disease protection may be related to the colour patterns observed in the species studied here, and future studies may help to elucidate these questions.

## 7. COMPETING INTERESTS

No competing interests declared

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**Table 1. Differences in heating time thermal constants (TAU) between three *Liolaemus* species studied.** Statistical values for gls, anova and *post-hoc* glht corresponding to the best model are shown, according to AICc values. Species are abbreviated by the initials (LG: *L. gununakuna*; LE: *L. elongatus*; LS: *L. shitan*). Significant values are shown in bold ( $\alpha = 0.05$ ).

<b>TAU ~ Species, weights = varIdent (form = ~ 1 Species)</b>													
gls coefficients					anova				glht posthoc				
	Value	S.E.	t	<i>p</i>		DF	F	<i>p</i>		Estimate	S.E.	z	<i>pr</i> (> z )
(Intercept)	8.302	0.523	15.862	0.000	(Intercept)	1	630.412	<.0001	LG-LE	1.929	1.507	1.280	0.388
Species: LG	1.929	1.507	1.280	0.214	<b>Species</b>	<b>2</b>	<b>7.4475</b>	<b>0.0036</b>	<b>LS-LE</b>	<b>-1.949</b>	<b>0.626</b>	<b>-3.113</b>	<b>0.004</b>
<b>Species: LS</b>	<b>-1.949</b>	<b>0.626</b>	<b>-3.113</b>	<b>0.005</b>	DF: 2,23				<b>LS-LG</b>	<b>-3.879</b>	<b>1.454</b>	<b>-2.667</b>	<b>0.018</b>

**Table 2. Comparison of sprint speed performance variables for the studied species.** Vmax: Maximum sprint speed; To; Temperature at which the species reaches Vmax; B80L; B80U; B80range: inferior, superior and temperature range at which lizards reach 80% of Vmax; B95L; B95U; B95 range: inferior, superior and temperature range at which lizards reach 95% of Vmax; Table shows statistical values for gls, anova and post-hoc glht corresponding to the best model according to AICc values for every variable. Species are abbreviated by the initials (LG: *Liolaemus gununakuna*; LE: *L. elongatus*; LS: *L. shitan*). Significant values are shown in bold.

<b>Vmax ~ Species, weights = varIdent (form = ~ 1 Species)</b>													
gls coefficients:					anova				glht posthoc				
	Value	S.E.	t	<i>p</i>		DF	F	<i>p</i>		Estimate	S.E.	z	<i>pr</i> (> z )
(Intercept)	2.478	0.079	31.239	0.000	(Intercept)	1	2583.504	<.0001	LG -LE	-0.388	0.155	-2.503	<b>0.032</b>
<b>SpLG</b>	<b>-0.388</b>	<b>0.155</b>	<b>-2.503</b>	<b>0.015</b>	Species	2	3.746	<b>0.030</b>	LS - LE	-0.203	0.100	-2.026	0.103
<b>SpLS</b>	<b>-0.203</b>	<b>0.100</b>	<b>-2.026</b>	<b>0.048</b>	DF: 2,54				LS -LG	0.184	0.147	1.258	0.412
<b>To~Species*SVL</b>													

gls coefficients:					anova				glht posthoc				
	Value	S.E.	t	<i>p</i>		DF	F	<i>p</i>		Estimate	S.E.	z	<i>pr</i> (> z )
(Intercept)	73.707	17.979	4.100	0.000	(Intercept)	1	7754.558	<.0001	LG -LE	-24.489	97.790	-0.250	0.962
SpLG	-30.518	18.932	-1.612	0.115	Species	2	1.862	0.169	LS - LE	-31.446	97.708	-0.322	0.937
<b>SpLS</b>	<b>-39.758</b>	<b>19.383</b>	<b>-2.051</b>	<b>0.047</b>	SVL	1	4.795	<b>0.035</b>	LS -LG	-6.957	7.651	-0.909	0.600
<b>SVL</b>	<b>-0.666</b>	<b>0.241</b>	<b>-2.760</b>	<b>0.009</b>	Species:SVL	2	0.690	0.508					
SpLG:SVL	0.436	0.252	1.728	0.091	DF: 2, 44								
<b>SpLS:SVL</b>	<b>0.589</b>	<b>0.257</b>	<b>2.295</b>	<b>0.027</b>									
<b>B80i ~ Species * SVL, weights = varident (form = ~ 1 Species)</b>													
gls coefficients:					anova				glht posthoc				
	Value	S.E.	t	<i>p</i>		DF	F	<i>p</i>		Estimate	S.E.	z	<i>pr</i> (> z )
(Intercept)	73.707	17.979	4.100	0.000	(Intercept)	1	11426.217	<.0001	LG -LE	-30.518	18.932	-1.612	0.229
SpLG	-30.518	18.932	-1.612	0.115	Species	2	9.675	<b>0.000</b>	LS - LE	-39.758	19.383	-2.051	0.093
<b>SpLS</b>	<b>-39.758</b>	<b>19.383</b>	<b>-2.051</b>	<b>0.047</b>	SVL	1	12.242	<b>0.001</b>	LS -LG	-9.240	9.359	-0.987	0.572
<b>SVL</b>	<b>-0.666</b>	<b>0.241</b>	<b>-2.760</b>	<b>0.009</b>	Species:SVL	2	2.925	0.065					
SpLG:SVL	0.436	0.252	1.728	0.091	DF: 2, 48								
<b>SpLS:SVL</b>	<b>0.589</b>	<b>0.257</b>	<b>2.295</b>	<b>0.027</b>									
<b>B80i ~Species * SVL</b>													
gls coefficients:					anova				glht posthoc				
	Value	S.E.	t	<i>p</i>		DF	F	<i>p</i>		Estimate	S.E.	z	<i>pr</i> (> z )
(Intercept)	73.707	133.855	0.551	0.585	(Intercept)	1	2865.446	<.0001	LG -LE	-30.520	134.110	-0.228	0.968
SpLG	-30.518	134.106	-0.228	0.821	Species	2	4.307	<b>0.020</b>	LS - LE	-39.760	134.000	-0.297	0.947
SpLS	-39.758	134.000	-0.297	0.768	SVL	1	4.709	<b>0.036</b>	LS -LG	-9.240	10.310	-0.896	0.609
SVL	-0.666	1.796	-0.371	0.713	Species:SVL	2	0.769	0.470					

SpLG:SVL	0.436	1.799	0.242	0.810	DF: 2,48								
SpLS:SVL	0.589	1.798	0.328	0.745									
<b>B80s ~ Species * SVL</b>													
gls coefficients:					anova				glht posthoc				
	Value	S.E.	t	<i>p</i>		DF	F	<i>p</i>		Estimate	S.E.	z	<i>pr</i> (> z )
(Intercept)	55.852	45.543	1.226	0.229	(Intercept)	1	42689.600	<.0001	LG -LE	-11.870	45.643	-0.260	0.959
SpLG	-11.870	45.643	-0.260	0.796	Species	2	0.750	0.479	LS - LE	-14.874	45.595	-0.326	0.936
SpLS	-14.874	45.595	-0.326	0.746	SVL	1	4.150	<b>0.050</b>	LS -LG	-3.004	3.711	-0.810	0.667
SVL	-0.241	0.611	-0.395	0.696	Species:SVL	2	0.510	0.606					
SpLG:SVL	0.167	0.612	0.273	0.786	DF: 2,39								
SpLS:SVL	0.212	0.612	0.346	0.732									
<b>B80s ~ Species * SVL, weights = varident (form = ~ 1 Species)</b>													
gls coefficients:					anova				glht posthoc				
	Value	S.E.	t	<i>p</i>		DF	F	<i>p</i>		Estimate	S.E.	z	<i>pr</i> (> z )
(Intercept)	55.852	7.586	7.363	0.000	(Intercept)	1	162132.780	<.0001	LG -LE	-11.870	7.900	-1.503	0.273
SpLG	-11.870	7.900	-1.503	0.143	Species	2	2.750	0.079	LS - LE	-14.874	7.983	-1.863	0.138
SpLS	-14.874	7.983	-1.863	0.071	SVL	1	9.140	<b>0.005</b>	LS -LG	-3.004	3.324	-0.904	0.623
<b>SVL</b>	<b>-0.241</b>	<b>0.102</b>	<b>-2.369</b>	<b>0.024</b>	Species:SVL	2	2.220	0.125					
SpLG:SVL	0.167	0.106	1.587	0.122	DF: 2, 39								
SpLS:SVL	0.212	0.106	1.994	0.055									
<b>B80Rango ~ Species * SVL</b>													
Coefficients:					anova				glht posthoc				
	Value	S.E.	t	<i>p</i>		DF	F	<i>p</i>		Estimate	S.E.	z	<i>pr</i> (> z )
(Intercept)	-17.855	93.047	-0.192	0.849	(Intercept)	1	1112.017	<.0001	LG -LE	23.809	93.222	0.255	0.960

SpLG	23.809	93.222	0.255	0.800	Species	2	2.629	0.084	LS - LE	25.266	93.149	0.271	0.955
SpLS	25.266	93.149	0.271	0.788	SVL	1	1.685	0.202	LS - LG	1.457	7.174	0.203	0.975
SVL	0.425	1.249	0.340	0.735	Species:SVL	2	0.115	0.891					
SpLG:SVL	-0.349	1.251	-0.279	0.782	DF: 2, 47								
SpLS:SVL	-0.382	1.250	-0.306	0.761									
<b>B95i ~ Species * SVL</b>													
gls coefficients:					anova				glht posthoc				
	Value	S.E.	t	p		DF	F	p		Estimate	S.E.	z	pr (> z )
(Intercept)	-51.914	107.139	-0.485	0.631	(Intercept)	1	5262.575	<.0001	LG -LE	96.636	107.349	0.900	0.606
SpLG	96.636	107.349	0.900	0.374	Species	2	3.478	<b>0.041</b>	LS - LE	88.949	107.259	0.829	0.653
SpLS	88.949	107.259	0.829	0.412	SVL	1	5.624	<b>0.023</b>	LS -LG	-7.688	8.398	-0.915	0.596
SVL	1.061	1.438	0.738	0.465	Species:SVL	2	1.004	0.376					
SpLG:SVL	-1.258	1.440	-0.873	0.388	DF: 2, 44								
SpLS:SVL	-1.138	1.439	-0.791	0.434									
<b>B95s ~ Species * SVL</b>													
gls coefficients:					anova				glht posthoc				
	Value	S.E.	t	p		DF	F	p		Estimate	S.E.	z	pr (> z )
(Intercept)	63.604	71.891	0.885	0.382	(Intercept)	1	15406.423	<.0001	LG -LE	-18.289	72.032	-0.254	0.961
SpLG	-18.289	72.032	-0.254	0.801	Species	2	1.666	0.204	LS - LE	-26.263	71.987	-0.365	0.920
SpLS	-26.263	71.987	-0.365	0.717	SVL	1	3.033	0.090	LS -LG	-7.975	5.837	-1.366	0.320
SVL	-0.383	0.965	-0.397	0.694	Species:SVL	2	1.321	0.280					
SpLG:SVL	0.252	0.966	0.261	0.796	DF: 2, 41								
SpLS:SVL	0.366	0.966	0.379	0.707									
<b>B95Rango ~ Species * SVL</b>													

gls coefficients:					anova				glht posthoc				
	Value	S.E.	t	p		DF	F	p		Estimate	S.E.	z	pr (> z )
(Intercept)	-47.945	246.920	-0.194	0.847	(Intercept)	1	838.2199	<.0001	LG -LE	50.014	246.939	0.203	0.974
SpLG	50.014	246.939	0.203	0.841	Species	2	2.2838	0.115	LS - LE	51.408	246.931	0.208	0.973
SpLS	51.408	246.931	0.208	0.836	SVL	1	1.6755	0.203	LS -LG	1.395	3.862	0.361	0.921
SVL	0.727	3.280	0.222	0.826	Species:SVL	2	0.165	0.848					
SpLG:SVL	-0.681	3.280	-0.208	0.837	DF: 2, 46								
SpLS:SVL	-0.707	3.280	-0.215	0.831									

**Table 3. Final body temperature and sprint speed after fixed heating time.** Differences between the three *Liolaemus* species studied in final body temperature without (Tf15, Tf25) and with restricted movement (Tf15r), and sprint speed (Vmax15, Vmax25), after fixed heating for all initial body temperatures,. Statistical values for gls, anova and post-hoc glht corresponding to the best model according to AICc values are shown. Species are abbreviated by the initials (LG: *L. gununakuna*; LE: *L. elongatus*; LS: *L. shitan*). Significant values are shown in bold ( $\alpha = 0.05$ ).

<b>Tf15 ~ Species</b>													
gls coefficients:					anova				glht posthoc				
	Value	S.E.	t	p		DF	F	p		Estimate	S.E.	z	pr (> z )
<b>(Intercept)</b>	<b>24.852</b>	<b>0.226</b>	<b>109.745</b>	<b>0.000</b>	<b>(Intercept)</b>	<b>1</b>	<b>12144.430</b>	<b>&lt;.0001</b>	<b>LE - LG</b>	<b>3.222</b>	<b>0.566</b>	<b>5.698</b>	<b>&lt;.0001</b>
SpeciesLG	0.589	0.369	1.596	0.121	<b>Species</b>	<b>2</b>	<b>16.770</b>	<b>&lt;.0001</b>	LS - LG	0.833	0.522	1.596	0.247
<b>SpeciesLS</b>	<b>-2.291</b>	<b>0.414</b>	<b>-5.533</b>	<b>0.000</b>	DF: 2, 31				<b>LS - LE</b>	<b>-2.389</b>	<b>0.575</b>	<b>-4.154</b>	<b>&lt;.0001</b>
<b>Tf25~ Species</b>													
gls coefficients:					anova				glht posthoc				
	Value	S.E.	t	p		DF	F	p		Estimate	S.E.	z	pr (> z )



<b>(Intercept)</b>	<b>29.147</b>	<b>0.144</b>	<b>201.813</b>	<b>0.000</b>	<b>(Intercept)</b>	<b>1</b>	<b>40789.080</b>	<b>&lt;.0001</b>	LE - LG	0.723	0.351	2.058	0.099
SpeciesLG	-0.305	0.248	-1.229	0.228	<b>Species</b>	<b>2</b>	<b>5.290</b>	<b>0.011</b>	LS - LG	-0.432	0.351	-1.229	0.436
<b>SpeciesLS</b>	<b>-0.766</b>	<b>0.252</b>	<b>-3.042</b>	<b>0.005</b>	DF: 2, 31				<b>LS - LE</b>	<b>-1.155</b>	<b>0.359</b>	<b>-3.218</b>	<b>0.004</b>
<b>Tf15r~ Species</b>													
gls coefficients:				anova				glht posthoc					
	Value	S.E.	t	p		DF	F	p		Estimate	S.E.	z	pr (> z )
<b>(Intercept)</b>	<b>24.049</b>	<b>0.306</b>	<b>78.574</b>	<b>0.000</b>	<b>(Intercept)</b>	<b>1</b>	<b>12690.220</b>	<b>&lt;.0001</b>	<b>LG-LE</b>	<b>-1.141</b>	<b>0.515</b>	<b>-2.214</b>	<b>0.068</b>
<b>SpeciesLG</b>	<b>-1.141</b>	<b>0.515</b>	<b>-2.214</b>	<b>0.039</b>	<b>Species</b>	<b>2</b>	<b>3.690</b>	<b>0.043</b>	LS - LE	0.356	0.515	0.691	0.768
SpeciesLS	0.356	0.515	0.691	0.498	DF: 2, 31				<b>LS - LG</b>	<b>1.497</b>	<b>0.586</b>	<b>2.554</b>	<b>0.029</b>
<b>Vmax15~ Species</b>													
gls coefficients:				anova				glht posthoc					
	Value	S.E.	t	p		DF	F	p		Estimate	S.E.	z	pr (> z )
<b>(Intercept)</b>	<b>1.777</b>	<b>0.109</b>	<b>16.358</b>	<b>0.000</b>	<b>(Intercept)</b>	<b>1</b>	<b>408.799</b>	<b>&lt;.0001</b>	<b>LE - LG</b>	<b>0.582</b>	<b>0.170</b>	<b>3.420</b>	<b>0.002</b>
<b>SpeciesLG</b>	<b>0.515</b>	<b>0.210</b>	<b>2.445</b>	<b>0.020</b>	<b>Species</b>	<b>2</b>	<b>7.334</b>	<b>0.003</b>	<b>LS - LG</b>	<b>0.728</b>	<b>0.298</b>	<b>2.445</b>	<b>0.036</b>
SpeciesLS	-0.178	0.163	-1.095	0.282	DF: 2, 31				LS - LE	0.145	0.308	0.472	0.881
<b>Vmax25~ Species</b>													
gls coefficients:				anova				glht posthoc					
	Value	S.E.	t	p		DF	F	p		Estimate	S.E.	z	pr (> z )
<b>(Intercept)</b>	<b>1.994</b>	<b>0.098</b>	<b>20.411</b>	<b>0.000</b>	<b>(Intercept)</b>	<b>1</b>	<b>412.829</b>	<b>&lt;.0001</b>	LE - LG	0.390	0.238	1.642	0.228
<b>SpeciesLG</b>	<b>0.495</b>	<b>0.168</b>	<b>2.946</b>	<b>0.006</b>	<b>Species</b>	<b>2</b>	<b>4.374</b>	<b>0.021</b>	<b>LS - LG</b>	<b>0.700</b>	<b>0.238</b>	<b>2.946</b>	<b>0.009</b>
SpeciesLS	-0.033	0.170	-0.193	0.848	DF: 2, 31				LS - LE	0.310	0.243	1.276	0.409

Fig. 1. Lizard species studied. A) *Liolaemus shitan*, B) *L. elongatus*, C) *L. gununakuna*.

Fig. 2. Collection sites of the studied species.

Fig. 3. Dorsal and Dorsolateral spots where colour measures were taken; head (1), nape (2), inter-scapular region (3), mid-dorsum (4), posterior region (between hind legs, 5), and tail base (6), cheek (7) shoulder (anti-humeral spot, 8), and flank (9).

Fig. 4. Reflectance curves averaged for the three *Liolaemus* species studied (LG: *L. gununakuna*, N = 7; LE: *L. elongatus*, N = 9; and LS: *L. shitan*, N = 3), showing the percentage reflectance measured for every wavelength value in nanometers (nm) within the visible spectre range. ANOVA results are shown in the left upper corner of the figure.

Fig. 5. A) Scatterplot of thermal time constants (TAU) for heating rates of the three *Liolaemus* species studied here and their variation in relation to body mass. Grey shadow represents 95% confidence interval. B). Boxplot of thermal time constants of heating rates measured for the three studied species. Different letters show significant differences between groups in the *post hoc* analysis. Sample size was 6 *L. gununakuna*, 10 *L. elongatus* and 10 *L. shitan*.

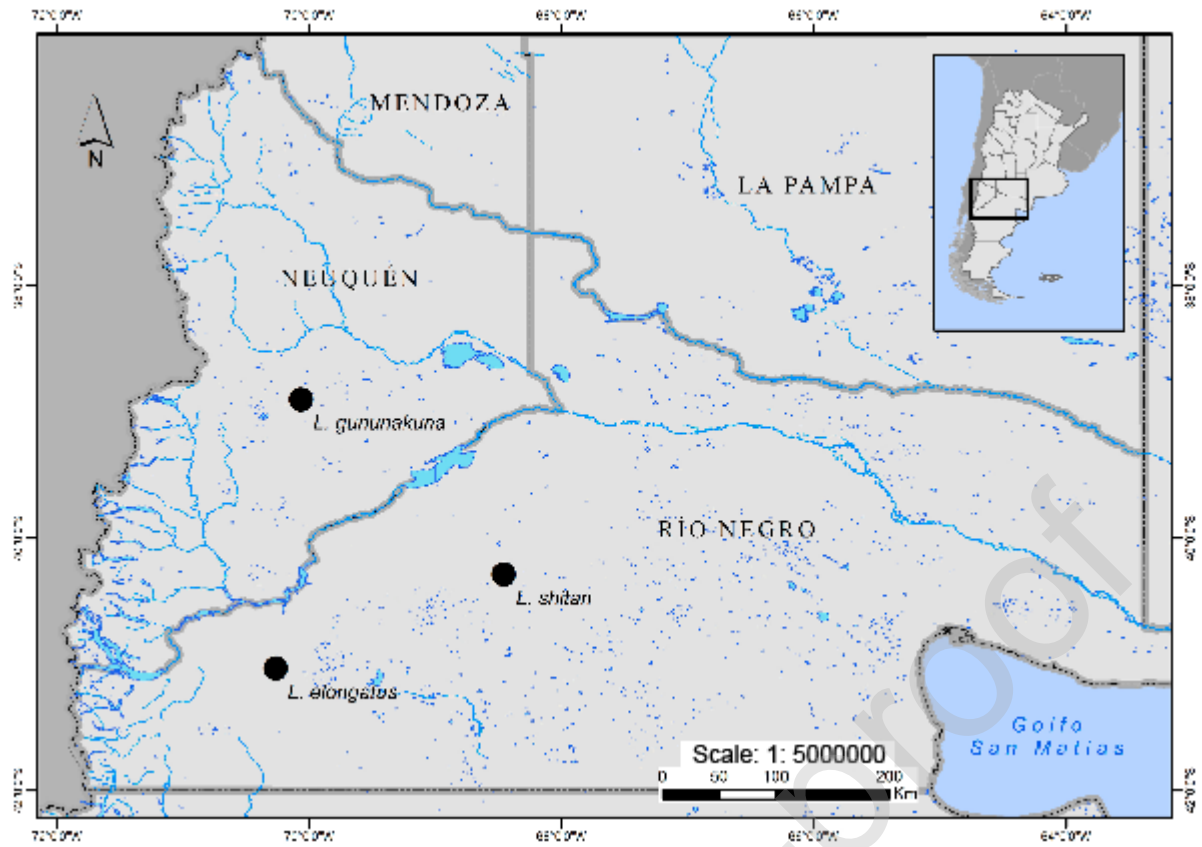
Fig. 6. Sprint speed curves of performance against temperature, for the three *Liolaemus* species studied here (LG: *L. gununakuna*, N = 19; LE: *L. elongatus*, N = 13; and LS: *L. shitan*, N = 27).

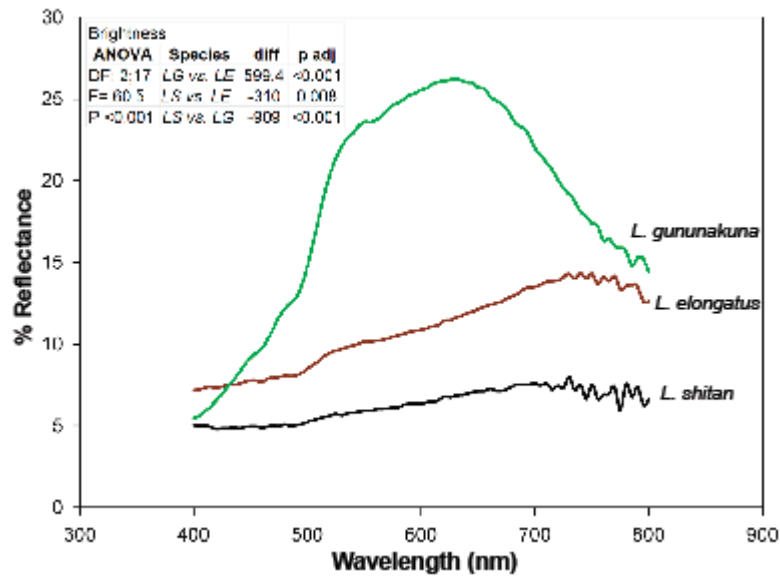
Fig. 7. Boxplot of final body temperature reached after 10 min heating for the three *Liolaemus* species studied, A) Initial temperature,  $T_i = 15\text{ }^{\circ}\text{C}$ , free movement, N/Species: 13 *L. gununakuna*, 9 *L. elongatus* and 12 *L. shitan*., B)  $T_i = 15\text{ }^{\circ}\text{C}$ , restricted movement, N/Species: 6 *L. gununakuna*, 11 *L. elongatus* and 6 *L. shitan*. C),  $T_i = 25\text{ }^{\circ}\text{C}$ , free movement, N/Species: 12 *L. gununakuna*, 11 *L. elongatus* and 11 *L. shitan*.

Fig. 8. Maximum sprint speed of the studied species measured after 10 min heating. A) Initial temperature is  $15\text{ }^{\circ}\text{C}$ , N/Species: 13 *L. gununakuna*, 9 *L. elongatus* and 12 *L. shitan*. C) . B) Initial temperature is  $25\text{ }^{\circ}\text{C}$ , N/Species: 12 *L. gununakuna*, 11 *L. elongatus* and 11 *L. shitan*. Different letters show significant differences between groups.









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