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Interaction of morphology, thermal physiology and burrowing performance during the evolution of fossoriality in Gymnophthalmini lizards

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Summary

1. The evolution of fossoriality in Squamata often leads to a phenotypic syndrome involving snake-like body form, relatively low preferred temperature (T_P) and low critical maximum temperatures (CT_{max}). However, how traits interacted among them and with the environment during the evolution of such syndrome remains poorly understood.

2. We studied this process in ten species of gymnophthalmid lizards from the Brazilian Caatinga representing one full transition from typical lacertoid species to fossorial snake-like (FSL) ones. We tested whether different morphotypes exhibited different burrowing performances in response to heat, and also different T_P and CT_{max}. Then, we estimated how changes in burrowing performance would relate to thermoregulation costs in terms of the number of daily hours for which preferred temperatures are available and of risk of overheating.

3. Fossorial snake-like species burrowed deeper, exhibited lower T_P but kept very high CT_{max}, comparable to lacertoid species. A better burrowing performance and lower T_P allowed increasing the daily amount of time during which T_P was accessible within the soil of the study region. In addition, temperatures above CT_{max} of the studied species were present down to 5 cm deep, suggesting that just burrowing does not protect against exposure to extreme temperatures in species that are surface-active during the day (all lacertoids and some FSL). Nonetheless, FSL species active at cool hours of the day exhibited lower CT_{max} than diurnal and syntopic, lacertoid and FSL species.

4. Based on our data and previous literature, we propose a sequential explanation for the acquisition of the fossorial syndrome in Squamata.

Key-words: burrowing performance, CT_{max}, preferred temperatures, fossoriality

Introduction

The evolution of fossoriality is an ecological shift of remarkable importance for the evolution of Squamates. More than 16 independent lineages within this group have evolved a fossorial life at different continents (Wiens, Brandley & Reeder 2006), leading to convergent modifications of morphology, behaviour and physiology. This interesting phenotypic syndrome is morphologically characterized by tiny or absent limbs, elongated bodies and relatively short tails (thus similar to snakes, e.g. Greer 1991; Wiens, Brandley & Reeder 2006; Bergmann & Irschick 2011). Fossorial snake-like (FSL) lizards typically use undulatory locomotion for burrowing through the loose soil (e.g. Gans 1975; Benesch & Withers 2002) and are often crepuscular/nocturnal (Meiri *et al.* 2013). In addition, FSL lizards frequently show lower preferred temperature (T_P) and critical maximum temperatures (CT_{max}) (e.g. Greer 1980; Andrews 1994; Clusella-Trullas,

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Blackburn & Chown 2011; Meiri *et al*. 2013; but see Withers 1981 for a different view). Despite the fact that evolution of snake-like morphotypes has attracted much scientific attention, the co-evolution of morphology, behaviour and thermal physiology during the acquisition of this 'fossorial syndrome' remains poorly understood.

Cost–benefit theory on behavioural thermoregulation predicts that low preferred temperature (T_P) may appear if costs for maintaining high body temperatures are too high (Huey & Slatkin 1976). Accordingly, the requirement of low temperatures of fossorial Squamates has been frequently interpreted under the assumption that FSL species inhabit relatively cool microenvironments in which it is costly to reach high body temperatures (Bury & Balgooyen 1976; Avery 1982; Andrews 1994; Lopez, Civantos & Martín 2002). However, this assumption is not guaranteed in the case of the evolution of FSL due to many reasons: first, lacertoid species may sand-swim too (Maladen *et al*. 2009), and it is not clear how morphology affects burrowing performance and the ability of using subterranean thermal gradients. Secondly, a better burrowing performance of FSL does not necessarily preclude them from foraging at superficial layers (e.g. Andrews 1994; Rocha & Rodrigues 2005) and use thermal gradients of the subsoil (Geiger 1950) to keep ancestral body temperatures (Porter *et al*. 1973; Huey 1982, Huey *et al*. 1989). Thirdly, several lineages of FSL lizards inhabit very hot environments/regions (e.g. Rodrigues 1996; Wiens, Brandley & Reeder 2006; Grizante, Brandt & Kohlsdorf 2012), and a few have shown comparable T_P and CT_{max} to lacertoid species (Withers 1981) and diurnal activity (Andrews 1994; Rocha & Rodrigues 2005). Fourthly, both the use of deep soil layers, and being nocturnal might favour low preferred T_P and reduce the importance of high CT_{max}. As we can see, understanding the evolution of this 'fossorial syndrome' requires relating morphology, behaviour and thermal physiology with associated thermoregulation costs during a full transition from lacertoid to FSL forms.

We studied the evolution of this syndrome in Gymnopthalmidae lizards (Fig. 1), where FSL morphotypes have evolved in arid environments of Brazil (Grizante, Brandt & Kohlsdorf 2012). Within that family, the Gymnopthalmini tribe comprises two relatively recently diverged sister clades (Wiens, Brandley & Reeder 2006). One is composed

of lacertoid species and another of FSL species (Pellegrino *et al*. 2001; Pellegrino, Rodrigues & Sites 2011). Most species are endemic of sandy regions within the Brazilian Caatinga, where both morphotypes co-occur under the same small bushes (Rodrigues 1996). This syntopy raises two critical advantages in examining the processes leading to the 'fossorial syndrome': (i) species can be compared in a geographic common garden, avoiding effects of geography (Grigg & Buckley 2013) during comparisons of thermal physiology; (ii) the description of the thermal environment inhabited by the species group is simpler, making it easier to estimate costs of thermoregulation associated with burrowing. A single evolution event may not be adequate for testing the adaptive nature of a trait (e.g. reviews in Garland & Carter 1994; Garland & Losos 1994); thus, we focused on the relationships among traits. We used ten of the thirteen recognized species of this group (five FSL in our sample) to test: (i) whether morphology explains thermal preference and tolerance and (ii) whether burrowing performance is related to morphology. We also estimated how an enhanced burrowing performance (i.e. burrowing deeper) would relate to costs of thermoregulation for a hypothetical ancestor of FSL species. Finally, we discuss how behaviour may have mediated the co-evolution of morphology and thermal physiology during such transition.

Materials and methods

SAMPLING SITES

During field trips, lizards were collected and soil temperatures measured at the site of captures. To find lizards, we raked the leaf litter of six Brazilian Caatinga sites: Saldanha (10°27′51·93″S, 42°35′40·97″W, municipality of Poção, Bahia State, 400 m a.s.l. in November 2009), Alagoado (9°29′19·65″S, 41°22′34·06″W, municipality of Casanova, Bahia State, 400 m a.s.l. in February 2010), Pedra Vermelha (municipality of Santo Inácio, Bahia state, 450 mm a.s.l. in September 2010), Gameleira de Assuruá (11°18′6·78″S, 42°39′28·11″W, municipality of Gentio d'Ouro, Bahia state 750 m a.s.l. in September 2010), Vacaria, (10°40′38·22″ S, 42°37′46·30″W, municipality of Xique-Xique, Bahia state, 400 m a.s.l.) and Catimbau (8°35′29·23″S, 37°14′44·32″W, municipality of Buique, Pernambuco state, 750 m a.s.l.). These sites comprise most of sandy spots in Brazilian Caatingas occupied by Gymnopthalmini FSL species. Lizards were collected in both seasons of the year (dry and wet, temperatures remain very similar throughout the year). We collected 15 individuals for each of the 10 lizard species studied (IBAMA license number: 17086-2).

FIELD OPERATIVE TEMPERATURES

Subsoil temperatures were measured at four sites during the field trips: Alagoado (6 days), Gameleira de Assuruá (12 days), Vacaria (2 days) and Catimbau (10 days). These sites comprise the latitudinal and altitudinal ranges known for the distribution of FSL species within Gymnopthalmini. Both latitudinal and altitudinal extremes were sampled in both seasons. Temperatures were registered by AA Logbox, Supernovus® dataloggers (precision 1 °C), with a silvered cylindrical aluminium probe (3 mm diameter by 3 cm long, *c*. 0·5 g). We sampled environmental temperatures at

Fig. 1. Right. Morphotypes studied represented by the species *Scriptosaura catimbau* (fossorial snake-like) and *Microblepharus maximiliani* (Lacertoid). Left. Typical vegetation. Photographs taken at 'Catimbau' sampling site by Agustín Camacho.

four different depths under bushes occupied by the studied lizards (surface and under leaf litter, depths of 5 and 10 cm). Temporal sampling resolution was 1/10 min. The emplacement of dataloggers was changed daily to increase temporal independence of temperature measures within each site. We calibrated the temperature dataloggers against living specimens of the different species studied (Bakken, 1992, see Appendix S1, Supporting information). Thus, the temperatures used herein can be considered operative temperatures for those species.

MAINTENANCE

Animals were kept in $15 \times 60 \times 40$ cm plastic terraria and separated by species. Groups were maintained as they were found together in nature under single bushes during fieldwork. All terraria were exposed to UV (L12:D12) and heating (L10:D14) light. The latter generated spatial thermal gradients that ranged from above to below the preferred temperatures of all lizard species examined. Night-time temperatures reached typical nocturnal temperatures at sampling sites (20–22 °C). All lizards were fed termites, avoiding known effects of diet type on T_P (Simandle *et al.* 2001). Water was directly sprayed into the terraria three times per week. Following recommendations by Garland and Carter (1994), the lizards were maintained for at least a week in terraria before physiological measurements were initiated. For each species, all experimental procedures were performed on different, partially overlapping subsamples of these 15 individuals, always including animals of both sexes and juveniles. All procedures were approved by the Ethics Committee of the Instituto de Biociências, Universidade de São Paulo. The number of individuals studied per species, their corresponding sampling site and measured traits are presented in Table S1 (Supporting information).

MORPHOLOGY

The studied species can be easily classified as either lacertoid or FSL according to their morphological descriptions (e.g. Rodrigues 1984, 1991a,b,c). FSL present much more elongated bodies, shorter tails and no more than six digits among all limbs when all are present (*Nothobachia ablephara*). All lacertoid species are four limbed with nine digits among all limbs.

THERMAL PHYSIOLOGY

We determined the preferred temperature (T_P) and thermal limits (CT_{max}) of each species. Each individual's T_P was represented by the median value of the 90% central distribution of body temperatures registered within a laboratory thermal gradient (Hertz, Huey & Stevenson 1993). The T_P and CT_{max} of each species were represented using the median of individuals' T_{Ps}, being a more robust descriptor of non-normal distributions. An automatic register (Field logger, NOVUS®) measured body temperatures of lizards confined during a single period of 11 h, within individual thermal gradients (60–50 to 12–15 °C). Lights were turned on from 7:30 to 18:30, and temperatures were measured after the gradient stabilized, from 8:00 to 19:30. Thermal gradients consisted of plastic boxes ($90 \times 15 \times 16$ cm) with an aluminium base. A 1-cm-deep substrate of plastic beads (1 mm diameter) allowed for easy locomotion for all species and prevented the loosening of adhesive tape used to attach thermocouples on lizards' bodies. A 60 W spot lamp provided heat under the hot end of the gradient. Under the other end was an ice bag, which was changed every 2·5 h, stabilizing the temperature of the cold end. A transparent acrylic lid sealed the top of the gradient and had a fine split through which the thermocouple passed to the register. The boxes remained inside a quiet room with air temperature between 25 and 27 °C

and illuminated from above with diffuse light from 7:30 AM to 6:30 PM. Lizards with thermocouples inserted in their cloaca altered their behaviour (they remained motionless or extremely agitated, often entangling with the thermocouples). Therefore, we attached the T-type thermocouples (<0·4 mm diameter, Physitemp Instruments, Inc., Clifton, NJ, USA) above the lizards' pelvic girdle using a double layer of surgical tape. Based on previous studies, differences in the core-surface temperatures of lizards with a body wall <3 cm thick (Porter *et al.* 1973), and thermal inertia for animals lighter than 10 g (Stevenson 1985), were considered depreciable. The study species never exceeded body diameter of half a centimetre and weight of <1·2 g. Thermocouple's temperatures were calibrated against a standardized Miller-Weber® fast-reading mercury thermometer (see Appendix S1).

CT_{max} was the last trait measured for any lizard. Based on classical definitions (Lutterschmidt & Hutchison 1997), CT_{max} was considered the median of the experimental temperatures that established the onset of spasms for each lizard. The onset of spasms was identified by a sudden body arching and/or rolling over the back. The lizards were heated individually at a rate of *c.* 1 °C min^{-1} inside narrow, cylindrical, aluminium chambers (600 mL, 6 cm diameter) that were submerged into a temperature-controlled water bath. We observed that this procedure allowed equilibrium between core and surface body temperatures, the latter being monitored with a fast-reading mercury thermometer (Miller-Weber®), kept in contact with the lizards during the whole experiment. Start temperature was always 28 °C. Lizard movement and temperature inside of the chamber were continuously observed through transparent plastic lids that sealed the chamber from above until the onset of spasms. Lizards were then liberated from the chamber and laid on their backs on a cool wet surface. When they started moving, they were released in their terraria. Lizards that recovered movement immediately, died or showed clear symptoms of bad health 24 h after the CT_{max} measurement were not analysed (six individuals in total). To control potential effects of daily cycles or body mass on CT_{max} (e.g. Ribeiro, Camacho & Navas 2012), both time of measurement and weight were interspersed during comparisons among morphotypes.

MEASUREMENT OF BURROWING PERFORMANCE

We estimated the species' burrowing performance as the median of the depths attained by individuals of that species in response to an experimental warming of sand. These lizards naturally burrow within the sand to hide or retreat (A. Camacho, pers. obs). Therefore, we simply allowed them to burrow in individual wooden boxes ($5 \times 3 \times 14$ cm) filled with filtered, washed and dried sand from the habitats of lizards. The boxes with burrowed lizards were then X-rayed, and the sand surface was subsequently warmed using 300 W lamps illuminating from above. The sand was heated until it reached 42 °C at 2 cm deep and over 60 °C at the surface (both well over the preferred thermal ranges of the Gymnopthalmini lizards studied). Temperature was monitored with the same Miller-Weber used before, inserted into the sand. Another scaled digital X-ray photograph was then taken, and depth was calculated from the obtained photograph using the free source program TRACKER V.3.2 (Cabrillo edu®, Aptos, CA, USA, https://www.cabrillo.edu/˜dbrown/tracker/). Lizards that came out of the sand during warming were not used in calculating the mean species depth.

MODELLING SURFACE AND SUBSOIL OPERATIVE TEMPERATURES FACED BY GYMNOPTHALMINI LIZARDS

Operative temperatures were interpolated between the different depths at which temperature was measured using the Gridfit

function (D'Errico 2006). This function is a noise-reducing surface modelling code, which generates smooth estimation based on experimental data. Herein, the 'smooth' power was set equal to 5. This value included most variation observed in field data, generating dynamics seen in the field data. Using this function, we modelled the average daily temperatures and the maximum temperatures registered for depths ranging from the surface to 9 cm deep (1-cm resolution), and also for all hours of the day (10-min resolution). For ease of calculation, the leaf litter layer was considered as having a 1 cm depth during interpolation. With the spatiotemporal model of average temperatures generated (Fig. S4, Supporting information), we calculated the number of daily hours in which a given temperature interval was available, as a function of the potential depth range usable within the soil. Using the maximum temperatures model (Fig. S5, Supporting information), we calculated a vertical profile of maximum temperatures. MATLAB scripts for generating the thermal model and calibration of temperature data are available upon request to RP.

CORRELATION OF MORPHOLOGY AND THERMAL PHYSIOLOGY

To test the relationship between morphology and thermal physiology (T_P, CT_{max}), we used a generalized least squares approach, estimating the importance of phylogenetic relationships by calculating the λ coefficient (Revell 2010). Phylogenetic relationships were directly obtained from the most recent molecular phylogeny obtained for this family (Pellegrino, Rodrigues & Sites 2011). This phylogeny includes branch lengths, calculated by Bayesian procedures over molecular and morphological data.

RELATIONSHIP BETWEEN THERMOREGULATION COSTS AND BURROWING PERFORMANCE

We quantified thermoregulation costs in terms of time and risk. Regarding time, we considered thermoregulation costs as inversely related to the amount of daily time that individuals of a given species would be able to reach operative temperatures within their T_P. The daily amount of hours during which T_P is available seems a reasonable measure of thermoregulatory costs because it explains rates of individual's body growth and population's persistence of lizard populations and is thus a good measure of thermoregulation costs (Sinervo & Adolph, 1994; Sinervo *et al*. 2010). Regarding risk, we considered higher thermoregulation costs for species whose burrowing performances confined them to depths in which operative temperatures may exceed their CT_{max}.

To know whether an increase in burrowing performance may increase costs of thermoregulation for a hypothetical ancestral Gymnopthalmini, we estimated ancestral T_P and CT_{max} using two different methods: maximum likelihood (Schluter *et al*. 1997) and phylogenetic independent contrasts (Felsenstein 1985). We calculated them with the function 'ace' (R package 'APE', Paradis, Claude & Strimmer 2004). Finally, we estimated the daily cumulative time during which operative temperatures within ancestral T_P were reachable and the maximum temperature reached during the sampling period. Both were represented as a function of achievable soil depth and visually inspected. We measured temperatures from the highest to the lowest elevations and latitude in which FSL species can be found, and the study region has remained within the tropics since before the evolution of this FSL lineage (Wiens, Brandley & Reeder 2006). Thus, it is reasonable to assume that the thermal soil profile has kept within the range of variation sampled for this study.

Results

RELATIONSHIP BETWEEN MORPHOLOGY, THERMAL PHYSIOLOGY AND PERFORMANCE

Morphology is related to T_P across the studied species ($\lambda = -0.44$; residual d.f. = 8; coeff = -3.018; SD = 0.422; t-value = -7.151; $P < 0.001$, Fig. 2a) but not to CT_{max} [Phylogenetic Generalized Least Squares (PGLS): $\lambda = 0.83$; residual d.f. = 8; coeff = -0.876; SD = 0.849; t-value = -1.031; $P = 0.332$, Fig. 2b]. Non-phylogenetic Generalized Least Squares (GLS) analyses exhibited similar results: morphology is related to T_P (residual d.f. = 8; coeff = -2.727; SD = 1.120; t-value = -2.435; $P < 0.040$) but not to CT_{max} (residual d.f. = 8; coeff = -1.12; SD = 0.499; t-value = -2.240; $P < 0.055$). *Calyptommatus* had lower CT_{max} than did any other syntopic lacertoid and FSL species (Table S1).
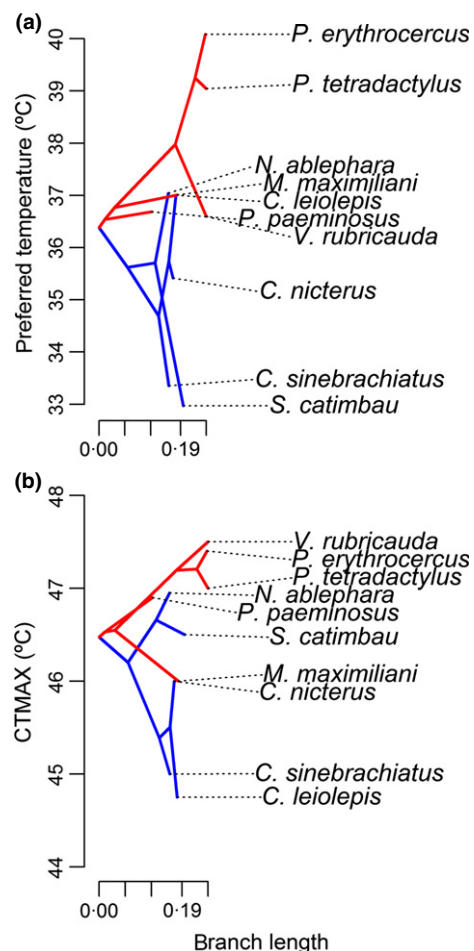

Fig. 2. Evolution of preferred and critical maximum temperatures in Gymnophthalmini lizards from the Brazilian Caatinga. Branch lengths indicate number of mutations per site. Blue (darker) lines represent phylogenetic relationships between fossorial snake-like species, whereas red (lighter) lines do for lacertoid species.

RELATIONSHIP OF MORPHOLOGY AND BURROWING PERFORMANCE

Fossorial snake-like species burrowed deeper than lacertoid species during experimental heating sessions (PGLS: $\lambda = -0.052$, residual d.f. = 8; coeff = 3·563; SD = 0·685; t-value = 5·194559; $P < 0.001$). Again, non-phylogenetic GLS analyses exhibited similar results (GLS: residual d.f. = 8; coeff = 3·538; SD = 0·727; t-value = 4·860; $P = 0.001$). Except for *N. ablephara*, which already burrowed very deep before warming, the median of the depth for all species of lizards was deeper after warming (Table S1). All FSL species and the lacertoid *Psilopthalmus paeminosus* reached below 7 cm in the sand, and the maximum depth reached by the other lacertoid species was *c.* 4 cm. One of the eight individuals observed of *Vanzosaura rubricauda*, four of six *Procellosaurinus tetradactylus* and one of eight *P. paeminosus* emerged out of the sand during warming, instead of burrow deeper to avoid the heat. These individuals were not included in the analysis. On the contrary, none of the FSL species tried to emerge from the sand during warming.

RELATIONSHIP BETWEEN BURROWING PERFORMANCE AND THERMOREGULATION COSTS FOR AN ANCESTOR OF FSL SPECIES

Both methods for estimating ancestral states of T_P gave broadly similar 95% confidence intervals of T_P and CT_{max} (ML: T_P: 34·8–38·6 °C; CT_{max}: 45·8–47·0 °C; IC: T_P: 35·5–37·2 °C; CT_{max}: 45·5–47·2 °C). Thus, we will hereafter refer to the range of 34–38 °C as the ancestral T_P, and 46 °C as ancestral CT_{max}. The ability of burrowing deeper does not decrease the amount of daily time in which ancestral T_P is available, even for high T_P (Fig. 3, lines with triangles, squares and circles on left axis). Both the highest T_P (triangles) and lowest ancestral T_P (squares) lead to less daily time during which T_P is available if burrowing abilities are absent. Strikingly, the amount of daily time in which ancestral T_P is available increases with better burrowing performance only for species with the lowest ancestral T_P. With respect to overheating costs (crosses), maximum operative temperatures ranged from 75 °C at the leaf litter surface to 38 °C up to 10 cm within the soil. Digging below 5 cm eliminates overheating costs for species with ancestral CT_{max}, as operative temperatures never reached 45 °C below that depth.

Discussion

T_P for both FSL and surface-active species are higher than previous global estimates for Squamates with similar ecological class (Clusella-Trullas, Blackburn & Chown 2011). Nonetheless, Gymnophthalmini lizards fit the general trend in which FSL species prefer lower temperatures compared to surface-active lizards. A widely held explanation for the lower preferred temperatures of fossorials lizards is that
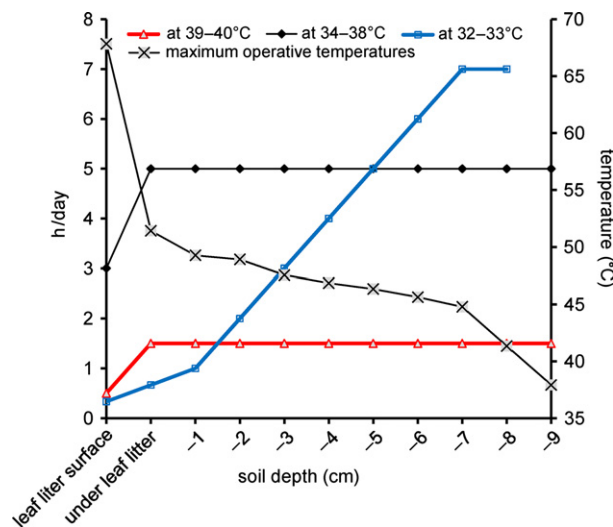

Fig. 3. Relationships between achievable soil depth, preferred temperatures and costs of thermoregulation. The left axis and lines (triangles, balls, squares) represent the number of daily hours of access to operative temperatures within three hypothetical intervals of ancestrally preferred temperatures for Gymnophthalmini lizards from the Brazilian Caatinga. The right axis and the line with 'crosses' symbols indicate the highest operative temperature modelled for each given soil depth.

maintaining lower temperatures is relatively less costly in the subsoil (Bury & Balgooyen 1976; Avery 1982; Andrews 1994; Lopez, Civantos & Martín 2002). Our results help understanding the thermoregulatory costs of evolving fossoriality. A better burrowing performance does not necessarily increase costs of maintenance of ancestral temperatures, even in the range of 39–40 °C, which can be considered high for reptiles (Clusella-Trullas, Blackburn & Chown 2011). It is the co-evolution of a lower T_P and better burrowing performance that decreases the costs for thermoregulation.

In contrast with T_P, we found that FSL species may reach very similar CT_{max} levels compared to relative lacertoid species. Studied species of both morphs showed very high CT_{max} levels (more than eight degrees above previous global estimations Clusella-Trullas, Blackburn & Chown 2011). Still, our CT_{max} levels for FSL species were similar to the ones exhibited by African FSL scincids in a previous study done at similar latitude (Withers 1981). A high CT_{max} would be expected for tiny animals dwelling in close contact with surfaces where operative temperatures reach extreme levels during the day, like these lizard species. Thermal ecology studies of FSL species have found milder operative temperatures, which may support the traditional explanation for the frequent low CT_{max} of FSL Squamates. However, most of those studies were done at higher latitudes (Bury & Balgooyen 1976; Papenfuss 1982), measuring more protected soils (e.g. under rocks, Lopez, Civantos & Martín 2002) at deeper layers (López, Salvador & Martín 1998), or during relatively cool periods of the year (Andrews 1994).

Our data, supported by previous literature, suggest a mechanistic explanation integrating traits and environment

during the evolution of the 'fossorial syndrome' in Squamata (Greer 1980; Andrews 1994; Clusella-Trullas, Blackburn & Chown 2011; Meiri *et al.* 2013). Firstly, in the extremely hot environment of our study area, the layers of leaf litter and loose sand facilitate spending time within preferred temperatures even for poor burrowers. The evolution of morphology can increase species' ability for burrowing (Benesch & Withers 2002; herein), allowing a situation in which the evolution of lower Tp is rewarded by more time in which Tp is available. In addition, operative temperatures within typical T_P variation for our FSL species (*c.* 25–35 °C) extend into the night within the subsoil of the studied region, thus allowing the extension of their activity time at night. Finally, for burrowing species in hot environments like those studied here, shifting to a nocturnal activity while resting during the day in deep sand allows the avoidance of operative temperatures above the ancestral CT_{max}. This might relax selective pressure over high CT_{max} levels. This explanation is tightly supported by our species study group: the diurnal forager FSL species *N. ablephara* (Rocha & Rodrigues 2005) possesses lower T_P than most lacertoid species but still keeps relatively high CT_{max}, especially compared to the syntopic FSL species, *Calyptommatus leiolepis*. Indeed, species from genus *Calyptommatus* are active at dusk/night (Rodrigues 1996; Rocha & Rodrigues 2005). Species of this genus show lower CT_{max} and T_P than diurnal and syntopic species of both, FSL and lacertoid species groups.

The evolution of Gymnopthalmini FSL has taken place during a complex interaction of environmental challenges (e.g. extreme temperatures), and opportunities (e.g. soil looseness and thermal inertia) and key traits (e.g. thermoregulatory burrowing behaviour). This set-up has been possibly favoured by a historical lack of competition in the fossorial environment (Wiens, Brandley & Reeder 2006). Studies on other species groups are necessary to verify whether there is a general sequential process leading to a 'fossorial syndrome' in Squamata.

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Appendix S1. Thermometer's calibration procedure and data of species and thermal environment.

Figure S1. Calibration of thermocouples with mercury thermometer.

Figure S2. Relationship of water temperatures measured by a mercury thermometer and the mean of the temperatures measured by the sensors used for measuring environmental temperatures during this study.

Figure S3. Calibration of datalogger sensors with body temperatures of several species of gymnopthalmids (*Calyptommatus leiolepis, Nothobachia ablephara, Vanzosaura rubricauda, Procellosaurinus tetradactylus, Psilophtalmus paeminosus*) over different substrates (sand and leaf litter).

Figure S4. Spatiotemporal profile of the distribution of mean temperatures measured under bushes inhabited by Gymnophthalmini lizards.

Figure S5. Same profile showing interpolation of maximum temperatures registered at the same study site.

Table S1. Phenotypic traits and burrowing performance of 10 species of Gymnopthalmini lizards.