Shifts in space and time: ecological transitions affect the evolution of resting metabolic rates in microteiid lizards

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ABSTRACT
Ecological diversification often encompasses exposure to new thermal regimes given by the use of specific spatial (microhabitat) and temporal (activity periods) niches. Empirical evidence provides links between temperature and physiology (e.g. rates of oxygen consumption), fostering predictions of evolutionary changes in metabolic rates coupled with ecological shifts. One example of such correspondence is the evolution of fossoriality and nocturnality in vertebrate ectotherms, where changes in metabolic rates coupled with niche transitions are expected. Because most studies address single transitions (fossoriality or nocturnality), metabolic changes associated with concomitant shifts in spatial and temporal components of habitat usage are underestimated, and it remains unclear which transition plays a major role for metabolic evolution. Integrating multiple ecological aspects that affect the evolution of thermosensitive traits is essential for a proper understanding of physiological correlates in niche transitions. Here, we provide the first phylogenetic multidimensional description of effects from ecological niche transitions both in space (origin of fossorial lineages) and in time (origin of nocturnal lineages) on the evolution of microteiid lizard (Gymnophthalmidae) metabolic rates. We found that evolution of resting metabolic rates was affected by both niche transitions, but with opposite trends. Evolution of fossoriality in endemic diurnal microteiids is coupled with a less thermally sensitive metabolism and higher metabolic rates. In contrast, a reduction in metabolic rates was detected in the endemic fossorial–nocturnal lineage, although metabolic thermal sensitivity remained as high as that observed in epigean species, a pattern that likely reduces locomotion costs at lower temperatures and also favors thermoregulation in subsuperficial sand layers.

KEY WORDS: Fossorial, Gymnophthalmidae, Metabolism, Nocturnal, Squamata, Temperature

INTRODUCTION
Shifts in ecological niches likely affect the thermal regimes experienced by organisms (Huey et al., 1989; Meiri et al., 2013) and are of particular relevance for vertebrate ectotherms, as temperature strongly influences the pace of physiological processes and life history traits (Bartholomew and Tucker, 1964; Harlow et al., 2010; Spotila et al., 1973; Tracy et al., 2010). Ecophysiological consequences of such shifts have been often investigated from the perspective of single ecological dimensions; for example, focusing on the physiological correlates of changes in microhabitat use or in activity periods (Autumn et al., 1999; Camacho et al., 2015; Hare et al., 2007, 2015; Hitchcock and Mcbrayer, 2006). Niche transitions involving multiple ecological components are nevertheless not rare. For example, fossoriality evolved in association with nocturnality in different vertebrate groups (Pough et al., 2016), and each transition is associated with specific physical changes in the environment experienced by individuals (Smith and Ballinger, 2001). Consequently, comparisons among species focusing on a single ecological dimension (e.g. either the fossorial or the nocturnal lifestyle) may confound conclusions about physiological correlates of ecological transitions.

Among physiological traits, metabolism is of distinctive relevance in scenarios concerning ecological shifts because of its sensitivity to temperature (Dawson, 1975; Litzgusa and Hopkins, 2003; Piercy et al., 2015) and clear associations with species’ ecology (Hare et al., 2007; Stuginski et al., 2017). Empirical evidence corroborates a clear relationship between metabolism and microhabitat type (Selďák, 2007; Wu et al., 2015), as well as associations between metabolism and the period of activity (see Autumn et al., 1999; Hare et al., 2006, 2007; Putnam and Murphy, 1982 for examples). Nevertheless, most studies explore ecological transitions as a single dimension, focusing either on the spatial or temporal scale of habitat usage, and lack a formal incorporation of the phylogenetic history when investigating the evolution of thermosensitive traits (Autumn et al., 1999; Hare et al., 2006, 2007; Camacho et al., 2015; Putnam and Murphy, 1982; Withers, 1981; Wu et al., 2015). Metabolic shifts during the evolution of fossoriality or nocturnality, for example, have been identified under this single-dimension approach (see Abe and Johansen, 1987; Autumn et al., 1999; Fusari, 1984; Johansen et al., 1980; Kearney and Porter, 2004; Putnam and Murphy, 1982), and it remains unclear whether effects from a given ecological transition might prevail over the other. For example, evolution of lower metabolic rates have been identified in nocturnal geckos (Autumn et al., 1999; Cortés et al., 1994; Putnam and Murphy, 1982), which characterize a temporal transition, as well as in fossorial lizards, which represent the spatial shift of becoming active in relatively colder and hypoxic subterranean environments (Abe and Johansen, 1987; Andrews and Pough, 1985; Fusari, 1984; Johansen et al., 1980). Given that each ecological shift comprises the occupation of a very specific thermal environment (see Autumn et al., 1999; Camacho et al., 2015; Smith and Ballinger, 2001), concomitant changes in microhabitat usage and activity periods likely represent unique evolutionary transitions affecting thermosensitive traits.

Given that both scales define the thermal environment experienced by a given organism, niche transitions involving multiple ecological components demand integrative hypothesis testing for changes in metabolism associated with shifts in space and time. For example, epigean lizards active during the day face higher mean temperatures that are usually more variable than those
experienced by fossorial or nocturnal species (Camacho et al., 2015). In turn, fossorial species active during the day likely experience temperatures that are lower than those experienced by epigean species but higher than the temperatures experienced by fossorial–nocturnal species (Camacho et al., 2015; Pianka and Vitt, 2006). Although facing a narrower temperature range, fossorial species active during the night have a lower risk of overheating during activity (Camacho et al., 2015). Predictions regarding how thermosensitive traits evolve during concomitant ecological shifts are therefore not straightforward, and research programs likely benefit from investigating lineages in which both fossoriality and nocturnality evolved.

Here, we address the evolution of resting metabolic rate (RMR) and its thermal sensitivity in endemic microteiid lizards from the Gymnophthalmini tribe (Squamata: Gymnophthalmidae). This lineage has evolved fossorial–diurnal and fossorial–nocturnal lifestyles from a presumably epigean–diurnal ancestor in the semi-arid Brazilian Caatingas (Rocha and Rodrigues, 2005). We applied a phylogenetic framework to identify the events of ecological shifts during diversification of this lineage, subsequently testing whether and to what extent these ecological transitions in both spatial and temporal dimensions of the habitat are coupled with changes in RMR and its thermal sensitivity. Given the empirical evidence available suggesting that fossoriality and nocturnality affect the species’ thermal niches and favor reduced metabolic rates, our a priori hypothesis postulates that nocturnal–fossorial gymnophthalmids exhibit the lowest metabolic rates and that ecological transitions also involved changes in thermal sensitivity of RMR in microteiids. Using such an approach, we innovate by integrating shifts in space and time during niche transitions in the study of metabolic evolution in microteiids.

**MATERIALS AND METHODS**

**Animals**

Seven endemic species of Gymnophthalmini lizards (Pellegrino et al., 2001) were collected during two field trips (June 2010 and April 2011) at the sand banks of the São Francisco River in the semi-arid Caatingas of the State of Bahia, northeastern Brazil (Fig. S1A; IBAMA/Brazil permits: 23033-2 and 17086-2). Lizards were manually collected by excavation using shovels or by hand, stirring the sand and leaf litter. Collected animals (adults of both sexes) were transported from the field to the Laboratory of Evolution and Integrative Biology (LEBI) at University of São Paulo (USP-Ribeirão Preto, São Paulo, Brazil) in plastic terraria filled with a 10 cm layer of sand substrate from the locality in which they had been collected. Lizards were maintained in an animal facility with light cycle and temperature adjusted using ultraviolet lights connected to a timer (12 h:12 h light:dark). Each terrarium had one end under the ultraviolet light, and the opposite end was humidified with periodic water misting. Such a design established a temperature-controlled gradient in the terrarium (34–38±1°C) that offered temperatures near the preferred temperatures measured in the laboratory (Camacho et al., 2015), allowing lizards to thermoregulate. Individuals were fed with termites three times a week, and water was offered ad libitum.

Ecological habits in Gymnophthalmini are diversified and include both cryptic epigean species active in leaf litter and fossorial snake-like species (Höfling and Renous, 2004; Rodrigues, 1991); our hypotheses predicting ecological associations of RMR were tested using at least two species representing each ecological habit: two epigean–diurnal species (Fanzosaura multiscutata (Amaral 1933) and Psilops paeminosus (Rodrigues 1991)), two fossorial–diurnal species (Nothobachia ablephora Rodrigues 1984 and Scriptosaura catimbau Rodrigues & dos Santos, 2008) and three fossorial–nocturnal species (Calliptommatus leiteolpis Rodrigues 1991, C. sibrebrachius Rodrigues 1991 and C. nicterus Rodrigues 1991) (Table S1). Both males and females were included in the present study because Gymnophthalmini lizards are difficult to locate in large numbers. Moreover, sexual dimorphism in RMR has been described as resulting from differences in body mass (Cullum, 1998) or reproductive state (Angilletta and Sears, 2000), but we did not identify pregnant females among the collected lizards.

Different localities were sampled because most species are endemic and differ in geographic distribution along the sand banks adjacent to the São Francisco River (Table S1, Fig. S1A). Whenever possible, we used individuals from each species that were collected at the same locality, in order to minimize possible geographic or population effects. Nevertheless, lizards were collected in a relatively constrained range, minimizing potential latitudinal effects (8°S to 11°S, Table S1). Despite the cryptic habitats of Gymnophthalmini species, we were able to test our hypotheses using almost one-third of the species described for this lineage. All procedures were approved and conducted in strict accordance with the aforementioned IBAMA permits and the Ethics Committee on Animal Use from University of São Paulo (CEUA/USP protocol number 06.1.1390.53.5).

**Resting metabolic rates**

The metabolic profile of small ectotherms involves measurements of relatively low levels of oxygen consumption, and intermittent-flow respirometry represents the most accurate method with animals as small as the gymnophthalmid lizards weighing less than 1 g (see Kristin and Gvoždík, 2012; Navas and Gomes, 2001). The rate of oxygen consumption during rest was used as an index of RMR (following standard practices; for example, see Watson and Burggren, 2016). Animals were recorded at three experimental temperatures: 24, 30 and 36°C. These experimental temperatures have been chosen based on field operative measurements obtained using copper models and dataloggers (NOVUS® model LogBoxAA) during field trips (see Fig. S1B), which are equivalent to operative temperatures published for Gymnophthalmini (Camacho et al., 2015).

All experiments were conducted 1 week after individuals arrived in the laboratory, in order to provide time for individuals to recover from possible stress effects owing to transportation from the field to the laboratory as well as to avoid possible acclimation effects. Measurements of RMR were obtained using 10 individual sealed acrylic chambers (105 ml) designed to optimize air flow along all concave extremities of the chamber (Fig. S1C). Each individual was weighed to the nearest 0.001 g prior to all experiments for mass-specific RMR estimation. Each chamber was filled with a thin sand layer so that animals could achieve a resting state. Preliminary tests performed with chambers filled with the thin sand layer and no lizard inside confirmed that the presence of substrate does not affect the chamber airflow dynamics or create any oxygen depletion. Experiments started with acclimating individuals for a period of at least 5 h prior to data acquisition in a BOD incubator (model 101M/3 series 080129, Eletrolab, São Paulo, Brazil), in order to assure they reached the experimental temperature, assumed a resting posture and recovered from any stress owing to previous manipulation, following standard protocols (for examples, see Gomes et al., 2004; Kiss et al., 2009). During this period, all metabolic chambers were constantly renewed with fresh outdoor air...
using an aquarium pump, in order to avoid hypoxia. To record RMR, each individual chamber was closed for 100 min, the period standardized from pilot experiments as being adequate to detect a reasonable signal without impairing the animals.

Oxygen levels dropped less than 20% during the 100 min, allowing a reliable measurement of metabolism without exposing individuals to hypoxic conditions. After this period, each chamber was opened individually to conduct the air from the chamber to the oxygen analyzer at a flow rate of 180 ml min\(^{-1}\). Air flow from each chamber was established by connecting a pump (Flowbar Multi Channel, Sable Systems, Henderson, NV, USA) to guide air flow individually from each chamber through a controlled air flow system with multiple channels (Multiplexer V3, Sable Systems) and an oxygen analyzer (FC-10a O\(_2\) Analyzer, Sable Systems). Before entering the oxygen analyzer, the air circulated through a filter containing three layers (drierite–ascarite–drierite) to remove water and carbon dioxide from samples before data acquisition. Oxygen consumption curves for each chamber were recorded and visualized in Expedata software (version 1.1.15, Sable Systems) through an interface (UI2, Sable Systems).

Oxygen consumption rates for each individual were estimated by calculating the integral of the area beneath the curve of oxygen depletion in relation to the baseline normoxia (Kiss et al., 2009). From each depletion curve, we calculated the integral of the area using Expedata software and then used it to estimate the resting metabolic rate of each individual according to the formula:

\[
\dot{V}_{O_2} = \int \frac{F}{(m \cdot t \cdot 100)} \cdot 60, 
\]

where \(\dot{V}_{O_2}\) is oxygen consumption rate per gram of an individual and per hour in standard conditions of temperature and pressure (STP) (ml O\(_2\) g\(^{-1}\) h\(^{-1}\)); \(F\) is the integral of the oxygen depletion curve (%); \(t\) is air flow (180 ml min\(^{-1}\)); \(m\) is the mass of an individual (g); \(t\) is the time for which chambers remained closed (min); 100 in the denominator is the conversion from O\(_2\) percentage to raw quantity (ml); and 60 in the denominator is the conversion from minutes to hours.

In order to standardize resting experimental conditions, all measurements were performed during the resting period of each species: diurnal animals were measured during the late afternoon and/ or night (18:00–22:00 h) and nocturnal species were measured during the late morning and/or early afternoon (10:00–14:00 h). All chambers were video monitored from a top-view perspective. If a lizard moved during the period when chambers were closed, its measurements were discarded and the experiment was repeated on a different day (only minor posture adjustments were tolerated, following Gomes et al., 2004; Kiss et al., 2009). Light conditions were standardized following day cycles using a scheduled photoperiod system inside the BOD incubator. During nocturnal tests, when light was off, we used a red lamp inside the BOD to allow video monitoring. Experiments were performed twice at each temperature, on different days. Within each temperature test, we calculated the mean values for each day for each species; subsequently, the mean values from these two means were designated as the RMR for each species. The order in which experiments were performed (regarding the choices of species and temperatures) was random and interleaved to avoid the days in which animals were fed, ensuring a post-absorptive state and eliminating effects of fasting in the estimation of RMR (Secor, 2009).

Rates of oxygen consumption were corrected by body mass and STP to calculate mass-specific rates, expressed as ml O\(_2\) g\(^{-1}\) h\(^{-1}\), and are hereafter referred to as RMR. After experiments, all individuals were euthanized with lethal doses of lidocaine 2%, fixed in 10% formaldehyde and preserved in 70% ethanol (approved by the Ethics Committee on Animal Use of University of São Paulo CEUA/USP protocol number 06.1.1390.53.5). Specimens were added to the Herpetological Collection of Ribeirão Preto (CHRP/USP).

### Ecological data

Gymnophthalmini species were classified as fossorial/epigean and as diurnal/nocturnal based on the primary microhabitat type and the activity period species are usually found in nature, following the literature and personal observations in the field (Table S1). For microhabitat classification, we used morphological specializations associated with sand swimming in Gymnophthalmini (Rodrigues, 1991) and snake-like characteristics such as elongated trunks and very reduced limbs to classify species as fossorial. Therefore, species that are primarily active below the surface (e.g. buried under sand layers) and exhibit morphological specializations for sand-swimming were classified as fossorial, whereas species that forage mainly on the ground and/or in leaf litter and exhibit a typical lacertoid (lizard-like) body shape were classified as epigean. Regarding activity period, species that forage mainly during daytime hours (morning to afternoon) were classified as diurnal, whereas those that forage at dusk or during nighttime hours were classified as nocturnal. Such classification followed personal observations in the field (M.B.-C., A.G.C. and T.K.) as well as patterns described by Noronha-de-Souza (2014) and Rocha and Rodrigues (2005).

### Statistical analyses

Prior to analyses, RMR was log\(_{10}\) transformed following standard practice (Watson and Burggren, 2016; Xiao et al., 2011), as some measurements of raw data violated the assumptions of a normal distribution and homoscedasticity. Indices corresponding to thermal sensitivity of RMR \((Q_{10})\), see below) were not transformed because they exhibited a normal distribution and homogeneity of variance. Hypotheses predicting thermal sensitivity of RMR and associations between niche transitions and variation in RMR and \(Q_{10}\) during the evolutionary history of Gymnophthalmini were tested using two complementary approaches: (1) we evaluated the thermal sensitivity of RMR; and (2) we subsequently tested for evolutionary associations between RMR, its thermal sensitivity and niche transitions. All statistical analyses were performed in the R (version 3.4.3) environment using RStudio (version 0.99.489; https://www.rstudio.com/).

### Thermal sensitivity of resting metabolic rates

Within each species, we tested whether values of RMR differed among experimental temperatures using ANOVA followed by Tukey post hoc analysis. We then determined the thermal sensitivity of RMR for each species by calculating \(Q_{10}\) values. The RMR thermal sensitivity was calculated over the total temperature range (24–36°C) and the smaller temperature intervals of 24–30°C and 30–36°C. We used the van’t Hoff equation (following Watson and Burggren, 2016) to calculate \(Q_{10}\) indices (which will be referred in the paper as \(Q_{24–36}, Q_{24–36}\) and \(Q_{30–36}\) according to the interval measured), as follows:

\[
Q_{10} = \left( \frac{RMR_2}{RMR_1} \right) ^ {\frac{10}{T_2 - T_1}},
\]

where RMR\(_1\) and RMR\(_2\) correspond to mean metabolic rates measured at \(T_1\) (relatively lowest temperature) and \(T_2\) (relatively highest temperature), respectively.
All $Q_{10}$ indices ($Q_{24-36}$, $Q_{24-30}$ and $Q_{30-36}$) were tested for ecological associations, as described in the following section. As a complementary approach, we tested different regression models (linear, logarithmic, quadratic and exponential) to identify which equation better describes the relationship between variation in resting metabolic rates and temperature, selecting those having the lowest corrected Akaike information criterion (AICc) (Burnham and Anderson, 2002) for each species model (Table S3). In these equation analyses, data on RMR were not log$_{10}$ transformed.

### Phylogenetic analyses on ecological associations

The second approach used comprises two sets of phylogenetic analyses. The first analysis consisted of implementing ancestral reconstructions to trace character history of discrete (ecological) and continuous (RMR and its thermal sensitivity) data through stochastic character mapping and maximum likelihood, respectively (Bollback, 2006; Revell, 2012). For ecological data, we used all Gymnophthalmini species for which we could obtain ecological information from the literature (microhabitat type and activity period; Table S2; total of 22 species), with the aim to produce a robust estimation of evolutionary niche transitions in the lineage. In the second phylogenetic analysis, we tested whether and to what extent RMR and its thermal sensitivity evolved in association to niche transitions, using phylogenetic generalized least-squares regressions (PGLS; Caper package; https://cran.r-project.org/web/packages/caper).

We used the phylogenetic hypothesis of Zheng and Wiens (2016), as it comprises divergence time for the clades, with the inclusion of *S. catimbau* as a sister-species of *N. ablephara* (following Laguna and Yonenaga-Yassuda, 2011; Pellegrino et al., 2011). We also performed complementary analyses using the phylogenetic hypothesis from Pellegrino et al. (2011), which places *P. paeminosus* and *V. multiscutata* as sister-species (Fig. S1D), in agreement with Camacho et al. (2015), who investigated thermal relationships of the same species studied here using the hypothesis proposed by Pellegrino et al. (2011). Results using either phylogenetic hypothesis were equivalent, so in the main text we discuss results provided by analyses implemented using the topology based on Zheng and Wiens (2016), and results based on Pellegrino et al. (2011) are provided in the supplementary material (Tables S4, S5).

### RESULTS

#### Thermal sensitivity of RMR in Gymnophthalmini

Most Gymnophthalmini species studied here exhibited mass-specific resting metabolic rates (RMRs) that were affected by temperature (Table 1, Fig. 1A). The fossorial–diurnal species (*N. ablephara* and *C. catimbau*) were not identified across the temperature range tested (Table 2).

The relationship between RMR and temperature in Gymnophthalmini was described by different mathematical functions, which had similar fit (Table S3). RMRs of all species increased with temperature, with the exception of fossorial–diurnal ones (*C. catimbau* and *N. ablephara*; see Fig. 1A; Table S3). Comparisons among AICc values suggested that almost all models provide similar empirical support fitting the data ($\Delta$AICc≤4; Table S3; Burnham and Anderson, 2002). Still, the model having the lowest AICc was assumed to represent the relationship between RMR and temperature for each studied species (Fig. 1A).

#### Evolutionary associations between metabolism and ecological shifts

Phylogenetic generalized least-squares (PGLS) corroborated the patterns identified in the ancestral reconstructions. When both diurnal and nocturnal species were considered together in comparisons between epigean and fossorial species, ecological associations between RMR and microhabitat were not detected.

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**Table 1. Mass-specific resting metabolic rates (RMR; ml O$_2$ g$^{-1}$ h$^{-1}$) obtained for Gymnophthalmini lizards**

<table>
<thead>
<tr>
<th>Species</th>
<th>Ecology</th>
<th>Mass (g)</th>
<th>$RMR$ 24°C</th>
<th>n</th>
<th>$RMR$ 30°C</th>
<th>n</th>
<th>$RMR$ 36°C</th>
<th>n</th>
<th>$Q_{24-36}$</th>
<th>$Q_{24-30}$</th>
<th>$Q_{30-36}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Calyptommatus leioplepis</em></td>
<td>FN</td>
<td>0.657±0.037</td>
<td>0.077±0.033</td>
<td>10</td>
<td>0.095±0.022</td>
<td>10</td>
<td>0.199±0.051</td>
<td>10</td>
<td>1.78</td>
<td>1.44</td>
<td>2.19</td>
</tr>
<tr>
<td><em>Calyptommatus sinebrachiatus</em></td>
<td>FN</td>
<td>0.725±0.050</td>
<td>0.046±0.004</td>
<td>10</td>
<td>0.057±0.005</td>
<td>10</td>
<td>0.094±0.008</td>
<td>10</td>
<td>1.81</td>
<td>1.47</td>
<td>2.23</td>
</tr>
<tr>
<td><em>Calyptommatus nicterus</em></td>
<td>FN</td>
<td>0.981±0.105</td>
<td>0.045±0.011</td>
<td>10</td>
<td>0.069±0.023</td>
<td>10</td>
<td>0.097±0.027</td>
<td>10</td>
<td>1.56</td>
<td>2.03</td>
<td>1.20</td>
</tr>
<tr>
<td><em>Nothobachia ablephara</em></td>
<td>FD</td>
<td>0.496±0.080</td>
<td>0.156±0.085</td>
<td>10</td>
<td>0.163±0.032</td>
<td>10</td>
<td>0.152±0.020</td>
<td>10</td>
<td>0.98</td>
<td>1.07</td>
<td>0.90</td>
</tr>
<tr>
<td><em>Scriptosaura catimbau</em></td>
<td>FD</td>
<td>0.579±0.057</td>
<td>0.155±0.033</td>
<td>15</td>
<td>0.153±0.042</td>
<td>15</td>
<td>0.122±0.018</td>
<td>10</td>
<td>0.81</td>
<td>0.97</td>
<td>0.68</td>
</tr>
<tr>
<td><em>Psilops paeminosus</em></td>
<td>ED</td>
<td>0.401±0.036</td>
<td>0.117±0.006</td>
<td>12</td>
<td>0.122±0.009</td>
<td>12</td>
<td>0.159±0.015</td>
<td>12</td>
<td>1.29</td>
<td>1.07</td>
<td>1.55</td>
</tr>
<tr>
<td><em>Vanossaura multiscutata</em></td>
<td>ED</td>
<td>0.554±0.025</td>
<td>0.116±0.028</td>
<td>10</td>
<td>0.152±0.020</td>
<td>10</td>
<td>0.235±0.019</td>
<td>10</td>
<td>1.80</td>
<td>1.56</td>
<td>2.06</td>
</tr>
</tbody>
</table>

Ecological classification: FN, fossorial–nocturnal; FD, fossorial–diurnal; ED, epigeal–diurnal; mean values for body mass (±s.e.m.), mass-specific resting metabolic rates in each experimental temperature (±s.e.m.), numbers of individuals measured in each experiment (n), and RMR thermal sensitivity ($Q_{10}$ indices, represented as $Q_{24-36}$, $Q_{24-30}$ and $Q_{30-36}$ according to the interval measured: 24–36°C, 24–30°C and 30–36°C, respectively).
(Table 3). However, analyses comprising only diurnal species provided evidence for increased values of RMR measured at 24°C in the fossorial–diurnal lineage, when compared with epigeal–

Table 2. ANOVA results comparing resting metabolic rates among three experimental temperatures (24, 30 and 36°C), with associated Tukey post hoc comparisons

<table>
<thead>
<tr>
<th>Species</th>
<th>F-value</th>
<th>P-value</th>
<th>Group</th>
<th>Difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C. leiolepis</strong></td>
<td>8.229</td>
<td>0.001</td>
<td>24–30°C</td>
<td>–0.027</td>
<td>0.977</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24–36°C</td>
<td>0.462</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30–36°C</td>
<td>0.489</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>C. sinebrachiatus</strong></td>
<td>9.427</td>
<td>&lt;0.001</td>
<td>24–30°C</td>
<td>0.016</td>
<td>0.192</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24–36°C</td>
<td>0.045</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30–36°C</td>
<td>0.029</td>
<td>0.044</td>
</tr>
<tr>
<td><strong>C. nicterus</strong></td>
<td>7.756</td>
<td>0.003</td>
<td>24–30°C</td>
<td>0.376</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24–36°C</td>
<td>0.421</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>30–36°C</td>
<td>0.044</td>
<td>0.922</td>
</tr>
<tr>
<td><strong>N. ablephara</strong></td>
<td>1.153</td>
<td>0.342</td>
<td>24–30°C</td>
<td>0.203</td>
<td>0.433</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>24–36°C</td>
<td>0.176</td>
<td>0.419</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30–36°C</td>
<td>–0.026</td>
<td>0.985</td>
</tr>
<tr>
<td><strong>S. catimbau</strong></td>
<td>2.333</td>
<td>0.123</td>
<td>24–30°C</td>
<td>0.002</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24–36°C</td>
<td>–0.280</td>
<td>0.181</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30–36°C</td>
<td>–0.283</td>
<td>0.163</td>
</tr>
<tr>
<td><strong>P. paeminosus</strong></td>
<td>4.138</td>
<td>0.028</td>
<td>24–30°C</td>
<td>0.050</td>
<td>0.680</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24–36°C</td>
<td>0.161</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30–36°C</td>
<td>0.111</td>
<td>0.151</td>
</tr>
<tr>
<td><strong>V. multiscutata</strong></td>
<td>8.748</td>
<td>0.001</td>
<td>24–30°C</td>
<td>0.232</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24–36°C</td>
<td>0.360</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30–36°C</td>
<td>0.127</td>
<td>0.342</td>
</tr>
</tbody>
</table>

*Group* corresponds to each experimental temperature; *difference* corresponds to differences in mean log-transformed RMR values (ml O2 g−1 h−1) from each pair in a comparison. Significance is at P<0.05 and values in bold correspond to statistically significant comparisons.

The evolution of nocturnality within the fossorial lineage, in contrast, imprinted a remarkable reduction in RMR (Table 3), and nocturnal species exhibited lower metabolic rates than any diurnal lizard from the tribe Gymnophthalmini (Table 1, Fig. 4). Results from phylogenetic regressions with RMR measured at 24 and 30°C were broadly similar, and provided evidence for a strong association between resting metabolism and activity period (Fig. 4), which explains more than 80% of the variation in RMR in Gymnophthalmini (Table 3). Interestingly, this association was not detected in the analyses performed for RMR measured at 36°C (Table 3).

The thermal sensitivity of RMR (Q10 indices, represented as Q24–36°C, Q24–30°C and Q30–36°C according to the interval measured) was neither associated with fossoriality nor with nocturnality when all species were analyzed together (Table 4). Nevertheless, analyses considering Q10 only within fossorial species in the total range of temperature tested (Q24–36°C) provided evidence for reduced thermal sensitivity of RMR in fossorial–diurnal species (Table 4, Fig. 1).

**DISCUSSION**

Ecological shifts in space and time are usually coupled with morphological, ecological and physiological changes in species experiencing new environmental conditions (Barros et al., 2011; Camacho et al., 2015; Grizante et al., 2012; Hare et al., 2007), and thus are recognized as major drivers of diversification events (Anderson and Wiens, 2017; Bars-Closel et al., 2017). Sometimes these transitions comprise spatial changes in microhabitat use that are coupled with temporal modifications in the activity periods, and our study shows that integration of spatial and temporal dimensions of
habitat usage contributes to the evaluation of metabolic evolution. Changes in RMR associated with the evolution of fossoriality or nocturnality have long been considered a key factor in understanding the evolution of thermosensitive traits during ecological transitions (Abe and Johansen, 1987; Andrews and Pough, 1985; Fusari, 1984; Gordon et al., 2010). Previous studies that addressed separately the spatial or temporal transitions in niche occupation by Scincidae and Anguidae lizards (Abe and Johansen, 1987; Andrews and Pough, 1985; Fusari, 1984; Gordon et al., 2010) found that fossoriality and nocturnality reduce RMR. However, the phylogenetic approach we applied here fosters the evaluation of both niche transitions to understand metabolic evolution in lizards. Our results suggest that the shift that led to the origin of fossorial–nocturnal Gymnophthalmimini species was associated with a notable reduction in RMR, a trend not perceived when evolution of fossoriality is considered alone. Moreover, fossorial–diurnal Gymnophthalmimini have significantly higher RMR, contrary to what has been described in the literature for fossorial species (Abe and Johansen, 1987; Johansen et al., 1980; Putnam and Murphy, 1982), and their values surpassed not only those of epigean–diurnal Gymnophthalmimids, but also those of other epigean–diurnal lizards (Andrews and Pough, 1985). Several factors likely explain the evolution of specific metabolic patterns (see Burton et al., 2011), and here we focus on thermal regimes and ecological traits in the Gymnophthalmimini.

**Metabolism, temperature and ecological associations with fossoriality and nocturnality**

The evolution of nocturnality coupled with a fossorial lifestyle in microteiids endemic to the extremely hot Brazilian sand dune fields allows maintenance of body temperatures closer to ancestral preferred temperatures at night, when surface temperature drops below preferred levels (Camacho et al., 2015). Lower RMRs reduce energy expenditure and water loss, which likely enhance survival in hot and dry environments that have a very low productivity (Mautz, 1979; Putnam and Murphy, 1982), such as the Brazilian Caatingas (Araújo et al., 2007). Nocturnal lizards may face thermoregulatory challenges, as they do not bask and often have less opportunities to exploit the thermal environment than the diurnal species (Porter et al., 1973). However, field operative temperatures in the Brazilian Caatingas, sampled in this study and by Camacho et al. (2015) suggest that fossorial–nocturnal species may not face low temperatures when active during the night because the subterranean environment buffers the thermal variation observed in the surface. Consequently, foraging in a thermally homogeneous underground and being active at relatively warmer temperatures during the night may contribute to energy saving in nocturnal *Calyptommatus* through reduction of metabolic rates.

Many physiological processes (e.g. digestion, see Secor, 2009) may be optimized in fossorial–nocturnal species that rest at warmer daily temperatures. The nocturnal *Calyptommatus* feed on large amounts of termites, which are soft prey (Barros et al., 2011) that provide low energy to the organism (Nagy et al., 1984), but represent an important energy and water resource for nocturnal lizards, especially in arid zones (Abensperg-Traun, 1994; Abensperg-Traun and Steven, 1997). Digestion of large food amounts may be optimized when animals rest at warmer temperatures (Adolph and Porter, 1993; Bustard, 1967; Secor, 2009), reducing the demand for elevated metabolic rates. Reduced

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Fig. 2. Ancestral state reconstruction of ecological transitions in Gymnophthalmini. Colors represent (A) the spatial shift in ecological categories from epigean (E) to fossorial (F), in green, and (B) the temporal shift in ecological categories from diurnal (D) to nocturnal (N), in blue. The colors in pie charts represent the proportional likelihood of that given ecological state at the node; the colors in the tips represent the ecological state of each species. Phylogenetic hypothesis and branch lengths modified from Zheng and Wiens (2016), with the addition of *S. catimbau* as a sister-species of *N. ablephara*, following Pellegrino et al. (2011) and Laguna and Yonenaga-Yassuda (2011). Branch length scale represents millions of years from present (time=0).
Fig. 3. Phylogenetic reconstruction of RMR at three experimental temperatures and its thermal sensitivity. (A) RMR at 24°C; (B) RMR at 30°C; (C) RMR at 36°C and (D) RMR thermal sensitivity (Q10) inferred in the total temperature interval used here (Q24–36 index). Colors represent the gradient from lower (cold colors) to higher values (warm colors) of RMR (A–C) and its thermal sensitivity (D). FN, fossorial–nocturnal lineage; FD, fossorial–diurnal lineage. Phylogenetic hypothesis and branch lengths modified from Zheng and Wiens (2016), with the addition of Pellegrino et al. (2011) and Laguna and Yonenaga-Yassuda (2011). Branch length scale represents millions of years from present (time=0).

RMRs have also been described in gecko lizards specialized in termites (Withers et al., 2000).

Lower RMRs are favored in seasonal environments (Salimon and Anderson, 2017), such as the Brazilian Caatingas, given that reduced energetic demands may minimize the effects of food scarcity in specific periods (Burton et al., 2011). Combined with smaller geographic ranges (as observed in Calyptommatus species; Camacho et al., 2017), restricted activity periods during the night also likely reduce the total energetic demand in fossorial–nocturnal microteiids, which probably spend less time exposed to potential predators, and reduce niche overlap (Gordon et al., 2010). Reduced RMRs may be adaptive in the fossorial microhabitats of the Caatingas also by allowing energy allocation for specific functions, such as growth and reproduction (Artacho and Nespolo, 2009; Burton et al., 2011).

However, RMRs in fossorial–diurnal microteiids were elevated, and the values we measured surpassed those of fossorial–nocturnal and epigean–diurnal microteiids, being even higher than those described for other epigean lizards in the literature (Andrews and Pough, 1985). Fossorial–diurnal species may benefit from the buffering effect of subterranean environments, which provides warmer temperatures for resting during the night but also maintains high temperatures at the subsuperficial layers at daytime (present study; Camacho et al., 2015). Given that metabolism correlates with temperature in most ectotherms (Dawson, 1975; Litzgusa and Hopkins, 2003; Piercy et al., 2015), and that resting and activity metabolic rates often evolve in association (Auer et al., 2017; Bennett, 1982), the relatively high temperatures experienced by fossorial–diurnal Gymnophthalmini during activity may have influenced the evolution of RMR, especially considering the locomotion mode of these species (Höfling and Renous, 2004). Specifically, these microteiids move by sand-swimming in
extremely hot environments, which likely increases energetic requirements and energy expenditure of individuals foraging in substrates with substantial resistance to displacement (Barros et al., 2011; Sedlácek, 2007; Wu et al., 2015). A sustained sand-swimming locomotion imposes elevated biomechanical costs and increased energetic demands in fossorial–diurnal species (Walton et al., 1990), and higher RMRs in these fossorial–diurnal microteiids may optimize burrowing performance and dispersal capacity, as observed in the diurnal *N. ablephara* (Camacho et al., 2015, 2017). Epigeal microteiids, in contrast, move intermittently through bushes and leaf litter (Höfling and Renous, 2004; Rodrigues, 2003), using a type of locomotion that is energetically more economical than sand-swimming (Gleeson and Hancock, 2002; Sedlácek, 2007; Weinstein and Full, 1999; Wu et al., 2015). Owing to the low cost of locomotion at night (see Hare et al., 2007), nocturnal microteiids that sand-swim may require less energy expenditure in comparison with the fossorial–diurnal species.

**Thermal sensitivity of resting metabolic rates**

Our results demonstrate that metabolic evolution is associated with niche occupation in microteiids endemic to the Brazilian Caatingas, exhibiting opposite patterns depending on the ecological category. Specifically, fossorial–nocturnal species have the lowest RMRs, whereas the largest values were recorded in fossorial–diurnal species; epigeal–diurnal species exhibited intermediate RMRs among the lizards we measured. Such RMR patterns seem unique to the Gymnophthalmini, as they do not resemble what has been previously described in the literature for fossorial squamates (Andrews and Pough, 1985; Fusari, 1984; Wu et al., 2015). The thermal sensitivity of RMR has been more often studied under single-dimension approaches that investigate separately the niche transitions in space (origin of fossorial lineages) and time (evolution of nocturnal species). When considered together, the results of such single-dimension studies may offer confounding conclusions about the evolution of thermosensitive physiological traits. For example,
Table 4. Results from phylogenetic generalized least-squares regressions (PGLS) between RMR thermal sensitivity ($Q_{10}$) and ecology (microhabitat use and activity period), as well as within each ecological category considering only diurnal or only fossorial species

<table>
<thead>
<tr>
<th>Thermal sensitivity ($Q_{10}$)</th>
<th>Parameter</th>
<th>$r^2$</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q$_{24-36}$</td>
<td>Microhabitat</td>
<td>0.026</td>
<td>0.134</td>
<td>0.728</td>
</tr>
<tr>
<td></td>
<td>Microhabitat: only diurnal</td>
<td>0.743</td>
<td>5.791</td>
<td>0.137</td>
</tr>
<tr>
<td></td>
<td>Activity period</td>
<td>0.343</td>
<td>2.614</td>
<td>0.166</td>
</tr>
<tr>
<td></td>
<td>Activity period: only fossorial</td>
<td>0.941</td>
<td>47.870</td>
<td>0.006</td>
</tr>
<tr>
<td>Q$_{24-30}$</td>
<td>Microhabitat</td>
<td>0.011</td>
<td>0.057</td>
<td>0.820</td>
</tr>
<tr>
<td></td>
<td>Microhabitat: only diurnal</td>
<td>0.401</td>
<td>1.343</td>
<td>0.366</td>
</tr>
<tr>
<td></td>
<td>Activity period</td>
<td>0.470</td>
<td>4.447</td>
<td>0.088</td>
</tr>
<tr>
<td></td>
<td>Activity period: only fossorial</td>
<td>0.670</td>
<td>6.097</td>
<td>0.090</td>
</tr>
<tr>
<td>Q$_{30-36}$</td>
<td>Microhabitat</td>
<td>0.078</td>
<td>0.426</td>
<td>0.542</td>
</tr>
<tr>
<td></td>
<td>Microhabitat: only diurnal</td>
<td>0.870</td>
<td>14.606</td>
<td>0.066</td>
</tr>
<tr>
<td></td>
<td>Activity period</td>
<td>0.232</td>
<td>1.514</td>
<td>0.232</td>
</tr>
<tr>
<td></td>
<td>Activity period: only fossorial</td>
<td>0.684</td>
<td>5.953</td>
<td>0.092</td>
</tr>
</tbody>
</table>

Q$_{24-36}$, RMR $Q_{10}$ within 24–36°C; Q$_{24-30}$, RMR $Q_{10}$ within 24–30°C; Q$_{30-36}$, RMR $Q_{10}$ within 30–36°C. Significance is at P<0.05.

some studies suggest that thermal sensitivity of RMR is relatively similar among diurnal and nocturnal species (Autumn et al., 1994), while others suggest a reduction in nocturnal (Al-sadoon and Abdо, 1989) or fossorial lizards (Loubourdis and Hailey, 1985). Microhabitat usage influences thermostorregulatory behavior (Smith and Ballinger, 2001), so the reduction of RMR thermal sensitivity we identified in fossorial–diurnal microteids may reflect enhanced thermostorregulatory opportunities at warm subterranean temperatures during the day (present study; Camacho et al., 2015) coupled with occupation of a less-competitive environment (Greenville and Dickman, 2009), weakening the pressure for rapid metabolic increments in fossorial–diurnal species (Hitchcock and Mcbrayer, 2006; Watson and Burggren, 2016). Epigean microteids, in contrast, likely face higher competition and predation risks when foraging at the surface, and might benefit from increased RMR thermal sensitivity that shortens activity periods and reduces the amount of time they are exposed to potential predators (Martin, 2001). During the night, thermostorregulatory opportunities are restricted (Huey et al., 1974; Wu et al., 2009), so an enhanced thermal sensitivity may optimize fast metabolic responses in fossorial–nocturnal species. Accordingly, fossorial–nocturnal microteids would benefit from a thermal sensitivity of RMR that is similar to that measured in epigean species owing to the mild and relatively homogeneous temperatures that characterize subterranean microhabitats from the Brazilian Caatingas during the night (present study; Camacho et al., 2015).

Conclusions
The unique evolutionary history of Gymnophthalmini radiation, comprising concomitant shifts in two ecological niches within a narrow time frame, fosters investigation of physiological and behavioral traits associated with these niche transitions. To our knowledge, this is the first time that evolution of RMR is addressed in a scenario of concomitant ecological shifts involving both spatial and temporal scales under a phylogenetic approach, as most studies have focused either on fossoriality or on nocturnality. Our results demonstrate that both microhabitat use and period of activity explain variation in RMR, and the thermal sensitivity of RMR changed mostly in the diurnal–fossorial lineage. Differences in RMR potentially reflect variation in the thermal regimes experienced, in addition to other ecological correlates. Additional parameters that potentially contribute to variation in Gymnophthalmini RMR include growth, diet, foraging mode and reproductive state (Andrews and Pough, 1985; Brown et al., 2004; Congdon et al., 1982; McNab, 2001), and further studies may consider such parameters in the investigation of evolution of thermosensitive traits during ecological transitions. The present study supports theoretical models predicting the evolution of thermal physiology coupled with temperatures experienced in the field (Angilletta et al., 2002; Huey and Kingsolver, 1989), and demonstrates that metabolic evolution is coupled with multidimensional aspects of niche transitions. Our results indicate that ecological diversification in microteids may be associated with opposite trends of physiological specialization, highlighting the importance of integrating temporal and spatial components of niche occupation to address the evolution of thermosensitive traits.

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Competing interests
The authors declare no competing or financial interests.

Author contributions

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References


