Agustín Camacho Guerrero

Evolução da fossorialidade nos lagartos da

tribo GYMNOPHTHALMINI

(GYMNOPHTHALMIDAE, SQUAMATA)

Evolution of fossoriality in lizards of the tribe GYMNOPHTHALMINI

(GYMNOPHTHALMIDAE, SQUAMATA)

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Epígrafe

“...The recognition by comparative biologists that they need systematists should do much to stimulate the work of systematists. In turn, systematists should do encourage comparative biologists to study groups for which phylogenetic information is already available or currently being gathered...”

Theodore Garland Jr, Raymond Huey e Alfred Bennet.

Phylogeny and coadaptation of thermal physiology in lizards: A reanalysis.

Evolution. (1991)

“Going to try, with a little help from my friends”

Ringo Starr,

Sgt. Pepper's Lonely Hearts Club Band (1967)

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- IV) **Two parameters for understanding thermal challenges: maximal temperatures and the range of spatial thermal gradients.** Não submetido.
- V) **Considerations for assessing maximum critical temperatures in small ectothermic animals: insights from leaf-cutting ants.** PLoS One.
- VI) **Limb reduction and range-size evolution in a lizard radiation (*Lerista*: Scincidae).** Aceito no Journal of Biogeography.

Introdução Geral

Para efeitos de análise de sua evolução, a fossorialidade tem sido definida como a habilidade de locomover-se e realizar a maioria das atividades diárias no subsolo (Wiens *et al.* 2006). Este hábito tem evoluído ao menos 19 vezes em lagartos de todos os continentes, exceto na Antártica, e em todas as famílias com exceção das que compõem o clado Iguania (Greer, 1991; Pough, 1997; Wiens *et al.* 2006). Evidências moleculares, morfológicas e paleontológicas sugerem que a origem das anfisbenas (Kearney & Stuart 2004, Muller *et al.* 2011, Wiens 2012) e as serpentes (Walls, 1944; Bellairs e Underwood, 1951; Skinner e Lee, 2009; Zaher *et al.* 2009; Wiens, 2012), os grupos mais diferenciados dentro do plano morfológico geral dos Squamata, (Gans, 1980, Vitt & Caldwell, 1998, Sites *et al.* 2011), esteve relacionada à aquisição de hábitos fossoriais. Por estes motivos, conhecer as causas e consequências da transição entre o morfótipo lacertiforme e o serpentiforme é fundamental para explicar a evolução da diversidade morfológica dentro dos Squamata.

A evolução da fossorialidade está associada à evolução de um dos três grandes tipos de répteis com morfótipo serpentiforme (isto é, corpo alongado e com diferentes níveis de redução de membros). Estes três grandes tipos podem ser considerados ecomorfos que representam conjuntos de espécies com marcadas diferenças de uso do ambiente e

morfologia. Espécies com o ecomorfo superficial têm evoluído caudas mais alongadas e tendem a explorar a superfície do solo fazendo uso frequente da vegetação herbácea, tais como espécies dos gêneros: *Ophiodes*, *Anguis*, *Chamaesaura*, *Lialis* (Wiens *et al.* 2006). Espécies com o ecomorfo fossorial caracterizam-se por ter corpo alongado, cauda geralmente mais curta, e por explorar o subsolo, a camada de humus e o folhiço. Exemplos seriam as espécies dos gêneros: *Calyptommatus*, *Anniella*, *Typhlosaurus*, e *Dibamus* (Wiens *et al.* 2006). O ecomorfo aquático representa um grupo extinto de répteis, os mossasauros, caracterizados por apresentar cabeças relativamente grandes, durante sua colonização dos mares no Cretáceo (Bellairs & Underwood, 1951; Lindgren *et al.* 2010, e estudos revisados nesse trabalho).

A evolução do ecomorfo fossorial foi inicialmente concebida como um processo ordenado, no qual o alongamento do corpo seria seguido de uma posterior redução dos membros e da cabeça (Gans, 1974; revisado em Greer, 1991). Porém, um trabalho recente em lagartos das famílias Anguidae (Wiens e Slingluff, 2001) refuta esta ideia, e estudos com as famílias Scincidae (Skinner e Lee, 2009) e Gymnophthalmidae (Grizante *et al.* 2012) mostram que as características mais típicas do morfótipo serpentiforme (redução de membros, dedos, e alongamento do corpo) podem evoluir tanto de forma correlacionada quanto em módulos de caracteres relativamente independentes.

Explicações gerais para a evolução dos morfótipos de vertebrados assumem um valor adaptativo para cada forma intermediária na evolução dos caracteres morfológicos (e.g. Kardong, 2006; Pough, 2008). No caso do

ecomorfo fossorial, diferentes autores têm hipotetizado diversas vantagens proporcionadas pelos novos morfótipos. Estas incluem: melhora na eficiência da locomoção ou também em solos soltos (Gans, 1975; Gans, 1980; Pianka e Vitt, 2003), uma melhor capacidade de alimentação sob o solo (Pianka e Vitt, 2003), e, por ultimo, permitir evitar ambientes com temperaturas extremas (Pianka e Vitt, 2003, Rodrigues *et al.* 2009).

Em resposta, alguns estudos funcionais têm analisado estas explicações. No caso da locomoção, Walton *et al.* (1990) não encontraram uma maior eficiência energética da locomoção serpentiforme quando comparados animais exercitados. Bergmann e Irschick (2010), encontraram uma associação negativa entre a redução do comprimento relativo dos membros e a velocidade máxima de corrida. Porém, sem influencias do tipo de substrato. Benesch e Whithers (2002) encontraram que espécies com morfótipo fossorial possuem uma maior disposição para escavar e maior velocidade na escavação na areia. Contudo, espécies de lagartos com morfótipo lacertiforme podem escavar bem em substrato solto (areia) dobrando os braços contra o corpo (Maladen *et al.* 2009). Por último, Renous *et al.* (1998) não avaliaram nenhuma vantagem adaptativa dos morfótipos serpentiformes, mas mostraram como o uso da musculatura axial ganha importância na locomoção durante a transição entre morfótipos lacertiformes e ecomorfos fossoriais.

Quanto à alimentação, Shine (1986) mostra que alguns pigopodídeos serpentiformes estão especializados em alimentar-se de aranhas que vivem em buracos. Já Andrews *et al.* (1987) e Pough *et al.* (1997) mostraram que lagartos com morfótipos serpentiformes levam um maior tempo de manejo de

presas. Apesar disso, Barros et al. (2011), mostraram que a dieta permanece conservada apesar da alta especialização do crânio desenvolvida durante a evolução de lagartos serpentiformes na família Gymnophthalmidae.

Em relação à fisiologia térmica, alguns estudos com scincídeos, anguídeos e anfisbenídeos têm sugerido que ecomorfos fossoriais sofrem restrições do ambiente subterrâneo na termoregulação e que apresentam baixos requerimentos térmicos (ex. Greer, 1980; Avery, 1982; Withers, 1981; Huey e Bennet, 1987).

Testar as teorias propostas sobre a evolução dos ecomorfos serpentiformes fossoriais através de comparações entre espécies com diferentes morfótipos envolve toda uma gama de dificuldades que abarcam problemas conceituais, experimentais e analíticos. O primeiro dos problemas conceituais deriva de que as teorias apresentadas acima relacionaram a evolução da morfologia com um melhor desempenho em casos de tarefas concretas (ex. evitar exposição a temperaturas ambientais extremas) e em outros casos, de funções gerais (ex. locomoção). Porém, o desempenho de organismos (*sensu* Irschick *et al.* 2008) pode ser medido apenas com relação à execução de tarefas concretas (ex. velocidade de fuga, velocidade em comer presas enterradas), não de uma função geral (ex. termoregulação, alimentação, etc.). Isto se deve a dois motivos, o primeiro é que uma função geral pode envolver inúmeras tarefas e cada uma dessas tarefas pode apresentar requerimentos morfológicos diferentes para um melhor desempenho, resultando em compromissos com implicações funcionais. Por exemplo, lagartos fossoriais potencialmente podem acessar mais presas no subsolo. Contudo, estes lagartos requerem de um maior tempo de manejo

das mesmas (Andrews *et al.* 1987; Pough *et al.* 1997). Como dizer então se a fossorialidade leva a um maior sucesso na alimentação? O segundo motivo é que o melhor desempenho em uma ou várias tarefas relacionadas a uma função, só conferirá um maior sucesso na realização dessa função se o ambiente favorecer os organismos com melhor desempenho (ex. de nada serve um melhor desempenho na captura de presas enterradas se não houver disponibilidade delas no ambiente, ou uma pressão por alcança-as).

Por estes motivos, averiguar se determinadas funções são melhor executadas por um determinado morfótipo faz necessários, ao menos, dois procedimentos. O primeiro é comparar o desempenho de organismos com morfótipos plesiomórficos e derivados em tarefas relacionadas a uma função concreta, se possível em diferentes contextos ambientais potencialmente importantes no ambiente de origem (Baum e Larson, 1991). O segundo, avaliar se o ambiente seleciona (Arnold, 1983), ou concede oportunidades, a organismos em função do seu desempenho nas tarefas avaliadas. Até o momento, estudos comparativos que visaram entender a evolução do morfótipo serpentiforme apenas compararam medidas fisiológicas e comportamentais (Withers, 1981; Shine, 1986), ou o desempenho de tarefas (Walton *et al.* 1990; Benesch e Withers, 2002), sem contrastar com medidas das potenciais pressões ou oportunidades existentes no ambiente de origem.

Um segundo problema conceitual é que, tal como enunciadas pelos seus autores, teorias sobre a evolução de morfótipos não explicam se as vantagens dos novos morfótipos se aplicaram durante a fixação das características na população inicial ou durante o estabelecimento e persistência do novo morfótipo em diferentes espécies e ambientes. Ambas

partes da história são importantes e complementares. Para gerar teorias mais completas e que expliquem quais funções foram beneficiadas pela evolução do morfótipo serpentiforme, é necessário relacionar avaliações de sucesso durante processos que acontecem em dois níveis (ex. intraespecífico e interespecífico). No nível intraespecífico, estudos do fitness individual (*sensu* Darwin, 1859) permitem avaliar o sucesso relativo de um morfótipo em deixar descendência (ex. Le Galliard *et al.* 2004). Associadas a esses estudos, avaliações do sucesso na execução de uma função permitem determinar se este está relacionado à eventual fixação do morfótipo na população. Por outro lado, estudos no nível interespecífico permitem observar como os fenótipos co-evoluem entre as espécies (ex. Huey & Bennet, 1987; Garland *et al.* 1991, capítulo 1). Deste modo, podemos saber se morfótipos promovem sucesso na execução de determinadas funções em determinados contextos ambientais (ex. Sites *et al.* 2011, capítulo 2). Além disso, estudos comparativos interespecíficos como o presente, também servem para subsidiar análises posteriores sobre o impacto da aquisição dos morfótipos no sucesso ecológico das espécies (seu potencial de persistência ao longo do tempo; Wilson, 1987), o qual está fortemente associado ao número total de indivíduos e tamanho da área de distribuição das espécies (Johnson, 1999; Kiessling e Aberhan, 2007; Williams *et al.* 2009), e potencialmente, com o sucesso na execução de funções importantes. No caso do ecomorfo fossorial que nos ocupa, estudos comparativos prévios, assim como esta tese, apenas realizaram comparações interespecíficas. Portanto, esses estudos, e o presente, não permitem explicar a fixação inicial do ecomorfo fossorial, a não ser sob a premissa de que as condições

ambientais relevantes para a evolução do ecomorfo*¹ em questão sejam hoje similares às do período onde ele se fixou (ex. Shine, 1986).

O segundo grupo de problemas é experimental, e o primeiro problema dentro deste grupo segue a continuação. Outros fenótipos (ex. comportamento) e o ambiente, além do desempenho e a morfologia, também podem ser determinantes do grau de sucesso que indivíduos de uma espécie têm durante a execução de uma função (Losos e Garland, 1994). Isto significa que, avaliar se espécies com diferentes morfótipos tem diferente sucesso na execução de uma função (ex. escapar de predadores) requer integrar medidas de desempenho, comportamento e morfologia das espécies durante a execução de tarefas relacionadas a uma função (ex. tamanho dos membros, velocidade e estratégia durante a fuga de predadores) e contrasta-las com avaliações das pressões seletivas e oportunidades do ambiente onde as espécies avaliadas ocorrem. No nosso exemplo, isto significaria avaliar a probabilidade de ter êxito na fuga de predadores (capítulo 2).

Em alguns casos, pode ser relativamente fácil avaliar o sucesso na execução de uma função. Por exemplo, é possível determinar experimentalmente se espécies com um determinado morfótipo conseguem se alimentar melhor ou pior de determinados tipos de presas (ex. Andrews *et al.* 1987; Pough *et al.* 1997) e estimar abundância destas presas na natureza. Em outros casos, as observações podem ser extremamente difíceis de realizar. Como exemplo, podemos imaginar as dificuldades de observar

¹ Ao longo do texto, as categorias morfótipo e ecomorfo estão sendo usadas para denominar morfótipo serpentiforme de um modo geral, e ao ecomorfo fossorial como um caso deste. O uso destas categorias segue apenas fins didáticos. O argumento explicado no parágrafo pode ser aplicado a qualquer descritor contínuo da morfologia.

pequenos lagartos de diferentes espécies, escapando de predadores ou termoregulando, sob o folhíço de arbustos da Caatinga. No entanto, nestes casos, existem alternativas à observação direta. Uma é comparar a distribuição das temperaturas ambientais com dados experimentais da capacidade dos animais de usar o ambiente e seus requerimentos térmicos. Outra alternativa provém do método do “paradigma”, (Baum e Larson 1991). Este método consiste em modelar estatisticamente a relação entre as características biológicas sob estudo (ex. comprimento dos membros) e o sucesso na execução de uma função, com base em um conjunto de premissas. Depois, pode-se prever o sucesso relativo de espécies com base nas características avaliadas. Esta alternativa pode ser explorada através de videogames (van Damme e van Dooren, 1999 e Beatty *et al.* 2004), uma vez que estes podem ser adaptados para avaliar o efeito que características dos indivíduos tem durante situações que imitem até certo ponto, as pressões seletivas e oportunidades oferecidas no ambiente. Deste modo é possível gerar um conjunto de observações suficientemente controladas e numerosas, que resultem em modelos empíricos da relação entre desempenho, morfologia, comportamento dos indivíduos e o sucesso durante a tarefa executada no videogame (ex. fugir de predadores). Uma vez gerados, estes modelos empíricos podem ser alimentados com dados das espécies sob estudo, permitindo prever o efeito relativo da morfologia, desempenho e comportamento na tarefa (ex. capacidade de fuga dos lagartos de ataques visualmente orientados no capítulo 2). Contudo, estes modelos ainda precisam ser validados com dados reais (Hutchison, 2012).

Um terceiro grupo de problemas deriva da análise de dados provenientes de diferentes espécies ou populações (aqui chamados dados comparativos). Os métodos estatísticos usados para analisar dados comparativos experimentaram uma revolução desde a invenção dos contrastes independentes (Felsenstein, 1985; Martins e Garland, 1991; Martins e Hansen, 1997; Garland *et al.* 2005; Revell, 2010). Métodos estatísticos convencionais assumem que dados interespecíficos são independentes, e isto faz com que os valores de *p* apareçam inflados. Isto se deve a que dados interespecíficos não são independentes, devido às interações filogenéticas existentes entre estes dados (Felsenstein, 1985). Por este motivo, recomenda-se o uso de contrastes independentes (Felsenstein, 1985) ou a estimativa paralela do efeito da filogenia e dos fatores (Blomberg *et al.* 2003; Revell, 2010). Para isto, são usados modelos de mínimos quadrados generalizados, os quais permitem levar em conta estruturas de correlação complexas entre os dados analisados (ex. Revell, 2010). Este sistema ainda não é ideal, pois para uma boa estimativa do efeito da filogenia são necessárias muitas espécies (mais de 20, segundo Blomberg *et al.* 2003; Freckleton *et al.* 2002). Porém, um estudo com poucas espécies (nove) têm demonstrado que regressões multivariadas significativas entre caracteres ainda podem ser encontradas usando filogenias com poucos caracteres (Heng *et al.* 2009).

A presente tese foi realizada sobre um conjunto de 10 espécies de lagartos da família Gymnophthalmidae, uma família praticamente desconhecida em termos ecofisiológicos (Avery, 1982). As espécies estudadas representam a quase totalidade das espécies do clado

Gymnophthalmini (Pellegrino *et al.* 2001) que ocupam a Caatinga. Este grupo de espécies apresenta importantes vantagens operacionais para estudos envolvendo os detalhes acima descritos. Para começar, espécies deste grupo podem ser claramente classificadas em dois grupos: Um deles é composto pelas espécies lacertiformes, pertencentes aos gêneros: *Psilophthalmus*, *Micrablepharus*, *Vanzosaura* e *Procellosaurinus*. O outro grupo é composto por espécies serpentiformes fossoriais, caracterizadas por corpos com um maior número de vértebras, membros muito reduzidos ou ausentes, crânio com forma de pá e hábitos fossoriais. Estas espécies pertencem aos gêneros *Calyptommatus*, *Nothobachia* e *Scriptosaura* (Sites *et al.* 2011; Roscito e Rodrigues, 2012; Figura 1 Capítulo 1). Exceto por *Vanzosaura rubricauda*, *Micrablepharus maximiliani* e *Procellosaurinus erythrocerus*, as espécies estudadas são endêmicas dos campos de areia que marginam o curso médio do rio São Francisco e que salpicam áreas de altitude na Caatinga (Rodrigues, 1996; Rodrigues e Santos, 2008). A pequena área de distribuição, especialmente das espécies com morfotipo fossorial, faz mais fácil de captar a variação geográfica nos parâmetros fenotípicos destas espécies e que potenciais diferenças fenotípicas (ex. na fisiologia termal) encontradas entre os morfótipos dificilmente possam ser explicadas por ter histórias biogeográficas diferentes, ou enfrentar diferentes climas (ex. como em Withers, 1981). Em cada uma das cinco localidades de estudo ocorre de um a dois pares de espécies sintópicas de representantes lacertiformes e serpentiformes fossoriais, o que implica que ambos enfrentam pressões derivadas do mesmo ambiente. Além disto, estas espécies podem ser abundantes localmente (Ex. Rodrigues, 1996) e se mostraram fáceis de

coletar, manipular e manter em cativeiro durante um projeto piloto e ao longo do projeto. Apenas dois gêneros de Gymnophalmini, *Tretioscincus* e *Gymnophthalmus*, ambos com distribuição amazônica, não foram estudados.

Contudo, a estrutura filogenética deste grupo representa a pior possível do ponto de vista da análise estatística filogenética (Felsenstein, 1988; Garland *et al.* 2005). Isto é porque os Gymnophthalmini representam apenas uma das duas únicas aquisições do ecomorfo fossorial conhecidas para a América do Sul (Pellegrino *et al.* 2001, Wiens *et al.* 2006). Deste modo, todas as espécies com ecomorfo fossorial são mais aparentadas entre si do que com as lacertiformes. Do mesmo modo, dez é um número considerado baixo de espécies para as análises estatísticas filogenéticas. Um baixo número de espécies torna as estimativas do efeito da filogenia potencialmente imprecisas (muito baixas, Blomberg *et al.* 2003). Ao mesmo tempo, o padrão filogenético destes dois grupos faz com que as diferenças encontradas entre morfótipos não possam ser estatisticamente atribuídas à morfologia nem a adaptação (Garland e Adolph, 1994), uma vez que espécies do mesmo morfótipo são mais aparentadas entre si. Estes problemas são aqui tratados do seguinte modo: Por um lado, as comparações entre morfótipos diferentes são feitas usando métodos estatísticos que permitem comparar seus resultados com e sem informações da filogenia. Deste modo, é possível verificar se os resultados são robustos ou derivados de estimativas imprecisas do efeito da filogenia. Além disso, neste estudo, as interpretações sobre a utilidade do morfotipo serpentiforme são baseadas em caracterizações do ambiente, as quais permitiram uma

apreciação das potenciais pressões seletivas atuando sobre as espécies estudadas. Além disso, visto que são amostradas quase todas as espécies e a maioria dos gêneros conhecidos do grupo, os resultados podem ser considerados como representantes da evolução dos fenótipos estudados entre os Gymnophthalmini da Caatinga. Por último, como este clado representa apenas uma das múltiplas vezes que o morfótipo serpentiforme evoluiu (ex. Wiens *et al.* 2006), os resultados deste estudo permitem apenas uma primeira visão da co-evolução entre morfologia, desempenho, comportamento, fisiologia e do potencial sucesso na execução de funções que podem ocorrer durante a evolução dos morfótipos serpentiformes fossoriais, e sem avaliar se estas constituem uma adaptação ou não.

Esta tese é composta por dois capítulos, e cada um avalia diferentes funções. No primeiro capítulo, analisamos a co-evolução do morfótipo com o desempenho escavador, a fisiologia térmica e os custos da função termoregulatória. No segundo capítulo, analisamos diferenças na velocidade de locomoção horizontal, no comportamento de fuga e na capacidade de alimentar-se de diferentes tipos de presas. A utilidade da velocidade, o morfotipo e o comportamento de fuga é avaliada frente à fuga de predadores visualmente orientados. A capacidade de alimentar-se de presas é contrastada com a disponibilidade das mesmas nas localidades de estudo.

Os artigos que compõem o apêndice permitiram atacar metodológica e conceitualmente os tópicos tratados na tese, entender melhor os seus resultados e abrir um caminho a seguir para continuar este estudo. Os apêndices I e II representam um primeiro contato com a biologia de lagartos Gymnophthalmídeos fossoriais, o qual permitiu definir os parâmetros

ambientais que podiam ser importantes para sua evolução e persistência nos habitats que ocupam. Nestes apêndices foram feitas umas primeiras análises exploratórias das temperaturas ambientais e a preparação dos métodos empregados durante a caracterização da ecologia, comportamento e fisiologia, em lagartos Gymnophthalmi.

O apêndice III constitui uma revisão da literatura que integra dois corpos de conhecimento: um que trata sobre as respostas dos animais ectotérmicos terrestres ao ambiente térmico e outro que descreve as variações do ambiente térmico na Terra. A partir desta revisão é proposto um primórdio de teoria que explica a interação dos organismos ectotermos terrestres e variações na temperatura ambiental.

O apêndice IV, ainda não submetido para publicação, exemplifica as vantagens de dois parâmetros pouco ou nada usados em estudos de ecofisiologia termal. Concretamente, as temperaturas extremas e a magnitude dos gradientes espaciais de temperatura.

O apêndice V consiste em um estudo do método de medição da resistência a altas temperaturas. Este foi necessário para informar as decisões metodológicas empregadas e a interpretação dos resultados na caracterização da temperatura crítica máxima nos lagartos Gymnophthalmi (capítulo 1).

Finalmente, os apêndice VI mostra uma associação entre o tamanho na área de distribuição e a evolução de ecomorfos fossoriais citada no capítulo II desta tese. Este estudo é fruto de uma viagem de curta duração à Austrália e representa o início da exploração das consequências biogeográficas da evolução dos ecomorfos fossoriais.

Como a maior parte dos dados ainda não foi publicada e esta está sob forma de tabelas, em alguns casos com milhares de entradas, estes não acompanham ao corpo da tese. Porém estão á disposição dos membros da banca, sob requisição.

Capítulo 1

Does fossoriality co-evolve with the requirement of lower temperatures?

Abstract

Burrowing snake-like reptiles are widely regarded as sensitive to high temperatures and impaired for achieving high body temperatures. However, no studies have explored the co-evolution of burrowing performance and thermal physiology in burrowing snake-like ecomorphs (BSLE). Herein, we perform such exploration, testing two premises derived from evolutionary physiology theory in this study case. First, morphology needs to correlate with differences in the capability of using of the soil's thermal gradient (i.e. burrowing performance). Second, differential use of the soil's gradient needs to be related to change in thermoregulation costs. For that, we tested if ecophysiological traits (burrowing performance, preferred (T_P) and critical maximum temperatures (CT_{MAX}) varied with ecomorph among ten Gymnophthalmid species. Then, we examined the premises contrasting the traits with a model of thermal variation within the soil of a region where BSLE have evolved. BSLE species burrowed deeper, but did not exhibit systematically lower CT_{MAX} or T_P . Examination of the premises showed that thermoregulation costs vary with burrowing performance but that achieving

ancestral body temperatures is not more difficult for species with better burrowing capacity within the study region.

Introduction

The evolution of the burrowing snake-like ecomorph (BSLE) is one of the most profound changes in the body plan of squamates (Bergman & Irshick, 2011). This evolutionary process involves a complex of changes in morphology, physiology and performance. Thus, it provides an opportunity of exploring the interaction between phenotypes and environment that leads to the evolution of complex phenotypes.

The acquisition of BSLE involves correlated processes of reduction in limb and tail length, body elongation and cranial modifications (e.g. Gans, 1975; Greer, 1991; 2002; Greer & Wadsworth, 2003; Shine & Wall, 2008; Wiens et al. 2006; Lee, 1998; Lee et al. 2008). Changes in behavior and performance includes increased burrowing performance (e.g. Benesch & Withers, 2002), leading to an increased use of the subsoil microenvironment. In this microenvironment, temperatures generally get cooler and more homogeneous as we go deeper, which has lead to the wide view that costs of maintenance of high preferred temperatures (T_P) are higher for BSLE, which in turn have evolved low T_P and critical thermal maximum CT_{MAX} (e.g. Brattstrom, 1965; Bury & Balgooyen, 1976; Greer, 1980; Withers, 1981, Avery, 1982; Fusari, 1984; Andrews & Pough, 1985; Huey & Bennet, 1987; Andrews, 1994; Lopez et al. 1998; Lopez et al. 2002; Rocha & Rodrigues, 2005; Sinervo et al. 2010; Clusella-Trullas et al. 2011). However, the co-

evolution of morphology, burrowing performance and thermal physiology has never been explored.

Evolutionary physiology theory raises, at least, three necessary premises for the co-evolution between morphology, performance and physiology across species. The first is that interspecific differences in morphology should be related to differences in locomotor performance and in turn, these differences should translate into differences in microhabitat use across species (links reviewed by Garland & Losos, 1994). BSLEs may be better burrowers than typically lacertoid lizards (e.g. Benesch & Withers, 2001). However, lacertoid ecomorphs may sand-swim or burrow quite well too (e.g. Maladen et al. 2009), making it unclear which part of the soil's thermal gradient are able to exploit. In addition, thermal gradients within the subsoil are more important in the first few centimetres (e.g. Geiger, 1950; Porter et al. 1973). Thus, it is not clear if an initial acquisition of snake-like morphologies generates any improvement over the use of the soils' thermal gradients.

A second premise is that costs of thermoregulation change across ecomorphs due to their differences in microhabitat use (e.g. like in Huey, 1974). For example, if costs for maintenance of ancestral preferred temperatures are lower for BSLE, then there should not be reason for expecting lower preferred temperatures associated to their evolution. To assess this premise, it is necessary to compare ancestral T_p of BSLEs with measures of the costs of thermoregulation.

While behavior often acts to increase the rate of evolutionary change, in other cases it can act to halt evolution (e.g. Huey et al. 2003). Accordingly, a third premise for the co-evolution of BSLE and thermal physiology is that

organisms have to acquire behaviors that exploit thermal opportunities offered by an evolved burrowing performance. For instance, BSLE may be better to reach cooler microhabitats, but if individuals maintain their use, of ancestral, high temperature microhabitats, then the co-evolution of low thermal requirements and morphology may become de-coupled. Many BSLE species are crepuscular or nocturnal (ex. Greer, 1980), suggesting that the co-evolution of BSLE and the requirement of low temperatures may be expected. However, tropical BSLE may be diurnal (Rocha & Rodrigues, 2005).

Herein, we use Gymnophthalmini lizards (Gymnophthalmidae) from Brazilian Caatingas to analyze the co-evolution between the acquisition of BSLE, burrowing performance and thermal physiology. This group comprises two monophyletic sister groups which correspond with lacertoid and BSLEs (Pellegrino et al. 2001, 2011). These species comprise the only acquisition of BSLE among Gymnophthalmini lizards (Pellegrino et al. 2001; 2011, Benozatti & Rodrigues, 2003). The study of this group has three advantages to understand co-evolutionary processes during the acquisition of the BSLE: first, Lacertoid and BSLEs are closely related and live in syntopy. Second, BSLEs and some lacertoid species of this group have very limited distributions (figure 1), permitting the estimation of the thermal environment that these species inhabit, and third, most of species are abundant, easily collected and maintained in laboratory.

Firstly, we tested whether thermal physiology co-evolves with morphology and then analyze two of the three premises derived from evolutionary physiology theory for the co-evolution of sets of traits: 1) if morphology also co-evolved with burrowing performance and therefore, BSLE

may use the soil's vertical thermal gradient differently from lacertoid species.

2) If better burrowing performance leads to a more restrictive or to a safer thermal environment. Implications of the third premise are discussed.

Methods

Fieldwork

During field trips, we collected lizards and measured soil temperatures. Lizards were collected by hand, detecting them by raking the leaf litter of 6 sites of Brazilian Caatingas: Saldanha (10°27'51.93"S, 42°35'40.97"W, mun. Poçoão, Bahia State, 400m a.s.l. in November 2009), Alagoado (9°29'19.65"S, 41°22'34.06"W, mun. Casanova, Bahia State, 400m a.s.l. in february 2010), Pedra vermelha (mun. Santo Inácio, Bahia state, 450mm a.s.l. in september 2010), Gameleira de Assuruá (11°18'6.78"S, 42°39'28.11"W, mun. Gentio d'Ouro, Bahia state 750m a.s.l. in September 2010), Vacaria, (10°40'38.22"S, 42°37'46.30"W, mun. of Xique-Xique, Bahia state, 400m a.s.l.) and Catimbau (8°35'29.23"S, 37°14'44.32"W, mun. of Buique, Pernambuco state, 750m a.s.l.). These sites comprise most of sandy spots occupied by Gymnophthalmi BSLEs at the Brazilian Caatingas. Lizards were collected at both seasons of the year (dry and wet). Fifteen lizards of each species were collected under the IBAMA license number (17086-2). All experimental procedures were done over different, partially overlapped subsamples of these 15 individuals, always including animals of both sexes and juveniles.

Temperatures of the subsoil 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 BSLE within the Gymnophthalmini. Both, latitudinal and altitudinal extremes were sampled in both seasons. Temperatures were registered by dataloggers AA Logbox, Supernovus® (precision 1°C), with a silvered, cylindrical aluminum probe (3 mm diameter by 3 cm long, 0.5 g). Given the low weight and high surface volume ratio of both, probe and species (snout vent length of most individuals was between 2-5 cm and weight between 0.2 and 1 g), we considered differences in thermal properties between lizards and the probe negligible when compared to the thermal variation registered (supporting online file). We sampled environmental temperatures at 4 different depths under bushes (surface, under leaf litter, 5cm and 10cm depth). Temporal sampling resolution was 1/10min. Every day, dataloggers' position was changed, so as to increase temporal independence of temperature measures within each site.

Captivity conditions

In the laboratory, animals were kept in 15x60x40 cm plastic terraria, separated by species but maintaining the groups found together in nature under single bushes during fieldwork. All terraria received UV (L12:D12) and heating (L10:D14) light. The later generated spatial thermal gradients in terraria that ranged from above to below the preferred temperatures of all lizard species examined. Temperatures at nights reached minima common at sampling sites (20-22 °C). All lizards were feed with termites, which were well accepted by all species and allowed standardizing known effects of diet type over T_P (Simandle et al. 2001a, and articles referred within). Water was

provided three times a week, directly sprayed into the terraria. Following recommendations by Garland and Adolph (1995), lizards were maintained for a week in terraria prior to initiate physiological measurements. The experimental procedures related here were approved by an official ethics committee at the Instituto de Biociências da Universidade de São Paulo. The number of individuals studied per species and trait, species, ecomorph, sampling sites and trait values are presented in the supporting information file (table S1).

Measurement of thermophysiological traits

Herein, we represent thermal physiology using two common descriptors of thermal physiology: preferred temperatures (T_P) and thermal limits (CT_{MAX}). We considered T_P as the mean value of the distribution of temperatures registered in unrestrained lizards within a laboratory thermal gradient (Licht, 1966, Lailvaux et al. 2003). We preferred this parameter to the mode (Crowley, 1987) because the distribution of T_P of the study species may show two modes. We also avoided the use of the median of an arbitrary segment of the distribution (Hertz et al. 1993) due to its subjectivity. Temperatures were measured on individual lizards during 11h (8:00 to 19:30). Lizards were individually confined within thermal gradients (60-50°C to 12-15°), inside of a quiet room with air temperature between 25-27°C and illuminated with diffuse light that was switched off 30 min before 19:00. Thermal gradients consisted of plastic boxes (90x15x16cm) with a base made of aluminum. The aluminum was covered by 1cm of plastic beads (1mm diameter). Beads allowed easy locomotion for all the species and prevented

abrasion of sand over the adhesive tape used for fixation of thermocouples on lizards' bodies. Under an end of the gradient, a 60W spot lamp heated the gradient from below. Under the other end, an ice bag was changed periodically at each 2.5 hours, maintaining temperature between 15-17 °C. A transparent acrylic lid closed the gradient from above and had a fine split through which the thermocouple passed in direction to a stand-alone, multiple entry datalogger (Novus Fieldlogger ®). During a pilot study, we saw that thermocouples inserted in lizards' cloaca changed importantly their behavior (they remained motionless or extremely agitated, leading them to entangle in the thermocouples). Differences in core-surface temperature in lizards with body wall less than 3 cm thick (Porter et al. 1973) and thermal inertia for animals lighter than 10g (Stevenson, 1985) have been considered depreciable. Gymnophthalmi lizards rarely exceed half a centimeter of body diameter and weight less than 1.2 g. Therefore, we adhered T type thermocouples (< 0.4 mm diameter) to the back of the lizard just above the pelvic cincture, using a double cover of surgical tape. Temperatures registered by thermocouples were calibrated against a mercury thermometer (supporting online material, figure S1).

We considered CT_{MAX} as the environmental temperature that inhibits righting response (Cowles and Bogert, 1944, Lutterschmidt & Hutchison, 1997). Lizards were heated individually (rate: 1°C/Min), inside of a cylindrical, aluminum chamber (600ml, 6cm diameter), submerged in a temperature controlled water bath. Start temperature was always 28°C degrees, which is within the preferred 50% thermal range of all the studied species. The chamber's temperature was measured using a fast-reading mercury

thermometer (Shult-zeiss®) in contact with the animals. Lizard movements and temperature inside of the chamber were continuously observed through transparent plastic lids that sealed the chamber from above. During a pilot, it was determined that loss of righting response in this group came when the animals bended their bodies over a side of the body, or rolled over their back. We thus used this observation to determine CT_{MAX} . When the animals exhibited one of these behaviors the temperature was read and the animals were released out of the chamber, lying on their backs on a cool surface. They were then cooled with fresh water and released in their terraria. Data from lizards that recovered movement immediately, died or showed clear symptoms of bad health 24h after the CT_{MAX} measurement where not analyzed (6 individuals in total). To control potential effects of daily cycles or body mass over CT_{MAX} (e.g. Ribeiro et al. 2012) during comparisons between ecomorphs, CT_{MAX} measurements were spread from 7:30 to 19:30 and all specimens were weighted before being warmed. None of these factors confounded CT_{MAX} comparisons among ecomorphs (Camacho, unpub. data). This was the last trait measured.

Measurement of burrowing performance

We estimated burrowing performance of lizards as the depth they attained in response to an experimental warming of sand. These lizards naturally burrow within the sand to hide or retreat (Camacho, unpub), so we simply placed lizards in individual wooden boxes (5x3x14 cm), filled with filtered, washed and dried sand from lizards' habitats, and allowed them to bury. With the lizards in the sand, we took calibrated digital X-ray

photographs and then warmed the sand surface using 300 W lamps. Sand was heated until the temperature of 42°C (well over preferred thermal ranges of *Gymnophthalmini* lizards studied) reached 2cm deep, as measured by a fast, Miller-Weber, mercury thermometer inserted in the sand. Another calibrated digital X-ray photograph was then taken and the depth was calculated measuring obtained photographs using the free source program Tracker V.3.2 (Cabrillo edu ®).

Modelling surface and subsoil environmental temperatures faced by *Gymnophthalmini* lizards

Environmental temperatures were interpolated between the different depths at which temperature was measured. For that, we used the gridfit function (D'Errico, 2005). This function is a noise reducing surface modelling code, which generates smooth estimation based on experimental data. Herein, we set the 'smooth' power equal to 5. This value included most variation observed in the field data and did not generate dynamics not observed in the field data. Using this function, we modelled the average daily temperatures reached at depths from the surface to 9 cm deep, at ten minute intervals, for all hours of the day. For easiness of calculation, data measured just under the leaf litter were considered as having the first depth section (=depth -1cm during interpolation). Due to this assignment, our thermal model extends to a depth of 9 cm. With the spatiotemporal grid of temperatures generated, 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. Matlab scripts for generating the thermal model and calibration of the temperature data are available under request.

Co-evolution of morphology and thermal physiology.

To test the relationship between ecomorph and thermal physiology (T_P , CT_{MAX}), we used a generalized least squares approach, estimating the importance of phylogenetic relationships through calculation of the lambda coefficient (Revell, 2010). The phylogeny was pruned from the last available phylogeny obtained for the family (Pellegrino et al. 2011). This phylogeny includes branch lengths, calculated by Bayesian procedures over molecular and morphological data.

Premise 1: Co-evolution of morphology and burrowing performance.

To test the relationship between ecomorph and performance, we followed the same procedure as the preceding paragraph.

Premise 2: relationship between burrowing performance, and thermoregulation costs.

We represented costs of thermoregulation in two ways: a) maintenance costs, which are inversely proportional to the daily time that individuals of a species would be able to find environmental temperatures within its T_P , b) potential heat shock costs, which represent the risk for individuals of these species of very small lizards of encountering environmental temperatures higher than its CT_{MAX} . To represent the thermal physiology of an ancestral Gymnophthalmi with the option to evolve

fossoriality, we estimated ancestral T_P and CT_{MAX} , using both, maximum likelihood (Schluter et al., 1997) and phylogenetic independent contrasts (Felsenstein, 1985), using the function `ace` of the R package “ape” (Paradis et al. 2004). Finally, we estimated the maintenance and heat shock costs of evolving better burrowing performance relating the achievable soil depth to maintenance costs and the occurrence of extreme temperatures.

Results

Co-evolution of morphology thermal physiology and performance.

Ecomorph did not co-evolved with T_P (Lambda: 1.04; Residual degrees of freedom=8; Coeff=1.794; sd=4.358; t-value=0.411; p=0.691), nor CT_{MAX} (PGLS: Lambda: 0.797; Residual degrees of freedom=8; Coeff=-0.813; sd=0.851; t-value=-0.955; p=0.367). Non-phylogenetic GLS analyses also gave non-significant results. Nonetheless, BSLE species of the genus *Calyptommatus* had lower CT_{MAX} than any other syntopic lacertoid and BSLE species (Table S1).

Premise 1: co-evolution of morphology and burrowing performance

Lacertoid species were less likely to burrow in response to warming: 1/8 of *Vanzosaura rubricauda*, 4/6 *Procellosaurinus tetradactylus* and 1/8 *Psilophthalmus paeminosus* escaped over the sand during warming. On the contrary, none of the BSLE tried to escape over the sand. All species of lizards reached deeper after warming (supporting material). Mean depth reached by each species was significantly related to ecomorph (Lambda= -0.395 (GLS: Residual degrees of freedom=8; Coeff=4.632; sd=0.351; t-

value=13.165; $p < 0.001$). All BSLE and only the lacertoid *Psilophthalmus paeminosus* reached deeper than 7 cm (supporting material).

Premise 2: relationship between burrowing performance, and thermoregulation costs.

Confidence intervals (95%) for ancestral states of T_P differed in range between the two methods of estimation used. Both methods gave broadly similar intervals for T_P and CT_{MAX} (ML: T_P : 26.8-31.5°C; CT_{MAX} : 45.7-47.0°C; IC: T_P : 28.4-30.0°C; CT_{MAX} : 45.5-47.2°C). T_P for an ancestral Gymnophthalmini were lower than that for most burrowing species studied here (see supporting material), which is a likely effect of the lower thermal preferences showed by species from higher altitudes of both ecomorphs (e.g. *Psilophthalmus paeminosus*, *Scriptosaura catimbau*, *Micrablepharus maximilliani*, see supporting online file). The spatiotemporal model of temperatures showed complex temperature dynamics of the first 9 cm within the subsoil. Mean subsoil temperatures within the T_P of most of the studied species extend to 9 cm or more and through the night (Fig.2). Maintenance costs monotonically decreased with achievable depth. However, the vertical distribution of maximum environmental temperatures showed a complex vertical curve (Fig. 3). For lizards unable to burrow deeper than 4-6 cm, the model indicates that remaining close to the leaf litter layer would provide a safer option to avoid critically high temperatures at the hottest hours. Maximal temperatures at 9 cm were closer to preferred temperatures of many of the species studied, but still high for an ancestral Gymnophthalmini.

Discussion

The first premise necessary for the co-evolution of morphology and thermal physiology holds for the studied species of gymnophthalmi lizards: changes in morphology were related to changes in burrowing performance which regulate the access to the subsoil's thermal gradient, strongly suggesting that Gymnophthalmi BSLEs have better access to subsoil's thermal gradient than their sister group of syntopic lacertoid species.

However, the second does not: maintenance costs of ancestral T_P were inversely related to achievable depth and heat shock costs might actually be higher within the sand than under the leaf litter. This is not to say that BSLE have lower maintenance costs than lacertoid ecomorphs, or that heat shock costs are always higher in the subsoil. Obviously, the three-dimensional thermal environment of a bush is more complicated than the spatiotemporal thermal model constructed by us, giving lizards other options for thermoregulation than going up or down in the soil. However, these results unequivocally show that the evolution of a burrowing lifestyle does not necessarily require lowering T_P , and that may still be pressures for high CT_{MAX} values for BSLE, at least at certain contexts (i.e. irradiated spots under bushes during the hottest hours of the day).

Based on global data from 396 species of reptiles, Clusella-Trullas et al. (2011) proposes a mean of 30°C for T_P of cryptozoic and burrowing reptiles, which was by far the lowest T_P of the ecological contexts compared in their work. Our data on T_P closely resemble that mean, suggesting that the evolution of fossoriality might be facilitated for species that already have low T_P . However, the species study group showed CT_{MAX} values about 9°C higher

than estimated by Clusella-Trullas et al. (2011). This suggests that species of both ecomorphs in our study group confront especially high thermal pressures over CT_{MAX} , compared to other cryptozoic and burrowing reptiles worldwide.

The examination of the third premise, contrasted with our results, shows a way in which many lizard species with potentiated burrowing performance might have evolved low CT_{MAX} . Our thermal model shows that the deeper within the subsoil, the longer environmental temperatures within ancestral T_P for Gymnophthalmini are accessible during the night. Thus, a better access to the subsoil environment may aid extending the activity period during the night without having to evolve lower T_P . Being active at night and expending the day inactive in deep soil may allow, among other things, decreasing pressures against encounters with extremely hot surfaces. Accordingly, whereas the diurnal lizards from genus *Scriptosaura* e *Nothobachia* show higher or equal CT_{MAX} values than lacertoid relatives, nocturnal species from genus *Calyptommatus* tend to show lower CT_{MAX} than syntopic species of both ecomorphs (Rocha & Rodrigues 2005, Camacho , unpublished data, suporting online file). Further, many cryptozoic and fossorial reptiles are crepuscular and nocturnal (i.e. Greer, 1980). Fossoriality has been proposed as an adaptation to avoid extreme temperatures existing in the surface of tropical soils (Rodrigues et al. 2010). However, our results suggest that a potentiated burrowing performance may be though as a pre-adaptation for nocturnality, which may lead to lower thermal pressures over CT_{MAX} , at least in in gymnophthalmids.

In conclusion, our results demonstrate, against the general view, that the subsoil environment may offer important thermoregulation options during

the evolution of BSLE and that they may be as thermally resistant as surface active lacertoid species.

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Legends

Figure 1. Phylogeny and approximated distribution areas of studied burrowing snake-like (red) and lacertoid (violet) ecomorphs within Gymnophthalmini lizards occurring at northeast Brazil. Circles show study sites. Blue colour represents sites around 800 m a.s.l and green those around 450 m a.s.l. 1= Catimbau, 2= Alagoado, 3= Saldanha, 4= Vacaria, 5= Pedra vermelha, 6= Gameleira. Distribution areas were generated using Maxent algorithm.

Figure 2. A spatiotemporal model of the distribution of mean temperatures measured under bushes inhabited by Gymnophthalmini lizards. Temperatures were measured at four isolated sandy habitats within the Brazilian Caatingas, at both seasons of the year.

Figure 3. Relationships between achievable soil depth and costs of thermoregulation. The left axis shows the number of daily hours of access to

environmental temperatures within hypothetical intervals of ancestrally preferred temperatures (APT) for Gymnophthalmini lizards from the Brazilian Caatingas. APT were calculated using Maximum likelihood (diamonds) and independent contrasts (x). Maximum temperatures line indicates the highest environmental temperature modelled for each given soil depth.

Figure S1. A. Relationships of temperatures measured with T type thermocouples and with a mercury thermometer within a water bath. B. Relationships of temperatures measured with data loggers and with a mercury thermometer in the same conditions as in A. C. Relationships of temperatures measured at dataloggers against Gymnophthalmini lizards of different species and weights, under different conditions of light (artificial and sun) and substrates (metallic, leaf litter and sand). Equations show relationships between raw (in black) and calibrated (in orange) temperatures against reference temperatures.

Figure 1

Figure 2.

Figure 3.

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Supporting online file

Does fossoriality co-evolve with the requirement of lower temperatures?

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Calibration procedures for thermocouples and dataloggers' sensors

Water temperatures within a controlled water bath were simultaneously measured using three different temperature registers. Those were: thermocouples used for estimation of body temperatures, dataloggers' sensors (PT100 wire model) used for measuring of environmental temperatures and a fast reading mercury thermometer. Inspecting the equation relating the temperatures measured in the thermocouples and the sensors (figures S1 and S2), we observed that only the temperatures from thermocouples needed calibration against the mercury temperatures. Thus, we used the equation in figure S1 to correct the temperatures measured by thermocouples.

After that, we used the thermocouples to register lizards' body temperatures during a second calibration of dataloggers's sensor temperatures against lizards's body temperatures, measured in two different microhabitats: sand and leaf litter, and in two light conditions: artificial and sunlight. Finally, by using the equation in figure S3, we calibrated the raw temperatures measured by the dataloggers' sensors against the mercury corrected temperatures, measured by the thermocouples within the lizards' bodies.

Figure S1. Calibration of thermocouples with mercury thermometers.

Figure S2. Relationship of sensor temperatures against a mercury thermometer in water.

Figure S3. Calibration of dataloggers's sensors with body temperatures of several species of gymnophthalmids (*Calyptommatus leiolepis*, *Nothobachia ablephara*, *Vanzosaura rubricauda*, *Procellosaurinus tetradactylus*, *Psilophtalmus paeminosus*) over different substrates (sand and leaf litter). Black dots represent raw temperatures registered by the dataloggers sensors and orange dots represent calibrated ones.

Table 1. Ecophysiological traits, number of specimens used and sampling localities of Gymnophthalmini lizards from Brazilian Caatingas. Abbreviations: N_ = number of specimens used for measuring each parameter, Tp= preferred temperatures, CTMAX= critical maximum temperature, Cdepth= depth reached by lizards before warming, Wdepth= depth reached by lizards after warming.

species	morph	weight(g)	N_Tp	Tp	N_CTMAX	CTMAX	N_depth	cdlength	wedgth	activity	localities
<i>Calyptomenoides fidelepis</i>	snake-like	0.9	7.0	32.2	12.0	44.7	6.0	5.8	7.3	nocturnal	Alagoado, Saltenha
<i>Calyptomenoides nicterus</i>	snake-like	0.7	9.0	31.0	9.0	45.8	8.0	3.3	8.1	nocturnal	Vazaria
<i>Calyptomenoides sinebrochionus</i>	snake-like	0.5	9.0	31.4	16.0	45.0	6.0	2.9	5.7	nocturnal	Pedra vermelha, Gamelêtra
<i>Scipiosaurus carinhau</i>	snake-like	0.6	9.0	26.7	8.0	46.4	8.0	3.7	6.0	diurnal	Catimbeau
<i>Notobatrachia albepihara</i>	snake-like	0.5	10.0	31.3	10.0	47.0	5.0	8.3	9.2	diurnal	Alagoado
<i>Procellosaurinus erythrocercus</i>	lacetoid	0.4	5.0	33.7	11.0	46.9	7.0	0.7	2.2	diurnal	Alagoado, Saltenha
<i>Procellosaurinus tetradactylus</i>	lacetoid	0.4	12.0	33.1	10.0	46.9	6.0	1.2	2.1	diurnal	Alagoado
<i>Vanzosaura rubricauda</i>	lacetoid	0.5	10.0	28.8	15.0	47.3	13.0	0.6	2.9	diurnal	Pedra vermelha, Vazaria, Saltenha, Catimbeau
<i>Microbelypharus mazzinikiani</i>	lacetoid	0.5	3.0	24.2	2.0	46.0	8.0	0.6	2.7	diurnal	Catimbeau
<i>Psilophthalmus poeninosus</i>	lacetoid	0.5	10.0	26.6	13.0	46.8	13.0	1.6	4.1	diurnal	Gamelêtra

Capítulo 2

Functional consequences of a snake-like body plan.

Abstract

The evolution of snakes and amphisbaenians are just two more instances of the widespread acquisitions of snake-like body plans among lizards. Further understanding of this process requires observing interactions between function and environment that may favor or restrict the evolution and persistence of species with a snake-like body plan. We combined virtual and experimental evaluations of the escape and feeding functions under contexts that help explain puzzling ecological and biogeographical traits of snake-like body plans in the Gymnophthalmidae family. Snake-like lizards exhibited lower anti-predatory success against visual predators, despite their behavior partially compensates that trend using opportunities brought by the environmental setting (leaf litter, loose soil). On the other hand, the feeding response of the snake-like lizards was superior in environments where buried prey was available. Contrary to previous authors, we propose that more than by better performance abilities, the evolution of snake-like lizards has been favored by opportunities given by specific environments.

Main text

The evolution of snakes and amphisbaenians are just two of the more than two tens of independent evolutionary acquisitions of the snake-like body plan among lizards (e.g. 1-3). This evolutionary process represents a major ecomorphological transformation of the tetrapod body plan, enticing biologists from many disciplines since more than a century (e.g. 4-12). Many of these studies agree in that the acquisition of a burrowing life is a fundamental step for the evolution of snake-like body plans in lizards (e.g. 2, 4, 15-19, but see 20). However, further understanding of this process requires knowing which functions and environmental settings may favor or restrict the evolution and persistence of species with a snake-like body plan (e.g. 2, 16).

Lizards with burrowing snake-like ecomorphs (BSLE) converge in having restricted distribution ranges in Australia and Brazil (7, unpub. data, respectively), suggesting BSLE present dispersive restrictions and confront higher extinction risk (8). To explain the persistence of BSLE, it has been argued that local selective pressures might actively preserve BLSE (9,10), which is in agreement with the observations of the higher abundance of these lizards in sandy soils of Brazil and Australia (11,12). Previously, BSLEs had been proposed as advantageous for efficient locomotion in loose substrates (15), or for feeding on buried prey (17, 21). However, the success of species with lacertoid and BLSE has never been compared during the execution of these functions.

We compared the success of lacertoid and burrowing snake-like ecomorphs (BSLE) performing two critical functions (escape from predators

and feeding). For that, we used the Gymnophthalmini lizards (Gymnophthalmidae, 22). This clade comprises two sister groups of burrowing snake-like ecomorphs and lacertoid species that have evolved in syntopy accross isolated patches of Quaternary sandy soils within the Brazilian Caatingas (11, Fig.S1).

To evaluate the success of the escape response, we first made separated measures of their escape behavior (to burrow, hide or run during escape trials), performance (sprint speed during horizontal escape in both hard and loose soil, and vertical escape through loose sand), and their morphology (relative length of body and tail, and tail colour). For details, see Suporting Online Material (SOM). BSLEs escaped burrowing more frequently than species of the lacertoid sister clade (PGLS: $n=10$, $\lambda=-0.188$, $\text{coeff}=1.107$, $t\text{-value}=10.020$, $p<0.001$). The context had a weaker, marginally significant effect over escape strategy ($n=10$, $\lambda=1$, $t=2.430$, $p=0.0454$): in the leaf litter, species tended to somewhat more hiding and burrowing and these differences were largely explained by phylogeny (SOM). BSLEs also exhibited lower running performance than lacertoid species (PGLS: $n=10$, $\lambda=-0.475$, $\text{coeff}=-52.299$, $t\text{-value}=-44.739$, $p<0.001$). We found significant effects of substrate type over horizontal escape performance of each ecomorph: sand favoured escape performance of BSLEs but impaired that of lacertoid species (phylogenetic t-test: $n=10$, $\lambda=-0.944$, $\text{coeff}=21.480$, $t=22.260$, $p<0.001$). With respect to horozintal escape, snake-like lizards were slower than lacertoid relative (phylogenetic t-test: $n=10$, $\lambda=-0.944$, $\text{coeff}=21.480$, $t=22.260$, $p<0.001$), a result which again presents a high phylogenetic signal. Our

results on performance (Fig.1a) agree with the general effect of relative limb size on speed in lizards (23-25) and it shows that sand may allow snake-like to perform better, but still much worse than lacertoid relatives. In this case, by burrowing during flight, behavior seemed to look for compensating a lower horizontal escape performance.

Videogames offer a way to model the effects of morphology and speed over antipredatory success, an exceptionally difficult task to do for multiple species *in vivo* (26, 27). Thus, we used a videogame, played by 145 persons in Brazil, Australia, Spain and U.S.A. to estimate the relative effects of behavior, performance and morphology, over the risk of being predated during visually directed predatory events (SOM). In short, the results of virtual predatory trials were described as a multinomial variable, in decreasing order of anti-predatory success: no catch, tail caught or body caught. The traits of the virtual lizards (morphology, behavior and performance) and of the virtual environment (background color) were stored in a dataframe. After that, an exhaustive model selection technique, based on both, Akaike's information criterion, selected after comparing 450 models the statistical combination of morphological, behavioral and performance traits that best explained the location of the attacks during the videogame trials. The best fitted model was:

$$\begin{aligned} \text{Anti-predatory success} = & \text{Speed} + \text{Relative tail size} + \text{tail contrast} \\ & + \text{relative body length:Speed} + \text{Relative tail size:Speed} + \text{Relative tail} \\ & \text{size:relative body length} + \text{Strategy:relative body length} + \text{Tail} \\ & \text{contrast:Relative tail size} \end{aligned}$$

Where “:” means interaction among terms of the equation. However, other 12 models showed AIC values within 2 points of difference. The

parameters of the selected model were fitted using a probit link function with the same data to generate it (results in table S1). A relatively major and positive effect of performance (speed) and a secondary negative effect of relative body elongation over anti-predatory success (see also Fig. 3). Striking empirical support for the model comes from Downes & Shine (28), who found that tail loss (and consequently, relative body lengthening) was responsible for increasing rates of visually directed predation in another species of small terrestrial lizard. The model also recovered the interaction of tail color and tail size, showing that colored tails help directing attacks away from the body. These results are again supported by findings that plastic models of lizards receive more attacks from birds at colored parts of the body (29). Finally, despite an optimistic simulation of burrowing behavior in which lizards do not decrease speed during burrowing, compensated only partially the effects of speed and body relative length (Fig.3).

We then fed the obtained model with the performance, morphological and behavioral traits of the study species and analysed the effect of morphotype evolution over escape success. Anti-predatory success was lower in BSLE, suggesting that their escape behavior is not enough to overcome the differences in morphological and locomotor traits existent between BSLE and typical lizards (Fig. 3a). As both, energetic (30) and escape costs (considering them as inversely related to anti-predatory success) are not lower in BSLE, we suggest, differently to Gans (15), that serpentiform locomotion is not necessarily more efficient than tetrapod locomotion, even in loose soil, and that the evolution of BSLE from tetrapods requires specific environments which reduces the probability of being

detected by visual predators (e.g. the leaf litter, loose soil) or options for behavioral strategies relying on a high speed.

To assess the relative success of Gymnophthalmini BSLEs, and also potential trade-offs, we evaluated the efficiency of lizards preying upon buried and non buried termites, and slow and fast crickets (SOM). BSLEs only differed from lacertoid sister taxa in that they ate more buried prey (PGLS: $n=10$, $\lambda=1.129$, $\text{coeff}=0.759$, $t=5.445$, $p=0.003$), a difference highly explained by phylogeny. Additionally, we estimated prey abundance and diversity along 122 measures of the proportion of prey items and prey categories existing over and within the subsoil. Both, vegetation (estimate= -0.849, $p<0.001$) and depth (estimate: 0.545, $p<0.001$) in the soil had significant effects and interaction (estimate= -0.314, $p=0.015$) over the diversity of potential prey items ($df: 127$ $R^2=0.359$, $F: 25.33$ $p<0.001$). The same happened for abundance of prey items: vegetation (estimate= -2.012, $p<0.001$) and depth (estimate: 1.228, $p<0.001$) in the soil had significant effects and interaction (estimate= -0.881, $p=0.003$) over the diversity of potential prey items ($df: 127$ $R^2=0.294$, $F: 19.12$, $p<0.001$). A visual examination of these results indicate that the leaf litter layer existent under the vegetation is the most profitable of the compared microenvironments in terms of feeding resources at the study region (Figure S5).

Integrating feeding performance and the availability of feeding resources underground measured at the localities they inhabit, snake-like lizards gained significantly more access to both, abundance (PGLS: $n=7$, $\lambda=0.070$, $\text{coeff}=0.154$, $t=4.470$, $p=0.006$) and diversity of prey (PGLS: $n=7$, $\lambda=0.774$, $\text{coeff}=0.182$, $t=3.302$, $p=0.021$), the two λ values

of these models show a great variation, a point which will be further discussed in the last paragraph. Our results support the hypothesis that the evolution of BSLE has been benefited by a greater access to hidden or buried feeding resources (17, 18). However, most feeding resources were found in the surface, which does not support a hypothesis of selective pressures favoring specialization for feeding underground. Accordingly, Gymnophthalmid lizards have maintained diet composition despite the important cranial changes associated to the evolution of BSLEs (31).

Taking all that into account, we argue that specific environments may favor the evolution and persistence of BSLE by both, relaxing selective pressures due to visual predation and bringing opportunities for feeding underground. That may partially explain the smaller distribution ranges in BSLE among Gymnophthalmids, (Camacho et al. unpublished) and australian scincids (7) and why they are so persistent and abundant within areas with sandy soils (11, 12).

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Fig. 1. Comparative A) horizontal escape speed among lacertoid and burrowing snake-like species of Gymnophthalmi lizards from Brazilian Caatingas. B) Comparison of predatory performance capturing buried termites (a maximum of five per trial). Species names, from left to the right: *Calyptommatus leiolepis*, *C. nicterus*, *C. sinebrachiatus*, *Scriptosaura catimbau*, *Nothobachia ablephara*, *Psilophthalmus paeminosus*, *Micrablepharus maximilianni*, *Vanzosaura rubricauda*, *Procellosaurinus tetradactylus*, *P. erythrocerus*. This last species was not submitted to the feeding experiment.

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Fig. 2. Attack location over virtual lizards during videogame trials played by humans. A) shows the relationship between attack location and lizards' speed. B) shows the relationship between attack location

and relative snout vent length, with an interaction with escape behavior. C Shows the effect of tail contrast, measured in RGB points of difference with the background. D shows the effect of relative tail size measured as proportion of the total body length. A loess function provided the smoothing between the average value of the dependent variable at each of categories representing attack location. The grey area represents the standard deviation of the dependent variable at each category.

Fig. 3. Comparative success in antipredator and feeding responses of syntopic lacertoid and burrowing snake-like species of Gymnophthalmini lizards. Left panel contains data from five species in each box plot. Right panel contains data from 4 lacertoid and 5 burrowing snake-like species.

Supporting online material

Functional advantages of the evolution of the snake-like body plan

Methods

Study sites

During field trips, we collected lizards at 6 sites of Brazilian Caatingas: Saldanha (10°27'51.93"S, 42°35'40.97"W, mun. Poçoão, Bahia State, 400m a.s.l. in November 2009), Alagado (9°29'19.65"S, 41°22'34.06"W, mun. Casanova, Bahia State, 400m a.s.l. in february 2010), Pedra vermelha (mun. Santo Inácio, Bahia state, 450mm a.s.l. in september 2010), Gameleira de Assuruá (11°18'6.78"S, 42°39'28.11"W, mun. Gentio d'Ouro, Bahia state 750m a.s.l. in September 2010), Vacaria, (10°40'38.22"S, 42°37'46.30"W, mun. of Xique-Xique, Bahia state, 400m a.s.l.) and Catimbau (8°35'29.23"S, 37°14'44.32"W, mun. of Buique, Pernambuco state, 750m a.s.l.). These sites represent most of sandy spots where Gymnophthalmiini BSLE and lacertoid species are syntopic at the Brazilian Caatingas. Lizards were collected at both seasons of the year (dry and wet). Fifteen lizards of each species per site were collected under the IBAMA license number (17086-2).

Species accounts

Gymnophthalmini species from Brazilian caatingas compose two monophyletic sister groups which correspond to lacertoid and BSLE, comprise the only acquisition of BSLE among Gymnophthalmini lizards (1,2). Both ecomorphs share the leaf litter existent under sparse vegetation at a few sandy spots within the Brazilian Caatingas (e.g. 3-5), from which all known BSLE and at least three lacertoid species (*Psilophthalmus paeminosus*, *Procellosaurinus tetradactylus*, *Procellosaurinus erythrocerus*) are endemic from that region. The open habitat they inhabit and the diurnal habits of most of them (e.g. 4) suggest important incidence of visually oriented predation (e.g. 6). The species study group comprises all known species of BSLE within Gymnophthalmini, except for *Calyptommatus confusionibus* (Rodrigues *et al.* 2001,7), and all the described lacertoid species of the group which maintain syntopic populations with BSLE species.

Captivity conditions of specimens

During field work, specimens were kept individually or in small groups in plastic bags with substrate from the exact place where they were collected. A lamp maintained thermal and light gradients within the bags. Animals were fed with termites and animals that came with the substrate, and also softly watered each two or three days. In the lab, animals were kept in 15x60x40 cm plastic terraria, separated by

species but maintaining the groups found together in nature under single bushes during fieldwork. All terraria received UV (L12:D12) and heating (L10:D14) light. The later generated spatial thermal gradients in terraria that ranged from above to under lizards preferred temperatures and temperatures at nights reached minima common at sampling sites (20-22 °C). All lizards were feed with termites, which were well accepted by all species and allowed standardizing known effects of diet type over T_P (8, and articles referred within). Water was provided three times a week, directly sprayed into the terraria. Following (9), lizards where maintained for a week in terraria prior to initiate physiological measurements. The experimental procedures related here were approved by an official ethics committee at the Instituto de Biociências da Universidade de São Paulo.

Due to limitations of the collecting permit (IBAMA number 17086-2), all experimental procedures were done over different, partially overlapped subsamples of 15 individuals, always including animals of both sexes and juveniles. Any specimen visually unhealthy (skinny or slow) was excerpted from the experiments.

Experimental procedures

Measurement of escape behavior, escape performance and estimation of success of the escape response.

First, the escape behavior of specimens was observed within 48h of collection, in standardised experimental conditions. The

experimental set up consisted of an opaque plastic box (measures 13x35x29 cm). The bottom of the box was covered by approximately 3 cm of sand and a half of it was also covered by leaf litter from some local patch inhabited by Gymnophthalmini lizards. During each trial, a lizard was drop at one of the two contexts generated: over sand or over sand plus a leaf litter layer. Its first escape response was considered what the animal did immediately after dropping it inside the box. The response was given the value 3, if the animal burrowed completely, 2, if the animal did leave an exposed part of the body, or only entered under the leaf litter layer; or 1, if the animal kept running after the first 2 or 3 steps. The escape strategy of each species within each context was considered the average of the strategies observed among all individuals of that species.

Escape performance is represented here by sprint speed. To measure it, lizards were chased within plastic enclosures (45x50x10cm), over two substrates: sand glued on a woodcard (9) and loose soil. To construct the loose soil arena, a sand layer of 2-3 millimeters was added to the sand glued. That was enough to allow lateral support for movement of BSLE and avoid that escaping lizards burrowed under the sand. Potential effects of different thermal optima and loss of motivation were addressed making lizards run in randomly ordered sequences of five different temperatures (22, 27, 32, 37, 42). Each lizard run at one temperature per day, during the normal activity hours of its species, with 2 daily runs per lizard, substrate and temperature (four runs/day/lizard in total). Trials were recorded using a

high speed video camera (Redlake MotionXtra HG-SE, 250 frames per second) illuminating with fluorescent lamps. This procedure generated 2054 videos which were analysed using the program tracker 3.0 (Cabrillo edu®). With that program, linear individual speeds were calculated as the distance run between 3 steps of rectilinear run. The best run for each lizard/substrate among all temperatures was used. Species escape performance for each substrate was calculated as the average of the highest individual speeds of a given species in a given substrate, across all temperatures measured.

To generate a model with which evaluate the success of the escape response, we constructed a videogame using the ActionScript3 programming language (Flash software). Voluntaries played individually, catching as many lizards as possible. For that, they relied only on visual clues and they were not informed (but allowed to learn along the way) about escape strategies of our virtual lizards. During a game session, 50 autotomic lizards run, one after the other, over a standardized distance with random direction and origin of the escape route, colour of the background, tail and body, relative length of body and tail, and speed. In this way we tried to imitate a diversity of predatory contexts. Half of the lizards presented the same characteristics but different escape behavior: they became invisible but remained still catchable in the middle of the standardized distance. In such way, we tried to model the quick and anti-predatory burrowing that Gymnophthalmini lizards showed during collection and escape behavior trials. One hundred and twenty six persons from Australia,

Brazil, Spain and United States played this game using different devices (mouse or touchpad in desktops, laptops and Ipads), thus generating a considerable amount of variability in predator efficiency. For each session, a table with the characteristics of each lizard, and the location of the attack (body, tail or failure) was generated and automatically sent to the e-mail address of one of the authors. The game is playable at <http://lizardgame.appspot.com/>. It requires login with a gmail account and it will send gaming session' results and player's nickname to A.C.G.

Measurement of feeding performance and availability of feeding resources.

We evaluated feeding performance within four prey contexts, buried, epigeal, slow and fast prey. For the first context, we placed, individually, lizards in plastic boxes containing 5 termites buried under two centimeters of clean sand from their habitats, a reachable depth by all the studied lizards (A.C. unpub. data). A previous observation confirmed that termites were unable to neither resurge from this deep nor dig into the sand. After 24h, we counted how many they ate. As control, for each lizard, we repeated this procedure with 5 termites left above the sand. For the slow-fast prey examinations we used captive raised hatchling crickets, which often present injured legs. In this case, we preferred to evaluate slow and fast prey at the same time because even if an ecomorph was worse to eat a class of prey, they would be able to prey over all of them along the 24 hours of the trial, if there was

no other better option. So, to increase our power to truly differentiate their capabilities to eat over these two prey contexts we put, in each box, 5 normal crickets and 5 injured ones. To stimulate lizards to eat all the food, we left lizards without food for 5 days before trials.

Estimating the gain promoted by feeding performance requires estimates of prey availability too. For that, we measured the abundance and diversity of potential prey at all combinations of two treatments with two levels each, (depth: over and under soil level, vegetation: under bushes or trees and at open sand) in 128 standardized samples distributed along the four sites. Each sample consisted in, using a shovel, quickly collecting a litre of leaf litter in a small area of less than 50cm over the ground, and then, within a distance of one meter, another litre of soil collected to a depth of 10 cm (shovel length) right under the leaf litter. The same day of collection, each sample was carefully examined by the first author in a white dish. Lizards are very generalistic predators (11), so we considered potential prey all the complete invertebrates smaller than 5 mm were counted, identified to traditional feeding categories (12), and preserved in alcohol. Failed predation observed in laboratory of pupae of 5mm in minimum diameter suggested difficulties for these lizards to prey over bigger prey. The relative feeding success of each species was calculated as the relative gain in number of prey items or prey types. It was calculated multiplying the average species' performance consuming buried prey by the relative amount of buried prey, in localities inhabited by each species. Availability for *Calyptommatus*

nicterus was estimated 40 km from the place they were collected, in an area inhabited by *C. sinebrachiatus*.

Analyses

All comparative analyses performed to test the relationship between morph and any trait, except when specifically described in the pertinent section, followed the same procedure in R software (13). These consisted in constructing a newick file of the Rodrigues clade with branch lengths, using the package “ape” (14). With the tree, we used the packages geiger (15) and nlme (16) to fit a linear model estimating the test statistic and the phylogenetic dependence at the same time. The latter is measured by Pagel’s lambda (17), and calculated via the function “corrPagel”, from the “ape” package, under restricted maximum likelihood and a Brownian model of evolution. Estimating both parameters at the same time has been found to be better than non phylogenetic fitting of linear models (18).

Analysis of escape strategy, escape speed and estimation of success of the escape response.

Escape behavior

After testing the relationship between ecomorph and escape behavior, we used a phylogenetic t-test (19) to test if the environmental

context (sand versus leaf litter) affected escape strategy, using the package “Phytools” (20).

Escape performance

After testing the relationship between escape performance and ecomorph, we tested the interaction between morph and escape context (loose versus glued sand) relating morph and the species' average difference in escape performance between the two contexts.

Modelling escape success during the videogame sessions

We ranked the results of each virtual trial, constructing an ordered multinomial variable of escape success with three levels (escape=2, tail attack=1, body attack=0). Then, we used the package “gmulti” (21) to generate the best fitting model for predation risk as a function of continuous variables (relative snout-vent length, relative tail length, tail contrast, speed) and a factor (escape strategy, with two levels) of the virtual lizards using the fitting function “polr” from the package MASS (22). Once generated, the best model was fitted to know the coefficients of each term. Finally, the model was fed with real morphological, performance and behavioral traits of the study species lizards to calculate the probability of species success in a visually oriented predatory event. The only morphological trait considered relevant by the model was relative snout vent length, which we took

from Grizante et al. (23, a work which included, among others, animals from our experiments). As performance trait, we used sprint speed (explained above). For behavior, we used a categorical factor with two levels: burrowing and not burrowing. We did not use hiding because the game shows a situation in which the lizard is chased over open sand, where these lizards rarely choose that escape strategy (Table S1). Once escape success was calculated for each species, we tested the relationship between morph and escape success. For simplicity, body colour was not analyzed, given the overall cryptic body colour among all the studied species. Size and speed was decoupled in this videogame, as it was the case for the studied ecomorphs.

Analysis of feeding performance and success of the feeding response in nature.

We tested the relationship between morph and feeding performance in the different experimental contexts experienced (buried, epigeal, slow and fast prey). As fast and slow prey were analysed in a single experiment, we analysed them together generating an index of prey electivity. This index was generated subtracting the proportion of slow prey ingested to the proportion of fast prey ingested. The index was calculated for all the individuals of each species and species' values were averaged.

Differences in the ecological distribution of the abundance and diversity of prey items were assessed fitting generalized least squares

of the effects of habitat (under vegetation or in the open) and microhabitat (surface or underground) over both, abundance and diversity of prey items.

We only found differences in feeding performance between morphs with respect to consumption of buried prey. Thus, we only estimated feeding success for feeding over buried prey. We calculated feeding success multiplying feeding performance of each species during experimental trials by the fraction of total prey abundance and diversity that was found underground.

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Table S2. Relative effects of the terms of a model of antipredatory success against visual predators. The coefficients were obtained fitting a multinomial ordered model, via probit transformation, over data from 4900 virtual lizard hunting trials played by 126 persons from 4 different continents.

Terms	Value	Std.Error	t-value
Speed	-0.36	0.0248963	-14.53
relative tail size	-2.10	0.0970386	-21.7
tail contrast	-0.00	0.0005308	-1.836
speed : relative body length	0.00	0.0003368	2.944
speed: relative tail size	0.09	0.0123086	7.357
relative tail size : relative body length	0.02	0.0034543	6.914
relative body length : behavior	-0.00	0.0007458	-7.074
relative tail size : tail contrast	0.00	0.0004883	2.05

Fig.S1. Aspect and phylogeny of the species study group. Photographs show typical microenvironments where these lizards inhabit within the Brazilian Caatingas. A shows a “Carrasco”, a low and dense vegetation type abundant at relatively high altitudes within the Caatinga. B shows a “Carnaubal” ,a vegetation type primarily composed by the palm carnauba “*Copernicia prunifera*” which happens at lower altitudes and next to water bodies. C shows the shrubby vegetation structure dominant at sandy soils all across the studied region.

Fig. S2. Escape behavior chosen by 10 species of lizards of two ecomorphs (lacertoid and snake-like) under standardized conditions in which all the escape options were always possible. Treatment represents the context in which the lizard was left to escape. These are the environmental contexts in which these lizards inhabit in nature. Box-plots show interspecific variation in the frequency of each escape behavior in each case. Horizontal lines within box-plots show the median of the frequency distribution.

Fig. S3. Distribution of feeding resources across a combination of microhabitats and position, with respect to the soils's surface.

Conclusões

Este estudo integrou observações funcionais, morfológicas, ecológicas e virtuais com objetivo de explicar a evolução do ecomorfo serpentiforme fossorial no nível interespecífico, em espécies da tribo Gymnophthalmini. Isto quer dizer que suas conclusões podem ajudar a explicar porque as espécies do clado Gymnophthalmini têm persistido até nossos dias nos habitats que ocupam através de sua capacidade de executar funções importantes para sua sobrevivência tais como a termoregulação, escape de predadores e alimentação. Os apêndices desta tese são artigos decorrentes das pesquisas realizadas durante a tese que permitiram atacar conceitual e metodologicamente o problema planteado por ela.

Quanto à termoregulação, a teoria termobiológica dos ectotermos (apêndice III) prediz que a combinação do comportamento, fisiologia e morfologia dos indivíduos tenderão a aumentar a proporção do tempo de vida realizado dentro das temperaturas preferenciais, assim como evitar as temperaturas críticas. Assim, manter o corpo dentro das temperaturas preferenciais por mais tempo e evitar choques de calor por contato com superfícies a temperaturas extremas aparecem como dois problemas fundamentais para a termoregulação em pequenos animais ectotermos terrestres (apêndice IV). Estes problemas podem ser estudados mais eficientemente através da comparação de gradientes espaciais de temperatura e da ocorrência de temperaturas extremas com características

da morfologia, fisiologia térmica e o comportamento de espécies sob estudo (apêndice V).

Quanto à manutenção de temperaturas preferenciais, este estudo demonstra que os ambientes onde os ecomorfos fossoriais são endêmicos apresentam importantes microgradientes térmicos no subsolo (apêndice V). Estes microgradientes permitem aumentar o número de horas diárias despendidas dentro das temperaturas preferenciais a animais com uma melhor capacidade de enterramento, característica apresentada pelos ecomorfos fossoriais estudados (capítulo 1). Deste modo, os dados levantados sugerem que os ambientes arenosos, secos e quentes onde os Gymnophthalmini fossoriais são endêmicos não ofereçam importantes restrições termoregulatórias associadas à evolução de ecomorfos fossoriais.

Quanto à evitação de temperaturas extremas, este estudo demonstrou que temperaturas letais para lagartos Gymnophthalmini (ex. acima de 45 °C) são freqüentes perto da superfície do solo em regiões habitadas por ecomorfos fossoriais dentro da família Gymnophthalmidae no Brasil (apêndices, I, II, V, capítulo 1). Além disso, a maioria dos artrópodes amostrados neste estudo se concentram na superfície (capítulo 2). Isto pode estar relacionado ao fato de que todas as espécies de Gymnophthalmini que forrageiam durante o dia nas regiões de estudo, incluindo as fossoriais (ex. *Nothobachia ablephara* e *Scriptosaura catimbau*) possuam altas temperaturas críticas máximas (capítulo 1). Deste modo, a evolução do morfotipo fossorial e a capacidade de enterramento não parece suficiente para evitar temperaturas extremas em Gymnophthalmini, como sugerido por Rodrigues et al. (2009). Porém, o subsolo destas regiões, no clima quente e

seco da Caatinga, mantém temperaturas dentro dos espectros preferenciais das espécies de Gymnophthalmini durante a noite. Este fato pode ter favorecido a evolução da noturnidade em lagartos com maior capacidade de enterramento, como é o caso de *Calyptommatatus* (Rocha & Rodrigues, 2005) e outros lagartos fossoriais, tais como no gênero *Bachia* (apêndice II) no Brasil e em lagartos australianos (Greer, 1980). Ao passar as horas mais quentes do dia enterrados em locais mais frios e forragear durante a noite, encontros com superfícies em temperaturas extremas provavelmente se reduzem. Isso pode explicar as temperaturas críticas máximas mais baixas em *Calyptommatatus* e *Bachia* (capítulo 1 e apêndice II, respectivamente) e outros lagartos fossoriais (Greer, 1980; Clusella-trullas et al. 2011).

Quanto ao escape de predadores, espécies com ecomorfo fossorial nos Gymnophthalmini possuíram menor velocidade de locomoção e maior capacidade e tendência a enterrar-se (apêndice II, capítulo 2). Além disso, a presença de uma capa de folhiço no ambiente onde estes animais têm evoluído faz com que lagartos dos dois ecomorfos comparados usem mais o comportamento de esconder-se ou enterrar-se para fugir de predadores (apêndice II, capítulo 2). Por último, as observações feitas com o jogo sugerem que enterrar-se é mais efetivo para animais mais lentos e de corpo alongado frente a predadores visualmente orientados (capítulo 2). Porém, o efeito de enterrar-se foi baixo comparado com a velocidade de fuga (capítulo 2). Por este motivo, espécies com o ecomorfo fossorial apresentaram sistematicamente um menor sucesso de fuga frente a predadores visualmente orientados (capítulo 2). Estes resultados sugerem que a evolução do comportamento de enterrar-se pode ter sido favorecida por

habitar ambientes que já apresentam ao mesmo tempo obstáculos para o escape em alta velocidade e opções de esconder-se. Ao mesmo tempo, a menor velocidade dos ecomorfos fossoriais sem areia e seu baixo sucesso de escape frente a predadores visualmente orientados sugere que seja difícil que estes sobrevivam sem ambientes que lhes permitam manter-se escondidos (ex. folhiço ou areia).

Em relação à captura de presas, espécies com o ecomorfo fossorial capturaram mais presas enterradas que lagartos lacertiformes, sem aparente diminuição do desempenho na captura de presas rápidas ou localizadas na superfície (capítulo 2). Isto fez com que tivessem acesso a uma maior quantidade e diversidade de presas, levando em conta uma estimativa de abundância de presas no subsolo, nas localidades onde estes lagartos habitam (capítulo 2). Porém, a quantidade de presas foi quase sempre maior na superfície, o que não sugere a existência de pressões seletivas para alimentar-se sob a terra.

Em resumo, habitats secos e quentes, com solo solto, folhiço e presas no subsolo parecem favorecer a persistência de lagartos com ecomorfos fossoriais, dadas suas características morfológicas, fisiológicas, comportamentais e de desempenho na fuga e na escavação. O fato da área de distribuição de ecomorfos fossoriais ser menor em lagartos *Lerista* australianos (apêndice VI) e gymnophthalmídeos brasileiros (fig. 1, capítulo I) sugerem que houve pouca dispersão posterior à aquisição deste ecomorfo. Isso faz pensar que as características ambientais que hoje favorecem a persistência destes ecomorfos possam ser relativamente similares às que permitiram ou favoreceram sua evolução. Porém, os motivos que levam à

fixação das características típicas do ecomorfo fossorial em lagartos ainda precisam de estudos intraespecíficos. À medida que descobrimos os correlatos funcionais e biogeográficos da aquisição do ecomorfo fossorial, abrem-se as portas para explicar a evolução desta dramática transformação ecomorfológica nos Squamata.

Resumo

A evolução da fossorialidade (capacidade de locomover-se no subsolo) em lagartos está associada à evolução ecomorfos serpentiformes fossoriais. Esta é uma das mais importantes transformações ecomorfológicas nos lagartos e, pelo tanto, seu entendimento é necessário para explicar a evolução da diversidade morfológica nos Squamata. Porém, a falta de dados comparativos funcionais impede um melhor entendimento de quais funções ou capacidades foram beneficiadas pela evolução de ecomorfos serpentiformes fossoriais, de como co-evoluíram a morfologia, comportamento e fisiologia durante a aquisição de ecomorfos fossoriais e de quais ambientes favorecem a evolução e persistência destes ecomorfos. Aqui são apresentados dados funcionais de 10 espécies de lagartos Gymnophthalmideos da tribo Gymnophthalmini. As espécies estudadas estão filogeneticamente divididas por dois grupos monofiléticos, os quais estão compostos por espécies lacertiformes ou serpentiformes fossoriais. Pares de representantes de ambos grupos convivem em umas poucas localidades de habitat arenoso da Caatinga.

Neste estudo foram avaliadas: a capacidade de termoregular mediante escavação, de escapar de predadores visualmente orientados e de alimentar-se em diferentes contextos e tipos de presas. Para isso foram mensuradas: 1) a profundidade da escavação durante um choque térmico experimental, 2) a tolerância e preferência térmicas, 3) a escolha do modo de fuga e sua dependência do ambiente na qual a fuga é realizada (areia e

folhiço), 4) a velocidade de escape e sua dependência da temperatura e substrato (areia solta e solo duro) durante escape na horizontal, 5) o desempenho na alimentação de presas epígeas e enterradas no substrato, assim como de presas rápidas e lentas. Além disto, para avaliar potenciais restrições ou oportunidades do ambiente sobre a termoregulação destes lagartos foi gerado um modelo espaço-temporal da distribuição e dinâmica das temperaturas ambientais enfrentadas pelos lagartos estudados. Para avaliar o sucesso de escape de lagartos fossoriais e lacertiformes foi gerado um videojogo que simulava as condições de fuga destes animais. Por último, a disponibilidade de presas acima e abaixo da vegetação foi estimada em regiões onde lagartos fossoriais são endêmicos e convivem com espécies lacertiformes.

Os resultados indicam que: 1) que a evolução de ecomorfos fossoriais na tribo Gymnophthalmini tem levado a uma maior eficiência no enterramento, a qual tem aumentado as opções para termoregular escavando. Porém, a capacidade de enterramento *per se* não tem co-evoluido com a fisiologia térmica, sugerindo que mudanças evolutivas do comportamento (aquisição de hábitos noturnos) é necessária para evitar temperaturas extremas. 2) Ecomorfos fossoriais são piores no escape a predadores visualmente orientados. 3) Ecomorfos fossoriais têm maior acesso a recursos alimentares enterrados que espécies lacertiformes. Estes resultados permitem explicar porque lagartos fossoriais da tribo Gymnophthalmini, tem áreas de distribuição menores, persistindo apenas em ambientes quentes, secos, e com solo solto e folhiço. Estes ambientes lhes

oferecem opções para termoregular, evitar predadores visualmente orientados e aproveitar a disponibilidade de alimento enterrado.

Abstract

The evolution of fossoriality (ability of underground locomotion) in lizards represents one of the three main evolutionary pathways for the acquisition of burrowing snake-like ecomorphs (BSLEs). This is a major ecomorphological transformation among lizards, which makes its understanding fundamental to explain the evolution of diversity within Squamata. However, the lack of comparative data on function of typically lacertoid and BSLEs prevents a better understanding of the functions that actually benefitted from the evolution of a BSLE, the co-evolution of morphology, behavior and physiology during that process, and what environments favour their evolution or persistence of BSLEs, behavior and physiology during this process. Herein, we provide functional comparative data for 10 species of Gymnophthalmidae lizards from the Gymnophthalmini tribe. The study species group splits into two sister groups of monophyletic branches, each one being composed by either, lacertoid or BSLE species. Different pairs of species of the two branches live in syntopy and are endemic at a few localities of sandy habitats within the Caatingas.

During this study it was measured: 1) the vertical burrowing performance, 2) thermal tolerance and preference, 3) escape behavior and its dependence of environment (leaf-litter and open sand), 4) escape speed and its dependence of temperature and substrate (loose versus glued sand), and 5) feeding performance over buried, epigeal, fast and slow prey. To estimate environmental opportunities and restrictions for the evolution and persistence

of BSLE, it was generated a model of the distribution and dynamics of soil temperatures. To compare relative success of escape to visual predators, it was generated a videogame which simulated escape conditions of these animals. Finally, the availability of prey over and under the soil was estimated in regions where these BSLEs and lacertoid species coexist. Results show that: 1) The evolution of BSLE led to better burrowing abilities in Gymnophthalmi, increasing options for thermoregulation within the sub-soil. However, a better burrowing ability did not co-evolve with thermal physiology, suggesting that a change in behavior (the acquisition of nocturnal habits) is necessary to relax thermal pressures existing over BSLEs. 2) BSLEs were worse avoiding visually oriented predators. 3) BSLEs have more access to buried feeding resources than their lacertoid relatives. These results allow explaining why Gymnophthalmi BSLEs have smaller distribution areas and have persisted only in warm, dry habitats with protective microenvironments (loose sand and leaf litter). These environments offer them special opportunities for thermoregulation, avoiding visual predators and profit on buried feeding resources.

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Apêndices

A new species of the lizard genus *Bachia* (Squamata: Gymnophthalmidae) from the Cerrados of Central Brazil

MIGUEL TREFAUT RODRIGUES¹, AGUSTÍN CAMACHO¹, PEDRO MURILO SALES NUNES¹,
RENATO SOUSA RECODER¹, MAURO TEIXEIRA JR.¹, PAULA H. VALDUJO², JOSÉ MÁRIO B.
GHELLERE¹, TAMÍ MOTT³ & CRISTIANO NOGUEIRA⁴

¹Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, Caixa Postal 11.461, CEP 05422-970, São Paulo, SP, Brazil. E-mail: mturodri@usp.br

²Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, Trav. 14, nº 321, Cidade Universitária, CEP 05508-900, São Paulo, SP, Brazil

³Universidade Federal de Mato Grosso, Av. Fernando Correa da Costa s/n, Coxipó, CEP 78060-900, Cuiabá, MT, Brazil

⁴Conservação Internacional do Brasil, programa Cerrado-Pantanal. SAUS Qd. 3 Lt. 2 Bl. C, Edifício Business Point, Salas 713–722, CEP 70070-934, Brasília, DF, Brazil

Abstract

A new species of *Bachia* of the *bresslaui* group, is described from Estação Ecológica Serra Geral do Tocantins, a recently created protected area in the Central Brazilian Cerrados of state of Tocantins. The new species is most similar to *Bachia psamophila* from which it differs in limb morphology and head and body scalation. As in *Bachia psamophila* the shovel-shaped snout of the new species is highly prominent, an adaptation related to its psamophilous habits.

Key words: *Bachia oxyrhina*, new species, Gymnophthalmidae, Brazil, Cerrado, Jalapão, Tocantins state, Estação Ecológica Serra Geral do Tocantins

Introduction

Fossorial gymnophthalmid lizards of the genus *Bachia* lack an external ear opening, have an elongate body and tail, present a distinctive eyelid and show substantial variation in levels of limb reduction (Colli *et al.* 1998; Kohlsdorf & Wagner 2006; Rodrigues *et al.* 2007). The genus was last reviewed on the basis of external attributes by Dixon (1973) who recognized 15 species and 12 subspecies which were allocated in four species groups: *bresslaui*, *dorbignyi*, *heteropa*, and *flavescens*. Since then, taxonomy of *Bachia* has been largely based on Dixon's review although the status of several species has been reevaluated and new species have been described (Hoogmoed & Dixon 1977; McDiarmid & DeWeese 1977; Avila-Pires 1995; Kizirian & McDiarmid 1998; Castrillon & Strussmann 1998; Rodrigues *et al.* 2007). These new contributions have increased the number of species in the genus to 20 (Rodrigues *et al.* 2007). Species of *Bachia* have also been the subject of molecular studies. In the study of Pellegrino *et al.* (2001) designed to investigate relationships among Gymnophthalmidae, three species (*Bachia bresslaui*, *B. flavescens* and *B. dorbignyi*) were sequenced for nuclear and mitochondrial genes and recovered as the basal genus of the tribe Cercosaurini. The genus was also recovered as the sister group of all cercosaurines by Castoe *et al.* (2004); these authors used the same species of *Bachia* and a slightly reduced data set than that used by Pellegrino *et al.* (2001), and assigned tribal rank (Bachini) to the genus based on its distinctive morphology. In a more recent study with larger taxon sam-

pling, Kohlsdorf and Wagner (2006) investigated the reversibility of digit loss in *Bachia*. They used formalin-fixed tissues to obtain a molecular phylogenetic hypothesis for the relationships of 15 taxa. The major taxonomic consequences of that paper were not further explored but indicate that Dixon's systematic arrangement needs to be reviewed. Some clades of the topology obtained by Kohlsdorf and Wagner (2006) received weak support but none of Dixon's four species groups were confirmed. Furthermore, their study also indicates that the status of several subspecies should be reconsidered, suggesting a morphological reanalysis of the genus. While waiting for such studies, as a matter of convenience, we still rely on Dixon's species groups to orient description and comparisons of new taxa, as will be the case herein after.

Species of *Bachia* of the *bresslaui* group are characterized by lanceolate, keeled, and imbricate dorsal and lateral body scales, quadrangular and juxtaposed ventrals, 2–2 femoral and 1–1 preanal pores, presence of interparietal, supraocular, and superciliary scales, and a varied number of digits on limbs. Seven species fit this description: *Bachia bresslaui*, *B. cacerensis*, *B. micromela*, *B. panoplia*, *B. psamophila*, *B. scolecoides*, and *B. pyburni*.

In a recent paper, two new Brazilian species of *Bachia* of the *bresslaui* group were described from sandy habitats in the Cerrados of the west bank of the Rio Tocantins, state of Tocantins (Rodrigues *et al.* 2007). In the course of a faunal survey in the newly created protected area Estação Ecológica Serra Geral do Tocantins, we obtained another undescribed species of the *bresslaui* group also in sandy habitats but from the east bank of the Rio Tocantins. Herein, we describe the new species and comment on its variation and habitat.

Material and methods

Length measurements were taken after fixation to the nearest mm with a ruler; scale counts and observations of other morphological characters were performed with a stereomicroscope Zeiss STEMI SV6. Scale nomenclature follows Dixon (1973). The hemipenis was prepared following the procedures described by Manzani and Abe (1988), modified by Pesantes (1994) and Zaher (1999). The retractor muscle was manually separated and the everted organ filled with stained petroleum jelly. Hemipenian calcareous structures were stained in an alcoholic solution of red alizarin for 24 hours, in a modification of the procedures described by Uzzel and Barry (1971) and Uzzel (1973). Hemipenian terminology follows Dowling and Savage (1960), Savage (1997) and Myers and Donnelly (2001, 2008). In the field temperature data were taken with a data logger model HOBO Pro; temperatures were recorded at continuous intervals of one hour. All specimens of *Bachia* used for comparisons are presently housed in MZUSP (Museu de Zoologia, Universidade de São Paulo) and CHUNB (Coleção Herpetológica da Universidade de Brasília).

Results

Taxon description

Bachia oxyrhina, sp. nov.

(Figs. 1, 2)

Holotype: MZUSP 98086 (Fig. 1), an adult male from Morro do Fumo, Estação Ecológica Serra Geral do Tocantins (10°51'58.41"S, 46°49'9.07"W), Mateiros municipality, state of Tocantins, Brazil, collected by the authors of this paper on February 15th, 2008. Field number PHV 2208.

Paratypes: MZUSP 98080 (11°6'7.92"S, 46°45'57.60"W), MZUSP 98082 (11°7'25.68"S, 46°47'26.88"W), MZUSP 98083 (Fig. 2A) (11°7'12.36"S, 46°47'12.48"W) (females); MZUSP 98081 (11°6'7.92"S, 46°45'57.60"W), and MZUSP 98084 (Fig. 2C) (11°18'41.29"S, 46°56'6.36"W) (males); all

collected by the authors of this paper between January 28th and February 4th 2008, all localities in Estação Ecológica Serra Geral do Tocantins, Almas municipality, state of Tocantins, Brazil.

Etymology: The specific name derives from the Greek “oxy” (sharp, spatulate, wedge shaped), and “rhino” (nose) being a reference to the pronounced, wedge shaped nose of this species, an adaptation to life in the sandy habitats where it occurs.

Diagnosis: (Table 1) A species of the *bresslaui* group having lanceolate dorsal and lateral body scales, quadrangular and juxtaposed smooth ventrals, tail scales lanceolate, imbricate, keeled, 1–1 femoral pores and 1–1 preanal pores in males (only preanal pores in females), interparietal, supraoculars and superciliaries present, 42–45 dorsals, 34–36 ventrals, and 29–30 scales around midbody. Snout highly prominent and wedge shaped, distinctively projecting over lower jaw. Fore limb and hind limb rudimentary, stiliform, ending in one apical scale. Five supralabials; fifth the largest and the highest, contacting or not parietal. Anterior portion of nasal scale fused with first supralabial. One enlarged temporal scale contacting or not postocular. Two supraoculars; second small, restricted to the lateral face of head, allowing extensive contact between parietal and first supraocular. Width of first supraocular less than 1/3 of the anterior margin of frontal.

TABLE 1. Comparative table of diagnostic characters in *Bachia* of the *bresslaui* group.

	<i>B. oxyrhina</i>	<i>B. bresslaui</i>	<i>B. cacerensis</i>	<i>B. micromela</i>
Prefrontals	absent	absent	absent	absent
Supralabials	five	six	six	six
First supralabial and nasal	fusionated	separated	separated	separated
Scales around midbody	29–30	33–35	35	38–40
Contact between supralabial and parietal	5 th supralabial	no contact	6 th supralabial, but not always in contact	5 th supralabial
Forelimb	one apical scale	one apical scale	four apical scales	one apical scale
Hindlimb	one apical scale	one apical scale	one apical scale	two apical scale

continued.

	<i>B. psamophila</i>	<i>B. panoplia</i>	<i>B. pyburni</i>	<i>B. scolecoides</i>
Prefrontals	absent	present in contact	present in contact	present and separated
Supralabials	six	six	six	six
First supralabial and nasal	separated	separated	separated	separated
Scales around midbody	35–38	43–47	41	36–40
Contact between supralabial and parietal	6 th supralabial	no contact	no contact	no contact
Forelimb	one apical scale	four reduced fingers	four free fingers	four reduced fingers
Hindlimb	four clawed toes	four reduced toes	four free toes	four reduced toes

Bachia oxyrhina can be immediately distinguished from *B. panoplia* and *B. pyburni* by the absence of prefrontals, present and in contact at midline in both latter species. In *Bachia scolecoides*, prefrontals are also present but widely separated and reduced in size. Species of *Bachia* of the *bresslaui* group without prefrontals are *B. bresslaui*, *B. cacerensis*, *B. micromela*, *B. psamophila*, and *B. oxyrhina*. Except for *Bachia oxyrhina* that has five supralabials, all other species of *bresslaui* group have six. *Bachia oxyrhina* is also unique in having the anterior portion of nasal scale fused to first supralabial and the lower number of scales around body (29–30 vs. 33–47 in other species). *Bachia oxyrhina* resembles more closely *B. psamophila* and *B. micromela*, especially the former by its pronounced and prominent wedge shaped snout. Besides the above mentioned

characters *Bachia oxyrhina* differs from the latter species by presenting the second pair of chin shields broadly separated at midline (respectively in slight and broad contact at midline in *B. psamophila* and *B. micromela*). In *Bachia bresslaui*, *B. scolecoides*, *B. panoplia*, and *B. pyburni* there is no contact between supralabials and parietal. Contact between supralabials and parietal is variable in *Bachia cacerensis*, *B. psamophila*, *B. micromela*, and *B. oxyrhina*. In one of the three known specimens of *Bachia cacerensis* there is slight contact between parietal and 6th labial. In *Bachia psamophila* the 6th supralabial is the largest and highest and contacts the parietal; in *B. micromela* it is the 5th supralabial that contacts the parietal. The condition varies in *Bachia oxyrhina* where either it is the 5th supralabial that contacts punctually the parietal or their contact is prevented by a slight contact between postocular and temporal. This is a different condition than the one observed in *Bachia psamophila* where there are six labials and an enlarged postocular separates the 5th supralabial from parietal. *Bachia cacerensis* is unique in the *bresslaui* group in having four unclawed finger-like apical scales in the forelimb; all other species, *B. oxyrhina*, *B. psamophila*, *B. bresslaui* and *B. micromela* have just one apical scale in the forelimb, in the last species ending in an ungual sheath. The hind limb of *Bachia oxyrhina*, like those of *B. bresslaui* and *B. cacerensis* ends with one apical scale, whereas there are two in *B. micromela* and four clawed toes in *B. psamophila*. A comparative list of characters are shown in Table 1.

Description of the holotype: (Fig. 1) Body elongate, with a slight cervical constriction on head, snout highly prominent and wedge shaped, tail longer than body. Rostral broad, prominent, contacting first supralabial, nasal and frontonasal. Viewed from above the rostral is about twice as wide as high; on lateral view it projects broadly anteriorly toward, forming a horizontal surface ventrally, at the level of ventral surface of the upper lips. Frontonasal trapezoidal, wider than long, wider posteriorly, contacting rostral, nasal, first supraocular and frontal. Prefrontals absent. Frontal pentagonal, longer than wide, with anterior margin straight, as wide as and in broad contact with frontonasal; lateral margins straight to slightly concave, in contact on each sides with first supraocular; posteriorly angulose, broadly contacting parietals and in short contact with interparietal. Frontal more than four times wider than anterior supraocular. Frontoparietals absent. Interparietal narrow, longer than wide, subrectangular, slightly wider posteriorly, shorter than frontal and parietals. Parietals very large, longer than wide, slightly longer and wider than frontal, roughly pentagonal, their anterior margin deeply indented and in broad contact with frontal, externally contacting first and second supraoculars, the postocular, the sixth supralabial, a large and long temporal and the dorsals; internally it contacts frontal and interparietal. Posterior borders of interparietal and parietals and dorsals coincide with a slight transverse cervical constriction in the occipital region. Two supraoculars, first largest, about three times longer than wide, contacting frontal, frontonasal, nasal, loreal, first superciliary, second supraocular and parietal. Second supraocular smaller, above second superciliary, longer than wide, separated from frontal by the slight contact between parietal and first supraocular. Two superciliaries, the first longer, its sutures coincide with that between supraoculars. Nasal large, longer than high, their suture with posterior part of first supralabial complete, anteriorly to nostril fused to first supralabial forming an incomplete nasolabial. Nasal portion of this incomplete nasolabial clearly viewed from above; above first and second supralabials. Nostril in the first third of lower margin of nasal, deeply indenting the suture with the posterior part of first supralabial. Loreal roughly squared, in contact with nasal, first supraocular, first superciliary, preocular, subocular and second and third supralabials. Frenocular absent. Five supralabials, third and fourth under the orbital region, fifth the highest and largest, contacting punctually parietal. One long subocular, widest posteriorly. Eyelid present with an undivided semitransparent disc. A large and elongate postocular between fourth and fifth supralabials and parietal. An enlarged, longer than wide, temporal scale between parietal and fifth labial, in broad contact with parietal. Ear opening absent. All head scales smooth and juxtaposed with scattered sensorial organs.

Mental roughly trapezoidal, wider than long, slightly longer than the ventral surface of rostral. Postmental heptagonal, as wide as long. Two pairs of chin shields, both contacting infralabials; the anterior pair smaller, in broad contact at midline; second pair broadly separated by an enlarged pair of symmetric flat and diagonally disposed elongate pregonals. Five infralabials, first the smallest, second, third and fourth about the same

size. Gulars smooth, imbricate, rounded posteriorly, in eight transversal rows; scales of gular rows increasing gradually in size toward interbrachial region. Interbrachial region with four scales, the central ones largest, twice longer than wide. Lateral scales of neck subrectangular, smooth, imbricate, slightly rounded posteriorly and longer than wide, disposed in regular transverse rows and becoming gradually similar to adjacent dorsal or ventral scales. Collar fold absent.

FIGURE 1. Lateral (A), ventral (B), and dorsal (C) views of the head of the holotype of *Bachia oxyrhina* **sp. nov.** (MZUSP 98086).

Dorsal scales imbricate and disposed in regular transversal rows; smooth, subrectangular and wider in occipital region, becoming progressively narrower, more elongate and rounded towards the level of the forelimbs and then on longer, hexagonal, lanceolate, strongly keeled, with lateral sides almost juxtaposed. Forty-five transverse rows between interparietal and the level of hind limbs. Lateral scales about the same size as dor-

sals but smooth and less acuminate; those closer to ventrals slightly wider. A distinctive area with granular scales surrounds the area of arm insertion and the posterior part of leg insertion. Thirty scales around midbody. Ventral scales smooth, longitudinally imbricate, laterally juxtaposed, almost squared just after the interbrachial row, becoming gradually longer than wide, rounded posteriorly, those after midbody narrower; 34 transverse rows from interbrachials (excluded) to preanals. Five scales in the posterior part of preanal plate, central one largest. One preanal and one femoral pore on each side.

FIGURE 2. *Bachia oxyrhina* **sp. nov.**: (A) Close-up of the head (MZUSP 98083), note the absence of suture between anterior part of nasal scale and first supralabial; (B) sulcate (left) and assulcate (right) sides of the left hemipenis; and (C) paratype in life (MZUSP 98084).

FIGURE 3. Habitat and track of *Bachia oxyrhina* sp. nov.: (A) general physiognomy of the habitats at EESGT where specimens were found; (B) aspect of an opened Cerrado (“campo sujo”) with scattered trees and areas of bare sand; (C) tracks in white sand.

FIGURE 4. Distributional records of *Bachia* species from Brazilian Cerrados: *Bachia bresslaui*, *B. cacerensis*, *B. micromela*, *B. psammophila*, and *B. oxyrhina* sp. nov.

Scales of tail similar to midbody dorsals, keeled, lanceolate, strongly imbricate.

Fore limbs rudimentary, stiliform, covered by smooth and imbricate scales, ending in a single apical scale.

Fore limb length equal to width of one row of lateral scales. Hind limb also stiliform but larger than fore limb, covered by smooth, large and imbricate scales ending by one apical scale; length equivalent to one and one-half scale rows. One femoral pore present at each side.

Background dorsal and lateral surfaces of body and tail almost uniformly cream to light brown with scattered melanophores. Ventral parts of body and tail immaculate cream.

Hemipenes partially everted, bilobed, sulcus spermaticus central in position, extending from the base of organ to lobes, sulcate and assulcate faces smooth and without papillae or spines.

Measurements of the holotype: Snout-vent-length: 65 mm; tail length (regenerated): 85 mm.

Variation: the type series is fairly homogeneous, showing little variation in number of dorsal scales (42–45), ventral scales (34–36), scales around body (29–30), and gular scale rows (6–8). Like the holotype all other specimens have the first supralabial partially fused with nasal, two superciliaries, five supralabials, five infralabials, five preanal scales, one preanal pore, and both fore and hind limb ending by one apical scale. All males have one femoral pore at each side; these are absent in females. In two specimens (MZUSP 98080 and 98081) the first supraocular is fused to the loreal on the left side. There is also some variation regarding contact between labials and parietal. Like in the holotype, in general the fifth supralabial punctually contacts the parietal except for some cases when there is a contact between postocular and temporal. This is the case in MZUSP 98081, on the right side of the holotype, where there is a slight contact between postocular and temporal, and in MZUSP 98082 and 98083 where this contact is larger. Maximum snout-vent length varies between 61–80 mm, the largest specimen is a female. Although the holotype is almost homogeneously immaculate cream, other specimens show in general two symmetrical dorsolateral light stripes two to two and one-half scales wide, and extending from the parietal scale to the end of tail, showing a darker overall color pattern. Between them there is a conspicuously wider but irregular brown dorsal stripe also extending from the posterior border of the interparietal scale to the tip of the tail. Longitudinal dark and light stripes are more conspicuous anteriorly where the light stripes are limited below and above by a darker border. Lateral parts of body are cream with a scattered light brown reticulum.

Hemipenes of one of the paratypes (MZUSP 98084) were partially everted during preservation and the left hemipenis was prepared (Fig. 2B). The organ is relatively small, extending along approximately four subcaudal rows (4 mm). It is slightly bilobed, with the distal tip of the retractor muscle divided. The hemipenial body is globose, slightly Y-shaped, ending in two small and symmetric lobes surrounded by a thin and fleshy fold. The sulcus spermaticus is broad, central in position, originates at the base of the organ, and proceed in a straight line towards the lobes. At the distal tip of the hemipenial body the sulcus is divided in two branches. In the lobes, branches of the sulcus run among folds in an S-shape condition, ending at their tip. Sulcate and assulcate faces of the organ are completely smooth, with no evident plicae, papillae, ridges, calyces, mineralized spines or spinules, even after immersion on an Alizarin Red solution for 24 hours. The absence of hemipenial ornamentation is similar to the condition reported for *Bachia trisanale* and *B. intermedia* (Presch 1978).

Distribution and natural history: The study area is situated near the borders of states of Tocantins, Maranhão, Piauí, and Bahia and is usually referred to as the Jalapão region. The Jalapão, comprising approximately 53300 km², consists of extensive quartzitic sand depressions resulting from erosion of arenitic plateaus of the Serra Geral and Chapada das Mangabeiras, and is drained by headwaters of the Tocantins and São Francisco river basins (SEPLAN 2003). Isolated and highly eroded sandstone relicts are frequent in the depositional plains, and represent evidence of the formerly continuous plateau (Ribeiro *et al.* in press). The Jalapão is considered one of the three best preserved areas in the Cerrado domain, Central Brazil (Cavalcanti & Joly 2002). Estação Ecológica da Serra Geral do Tocantins (EESGT) is a conservation unit created in 2001 that protects 7163.06 Km² of the depositional plains and part of the Serra Geral, being the second largest federal protected area in the Cerrado. Vegetation in Jalapão and EESGT is typical of the Cerrado domain, and is characterized by extensive grasslands, open savannas and typical “cerrado” savannas (campo sujo, campo cer-

rado and cerrado *sensu stricto*, respectively, Oliveira-Filho & Ratter 2002) (Figs. 3A, 3B), interspersed by palm marshes (dominated by *Mauritia flexuosa* palms) and gallery forests along water courses.

Two main regions were sampled at EESGT: a southern, lower portion, located mostly at Almas municipality, state of Tocantins, and a northeastern portion, located at Formosa do Rio Preto municipality, in state of Bahia, in the higher portions of EESGT on the Serra Geral plateau. Nine pitfall-trap lines, containing five sets of traps each, were installed in each region. Each set of traps consisted in four 35 liter buckets arranged in Y shape and connected by drift fences. A total of 180 buckets were used in each site, which remained opened for nine consecutive days at the southern (27/January to 04/February/2008) and at the northeastern sites (08–16/February/2008), totaling an effort equivalent to 3240 buckets/day. The traps covered the main physiognomies present in EESGT region, ranging from grasslands, with few scattered small trees, to typical Cerrado, with discontinuous herbaceous layer and leaf litter patches. Palm marshes and gallery forests were also sampled. In addition, active search was performed around the lines and along the roads that cross the EESGT. Although we saw tracks of *Bachia oxyrhina* (Fig. 3C) along the different sampled habitats at the southern part of EESGT, except in palm marshes and gallery forests, only four specimens were collected in pitfall traps, all at lower elevations in the south-central part of the station (Fig. 4). Two additional individuals were collected manually by digging through the sand surface, one in the southern site, and another in the central part of the station, adjacent to “Morro do Fumo”, one of the relictual and highly eroded sandstone plateaus of the area. Although sandy soil grasslands and denser savanna habitats, similar to those of the southern site were sampled with similar effort in the northeastern part of the station, no specimens or tracks were found. The absence of *Bachia oxyrhina* at the northeastern site and characterized by higher elevations, suggests that the species is restricted to the southern part of the Jalapão. This is reinforced by the absence of the species in all other previous herpetological surveys conducted at the northern part of Jalapão region (Vitt *et al.* 2002; Vitt *et al.* 2005; Mesquita *et al.* 2006; Nogueira 2006). The single previous lizard sampling within EESGT was conducted in March–April 2003 by one of us (CN) as part of a study on Cerrado lizard diversity (Nogueira 2006; Nogueira *et al.* in press), in the northernmost limits of the protected area, all north of the Morro do Fumo site, in depressions associated with the Rio Novo drainage, one of the main watercourses in the Jalapão. Using the same overall sampling method (35 liter bucket traps with drift fences, in different habitat types), 490 lizard specimens of 14 species were collected (see results in Nogueira *et al.* in press), after 3600 bucket/days of pitfall trapping and manual collection in fifteen days of fieldwork. In this previous sampling, no specimens of *Bachia* were obtained. Furthermore, no tracks were observed at these sites within the Rio Novo depressions, suggesting that the new species is rare or absent outside the southern portion of EESGT, which was previously unsampled.

Bachia oxyrhina seems to inhabit the superficial layer of sandy soils, as many tracks can be easily found at exposed sandy soils in the station. Frequently, tracks were observed on open ground heading from one bush to another, indicating sub-superficial ground locomotion. During the survey, we measured temperatures hourly using a HOBO data logger, with a sensor placed 1 cm deep in the exposed sand, next to one of the lines where two specimens of *Bachia oxyrhina* were captured in pitfall traps. Mean temperature was 26.32°C, ranging from 21.33°C to 42.46°C (sd = 4.83°C, n = 192). These data represent a gross estimate of the temperatures that the species may encounter in the sand.

One *Scotinus* sp. larva (Tenebrionoidea, Coleoptera) was regurgitated by the holotype during fixation.

The fact that only four individuals were obtained with pitfall-traps, despite the relatively large effort, is not surprising given that individuals of the genus *Bachia* are usually not abundant in herpetological surveys (Colli *et al.* 1998; Nogueira 2006; Pavan 2007; Rodrigues *et al.* 2007). However, as many tracks were seen through great part of the station it is difficult to say if this species is rare. Considering that it was raining during part of the time that the pitfalls remained open, we prefer do not use our pitfall sampling results to infer the real density of this species.

Discussion

In the absence of an explicit phylogeny it is impossible to be certain regarding the relationships of *Bachia oxyrhina*. The molecular tree obtained by Kohsdorf and Wagner (2006) includes 15 taxa, but only *Bachia bresslaui* and *Bachia scolecoides* of the *bresslaui* group. *Bachia bresslaui* was recovered at the base of the tree whereas *Bachia scolecoides* was nested at the most derived node, sister to *B. huallagana* from the *dorbignyi* group. These two last species were also recovered as sister to *Bachia allenii* from the *heteropa* group in the topology (*B. allenii* (*B. scolecoides* + *B. huallagana*)), and both nodes were strongly supported. These results indicate that *Bachia scolecoides* could be misplaced in the *bresslaui* group. In fact, *Bachia scolecoides*, *Bachia pyburni*, and *Bachia panoplia* are the only species with prefrontal scales included in the group. Species of the *bresslaui* group lacking prefrontals are *Bachia oxyrhina*, *Bachia bresslaui*, *Bachia cacerensis*, *Bachia micromela* and *Bachia psamophila*. Patterns of limb morphology are distinctive between these species. *Bachia cacerensis* is unique in having four unclawed finger-resembling apical scales on the fore limb. *Bachia bresslaui*, *Bachia micromela*, *Bachia oxyrhina*, and *Bachia psamophila* have a very different fore limb ending in a single apical scale. Differences are more pronounced in the hind limb where *Bachia psamophila* is the only species having four clawed toes; in *Bachia micromela* the hind limb ends in two apical scales, and only one in *Bachia bresslaui* and *Bachia cacerensis*. Despite the differences in hind limb morphology *Bachia oxyrhina* phenotypically is more similar to *Bachia psamophila*. In both species the second pair of chin shields is separated or in slight contact at midline, whereas they are in broad contact in the other species. However, the most striking similarity between *Bachia psamophila* and *Bachia oxyrhina* is their wedge shaped snout, a clear adaptation to subaerial locomotion in sandy habitats (Rodrigues 1991; Rodrigues *et al.* 2007). The fact that two fossorial species with a highly projecting snout and a distinctive hind limb morphology live in geographically close and ecologically similar habitats is intriguing. Are they in fact closely related? Do they have similar microhabitat preferences and modes of locomotion or do they forage differently in similar habitats? Although distinctive patterns of limb reduction in species of *Bachia* living in the same habitat have been generally hypothetically associated with different locomotor strategies caused by the use of different microhabitats (see Kohsdorf & Wagner 2006), other causes can be invoked to account for this pattern.

Bachia cacerensis, *B. bresslaui*, *B. psamophila*, *B. micromela*, and *B. oxyrhina* are associated with distinct geographic regions of the Brazilian Cerrados (Fig. 4). *Bachia bresslaui* is widely distributed, *Bachia cacerensis* is restricted to the Pantanal area and the three other species are restricted to sandy habitats in the central part of state of Tocantins. *Bachia oxyrhina* is known only from sandy habitats at Estação Ecológica Serra Geral do Tocantins, a protected area in the Cerrados on the headwaters of tributaries from the east bank of the Rio Tocantins. *Bachia psamophila* and *Bachia micromela* were described recently from sandy habitats on the west bank of the Rio Tocantins. It is interesting to note that the last three species were recently collected and are possibly endemic to isolated sandy habitats. The possible restriction to isolated blocks of sandy depositional soils would agree to former descriptions of Cerrado lizard abundance and distribution patterns, largely dependent on the patchy horizontal structure of natural habitat mosaics (see Colli *et al.* 2002; Nogueira 2006; Pavan 2007; Nogueira *et al.* in press.), and strongly influenced by drastic variations in vegetation structure, topography and elevation. However, further detailed sampling is still necessary to understand the limits of the local distribution of *Bachia* in the Jalapão region, characterized by the complex contact of plateaus and depositional depressions. Although similar sandy habitats in the Caatingas of northeast Brazil have been recognized as an area rich in endemic squamates (Rodrigues 1996; Rodrigues & Juncá 2002), this has not been the case for the Cerrados (but see indications in Colli *et al.* 2002; Colli *et al.* 2003). The recently discovered new species of *Bachia* from sandy areas within the Cerrados suggests that these habitats may be richer than previously thought and should be the target of special concern in surveys and conservation measures.

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Appendix 1. Specimens examined

Bachia bresslaui: MZUSP 4737 São Paulo, Brazil; MZUSP 10300 Utiariti, Mato Grosso, Brazil; MZUSP 78211 Bataguçu, Mato Grosso do Sul, Brazil; MZUSP 91658–91659 Brasília, Distrito Federal, Brazil; MZUSP 94473–94474 Parque Nacional Grande Sertão Veredas, Formoso, Minas Gerais, Brazil; MZUSP 95396–95399 Sonora, Mato Grosso do Sul, Brazil; *Bachia micromela*: MZUSP 91315–91318, 91030–91031 Guaraí, Tocantins, Brazil; *Bachia oxyrhina*: MZUSP 98080–98084 EESGT, Almas, Tocantins, Brazil; MZUSP 98086 Estação Ecológica Serra Geral do Tocantins, Mateiros, Tocantins, Brazil; *Bachia panoplia*: MZUSP 57329, 57561–57562, 57638–57639, 57852, 58814 Manaus, Amazonas, Brazil; *Bachia psamophila*: CHUNB 24209, MZUSP 95079–95080 UHE Luis Eduardo Magalhães, Tocantins, Brazil; *Bachia scolecoides*: MZUSP 3289–3295, 3337–3344 Rio Teles Pires, Mato Grosso, Brazil; MZUSP 38374 Sinop, Mato Grosso, Brazil; MZUSP 82525–82526 Fazenda Iracema, Claudia, Mato Grosso, Brazil.

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A new species of *Bachia* Gray, 1845 (Squamata: Gymnophthalmidae) from the Eastern Brazilian Cerrado, and data on its ecology, physiology and behavior

MAURO TEIXEIRA JR, RENATO SOUSA RECODER, AGUSTÍN CAMACHO,
MARCO AURÉLIO DE SENA, CARLOS ARTURO NAVAS & MIGUEL
TREFAUT RODRIGUES

Respostas dos animais ectotermos terrestres à variação microclimática

Responses of terrestrial ectothermic animals to microclimatic variation

Agustín Camacho

Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo

Resumo. Entender de maneira útil os efeitos da variação climática sobre os seres vivos requer de, ao menos, três passos: 1) Conhecer os princípios que explicam a variação dos parâmetros climáticos e as respostas dos seres vivos a esta variação. 2) Detectar os padrões de variação do clima nas escalas em que este interage com os organismos, e os padrões de resposta dos seres vivos. 3) Sintetizar teorias preditivas a partir deste conhecimento. Estudos dos efeitos da temperatura em animais ectotermos terrestres (AET) são extremamente abundantes, o que faz possível e necessário avançar sua síntese teórica. Neste texto, aplico os três passos referidos para propor um primórdio de teoria preditiva dos efeitos da temperatura sobre a biologia dos AETs em diferentes níveis organizacionais. Este mesmo procedimento pode ser aplicado a outras variáveis climatológicas ou características dos ectotermos não tratadas aqui, em um caminho de síntese teórica de abrangência cada vez maior.

Palavras-chave. *Mudanças ambientais, microclima, fisiologia termal.*

Abstract. Understanding the effects of microclimatic variation on living beings requires, at least, three steps: 1) Knowing the principles for variation of climatic parameters and the responses of living beings to these variations. 2) Detecting climatic variation patterns in the scales within climate interacts with living beings, and response patterns of living beings. 3) Synthesizing predictive theories from this knowledge. Studies about the effects of temperature and AETs are extremely abundant and it is necessary to advance in the direction of producing a theoretical synthesis. In this paper, I apply the above mentioned steps for proposing a primordial theory about temperature effects on AETs, along several organizational levels. The same procedure may be applied to other climatic variables or ectotherm's characteristics in a path of theoretic synthesis of increasing generality.

Keywords. *Environmental change, microclimate, thermal physiology.*

Contato do autor:

agustincamacho@usp.br

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Próximos à superfície terrestre, a amplitude das variações térmicas em diversas escalas espaço-temporais podem superar o espectro onde os seres vivos conseguem se reproduzir (Tansey e Brock 1972; angilletta 2009, Geiger, 1950, e estudos revisados em Panikov e col., 2006). É nestas escalas que o clima interage diretamente com os seres vivos. Por este motivo, para entender os efeitos do clima sobre os AET, precisamos conhecer a variação do clima e das características dos organismos nestas escalas.

Segundo, Holmes e Dingle (1965), microclimatologia é a disciplina encarregada de estudar a dinâmica dos parâmetros físicos do clima (ex. temperatura, umidade relativa) em escalas em que estes são marcadamente afetados por elementos da superfície terrestre (ex. florestas, acidentes geográficos), ou bem como pelos tipos de superfícies existentes sobre ela (ex. gelo, areia, rochas). Nestas escalas, o efeito da radiação e condução da energia domina sobre o das temperaturas das grandes massas de ar (Geiger, 1950).

Há pelo menos um século que uma formidável força de trabalho científico tem-se focado em descobrir padrões e efeitos das variações microclimatológicas sobre diversos

aspectos da biologia dos seres vivos. Entre os parâmetros físicos mais estudados, destaca-se a temperatura (ex. (Angilletta, 2009; Ashton e Feldman, 2003; Huey e Slatkin, 1976; Bartholomew, 1964; Carnaval e col. 2009; Cowles e Bogert, 1944; Geiger, 1950; Hertz e col. 1993; Huey e col. 2003; Huey e Stevenson, 1979; Kearney e Porter, 2004; Kearney e Porter, 2004; Kingsolver e Huey, 1998; Kraus, 1911; Navas, 2006; Porter, e col. 2010; Huey e Slatkin 1976; Root e col. 2005; Sunday e col. 2010; Vitt e Sartorius, 1999; Vitt, e col. 1998). Esses estudos tem resultado no acúmulo de modelos estendendo-se por diferentes escalas espaço-temporais e níveis de organização biológica (ex. Grant e Porter, 1992, Porter e col., 1973). Alguns destes modelos permitem prever de maneira espacial os efeitos do aumento da temperatura sobre a persistência e distribuição das espécies (ex, Sinervo e col., 2010, Cassemiro e col., 2012, Costa e col., 2012)

Revisões sobre a relação entre as características do microclima e os seres vivos também são muitas e começaram a aparecer há mais de meio século (Angilletta, 2002; 2003; 2009; Chown e Terblanche, 2006; Cloudsley-

-Thompson, 1962; Geiger, 1950; Navas e Carvalho, 2009; Withers, 1992), inclusive em português (Rocha e col., 2009; Closes e Kohlsdorf, 2012; Assis, 2012; Ribeiro e Moreira, 2012; Costa e col., 2012; Titon e Gomes, 2012). Entretanto, o firme avanço da pesquisa nesta área, requer de contínuos esforços pela sumarização dos resultados obtidos. Além disto, ainda faltam sínteses teóricas preditivas com estrutura formalizada que atinjam diferentes escalas e níveis de organização.

Para serem úteis ao avanço da ciência e sociedade, facilitando a transmissão do conhecimento estabelecido e guiando novos experimentos, teorias devem cumprir com alguns requerimentos. De acordo com Scheiner (2010), uma teoria biológica formal deveria conter os seguintes elementos: 1) domínio de aplicação: parcela da realidade a qual se aplica as predições da teoria (Scheiner, 2010; Weber, 1999). 2) princípios: enunciados gerais, de cuja validade depende do resto da teoria (Scheiner, 2010). 3) premissas: enunciados com o mesmo papel que os princípios, que são considerados como verdadeiros provisoriamente, portanto, podem ser refutados (Popper, 1959; Scheiner, 2010). Exemplo de uma teoria assim é a teoria da evolução de Darwin (Scheiner, 2010).

Teorias com esta estrutura têm poder preditivo e permitem transformar fatos em um objeto de estrutura explícita, operável e passível de difusão e transferência a outros segmentos da sociedade. Além disso, teorias assim favorecem a crítica, a integração, e a determinação do que é preciso para estendê-la dentro de teorias de domínio mais abrangente (Scheiner, 2010).

Este trabalho tem como foco avançar na construção de uma síntese teórica sobre o impacto do microclima em populações de animais ectotermos não estritamente aquáticos (aqui referidos como AETs). Para isso, dividi o presente texto em três fases:

1) Resumo dos princípios que explicam a variação do parâmetro microclimático de interesse e as respostas dos seres vivos a esta variação.

2) Descrição de padrões termobiológicos, combinando padrões de variação microclimática e de resposta dos seres vivos em diferentes níveis de organização.

3) Síntese destes princípios, processos e padrões em uma teoria preditiva, formalmente estruturada e testável.

Para a realização desta síntese foram apenas escolhidos representantes dos elementos que a teoria abrange. Entre os parâmetros microclimáticos, escolhi a temperatura, motivado pela grande quantidade de estudos realizados sobre sua variação e influência. Dos fatores que afetam a temperatura experimentada pelos seres vivos, apenas alguns foram escolhidos, os quais atuam sobre diferentes estágios da transferência de energia desde sua chegada e distribuição na superfície terrestre, até os indivíduos (ex. latitude e altitude, topografia, cavernas e presença de elementos sobre a superfície terrestre, propriedades dos materiais irradiados pelo sol e propriedades do corpo dos próprios indivíduos).

Princípios básicos de fisiologia térmica em AETs

Os processos fisiológicos necessários para a vida do

Princípios básicos de Termodinâmica: como se geram os gradientes de temperatura

Para entender a formação dos gradientes de temperatura são necessários alguns princípios básicos e definições de termodinâmica. Consulte Moran e Shapiro (2006) ou Lienhard e Lienhard (2011), para maiores detalhes.

A temperatura é uma medida da quantidade de energia dos corpos. A energia de um corpo pode aumentar através da sua transferência a partir de outros, mais quentes. Calor é o processo de transferência de energia que provoca variações de temperatura. Neste texto, a expressão gradiente de temperatura representa qualquer variação espacial de temperatura. Variações temporais de temperatura serão representadas pela expressão ciclos de temperatura.

Existem três formas diferentes de transferência de energia por calor: Radiação, transferência de energia através de um meio em forma de partículas ou ondas. Todos os corpos intercambiam energia por radiação, principalmente influenciados pela sua temperatura. Convecção: transporte de energia feito através do movimento das partículas em um fluido. Condução: transmissão de energia entre corpos em contato direto, sem que haja transporte de massa (Moran e Shapiro, 2006). Condutividade térmica (CT) é um parâmetro que representa a facilidade com que a energia é transmitida por condução através de um material. Quanto maior é a CT, menores serão os gradientes de temperatura formados por condução térmica (Lienhard e Lienhard, 2011). Os diferentes modos de transferência de energia atuam sinergicamente e de acordo com a segunda lei da termodinâmica (Lienhard e Lienhard, 2011). Isto é, em ausência de fatores externos, a transferência de energia será feita de corpos mais quentes para os mais frios. Capacidade térmica é a relação entre a quantidade de energia que um corpo intercambia com o ambiente e as mudanças de temperatura que sofre, derivadas deste intercambio elementos com maior capacidade térmica tendem a demorar mais para aquecer-se e esfriar-se.

conjunto dos AETs acontecem em um espectro conhecido que vai de -39 a 110 graus (Tansey e Brock 1972; Angilletta 2009, Geiger, 1950, Panikov e col., 2006 e estudos revisados nesse trabalho). Dentro das margens de tolerância térmica dos AETs, a relação entre a temperatura e o desempenho de variáveis fisiológicas termodependentes, seja para um indivíduo ou conjunto destes, pode ser representada como uma curva com forma de seno. Na fase ascendente da curva, a velocidade de muitas reações e processos fisiológicos aumenta. Porém, em certo nível de temperatura, o desempenho diminui rapidamente, sem que ainda estejam claros os processos bioquímicos associados a esta diminuição. Diferentes espécies têm diferentes curvas para as mesmas variáveis (Angilletta, 2009), ao mesmo tempo, em uma mesma espécie, diferentes variáveis podem ter diferentes parâmetros em suas curvas de sensibilidade (Du e col., 2000). Uma explicação detalhada em português des-

tes parâmetros se encontra em Katzenberger e col., (2012), neste mesmo número especial.

Temperaturas mais altas aceleram os processos de desenvolvimento em ectotermos (Gillooly e col. 2002), desde que não superem o valor máximo das suas curvas específicas de desempenho.

AETs podem modificar sua temperatura corporal via termorregulação comportamental, como, por exemplo, buscando lugares mais quentes ou mais frios, mudando posturas, etc. Porém, algumas espécies de animais consideradas tradicionalmente AETs podem também produzir energia metabólica (Bartholomew e Casey, 1977; Bartholomew, 1964).

Em gradientes experimentais no laboratório, AETs apresentam temperaturas preferenciais (Tprfs) (Licht, Dawson, Shoemaker, e Main, 1966) que podem representar o compromisso entre as T_{opt} dos diferentes processos fisiológicos que acontecem nos indivíduos. Geralmente, Tprfs são próximas a T_{opt}, um pouco mais baixas (Martin e Huey, 2008). A Tprf de um indivíduo ou espécie pode mudar com o tempo (Angilletta, 2006; Trullas e col. 2007). Porém, com suficiente número de observações, é possível determinar uma faixa de temperaturas preferenciais características de um indivíduo, população ou espécie. A amplitude destas faixas pode variar de maneira importante em indivíduos de certas espécies, o que tem sido particularmente evidente em anfíbios (Kour e Hutchison, 1970).

As características termobiológicas de muitos AETs podem mudar com o tempo em processos de ajuste fisiológico a novas condições (Hutchison, 1976; Hutchison e Maness, 1979). Este processo, conhecido como aclimação, pode acontecer como consequência da exposição a uma temperatura durante períodos de variadas magnitudes (Angilletta, 2006). Por regra geral, aclimação tende a aumentar o valor do desempenho às temperaturas expostas (Hazel, 2002; Packard e col., 2001).

Variação da temperatura em duas dimensões: tempo e espaço

Em decorrência dos intercâmbios de energia entre os corpos, a temperatura varia ao longo do tempo e do espaço de acordo com o balanço da entrada e saída de energia, em função do efeito de diferentes fatores.

No tempo, a entrada de radiação solar sobre a superfície terrestre experimenta, ao menos, ciclos em três escalas temporais diferentes: diária, anual (Strahler e Strahler, 1987) e a infra-anual (Friis-Christensen e Lassen, 1991). Os fatores que geram esta variação são: a posição da superfície terrestre em relação ao sol, o ângulo do eixo terrestre e distância da terra com relação ao sol e os ciclos solares (Strahler e Strahler, 1987, Friis-Christensen e Lassen, 1991). Na escala diária, a temperatura de uma superfície exposta ao sol é determinada, maioritariamente, pela quantidade de radiação incidente por unidade de superfície. Durante a noite, a temperatura diminui pela radiação emitida pela própria superfície em direção ao espaço (Geiger, 1950). Processos de evaporação e intercâmbio de energia aportada pelas capas inferiores do solo, aquecidas

durante o dia (Geiger, 1950), são também importantes no controle da temperatura de uma superfície durante a noite. A magnitude do intercâmbio de energia é muito maior na presença de luz solar (Geiger, 1950). Por este motivo, o ambiente térmico é mais homogêneo à noite, na superfície terrestre. Na escala anual, a diferente inclinação do eixo terrestre afeta à intensidade e número de horas de radiação solar na superfície terrestre (Geiger, 1950). Infra-anualmente, existem ciclos relacionados com a radiação proveniente do sol, porém seu efeito nos AETs não é suficientemente conhecido como para ser revisado aqui.

Um fator térmico temporal, não necessariamente cíclico é a nebulosidade do céu. Durante o dia, as nuvens diminuem a fração da radiação que chega à superfície terrestre, diminuindo a intensidade das variações de temperatura entre o sol e a sombra. Durante a noite, a presença de nuvens reduz a transmissão de energia desde a superfície para capas mais altas da atmosfera, desacelerando a diminuição da temperatura ambiental perto da superfície terrestre (Geiger, 1950).

Frequentemente em ecossistemas terrestres, AETs só podem realizar suas atividades em porções específicas da variação térmica total que ocorre em seus habitats. Por este motivo, horários e épocas de atividade de muitos AETs estão necessariamente reduzidos a períodos nos quais estes segmentos podem ser atingidos (ex. durante a noite, no verão, etc.; John-Alder, Morin, e Lawler, 1988; Porter e col., 1973).

A existência de variações espaciais de temperatura permite aos AETs regular comportamentalmente a temperatura corporal, mudando de lugar conforme suas necessidades (Hutchison e Maness, 1979; Porter e col., 1973). De acordo com Hutchison e Maness, (1979), podemos concluir que a capacidade dos AETs de aproveitar a diversidade espaço-temporal de ambientes térmicos para regular suas atividades e ciclos de vida, influenciará de maneira decisiva o número de horas que estes conseguem manter-se em suas TPRFs ao longo de suas vidas. Seguindo esta dedução, Sinervo e col. (2010) relacionaram a extinção de populações de lagartos de todo o mundo com a redução do tempo que o ambiente disponibiliza TPRFs às espécies estudadas, em resposta a um excessivo aumento da temperatura nas épocas reprodutivas.

Fatores, processos e padrões termobiológicos relacionados ao intercâmbio de radiação do ambiente com o sol e o espaço

Latitude

Com o aumento da latitude, aumenta o número diário de horas de sol no verão e diminui no inverno. Ao mesmo tempo, a intensidade de radiação por unidade de superfície diminui em todas as épocas do ano (Strahler e Strahler, 1987). Isto leva a um aumento da variabilidade anual da temperatura e um aumento da variabilidade diária da temperatura em direção à latitudes médias. O maior tempo diário de radiação em latitudes mais altas permite que superfícies irradiadas possam alcançar temperaturas bastante altas para a maioria dos ectotermos, mesmo em

altas latitudes (ex. 55°C na superfície da areia a 42°N, Geiger, (1950). Ao mesmo tempo, o maior tempo de déficit de entrada de energia solar faz com que as temperaturas mínimas absolutas diminuam muito (Strahler e Strahler, 1987). Em resposta a estas condições, populações de AETs de latitudes mais altas tendem a ter sincronizados seus ciclos de desenvolvimento com as estações do ano e são comuns respostas comportamentais e fisiológicas que permitem evitar o frio ou suportá-lo (ex. busca de micro-habitats mais resguardados, migrações, fases de repouso, ou tolerância ao congelamento) (Bale, 2002; Claussen e col., 1990; Sinclair e col., 2003). Espécies de AETs de latitudes mais altas tendem a possuir T_{max} e T_{min} mais extremas conforme aumenta a latitude, porém, paralelamente ao que acontece com variações altitudinais, o efeito da latitude é maior nas T_{min} do que nas T_{max} das distintas espécies (Sunday e col., 2010). No nível de comunidade, padrões latitudinais de distribuição e riqueza de espécies também podem estar relacionados à latitude em AETs (Cushman e col., 1993). Os efeitos da temperatura e outros fatores ambientais relacionados à latitude podem ser observáveis mesmo quando controlando os efeitos gerados por fatores históricos (Buckley e Jetz, 2007, Morales-Castilla e col. 2010).

Altitude

Ao aumentar a altitude, a densidade e temperatura do ar diminuem (Geiger, 1950), consequentemente, aumentam a radiação recebida do sol e a radiação expulsa durante a noite e na sombra (Geiger, 1950). Isto implica que ciclos de aquecimento e esfriamento podem ser muito rápidos e alcançar valores muito extremos em grandes altitudes (ex. Carvajalino-Fernandez, 2010). Ciclos tão rápidos tendem a diminuir o número que os AETs experimentam dentro de qualquer espectro de temperaturas menor do que a amplitude diária local.

Para compensar estes problemas, alguns AETs podem mudar seu comportamento termorregulatório (ex. orientação ao sol; Samietz e col., 2005), ou seus microhabitats preferenciais de maneira a obter as temperaturas adequadas (maior uso de superfícies rochosas e do solo; Adolph, 1990). Outros AETs podem aproveitar gradientes térmicos existentes em microhabitats com maior inércia térmica, como é o caso de algumas salamandras em poças de elevada altitude (Heath, 1975). Para artrópodes, a convecção é o principal modo de transferência de energia com o ambiente (Porter e Gates, 1969). Devido ao declínio de pressão atmosférica, a convecção perde seu poder de esfriá-los fazendo com que estes consigam manter suas temperaturas muito mais tempo (Casey, 1992; Dillon, 2006; Porter e Gates, 1969).

Em resposta ao clima térmico provocado pela altitude, o tempo necessário até a fase adulta dos indivíduos pode aumentar (Angilletta, 2004; Angilletta e Dunham, 2003). Indivíduos de uma mesma espécie procedentes de maiores altitudes podem apresentar maior espectro de tolerância térmica, com efeito mais acusado sobre a CTMIN (ex. besouros, Gaston e Chown, 1999). Porém, os indivíduos podem mudar seu comportamento termoregulatório, assim como sua velocidade de crescimento em função das

condições térmicas da ladeira que habitam (ex. gafanhotos, Weiss e col., 2011).

Quando considerando o nível de população, valores de TPRF e TOPT e TCMAX parecem ser menos influenciadas pela elevação do que a CTMIN, quando comparando populações da mesma espécie de AETs, de diferentes altitudes (Christian e col., 1988; Marquet e col., 1989).

No nível de espécie, é possível observar o mesmo padrão (Christian e col., 1988; Marquet e col., 1989, Gaston e Chown, 1999). Um caso extremo deste ajuste da CTMIN é apresentando por uma espécie de anuro tropical, possuidor de resistência ao congelamento diário parcial (Carvajalino-Fernandez, 2010). Neste nível, as diferentes faixas de temperatura em montanhas influenciam a distribuição de espécies de diversos AETs (Huang e col., 2006; Monasterio e col., 2009; Navas, 2002). Presumivelmente favorecida pela confluência de temperaturas favoráveis para um maior número de espécies,

No nível de comunidade, a riqueza de AETs pode experimentar picos a altitudes médias, mas tende a diminuir fortemente em grandes altitudes (McCain, 2010; Navas, 2002).

Topografia

Geiger (1950) descreve como a ondulação do terreno influencia a circulação do ar e a intensidade e o tempo de radiação que o solo recebe. Em noites sem vento forte, massas de ar mais fresco tendem a ocupar regiões baixas da topografia local, enquanto que massas de ar mais quentes tendem a subir. Isto leva a formação de “poças” de ar frio em depressões ou junto a objetos que impedem este movimento de massas de ar. Estas poças se formam regularmente, sendo de grande importância na distribuição topográfica de geadas em regiões frias. Por este mesmo processo, regiões altas de ladeiras mantêm-se mais quentes à noite, comparadas com fundos de vale e platôs. A diferença de temperaturas entre o fundo do vale e estas regiões altas pode superar os 10°C nas temperaturas mínimas.

A orientação norte-sul das ladeiras afeta ao tempo que estas recebem radiação (maior em ladeiras orientadas ao equador), enquanto que a orientação leste-oeste determinará o horário de incidência de radiação solar (mais cedo quanto mais orientada ao leste).

A inclinação das ladeiras afeta a quantidade de radiação recebida do sol, ou emitida ao céu por unidade de superfície. A quantidade de radiação recebida depende do ângulo de incidência dos raios solares sobre a ladeira. A quantidade de radiação emitida aumenta conforme a ladeira fica horizontal (Geiger, 1950).

Decorrente da existência de espécies com diferentes necessidades térmicas espera-se que diferentes espécies de AETs ocupem ladeiras de diferente orientação, como acontece em artrópodes (Tolbert, 1975). Entretanto, para alguns répteis de áreas temperadas, a orientação da ladeira não teve grande poder preditivo sobre sua distribuição (Guisan e Hofer, 2003).

Superfícies irradiadas

Na superfície terrestre, em dias ensolarados, a pri-

meira superfície alcançada pelos raios do sol recolhe a maior parte da energia enviada pelo sol (ex. nuvens, dossel arbóreo ou solo; Geiger, 1950). Depois de alcançar esta superfície, a energia se transmite para capas inferiores ou superiores através de diferentes processos (ex. radiação, condução, convecção), gerando gradientes de temperatura ao redor da superfície irradiada (Geiger, 1950).

Conforme nos aproximamos de superfícies irradiadas pelo sol, a temperatura tende a aumentar exponencialmente, em direção a estas superfícies (Geiger, 1950). Durante a noite, as superfícies irradiam energia para o espaço esfriando-se mais rápido que o ar justo acima delas. Nas horas mais frias da noite e nas mais quentes do dia, em superfícies não cobertas e em ausência de vento, formam-se perfis térmicos. Em ambos os perfis, as temperaturas mais extremas acontecem na superfície. Conforme nos separamos da superfície, os ciclos diários de variação térmica diminuem e os extremos de temperatura podem acontecer atrasados com relação à superfície irradiada (Geiger, 1950).

Durante a noite, estes gradientes verticais de temperatura podem repetir-se se houver uma segunda superfície radiante (ex. o dossel arbóreo). Nestes casos, a diminuição de temperatura tende a ser menor nas superfícies inferiores (Geiger, 1950). Os gradientes verticais de temperatura provocados por radiação na superfície terrestre podem estender-se por algumas centenas de metros sobre a superfície, mas, geralmente, por poucos metros no subsolo (Geiger, 1950).

Quanto menor a CT do material que compõe a superfície irradiada ou radiante, menos energia é transmitida através dela. Por este motivo, materiais com menor CT sofrem maior variação diária de temperatura na sua superfície e isolam mais as capas de substrato existentes sob ela (Geiger, 1950). Ao mesmo tempo, quanto maior a CT, maior a profundidade do solo que uma geada pode penetrar (Geiger, 1950). Microhabitats podem diferir em termos de CT. Por exemplo, de menor a maior condutividade, podemos ordenar: ar, folhicho, madeiras leves, areia seca, terra, cascalho, solo coberto de erva, solo argiloso úmido, água, gelo, rocha calcária, granito, concreto e ferro (Geiger, 1950; Lienhard e Lienhard, 2011).

Sobre a superfície de um material, a convecção domina a transmissão de energia, devido à baixa condutividade do ar (Geiger, 1950). Abaixo da superfície, a transmissão de energia é feita por condução e convecção do ar e água existentes no material (Geiger, 1950). O conteúdo de ar diminui a CT da superfície do solo (Geiger, 1950). A água aumenta fortemente a CT do solo, podendo multiplicar até por 12 vezes a condutividade da areia seca (Geiger, 1950). Por estes motivos, deveria ser mais fácil termorregular sob solos arejados e secos, do que sob solos compactos ou úmidos.

Gradientes de temperatura permitem e obrigam os AETs a distribuir-se por eles com relação às suas preferências ou tolerâncias. Deste, modo é possível prever a posição de AETs combinando apenas o conhecimento de suas preferências e tolerâncias térmicas com modelos da distribuição microambiental de temperaturas ao lon-

go do dia e do ano (ex. lagartos de deserto; Porter e col., 1973). Gradientes noturnos permitem que AETs possam termorregular também à noite, seja em diferentes capas da atmosfera (ex. insetos voadores (Chapman e col., 2011)), ou em refúgios sob a superfície (ex. iguanas terrestres e lagartos de deserto (Christian e Porter, 1984; Porter e col., 1973)). A posição de repouso, de diversos vertebrados ectotermos em florestas tropicais fica relativamente elevada na vegetação. Esta posição tem sido principalmente interpretada como forma de evitar predadores por alguns autores (ex. Clark e Gillingham, 1990; Martins, 1993). A vista destes fatos é razoável pensar que possa ter também uma função para evitar que estas desçam excessivamente ou para reduzir gastos energéticos durante o repouso (como em Christian e Porter, 1984).

Características físicas dos materiais que rodeiam um corpo também podem afetar a quantidade de energia absorvida por este e os horários em que há calor disponível para termorregular. Por isso, AETs podem regular sua temperatura corporal através da escolha de determinados microhabitats. A seguir, mostrarei como a temperatura pode variar nestes microhabitats e relacionaremos esta variação com diferentes aspectos da biologia dos AETs.

Fatores, processos e padrões termobiológicos derivados de irregularidades da superfície terrestre

A superfície terrestre apresenta irregularidades (ex. estruturas dispostas sobre ela e orifícios) que podem influenciar profundamente o ambiente térmico local.

Estruturas derivadas da vegetação e rochas

Estruturas naturais abundantes na superfície terrestre são capas de folhas (ex. as capas de um dossel arbóreo, ervas ou material vegetal morto sobre o solo), troncos e rochas. Todos eles são opacos e tem relativamente baixa CT pelo que protegem de raios do sol e suas superfícies se aquecem rapidamente. Capas de folhas possuem pouca massa e estão rodeadas por ar, minimizando a transmissão de energia através delas por radiação e condução (Geiger, 1950). Troncos e rochas, apresentam maior CT que capas de folhas. Isto faz com que necessitem maior quantidade de energia para aumentar sua temperatura e que funcionem como armazenadores de energia térmica durante o esfriamento do ambiente. Vejamos agora alguns padrões e processos térmicos relacionados a estes elementos.

Em uma floresta, os ciclos de variação térmica diária seguem aproximadamente o seguinte padrão: raios solares atingem o solo mais tarde, e por menos tempo do que na copa e nos troncos. Por isso, a temperatura na região do tronco-solo tenderá a ser mais elevada do que na copa, nas horas centrais do dia. A continuação, estas igualam-se até que a temperatura do solo cai por baixo antes do pôr-do-sol, uma vez que a radiação também se perde antes na região baixa da floresta (Geiger, 1950). Em relação aos gradientes de temperatura, Geiger (1950) argumenta que as máximas e mínimas temperaturas diárias são alcançadas na metade inferior do dossel de uma floresta. Porém, em florestas tropicais, o folhicho talvez alcance os valores

mais altos durante o dia (50°C Mesquita 2005). Acima das árvores gera-se por evaporação da água esfriamentos do ar que podem ser mensurados a alguns metros de altura sobre a superfície das folhas. Na base, a ascensão de seiva nas plantas pode transportar energia desde o solo, podendo provocar diferenças de 15°C, entre lugares de seiva queta e seiva ascendente.

Clareiras e bordas influenciam o horário e tempo de radiação sobre as superfícies das regiões inferiores das florestas (Geiger 1950). A variabilidade da temperatura do solo e ar em clareiras aumenta em função da relação diâmetro da clareira/altura das árvores (Geiger 1950). Do mesmo modo acontece com a penetração do efeito da clareira na floresta adjacente (Spittlehouse e Stathers, 1990).

Em ausência de vegetação protetora, as superfícies de material vegetal morto experimentam temperaturas extremas em sua superfície (Geiger, 1950).

Esta ampla heterogeneidade espaço-temporal de temperaturas obrigará a qualquer AET a ocupar locais onde suas temperaturas preferenciais ocorram. Por este motivo, mesmo tendo em conta apenas seu efeito sobre as relações terminais dos AETs, a vegetação tem um grande potencial de partilhar a distribuição espaço-temporal destes. Apenas diferenças na TPRF ou massa corporal podem ser suficientes para provocar diferenças na distribuição de AETs com relação à estrutura da vegetação (Asplund, 1974; Hillman, 1969).

Ectotermos de florestas são, em algumas ocasiões, considerados como termorreguladores passivos que deixam flutuar sua temperatura com a do ambiente (ex. Huey e Webster, 1976; Vitt e col., 1998; Huey e col., 2009). Entretanto, nestes habitats, também é possível encontrar termorreguladores bastante precisos, mesmo com temperaturas de atividade relativamente altas (ex. Vitt e Colli, 1994; Vitt, 1992). Ectotermos com preferências térmicas altas podem usar as clareiras e bordas para termoregular, ao tempo que animais de temperaturas em atividades mais baixas as evitam (ex. Dixo e Martins, 2008; Vitt, e col., 1998).

A distribuição preferencial que muitos grupos de ectotermos apresentam, e padrões estáveis de riqueza e composição de espécies relacionados à presença de clareiras ou bordas (Shure e Phillips, 1991; Vitt, e col., 1998) Wermelinger e col., 2007), e a estrutura da vegetação (ex. no folhíço, dossel ou troncos das árvores; Vitt, e col., 2003; Vitt e col., 1998; Wermelinger e col., 2007; em manchas abertas de vegetação; Nogueira e col., 2005) está possivelmente de acordo com suas preferências e tolerâncias térmicas. Porém, poucos estudos demonstram de maneira explícita a influência de variações térmicas, relacionadas à vegetação, na distribuição de AETs (mas veja Monasterio e col., (2009) e O'Neill e col., (1990)). Florestas estão entre os ecossistemas mais diversos do planeta (Myers e col., 2000). Possivelmente, a ampla heterogeneidade térmica existente dentro delas favorece esta alta diversidade biológica.

AETs podem usar troncos e rochas para termoregular. Isto é possível mudando sua posição ou escolhendo lugares de oviposição, com relação aos gradientes térmi-

cos gerados entorno deles (ex. como feito por lagartos em Adolph, 1990; Huey e col., 1989; López e col., 1998; e Vitt e col., 1996; ou larvas de besouros em Geiger 1950). Rochas pequenas podem aquecer rapidamente e ajudar a termoregular nas primeiras horas do dia a AETs refugiados sob elas (López e col., 1998). Rochas com mais de 10 cm de grossura podem diminuir as variações de temperatura no subsolo diretamente em contato com elas, facilitando a termorregulação de AETs que as utilizam como refúgio. Espécies fossoriais podem selecionar pedras maiores em horas avançadas do dia (Huey, 2011; López e col., 1998). Troncos de regiões temperadas podem gerar gradientes espaciais de temperatura de ao menos 20°C (Geiger, 1950).

Orifícios na superfície terrestre: cavernas

Ao contrário de objetos na superfície terrestre, orifícios na superfície terrestre (ex. cavernas, tocas) levam à homogeneização das temperaturas em seu interior (Geiger, 1950; Williams, Tieleman e Shobrak, 1999). Dentro das cavernas, em ausência de fluxos horizontais de ar, as temperaturas se estratificam verticalmente, sendo mais frias as regiões mais baixas. Se estas tiverem duas saídas, fortes ventos podem atravessá-las (Geiger, 1950), afetando a estratificação da temperatura. Os estudos levantados sugerem que AETs podem ser obrigados à atividade em temperaturas mais baixas do que os ótimos para a locomoção (Rogowitz e Sánchez-Rivoledda, 1999) e que a inversão dos gradientes térmicos dentro delas podem ser importantes controladores do período de atividade dentro delas (Resatarits, 1986; Rogowitz e col., 2001). Possivelmente relacionado a estes problemas de homogeneidade térmica e sub-otimicidade das temperaturas para AETs colonizadores, cavernas são ecossistemas com menor riqueza de espécies que os habitats exteriores onde se localizam. Nestes habitats, a heterogeneidade térmica não parece ser tão importante para explicar a riqueza de espécies de cavernas (Christman e Culver, 2001; Culver e col., 2004).

Processos e padrões termobiológicos relacionados ao corpo de AETs

Características físicas dos corpos (ex. tamanho e cor) podem afetar de maneira importante o intercâmbio de energia entre corpos e o ambiente (Lienhard e Lienhard, 2011). Por isto, espera-se que estas características influenciem a temperatura corporal experimentada por AETs.

Tamanho

Para um nível de radiação determinado, corpos de maior tamanho são capazes de absorver mais energia por unidade de radiação em relação à massa do corpo. Ao mesmo tempo, devido a um aumento em sua capacidade térmica, demorarão mais tempo que pequenos corpos em alcançar a temperatura de equilíbrio com o ambiente (Stevenson, 1985). Por estes motivos, dada suficiente entrada de energia por radiação, corpos maiores alcançarão temperaturas mais altas do que corpos pequenos. Ao mesmo tempo, corpos maiores serão mais difíceis de aquecer e esfriar. Este processo pode levar a um problema metodo-

lógico na determinação de temperaturas críticas de AETs (Ex. Ribeiro e col 2012).

Em resposta a estes processos, os indivíduos podem regular o comportamento de termorregulação. Em situações onde a perda de energia é mais rápida que o ganho, enquanto que indivíduos maiores podem precisar regular sua temperatura por mais tempo (ex., tartarugas aquáticas que se aquecem em terra, Lefevre e Brooks, 1995). Em animais muito grandes, a grande produção bruta de energia metabólica (ex. durante o exercício) e a relativamente inferior taxa de perda do mesmo pode levar à manutenção de uma temperatura relativamente estável, quando comparada com o médio. Se a taxa de perda é similar ou inferior ao ganho, em regiões de alta radiação, tamanhos de corpos maiores permitem passar mais tempo sem termorregular e manter a temperatura corporal em habitats mais sombreados (ex. lagartos tropicais, Asplund, 1974; Hillman 1969).

Vantagens termorregulatórias aparentemente explicam as relações intraespecíficas entre o tamanho do corpo e temperatura ambiental em répteis de todo o mundo (Ashton e Feldman, 2003). Entretanto, outros mecanismos possíveis também têm sido propostos por Angilletta (2004) e Angilletta e Dunham (2003).

Cor

A cor externa de um corpo afeta à quantidade de radiação que este pode absorver por unidade de tempo e, portanto, à temperatura máxima que este pode alcançar e sua taxa de aquecimento (Lienhard e Lienhard, 2011). (Geiger, 1950) mostra diferenças de aproximadamente 6 graus na temperatura de pequenos objetos de madeira, em similares condições experimentais, pintados com cores desde o branco ao negro. A revisão de Trullas e col., (2008) suporta a hipótese de que o melanismo é mais freqüente em regiões frias e que é relativamente benéfico para espécies diurnas de áreas mais frias. Segundo estes autores, a relação entre fisiologia, melanismo e comportamento também tem certo suporte, mas ainda requer mais estudos. Porém, nem todas as variações de cor levam a aumentos nas taxas de aquecimento de AETs, como exemplificado por Crisp e col., (2011).

Interações

Os diferentes fatores termobiológicos interagem entre si, gerando uma superfície de variação térmica extremamente irregular e dinâmica sobre a terra.

Fatores termobiológicos podem interagir de maneira aditiva, fazendo com que a temperatura experimentada pelos AETs alcance valores mais extremos; ou não aditiva, suavizando o ambiente térmico. Por exemplo, ladeiras inclinadas e orientadas aos pólos podem suavizar a temperatura ambiental em regiões tropicais, porém, tenderão a aumentar os extremos de temperatura em regiões circumpolares.

A relação entre dois fatores pode não ser linear. Por exemplo, conforme aumenta a latitude o efeito da orientação das ladeiras sobre as diferenças nas temperaturas ambientais também aumenta. Porém, a partir de certa

latitude também ascende a proporção de radiação difusa (procedente da reflexão do ar) com relação à radiação direta do sol (Geiger, 1950). Por este motivo, as diferenças de temperaturas entre ladeiras orientadas ao equador e aos pólos seguem uma relação parabólica com a latitude.

Diferentes fatores termobiológicos podem desencadear o mesmo padrão de variação térmica. Por exemplo, tanto latitude quanto altitude levam a um aumento da amplitude térmica, principalmente por diminuição das temperaturas mínimas absolutas (ex. Sunday e col 2010). Ao mesmo tempo, estes fatores podem ter influências opostas sobre outras características térmicas. Como exemplo, a magnitude do ciclo diário de temperaturas aumenta com a altitude e diminui com a latitude.

As características dos AETs (comportamento, morfologia e fisiologia) também podem interagir favorecendo ou evitando mudanças nas outras. Por exemplo, mudanças no comportamento (ex. uso de microhabitats) permitem evitar variação na temperatura de atividade, ao longo de gradientes térmicos gerados pela altitude (Huey e col., 2003).

Os padrões de variação térmica interagem com as características comportamentais, morfológicas e fisiológicas dos diferentes grupos taxonômicos (Trullas e col 2008, Asplund, 1974; Hillman 1969). Estas interações poderiam determinar qual grupo terá maior sucesso na colonização de gradientes de variação térmica provocados pelo mesmo fator (ex., diferenças latitudinais no sucesso colonizador de gradientes altitudinais entre anuros e lagartos sulamericanos, Navas, 2002).

Síntese

A transmissão de energia térmica acontece ao longo de um contínuo, desde o ambiente até os indivíduos e vice-versa. Porém, para fins didáticos, os processos e padrões termobiológicos que afetam aos ectotermos terrestres poderiam ser categorizados em três grandes grupos:

- 1) Os relacionados à intensidade e quantidade dos intercâmbios de energia dos ecossistemas (ex. derivados da topografia, altitude e latitude).

- 2) Os relacionados à distribuição espaço-temporal da energia que chega a superfície terrestre (derivados de propriedades físicas do micro-ambiente e irregularidades da superfície terrestre).

- 3) Os relacionados ao intercâmbio de energia entre o corpo dos AETs e o microambiente (derivados de propriedades físicas e fisiológicas dos corpos dos AETs).

As respostas dos AETs perante variações em um parâmetro climatológico podem acontecer em diferentes níveis organizacionais (ex. no comportamento ou fisiologia no nível dos indivíduos, na distribuição espacial, no nível das populações, ou na riqueza de espécies, no nível das comunidades).

Parece possível descrever os processos e padrões termobiológicos com um número limitado de fatores, passíveis de serem analisados matematicamente (ex., entrada de energia, propriedades físicas do ambiente e do corpo dos indivíduos, sensibilidade fisiológica à temperatura, capacidade termorregulatória, etc. (ex. como feito por

Grant e Porter, 1992, e Porter e col., 1973).

Finalmente, fatores térmicos podem interagir de maneiras aditiva ou não aditiva e linear ou não linear para afetar a temperatura ambiental e a experimentada pelos ectotermos.

Baseando-se no que foi apresentado nesta revisão, é possível sintetizar uma teoria preditiva sobre o efeito da temperatura nos ectotermos.

De acordo com a escala apresentada por Scheiner (2010), e mesmo em estado rudimentar, esta teoria já prediz relações gerais em mais níveis de organização do que teorias centrais da biologia, tais como a teoria da evolução de Darwin, a teoria da célula, e a teoria dos organismos, ambas propostas por Scheiner (2010).

O processo aplicado aqui pode ser repetido para outros parâmetros microclimáticos e características dos seres vivos (como os comentados por Titon e Gomes, 2012; Assis, 2012 ou Closel e Kolhsdorf, 2012), ou grupos não tratados neste texto (ex. endotermos). Deste modo, poder-se-ia organizar uma teoria bioclimática dos seres vivos preditiva, baseada em dados, e de fácil entendimento, aplicação e crítica.

Teoria termobiológica dos ectotermos

Domínio de aplicação

Respostas dos seres vivos ectotermos à variação térmica.

Premissa

O tempo em que ectotermos passam dentro de suas temperaturas preferenciais aumentaria sua aptidão. Esta premissa é suportada pela relação entre taxas de crescimento populacional (uma medida geral de aptidão) e temperatura em ectotermos (Huey e Berrigan, 2001), assim como por estudos de fisiologia reprodutiva em Peixes (Ribeiro e Moreira, 2012).

Predições

1. Comportamento, fisiologia e morfologia dos indivíduos tenderão a aumentar a proporção do tempo de vida realizado dentro das Tprf, assim como evitar as TC;
2. Animais ectotermos que desenvolvam sua atividade mais perto de superfícies irradiadas pelo sol e com períodos de atividade mais próximos ao meio dia, serão termoreguladores mais ativos;
3. A distribuição e tamanho das populações de ectotermos ao longo de gradientes e ciclos de temperatura será explicada pelo sucesso relativo dos indivíduos de permanecer dentro de suas Tprf, e de evitar TC. Por este motivo, dentro dos referidos gradientes, o espaço-tempo disponível para termorregular em um determinado local determinará um limite superior para o tamanho das populações de AETs;
4. A interação não aditiva de fatores térmicos terá efeito positivo na riqueza de espécies de AETs de um local. Efeitos aditivos terão efeito negativo;
5. Quanto maior for a intensidade e estabilidade espaço-temporal das variações térmicas, maior será seu poder de predição sobre a posição das espécies de AETs

ao longo destas.

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Two parameters for identifying thermal challenges for small terrestrial ectotherms: thermal extremes and spatial thermal ranges

Autores:

Agustín Camacho, Miguel Trefaut Rodrigues, and Carlos Navas

Considerations for Assessing Maximum Critical Temperatures in Small Ectothermic Animals: Insights from Leaf-Cutting Ants

Pedro Leite Ribeiro*, Agustín Camacho, Carlos Arturo Navas

Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil

Abstract

The thermal limits of individual animals were originally proposed as a link between animal physiology and thermal ecology. Although this link is valid in theory, the evaluation of physiological tolerances involves some problems that are the focus of this study. One rationale was that heating rates shall influence upper critical limits, so that ecological thermal limits need to consider experimental heating rates. In addition, if thermal limits are not surpassed in experiments, subsequent tests of the same individual should yield similar results or produce evidence of hardening. Finally, several non-controlled variables such as time under experimental conditions and procedures may affect results. To analyze these issues we conducted an integrative study of upper critical temperatures in a single species, the ant *Atta sexdens rubropilosa*, an animal model providing large numbers of individuals of diverse sizes but similar genetic makeup. Our specific aims were to test the 1) influence of heating rates in the experimental evaluation of upper critical temperature, 2) assumptions of absence of physical damage and reproducibility, and 3) sources of variance often overlooked in the thermal-limits literature; and 4) to introduce some experimental approaches that may help researchers to separate physiological and methodological issues. The upper thermal limits were influenced by both heating rates and body mass. In the latter case, the effect was physiological rather than methodological. The critical temperature decreased during subsequent tests performed on the same individual ants, even one week after the initial test. Accordingly, upper thermal limits may have been overestimated by our (and typical) protocols. Heating rates, body mass, procedures independent of temperature and other variables may affect the estimation of upper critical temperatures. Therefore, based on our data, we offer suggestions to enhance the quality of measurements, and offer recommendations to authors aiming to compile and analyze databases from the literature.

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* E-mail: pedrolribeiro@gmail.com

Introduction

Climate warming has stimulated integrative studies aiming to assess or predict the impact of environmental temperatures on faunas. This complex problem has been addressed from several perspectives, one of which is how thermal climatic events affect individual performance and, in turn, may cause population declines [1,2]. Most such studies have focused on various views of the thermal tolerances of ectothermic animals because they represent the vast majority of animal species and many are known to have thermally dependent behavioral and physiological functions. Therefore, a main tenet is that measures of thermal constraints, studied in parallel with thermal preferences and environmental temperatures, provide information about the vulnerability of organisms to changing temperatures [3–25]. From this perspective, vulnerability (an inference about ecological performance) may be expressed as a correlate of the difference between the expected body temperature in a given scenario (an autoecological parameter) and indicators of maximal thermal tolerance (a physiological parameter) [6,26]. Relying on diverse

indicators of thermal limits (see definitions in [27], Table 3.4 and [1], Table 1), this approach to assess vulnerability has been applied to several contexts and systematic groups [28–35]. However, if heating rates influence upper thermal limits, these limits may differ among species when assessed at a given heating rate, yet be similar when ecologically relevant heating rates are considered for each species. Therefore, analyses of vulnerability based on upper thermal limits, and general considerations regarding ecological implications of critical temperatures, require considerations about heating rates [1]. In addition experimentally determined thermal limits rely on two major (but usually tacit) assumptions. One assumption is that these limiting values reflect the maximum temperature tolerated by animals before they suffer permanent physiological damage (because damage would indicate that these limits were actually surpassed in the test and therefore the estimate was more a lethal rather than a critical upper limit). Another assumption is that thermal limits are reproducible traits of individuals, so that the average values from several individuals define the thermal tolerances of a population. However, these major assumptions require experimental validation. Finally,

Table 1. Heating rates treatments and sample size.

Group	Heating rate	Number of ants that recovered after 2 hours of CTMax test
1	2°C/1 min	59
2	1°C/1 min	30
3	0.66°C/1 min	30
4	0.5°C/1 min	30
5	0.4°C/1 min	30
6	0.33°C/1 min	30
7	0.29°C/1 min	30
8	0.25°C/1 min	37
9	0.22°C/1 min	31
10	0.2°C/1 min	39
11	0.18°C/1 min	33
12	0.16°C/1 min	30

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hidden and uncontrolled sources of variation, e.g., protocols (time of the day, end point and others) and experimental procedure (time under test conditions, manipulation and others) may add complications to the interpretation of data through unplanned effects on physiology. The aims of this paper are to test in a single experimental model the 1) influence of heating rates in the experimental evaluation of upper critical temperature, 2) assumptions of absence of physical damage and reproducibility, and 3) sources of variance often overlooked in the thermal-limits literature; and 4) to introduce some experimental approaches that may help researchers to separate physiological and methodological issues.

The thermal tolerance of animals is a plastic, environmentally-induced trait that responds to planned experimental sources of variance [18,36–45], including past thermal history [37], acclimation [36,39,40] and acclimatization [46]. It also varies with ontogenetic stage [47] and with seasonal and daily biological cycles [48–50]. These known sources of variation need to be recognized if thermal tolerances are calculated. Thus, researchers must control or standardize measures in terms of ontogeny, reproductive state, season and thermal history. However, less obvious sources of variance may enhance intra-specific variation and produce a failure to detect differences among species (increase Type II error), or may exaggerate the relevance of minor differences. Critical temperatures may be influenced by the mass of tested individuals [51], the heating rates applied [18,51–54] and indicators of experimental endpoint such as lack of response, muscular spasms [55], or thermolimit respirometry [56]. In addition, the analysis of critical temperatures may be based on ramping or static methods (e.g. knock-down critical temperatures (see [57])). Given this methodological diversity, both uncontrolled sources of variation in physiological states and methodological issues may affect the determination of thermal limits in ectothermic animals. However, several important sources of variation have been studied largely independently and in different taxa and a single-species integrative analyses are much needed.

We chose ants of the same colony as an ideal model in our study, because large numbers of individuals are available, remarkable differences in body size exist among individuals possessing similar genetic makeup, and colonies can be maintained in captivity under controlled conditions. To assess upper critical temperatures, we followed classical approaches for studying the

parameter known as the critical thermal maximum (CTMax). The CTMax figures among the first parameters proposed to link animal thermal physiology and ecology [58]. It has been widely used to investigate thermal limits in ectothermic animals [59] and has been applied in the context of climate warming [16,24,60–62]. We investigated the effects of heating rates on the CTMax, and assess damage and reproducibility of thermal limits by focusing on whether tests had an impact on subsequent measures. If an impact occurred, we sought to determine whether usually overlooked sources of variance (duration of experiments, procedures other than thermal treatment, daily rhythms and body mass) could be responsible for the observed variation in the CTMax. For example, the duration CTMax experiments is inevitably correlated with heating rates and with the magnitude of the temperature turning out to be the CTMax for a given species (e.g., if heating rates are constant, greater CTMax require longer experiments). Therefore, researchers must know whether effects on CTMax are truly derived from heating rates themselves or from collateral effects of extended manipulation. Daily rhythms and mass-dependent responses were additional concerns addressed by the study. We also present detailed protocols and analyses that shall help researchers to tell apart effects of some sources of variation. Our results generated a series of recommendations that contribute to current debate and are useful for investigations of organismal upper thermal limits, independently of the method used.

Results

Heating rates determined final CTMax readings. Within the range of heating rates explored (2°C/min to 0.16°C/min [1°C per 6 min]), faster heating led to a higher CTMax (ANCOVA: $F_{3,400} = 35.5695$, $p < 0.001$, Figure 1a). This effect was due to heating rates *per se*, and not to extended experiments as a byproduct of slower heating rate (see results on a Procedure-control below).

Our test of reproducibility compared the CTMax of marked individuals measured more than once, always recovering under normal colony life between tests. These tests indicated that CTMax values are not reproducible in *Atta sexdens rubropilosa*. Exposure to a first measure of CTMax caused a decrease in two subsequent CTMax readings, one performed 24 h after the first measure and a third one 24 h after the second one (ANCOVA: $F_{2,40} = 8.5104e+25$, $p < 0.001$, Figure 1b). However, the effect was not cumulative, and second and third CTMax measures were comparable (Tukey test: $p = 0.985$). An additional test of reproducibility of CTMax was made six days after a first measure. This test also led to reduced CTMax, although the observed reduction was lower in magnitude than those observed in reproducibility tests after 24 h and 48 h (ANCOVA: $F_{3,71} = 17.745$, $p < 0.001$, Figure 1b). The above results seem derived from exposure to critical temperatures and not from responses of tested individuals to any other aspect of the experiment. This is so because a sub-critical temperature control test (temperatures elevated to high, yet subcritical values, see materials and methods) did not alter subsequent CTMax readings after 48 h (ANCOVA: $F_{3,46} = 0.0012$, $p = 0.973$, Figure 1b). Similarly, CTMax tests controlling for experimental procedures other than thermal treatment, and focusing mainly on the period of time under experimental manipulation (see procedure-control test in Material and Methods) produced values of CTMax similar to those performed in the absence of such control. Procedure-control tests generated CTMax data comparable to that generated in the first CTMax performed (ANCOVA: $F_{3,99} = 1.338$, $p = 0.250$) and the test exploring long-time recovery (ANCOVA:

Figure 1. Results of CTmax tests. a) CTmaxs measured at different heating rates from 2°C/min to 0.16°C/min. b) CTmax estimates corresponding to different contrasts: i) the contrast between the first exposure*, and second and third exposures, to determine reproducibility under short-time recovery (each exposure with a 24 h interval); ii) the contrast between the first exposure* and the six-day recovery, to determine reproducibility under long-time recovery (144 h); iii) The contrast between the first exposure* and the CTmax after subcritical exposure and the manipulation control. c) Correlation between body mass and CTmax. d) CTmax measured at different times of the day. Bars indicate SDs. *The results from the first days of the short- and long-time recovery experiments are plotted together.

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$F_{3,82} = 1.296$, $p = 0.258$, Figure 1b). In summary, we observed reduced reproducibility of CTmax only in tests that actually exposed individuals to upper critical temperatures, not in any control test comparable regarding manipulation and procedure, but that did not exposed animals to CTmax values.

Daily cycles did not influence the CTmax (ANCOVA: $F_{3,223} = 1.464$, $p = 0.162$, Figure 1d). In contrast, ant body mass affected upper thermal tolerances. Larger individuals ants tolerated higher temperatures than smaller (linear regression R^2 : $F_{1,839} = 187.6$, $R = 0.182$, $p < 0.001$, Figure 1c). The interaction between body mass and heating rate was not significant (ANCOVA: $F_{3,400} = 0.032$, $p = 0.954$), as would have been expected if thermal inertia were an issue.

Discussion

Our data confirm that heating rates are influential in the estimates of critical temperatures [51–54,63]. The trends found in *Atta sexdens rubropilosa* are likely general for the species (not necessarily in absolute values, which may vary among colonies [3], but on main patterns and correlations. In addition, these trends are generally compatible with those presented by Rezende et al. [18], but we do not postulate any specific mechanism as a causal agent of the pattern observed. Our argument is that thermal increase in ectothermic animals will produce two simultaneous phenomena. First, both exposure to temperature and manipulation

may affect the physiological performance of test animals for diverse reasons, among them the explanations hypothesized by Rezende et al. [18]. From this viewpoint, it is probable that longer experiments will reduce the health of animals under experimental conditions and will reduce tolerance (through mechanisms including but not limited to desiccation, loss of energy reserves and oxygen-limited thermal tolerance; see Portner et al. [64], Peck et al. [54] and Rezende et al. [18]). Oxygen-limited thermal tolerance may have influenced the trends found in *Atta sexdens rubropilosa*, because the observed reduction in thermal tolerance was related only to exposure to upper critical temperatures, and not to any other aspect of experimental manipulation. On the other hand, exposure to increasingly high temperatures may activate physiological responses that enhance thermal tolerance and that have different temporal courses. Thus, trends enhancing or decreasing thermal tolerance are possible, even more as experiments become longer (i.e., as heating rates become slower or CTmax values turn out to be higher). Accordingly, the dominant trend for a given group of experimental animals will depend on both taxon-specific and experiment-specific considerations. Under this model, it would not be possible to anticipate the impact of heating rates in a new organism to be tested. However, given nuances of individual variation, slow heating rates leading to longer experiments may be associated with higher variances (as seen in Chown et al. [51]).

If heating-rate effects cannot be anticipated, authors targeting baseline thermal protection may prefer fast acute heating rates. In

addition, fast heating rates are less likely to impose a lag in the homogenization of the body temperature, at least in small aquatic animals, such small fish [65]. However, the relationship between the heating power of the equipment, the size of the experimental animals, and the thermal conductivity of the media (e.g. air versus water) needs to be explored to guarantee uniform heating rates. This is more of a problem in aerial tests because the thermal conductivity of air is more than 20 times smaller than that of water. Although experiments can be fine-tuned in preliminary tests, some species of interest may provide only limited samples. In such cases, we suggest researchers opt for choose heating rates compatible with homogeneous heating in tested individuals.

A final important comment on heating rates addresses the dominant assumption that a higher baseline critical temperature (e.g., CTMax) confers greater thermal tolerances in the field [4,27]. This very assumption supports the premise that safety ranges can be deduced from differences between critical temperatures and actual or predicted field temperatures. Support for this assumption emerges from the observation that species exposed to very high temperatures have particularly high critical temperatures [22,62,66–69] and, more recently, from broad meta-analysis [18,70]. However, the current global scenario requires inference about ecological critical temperatures, that is, the thermal tolerance at typical (or predicted) rates of field temperature change [1]. Baseline and ecological critical temperatures are not necessarily the same, and we lack empirical studies generalizing the relationship between these two parameters (but see Rezende et al. [18,70]). For example, two species differing in acute CTMax may exhibit similar values if compared at the heating rates typical of their microhabitat. This largely unexplored area requires additional data.

The key assumption of reproducibility of thermal limits has received minimal attention in the literature. A related assumption is that critical thermal limits refer to temperatures withstood by animals without suffering permanent damage [58,59], a post-experimental state usually validated through simple behavioral observations. If these two assumptions are met, upper critical temperatures would be truly reproducible, generate similar values across tests, or eventually would indicate heat hardening [71,72]. Alternative results have been observed in *Embioptera* [73] and in the juveniles of three species of arachnids, *Rhipicephalus sanguineus*, *Ixodes scapularis* and *Amblyomma americanum* [74]. Likely, hardening has variable temporal scales and vary according to thermal treatment, heating protocol and species. Independently of this consideration, our results suggest that critical temperatures are not necessarily reproducible among individuals, not because they are transient but because exposure to upper critical temperatures cause long lasting deleterious effects on the animals, even if recuperation is apparent through behavioral observations. Accordingly, the end point of the experiments as performed may have overestimated tolerances in our experiments, and overall, in the literature. This issue requires attention because it challenges the practice of evaluating CTMax recovery from simple post-test behavioral observations. The deleterious effects of a single CTMax tests were evident six days after that tests and reduced critical temperatures by more than 3°C. In addition, the variance in critical temperature was more than 7-fold higher in tests 2 (24 h after first tests) and 3 (48 h after first test) than in test one. This finding suggests that exposure to upper thermal limits affected individuals in different way, favors a simple dichotomic model (hardening versus health, see next paragraph), and poses straightforward practical implications: 1) even if recuperation is apparent, upper critical temperature experiments may overestimate tolerances; 2) simple behavioral tests are not ideal to demonstrate recuperation. Ideally, test should

be repeated controlling for time in captivity and manipulation, but this may not be practical under many circumstances. Then, longer post-experimental observations and alternative species-specific options should be considered; and 3) if extrapolations are made from laboratory tests to the field, the frequency of near-critical events may affect ecological performance. One single event may be critical reducing tolerance to subsequent exposure.

Body mass is an important source of variance that is often unconsidered or not clearly associated with either physiological or methodological issues. Our results suggest that in *Atta sexdens rubropilosa* upper thermal limits are affected by body mass, and that this is not an artifact of mass-procedure interactions. The interaction between body mass and heating rate may be illustrative in this context: If this interaction explains variance in thermal limits, it is likely that experimental correlates of body size, such as heating rates or dehydration rates (e.g., Rezende et al. [18]) are complicating factors. In knock down experimental designs, heating rates may vary among individuals of different sizes, thereby increasing variance and reducing the power of the analysis. Although body size affects the CTMax of *A. s. rubropilosa*, these results cannot be obviously correlated with the ecology or behavior of ant castes. For example, we did not use the smallest ants, which are rarely exposed to high temperatures. So far studies in different species corroborate tendencies towards an increase [54] or decrease [75] of CTMax with body size; given the physiological, phylogenetic, methodological and scale issues involved, this diversity shall not be surprising.

Daily rhythms are not critical for determining the thermal limits of *Atta sexdens rubropilosa*. If sufficient numbers of individuals are available, assessments like ours may support the idea that testing at various times of the day will not increase the variance of the results. However, this trend is unlikely to be general because circadian clocks play a role in the expression of heat shock proteins [76] and because daily rhythms are a factor in the critical temperatures of other species, for example *Rana clamitans* [50]. If cyclic components of critical temperatures are more important in some models than in others, the only options are to make preliminary tests that verify this possibility or to take a conservative approach that aims to use measures according to ecologically relevant criteria, e.g., the time of day at which the maximum exposure to warm temperatures is likely to occur.

Protocols similar to those proposed in this paper would be useful to detect or rule out manipulation effects (independently of physiological mechanisms) and to better understand size-related effects on tests aiming to assess upper critical temperatures. In addition, these considerations may help authors interested in building upon published literature, for common ground is necessary [70]. The best data in this context would be collected according to the same standard protocols. However, if this is not possible, comparisons will require careful reading of the methods in each paper and the construction of a detailed dataset that includes the heating rate, time of day, season and actual geographic origin of the individuals included (not necessarily the native range). Special attention needs to be given to body mass and heating rates. Body mass needs to be considered for it may reflect both experimental artifacts and true physiological traits. Heating rates are likely to vary among samples, so that the interaction between body mass and heating rate needs to be explored within the limits of the data. In addition, because daily rhythms may be more pronounced in some species than in others, no general suggestions can be anticipated, and experimental controls for the time of day should be used. Finally, the criteria for endpoints and for pos-tests behavioral observation of experiments may generate different results.

Materials and Methods

Colonies

We used one laboratory colony of *Atta sexdens rubropilosa* containing approximately 25,000 individuals. We opted to use one colony because the physiological and methodological correlates of critical temperatures could be better analyzed when minimizing genetic variation in the sample. This colony was kept under natural photoperiod (from 10 h31 min D: 13 h29 min L to 11 h14 D: 12 h46 L) and at room temperature which varied throughout the experiment from 22°C to 27°C. It was regularly fed with leaves of *Acalypha* sp. *ad libitum*. The presence of immature forms was frequently monitored as the best possible indication of colony and queen health. At the time of the experiments, the colony was approximately 4 years old, appeared vigorous and displayed pots full of fungus and intense foraging activity. In addition, one mature natural colony was used to test for daily rhythms. This colony was located on the campus of the University of São Paulo. It is probable that this natural colony contained over 1,000,000 individuals.

Critical Thermal Maximum

Our equipment was designed by Sable Systems (Las Vegas, USA) and consisted of a hotplate pelt with a programmable heating rate controlled by a computer interface. The temperature was monitored by two channels that measured the temperature independently and were simultaneously connected to a TC2000 Thermocouple Meter (Sable Systems). Because our general approach involved several hundred tests, we did not use of temperature sensors in the bodies of the ants. The system permitted ten ants to be tested simultaneously. Two ants were tested in each of five separate containers. We used only ants cutting or carrying leaves, regardless of body size. The temperature was increased at various rates (see Heating Rates). During the experiments, the ants were observed continuously and in the same order. Individual ants were rapidly turned upside down. The experiment ended when an individual could no longer return to the normal position within 5 seconds. The temperature was then recorded, and the ant was immediately placed in a small container at 25°C for recovery. Data points were only considered valid if an ant displayed normal activity two hours after a CTMax test. The ant was then weighed on a precision balance.

Heating Rates

The equipment include a temperature controller allowing to control for heating rates and was calibrated to define the actual rate of warming of aluminum containers (6.2 cm in diameter and 2.4 cm deep) used to measure the CTMax of an ant. We measured the CTMax of 409 ants tested at 12 heating rates (Table 1).

Daily Cycles

We used an adult colony of *Atta laevigata*. The mature colony exhibited vigorous trails and thousands of foraging ants. This is a phylogenetic related species to *Atta sexdens rubropilosa* and occupies a very similar niche [77]. At the time the experiments were carried out mature natural colonies of *Atta sexdens rubropilosa* were unavailable on campus, and proximity to the laboratory was essential to minimize the time lag between capture and experiments. We preferred a natural colony for these tests to ensure the daily rhythms occurring in the field without interference from artificial lighting, feeding or maintenance schedules and to provide a natural temperature cycle. In this experiment, the heating rate used was always 1°C each

2 minutes. The experiment was conducted between 28/10/2010 and 12/01/2011 only on days without rain (these ants do not forage on rainy days) when ants were available (ants do not leave the nest on very hot days, e.g., >30°C). The ants were collected at the following times of day: 0:30, 3:30, 6:30, 9:30, 12:30, 15:30, 18:30 and 21:30. Three tests with 10 ants each were made at each time of day and on different days with this approach the time-lag between capture and experiment was always shorter than 10 min. In all, 232 ants were tested. We collected only ants returning to the nest with a piece of vegetation.

Reproducibility of CTMax

In this experiment, we measured the CTMax of 76 ants. We followed the standard protocol with a heating rate of 0.5°C/min. The ants were returned to the colony after the test. All ants were marked on the head with a *PenTouch* pen (Sakura Color products corp, Osaka, Japan) for identification as tested or not-tested. This system does not allow ant individual recognition, but minimizes manipulation, time out of the colony and lost of chemical recognition marking, experimental priorities in these tests. After 24 hours, the CTMax of 41 marked ants was measured with the same protocol (0.5°C/min). After 24 additional hours (48 hours after the first test), 24 ants that had two marks (one from the first test and another from the second) were collected and again examined using the CTMax test protocol. No other protocol is viable because individual *Atta* deteriorate rapidly after 24 hours of isolation from the colony [42]. However, we investigated the possibility that replacing ants in the colony would enhance recovery. In this experiment, the CTMax values of 58 ants were measured following the standard protocol, but the second test (after the first CTMax exposure) occurred 144 hours later. We recovered 17 ants for this trial.

To control for experimental manipulation, we used 70 ants from the same colony. These ants were exposed to the protocol described above, but the hotplate remained off. In these tests, a fixed time (60 min) compatible with the actual assessment of the CTMax determined the end of the experiments. In 28 ants, we could apply this control twice before the actual readings of the CTMax (1°C every 2 minutes). To control for exposure to high but submaximal temperatures, we used 50 ants tested following the standard protocol with a heating rate of 0.5°C/min, but we used 35°C as an experimental endpoint. After 24 h, 27 ants that had been exposed to submaximal temperatures were subjected to normal CTMax assessment. The body mass was also measured again.

Following Berthou [78] we performed an ANCOVA in which heterogeneity of variance among treatments was controlled using logarithmic transformation. Due to the non normality of the obtained data, the reproducibility experiment was analyzed using permutation ANCOVA, following Manly's permutation method [79]. All analyses were done using R software (version 2.0.1)

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Author Contributions

Conceived and designed the experiments: PR AC CN. Performed the experiments: PR AC. Analyzed the data: PR AC CN. Contributed reagents/materials/analysis tools: PR AC CN. Wrote the paper: PR AC CN.

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ORIGINAL
ARTICLE

The relationship between limb reduction, body elongation and geographical range in lizards (*Lerista*, Scincidae)

Michael S. Y. Lee^{1,2*}, Adam Skinner^{1,2} and Agustín Camacho³

¹School of Earth and Environmental Sciences, University of Adelaide, Adelaide, SA 5005, Australia, ²Earth Sciences Section, South Australian Museum, Adelaide, SA 5000, Australia, ³Instituto de Biociências, University of São Paulo, São Paulo-SP, CEP: 05508-090, Brazil

ABSTRACT

Aim The relationship between changes in body form (limb reduction and body elongation) and geographical range size was investigated across 68 species of *Lerista*, a species-rich clade of Australian scincid lizards that exhibits extensive interspecific variability in both body form and range size.

Location *Lerista* occurs across the entire Australian mainland, with diversity concentrated in arid and semi-arid regions.

Methods Geographical range size was estimated directly from c. 14,000 museum specimens using bioclimatic modelling in MAXENT. Body form was quantified using principal components analysis of morphometric variables. Comparative analyses testing for a correlation between these two variables used a full Bayesian approach that accounts for uncertainties in trait optimization as well as in tree topology and branch lengths.

Results A serpentine body form (elongated with reduced limbs) was significantly associated with smaller geographical range size, in both phylogenetically corrected and uncorrected analyses – but only if species from single localities (whose ranges could not be modelled using the above methods) were excluded.

Main conclusions These results suggest a general predictive relationship between body form and geographical range size in lizards: elongate, limb-reduced lizards tend to exhibit more restricted geographical ranges that may reflect reduced dispersal ability and may also predispose them to greater vulnerability of extinction.

Keywords

Australia, Bayesian inference, bioclimatic modelling, geographical range size, *Lerista*, limb reduction, lizards, reptiles, Scincidae, squamates.

*Correspondence: Michael S.Y. Lee, Earth Sciences Section, South Australian Museum, North Terrace, Adelaide, SA 5000, Australia. E-mail: mike.lee@samuseum.sa.gov.au

INTRODUCTION

Geographical range size varies widely among species and clades, but the evolutionary and ecological mechanisms underlying this variation remain contentious. One important facet of current debate concerns whether geographical range size is phylogenetically conserved ('heritable') over broad evolutionary time-scales. Do closely related species tend to share similar range sizes (e.g. Hunt *et al.*, 2005; Mouillot & Gaston, 2007; Borregaard *et al.*, 2012), and if range size is phylogenetically conserved, what mechanisms are responsible for such phylogenetic conservatism? Similarities in geographical range size among closely related species might be attributable to shared (inherited) biological

attributes, such as dispersal ability or niche breadth (e.g. Böhning-Gaese *et al.*, 2006). Alternatively, they could result from extrinsic factors – for instance, species from different clades could occupy different landmasses, and so could be affected by different (e.g. narrow or wide) geographical barriers (e.g. Machac *et al.*, 2011). For certain broad comparisons, the observed patterns and their causes appear intuitive; for instance, on a global scale, geographical range size is typically much larger in birds than in freshwater fishes, in pelagic organisms than in spring-dwellers, and in species with planktonic rather than directly developing larvae, almost certainly reflecting differences in organismal or habitat attributes affecting dispersal ability (e.g. Brown *et al.*, 1996; Gaston, 2003). A more

intriguing question is whether consistent phylogenetic patterns in geographical distribution persist when comparisons are made across closely related taxa. Such taxa are less likely to exhibit major biological and/or geographical differences that readily explain differing geographical range sizes. Analyses of relatively recent clades are therefore needed to assess the extent to which geographical distribution varies systematically at lower phylogenetic scales, and to elucidate more subtle evolutionary and ecological mechanisms that influence the evolution of range size. While these factors are potentially clade-specific, examination of multiple clades which each exhibit similar variation might reveal general correlations.

The scincid lizard clade *Lerista* possesses numerous features that make it ideal for investigating the fine-scale heritability of geographical range size and its causes. First, all species are endemic to Australia, eliminating the broadest-scale (continental) influences on geographical range size (e.g. Machac *et al.*, 2011). Second, it is a highly diverse clade containing species that display an extensive array of geographical distributions (e.g. Greer, 1989) – the c. 91 described species have known ranges that vary from single localities (e.g. *L. robusta*) to half a continent (e.g. *L. bipes*). Third, the alpha taxonomy of the group (Cogger *et al.*, 1983) is being actively investigated, improving the reliability of the museum records from which geographical distributions are constructed (e.g. Amey *et al.*, 2005; Smith & Adams, 2007). Fourth, a recent, detailed evolutionary tree for the group (Skinner *et al.*, 2008) provides a phylogenetic framework for evolutionary inferences. Fifth, *Lerista* has been sampled reasonably well throughout Australia, while there exists a detailed environmental database, including climate and soil data, for the whole continent. Thus, it is possible to generate reliable distribution maps, refined by the use of environmental modelling. Sixth, all species are broadly similar in both general anatomy and body size (to the extent that they are placed in the same genus), but vary substantially in gross body shape, permitting explicit investigation of the potential effects of body elongation and limb reduction on geographical range size evolution. Finally, although differences in body form are associated with different foraging modes (highly elongated species are predominantly fossorial, whereas shorter-bodied species are surface-active), surveys of stomach contents indicate that the examined species have essentially the same generalist diet (Pianka, 1986; Pough *et al.*, 1997). Any relationship between body form and range size is therefore unlikely to be confounded by specialization on habitat-restricted prey. Indeed, preliminary observations suggest that highly limb-reduced species of *Lerista* tend to have smaller ranges.

We assess here whether body form (i.e. the degree of body elongation and limb reduction) is correlated with geographical range size in *Lerista*, employing Bayesian methods that explicitly account for uncertainties in both phylogeny and character optimization.

MATERIALS AND METHODS

Analysis of body form

We measured four morphometric variables for 68 species of *Lerista* (see Skinner & Lee, 2009): snout–vent length, forelimb and hindlimb lengths (measured from the axilla and groin, respectively, to the tip of the longest digit), and head width (at the widest point of the head). Tail length was not used due to missing data caused by tail loss, and '*L. muelleri*' was excluded, because this taxon has recently been recognized as a species complex (Smith & Adams, 2007). The first three variables were size-corrected by dividing by head width, because although snout–vent length is the most commonly used body size proxy in squamate reptiles, it is poorly correlated with body mass (size) if there is great variation in body shape (Pincheira-Donoso *et al.*, 2011). The size-corrected data were subjected to principal components analysis, employing the correlation matrix, using R COMMANDER (Fox, 2005).

Range size

Geographical range sizes for the 68 species of *Lerista* included in the analyses were estimated using collection locality data for specimens held in Australian state and territory museums. We modelled ranges using MAXENT in R 2.14.1 (R Development Core Team, 2011), following recommendations in the vignette of the R package DISMO (Hijmans *et al.*, 2012), and calculated areas using a custom R script. MAXENT has proved itself a reliable algorithm when modelling with different sample sizes (Pearson *et al.*, 2007). Environmental modelling of species distributions was performed using a raster database including both climatic and soil layers. The database contained all of the climatic variables, plus elevation, available from the WorldClim database (<http://www.worldclim.org/>), and all of the soil-derived attribute layers available from the Harmonized World Soil Database (<http://www.iiasa.ac.at/Research/LUC/External-World-soil-database/HTML/>). The resolution of these databases is measured by the dimensions of the sides of the square cells that compose the raster layers. For this work, we used the highest resolution available for soil layers (5 arc-minutes). Distributions were modelled using the package DISMO in R (Hijmans *et al.*, 2012), following guidelines in the vignette. We used the area under the receiver operating characteristic curve (AUC) to assess the accuracy of model predictions in discriminating between places where the species is present and places where it is absent. Values over 0.5 indicate that the discriminations are better than random (Hanley & McNeil, 1982).

Generating reliable distribution sizes from museum records presents the following problems: (1) errors may be present in the locality or taxonomic data; (2) low sampling effort may underestimate the distribution size of species; (3) wide-ranging species may have been present in only a small portion of the modelled distribution generated by their records; (4) endemic species of very restricted areas (e.g. single localities) will be difficult to model using estimation algo-

rithms. Thus, we chose a conservative strategy to model distribution sizes that attempted to address these issues.

Our steps were as follows:

1. Specimens and localities were personally revised by A.S. and obvious errors corrected (e.g. zero-value coordinates, implausible outliers, records at sea or 'mirrored' in the Northern Hemisphere).
2. We generated a distribution map of the each species based on sampling records. In this way, we could check if species with smaller ranges tended to be confined to regions with low sampling effort (e.g. remote arid areas), which would be consistent with sampling artefacts. This was not the case – most species with limited ranges tended to be found in coastal regions which are generally better sampled than inland regions (see Wilson & Swan, 2010).
3. To avoid over-extrapolation of species ranges, for species with five or more locality records, we calculated the projected area of the initial distribution rectangle, using the highest and lowest longitudinal and latitudinal record of each species. The projected area (in raster cells) was then cropped using the range models generated in MAXENT (see previous paragraph), using the package RASTER (Hijmans & van Etten, 2012). In this way, we used the distribution models to remove areas with unsuitable habitat from within the latitudinal and longitudinal extent of each species distribution. MAXENT has been demonstrated to perform well with samples as small as five records (Pearson *et al.*, 2007).
4. For species with records from 2–4 localities, we calculated the projected area (in raster cells) of the initial distribution rectangle (without cropping from MAXENT).
5. Finally, for species known from a single locality, which are insufficient to generate distribution rectangles, we simply used the area of the raster cell. The R scripts for generating MAXENT models and calculating the distribution range sizes are available on request to A.C.

Phylogenetic analysis

Nucleotide sequence data for one nuclear locus (ATP synthase subunit β intron) and three mitochondrial loci (12S rRNA, 16S rRNA, ND4 and adjacent regions) are available for the above 68 species. We used the alignment, partitioning scheme and substitution models employed in Skinner *et al.* (2008). The trait correlation analyses described below only require relative (not absolute) branch lengths, but rather than use an arbitrary root age, we used the same calibration as Skinner *et al.* (2008), so the trees could also be interpreted as chronograms. The data were analysed using Bayesian Markov chain Monte Carlo (MCMC) phylogenetic methods in MRBAYES 3.2 (Ronquist *et al.*, 2012). Stepping-stone estimates of marginal likelihoods strongly ($10.9 \log L$ units) favoured the relaxed clock with independent gamma rates over a strict clock, so the former was employed. Four separate runs of 30 million generations were performed, each employing four chains, with sampling every 2000 generations; the first 10 million generations were discarded as burn-in, leaving 10,000 trees (per run) for

analysis, from which a majority-rule consensus tree was obtained. Full details (alignment, partitions, nucleotide models, chain length and number, heating, burn-in and clock parameters) are in the MRBAYES file (Appendix S2).

Correlation between geographical range size and body form

Phylogenetically uncorrected and corrected analyses were employed to evaluate the relationship between geographical range size and body form. In the phylogenetically uncorrected analyses (which effectively assume a star phylogeny), species were (naïvely) treated as independent data points. Because inferred geographical distribution is likely to have much higher error (extrapolated using complex models, often from sparse sampling) than measures of limb reduction, least-squares (LS) regressions were used. LS regression results are also directly comparable to the phylogenetically corrected analyses.

The phylogenetically corrected analyses directly employed the pool of sampled trees: using any single (e.g. consensus) tree was inappropriate due to uncertainty over many (especially basal) nodes in the *Lerista* phylogeny (Fig. 1; see also Skinner *et al.*, 2008). Analyses were performed using the CONTINUOUS module in BAYESTRAITS (Pagel, 1997, 1999; Pagel & Meade, 2007), which implements the generalized least-squares model, analytically equivalent to independent contrasts (Garland *et al.*, 2005). Eight (2^3) different analyses were performed: (1) with single-locality species either excluded or included; (2) with the lambda parameter, which models the variance attributable to phylogenetic conservatism or 'heritability', either freely estimated or fixed to zero; and (3) with the covariance parameter, which describes the variance attributable to the correlation between geographical range size and body form, either freely estimated or fixed to zero. Analyses were run for 11 million generations, with a sampling frequency of 10,000 generations and a burn-in of 1 million generations. To assess convergence, each analysis was replicated four times. Bayes factors were used for inferring whether the lambda or covariance parameters improved model fit; we used Kass & Raftery's (1995) criterion of twice the marginal $\log_e$ -likelihood differences (herein abbreviated BF_{KR}). Marginal $\log_e$ -likelihoods were estimated using the Bayes Factor function in TRACER 1.5 (Rambaut & Drummond, 2009). Regression equations were calculated from the BAYESTRAITS parameter output (using the formula in Pagel, 1999, p. 10) for the analyses employing the best-fitting models.

RESULTS

Analysis of body form

The first principal component (PC1) explained 77% of the variation and was an index of limb reduction, with high and similar loadings for (size-corrected) snout–vent length (+0.62), forelimb length (−0.58) and hindlimb length (−0.53). The full morphometric data and PC1 loadings for each species are presented in Appendix S1.

Figure 1 Phylogeny of 68 species of the Australian lizard genus *Lerista*. Majority-rule Bayesian consensus tree, with posterior probabilities at nodes; the time-scale is in million years (Myr). Geographical range size of each species ($\log_{10} \text{ km}^2$) is indicated; note substantial lability, but also some apparent degree of phylogenetic conservatism.

Range size

The *Lerista* species considered here are represented by more than 14,000 specimens with locality data in Australian museums, with the sample size for each species ranging from four (for *L. eupoda*) to 1290 (for *L. bipes*).

This produced a total of 1663 locality records across all 68 species (median = 42, range = 1–168) for distribution modelling. All AUC values obtained were above 0.78, suggesting good general accuracy of the models generated. The resultant estimates of geographical range size are presented in Appendix S1. As these exhibited hetero-

scedasticity they were log-transformed (Fig. 1) before analyses.

Phylogenetic analysis

The majority-rule consensus tree from the Bayesian analysis (Fig. 1) is highly concordant with a previous analysis that used the same genes but with slightly more extensive taxon sampling (Skinner *et al.*, 2008). In both analyses, many of the basal clades of *Lerista* are poorly supported.

Correlation between geographical range size and body form

In the phylogenetically uncorrected analyses, when single-locality species were excluded, there was a significant negative relationship between geographical range size and body form ($P = 0.0322$; $r^2 = 0.078$, $F = 4.822$ with 57 d.f.), i.e. distributions were smaller for serpentine species (Fig. 2, thick dashed line). However, when single-locality species were included, this relationship was insignificant ($P = 0.289$, $r^2 = 0.017$, $F = 1.141$ with 66 d.f.; Fig. 2, thin dashed line). In the phylogenetically corrected analyses (Table 1), all eight analyses converged on essentially identical solutions for all four replicate runs, with effective sample sizes for all parameters

> 35,000. Estimating lambda (i.e. allowing for phylogenetic conservatism) always greatly improved model fit (BF_{KR} 13.22–23.94: compare analyses 1 vs. 3, 2 vs. 4, 5 vs. 7, 6 vs. 8). Lambda was estimated for both variables simultaneously (see Pagel, 1999) and was always large (*c.* 0.7; theoretical range 0–1), with the lower limit of the 95% highest posterior density (HPD) never falling below 0.45. Thus, there is a significant tendency for closely related species to be more similar in geographical range size (Fig. 1) and body form (Figure 1 in Skinner *et al.*, 2008) despite high lability in both traits. The following discussion therefore focuses on those analyses in which lambda was estimated (analyses 3, 4, 7 and 8).

When species from single localities were excluded, there was positive evidence that higher PC1 scores (i.e. elongate, limb-reduced body forms) were correlated with smaller geographical distributions ($BF_{KR} = 3.24$). The covariance, a measure of trait correlation, was estimated at -3.82 , and neither the 95% HPD (-2.92 , -4.77) nor even the entire sampled interval (-1.16 , -7.82) approached zero. However, when single-locality species were included, the evidence for correlated evolution was marginal ($BF_{KR} = 0.16$). The covariance between body form and geographical range size was much lower (-1.23), the 95% HPD approached zero (-0.19 , -2.37), and the sampled interval included zero ($+3.08$, -5.04).

Figure 2 Relationship between modelled range size ($\log_{10} \text{ km}^2$) and limb reduction (indexed by the first principal component, PC1) in the Australian lizard genus *Lerista*; least-squares regressions with modelled range size as the dependent variable. Dots along the horizontal row at the bottom (blue) represent taxa known from single localities whose ranges could not be accurately modelled. Solid lines are phylogenetically corrected generalized least-squares regressions (integrated across Bayesian tree samples; Pagel & Meade, 2007); dashed lines are uncorrected least-squares regressions. Thick lines (red) are analyses excluding single-locality taxa ($n = 59$; both gradients significantly less than 0: Table 1, analyses 9 and 10); thin lines (blue) are for analyses including single-locality taxa ($n = 68$; both gradients indistinguishable from 0: Table 1, analyses 11 and 12). Regression coefficients are given in Table 1; F -values are given in the main text.

Table 1 Model comparisons (analyses 1–8) and regressions using selected models (analyses 9–12; see also Fig. 2) of range size against limb size in Australian lizards of the genus *Lerista*. When single-locality species were excluded (1–4), leaving 59 species for analysis, there were significant improvements by including parameters accounting for phylogenetic conservatism (lambda) and correlation between the two variables (covariance). When single-locality species were included, and all 68 species were analysed (5–8), only the lambda parameter was supported. Similarly, in regression analyses, the gradient (relationship between range size and limb size) was significantly negative when single-locality species were excluded (9,10) but not when they were included (11,12).

Bayesian analysis: model comparisons	Lambda (95% HPD) [phylogenetic conservatism]	Covariance (95% HPD) [correlation between characters]	Log <i>L</i> (marginal)	<i>BF</i> _{KR} comparison (2 × Δlog <i>L</i>)
1–4: Single-locality taxa excluded (<i>n</i> = 59)				
1. lambda 0, covariance 0	0; fixed	0; fixed	–200.04	–27.18
2. lambda 0, covariance estimated	0; fixed	–4.44 (–3.49, –5.36)	–197.66	–22.42
3. lambda estimated, covariance 0	0.75 (0.56, 0.92)	0; fixed	–188.07	–3.24
4. lambda estimated, covariance estimated	0.73 (0.54, 0.91)	–3.82 (–2.92, –4.77)	–186.45	0 (best)
5–8: Single-locality taxa included (<i>n</i> = 68)				
5. lambda 0, covariance 0	0; fixed	0; fixed	–255.56	–15.22
6. lambda 0, covariance estimated	0; fixed	–2.99 (–2.15, –3.90)	–254.56	–13.22
7. lambda estimated, covariance 0	0.70 (0.47, 0.90)	0; fixed	–248.03	–0.16
8. lambda estimated, covariance estimated	0.69 (0.45, 0.90)	–1.23 (–0.19, –2.37)	–247.95	0 (best)
Regression	intercept	gradient	significance	
9. Single-locality taxa excluded (<i>n</i> = 59), phylogenetically corrected	4.426	–0.225	<i>BF</i> _{KR} = 3.24	
10. Single-locality taxa excluded (<i>n</i> = 59), uncorrected	4.466	–0.197	<i>P</i> = 0.032	
11. Single-locality taxa included (<i>n</i> = 68), phylogenetically corrected	3.975	–0.077	<i>BF</i> _{KR} = 0.16	
12. Single-locality taxa included (<i>n</i> = 68), uncorrected	3.975	–0.137	<i>P</i> = 0.289	

HPD, highest posterior density; log *L*, log-likelihood; *BF*_{KR}, Kass & Raftery's (1995) criterion of twice the difference in marginal log-likelihoods.

The phylogenetically corrected regressions calculated from the BAYES TRAITs parameter estimates (Fig. 2, solid lines) are very similar to the corresponding phylogenetically uncorrected regressions (Fig. 2, dashed lines).

DISCUSSION

We analysed geographical range size variability across closely related species using Bayesian approaches that integrate uncertainties both in model parameters and in phylogeny, and tested whether range size is consistently associated with body form. We found that elongate, limb-reduced species of *Lerista* have significantly smaller ranges, but only when single-locality species are excluded; when single-locality species are included, the relationship is much weaker and ceases to be statistically significant (Fig. 2). There might, however, be valid reasons justifying the exclusion of single-locality species. First, compared with species known from multiple localities, single-locality species are more likely to be rare and poorly sampled; if so, their known ranges might be particularly unrepresentative. Second, even if the known ranges of single-locality species are sampled as adequately as for other species, it was impossible to model the inferred ranges of these single-locality species using the same techniques used for other species, making the former data points methodologically inconsistent. Third, more detailed sampling and

phylogeographical analyses could eliminate some single-point species, either by revealing they have wider ranges, or by demonstrating that they only represent local populations of species that are more widely distributed. For example, the single-locality species *L. puncticauda* is nested within the more widely occurring *L. desertorum* (A. Skinner, in prep.), raising questions about its taxonomic status. Additional research could of course confirm that single-locality taxa such as *L. puncticauda* should be recognized as species; however, even in these cases, taxa will have originated relatively recently (considering the absence of reciprocal monophyly), so that geographical range size is perhaps more likely to be determined by the mode of speciation than the ecological factors that control distributional limits for older taxa. An additional consideration is that elongated, limb-reduced taxa may be more likely to contain undiscovered species, and so have actual geographical ranges that are smaller than those estimated in our analyses. Among the 68 species of *Lerista* described in the past half-century (see Cogger *et al.*, 1983; Amey *et al.*, 2005; Smith & Adams, 2007), 40 (nearly 60%) have fewer than three digits for the forelimb or both limbs. Assuming this pattern of species discovery continues, and that species discovery primarily involves 'splitting' of existing taxa, estimates of geographical range size for limb-reduced species would be expected to further decline with improved taxonomic understanding, reinforcing the inferred relation-

ship between body form and range size. If these arguments are accepted, there are reasons to prefer the results from analyses that exclude single-point ranges, which indicate that species displaying a serpentine body form have narrower distributions.

The analyses presented here are correlative, and do not directly evaluate the possible mechanisms responsible for a relationship between geographical range size and body form. Elongate, limb-reduced species of *Lerista* could be more affected by environmental patchiness if they are less likely to move long distances, more substrate-specific (e.g. sand-swimmers), or more cryptic in habit (e.g. see Greer, 1989). These traits could influence their dispersal ability and thus shape their geographical range sizes over evolutionary timescales. Species with lower dispersal ability may be expected to display higher rates of local (population) extinction and lower rates of recolonization, restricting the number of potentially suitable sites occupied at any particular time, thus reducing geographical range. Moreover, dispersal limitation may facilitate the genetic differentiation of local populations, promoting speciation and the associated partitioning of ancestral distributions, again reducing geographical range. The potential for dispersal ability to affect geographical range size has been demonstrated for *Sylvia* warblers, where species lacking ecomorphological adaptations for long-range flight have smaller ranges (Böhning-Gaese *et al.*, 2006). Assessing whether the association between body form and geographical range in *Lerista* is mediated by dispersal limitation will depend on future ecological studies of a range of limb-reduced and surface-active species involving, for example, radio-tracking and behavioural tests of substrate preferences and performances. The relationship between limblessness and geographical range size also has conservation implications: for instance, the only two *Lerista* species on the International Union for Conservation of Nature's *Red List of Threatened Species* (<http://www.iucnredlist.org/>) are highly elongate, limb-reduced species with very restricted ranges, namely *L. allanae* and *L. vittata*.

Geographical range size is an important attribute of species, and the boundaries of species' ranges are likely to be influenced by a variety of factors, including physiological tolerances, competition, and past and present climatic and geographical barriers (e.g. Geber, 2011). However, with the notable exception of body size (e.g. Olifiers *et al.*, 2004), there have been few quantitative analyses of morphological attributes that could also play a significant role (Böhning-Gaese *et al.*, 2006). Given the large number of possible confounding factors, demonstrating a relationship between range size and morphology requires a large sample of species possessing morphological traits that display high homoplasy (leading to replicated changes), as well as great variation in geographical range size. *Lerista* fulfils all criteria, and our analysis suggests – with the above caveats – that body elongation and limb reduction are associated with more restricted distributions.

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SUPPORTING INFORMATION

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

Appendix S1 Geographical range size and body form (PC1) data for 68 species of *Lerista*.

Appendix S2 MRBAYES 3.2 file with molecular alignment and analytical settings.

BIOSKETCHES

Michael S. Y. Lee is a senior research scientist at the South Australian Museum and University of Adelaide. He investigates macroevolutionary questions at the interface of phylogenetics, molecular biology and palaeontology.

Adam Skinner is a research fellow at the University of Adelaide. He works on reptile evolution and systematics, and is currently focusing on the developmental genomics of limb reduction.

Agustín Camacho is a research fellow at the University of São Paulo. He works on the evolutionary ecology of Neotropical reptiles.

Author contributions: A.S. gathered the primary distribution data and morphometric measurements; A.C. performed the bioclimatic analyses; M.L. performed the phylogenetic, morphometric and correlation analyses; M.L. wrote the paper with input from A.S. and A.C.

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