

Evidence from *Tarentola mauritanica* (Gekkota: Phyllodactylidae) helps validate thermography as a tool to infer internal body temperatures of lizards

Frederico M. Barroso^{a,b,1,*}, Gabriel Riaño^{a,1}, Marco Sannolo^a, Miguel A. Carretero^{a,b}, Catarina Rato^{a,b}

^a CIBIO, Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Campus de Vairão, Rua Pedro Armando Quintas n°7, Vairão, 4485 - 661, Vila do Conde, Portugal

^b Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, R. Campo Alegre, s/n, 4169 - 007, Porto, Portugal

ARTICLE INFO

Keywords:

Thermography
Tarentola mauritanica
 Infrared thermal imaging
 Regional heterothermy
 Lizard body temperature

ABSTRACT

Infrared (IR) thermal imaging has become an increasingly popular tool to measure body temperature of animals. The high-resolution data it provides with short lag and minimum disturbance makes it an appealing tool when studying reptile thermal ecology. However, due to the common phenomenon of regional heterothermy and surface-to-core temperature gradients, it is essential to select the appropriate body part to measure and provide calibrations to accurately infer internal body temperatures. This work follows from a previous study on lacertid lizards to assess the reliability of thermography-measured body temperatures, from several body locations, as a proxy for internal body temperature in lizards. This study focuses on the Moorish gecko, *Tarentola mauritanica*, due to its distant phylogenetic relationship and its different ecology and morphology from the previously tested species. A total of 60 adult geckos of both sexes and of a range of sizes were tested in thermal gradients and subjected to a sequence of randomly assorted treatments of heating and cooling. The temperatures of the animals were periodically measured with a thermal camera at six different body parts and, immediately after, the cloacal temperature was then measured with a thermocouple probe. Body parts' temperatures, obtained thermographically, were regressed against cloacal temperature using OLS regression and the pairwise correlations were tested using Spearman coefficients. Relationships among all body parts and between all body parts and the cloaca were strong in all cases ($R^2 > 0.87$, Spearman Correlation > 0.95). The observed pattern was very similar to those previously obtained from lacertid lizards. Ultimately, the eye proved to provide the best overall proxy for internal temperature, when accounting for both the slope and intercept of the regression. Hence, this study provides further support for the establishment of the eye as the standard location to infer internal body temperatures of lizards through thermography.

1. Introduction

The field of thermal ecology has, through the years, provided abundant important insights into the ecology, physiology and diversity of reptiles (Angilletta et al., 2002; Huey and Stevenson, 1979; Savage et al., 2004). The literature is laden with studies demonstrating the fundamental role of temperature for the ecophysiology of reptiles (e.g. Seebacher and Franklin, 2005) and on how body temperatures are maintained through behavioural adjustments, physiology and

microhabitat selection (Angilletta, 2010; Brown et al., 2004; Goller et al., 2014). This ability allows reptiles to optimise a range of metabolic functions (Angilletta et al., 2004; Angilletta, 2010; Huey and Stevenson, 1979) such as locomotion, digestion, growth and reproduction, without the high costs of endogenous heat production endured by endotherms (Huey and Slatkin, 1976).

The pivotal role that temperature plays in the ecology of reptiles led to the early origin of now well-established protocols for measuring internal body temperatures. Cloacal probes, in use since the onset of this

* Corresponding author. CIBIO, Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Campus de Vairão, Rua Pedro Armando Quintas n°7, Vairão, 4485 - 661, Vila do Conde, Portugal.

E-mail address: frederico.m.barroso@gmail.com (F.M. Barroso).

¹ These authors contributed equally to this work.

<https://doi.org/10.1016/j.jtherbio.2020.102700>

Received 31 January 2020; Received in revised form 30 July 2020; Accepted 3 August 2020

Available online 6 September 2020

0306-4565/© 2020 Elsevier Ltd. All rights reserved.

area of research (Angilletta, 2010), are a reliable tool that provide easily reproducible data with great accuracy and precision (e.g. Hibok 18 thermometer and thermocouple probe, used in this study, has an accuracy of $\pm 0.2\%$ and precision of $0.1\text{ }^{\circ}\text{C}$). However, as technology progresses, there has been an increasing reliance on new tools and protocols such as temperature-sensitive RFID (radio) tags (Roark and Dorcas, 2000), implantable data loggers (Campos and Magnusson, 2013), infrared thermometers (pyrometers) (Carretero, 2012; Chukwuka et al., 2019; Hare et al., 2007) and infrared thermal imaging (Bosch, 1983; Burns et al., 2015; Jones and Avery, 1989) to measure body temperatures. These allow high-resolution temperature data to be recorded with great speed and short lag while also minimising the animal disturbance (Barroso et al., 2016; Sannolo et al., 2014; Tattersall and Cadena, 2010).

Several studies have described the potential uses and advantages of said tools (Berg et al., 2015; Tattersall and Cadena, 2010; among others). Such is the case for infrared thermal imaging (hereafter referred to as thermography) where authors like Kastberger and Stachl (2003), Luna et al. (2013), Tattersall and Cadena (2010), and Virens and Cree (2019) demonstrated the potential uses of thermography for a range of studies in several taxa. However, few are the studies which evaluate the reliability of the data collected by this technology or its comparability to temperature data collected by other tools. An example would be the study by Carretero (2012) who demonstrated that temperatures of small lacertid lizards measured at a distance of 20 cm using a pyrometer (infrared thermometer) showed a non-isometric bias and a weak correlation to those obtained by cloacal probe, thus showing that pyrometry may not be a very reliable method of inferring internal body temperature. A more recent study by Chukwuka et al. (2019) with geckos obtained contrary conclusions to those reported by Carretero (2012) regarding the reliability of pyrometers but, in this case, measurements were taken at a distance of 5 mm, which required the animals to be handled. Indeed, these new tools are now widely used to produce increasingly complex results (e.g. Tattersall et al., 2016). As such, there is an urgent need to validate the use of these new instruments, as well as to develop up-to-date, standardized methodologies in order to provide reliable as well as comparable results.

Two previous studies, by Barroso et al. (2016) and Sannolo et al. (2014), have shown that thermography can be reliably used to infer internal body temperature of small to large lacertid lizards. In Barroso et al. (2016), the authors capitalised on the phenomenon of regional heterothermy - a widespread phenomenon among lizards, initially described by Heath (1964) - to investigate which body part would consistently provide the best direct proxy for the internal body temperature (taken as the cloacal temperature). Barroso et al. (2016) concluded that the eye provides such opportunity and suggested possible explanations. These include the presence of large venous sinuses behind the eye, a common feature among many squamate groups including lacertids (Bruner, 1907), iguanids (Porter and Witmer, 2015) and even snakes (Bruner, 1907). As a result, lizards may in fact show particular care and precision in regulating head/brain temperature (King and Green, 1999; Porter and Witmer, 2015; Tattersall et al., 2006). Hence, if such a pattern was maintained across a range of phylogenetically distant reptile groups, the eye could prove to be a strong candidate to be set as a new standard location to measure internal body temperature of squamates through thermography.

Therefore, this study aims to further validate the use of thermography to infer internal body temperature and provide appropriate guidelines, now using the Moorish gecko *Tarentola mauritanica* (Gekota, Phyllodactylidae) as a model. This is a small-sized species (maximum recorded snout-vent length of 86 mm in the Iberian Peninsula), resulting in small thermal inertia (Bell, 1980) while, being a gecko, it is also phylogenetically distant from lacertids, iguanids and snakes (Pyron et al., 2013). As with most geckos, it also lacks eyelids, having a scale over the eye (the brille) instead (Bellairs, 2009) and it shows less marked occurrence of regional heterothermy (see Results). All these characteristics, along with the combination of diurnal and

crepuscular activities and thus, alternating between heliothermic and thigmothermic strategies (Arad et al., 1997; Gil et al., 1994; Rato and Carretero, 2015; Simbula et al., 2019), makes it as distant as possible to a lacertid and hence, a good model to further test whether the pattern observed by Barroso et al. (2016) could potentially be ubiquitous among lizards.

Ultimately, the main goal of this study is to provide yet another stepping-stone towards the development of a unified protocol for the use of thermography as a non-invasive tool to measure body temperatures in reptile thermal ecology studies.

2. Methods

2.1. Sampling and animal maintenance

A total of 60 adults of *T. mauritanica* were tested in this study with a ratio of 31 males to 29 females, representing distinct body lengths (mean male SVL \pm SD = 67.64 ± 7.99 mm; mean female SVL \pm SD = 63.69 ± 4.62 mm) and body masses (mean male mass \pm SD = 7.53 ± 2.18 g; mean female mass \pm SD = 5.78 ± 0.92 g). *Tarentola mauritanica* was chosen due to its crepuscular and diurnal pattern of activity as well as its bimodal thermoregulatory strategy (i.e. heliothermy and thigmothermy, both observed in this species) (Rato and Carretero, 2015).

Animals were captured from four different populations (Torres Vedras: 39.09°N , 9.25°W ; Évora: 38.56°N , 7.91°W ; Portimão: 37.13°N , 8.54°W ; Ayamonte: 37.21°N , 7.38°W) all belonging to the same mitochondrial lineage in order to avoid phylogenetic effects (Rato et al., 2012, 2016), but encompassing a wide array of environmental conditions from the Iberian Peninsula (Rato and Carretero, 2015). Geckos were collected in spring 2019 and kept in captivity in couples with water and food provided *ad libitum* throughout the period of captivity. The experiments were performed several months before the onset of the reproductive season to ensure all females were not in oogenesis or gravid.

2.2. Experimental settings

Between the 25th of November and the December 13, 2019, six experimental sets were carried out. For each one, a total of seven thermal gradients ($\pm 16\text{--}55\text{ }^{\circ}\text{C}$) set up in acrylic tanks ($100 \times 30 \times 40$ cm) with one 150 W heat lamp placed at one end about 25 cm from the bottom and with an elevated aluminium plate running the full length of the terrarium as a refuge were set up following Rato and Carretero (2015). The heat lamp provided the opportunity for the animals to heat up heliothermically while also heating up the aluminium plate which in turn provided the opportunity for the animals to heat up thigmothermically. Therefore, with this set up, animals were able to vary between the two heating strategies as they have been reported to do in natural conditions (Arad et al., 1997).

Water was sprayed in the cold area before each experimental set started. This had the dual purpose of helping cool down the cooler side of the gradient, while also providing access to water as the level of hydration has been shown to affect thermoregulatory behaviour in some lizards (Sannolo and Carretero, 2019).

Males and females were previously labelled with dental floss loosely tied around the waist, and one couple was released per thermal gradient. The floss facilitated capture of the animal while minimising direct contact with it, which could interfere with the individual's temperature. Additionally, markings on the floss allowed the male and female individuals to be easily identified without the need to manipulate the animal. Order of individuals in the whole experimental setup was randomized, so individuals from different populations were tested in the same experimental set. Once all animals were in the thermal gradients, and in order not to just have warm or cold measurements, but to also ensure intermediate body temperatures were obtained, we used the android app *True Random Generator*[®] (downloaded from Google

Appstore, 2019) to randomize the heat lamp state (on or off). In preliminary tests, it was observed that *T. mauritanica* heated up or cooled down quickly (from 18 °C to 30 °C in less than 3 min, from 30 °C to 23 °C in less than 4 min; authors, pers. obs.). Hence, 1 min before the measurement each heat lamp was switched on or off according to the randomization. Heat lamp state was maintained until the following randomization event. This was done in order to obtain a uniform distribution of body temperatures over the widest possible temperature range. Measurements were carried out every 20 min until six recordings per individual were achieved. The experiments were performed in a large, air-conditioned room with air temperature set to 18 °C.

For the thermal pictures, a FLIR T335 thermal camera (sensitivity: < 0.05 °C; accuracy: \pm 2%; IR image resolution: 320 \times 240 pixels; Flir Systems Inc., Wilsonville, Oregon, USA) was used to simultaneously take an IR and a regular photo of each lizard's entire body (assuming a skin emissivity = 0.96). IR camera was handheld and photos were shot at 30–40 cm distance from the animals. This approach allowed maintaining the same resolution in every IR image, irrespective of body size. Immediately (<20 s) after photographing each animal, the subject was captured and its cloacal temperature measured with a contact thermometer (Hibok 18, precision: 0.1 °C, accuracy: \pm 0.2%) fitted with a k-type thermocouple probe. The reading was obtained by inserting the probe a few millimetres into the cloaca of the animal.

In order to reduce possible measurement biases, FMB took the thermal pictures and GMR the cloacal temperatures, using the floss to catch the individual, avoiding direct contact as much as possible to prevent heat exchange between the animal and the measurer. At the end of each set of gradients, all subjects were weighed in a precision balance (Sartorius M-Pact AX224, Sartorius AG, Goettingen, Germany). After all the experiments, snout-vent length (SVL) was measured for each individual.

All IR images were processed using the software FLIR Tools 2.1 (Copyright, 2014 FLIR Systems, Inc; **Error! Hyperlink reference not valid.** www.flir.com). The Spotmeter tool was used to extract the temperature dorsally at the centre of six body locations (Fig. 1): snout, eye, head, dorsum (centrally), leg (right hind knee articulation) and base of the tail (above the cloaca), as in Barroso et al. (2016). Whenever a body location was isothermic with the background (i.e. indistinguishable from the surroundings in the IR photo), the corresponding regular photo was used to try to locate the body part. The emissivity was set to 0.96 and the distance of measurement to 30 cm, while the ambient temperature and humidity were measured by the camera at the time of recording. For consistency, FMB processed all IR photos.

2.3. Statistical analysis

We investigated the potential effect of trial number (six repetitions) and heating status (on or off) using a two-way interaction mixed-effects model (Mod1), with the gecko's individual identity set as random factor to account for the repeated measures and trial and heating status as fixed factors (nlme R package, Pinheiro et al., 2019). To investigate the effect of individual variables on geckos' body temperature, we fitted a mixed-effects model (Mod2) with body temperatures as the dependent variable, and the first-order interaction of body position (seven levels) and sex (two levels) as fixed effects. Individual identity was used as the random effect. In both models, body mass was used as a covariate to account for the potential effect of body size (i.e. thermal inertia) on body temperatures. The statistical significance of single-level fixed factors was evaluated using ANOVA tables, while both random and fixed effects were evaluated using likelihood ratio tests (LRT) and by examining the summary tables of the models (*t*-value). Post hoc tests on multilevel factors were carried on using the lsmeans R package (Lenth, 2016).

Given the non-linearity of body temperature data (Shapiro-Wilkson test = 0.9, $P < 0.0001$) and the repeated measure design of the experiment, we analysed the relationship between cloacal temperatures (contact thermometer) and the temperatures of other body parts

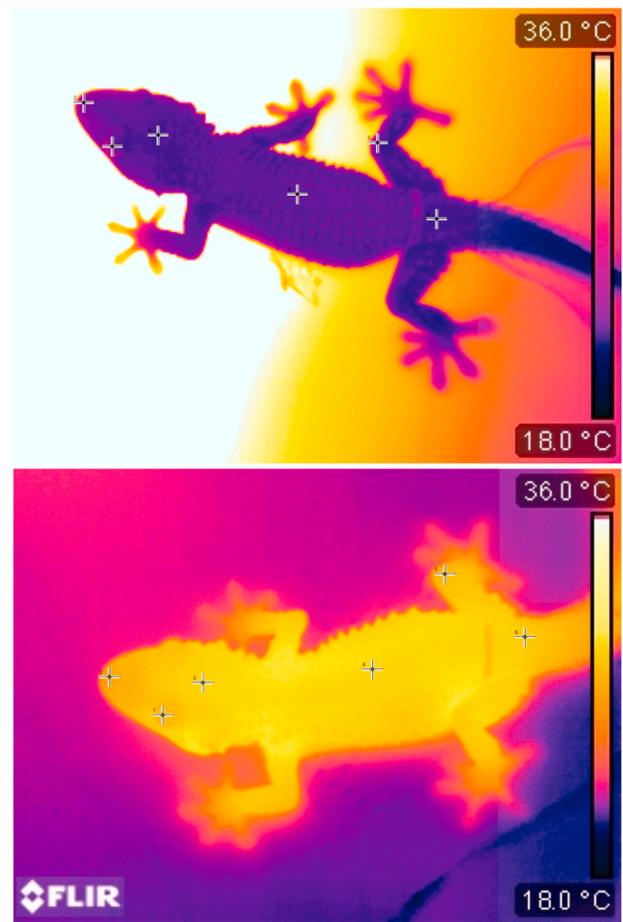


Fig. 1. Infrared thermal photos of *T. mauritanica* showing the body parts measured and the low regional heterothermy observed both during heating (top) and cooling (bottom).

(infrared camera) following the method of Barroso et al. (2016). Hence, the relationship between the cloacal temperature and each of the other body parts temperatures was investigated using OLS with resampling (lmodel2 R package, Legendre, 2018, 999 resampling). Calibration parameters and functions values were extracted to compare the relationship between cloacal temperatures and the other body parts' temperatures.

Finally, in order to better represent the relationships between the different body temperatures and to allow comparisons between geckos and lacertids, Spearman correlation and Multidimensional Scaling (vegan R package, Oksanen et al., 2019) were performed for *T. mauritanica*'s temperatures. The same analyses were also applied to the data from Barroso et al. (2016) for lacertids and compared with the results obtained for this study. All analyses were performed using the R software, version 3.6.0 (R Core Team, <https://www.r-project.org>).

3. Results

Regarding the potential effects of procedures on body temperature (Mod1), we found significant differences in body temperatures depending on the trial (chi-squared = 286.2, $P < 0.0001$), while the heating status was not significant ($P > 0.05$). More specifically, post hoc tests with Tukey correction for multiple comparisons showed that average body temperatures significantly differed between the first trial and all others, and between the last trial and all others (Supplementary Table S1), showing that the first and last trial tended to underestimate geckos' body temperature.

In the second model, in which we tested for the effect of individual

variables (sex, mass and body position) on body temperature, we found a significant contribution of individual variability, as expressed by the random effect (LRT = 502.4, $P < 0.001$). Contrarily, body mass was not significant ($P = 0.42$), despite the fact that males were heavier than females (mean $M = 7.587$ g, mean $F = 5.769$ g; Mann-Whitney test = 35455, $P < 0.0001$). There was also no significant difference between sexes ($P = 0.66$; Fig. 2) in body temperature for any of the body parts considered (mean \pm SD cloacal temperature $M = 22.9 \pm 5.01$ °C; $F = 22.4 \pm 4.80$ °C; see Table 1 for mean temperatures of remaining body parts and Fig. 2 for graphical representation).

Body temperature significantly differed depending on the position considered (chi-squared = 16.27, $P = 0.012$). However, statistically significant differences were detected only between the snout and cloaca (t -ratio = 3.446, $P = 0.0104$) and between the snout and dorsum (t -ratio = 3.016, $P = 0.041$).

Nonetheless, when comparing regressions between cloaca and the other body parts, the leg provided the best R^2 (0.97) (Table 1 and Fig. 3) which also showed the lesser line angle with cloacal temperature (0.84°). Slope values tended to increase from snout to tail, while intercept was less predictable (Table 1, Fig. 3). The best (i.e. closer to zero) average intercept was provided by the eye which, however, showed also the second widest 95% confidence interval for this parameter (Table 1). Similarly, the eye also provided the best (i.e. closer to 1) slope values (Table 1, Fig. 3), but again was affected by the second greatest variability (Table 1, Fig. 3). Nonetheless, despite the comparatively wide 95% CIs of the eye's slope and intercept, a strong association ($R^2 = 0.924$) for this regression was still obtained.

Results from the OLS regression and from the correlation analysis for *T. mauritanica* showed strong association between all measured variables (Table 1, Fig. 3 and Supplementary Table S2), with the lowest value having an R^2 of 0.871 and a correlation score of 0.956 (Table 1 and Supplementary Table S2). Interestingly, both the correlation analysis and MDS plots were congruent with cloacal temperature as the most different measurement and with all the other temperature measurements clustered together (Supplementary Fig. S1). However, in the correlation analysis head and dorsum were clustered the closest, followed by tail and leg, with eye and snout forming another cluster, whilst in the MDS (stress value = 0.1144) dorsum was closer to tail and to

similar distances from leg and head (Supplementary Fig. S1). Remarkably, eye and snout, respectively, were the most different measurements within that cluster.

On the other hand, data on three lacertid species from Barroso et al. (2016), here subjected to Spearman correlation and MDS analyses, showed a different pattern from *T. mauritanica*. Namely, while cloacal temperature was the most differentiated measurement for both methods, the rest of measurements showed more heterogeneity in the lacertids (Supplementary Figs. S2–S4). In the correlation analysis, all measured variables were similar, with strong correlation scores (Supplementary Tables S3–S5). However, eye and especially snout showed consistently lower scores than the rest. The difference is only slight and the correlation scores are high nonetheless (>0.87), hence the biological significance of this difference is arguable especially in light of the OLS results reported by Barroso et al. (2016).

4. Discussion

An increasing number of studies now acknowledge the need to validate the use of infrared thermography to measure body temperature of reptiles (Barroso et al., 2016; Luna et al., 2013; Sannolo et al., 2014). Nonetheless, most are focused on phylogenetically closely related lizards (e.g. belonging to the Family Lacertidae), with similar body shape and anatomy, physiology and ecology. Hence, devising a generalized validation of this tool for all lizards, from a small subset of species, is unreliable at best. Thus, the need has arisen to test thermography over a broader range of squamate groups moving towards a unified standard methodology for the use of thermography to measure body temperature of reptiles.

The present study found very strong correlations (OLS regression $R^2 > 0.87$, Spearman Correlation > 0.95) between the cloacal temperature and the thermography-measured temperatures of several body locations in *T. mauritanica*, as also described for lacertids in Barroso et al. (2016). In fact, this study found stronger correlations than those obtained for the lacertids, data of which were also analysed further in this study (Supplementary Tables S2–S5) (OLS regression $R^2 > 0.83$, Spearman Correlation Scores > 0.91).

Furthermore, when the R^2 of the OLS models are compared between

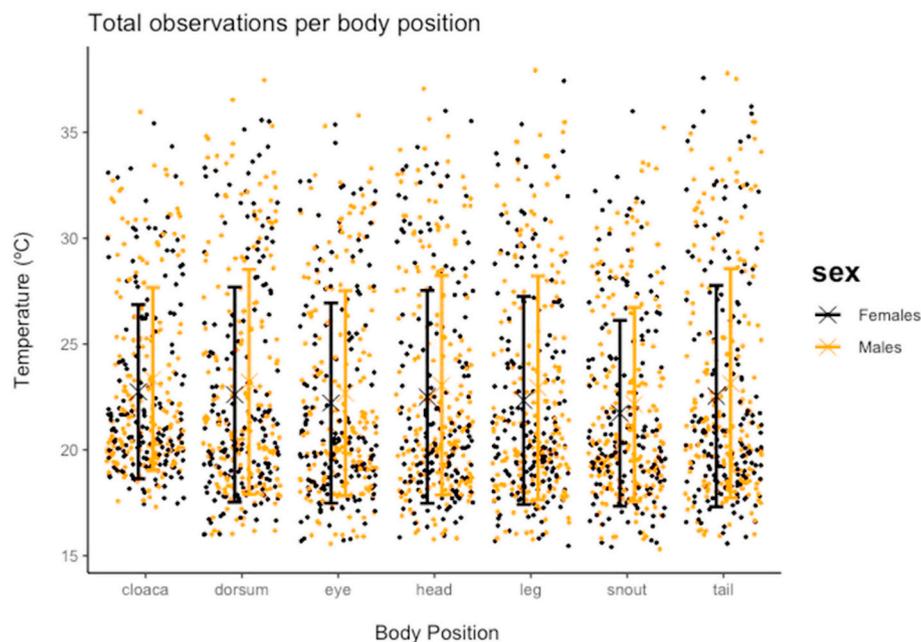


Fig. 2. Graph showing the scatter of the temperatures obtained for each body position for both males and females. The mean and standard deviation of the temperature of each body part are also shown further emphasizing the reported similarity between the sexes as well as between the different body parts (i.e. low regional heterothermy).

Table 1

Results of the OLS regressions between cloacal and the other body parts of *Tarentola mauritanica*. **Bold** values indicate the body position that provided the best fit (highest R^2 ; slope closer to 1; intercept closer to 0). Underlined values indicate the narrowest 95% confidence intervals. A column with the mean \pm SD temperature was also included to emphasize the similarity of average temperatures between sexes as well as between body parts. For reference and ease of comparison, the mean \pm SD of cloacal temperatures of male and female geckos were 22.9 ± 5.01 °C and 22.4 ± 4.80 °C respectively.

| Fig. 3 Graph | Body Part | R^2 | Slope | Slope 95% CI | Intercept | Inter. 95% CI | Temperature Mean \pm SD (°C) |
|--------------|-----------|--------------|--------------|------------------------------|---------------|--------------------------------|--|
| A | Eye | 0.924 | 1.087 | 1.054 1.120 | -2.689 | -3.462 -1.917 | M: 22.71 ± 4.83 F: 22.22 ± 4.73 |
| B | Snout | 0.871 | 0.879 | 0.844 0.915 | 3.817 | 3.022 4.611 | M: 22.16 ± 4.58 F: 21.77 ± 4.4 |
| C | Head | 0.948 | 1.176 | 1.147 1.205 | -4.351 | -5.026 -3.677 | M: 23.11 ± 5.17 F: 22.6 ± 5.05 |
| D | Dorsum | 0.964 | 1.208 | 1.184 1.232 | -4.944 | -5.515 -4.374 | M: 23.27 ± 5.29 F: 22.72 ± 5.12 |
| E | Leg | 0.970 | 1.242 | <u>1.219</u> <u>1.265</u> | -5.827 | <u>-6.361</u> <u>-5.293</u> | M: 22.99 ± 5.26 F: 22.45 ± 4.95 |
| F | Tail | 0.940 | 1.172 | 1.141 1.203 | -4.384 | -5.111 -3.658 | M: 23.17 ± 5.42 F: 22.65 ± 5.25 |

those of *T. mauritanica* to the more closely sized lacertid, *Podarcis virescens*, in Barroso et al. (2016), the increased strength of correlations found here becomes even more obvious. The similar size and mass of these two species should mean a similar degree of thermal inertia as well as a similar distance between the different body parts tested. This result is, nonetheless, contrary to what could be expected based on the behaviour and ecophysiology of these species. *Tarentola mauritanica*'s apparently more flexible approach at thermoregulation, perhaps due to its diurnal, and crepuscular habits, would indicate a lesser concern towards very precise control of body temperatures. This, in theory, results in less evident regional heterothermy leading to less consistent and more unpredictable body temperature gradients (Webb et al., 1972). The latter should have produced weaker correlations between temperatures of different body parts of *T. mauritanica*, contrary to what is found here.

For the previously outlined reasons, regional heterothermy in *T. mauritanica* should be less marked than what was observed in *P. virescens*. This pattern was clear when observing a specimen through a thermal camera (Fig. 1). This is further supported by the strong correlations found between the temperatures of all body parts (Supplementary Table S2).

Perhaps *T. mauritanica* does in fact thermoregulate precisely but dedicates less resources to maintaining body temperature gradients, aided by its low thermal inertia. A different experimental set up would be necessary to test this. Alternatively, the morphological and physiological mechanisms responsible for maintaining body temperature gradients, found in many other lizard groups, may simply be absent or less prominent in geckos (Webb et al., 1972). Many of these mechanisms, such as heart rate hysteresis and peripheral circulation controls, are widely studied among lacertids, agamids and iguanids (Bruner, 1907; Heath, 1966; Porter and Witmer, 2015) yet there is a lack of literature regarding these conditions in geckos (Bauer, A., pers. com.) (but see Grimmond and Evetts, 1981; and Webb et al., 1972).

Most of such phenomena rely, to some extent, in adaptations of the circulatory system. The work of Mahendra (1942) on the anatomy of the gecko *Hemidactylus flaviviridis* (Family Gekkonidae) hypothesizes about geckos having a more basal form of circulatory system among squamates (but see Porter and Witmer, 2020). If also applicable to *Tarentola*, this could explain the apparent lack of precise control over surface body temperature gradients observed. However, the otherwise extensive study lacks a description of the cephalic and peripheral circulation, such as what is available for other groups as lacertids (Bruner, 1907) and iguanids (Porter and Witmer, 2015). Blood circulation would be a major factor influencing the control of regional heterothermy (Heath, 1966; Webb et al., 1972), along with thermal inertia. Furthermore, other studies have also shown the presence of complex vascularization patterns in the feet of geckos (Russel, 1981) which may prove relevant for the control of thigmothermic heat transfer.

Despite the strong correlations observed between temperatures of all

body parts, the MDS plots and the correlation matrices of the *T. mauritanica* clusters the surface temperatures together and the cloacal temperature appears as an outgroup (Supplementary Fig. S1). The same is partially true for the different lacertid species, with the exception of *Timon lepidus*, the largest species tested (Supplementary Figs. S2–S4). Furthermore, for *T. mauritanica* the observed arrangement of relationships follows a geographic pattern with the physically closer points having more similar temperatures amongst themselves than to more distant body parts. This non-arbitrary pattern of temperature gradients does support the presence of some regional temperature control, albeit the very small absolute differences observed in *T. mauritanica* (Fig. 2; Table 1) would suggest this control may not be very precise.

At a broader scale, this study found that the first and last trials of each experimental set significantly lower body temperatures than the others. However, caution must be taken to allow for sufficient time for the animal to acclimate to the experimental set up as this may have been the cause for the lower temperatures observed in trial 1. Equally, attention must also be paid not to overexpose the animals to very long periods in the gradients as they may become stressed (e.g. over handling, dehydration, too much energy spent thermoregulating) and decrease their thermoregulatory precision or set point, which could explain the lower temperatures observed in trial 6.

In the case of *T. mauritanica*, the relatively large size of its eyes, compared to that of other lizards, should have made this structure a relatively easy one to target in IR photos. However, this was not always the case. Likely due to the presence of a scale covering the eye - the brille - a structure common to most geckos (Bellairs, 2009), eyes were often isothermal with the surrounding area and thus difficult to distinguish. Furthermore, we were unable to find any literature on the detailed vascularization of gecko's cephalic region, thus we are not certain how well the eye is vascularised and whether there is a large storage of blood (i.e. sinus orbitalis), and thus of temperature, coming from the brain, as is common in many other squamate groups (Bruner, 1907; Porter and Witmer, 2015). Further research should dedicate some effort towards describing the pattern of cephalic circulation of geckos and its importance to thermoregulation as well as to investigate whether the presence of the brille or the fact that geckos frequently lick their eyes (Bustard, 1963) would significantly affect heat exchange with the environment (e.g. through evaporative cooling).

Ultimately, the results obtained show that body surface temperatures of *T. mauritanica* measured through thermography can reliably be used to infer internal body temperature. The minute differences in temperatures between all body parts (Fig. 2) and the high R^2 values obtained by all the OLS regressions (Table 1), suggest that any body part could be used to infer internal body temperature with equivalent results, given the appropriate correction is applied. Nonetheless when also considering the intercept and degree of slope of the models (Table 1, Fig. 3), to account for the presence of biases in the subsequent inferences, as well

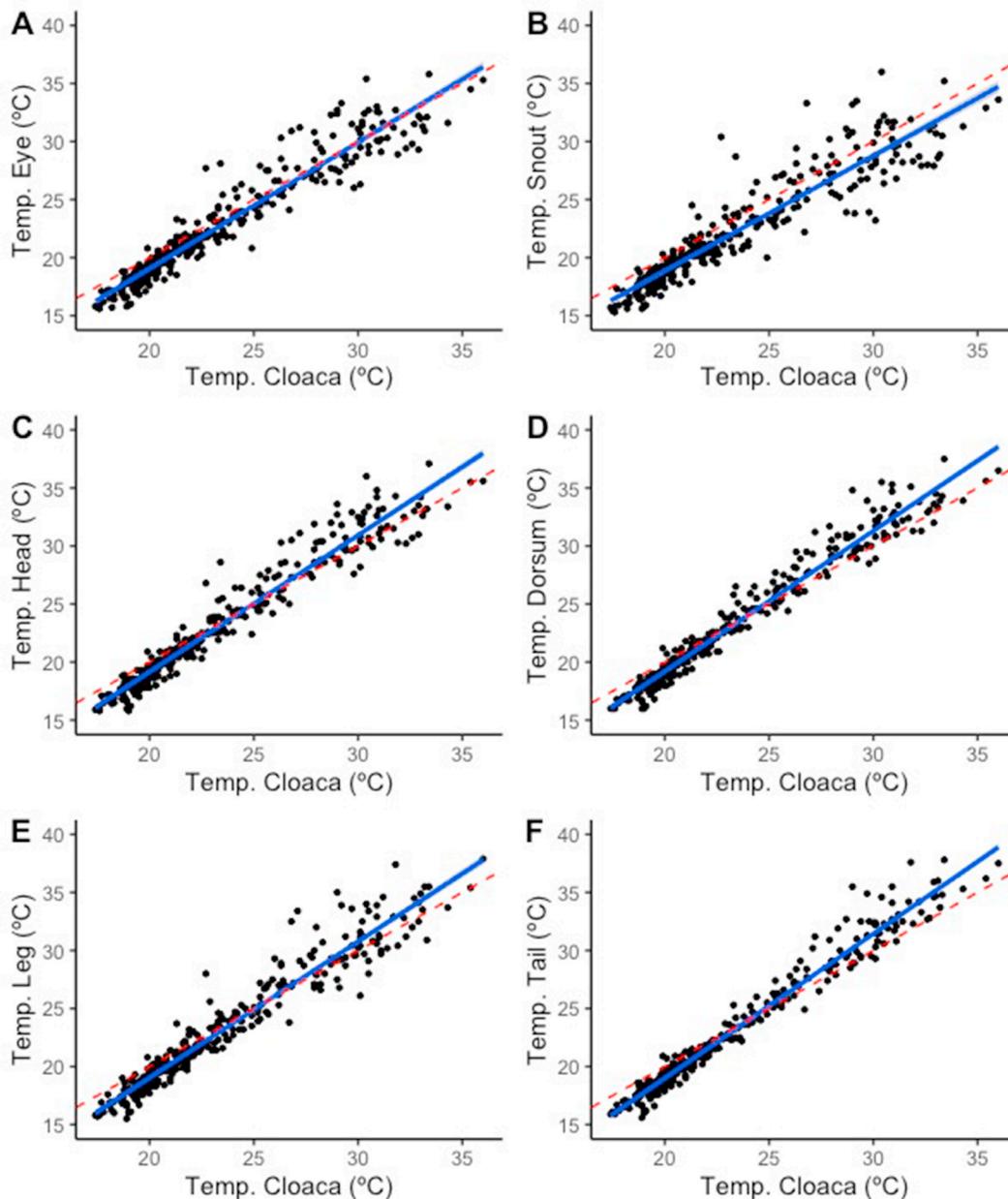


Fig. 3. Graphical representation of the OLS regression lines (blue) and corresponding 95% confidence intervals (grey shading - very close to the blue lines given the high fits of the models) between the thermography-measured body temperatures (y-axes) and the thermometry-measured cloacal temperature (x-axes). The dotted red lines indicate the isothermal line ($y = x + 0$).

as the results obtained by Barroso et al. (2016), then the eye presents as the most ubiquitously suitable candidate body location to infer internal temperature.

5. Conclusion

From this study we further emphasize the validity of IR thermography as a tool to obtain high-resolution temperature data which, given the appropriate calibrations, may still provide comparable results to those obtained by traditional, more invasive methods. Hence, we suggest this tool will prove increasingly useful in the field thermal ecology of ectotherms.

Furthermore, this study has also shown that, through a simple set-up and randomization of heating treatment, it is relatively simple and fast to obtain a sample of temperature data, over a representative thermal range, which can then be used to calibrate the tool for the species of

interest. Further studies should focus on testing this tool over a wider variety of squamates, particularly in those groups with larger body size ranges (e.g. varanids) and/or with larger distances between the eye and the cloaca (e.g. snakes) in order to fully validate the proposed methodology. Furthermore, future work is needed to determine the importance of evaporative cooling from the chosen body part and its subsequent effect on the temperature readings while some efforts should also be diverted towards establishing appropriate emissivity values for the tissues from which readings are taken (e.g. skin types, osteoderms, eye, etc).

Ultimately, this study provides further support towards establishing the eye as the standard location to infer internal body temperature of lizards from thermography measured temperatures, as previously suggested by Barroso et al. (2016). Nonetheless it is still important to consider all the limitations revised in Barroso et al. (2016) including the pixel size effect (Faye et al., 2016), the distance between the camera and

the subject (Faye et al., 2016), the need for species-specific calibrations, the need to establish appropriate emissivity values, the effect of evaporative cooling (particularly in the eye and snout), etc. when considering the use of IR cameras to infer internal body temperatures of lizards.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of competing interest

There are no conflicts of interests to declare.

Acknowledgements:

We thank Silva-Rocha, I., Marques, V., Gomes, V., González de la Vega, J. P., Fraile, A., Sousa, L. and Ceriáco, L. for their help with collecting the animals in the field. Special thanks to Kaliontzopoulou, A. and Enriquez-Urzelai, U. for their useful advice in the statistical part and to Bauer, A., for his invaluable suggestions of gecko circulation literature. We also thank Instituto da Conservação da Natureza e das Florestas (27497/2019/DRNCN/DGEFF) and Consejería de Agricultura, Ganadería y Pesca y Desarrollo Sostenible de la Junta de Andalucía (SGIB/AF) for sampling permits. Animals were kept in captivity under the protocol approved by the Committee of Animal Experimentation of the University of Porto (Portugal) under the Directive 2010/63/EU of the European Parliament. This study was carried out under the project PTDC/BIA-REP/27958/2017 funded by Fundação para a Ciência e a Tecnologia (FCT; Portugal). MS is supported by the project PTDC/BIA-CBI/28014/2017; CR by a FCT postdoctoral fellowship (DL57/2016/CP1440/CT0005), GMR by the project PTDC/BIA-REP/27958/2017, and FMB by a PhD grant (SFRH/BD/147535/2019), all from FCT.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2020.102700>.

References

Angilletta Jr., M.J., 2010. Thermal Adaptation. Oxford University Press, Oxford.

Angilletta Jr., M.J., Niewiarowski, P.H., Navas, C.A., 2002. The evolution of thermal physiology in ectotherms. *J. Therm. Biol.* 27, 249–268.

Angilletta Jr., M.J., Steury, T.D., Sears, M.W., 2004. Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. *Integr. Comp. Biol.* 44, 498–509.

Arad, Z., Schwarzbach, A., Werner, Y.L., 1997. Temperature selection in the Moorish gecko, *Tarentola mauritanica*. *Amphib. Reptile Conserv.* 69, 269–282.

Barroso, F.M., Carretero, M.A., Silva, F., Sannolo, M., 2016. Assessing the reliability of thermography to infer internal body temperatures of lizards. *J. Therm. Biol.* 62, 90–96.

Bell, C.J., 1980. The scaling of thermal inertia of lizards. *J. Exp. Biol.* 86, 79–85.

Bellairs, A.D., 2009. The eyelids and spectacle in geckos. *Proc. Zool. Soc. Lond.* 118, 420–425.

Berg, W., Theisinger, O., Dusmann, K.H., 2015. Evaluation of skin temperature measurements as suitable surrogates of body temperature in lizards under field conditions. *Herpetol. Rev.* 46, 157–161.

Bosch, H.A.J., 1983. Snout temperatures of reptiles, with special reference to the changes during feeding behaviour in *Python molurus bivittatus* (Serpentes, Boidae): a study using infrared radiation. *Amphib. Reptile Conserv.* 4, 49–61.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a metabolic theory of ecology. *Ecology* 85, 1771–1789.

Bruner, H.L., 1907. On the cephalic veins and sinuses of reptiles, with description of a mechanism for raising the venous blood-pressure in the head. *Am. J. Anat.* 7, 1–117.

Burns, T.J., McCafferty, D.J., Kennedy, M.W., 2015. Core and body surface temperatures of nesting leatherback turtles (*Dermodochelys coriacea*). *J. Therm. Biol.* 51, 15–22.

Bustard, H.R., 1963. Gecko behavioral trait: tongue wiping spectacle. *Herpetologica* 19, 217–218.

Campos, Z., Magnusson, W.E., 2013. Thermal relations of dwarf caiman, *Paleosuchus palpebrosus*, in a hillside stream: evidence for an unusual thermal niche among crocodylians. *J. Therm. Biol.* 38, 20–23.

Carretero, M.A., 2012. Measuring body temperatures in small lacertids: infrared vs. contact thermometers. *Basic Appl. Herpetol.* 26, 99–105.

Chukwuka, C., et al., 2019. Accuracy of an inexpensive, compact infrared thermometer for measuring skin surface temperature of small lizards. *J. Therm. Biol.* 84, 295–301.

Faye, E., Dangles, O., Pincebourde, S., 2016. Distance makes the difference in thermography for ecological studies. *J. Therm. Biol.* 56, 1–9.

Gil, M.J., Guerrero, F., Pérez-Mellado, V., 1994. Diel variation in preferred body temperature of the Moorish gecko *Tarentola mauritanica* during summer. *Herpetol. J.* 4, 56–59.

Goller, M., Goller, F., French, S.S., 2014. Heterogeneous thermal environment enables remarkable behavioral thermoregulation in *Uta stansburiana*. *Evol. Ecol.* 4, 3319–3329.

Grimmond, N.M., Evetts, P.M., 1981. The effects of temperature on heat exchange and oxygen consumption in two sympatric New Zealand lizards. In: *Contributions to Thermal Physiology. Satellite Symposium of the 28th International Congress of Physiological Sciences*, Pecs, Hungary, pp. 257–259. <https://doi.org/10.1016/B978-0-08-027354-9.50056-9>, 1980.

Hare, J.R., Whitworth, E., Cree, A., 2007. Correct orientation of a hand-held infrared thermometer is important for accurate measurements of body temperatures in small lizards and tuatara. *Herpetol. Rev.* 38, 311–315.

Heath, J.E., 1964. Head-body temperature differences in horned lizards. *Physiol. Zool.* 37, 273–279.

Heath, J.E., 1966. Venous shunts in the cephalic sinuses of horned lizards. *Physiol. Zool.* 37, 273–279.

Huey, R.B., Slatkin, M., 1976. Cost and benefits of lizard thermoregulation. *Q. Rev. Biol.* 51, 363–384.

Huey, R.B., Stevenson, R.D., 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *Am. Zool.* 19, 357–366.

Jones, S.M., Avery, R.A., 1989. The use of pyroelectric vidicon infra-red camera to monitor the body temperature of small terrestrial vertebrates. *Funct. Ecol.* 3, 373–377.

Kastberger, G., Stachl, R., 2003. Infrared imaging technology and biological applications. *Behav. Res. Methods* 35, 429–439.

King, D., Green, B., 1999. *Goannas: the Biology of Varanid Lizards*. UNSW Press, Sydney, Australia.

Legendre, P., 2018. lmodel2: Model II Regression. R Package Version 1.7-3. <https://CRAN.R-project.org/package=lmodel2>.

Lenth, R.V., 2016. Least-squares means: the R package lsmeans. *J. Stat. Software* 69, 1–33. <https://doi.org/10.18637/jss.v069.i01>.

Luna, S., Lanuza, G.P., Font, E., 2013. Use of an infrared thermographic camera to measure field body temperatures of small lacertid lizards. *Herpetol. Rev.* 44, 59–62.

Mahendra, B.C., 1942. Contributions to the bionomics, anatomy, reproduction and development of the Indian house-gecko, *Hemidactylus flaviviridis* Rüppel Part III. The heart and the venous system. *Proc. Indian Acad. Sci.* 15B5, 231–252.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szocs, E., Wagner, H., 2019. *Vegan: Community Ecology Package*. R Package Version 2, pp. 5–6.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2019. *nlme: Linear and Nonlinear Mixed Effects Models*. R Package Version 3.1-140. <https://CRAN.R-project.org/package=nlme>.

Porter, W.R., Witmer, L.M., 2015. Vascular patterns in iguanas and other squamates: blood vessels and sites of thermal exchange. *PLoS One* 10, 1–27.

Porter, W.R., Witmer, L.M., 2020. Vascular patterns in the heads of dinosaurs: evidence for blood vessels, sites of thermal exchange, and their role in physiological thermoregulatory strategies. *Anat. Rec.* 303, 1075–1103.

Pyron, R.A., Burbrink, F.T., Wiens, J.J., 2013. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evol. Biol.* 13, 1–53.

Rato, C., Carretero, M.A., 2015. Ecophysiology tracks phylogeny and meets ecological models in an Iberian gecko. *Physiol. Biochem. Zool.* 88, 564–575.

Rato, C., Carranza, S., Harris, D.J., 2012. Evolutionary history of the genus *Tarentola* (Gekkota: Phyllodactylidae) from the Mediterranean Basin, estimated using multilocus sequence data. *BMC Evol. Biol.* 12, 14.

Rato, C., Harris, D.J., Carranza, S., Machado, L., Perera, A., 2016. The taxonomy of the *Tarentola mauritanica* species complex (Gekkota: Phyllodactylidae): Bayesian species delimitation supports six candidate species. *Mol. Phylogenet. Evol.* 94, 271–278.

Roark, A.W., Dorcas, M.E., 2000. Regional body temperature variation in corn snakes measured using temperature-sensitive passive integrated transponders. *J. Herpetol.* 34, 481–485.

Russel, A.P., 1981. Arteries of the antebrachium and manus of the tokay (*Gekko gekko*) (Reptilia: Gekkonidae). *Can. J. Zool.* 59, 573–582.

Sannolo, M., Carretero, M.A., 2019. Dehydration constraints thermoregulation and space use in lizards. *PLoS One* 14 (7), e0220384.

Sannolo, M., Mangiacotti, M., Sacchi, R., Scali, S., 2014. Keeping a cool mind: head-body temperature differences in the common wall lizard. *J. Zool.* 293, 71–79.

Savage, V.M., Gillooly, J.F., Brown, J.H., West, G.B., Charnov, E.L., 2004. Effects of body size and temperature on population growth. *Am. Nat.* 163, E429–E441.

Seebacher, F., Franklin, C.E., 2005. Physiological mechanisms of thermoregulation in reptiles: a review. *J. Comp. Physiol. B* 175, 533–541.

Simbula, G., Luiselli, L., Vignoli, L., 2019. Lizards and the city: a community study of Lacertidae and Gekkonidae from an archaeological park in Rome. *Zool. Anz.* 283, 20–26.

Tattersall, G.J., Cadena, V., 2010. Insights into animal temperature adaptations revealed through thermal imaging. *Imag. Sci. J.* 58, 261–268.

Tattersall, G.J., Cadena, V., Skinner, M.C., 2006. Respiratory cooling and thermoregulatory coupling in reptiles. *Respir. Physiol. Neurobiol.* 154, 302–318.

Tattersall, G.J., Leite, C.A.C., Sanders, C.E., Cadena, V., Andrade, D.V., Abe, A.S., Milsom, W.K., 2016. Seasonal reproductive endothermy in tegu lizards. *Sci. Adv.* 2016 (2), 1–7.

Virens, J., Cree, A., 2019. Pregnancy reduces critical thermal maximum, but not voluntary thermal maximum, in a viviparous skink. *J. Comp. Physiol. B.* <https://doi.org/10.1007/s00360-019-01230-y>.

Webb, G.J.W., Johnson, C.R., Firth, B.T., 1972. Head-body temperature differences in lizards. *Physiol. Zool.* 45 (2), 130–142.