University of São Paulo
"Luiz de Queiroz" College of Agriculture
Center of Nuclear Energy in Agriculture

Behavioral thermal tolerance: implications for ecology and conservation of amphibians and reptiles

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Thesis presented to obtain the degree of Doctor in Science.
Area: Applied Ecology

Piracicaba
2022

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Behavioral thermal tolerance: implications for ecology and conservation of amphibians and reptiles

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Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP

Díaz Ricaurte, Juan Camilo

Behavioral thermal tolerance: implications for ecology and conservation of amphibians and reptiles / Juan Camilo Díaz Ricaurte - - Piracicaba, 2022.

203 p.

Tese (Doutorado) -- USP / Escola Superior de Agricultura "Luiz de Queiroz". Centro de Energia Nuclear na Agricultura.

1. Comportamento animal 2. Ecologia térmica 3. Ectotermos 4. Fisiologia animal 5. História natural 6. Mudanças climáticas 7. Respostas comportamentais 8. Termorregulação I. Título

I dedicate my PhD thesis to my son Juan Martin for teaching me every day to see life in the simplest way. Likewise, for being my inspiration at all times.

I love you son!

Dedico minha tese de doutorado a meu filho Juan Martin por me ensinar todos os dias a ver a vida da maneira mais simples. Da mesma forma, por ser a minha inspiração em todos os momentos.

Te amo filho!

AGRADECIMENTOS

"A vida acadêmica não é fácil." Em muitas ocasiões ouvi essa frase, mas apenas há alguns anos atrás comecei a entendê-la. É muito curioso como sua vida pode mudar tomando um caminho que você não sabe se é para você ou não. Um Doutorado é isso. Um caminho sobre o qual você não sabe absolutamente nada. Você não sabe se será o que você procura, o que o preenche, o que oferece realização. Ou pelo menos, eu não sabia disso no começo. Mas, mesmo sem saber nada disso, e com muita incerteza, o que acho que nos move é o amor pelo que fazemos, o amor pelo crescimento, o amor pela arte. Muitos de nós (inclusive eu) no início de um doutorado achamos que é só pesquisar, fazer algumas análises e se especializar em uma área específica. O que a gente não sabe é que o aprendizado vai muito além disso (pelo menos é o que eu acho). Tanto o aprendizado quanto o custo são muito altos. E refiro-me a custos em tempo, dinheiro (para quem não recebe bolsa) e, sobretudo, custos emocionais.

O processo de fazer doutorado toca todas as fibras do nosso ser, do nosso corpo e principalmente da nossa mente. Ele nos confronta com nossos medos e fraquezas mais fortes e ainda mais nos confronta com situações que exigem nosso maior esforço. Por isso considero que o doutorado vai além do título. Porque no final das contas, muitos têm títulos, mas sabem pouco sobre seu propósito. Desde o meu ponto de vista, e como sempre compartilhei com colegas, alunos e outras pessoas, como cientistas devemos passar as informações pelo melhor canal possível, e para isso nem sempre é suficiente conhecer os termos de uma pesquisa e/ou outros detalhes que como especialistas em uma área, já estão familiarizados. Acredito que humanidade, empatia, ética, honestidade, paciência e, acima de tudo, responsabilidade social e acadêmica são necessários. Ter esses pilares em nossa formação vai nos ajudar a mostrar que a ciência é para todos e não apenas para alguns. Somos todos cientistas, embora muitos não percebam isso. Acho que simplesmente à medida que crescemos ou "amadurecemos", perdemos a curiosidade pelo simples, pelas respostas simples. Afinal, "simples é mais poderoso", não é? No final das contas, como mencionei no início, é o amor pela arte que nos leva a buscar não apenas o maior grau acadêmico, mas também nos leva a continuar alimentando nossa curiosidade sobre o mundo. Com tudo o que um doutorado acarreta, acredito que qualquer pessoa, quem quer que seja, acredite no que acredita, busque o que busca, encontrará motivos suficientes para reconhecer o seu propósito na vida.

São muitas coisas que me vêm à mente quando penso no processo do doutorado. Reflito constantemente sobre todas as situações e aprendizados que experienciei durante esse processo, assim como penso nas muitas pessoas que, direta ou indiretamente, me deram algo de si, que me ensinaram, cada um à sua maneira e no seu tempo, a continuar lutando pelos meus sonhos, a continuar aprendendo, a ser uma pessoa e um cientista melhor a cada dia.

Pois bem, depois de muito tempo comecei a ver o doutorado como uma fase da minha vida. Por fase, é claro, estou me referindo a uma etapa importante que marcou e lançou as bases do que sou hoje como pesquisador, pessoa e cidadão. Ao escrever estas palavras, sinto-me feliz por poder registar nesta parte da minha tese a todas as pessoas e instituições que tornaram possível este processo académico e pessoal. Considero-me uma pessoa de muita sorte por ter tido pessoas incríveis, que me ajudaram a crescer em vários aspectos, começando pelo aspecto pessoal. Depois de todo esse tempo de aprendizado, está claro para mim que onde estamos agora é um reflexo dos nossos pensamentos e ações do passado, portanto, se quisermos mudar nosso futuro, temos que esclarecer nossas metas e objetivos hoje e colocá-los em ação. Ou diz alguém que uma vez, conversando com alguém, mencionou que faria doutorado no Brasil. E olha que aqui estou eu. Todos podemos!

Agradeço à minha família especialmente à minha avó Fabiola por ter me apoiado emocionalmente durante esse processo. Obrigado por acreditar em mim. Sem vocês tudo teria sido mais difícil.

Agradeço a Marco Rada e Lina Rubio por terem me recebido na sua casa nos primeiros meses da minha vida no Brasil.

Agradeço à família Coelho, especialmente à Maria e Osvaldo Coelho por terem me oferecido o calor e amor de seu lar. Ter me sentido como em casa fez tudo muito mais fácil. Realmente estou muito agradecido com vocês. Levarei vocês no meu coração o resto da minha vida.

À minha companheira de vida Caroline Molina, por estar em minha vida. Ninguém imagina o quão importante você foi nesse processo. Agradeço pela paciência nos meus dias de estresse, pela atenção, pelo amor, pela incondicionalidade, por sempre ter tentado me oferecer o melhor e sobretudo por ter sido a pessoa que me motivou desde o início a fazer

ciência, e isso muito antes do doutorado. Pelas milhares de risadas e momentos de “gadejo” que tanto me ajudaram nos meus dias mais difíceis. Apenas mil obrigado meu amor. Eu te amo!

Agradeço ao meu filho Juan Martin por me inspirar com a sua inocência, curiosidade e determinação. Sacrificamos muito por este momento. Isso foi em grande parte sobre você. Agradeço também a Ana Maria Galván por ter me apoiado incondicionalmente para que eu crescesse como profissional, por ter cuidado do nosso bebê durante todo este tempo e principalmente por ter facilitado a comunicação com ele. Obrigadão mesmo!

Agradeço ao meu querido Professor Dr. Marcio Martins que aceitou me orientar, que me mostrou o que é fazer ciência com amor, respeito, paixão e acima de tudo com muita ética e honestidade. Agradeço por sua paciência e pelas muitas vezes que você me recebeu na sua sala e respondeu às centenas de perguntas que eu tinha. Obrigado mesmo por acreditar em mim, por me apoiar incondicionalmente, pelo tempo investido, pelo respeito, risadas, pelos conselhos e por sempre ser um grande ser humano comigo. Espero que nossa amizade e relacionamento acadêmico continuem para sempre. Você me mostrou o que é ser um bom cientista! Obrigado!

Agradeço também ao Dr. Cristiano Nogueira e ao Dr. Agustín Camacho por terem me incentivado desde o início a embarcar neste processo acadêmico. Vocês dois foram a ponte para eu chegar onde estou agora. Muito obrigado pelos conselhos e apoio.

Agradeço ao meu grande amigo Filipe Serrano por me inspirar, por me desafiar a fazer sempre melhor, por ter viajado comigo no caminho da ciência, por estar sempre presente quando precisei, pelas risadas, pelas boas conversas, pelo apoio emocional. Por ser meu irmão acadêmico todos os dias. Por aquele "y vos qué" que tanto bem me fez. Quero agradecer o quanto você me ajudou. Para mim, sempre será uma honra explorar a ciência contigo, Viejo!

Agradeço ao meu amigo Dr. Hector Benatti pelas boas conversas, pela admiração e por sempre me incentivar com suas palavras a ser um melhor cientista. Jamais esquecerei nossos almoços e conversas sobre a vida. As tuas palavras estarão sempre no meu coração!

Agradeço ao Professor Dr. Fernando Ribeiro quem me inspirou como cientista a cada momento pela sua tranquilidade, paz e respeito pelas outras pessoas. Os seus comentários e conselhos acadêmicos sempre me apontaram para um caminho melhor. Obrigado!

Agradeço à Dra. Silvia Regina Travaglia Cardoso e ao Dr. Giuseppe Puorto por terem me auxiliado na gestão das autorizações ou uso dos animais do Museu Biológico do Instituto Butantan. Sem vocês dois, não teria sido possível fazer os experimentos ou coletar os dados nesta tese. Agradeço também aos técnicos do Museu Biológico do Instituto Butantan, por terem me apoiado durante parte da fase experimental deste trabalho.

Agradeço a todos os meus colegas do LabVert, que me apoiaram em campo durante as viagens de campo. Agradeço também aos técnicos da Estação Ecológica Águas de Santa Bárbara, em especial ao Marcos Antônio Soler (chamado Marcão de carinho), que sempre me deu sua amizade, apoio logístico e apoio incondicional durante minha permanência na estação. Agradeço ao Programa de Pós-Graduação em Ecologia Aplicada (PPGI - EA) da ESALQ, à Universidade de São Paulo, e em especial à Mara Casarin, Secretária do PPGI-EA, pelo profissionalismo, dedicação e competência, e acima de tudo por sempre ter me ajudar. É um prazer encontrar pessoas como você. Você é incrível!

Por último, mas não menos importante, e tomando algumas palavras do Snoop Dogg, quero me agradecer por acreditar em mim. Quero agradecer a mim mesmo por fazer todo esse ótimo trabalho. Quero agradecer a mim mesmo por não ter dias de folga. Quero agradecer a mim mesmo por nunca ter desistido, apesar das adversidades. Quero agradecer a mim mesmo por sempre ter dado e ter tentado dar mais sem receber. Quero me agradecer por ser eu em todos os momentos e bem quero me agradecer principalmente por acreditar que era possível, mesmo quando as previsões não eram boas. Acho que agradecer a nós mesmos pelo que fazemos é algo que tenho certeza que precisamos na vida diária.

Obrigado a todos!

ACKNOWLEDGMENTS

"Academic life is not easy." I have heard this phrase many times, but it was only a few years ago that I began to understand it. It's very curious how your life can change by taking a path that you don't know if it's for you or not. That's what a PhD is. A pathway you know absolutely nothing about. You don't know if it will be what you seek, what fulfills you, what offers achievement. At least, I didn't know it at first. But even without knowing any of this, and with a lot of uncertainty, what I think moves us is the love for what we do, the love for growing, the love for art. Many of us (including me) at the beginning of a PhD think it's just research, doing some analysis and specializing in a specific area. What we don't know is that learning goes far beyond that (at least that's what I think). Both the learning and the cost are very high. And I am referring to costs in time, money (for those who do not receive a scholarship) and, above all, emotional costs.

The process of obtaining a PhD touches every fiber of our being, our body and especially our mind. He confronts us with our strongest fears and weaknesses and even more confronts us with situations that demand our greatest effort. That's why I believe that the doctorate goes beyond the title. Because at the end of the day, many have titles but know little about their purpose. From my viewpoint, and as I have always shared with colleagues, students and other people, as scientists we must transfer information through the best possible way, and for that it is not always enough to know the terms of research and/or other details that as experts in an area are already familiar. I believe that humanity, empathy, ethics, honesty, patience and, above all, social and academic responsibility are necessary. Having these pillars in our training will help us show that science is for everyone and not just for a few. We are all scientists, although many do not perceive it. I think just as we grow up or "mature" we lose the curiosity for the simple, the simple answers. In the end, "simple is more powerful", isn't it? At the end of the day, as I mentioned at the beginning, it's the love of art that drives us to pursue not only the highest academic degree, but also drives us to continue nurturing our curiosity about the world. With all that a PhD entails, I believe that anyone, whoever they are, believe what they believe, pursue what they seek, will find reason enough to recognize their purpose in life.

There are many things that come to my mind when I think about the PhD process. I constantly reflect on all the situations and learnings I experienced during this process, as well

as I think of the many people who, directly or indirectly, gave me something of themselves, who taught me, each in their own way and in their own time, to continue fighting for my dreams, to keep learning, to be a better person and scientist every day.

Well, after a long time I started to see the PhD as a phase of my life. By phase, of course, I am referring to an important stage that marked and laid the foundations for what I am today as a researcher, person and citizen. In writing these words, I am happy to be able to register in this part of my thesis all the people and institutions that made this academic and personal process possible. I consider myself a very lucky person to have had amazing people, who helped me grow in many ways, starting with the personal aspect. After all this time of learning, it's clear to me that where we are now is a reflection of our past thoughts and actions, so if we want to change our future, we have to clarify our goals and objectives today and put them into action. Says someone who once, talking to someone, mentioned that he would do a PhD in Brazil. And look, here I am. We all can!

I thank my family, especially to my grandma Fabiola for supporting me emotionally during this process. Thanks for believing in me. Without you everything would have been more difficult.

I thank Marco Rada and Lina Rubio for having welcomed me into their home during the first months of my life in Brazil.

I thank the Coelho family, especially Maria and Osvaldo Coelho for offering me the warmth and love of their home. That home feeling made everything so much easier. I am really grateful to you. I will carry you in my heart for the rest of my life.

To my life partner Caroline Molina, for being in my life. No one can imagine how important you were in this process. I thank you for your patience in my stressful days, for your attention, for your love, for your unconditionality, for always trying to offer me the best and above all for being the person who motivated me to do science from the beginning, and that long before my PhD. For the thousands of laughs and “gadejo” moments that helped me so much on my most difficult days. Just a thousand thanks my love. LoveU!

I thank my son Juan Martin for inspiring me with his innocence, curiosity and determination. We sacrificed a lot for this moment. This was largely about you. I also thank Ana Maria Galván for having unconditionally supported me so that I could grow as a professional, for taking care of our baby during all this time and mainly for having facilitated communication with him. Really thank you!

I thank my dear Professor Dr. Marcio Martins who agreed to guide me, who showed me what it means to do science with love, respect, passion and above all with great ethics and honesty. Thank you for your patience and for the many times you welcomed me into your office and answered the hundreds of questions I had. Thank you for believing in me, for supporting me unconditionally, for the time invested, for the respect, laughter, advice and for always being a great human being with me. I hope our friendship and academic relationship will continue forever. You showed me what it's like to be a good scientist! Thank you!

I also thank Dr. Cristiano Nogueira and Dr. Agustín Camacho for encouraging me from the beginning to embark on this academic process. You two were the bridge for me to get to where I am now. Thank you very much for the advice and support.

I thank my great friend Filipe Serrano for inspiring me, for challenging me to always do better, for having traveled with me on the path of science, for always being there when I needed it, for the laughs, for the good conversations, for the emotional support. For being my academic brother every day. For that "y vos qué" that did me so much good. I want to thank you for how much you helped me. It will always be an honor for me to explore science with you, Viejo!

I thank my friend Dr. Hector Benatti for the good conversations, for his admiration and for always encouraging me with his words to be a better scientist. I will never forget our lunches and conversations about life. Your words will always be in my heart!

I thank Professor Dr. Fernando Ribeiro who inspired me as a scientist at every moment for his tranquility, peace and respect for other people. His academic comments and advice have always pointed me to a better path. Thank you!

I thank Dr. Silvia Regina Travaglia Cardoso and Dr. Giuseppe Puorto for helping me manage the permit to use of animals at the Biological Museum of the Butantan Institute. Without the two of you, it would not have been possible to do the experiments or collect the data in this thesis. I would also like to thank the technicians of the Museu Biologico at Instituto Butantan for supporting me during part of the experimental phase of this work.

Thanks to all my colleagues at LabVert who supported me in the field during field trips. I also thank the technicians of Águas de Santa Bárbara Ecological Station, especially Marcos Antônio Soler (affectionately named Marcão), who always gave me his friendship, logistical support and unconditional support during my stay at the station.

I thank the Graduate Program in Applied Ecology (PPGI - EA) at ESALQ, the University of São Paulo, and especially Mara Casarin, Secretary of the PPGI-EA, for her professionalism, dedication and competence, and above all for always have help me. It's a pleasure to meet people like you. You are awesome!

Last but not least, and taking a few words from Snoop Dogg, I want to thank myself for believing in me. I want to thank myself for doing all this great work. I want to thank myself for not having days off. I want to thank myself for never giving up despite adversity. I want to thank myself for always giving and trying to give more without receiving. I want to thank myself for being me at all times and I want to thank myself especially for believing that it was possible, even when the predictions weren't good. I think thanking ourselves for what we do is something I'm sure we need in daily life.

Thank you all!

"If you have an idea that you genuinely think is good, don't let some idiot talk you out of it." — ***Stan Lee***

*"Make everything as simple as possible, but not simpler." —****Albert Einstein***

Be brave, be curious, be determined, overcome the odds. It can be done"/ "Work gives you meaning and purpose and life is empty without it" — ***Stephen Hawking***

SUMMARY

RESUMO

Tolerância térmica comportamental: implicações para a ecologia e conservação de anfíbios e répteis

Animais ectotérmicos dependem das condições ambientais no seu habitat (por exemplo, temperatura) para levar e manter as suas funções fisiológicas. Devido ao aquecimento global atual e a alta diversidade no Neotrópico em perigo pelo aumento das temperaturas, faz com que o entendimento da sua ecologia térmica, junto com aspectos da sua história de vida e relações filogenéticas sejam necessárias para a sua conservação. Nesta tese eu estimei experimentalmente as tolerâncias térmicas comportamentais de 67 espécies de anfíbios e répteis Neotropicais. Adicionalmente explorei como estas se relacionam em diversos aspectos ecológicos, fisiológicos e macroevolutivos. No capitulo 1 eu validei o método experimental para estimar tolerâncias térmicas comportamentais em serpentes e investiguei se condições de cativeiro afetam essas tolerâncias térmicas em jararacas. No capitulo 2, investiguei se tolerâncias térmicas comportamentais ajudam a explicar os padrões de distribuição e alguns aspectos de história natural de sapos Neotropicais. No capitulo 3, usei uma abordagem macroevolutiva para explorar a relação entre as tolerâncias e características ambientais em um grupo monofilético de Jararacas Neotropicais. Por fim, no capitulo 4, eu estimei e compilei um banco de dados de tolerâncias térmicas voluntarias de 53 espécies de anfíbios e répteis para fornecer uma linha de base para a compreensão das tolerâncias térmicas dos vertebrados ectotérmicos. Os capítulos foram especificamente pensados e executados para descrever, em uma perspectiva ampla, as implicações das tolerâncias térmicas na ecologia, evolução e história de vida de ectotermos Neotropicais. Os resultados de cada capitulo apresentam pontos importantes para o entendimento, interpretação e uso das tolerâncias térmicas em estudos ecológicos, fisiológicos e filogenéticos. Isso deve resultar em uma abordagem mais completa e em um entendimento mais geral sobre os requerimentos e estratégias tanto comportamentais quanto fisiológicas, assim como a criação de cenários mais realistas das mudanças climáticas nos vertebrados ectotérmicos.

Palavras-chave: Comportamento animal, Ecologia térmica, Ectotermos, Fisiologia animal, História natural, Mudanças climáticas, Respostas comportamentais, Termorregulação

ABSTRACT

Behavioral thermal tolerance: implications for ecology and conservation of amphibians and reptiles

Ectothermic animals depend on the environmental conditions in their habitat (eg temperature) to carry and maintain their physiological functions. Due to current global warming and the high diversity in the Neotropics endangered by rising temperatures, the understanding of its thermal ecology, along with aspects of its life history and phylogenetic relationships are necessary for its conservation. In this thesis I experimentally estimated the behavioral thermal tolerances of 67 species of Neotropical amphibians and reptiles. Additionally, I explored how these are related in different ecological, physiological and macroevolutionary aspects. In Chapter 1 I validated the experimental method for estimating behavioral thermal tolerances in snakes and investigated whether captive conditions affect these thermal tolerances in pitvipers. In Chapter 2, I investigated whether behavioral thermal tolerances help to explain distribution patterns and some aspects of the natural history of Neotropical frogs. In Chapter 3, I used a macroevolutionary approach to explore the relationship between tolerances and environmental characteristics in a monophyletic group of Neotropical pitvipers. Finally, in Chapter 4, I estimated and compiled a database of voluntary thermal tolerances from 53 species of amphibians and reptiles to provide a baseline for understanding the thermal tolerances of ectothermic vertebrates. The chapters were specifically designed and executed to describe, in a broad perspective, the implications of thermal tolerances on the ecology, evolution and life history of Neotropical ectotherms. The results of each chapter present important points for the understanding, interpretation and use of thermal tolerances in ecological, physiological and phylogenetic studies. This should result in a more complete approach and a more general understanding of both behavioral and physiological requirements and strategies, as well as the creation of more realistic scenarios of climate change in ectothermic vertebrates.

Keywords: Animal behavior, Animal physiology, Behavioral responses, Climate change, Ectotherms, Natural history, Thermal ecology, Thermoregulation

1. INTRODUCTION / INTRODUÇÃO

1.1. English

Climate change is currently affecting the distribution patterns and ecological aspects of species around the world (PARMESAN & YOHE, 2003; CHEN et al., 2011; BELLARD et al., 2012; SKOGEN et al., 2018). High environmental temperatures are one of the main consequences of climate change on the loss of biodiversity (THOMAS et al., 2004; GEDAN & BERTNESS, 2009; NEWBOLD, 2018; WARREN et al., 2018). Vertebrates are one of the groups most affected by the increase in environmental temperatures (CEBALLOS et al., 2015; NEWBOLD, 2018; WARREN et al., 2018) and more specifically ectothermic vertebrates (CEBALLOS et al., 2015) which are organisms whose physiology is highly dependent on environmental conditions. For instance, the richness of amphibians and reptiles has been more affected than in birds and mammals, due not only to the effects of climate change but also to anthropogenic factors such as habitat loss (WARREN et al., 2013; NEWBOLD, 2018). This indicates that for amphibians and reptiles there is a disproportionate loss of their communities around the world (NEWBOLD, 2018).

The Neotropics are one of the regions with the highest species richness, hosting around a third of the biodiversity hotspots worldwide (MITTERMEIER et al., 2005; ANTONELLI & SANMARTION, 2011; ULLOA et al., 2017; ANTONELLI et al., 2018a, 2018b). This region extends from central Mexico to southern South America to Argentina, including the Caribbean and the West Indies (MORRONE, 2014). It comprises a great variety of biomes and ecoregions, types of habitats that range from dry and humid forests, including a diversity of thermal floors such as the Andes mountain range, to tropical forests and the Amazon region (MORRONE, 2014). The Neotropical region is then one of the richest in vertebrates (JENKINS et al., 2013; MOURA et al., 2016) with some exceptionally diverse groups in this region such as amphibians and reptiles (JENKINS et al., 2013; CATENAZZI & MAY, 2021). Amphibians and reptiles that inhabit the Neotropics are characterized by high species richness, and tend to thrive more in hot and humid climates, but are more sensitive to climate change (STUART et al., 2004; BUCKLEY & JETZ, 2007; NOGUEIRA et al., 2011; CATENAZZI & MAY, 2021). For instance, the most generalized declines in amphibians have occurred to a great extent in the Neotropical region where climate change is having important implications (POUNDS et al., 1999; KIESECKER et al., 2001; MENÉNDEZ-GUERRERO et al., 2020). In this sense, it is necessary to understand the mechanisms by which amphibians and reptiles are responding or not to climatic changes and variability in their habitats as a result of climate

change. This is essential to understand the ecological, physiological, and behavioral strategies, as well as the climatic vulnerability of these neotropical organisms in the face of global warming.

To understand how amphibians and reptiles respond to the effects of climate change such as high environmental temperatures, it is necessary to assess their thermal ecology from thermal performance curves that include behavioral and physiological thermal tolerance indices (KINGSOLVER & HUEY, 2008; ANGILLETTA, 2009), as well as other fundamental measures of thermal performance such as preferred body temperatures (LICHT, 1965; BRATTSTROM, 1979; ANGILLETTA et al., 2002). The latter are those temperatures selected behaviorally (e.g., basking behavior) by individuals in order to maintain their body temperature within a preferred range of temperatures to optimize their physiological functions (LILLYWHITE, 1970, 1971; MITCHELL & BERGMANN, 2016; GUEVARA-MOLINA et al., 2020; ROZEN-RECHELS, 2020). However, if body temperatures rise above this range, an individual can reach two thresholds of thermal tolerance, the first behavioral and the second physiological (CAMACHO & RUSCH, 2017).

Behavioral thresholds leads the individual to an immediate behavioral change in response to heat and is known as Voluntary Thermal Maximum (VT_{Max}; COWLES & BOGERT, 1944; e.g. see CAMACHO et al., 2018; GUEVARA-MOLINA et al., 2020; DIAZ-RICAURTE & SERRANO, 2020a, b; DIAZ-RICAURTE et al., 2020, 2021). A clear example of an individual expressing a VT_{Max} is when leaving a shelter that is warming up and moving to a place with a lower temperature (see CAMACHO et al., 2018; GUEVARA-MOLINA et al., 2020; DIAZ RICAURTE & SERRANO, 2020a, b; DIAZ-RICAURTE et al., 2020, 2021). However, if an individual does not respond to its VT_{Max} and its body temperature continues to rise, it will reach the second, but now physiological, threshold, known as Critical Thermal Maximum (CT_{Max}, COWLES & BOGERT, 1944). The CT_{Max} leads to a collapse of motor functions, onset spasms and stiffness, and death in a short time (COWLES & BOGERT, 1944; LUTTERSCHMIDT and HUTCHISON 1997a; 1997b; REZENDE et al., 2014). The preferred body temperatures, VT_{Max} and CT_{Max} can be measured under controlled conditions (CAMACHO & RUSCH, 2017). Specifically, the VT_{Max} has been widely estimated in lizards (HERTZ, 1979; KINGSBURY, 1993; CADENA & TATTERSALL, 2009; CAMACHO et al., 2018; DIAZ-RICAURTE & SERRANO, 2020a; DIAZ-RICAURTE et al., 2021) and recently in amphibians (GUEVARA-MOLINA et al., 2020a, b; DIAZ-RICAURTE et al., 2020, 2021) and snakes (DIAZ-RICAURTE & SERRANO, 2020b; DIAZ-RICAURTE et al., 2021). In turn, the VT_{Max} has been shown as an applicable behavioral index, which does not lead

individuals to death under experimental conditions and which allows obtaining eco-physiological information to be included in the predictions of the climatic vulnerability of ectothermic vertebrates. Thus, it is an index that can be integrated with both biological and experimental factors to make inferences about the ecological requirements of ectothermic vertebrates under changing climates (WILLIAMS et al., 2008).

The main purpose of this thesis was to estimate the behavioral thermal tolerances in Brazilian amphibians and reptiles, as well as to document how from these behavioral tolerances it is possible to integrate various aspects of the life history of Amphibians and Reptiles and finally to evaluate the implications of these measures in their conservation. Using amphibians and reptiles from both the Cerrado and the Brazilian Atlantic Forest, this thesis is divided into four chapters, of which three have already been published in international journals (see below) and one is currently in preparation for publication. From a multi and interdisciplinary approach, this thesis opens the first door to understand how physiological, ecological and life-history aspects such as natural history can be used to understand the short and medium-term vulnerability faced by Neotropical amphibians and reptiles. Finally, the objectives achieved in this, can potentially be applied to other ectotherms. Below, I present how this thesis is organized, followed by a brief description of each chapter.

Chapter 1: Short-term captivity does not affect immediate voluntary thermal maximum of a neotropical pitviper: Implications for behavioral thermoregulation.
Published in ***Journal of Experimental Zoology Part A. Ecological and Integrative Physiology.***

Reference: **Diaz-Ricaurte, J. C**., Serrano, F. C. 2021. Short-term captivity does not affect immediate voluntary thermal maximum of a neotropical pitviper: Implications for behavioral thermoregulation. *Journal of Experimental Zoology Part A. Ecological and Integrative Physiology,* 335(2), 199–206. https://doi.org/10.1002/jez.2433

In this chapter, we show for the first time the measurement of Voluntary Thermal Maximum (VT_{Max}) in a species of pitviper (*Bothrops pauloensis*) under conditions of captivity. We compared with field measurements and found that there are no effects of the short periods of captivity on the VT_{Max}, also validating the method for the first time to be used in snakes.

Chapter 2: Behavioral thermal tolerance predicts distribution pattern but not habitat use in sympatric Neotropical frogs.
Published in ***PLoS ONE***.

Reference: **Diaz-Ricaurte, J. C**., Serrano, F., Guevara-Molina, E. C., Araujo, C., Martins M. 2020. Behavioral thermal tolerance predicts distribution pattern but not habitat use in sympatric Neotropical frogs. PLoS ONE, 15(9), e0239485. https://doi.org/10.1371/journal.pone.0239485

In this chapter, we estimate the VT_{Max} in two species of Brazilian toads (i.e., *Physalaemus cuvieri* and *Physalaemus nattereri*), finding that the behavioral thermal tolerances can help explain not only distribution patterns of the species but also natural history aspects and habitat use. Additionally, after the publication of the article, we created a scientific dissemination video considering the main results of the research. The video was focused on lay people and children's community.

Chapter 3: On the brink of change? Behavioral thermal tolerance, thermal niche breadth and environmental features of South American pitvipers.

Reference: **Diaz-Ricaurte, J. C.**, Serrano, F. C., Camacho, A, Nogueira, C. C., Travaglia-Cardoso, S. R., Puorto, G., Martins M. On the brink of change? Behavioral thermal tolerance, thermal niche breadth and environmental features of South American pitvipers.

In this chapter, we explore the relationship between behavioral thermal limits (i.e., VT_{Max} and environmental features (e. g., topographic heterogeneity, precipitation and maximum and minimum environmental temperatures) in a monophyletic group of South American pitvipers (the genera *Bothrops* + *Bothrocophias*). We found that mean VT_{Max} values were similar between species. Additionally, we found that overall distribution of occurrences and environmental temperatures was similar for almost all species, and almost all species occur in forested areas. Our results suggest that environmental features do not predict VT_{Max} in South American pitvipers, but also that there may be complex factors at play regarding differences among species.

Chapter 4: VTMaxHerp: a data set of Voluntary Thermal Maximum temperatures of amphibians and reptiles from two Brazilian hotspots
Published in ***Ecology***.

Reference: **Diaz-Ricaurte, J. C**., Serrano, F. C., Martins, M. 2022. VTMaxHerp: a data set of Voluntary Thermal Maximum temperatures of amphibians and reptiles from two Brazilian hotspots. ***Ecology***, 103(3): e3602. https://doi.org/10.1002/ECY.3602

In this chapter, we offer for the first time an extensive database of the VT_{Max} of amphibians (25 species) and reptiles (28 species) of the Cerrado and the Brazilian Atlantic Forest. This dataset provides a baseline for understanding thermal tolerances and requirements of Neotropical ectothermic vertebrate species, which might be useful for future research on the impact of climate change.

1.2. Português

As mudanças climáticas estão afetando atualmente os padrões de distribuição e aspectos ecológicos das espécies em todo o mundo (PARMESAN & YOHE, 2003; CHEN et al., 2011; BELLARD et al., 2012; SKOGEN et al., 2018). As altas temperaturas ambientais são uma das principais consequências das mudanças climáticas sobre a perda de biodiversidade (THOMAS et al., 2004; GEDAN & BERTNESS, 2009; NEWBOLD, 2018; WARREN et al., 2018). Os vertebrados são um dos grupos mais afetados pelo aumento das temperaturas ambientais (CEBALLOS et al., 2015; NEWBOLD, 2018; WARREN et al., 2018) e mais especificamente vertebrados ectotérmicos (CEBALLOS et al., 2015) que são organismos cuja fisiologia é altamente dependente nas condições ambientais. Por exemplo, a riqueza de anfíbios e répteis foi mais afetada do que em pássaros e mamíferos, devido não apenas aos efeitos das mudanças climáticas, mas também a fatores antropogênicos, como perda de habitat (WARREN et al., 2013; NEWBOLD, 2018). Isso indica que, para anfíbios e répteis, há uma perda desproporcional de suas comunidades ao longo do mundo (NEWBOLD, 2018).

Os Neotrópicos são uma das regiões com maior riqueza de espécies, hospedando cerca de um terço dos hotspots de biodiversidade em todo o mundo (MITTERMEIER et al., 2005; ANTONELLI & SANMARTION, 2011; ULLOA et al., 2017; ANTONELLI et al., 2018a, 2018b). Esta região se estende do centro do México ao sul da América do Sul e à Argentina,

incluindo o Caribe e as Índias Ocidentais (MORRONE, 2014). Compreende uma grande variedade de biomas e ecorregiões, tipos de habitats que vão desde florestas secas e úmidas, passando por uma diversidade de pisos térmicos como a cordilheira dos Andes, até florestas tropicais e a região amazônica (MORRONE, 2014). A região Neotropical é então uma das mais ricas em vertebrados (JENKINS et al., 2013; MOURA et al., 2016) com alguns grupos excepcionalmente diversos nesta região, como anfíbios e répteis (JENKINS et al., 2013; CATENAZZI & MAY, 2021). Anfíbios e répteis que habitam os Neotrópicos são caracterizados por alta riqueza de espécies e tendem a se desenvolver mais em climas quentes e úmidos, mas são mais sensíveis às mudanças climáticas (STUART et al., 2004; BUCKLEY & JETZ, 2007; NOGUEIRA et al., 2011; CATENAZZI & MAY, 2021). Por exemplo, os declínios mais generalizados em anfíbios ocorreram em grande medida na região Neotropical, onde as mudanças climáticas estão tendo implicações importantes (POUNDS et al., 1999; KIESECKER et al., 2001; MENÉNDEZ-GUERRERO et al., 2020). Nesse sentido, é necessário compreender os mecanismos pelos quais anfíbios e répteis estão respondendo ou não às mudanças climáticas e à variabilidade em seus habitats em decorrência das mudanças climáticas. Isso é essencial para entender as estratégias ecológicas, fisiológicas e comportamentais, bem como a vulnerabilidade climática desses organismos neotropicais frente ao aquecimento global.

Para entender como anfíbios e répteis respondem aos efeitos das mudanças climáticas, como altas temperaturas ambientais, é necessário avaliar sua ecologia térmica a partir de curvas de desempenho térmico que incluem índices de tolerância térmica fisiológica e comportamental (KINGSOLVER & HUEY, 2008; ANGILLETTA, 2009), como bem como outras medidas fundamentais de desempenho térmico, como as temperaturas corporais preferidas (LICHT, 1965; BRATTSTROM, 1979; ANGILLETTA et al., 2002). As últimas são aquelas temperaturas selecionadas comportamentalmente (e. g., comportamento de assoalhamento) por indivíduos a fim de manter sua temperatura corporal dentro de uma faixa de temperaturas preferidas, para eventualmente otimizar suas funções fisiológicas (LILLYWHITE, 1970, 1971; MITCHELL & BERGMANN, 2016; GUEVARA-MOLINA et al., 2020; ROZEN-RECHELS, 2020). No entanto, se a temperatura corporal subir acima dessa faixa, um indivíduo pode atingir dois limiares de tolerância térmica, o primeiro comportamental e o segundo fisiológico (CAMACHO & RUSCH, 2017).

O limiar ou “threshold” comportamental leva o indivíduo a uma mudança comportamental imediata em resposta ao calor, e é conhecido como Máximo Térmico Voluntário (VT_{Max}; COWLES & BOGERT, 1944; e.g. see CAMACHO et al., 2018;

GUEVARA-MOLINA et al., 2020; DIAZ-RICAURTE & SERRANO, 2020a, b; DIAZ-RICAURTE et al., 2020, 2021). Um exemplo claro de um indivíduo expressando um VT_{Max} é quando sai de um abrigo que está se aquecendo e se muda para um local com temperatura mais baixa (ver CAMACHO et al., 2018; GUEVARA-MOLINA et al., 2020; DIAZ-RICAURTE & SERRANO, 2020a, b; DIAZ-RICAURTE et al., 2020, 2021). No entanto, se um indivíduo não responde ao seu VT_{Max} e sua temperatura corporal continua subindo, ele atingirá o segundo, mas agora fisiológico, limiar, conhecido como Máximo Térmico Crítico (CT_{Max}, COWLES & BOGERT, 1944). O CT_{Max} leva a um colapso das funções motoras, início de espasmos e rigidez e morte em um curto espaço de tempo (COWLES & BOGERT, 1944; LUTTERSCHMIDT & HUTCHISON 1997a; 1997b; REZENDE et al., 2014). As temperaturas corporais preferidas, VT_{Max} e CT_{Max} podem ser medidas sob condições controladas (CAMACHO & RUSCH, 2017). Especificamente, o VT_{Max} foi amplamente estimado em lagartos (HERTZ, 1979; KINGSBURY, 1993; CADENA & TATTERSALL, 2009; CAMACHO et al., 2018; DIAZ-RICAURTE & SERRANO, 2020a; DIAZ-RICAURTE et al., 2021) e recentemente em anfíbios (GUEVARA-MOLINA et al., 2020a, b; DIAZ-RICAURTE et al., 2020, 2021) e serpentes (DIAZ-RICAURTE & SERRANO, 2020b; DIAZ-RICAURTE et al. 2021). Por sua vez, o VT_{Max} tem se mostrado um índice comportamental aplicável, que não leva indivíduos à morte em condições experimentais e que permite a obtenção de informações ecofisiológicas para serem incluídas nas previsões de vulnerabilidade climática de vertebrados ectotérmicos. Assim, é um índice que pode ser integrado com fatores biológicos e experimentais para fazer inferências sobre as necessidades ecológicas de vertebrados ectotérmicos sob mudanças climáticas (e. g., WILLIAMS et al., 2008; CAMACHO et al., 2018; DIAZ-RICAURTE et al., 2020).

O objetivo principal desta tese foi estimar as tolerâncias térmicas comportamentais em anfíbios e répteis brasileiros, bem como documentar como a partir dessas tolerâncias comportamentais é possível integrar vários aspectos da história de vida de anfíbios e répteis e finalmente para avaliar as implicações dessas medidas na sua conservação. Utilizando anfíbios e répteis do Cerrado e da Mata Atlântica brasileira, esta tese está dividida em quatro capítulos, dos quais três já foram publicados em revistas internacionais (veja abaixo) e um está em preparação para publicação. A partir de uma abordagem multi e interdisciplinar, esta tese abre a primeira porta para entender como aspectos fisiológicos, ecológicos e de história de vida como a história natural podem ser usados para entender a vulnerabilidade de curto e médio prazo enfrentada por anfíbios e répteis Neotropicais. Finalmente, os objetivos

alcançados neste, podem ser potencialmente aplicados a outros animais ectotérmicos. Abaixo, apresento como esta tese está organizada, seguida de uma breve descrição de cada capítulo.

Capítulo 1: O cativeiro de curto prazo não afeta o máximo térmico voluntário imediato de uma jararaca Neotropical: implicações para a termorregulação comportamental.

Publicado na ***Journal of Experimental Zoology Part A. Ecological and Integrative Physiology.***

Referência: **Diaz-Ricaurte, J. C**., Serrano, F. C. 2020. Short-term captivity does not affect immediate voluntary thermal maximum of a neotropical pitviper: Implications for behavioral thermoregulation. *Journal of Experimental Zoology Part A.* ***Ecological and Integrative Physiology****,* 335(2): 199–206. https://doi.org/10.1002/jez.2433

Neste capítulo, mostramos pela primeira vez a medição do Máximo Térmico Voluntário (VT_{Max}) em uma espécie de jararaca (*Bothrops pauloensis*) em condições de cativeiro. Comparamos com medidas de campo e descobrimos que não há efeitos dos curtos períodos de cativeiro na VT_{Max}, validando também o método pela primeira vez para ser usado em serpentes.

Capítulo 2: A tolerância térmica comportamental prevê o padrão de distribuição, mas não o uso do habitat em sapos Neotropicais simpátricos.

Publicado na ***PLoS ONE***.

Referência: **Diaz-Ricaurte, J. C**., Serrano, F., Guevara-Molina, E. C., Araujo, C., Martins M. 2020. Behavioral thermal tolerance predicts distribution pattern but not habitat use in sympatric Neotropical frogs. ***PLoS ONE***, 15(9): e0239485. https://doi.org/10.1371/journal.pone.0239485

Neste capítulo, estimamos a VT_{Max} em duas espécies de sapos brasileiros (*Physalaemus cuvieri* e *Physalaemus nattereri*), descobrindo que a VT_{Max} pode ajudar a explicar não apenas os padrões de distribuição das espécies, mas também aspectos da história natural e uso do habitat. Adicionalmente, posterior à publicação do artigo, criamos um vídeo de divulgação cientifica considerando os resultados principais da pesquisa. O vídeo foi focado para pessoas leigas e comunidade infantil.

Capítulo 3: À beira da mudança? Tolerância térmica comportamental, amplitude do nicho térmico e características ambientais de jararacas Sul-Americanas.

Referência: **Diaz-Ricaurte, J. C.**, Serrano, F. C., Camacho, A, Nogueira, C. C., Travaglia-Cardoso, S. R., Puorto, G., Martins M. On the brink of change? Behavioral thermal tolerance, thermal niche breadth and environmental features of South American pitvipers.

Neste capítulo, exploramos a relação entre limites térmicos comportamentais (ou seja, VT_{Max} e características ambientais (p. ex., heterogeneidade topográfica, precipitação e temperaturas ambientais máximas e mínimas) em um grupo monofilético de jararacas Sul-Americanas (gêneros *Bothrops* + *Bothrocophias*). descobrimos que as tolerâncias térmicas eram semelhantes entre as espécies. Além disso, descobrimos que a distribuição geral de ocorrências e temperaturas ambientais era semelhante para quase todas as espécies, e quase todas as espécies ocorrem em áreas florestais. Nossos resultados sugerem que as características ambientais não preveem o VT_{Max} nas espécies de Jararacas da América do Sul, mas também que pode haver fatores complexos em jogo no que diz respeito às diferenças entre as espécies.

Capítulo 4: VTMaxHerp: um conjunto de dados de temperaturas máximas térmicas voluntárias de anfíbios e répteis de dois hotspots brasileiros.
Publicado na ***Ecology.***

Referência: **Diaz-Ricaurte, J. C**., Serrano, F. C., Martins, M. 2022. VTMaxHerp: a data set of Voluntary Thermal Maximum temperatures of amphibians and reptiles from two Brazilian hotspots. ***Ecology***, 103(3): e3602. https://doi.org/10.1002/ECY.3602

Neste capítulo, oferecemos pela primeira vez um extenso banco de dados das VT_{Max} de anfíbios (25 espécies) e répteis (28 espécies) do Cerrado e da Mata Atlântica brasileira. Este conjunto de dados fornece uma linha de base para a compreensão das tolerâncias térmicas e requisitos de espécies de vertebrados ectotérmicos Neotropicais, o que pode ser útil para pesquisas futuras sobre o impacto das mudanças climáticas.

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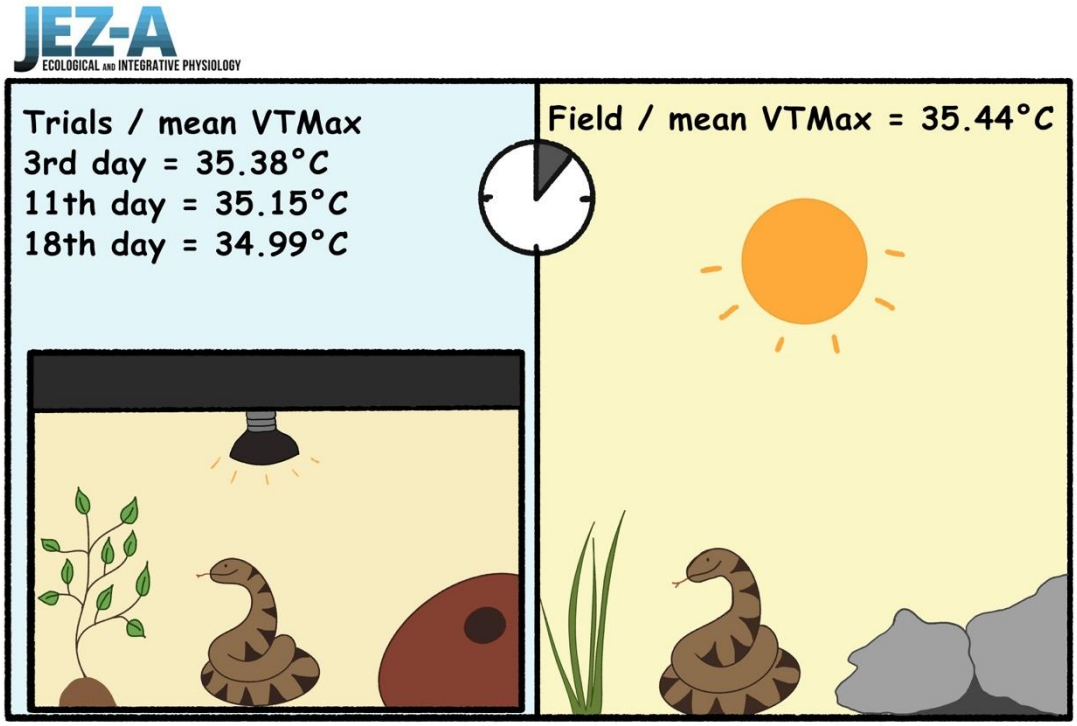
2. CHAPTER 1. SHORT-TERM CAPTIVITY DOES NOT AFFECT IMMEDIATE VOLUNTARY THERMAL MAXIMUM OF A NEOTROPICAL PITVIPER: IMPLICATIONS FOR BEHAVIORAL THERMOREGULATION



Published

DIAZ-RICAURTE JC, SERRANO FC. 2020. Short-term captivity does not affect immediate voluntary thermal maximum of a neotropical pitviper: Implications for behavioral thermoregulation. ***Journal of Experimental Zoology Part A. Ecological and Integrative Physiology***, 335(2): 199–206. https://doi.org/10.1002/jez.2433

Capítulo 1: O cativeiro de curto prazo não afeta o máximo térmico voluntário imediato de uma jararaca neotropical: implicações para a termorregulação comportamental

Resumo

Animais ectotérmicos dependem da temperatura para manter suas funções fisiológicas e, por meio de mudanças comportamentais, podem evitar o superaquecimento em seus habitats. O Voluntary Thermal Maximum (VT_{Max}) é um índice de tolerância térmica comportamental que representa a temperatura máxima tolerada pelos indivíduos antes de se mudarem ativamente para um local mais frio. No entanto, se e como o VT_{Max} pode mudar após a captura e em cativeiro permanece pouco estudado. Nosso estudo tem como objetivo investigar se as medidas feitas em cativeiro são um bom proxy para a tolerância térmica de indivíduos selvagens. Uma vez que a história térmica demonstrou afetar a resposta comportamental e os parâmetros fisiológicos, testamos aqui a hipótese de que o VT_{Max} da víbora Neotropical *Bothrops pauloensis* varia ao longo do período de cativeiro. Medimos o VT_{Max} de indivíduos imediatamente após a captura e em três tentativas durante um curto período em cativeiro. As medições foram feitas registrando a temperatura corporal interna na qual eles saíram de uma configuração experimental de caixa de aquecimento. Contrariamente à nossa hipótese, o VT_{Max} não foi significativamente afetado pelo tempo em cativeiro, mas houve variação interindividual. Também não houve diferenças significativas entre as medidas em campo (na captura) e as medidas em cativeiro, apesar do pequeno tamanho do efeito. Nossos resultados indicam que a tolerância térmica comportamental dessa população de serpentes não é afetada por um curto período de cativeiro. Além disso, um VT_{Max} invariante pode indicar baixa plasticidade fenotípica, uma vez que os indivíduos não parecem ajustar sua tolerância à exposição de curto prazo a temperaturas mais altas e vulnerabilidade potencial a ameaças como o aquecimento global. Esperamos que nossos resultados possam contribuir para o entendimento do efeito do cativeiro na tolerância térmica em repteis Neotropicais, permitindo uma compreensão sobre sua fisiologia e ecologia térmicas.

Palavras-chave: Comportamento animal, fisiologia térmica, tolerância térmica comportamental, temperatura corporal, *Bothrops*, variação interindividual.

Chapter 1: Short-term captivity does not affect immediate voluntary thermal maximum of a neotropical pitviper: implications for behavioral thermoregulation

Abstract

Ectothermic animals depend on temperature to maintain their physiological functions and through behavioral changes, they can avoid overheating in their habitats. The Voluntary Thermal Maximum (VT_{Max}) is a Behavioral Thermal Tolerance index that represents the maximum temperature tolerated by individuals before actively moving to a colder place. However, if and how VT_{Max} might change after capture and in captivity remains understudied. Our study aims to investigate if measurements taken in captivity are a good proxy for thermal tolerance of wild individuals. Since thermal history has been shown to affect behavioral response and physiological parameters, herein we test the hypothesis that the VT_{Max} of the Neotropical viper *Bothrops pauloensis* varies throughout the captivity period. We measured the VT_{Max} of individuals immediately after capture and in three trials during a short-term period in captivity. Measurements were done by recording the internal body temperature at which they exited a heating box experimental setup. Contrary to our hypothesis, the VT_{Max} was not significantly affected by time in captivity but there was inter-individual variation. There were also no significant differences between field-measured (at capture) and the measurements in captivity, in spite of a small effect size. Our results indicate that the Behavioral Thermal Tolerance of this snake population is not affected by a short-term captivity period. Furthermore, an invariant VT_{Max} might indicate low phenotypic plasticity, since individuals do not appear to adjust their tolerance to short-term exposure to higher temperatures, and potential vulnerability to threats such as global warming. We expect that our results can contribute to understanding the effect of captivity on thermal tolerance in Neotropical squamates, allowing for insights on their thermal physiology and ecology.

Keywords: Animal behavior, behavioral thermal tolerance, body temperature, *Bothrops*, inter-individual variation, thermal physiology.

2.1. Introduction

The ability to withstand environmental stress is crucial for species' long and short-term survival in their habitats (FISCHER et al., 2010). Ectothermic animals depend on environmental conditions (e.g. temperature, humidity) to maintain their physiological functions (STEVENSON, et al., 1985; PETERSON, 1987; ROW & BLOUIN-DEMERS, 2006). In natural environments, ectotherms are exposed to daily thermal fluctuations and through behavioral adjustments (e.g. moving or changing their body posture; BRATTSTROM, 1979) they can maintain their body temperatures within a range of preferred body temperatures (LILLYWHITE, 1980). Upper environmental temperatures can lead to an individual's body temperature being above this range, leading to overheating to which the individual behaviorally by moving to a colder place to lower its body temperature (COWLES & BOGERT, 1944). This behavioral thermal limit is known as the Voluntary Thermal Maximum (VT_{Max}, COWLES & BOGERT, 1944). However, if the individual fails to respond to its VT_{Max} and its body temperature continues increasing, it will reach its physiological thermal limit, known as Critical Thermal Maximum (CT_{Max}, COWLES & BOGERT, 1944). When CT_{Max} is reached, there is functional collapse followed by death at its lethal temperature (LUTTERSCHMIDT & HUTCHISON, 1997a, b; COWLES & BOGERT, 1944; REZENDE, et al., 2014).

Even though VT_{Max} has been documented for lizards (e.g. BRATTSTROM, 1965; CAMACHO et al., 2018; DIELE-VIEGAS et al., 2018; DIAZ-RICAURTE & SERRANO, 2020) and recently for frogs (GUEVARA-MOLINA, et al., 2020; DIAZ-RICAURTE, et al., 2020), the behavioral and physiological thermal tolerance of snakes are understudied (Lillywhite, 1980; Webb & Shine, 1998; Huang, Huang, Chen, & Tu, 2007). The VT_{Max} is an index measurable under experimental conditions and allows for estimating the behavioral thermal tolerance limits of individuals without exposing them to their physiological critical thermal limits (COWLES & BOGERT, 1944; CAMACHO & RUSCH, 2017; CAMACHO et al., 2018). Understanding the dynamics of physiological thresholds at the individual level is essential to assess the vulnerability of species to changing climate conditions (WILLIAMS, et al., 2008). Therefore, by estimating thermal tolerance indexes - such as VT_{Max} - and how they are influenced by experimental factors, we are able to better understand the thermal tolerance of populations and how these respond to changing climatic conditions.

As temperature is tightly linked to ectotherm physiology, it can also affect behavioral consistency (LILLYWHITE, 1987). Thus, within the lifetime of an individual, thermal history (previously encountered stressful short periods of exposure to non-lethal temperatures) might

alter its thermal physiology (BAHRNDORFF et al., 2009; AUBRET & SHINE, 2010). Due to thermal stress, cellular protective mechanisms enable a heat shock response, which subsequently increases thermal tolerance (FISCHER et al., 2010; SOBEK et al., 2011). Understanding how thermal tolerance changes with thermal history (i.e. phenotypic plasticity) is important because it allows for establishing a predictable relationship between thermal tolerance and fitness (BRODIE & RUSSELL, 1999), especially since low plasticity might render populations vulnerable to changing thermal environments (FISCHER et al., 2010). The impact of thermal history is therefore important to consider in behavioral thermal tolerance but has rarely been tested (HERTZ et al., 1993; MARTIN & HUEY, 2008).

One of the challenges of measuring changes in thermal tolerance is assessing how it is altered by time in captivity (Hoffmann et al., 2013; REZENDE et al., 2014), since exposure to constant thermal conditions - usually several weeks -in the laboratory can impact critical thermal tolerances of ectotherms (e.g. frogs: BRATTSTROM & LAWRENCE, 1962; Pintor et al., 2016; lizards: KOUR & HUTCHISON, 1970, PATTERSON, 1999; and snakes: JACOBSON & WHITFORD, 1970). This has implications on estimates of thermal tolerance and how well they represent natural tolerance, especially since on-field estimates are scarce (HUTCHISON & MANESS, 1979; PINTOR et al., 2016). Therefore, testing wild-caught individuals and assessing if captivity and thermal history affect their thermal tolerance has been suggested to improve and validate physiological studies where animals are tested in captivity (PINTOR et al., 2016, STILLMAN, 2019).

Here, we address the knowledge gap of how behavioral thermal tolerance is affected by captivity. We provide the first values of the Voluntary Thermal Maximum of a Neotropical pitviper, *Bothrops pauloensis*, and investigate if it changes throughout a short-term captivity period, testing for inter-individual variation. Since thermal history has been shown to affect behavioral response and physiological parameters, our hypothesis is that behavioral thermal tolerance is positively affected by captivity. Thus, we expect VT_{Max} values to increase between successive trials throughout the captivity period. We hope that our results can contribute to understanding the effect of captivity on thermal tolerance in Neotropical squamates, allowing for insights on their thermal physiology and ecology.

2.2. Materials and Methods

2.2.1. Study species

The Neotropical pitviper *Bothrops pauloensis* belongs to the *B. neuwiedi* group, which comprises nine species distributed in the dry open areas of South America, such as Cerrado

savannas and semi-arid Caatinga (ALENCAR et al., 2016; NOGUEIRA et al., 2019). *Bothrops pauloensis* is a Cerrado endemic species, occurring in eastern Bolivia, Brazil, and Paraguay at intermediate to high elevation (NOGUEIRA et al., 2019). It is frequently found in open savanna and grassland habitats (VALDUJO et al., 2002). Even though several individuals have been reported active before dusk (SAWAYA et al., 2008), during the day it is found inactive inside burrows to avoid solar radiation (VALDUJO et al., 2002).

2.2.2. Obtaining and maintenance of individuals

In December 2019, we captured five individuals (three females and two males) of *Bothrops pauloensis* at the Santa Bárbara Ecological Station (Santa Bárbara municipality, São Paulo, Brazil; 22.9488°S, 43.2862°W; WGS84, elevation 590 m a.s.l.). The five individuals had an average ± standard deviation snout-to-vent length (SVL) of 499.8 ± 58.1 mm, tail length (TL) of 58.7 ± 15.3 mm and weight of 112 g ± 42.07 g. To investigate the effect of time of captivity on the VT_{Max}, we measured them on three trials during an 18 day period: on the 3rd, 11th day, and 18th days of captivity. Throughout the captivity period, the individuals were individually kept in a 50 x 30 x 35 cm plastic box at University of São Paulo (Brazil), natural photoperiod and room temperature between 23 °C and 26 °C. All individuals were kept unfed and with access to water *ad libitum* during the captivity period, and were released back to the same exact site of capture after the experiments. This study was conducted under a permit by Comissão de Ética no Uso de Animais (CEUA #2325141019) of Instituto Butantan; Permit by Instituto Florestal at Estação Ecológica de Santa Bárbara (permit #260108-008.476/2014) and permit by ICMBio-SISBIO (permit#50658-3) to collect snake specimens.

2.2.3. Voluntary thermal maximum (VT_{Max}) measurements

The experimental setup consisted of a transparent plastic box (50x50x30) attached to a dark metal box wrapped in a thermal resistance for heating (hereafter "heating box"). We placed a thin thermocouple (type T, Omega ®) in the vent of each individual to record its body temperature during the experiment (CAMACHO & RUSCH, 2017; DIAZ-RICAURTE & SERRANO, 2020). The individuals were moved to the plastic box and allowed to move freely into the heating box, with the thermal resistance off. As soon as the individual moved into the heating box, we started to gradually increase the box temperature and to record the individual's body temperature and heating rate. We measured each animal separately. During heating, the box warming rate was kept constant to ensure that box temperature did not

exceed the temperature of the individual by more than 5–6 degrees Celsius, to allow for the animal to thermoregulate until it made the decision to leave the box. This was done by placing a different type T thermocouple on the surface of the heating box to record its temperature. Both thermocouples were calibrated and connected to a FieldLogger PicoLog TC-08 recording temperature data every 10 seconds. We considered the VT_{Max} as the last body temperature recorded at the time the individual left the heating box.

2.2.4. Statistical analysis

We used a linear mixed-effect model to investigate if VT_{Max} differed between days in captivity, adding the potential effects of heating rate and SVL, and considering individual ID nested in 'days in captivity' as a random factor to account for non - independence among trials. This model was built using the REML ("restricted maximum likelihood"). The linear mixed-effect model approach is preferable to a repeated-measures ANOVA since it allows for ordered trials, and because it has shown adequate control of the Type I error rate even with low sample sizes (OBERFELD & FRANKE, 2013) and robustness to violations of distributional assumptions (SCHIELZETH et al., 2020). Since SVL and weight were strongly correlated ($r = 0.91$, $p < 0.001$), we reduced model complexity by using only SVL since it does not change with reproductive or feeding status. Additionally, we calculated the standardized effect sizes. Standardized effect sizes are defined as the difference between two means divided by the pooled standard deviation for those means (COHEN, 1990) and can be used to compare studies which differ in sample sizes and are useful for meta-analyses (NAKAGAWA, 2004; COLEGRAVE & RUXTON, 2003). This approach is preferable to post-hoc power analysis (COLEGRAVE & RUXTON, 2003). For the linear mixed-effect model we calculated the effect sizes of the fixed effects with Cohen's d.

To investigate for potential acclimation prior to our first measurements on the 3rd day of captivity, we used an unpaired t-test to compare them to baseline VT_{Max} values from six individuals (three females and three males; SVL = 497.0 ± 181.0 mm; TL = 91.8 ± 12.4 mm; body mass = 83.2 ± 23.2 g) previously caught from the same population from 2018 to 2019 and measured immediately after capture (field-measured individuals). We used a two-tailed F-test to check the equality of the variances of the two samples. To estimate the t-test's effect size and their 95% confidence intervals, we used Hedge's g rather than Cohen's d since we had independent and small sample sizes (LAKENS, 2013). Herein, standardized effect sizes

represent a measure of the magnitude of the difference in VT_{Max} mean values between individuals in captivity and individuals measured after capture in the field.

Furthermore, we used an ANOVA and a Tukey's HSD Post-Hoc Test to further check for inter-individual variation in VT_{Max} by pairwise comparisons of the mean VT_{Max} between only the individuals in captivity. We computed Cohen's d effect sizes using the individual means and ANOVA output by dividing each mean difference by the square root of the mean square residuals (which is the pooled within-groups standard deviation). All statistical analyses were performed in R 3.5.2 using the ´ggplot2´, 'lme4', 'lmerTest' and 'stats' packages (R CORE TEAM, 2019). Even though the standards for evaluating significance of fixed effects have been questioned and discussed (Bates et al., 2015), we used the REML-fitted model and a Satterthwaite approximation to degrees of freedom, since these have been shown to be robust even for low samples, to consider p-values < 0.05 as significant (LUKE, 2017).

2.3. Results

The mean VT_{Max} were similar between the 3rd, 11th and 18th days in captivity (n = 5), (Table 1), with mean heating rates of 0.40 ± 0.1, 0.51 ± 0.19 and 0.57 ± 0.37, respectively. The field-measured individuals (n = 6) had a mean VT_{Max} of 35.44 ± 1.95, with a mean heating rate of 0.17 ± 0.09. The detailed experimental values of each individual can be seen in Supplementary material. The variance of the two samples of individuals (measured in captivity vs. field-measured) were not significantly different (F = 1.33, df = 5, p = 0.08). There were no significant differences of VT_{Max} between the individuals measured on the 3rd day of captivity and field-measured individuals (t = 0.05, df = 9, p = 0.96). We obtained a Hedges' g of 0.032 (CI 95% = -1.155 – 1.219) for the t-test, which suggests a small effect size. The linear mixed-effect model (Table 2) showed that neither time in captivity p = 0.09) nor SVL (p = 0.35) were significant, with only heating rate (p < 0.05) having a positive significant effect on the VT_{Max} of *B. pauloensis*. The effect sizes of the fixed effects were medium, with Cohen's d values larger than 0.4 (Table 2). There was also a small increase in heating rate between trials, in spite of the VT_{Max} remaining relatively constant. In spite of intra-individual differences between trials not being significant, there was significant inter-individual variation. The ANOVA (df = 4, F = 13.01 p < 0.001) and its Tukey's HSD Post-Hoc Test showed differences in overall VT_{Max} values between individuals (Figure 1; Table S1).

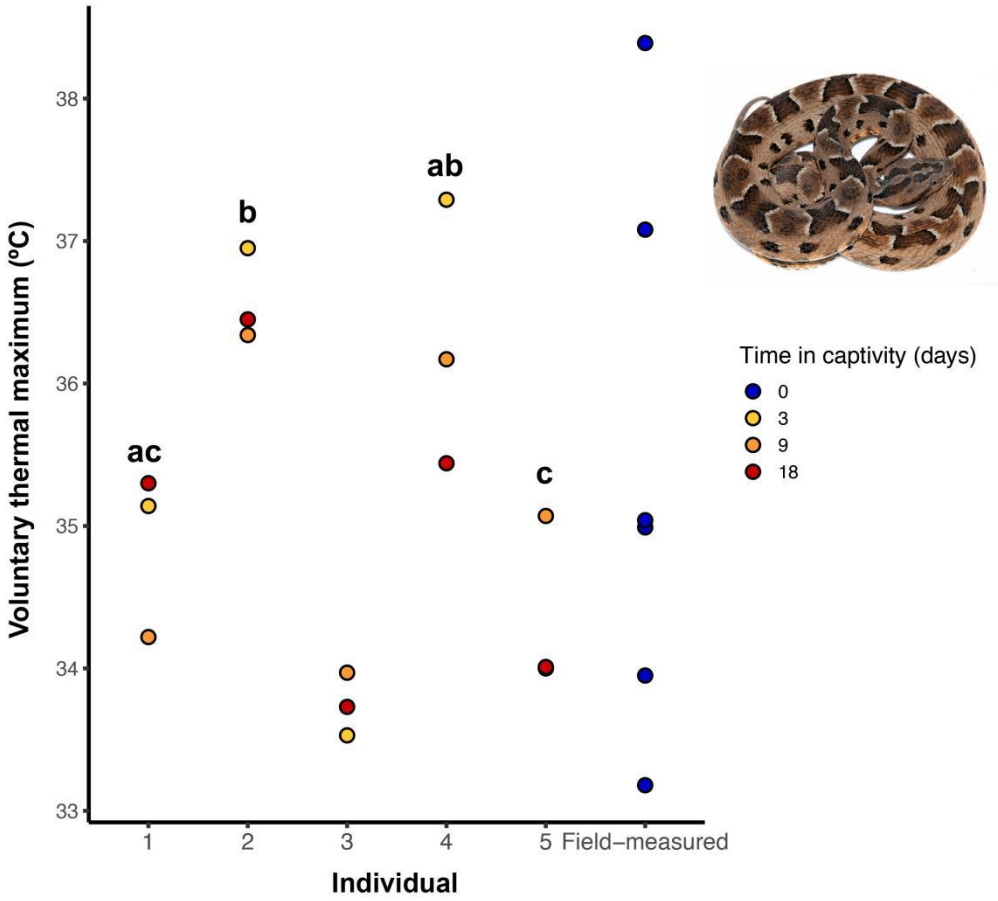


Figure 1. A) Comparison of the VT_{Max} of the *B. pauloensis* individuals (n = 5) measured on the 3rd (yellow), 11th (orange), and 18th (red) day of captivity, with the VT_{Max} of individuals measured immediately after capture (n = 6) (blue). The individual in the figure is an adult *B. pauloensis* (♀, SVL = 500 mm). Dissimilar letters indicate a significant difference ($p < 0.05$) in mean values of VT_{Max}, as obtained from Tukey's HSD Post-Hoc Test.

This was further confirmed by the estimated 95% intervals of the among - individual variance (Figure S1), which indicates that differences in mean values for individuals (random intercept) are related to small individual effects of time in captivity (random slope). Overall, the effect sizes of the Tukey's HSD Post-Hoc Test pairwise comparisons strongly agreed with the mean differences, with large effect sizes corresponding to large mean differences (Table S2).

Table 1. VT_{Max} and biometric values of *B. pauloensis* individuals measured on the 3rd, 11th and 18th days in captivity and immediately after capture (field-measured).

Individual	**VT_{Max} (ºC)**	**Weight**	**SVL**	**Tail**

(sex)	3rd day	11th day	18th day	Field-measured	(g)	(mm)	length (mm)
1 (♂)	35.14	34.22	35.3	-	70	440	56
2 (♀)	36.93	36.34	36.45	-	120	500	39.5
3 (♂)	33.53	33.97	33.73	-	90	510	77
4 (♀)	37.29	36.14	35.44	-	180	590	50
5 (♀)	34.00	35.07	34.01	-	100	459	71
Mean ± s.d.	35.38 ± 1.69	35.15 ± 1.08	34.99 ± 1.12	-	112 ± 42.1	499.8 ± 58.1	58.7 ± 15.3
6 (♀)	-	-	-	34.99	59	590	110
7 (♀)	-	-	-	33.95	78	558	93
8 (♂)	-	-	-	35.04	117	139	101
9 (♀)	-	-	-	33.18	95	625	88
10 (♂)	-	-	-	37.08	67	490	84
11 (♂)	-	-	-	38.39	98	580	75
Mean ± s.d.				35.44 ± 1.95	85.7 ± 21.6	497 ± 181	91.83 ± 12.5

Table 2. Summary of the fitted linear mixed-effects model showing the effect of different variables on the VT_{Max} of *B. pauloensis.* * indicates statistical significance. Effect sizes calculated with Cohen's d where 0.5 represents a medium effect size.

Variable	Estimate	Std.Error	t-value	df	p-value	Effect size
(Intercept)	29.928	4.668	6.412	3.70	0.004*	-
Heating rate	1.727	0.737	2.344	8.44	0.046*	0.61
Snout-Vent length	0.009	0.009	1.069	3.48	0.353	0.45
Time in captivity	-0.045	0.023	-1.947	6.53	0.095	0.51

2.4. Discussion

Our results show that Voluntary Thermal Maximum (VT_{Max}) remained constant between three trials over a 18 day period in captivity, thus not confirming our hypothesis that VT_{Max} increases throughout a short-term captivity period. There were also no significant differences between field-measured (at capture) and the first measurements in captivity. This suggests that short-term acclimation likely does not take place between capture and the 3rd day of captivity, despite the expectation that recently-captured individuals can endure higher temperatures to avoid exposing themselves to potential predators (CAMACHO et al., 2018). Thus, our results seem to indicate that behavioral thermal tolerance for this snake population is not affected by the stress of capture or a short-term period in captivity, which validates measurements of VT_{Max} taken after several days of captivity. However, the small effect size indicates that this hypothesis requires further testing to be fully supported.

While neither days in captivity nor body size affected VT_{Max}, heating rate significantly increased VT_{Max}. This differs from other studies on VT_{Max}, where both heating rate and body mass were significant factors for lizards (CAMACHO & RUSCH, 2017), while the VT_{Max} of another Neotropical lizard species was significantly affected by time after capture but not by body mass nor acclimation to a constant temperature (ROSSO, 2020). For anurans, neither heating rates nor SVL seem to impair VT_{Max} (DIAZ-RICAURTE et al., 2020; GUEVARA-MOLINA et al., 2020). Since VT_{Max} has also been shown to be related with life history (e.g. habitat use; DIAZ-RICAURTE et al., 2020), further studies are warranted to investigate how upper thermal limits are constrained by both experimental and ecomorphological aspects

across different ectotherm groups. However, this can only be tested by using standardized and comparable methodology (PINTOR et al., 2016).

Our results also seem to suggest that there is a noticeable inter-individual variation in thermal tolerance, with an idiosyncratic thermal profile for each individual. These differences in inter-individual thermal tolerance, albeit small, might be important for population dynamics (ANDERSON et al., 2011). In reptiles, body temperature and thermoregulation performance are linked to physiological processes such as growth, reproduction, and ultimately fitness (HUTCHINSON & MANESS, 1979; GRIBBINS et al., 2006). If unable to lower their body temperature, individuals with lower VT_{Max} might reach their critical thermal maximum sooner, thus leading to differences in inter-individual survival (HUEY & KINGSOLVER, 1993). Furthermore, data on inter-individual variation is relevant to consider in studies on population and thermal ecology, since using a single value to represent the voluntary thermal maximum of a population might misrepresent tolerances and thus misestimate the impact of potential threats such as global warming.

Bothrops pauloensis occurs in Cerrado open dry savannas (NOGUEIRA et al., 2019), with both high (up to 35–37 ºC in the study area, *pers. obs.*) and highly variable environmental temperatures. This requires it to have behavioral (e.g. occupying burrows) and physiological thermoregulatory strategies that allow its populations to survive and persist (HUEY & SLATKIN, 1976; LELIÈVRE et al., 2011). Thermal tolerance has been shown to depend on previous thermal history (KRISTENSEN et al., 2008; AUBRET & SHINE, 2010), such that ectotherms withstand higher temperatures after short periods of exposure to non-lethal temperatures (e.g. BAHRNDORFF et al., 2009; FISCHER et al., 2010; SOBEK et al., 2011). This was not the case of *B. pauloensis*, whose thermal tolerance did not increase after exposure to their VT_{Max}. Our observations seem to indicate a low phenotypic plasticity in this population of *B. pauloensis* since they do not appear to adjust their tolerance to higher temperatures, at least for short-term periods after exposure to VT_{Max}. Therefore, it appears to confirm previous findings that species living in hot environments have low plasticity in their heat tolerance (Stillman, 2003). Since the ability to adjust thermal tolerance has been shown to promote survival in terrestrial ectotherms (CHIDAWANYIKA & TERBLANCHE, 2011; SUNDAY et al., 2011; SEEBACHER et al., 2015), it may imply that thermal extremes pose a significant threat for *B. pauloensis*, as suggested for other species (STILLMAN, 2019). While studies on other populations could elucidate if thermal limits are invariant throughout its distribution, it is possible that *B. pauloensis* could be affected by global warming because it is distributed within a latitude range (between 20º and 40°) in which ectotherms are more

susceptible to changes in temperature (CLUSELLA-TRULLAS et al., 2011; HOFFMAN et al., 2013).

Despite numerous ecophysiological studies about the acclimation responses in ectotherms, these rarely account for behavioral thermal tolerances. Using behavioral thermal tolerances, such as the VT_{Max}, allows for the integration of thermoregulatory behavior, which usually happens before critical limits are reached (SINCLAIR et al., 2016; CAMACHO et al., 2018; GUEVARA-MOLINA et al., 2020). Furthermore, estimating behavioral thermal limits is critical to understand how temperature affects the behavior of individuals in their natural habitat and in captivity. Although the use of a low number of individuals of a single species limits the extent of the implication of our results, similar studies are scarce for ectothermic vertebrates (see CAMACHO et al., 2018). Even though Neotropical pitvipers of the *Bothrops* genus are widespread and occur in several distinct ecoregions (e.g. Amazon forest to the Brazilian Pampas), it is possible that other species from the *B. neuwiedi* group show a similar profile of thermal response to captivity due to their similar habitats and recent diversification (3–6 million years; ALENCAR et al., 2016; HAMDAN et al., 2019). However, factors such as microclimatic environmental variation might also play a role in determining thermal tolerance, as shown for CT_{Max} (DEWITT & SCHEINER, 2004; FRANKEN et al., 2018). Since snakes of this group are usually captured in remote areas, this allows for measurements to be taken in controlled conditions after individuals are collected and transported to laboratories.

Notwithstanding our novel observations, our study has some limitations regarding its nonsignificant results. Our results were based on a small sample size, with low effect sizes and confidence intervals overlapping zero for the comparison of field and captive individuals; however, the confidence intervals indicate a small magnitude of effects. This small effect size might be due to the small sample size, the small differences in the mean VT_{Max} values of individuals between field-capture and captivity, a large natural intra-population variance, or even a combination of them. This indicates caution in fully supporting the neglectable effect of time in captivity on the Voluntary Thermal Maximum (i.e. VT_{Max}) of *B. pauloensis*. Although further testing may be necessary before the effect of captivity on thermal tolerance is convincingly understood in pitvipers, we suggest that other interactive factors, such as time of the day when VT_{Max} is measured (DIAZ-RICAURTE et al., 2020) or reproductive status (LADYMAN et al., 2003), might play a role. Studies with snake species are usually limited by the difficulty of finding individuals and our study reflects a comprehensive sampling effort over a two-year period and thus a reasonably representative sample of this population. Thus,

since our results are the first estimates of behavioral thermal tolerance in Neotropical snakes, our findings can be a baseline for tests with larger sample sizes aiming to understand ectotherm thermal tolerances. Thus, we encourage future studies to estimate behavioral thermal tolerances to improve understanding of the vulnerability of these species to future global warming. We also hope this study promotes future observations on thermal ecology and the impact on captivity on ectotherms, especially Neotropical snakes.

Acknowledgments

We thank Marcio Martins, João Paulo dos Santos Vieira de Alencar, Carolina Farhat, Luciana Furtado and Patrícia Marques for their help in the capture and maintenance of individuals. We also thank Agustín Camacho for the initial idea and conceptualization of the methodology, and Gustavo Agudelo-Cantero and Matthijs Hollanders for valuable comments and suggestions on the manuscript.

Competing Interests

No potential conflict of interest was reported by the authors.

Author Contributions

Conceptualization: JCDR; Methodology: JCDR; Formal analysis: JCDR, FCS; Resources: JCDR; Data curation: JCDR, FCS; Writing - original draft: JCDR; Writing - review and editing: JCDR, FCS; Visualization: JCDR, FCS; Supervision: JCDR, FCS; Funding acquisition: JCDR, FCS.

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001”. Also, this study was financed by Fundação de Amparo à Pesquisa do Estado de São Paulo (Grant #2018/14091-8).

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Supplementary Material

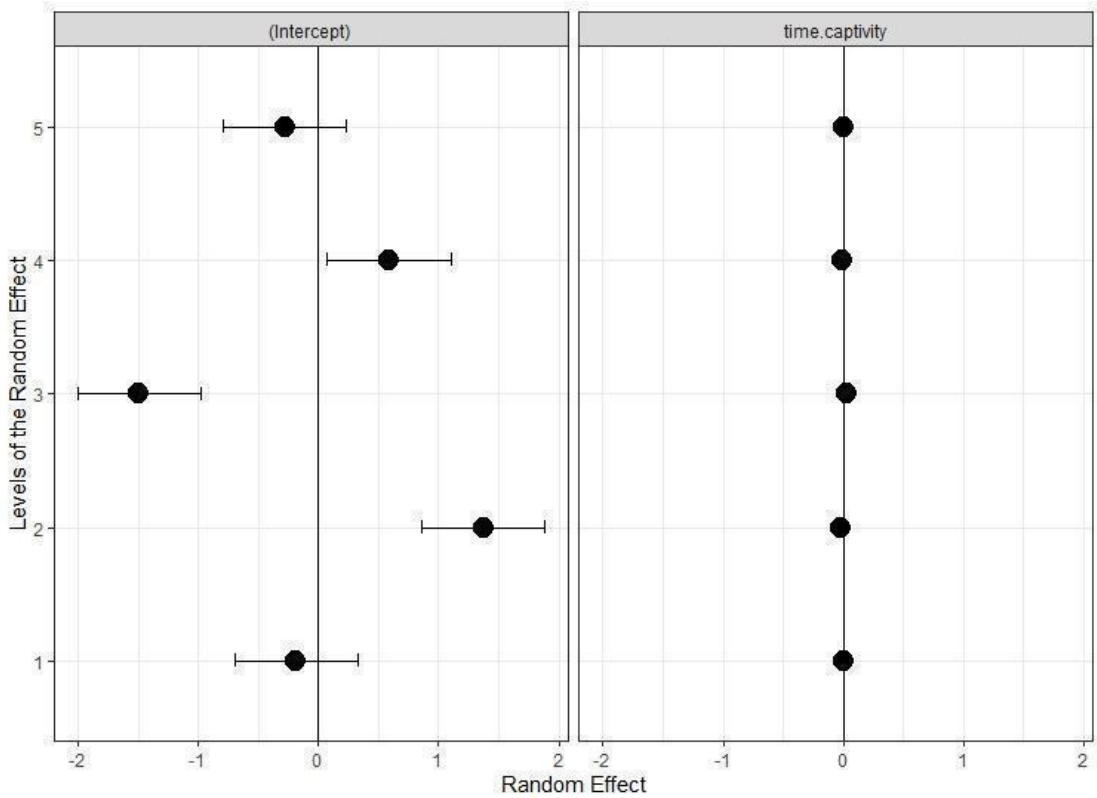


Figure S1. Caterpillar plot of the among - individual variance of $VT_{Max.}$ for the *B. pauloensis* individuals measured in captivity (n = 5). These plots arrange the random effects by the mean prediction intervals of individuals, with horizontal lines indicating the 95% prediction intervals. The differences in mean values for individuals (random intercept) are poorly related to small individual effects of time in captivity.

Table S1. p-values for the difference in mean values of VTMax between individuals, as obtained from Tukey's HSD Post-Hoc Test*

		Individual				
		1	2	3	4	5
Individual	1					
	2	0.0353024	0			
	3	0.1997368	0.0011194	0		
	4	0.0867919	0.9749248	0.002461	0	
	5	0.8062411	0.0066974	0.7082848	0.0160281	0

*$p < 0.05$ (red) indicates significant differences between individuals, while $p > 0.05$ indicates non-significant differences.

Table S2. Effect sizes of mean differences between individuals, as obtained from Tukey's HSD Post-Hoc Test*

		Individual				
		1	2	3	4	5
Individual	1	0				
	2	1.32525229	0			
	3	0.89834296	0.85162002	0		
	4	1.10263086	0.22262143	2.00097382	0	
	5	0.41381396	1.73906625	0.484529	1.51644482	0

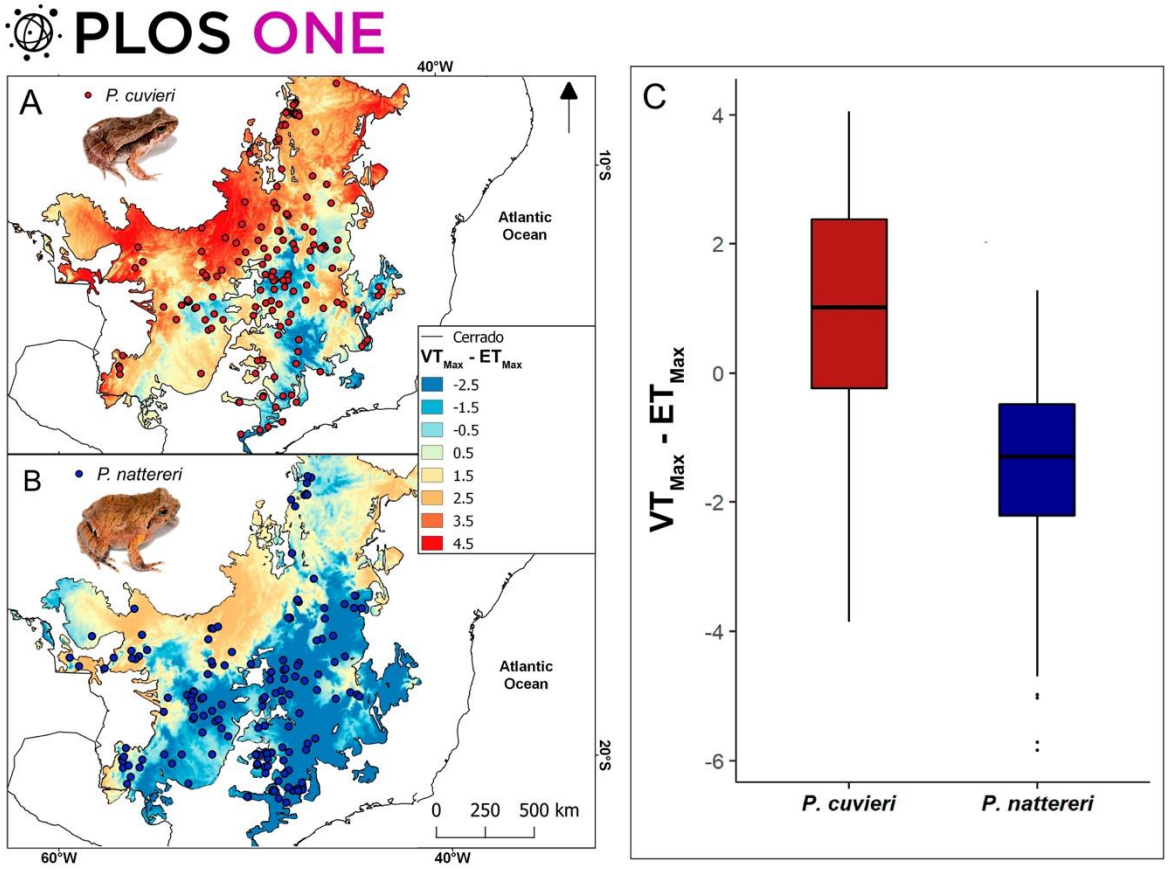
*0.2 = Small effect (cannot be discerned by the naked eye); 0.5 = Intermediate effect; >0.8 = Large Effect (can be seen by the naked eye)

Table S3. Physiological data summary of individuals of *B. pauloensis.*

Experiment data	Number of measurement	Individual ID	Field=F; Captivity=C	Time of captivity (days)	Sex	weight (g)	SVL (mm)	TL (mm)	Start time	Final time	Time of experiments (min)	Initial body temperature (ºC)	Final body temperature (ºC)	Vtmax (ºC)	Heating rate (ºC/min)
10/03/18	1	6	F	0	F	59	590	110	16:12	17:05	53	25.39	34.99	34.99	0.181
10/03/18	1	7	F	0	F	78	558	93	20:21	21:36	75	29.26	33.95	33.95	0.063
17/03/18	1	8	F	0	M	117	139	101	16:30	17:19	49	29.64	35.04	35.04	0.110
23/10/18	1	9	F	0	F	95	625	88	19:24	20:12	48	31.56	33.18	33.18	0.135
25/10/18	1	10	F	0	M	67	490	84	22:15	23:11	56	24.6	37.08	37.08	0.223
24/04/19	1	11	F	0	M	98	580	75	15:23	15:51	26	30.3	38.39	38.39	0.311
30/11/19	1	1	C	3	M	70	440	56	14:46	15:00	14	28.1	35.14	35.14	0.503
30/11/19	1	2	C	3	F	120	500	39.5	14:07	14:29	22	27.2	36.95	36.95	0.443
30/11/19	1	3	C	3	M	90	510	77	11:39	12:02	23	27.14	33.53	33.53	0.278
30/11/19	1	4	C	3	F	180	590	50	13:22	13:44	22	26.8	37.29	37.29	0.477
30/11/19	1	5	C	3	F	100	459	71	12:47	13:11	24	26.7	34	34	0.304
09/12/19	2	1	C	11	M	70	440	56	13:29	13:43	14	26.04	34.22	34.22	0.584
09/12/19	2	2	C	11	F	120	500	39.5	11:36	11:49	13	26.48	36.34	36.34	0.758
09/12/19	2	3	C	11	M	90	510	77	12:58	13:14	16	26.79	33.97	33.97	0.449
09/12/19	2	4	C	11	F	180	590	50	09:00	09:39	39	26.4	36.17	36.17	0.251
09/12/19	2	5	C	11	F	100	459	71	12:09	12:56	45	25.34	35.07	35.07	0.541
18/12/19	3	1	C	18	M	70	440	56	14:44	14:51	7	26.67	35.3	35.3	1.233
18/12/19	3	2	C	18	F	120	500	39.5	13:17	13:35	17	26.85	36.45	36.45	0.565
18/12/19	3	3	C	18	M	90	510	77	14:00	14:21	21	25.24	33.73	33.73	0.404
18/12/19	3	4	C	18	F	180	590	50	11:18	11:44	26	25.96	35.44	35.44	0.365
18/12/19	3	5	C	18	F	100	459	71	15:11	15:37	55	25.85	34.01	34.01	0.314

Table S4. Physiological data of the individuals measured in this study. For more details see supplementary material in https://doi.org/10.1002/jez.2433

3. CHAPTER 2. DOES BEHAVIORAL THERMAL TOLERANCE PREDICT DISTRIBUTION PATTERN AND HABITAT USE IN TWO SYMPATRIC NEOTROPICAL FROGS?



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DIAZ-RICAURTE, JC; SERRANO, F; GUEVARA-MOLINA, EC; ARAUJO, C; MARTINS M. 2020. Behavioral thermal tolerance predicts distribution pattern but not habitat use in sympatric Neotropical frogs. ***PLoS ONE***, 15(9): e0239485. https://doi.org/10.1371/journal.pone.0239485

Capítulo 2: A tolerância térmica comportamental prevê o padrão de distribuição, mas não o uso do habitat em sapos neotropicais simpátricos

Resumo

As temperaturas ambientais são uma restrição importante na abundância e diversidade de ectotérmicos, influenciando sua distribuição e história natural. Comparar as tolerâncias térmicas com as temperaturas ambientais é uma maneira simples de estimar as restrições térmicas nas distribuições de espécies. Investigamos os efeitos potenciais da tolerância térmica nos padrões de distribuição local (habitat) e global dos anuros e respostas comportamentais associadas. Testamos as diferenças no Máximo Térmico Voluntário de duas espécies de rãs simpátricas do gênero *Physalaemus* na ecorregião do Cerrado. Para cada espécie, construímos modelos para avaliar os efeitos do período do dia, duração do experimento, massa corporal inicial, temperatura corporal inicial e taxa de aquecimento no VT_{Max}. Mapeamos a diferença entre VT_{Max} e a temperatura máxima diária (VT_{Max} - ET_{Max}) para cada ponto de ocorrência. *Physalaemus nattereri* teve um VT_{Max} significativamente maior do que *P. cuvieri*. Para *P. nattereri*, o modelo incluindo apenas o período do dia foi escolhido como o melhor para explicar a variação no VT_{Max}. Para *P. cuvieri*, nenhum modelo foi selecionado como o melhor para prever VT_{Max}. Os valores de VT_{Max} - ET_{Max} foram significativamente diferentes entre as espécies, com *P. nattereri* encontrado principalmente em localidades que atingem temperaturas máximas inferiores a seu VT_{Max} e *P. cuvieri* apresentando o padrão inverso. Em relação ao uso do habitat, descobrimos que *P. cuvieri* é ligeiramente mais abundante em habitats abertos do que em habitats não abertos, enquanto *P. nattereri* mostra o padrão inverso. A diferença nos valores VT_{Max} entre essas duas espécies pode estar relacionada a seus diferentes tamanhos de corpo, mas, adicionalmente, pode refletir sua história natural, especialmente a forma como usam seus habitats, e restrições filogenéticas (as espécies estudadas estão em diferentes clados dentro de *Physalaemus*). Nosso estudo indica que as diferenças na tolerância térmica comportamental podem ser importantes na formação de padrões de distribuição locais e regionais. Além disso, o uso de habitat em pequena escala pode revelar uma ligação entre a tolerância térmica comportamental e as estratégias de história natural.

Palavras-chave: Brasil, Comportamento animal, Distribuição geográfica, Ecofisiologia, Habitats, Parâmetros fisiológicos, Sapos, Temperatura corporal.

Chapter 2: Behavioral thermal tolerance predicts distribution pattern but not habitat use in sympatric Neotropical frogs

Abstract

Environmental temperatures are a major constraint on ectotherm abundance and diversity, influencing their distribution and natural history. Comparing thermal tolerances with environmental temperatures is a simple way to estimate thermal constraints on species distributions. We investigate the potential effects of thermal tolerance on anuran local (habitat) and global distribution patterns and associated behavioral responses. We tested for differences in Voluntary Thermal Maximum of two sympatric frog species of the genus *Physalaemus* in the Cerrado ecoregion. For each species, we constructed models to assess the effects of period of day, duration of experiment, initial body mass, initial body temperature and heating rate on the VT_{Max}. We mapped the difference between VT_{Max} and maximum daily temperature (VT_{Max} - ET_{Max}) for each occurrence point. *Physalaemus nattereri* had a significantly higher VT_{Max} than *P. cuvieri*. For *P. nattereri*, the model including only period of day was chosen as the best to explain variation in the VT_{Max}. For *P. cuvieri*, no model was selected as best to predict VT_{Max}. The VT_{Max} - ET_{Max} values were significantly different between species, with *P. nattereri* mostly found in localities that attain maximum temperatures lower than its VT_{Max} and *P. cuvieri* showing the reverse pattern. Regarding habitat use, we found *P. cuvieri* to be slightly more abundant in open habitats than in non-open habitats, whereas *P. nattereri* show the reverse pattern. The difference in VT_{Max} values between these two species might be related to their different body sizes, but additionally might reflect their natural history, especially the way they use their habitats, and phylogenetic constraints (the species studied are in different clades within *Physalaemus*). Our study indicates that differences in behavioral thermal tolerance may be important in shaping local and regional distribution patterns. Furthermore, small-scale habitat use might reveal a link between behavioral thermal tolerance and natural history strategies.

Keywords: Animal behavior, Body temperature, Brazil, Ecophysiology, Frogs, Geographic distribution, Habitats, Physiological parameters.

3.1. Introduction

Environmental temperatures are a major constraint on ectotherm abundance and diversity, influencing their distribution and natural history (MALCOLM et al., 2006; POST et al., 2008; CAMACHO et al., 2018). Several studies have explored environmental constraints on ectothermic vertebrates at regional and global scales (MALCOLM et al., 2006; BUCKLEY et al., 2008). The physiological performance of individuals can be negatively affected by high environmental temperatures (CURRIE & FRITZ, 1993), which can lead to declining populations and/or local extinctions (ALLEN et al., 2002; PÖRTNER & FARRELL, 2008). Thus, knowing species thermal tolerance and exploring how environmental temperatures might affect their physiology and restrict their distribution is of primary concern for long-term conservation, especially under current global warming crisis (e.g. (SINERVO et al., 2010; STILLMAN, 2019), as well as habitat disturbance causing microclimate changes (e.g. habitat fragmentation; (URBINA-CARDONA et al., 2006).

However, thermal tolerances are rarely taken into account in studies that focus on local distribution and habitat use. For instance, many studies infer potential distribution of species using solely environmental temperatures from occurrence localities to model their niche (HILLMAN, 1969; PORTER et al., 1973; BARNES et al., 2015; FREITAS et al., 2015). While the broad geographical range of a species most likely reflects its thermal tolerance (e.g. GOUVEIA et al., 2014; SUNDAY et al., 2014), local factors might also play a role in shaping abundance and distribution. At a local scale, high environmental temperatures and its daily variation in the microhabitats of small ectotherms (e.g., anurans and lizards) impose physiological constraints on their activity patterns and habitat use (PORTER et al., 1973). For example, in habitats where direct sunlight is limited, the variation in temperatures is lower than in open habitats, suggesting a possible interplay between thermal tolerance and habitat use (RECODER et al., 2018). However, studies that relate how thermal tolerances affect habitat use and distribution are scarce.

Thermal tolerances can be behavioral, when an animal moves or adjusts its body posture to thermoregulate, or physiological if it does not move but uses other strategies such as increased respiration rates (CAMACHO et al., 2018). Behavioral and physiological thermal tolerances impact not only species ranges, but also the distribution and abundance patterns of their populations (CAMACHO et al., 2018). Identifying thermal tolerance thresholds (i.e. measurable thermal limits) outside the range of preferred body temperatures (PBT) for thermoregulation (see CAMACHO & RUSCH, 2017) allows for the identification of temperatures that directly affect the behavioral and physiological thermal tolerance of

ectothermic organisms. One of the thresholds related to PBT is the Voluntary Thermal Maximum (VT_{Max}), which represents a behavioral thermal tolerance measure. VT_{Max} is the maximum temperature that an organism will endure before trying to move to a place with a lower temperature, thus trying to maintain its body temperature within its range of PBT (COWLES & BOGERT, 1944; CAMACHO & RUSCH, 2017; CAMACHO et al., 2018). If an individual fails to respond to its VT_{Max}, an increase in body temperature will expose it to its physiological thermal limit (i.e. its Critical Thermal Maximum), which can lead to functional collapse and consequently death due to overheating (COWLES & BOGERT, 1944; REZENDE et al., 2014). Therefore, the behavioral response to upper limits might represent a more informative ecological threshold to identify thermal constraints on habitat use and geographic distribution (SINERVO et al., 2010; BARNES et al., 2015; CAMACHO et al., 2018). Contrary to the Critical Thermal Maximum, the exposure to the VT_{Max} does not induce an immediate loss of locomotion (LUTTERSCHMIDT & HUTCHISON, 1997; CAMACHO et al., 2018). Therefore, VT_{Max} can more realistically portray changes in species behavior associated with their natural history.

Behavioral thermal tolerances can be influenced by factors such as reproductive status, sex, photoperiod, and hydration state (CAMACHO & RUSCH, 2017; GUEVARA-MOLINA et al., 2020). Additionally, thermal tolerances such as the VT_{Max} might decrease with body size: due to thermal inertia, larger animals might have slower heating and cooling rates than small animals, which increases the exposed time to stressful thermal conditions (PORTER & GATES, 1969; LUNGHI et al., 2016). Thus, understanding the effects of these variables on the VT_{Max} might help to evaluate its impact on habitat use and geographic distribution.

Herein we address the question: Does VT_{Max} determine habitat use and regional distribution patterns in a pair of congeneric frogs, *Physalaemus cuvieri* and *P. nattereri*, which are widely sympatric in the savannas of Central Brazil? Our hypothesis is that, for being a measure that reflects avoidance of stressful thermal conditions, VT_{Max} determines both habitat use and geographic distribution in these species. If VT_{Max} decreases with body size (see above; PORTER & GATES, 1969; LUNGHI et al., 2016), we predict that VT_{Max} is lower in the larger species (*P. nattereri*). Furthermore, if VT_{Max} determines habitat use and geographic distribution, we predict that (i) the species with lower VT_{Max} is less abundant in open habitats, with higher environmental temperatures, and that (ii), regarding geographic distribution, both species occur mostly in localities where the maximum environmental temperature is below their VT_{Max}. We expect that our results can contribute to assess the vulnerability of Neotropical frogs to climate change by integrating their behavioral thermal

tolerances with their habitat use and distribution patterns, in order to identify areas with potential stressful climatic conditions to their populations.

3.2. Materials and Methods

3.2.1. Focal species

Most species of the genus *Physalaemus* have sympatric populations along extensive areas, such as *Physalaemus nattereri* (AQUINO et al., 2004) and *Physalaemus cuvieri* (MIJARES et al., 2010) (see FROST, 2020), which are widespread in central South America (AQUINO et al., 2004; MIJARES et al., 2010). These species belong to different clades within *Physalaemus* (*P. signifer* and *P. cuvieri* clades, respectively; LOURENÇO et al., 2015). *Physalaemus nattereri* has a stout body, a moderate to large size (adult snout-to-vent length of 29.8–50.6 mm) and is endemic to the Cerrado, whereas *P. cuvieri* has a slenderer body, a smaller size (snout-to-vent length of adults 28–30 mm) and occurs throughout the Cerrado, in southern portions of the Amazon Forest and in the Atlantic Forest (NASCIMENTO et al., 2005). Although the populations traditionally assigned to *P. cuvieri* (see FROST, 2020) may include more than one cryptic species (see LOURENÇO et al., 2015), most of the distribution of *P. cuvieri* in the Cerrado correspond to a single lineage (Lineage 2 in LOURENÇO et al., 2015). These two species also differ in their biology. While *P. cuvieri* uses several aquatic habitats for reproduction and seeks shelter during the day in previously-dug burrows, *P. nattereri* breeds mostly in temporary puddles and buries itself in the soil during the day aided by metatarsal tubercles (S1 Fig) on its hind feet (BRASILEIRO et al., 2005; NASCIMENTO et al., 2005; GIARETTA & FACURE, 2006).

3.2.2. Physiological Parameters

3.2.2.1. Capture and maintenance of individuals

Fieldwork was carried out at Estação Ecológica de Santa Bárbara (22°49’2.43"S, 49°14’11.29"W; WGS84, 590 m elevation), one of the few remnants of Cerrado savannas in the state of São Paulo, Brazil, with a total area of 2,712 ha (MELO & DURIGAN, 2011). The climate is Humid subtropical (KÖPPEN 1948), with temperatures averaging 24°C and 16°C during January and July, the hottest and coldest months, respectively. The average annual rainfall is 1100–1300 mm, with marked dry and wet seasons (approximately April to September and October to March, respectively; (MELO & DURIGAN, 2011). The landscape

not only consists of open grassland and savanna-type formations, such as 'campo sujo' and 'campo cerrado', but also of non-open vegetation types such as 'cerrado *strictu sensu*' (dense savanna) and 'cerradão' (cerrado woodland). Between 24 and 28 September 2018, we captured 14 individuals of *P. nattereri* and 20 of *P. cuvieri* in pitfall traps with drift fences (CORN, 1944; CECHIN & MARTINS, 2000) and these individuals were housed individually in plastic boxes at room temperature. This study was conducted under a permit by Comissão de Ética no Uso de Animais (CEUA #2325141019) of Instituto Butantan. All animals were alive after the experiments described below and were released the following morning at the site of capture.

3.2.2.2. Measurements of the Voluntary Thermal Maximum (VT_{Max})

To obtain the VT_{Max} for each species, we measured each individual at 100% hydration level less than 24 hours after capture. To reach maximum hydration level, each individual was placed in a cup with water *ad libitum* one hour prior to the experiment. Then, its pelvic waist was pressed to expel the urine and to obtain its 100% hydration level in relation to its standard body mass. We heated each individual inside a metal box wrapped in a thermal resistance for heating. The box had a movable lid, allowing the animal to easily leave the box when needed. A thin thermocouple (type-T, Omega®) was located in the inguinal region of each individual to record its body temperature during the heating (GUEVARA-MOLINA et al., 2020). Another type-T thermocouple was placed inside (on the surface) of the box to record heating rate of individuals. A dimmer previously connected to the box allowed to control that its temperature not exceeded 5–6°C the temperature of the individual, allowing the thermoregulation of individuals, and avoided thermal shock and/or a premature exit of the box by the frog (i. e., before VT_{Max} is reached; GUEVARA-MOLINA et al., 2020). The thermocouples were calibrated and connected to a FieldLogger PicoLog TC-08 to record temperature data every 10 seconds. The VT_{Max} of each individual was recorded as its last body temperature at the time of leaving the box. Once its final body mass was measured, it was taken to a container with water for recovery. Furthermore, to control for a potential effect of photoperiod on behavioral thermal tolerances, we tested if the VT_{Max} differed between different times of the day by testing half of the individuals of each species in different periods: 10:00 to 17:00 (daytime) and 19:00 to 00:00 (nighttime).

3.2.3. Statistical analyzes

We used Mann-Whitney U tests to compare the VT_{Max}, and experimental variables between species. Experimental variables were: period (day or night), duration of experiment, initial body mass, initial body temperature, and heating rate. To test for the effect of possible confounding experimental variables on the VT_{Max}, we constructed generalized least squares models for each species. We used the corrected Akaike Information Criterion (AICc) to select the model that best represented the effects of factors and their interactions on the VT_{Max} of each species. Differences of two units in AIC (ΔAICc) were not considered to be different (WANG & LI, 2006). We considered the model with weighted AIC (wAICc) values close or equal to 1 to represent the strongest model. All statistical analyzes and plotting were performed in R 3.5.0 (R Core Team 2018), with the nlme (PINHEIRO & BATES, 2000), ggplot2 (WICKHAM, 2016) and AICcmodavg (MAZEROLLE & MAZEROLLE, 2019) packages.

3.2.4. Distribution and habitat

We used vouchered occurrence data for *P. cuvieri* (N = 163) and *P. nattereri* (N = 164) in the Cerrado from a distribution database built for another study VALDUJO et al., 2012). We calculated and mapped the difference between the VT_{Max} and maximum environmental temperature (ET_{Max}; Bio 5; 30 seconds or ~1 km resolution from WorldClim Vr. 2.0; (FICK & HIJMANS 2017), for each occurrence point of each species in Cerrado; the VT_{Max} was that obtained at Estação Ecológica de Santa Bárbara. We used a Mann-Whitney U test to compare VT_{Max} - ET_{Max} of species occurrence records. All maps and GIS procedures were made in QGIS 3.12 (QGIS 2020). We tested for differences between species in habitat use by comparing abundances in open (‘campo cerrado’, ‘campo sujo’, and ‘campo limpo’) and non-open habitats (gallery forest, ‘cerradão’ and cerrado *stricto sensu*; Ribeiro & Walter 1998) for communities within Cerrado where both species occur in sympatry, available in the literature (MOTTA, 1999; THOMÉ, 2006; OLIVEIRA, 2012; ARAUJO & ALMEIDA-SANTOS, 2013; DULEBA, 2013; RAMALHO et al., 2014). We used PAST (HAMMER et al., 2001) to test for differences between the proportion of each species in open and non-open habitats with chi-square and Fisher Exact tests, the latter when at least one cell was < 5.

3.3. Results

3.3.1. Voluntary Thermal Maximum (VT_{Max}) and experimental conditions

We found that VT_{Max} was significantly lower for *P. cuvieri* than for *P. nattereri* (Table 1; w = 51, p = 0.0013). We also found significant differences in initial body mass (Table 1; w = 0, p < 0.0001) between species, with *P. nattereri* being heavier. We did not find significant differences in start body temperatures (Table 1; U = 112, p = 0.3359), period of day (Table 1; U= 0.12, df = 32, p = 0.9051), duration of the experiment (Table 1; U = 128, p = 0.6872) and heating rate (Table 1; U = 123.5, p = 0.5752) between species (see S1, S2 and S3 Tables).

Table 1. Variation of the VT_{Max} and predictor variables for *P. cuvieri* and *P. nattereri* from Estação Ecológica de Santa Bárbara, state of São Paulo, Brazil. Predictor variables are: period of day (day and night), initial body temperature (ST), duration of experiment (DOE), initial body mass (IBM), and heating rate (HRA).

Variable	***Physalaemus cuvieri***		***Physalaemus nattereri***	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**
VT_{Max}	30.20 ± 1.69 °C	27.48 – 33.13 °C	32.74 ± 2.14 °C	29.59 – 36.71 °C
Day	29.62 ± 1.48 °C	27.48 – 31.94 °C	34.18 ± 1.62 °C	32.09 – 36.71 °C
Night	30.69 ± 1.76 °C	28.14 – 33.13 °C	31.74 ± 1.96 °C	29.59 – 34.97 °C
DOE	27.85 ± 18.17 min	6 – 86 min	26.72 ± 20.07 min	6 – 81 min
ST	25.79 ± 1.18 °C	22.95 – 27.0 °C	26.41 ± 2.30 °C	22.73 – 30.58 °C
IBM	2.15 ± 0.72 g	1.19 – 3.82 g	7.27 ± 7.52 g	4.86 – 32.45 g
HRA	0.07 ± 0.07 °C/min	0.01 – 0.38 °C/min	0.12 ± 0.21 °C/min	0.06 – 0.84 °C/min

We compared six models for both species using the AIC selection criteria. For *P. nattereri*, the model including only period (day or night) was chosen as a better explanation of variation in the VT_{Max} (Table 2), with higher values attained during daytime. For *P. cuvieri*, we retained the simpler null model, which showed a higher wAICc, which indicates that no variable explains the variation of the VT_{Max} of this species (Table 3).

Table 2. Effect of period, start body temperature, duration, initial body mass, and heating rate on the Voluntary Thermal Maximum (VT_{Max}) of *P. nattereri* from Estação Ecológica de Santa Bárbara, state of São Paulo, Brazil.

Model	Variables	Value	Std.Error	t-value	AICc	wAICc	ΔAICc
VI	**Intercept**	34.245	0.7844	43.66	63.1	0.66	0.000
	Period	-2.3937	1.0072	-2.377			
I	**Intercept**	32.8771	0.5729	57.384	65.13	0.24	2.04
V	**Intercept**	33.48146	6.78518	4.934	67.13	0.09	4.03
	Period	-2.35356	1.09774	-2.144			
	Start body temperature	0.02784	0.24653	0.113			
IV	**Intercept**	33.402492	7.257777	4.602	72.18	0.01	9.08
	Period	-2.375403	1.192946	-1.991			
	Start body temperature	0.027744	0.258758	0.107			
	Duration	0.002234	0.029308	0.076			
III	**Intercept**	34.11138	7.23078	4.718	77.08	0	13.98
	Period	-2.9531	1.29369	-2.283			
	Start body temperature	-0.03477	0.2628	-0.132			
	Duration	0.01298	0.03072	0.422			
	Initial body mass	0.0873	0.08377	1.042			
II	**Intercept**	40.97635	8.8114	4.65	83.03	0	19.94
	Period	-4.69461	1.85994	-2.524			
	Start body temperature	-0.28142	0.31754	-0.886			
	Duration	0.04667	0.03908	1.194			

Initial body mass	0.13547	0.08914	1.52
Heating rate	-5.52697	4.19531	-1.317

Table 3. Effect of period, start body temperature, duration, initial body mass, and heating rate on the Voluntary Thermal Maximum (VT_{Max}) of *P. cuvieri* from Estação Ecológica de Santa Bárbara, state of São Paulo, Brazil.

Model	Variables	Value	Std.Error	t-value	AICc	wAICc	ΔAICc
I	**Intercept**	30.293	0.3788	79.98	81.52	0.48	0
VI	**Intercept**	29.69	0.5352	55.478	81.89	0.400	0.370
	Period	1.0964	0.7337	1.494			
V	**Intercept**	28.01163	8.54933	3.276	84.99	0.08	3.47
	Period	1.0601	0.7799	1.359			
	Start body temperature	0.06593	0.3355	0.196			
IV	**Intercept**	24.27443	8.89011	2.73	86.91	0.03	5.39
	Period	1.35975	0.81681	1.665			
	Start body temperature	0.15946	0.33822	0.471			
	Duration	0.02977	0.02327	1.279			
III	**Intercept**	24.16542	9.23459	2.617	91.07	0	9.55
	Period	1.4054	0.89665	1.567			
	Start body temperature	0.16829	0.35657	0.472			
	Duration	0.03163	0.02728	1.16			
	Initial body mass	-0.09116	0.62658	-0.145			
II	**Intercept**	24.89384	9.69648	2.567	95.73	0	14.21
	Period	1.38928	0.92409	1.503			
	Start body temperature	0.14817	0.37081	0.4			
	Duration	0.03157	0.02811	1.123			

Initial body mass	-0.05353	0.65185	-0.082
Heating rate	-2.10171	5.68863	-0.369

3.3.2. Distribution and habitat

Overall distribution of occurrences was similar for the two species, occupying mainly the central and southern portions of the Cerrado (Figure 1; S4 Table). Thus, the distribution of environmental temperatures was similar for both species. However, because the VT_{Max} was different between species, the resulting distribution of VT_{Max} - ET_{Max} values was markedly different (Figure 1A–B). The north central portion of the Cerrado showed much higher environmental temperatures than the VT_{Max} of *P. cuvieri* (Figure 1A), while this region is mostly below the VT_{Max} of *P. nattereri* (Figure 1B). Furthermore, VT_{Max} - ET_{Max} values were found to be significantly different between species ($U = 2249$, $p < 0.001$; Figure 1C). *Physalaemus nattereri* is mostly found (~ 80%) on localities that attain maximum temperatures equal to or lower than its VT_{Max}, whereas *P. cuvieri* seems to be mostly distributed (~ 60%) in localities with temperatures higher than its VT_{Max} (Figure 1C).

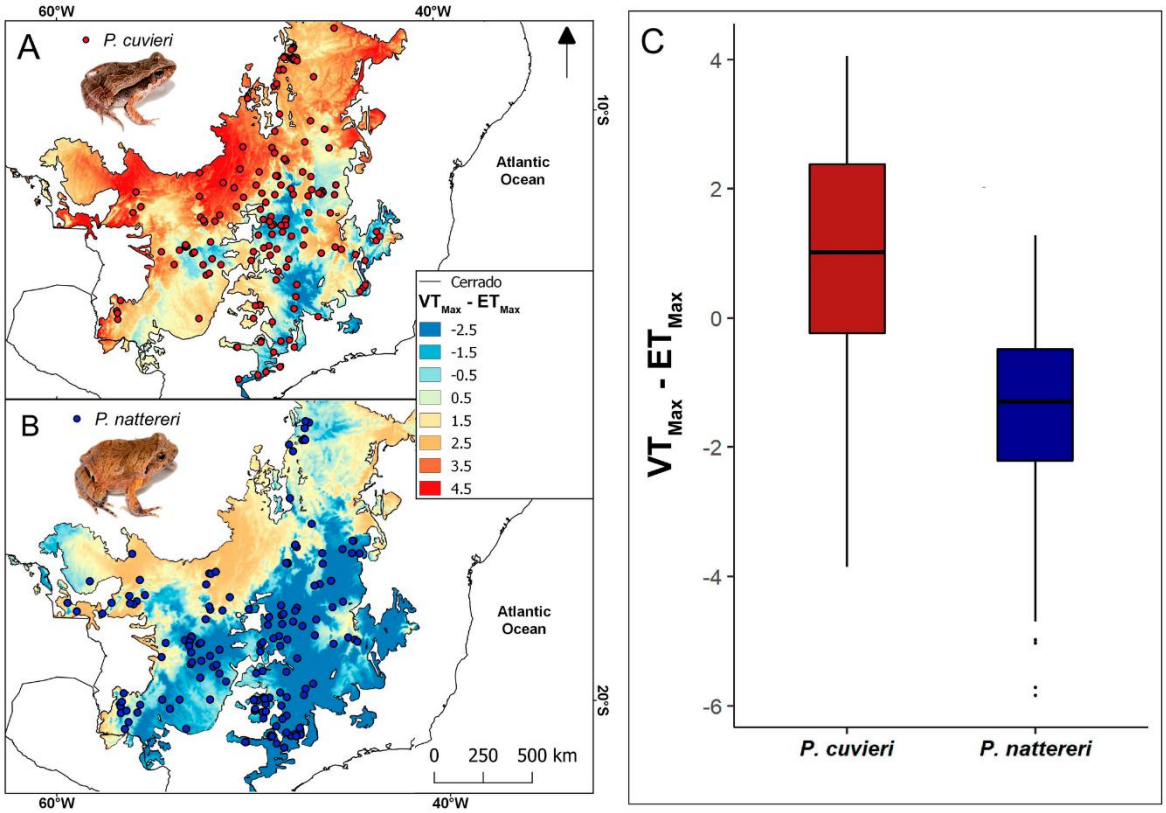

Figure 1. Geographical distribution of the studied species and VT_{Max} - ET_{Max} values throughout their distribution. (A) Distribution of *Physalaemus cuvieri*; (B) distribution of *Physalaemus nattereri*; and (C) comparison of VT_{Max} - ET_{Max} values at occurrence points between these species in the Cerrado.

We obtained abundance data for five additional localities in southern Cerrado, most of them from protected areas (Figure 2; see also S2 Figure). In only two localities (MOTTA, 1999; THOMÉ, 2006 + this study) we found significant differences between the proportion of each species in open and non-open habitats (S5 Table); in both cases, *P. cuvieri* was proportionally more abundant than *P. nattereri* in open areas. Considering the pooled abundances of these six studies, *P. cuvieri* was nearly twice more abundant in open (N = 2317 individuals) than in non-open areas (N = 1201), while *P. nattereri* was similarly abundant in open (N = 469) and non-open areas (N = 506; S5 Table). Furthermore, *P. cuvieri* was more abundant in open areas than in non-open areas in three localities and *P. nattereri*, in two localities, whereas both species were more abundant in non-open areas in two localities each (S5 Table).

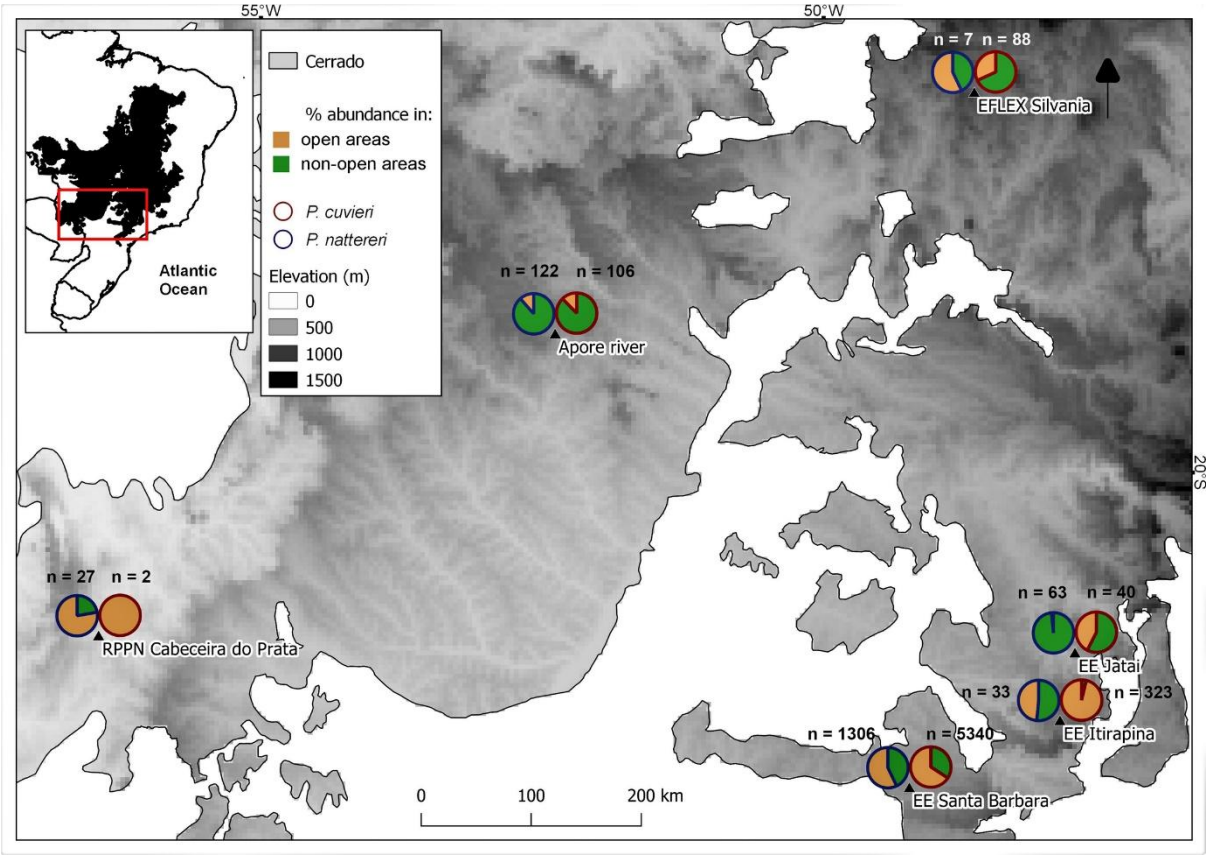

Figure 2. Relative abundance (in %) of *P. cuvieri* (blue circles) and *P. nattereri* (red circles) in open (brown) and non-open (green) areas in Cerrado (see S5 Table). The localities are: Floresta Nacional (FLONA) de Silvânia (GO), Reserva Particular do Patrimônio Natural (RPPN) Cabeceira do Prata (MS), Estação Ecológica (EE) Jataí (SP), Estação Ecológica de Itirapina (SP), Estação Ecológica de Santa Bárbara (SP), and Aporé River (GO and MS). Sources of data: (MOTTA, 1999; THOMÉ, 2006; OLIVEIRA, 2012; ARAUJO & ALMEIDA-SANTOS, 2013; DULEBA, 2013; RAMALHO et al., 2014). Detailed data on the abundance of the frogs in different vegetation types are in S5 Table.

3.4. Discussion

Our results show that the Voluntary Thermal Maximum (VT_{Max}) is higher for *P. nattereri* than for *P. cuvieri*, contrary to our first prediction that larger body size (and an expected slower cooling rate) would reflect in a lower VT_{Max}. Additionally, no difference in heating rate was found between species and only *P. nattereri* showed a significant difference on its VT_{Max} between day and night. Regarding habitat use, in general, we found the species with lower VT_{Max}, *P. cuvieri*, to be more abundant in open habitats than in non-open habitats, which does not support our prediction that the species with the lower thermal tolerance should be less abundant in habitats with higher environmental temperatures. Lastly, in spite of both

species being widespread in Cerrado, they showed different patterns of VT_{Max} - ET_{Max} values throughout their ranges, with only *P. nattereri* having most of its records in localities with temperatures below its VT_{Max}. Thus, only for *P. nattereri* did we confirm our prediction that regional distribution comprises mostly localities with environmental temperatures below the VT_{Max}.

Regarding the lower VT_{Max} values in the nocturnal period for *P. nattereri*, this result warrants future studies exploring variation in behavioral thermal tolerances in diurnal and nocturnal species in both periods of the day. Indeed, a higher VT_{Max} during the day could reflect physiological adjustment of its thermal safety margin (see Guevara-Molina et al., 2020), thus helping to protect the frog from extreme, potentially deleterious temperatures.

The difference in VT_{Max} values between these two frog species might be related to their different body sizes (TRACY, 1976; TRACY et al., 2010) but additionally might reflect their physiology and natural history. For instance, although there was no difference in heating rate between the species, *P. nattereri* might still cool slower when exposed to high temperatures because of its larger body size. As for differences in natural history, *P. nattereri* burrows in the soil (BRASILEIRO et al., 2005; GIARETTA & FACURE, 2006), which may allow it to quickly reduce its body temperature, since the soil is a good thermal insulant (PIANKA, 1986). On the other hand, *P. cuvieri* uses pre-existing cavities as diurnal refuge (e. g. see Bastos et al., 2003), which, in spite of also being below ground level (S2 Fig), are more exposed to variations in external environmental temperatures. Yet, despite having a lower VT_{Max}, most of the localities of *P. cuvieri* in Cerrado have temperatures above its VT_{Max}. This suggests that other aspects of its thermal ecology might be playing a role in avoiding thermal stress, such as a reduced daily activity time or physiological traits regulating hydration state.

As wet skin ectotherms, hydration level can also influence the temperatures tolerated and selected by individuals for thermoregulation in their habitats (ANGILLETA et al., 2003; NAVAS et al., 2008; ARTACHO et al., 2015; ANDERSON & ANDRADE, 2017). This has been observed for other frog species (e. g. *Lithobates catesbeianus*; Guevara-Molina et al., 2020), with individuals decreasing their VT_{Max} in response to dehydration, and some even losing their behavioral response to the VT_{Max}. Even though we controlled for hydration when measuring VT_{Max}, individuals in the wild rarely are at their optimal hydration level and thus desiccation might influence local frog distribution (WATLING & BRAGA, 2015). Desiccation has been shown to be correlated with substrate use (YOUNG et al., 2005) and with dispersal probability throughout the landscape (WATLING & BRAGA, 2015). Additionally, closely related frog species may vary in their response to desiccation along

thermal gradients, with some species showing greater resistance to water loss at lower temperatures, and others at higher temperatures (BEUCHAT et al., 1984). Therefore, knowing the interaction between VT_{Max} and hydration state of individuals in their environments can help to understand patterns and/or limits in their distribution (WATLING & BRAGA, 2015; BROWN et al., 2011; TINGLEY & SHINE, 2011; TITON & GOMES, 2017).

We found that *P. cuvieri*, the species with the lower VT_{Max}, was in general more abundant in open habitats, despite our second prediction that the species with the lower VT_{Max} should be less abundant in warmer habitats (up to 35–37 ºC in open habitats versus 32–35 ºC in non-open habitats in our study area; pers. obs.). On the other hand, *P. nattereri*, which showed a higher VT_{Max}, was in general similarly abundant in open and non-open habitats. These results may reflect clade-related physiological constraints and further studies on the relationship of VT_{Max} with habitat use should include additional species from both clades within the genus *Physalaemus* to which these species belong (Lourenço et al., 2015). Although competition could also lead to differences in habitat use, especially in closely related species, we found no evidence of competition between our focal species in cerrado habitats (e.g. extensive niche overlap associated with limited resources, negative correlations between abundances; MORIN, 2011).

Even though we found a relatively high variation in the data on habitat use for both species, the difference in the use of open and non-open habitats between species seems to be reflected in the overall patterns of their distribution throughout the Cerrado regarding their VT_{Max}. Indeed, *P. cuvieri* is in general more abundant in open and warmer habitats and occurs mostly in areas that attain maximum temperatures higher than its VT_{Max}, whereas *P. nattereri* tends to be abundant in both open and non-open (and cooler) areas and occurs mostly in areas that attain maximum temperatures below its VT_{Max}. Although geographic biases in sampling effort could affect these results, our study species usually are extremely abundant and conspicuous in localities where they occur, making them very easy to detect in inventories. Thus, we are confident that the records in the maps of figure 1 correspond to their actual distribution in the Cerrado. We highlight the importance of considering different spatial scales — geographic range and habitat use, as proposed by de CANDOLLE (1820) — because these allow to quantify how species distribution may reflect different aspects of their niches.

Despite numerous ecophysiological studies comparing how environmental temperatures influence habitat use of species (HILLMAN, 1969; BARNES et al., 2015), these rarely account for thermal tolerances. Using behavioral thermal tolerances, such as the VT_{Max},

allows for the integration of thermoregulatory behavior, which usually happens before critical limits are reached (WILLIAMS et al., 2008; SINCLAIR et al., 2016; CAMACHO et al., 2018). Furthermore, integrating the VT_{Max} with natural history and geographic distribution data can be critical to understand how future scenarios of global warming might impact distribution (CAREY & ALEXANDER, 2003; PARMESAN, 2007), especially for amphibians which are already under a global decline worldwide (ALFORD et al., 2001; BLAUSTEIN et al., 2010). Our study indicates that differences in behavioral thermal tolerance may be important in shaping local and regional distribution patterns. Furthermore, small-scale habitat use might reveal a link between behavioral thermal tolerance and natural history strategies. Further studies using additional sympatric species of the genus *Physalaemus* (e. g. *P. centralis*, from the same clade of *P. cuvieri*, and *P. marmoratus*, from the same clade of *P. nattereri*) could help to elucidate if those differences are due to body size variation or if tolerances are phylogenetically conserved. We hope this study stimulates future mechanistic studies on amphibian thermal ecology and on the impact of global warming on species distribution.

3.5. Additional publications about chapter 2

Video S1. Scientific dissemination video published on December 1, 2021 on the YouTube platform. The video shows the most relevant results of the investigation of this chapter. Additionally, the video was produced with the collaboration of the Papo de Sapo scientific dissemination team. Below is the video link: https://www.youtube.com/watch?v=fg_1jjUmEBc

Acknowledgements

We thank Instituto Florestal for allowing our work at Estação Ecológica de Santa Bárbara (permit #260108-008.476/2014; ICMBio-SISBIO for the permit to collect frog specimens (permit #50658-3). Paula Valdujo kindly provided occurrence data for both species. The comments and suggestions from an anonymous reviewer resulted in changes that enhanced the scientific quality of our manuscript. We thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for research grants (#2013/50741-7, #2015/21259-8, and #2018/14091-1). MM thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for a research fellowship (#306961/2015-6).

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Supporting Information

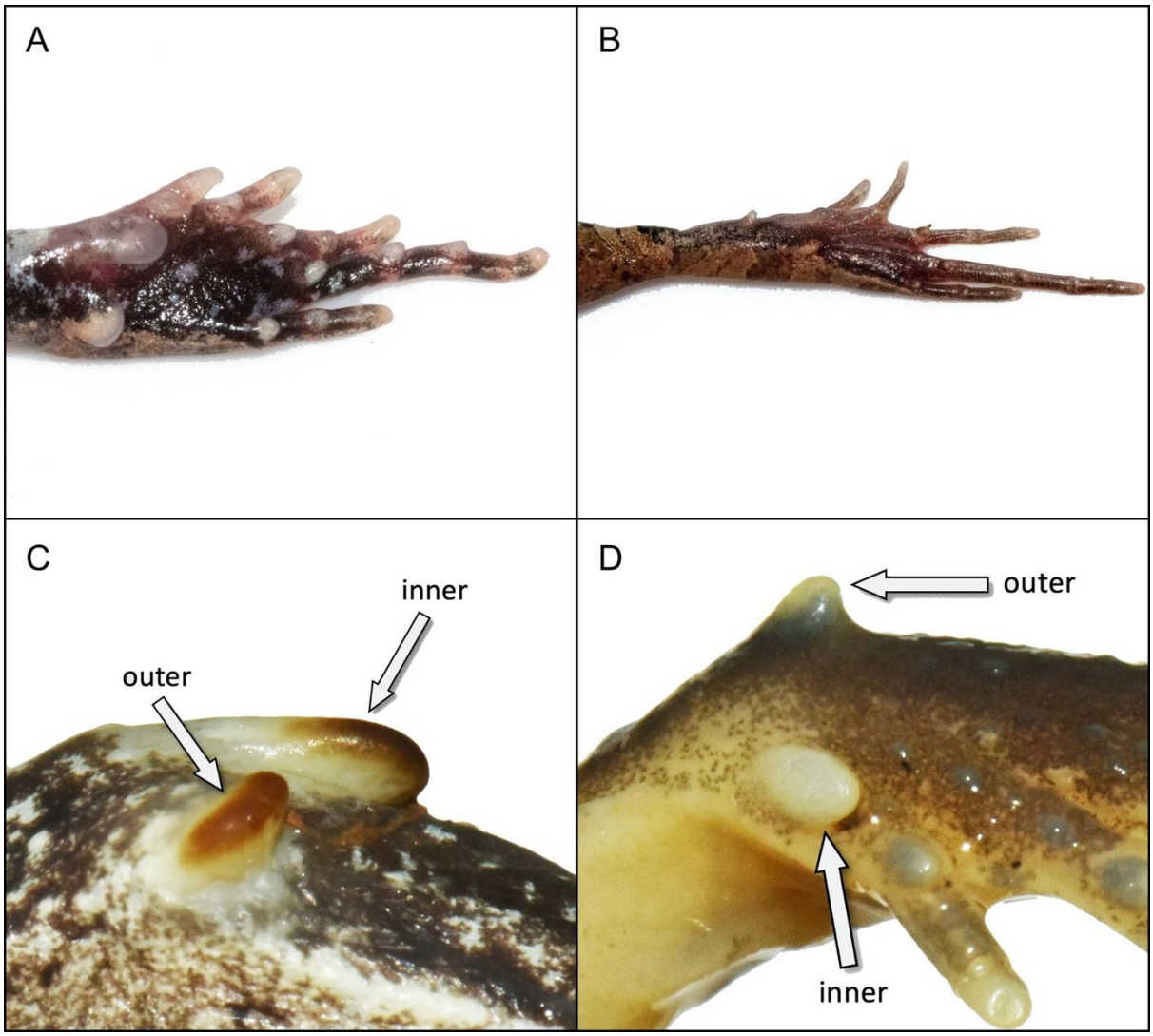


Figure S1. Detail of hind feet of *Physalaemus* species in the study. *P. nattereri* (A–B) and *P. cuvieri* (C–D), showing the inner and outer metatarsal tubercles in the detail. Note the much larger and strongly keratinized tubercles in *P. nattereri.* Photos not to scale.

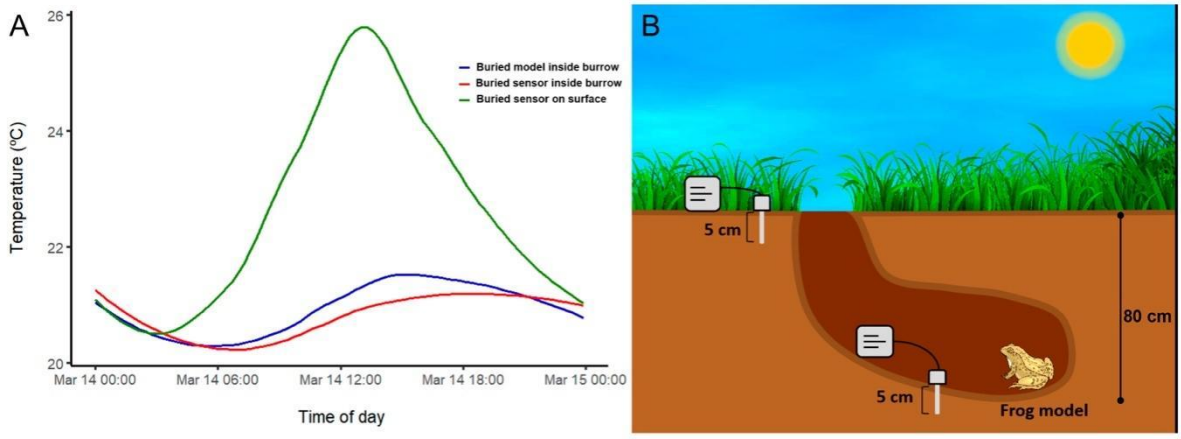


Figure S2. Temperature during a 24-hour cycle measured in the field. A) Temperature measured with sensors buried in the soil at superficial soil (green) and below ground level (red) and in a frog-sized plaster model (blue). B) Illustration of the measurement setup.

Table S1. Physiological data of species. Data on each individual tested for Voluntary Thermal Maximum (VT_{Max}) in this study.

Species	Individual	Period(Day/Night)	Time	Initial temperature	VTMax (ºC)	Time of experiments	Weight	Heating rate
Physalaemus nattereri	1	D	15:45	29.32	36.71	41	6.28	0.18
Physalaemus nattereri	2	D	16:29	30.58	34.74	44	6.4	0.095
Physalaemus nattereri	3	D	17:27	23.82	33.89	12	7.24	0.839
Physalaemus nattereri	4	D	17:49	30.33	33.03	6	10.65	0.45
Physalaemus nattereri	5	N	18:00	22.73	33.67	32	7.41	0.342
Physalaemus nattereri	6	D	11:32	24.96	32.09	48	7.51	0.149
Physalaemus nattereri	7	D	16:47	25.03	35.01	62	4.94	0.161
Physalaemus nattereri	8	N	18:09	26.53	33.07	81	5.18	0.081
Physalaemus nattereri	9	N	19:42	26.33	34.97	36	19.27	0.24
Physalaemus nattereri	10	N	20:27	27.32	32.45	41	32.45	0.125
Physalaemus nattereri	11	N	21:13	25.06	29.59	71	4.86	0.064

Physalaemus nattereri	12	N	22:29	27.65	30.38	44	5.94	0.062
Physalaemus nattereri	13	N	23:19	26.24	30.01	36	7.63	0.105
Physalaemus nattereri	14	N	00:03	26.52	30.67	34	7.9	0.122
Physalaemus cuvieri	12	D	08:08	23.58	28.04	30	1.29	0.15
Physalaemus cuvieri	13	D	09:10	26.39	31.94	29	2.27	0.19
Physalaemus cuvieri	14	D	09:46	25.95	31.28	56	2.66	0.1
Physalaemus cuvieri	15	D	10:57	24.27	30.07	32	2.01	0.18
Physalaemus cuvieri	16	D	11:34	23.99	28.63	56	2.1	0.08
Physalaemus cuvieri	17	D	12:32	26.48	30.12	47	1.53	0.08
Physalaemus cuvieri	18	D	13:28	25.23	29.17	86	1.99	0.05
Physalaemus cuvieri	19	D	15:00	27	27.48	45	3.82	0.01
Physalaemus cuvieri	20	D	15:50	26.02	30.48	37	2.23	0.12
Physalaemus cuvieri	1	N	18:26	22.95	32.07	54	3.29	0.17
Physalaemus cuvieri	2	N	20:30	25.78	30.98	49	3.39	0.11
Physalaemus cuvieri	3	N	21:27	26.96	31.58	46	3.39	0.1
Physalaemus cuvieri	4	N	22:19	26.98	28.14	6	1.19	0.19
Physalaemus cuvieri	5	N	22:35	25.38	28.35	24	2.43	0.12
Physalaemus cuvieri	6	N	23:10	26.28	31.26	39	2.53	0.13
Physalaemus cuvieri	7	N	23:58	26.12	33.05	55	2.41	0.13
Physalaemus cuvieri	8	N	00:58	26.02	28.9	17	1.9	0.17
Physalaemus cuvieri	9	N	01:21	26.85	31.4	31	2.09	0.15
Physalaemus cuvieri	10	N	01:57	26.68	29.79	24	3.19	0.13
Physalaemus cuvieri	11	N	02:25	26.32	33.13	18	1.78	0.38

Table S2. Temperature data of *P. cuvieri* during experiments. For more details see supplementary material in https://doi.org/10.1371/journal.pone.0239485.s005

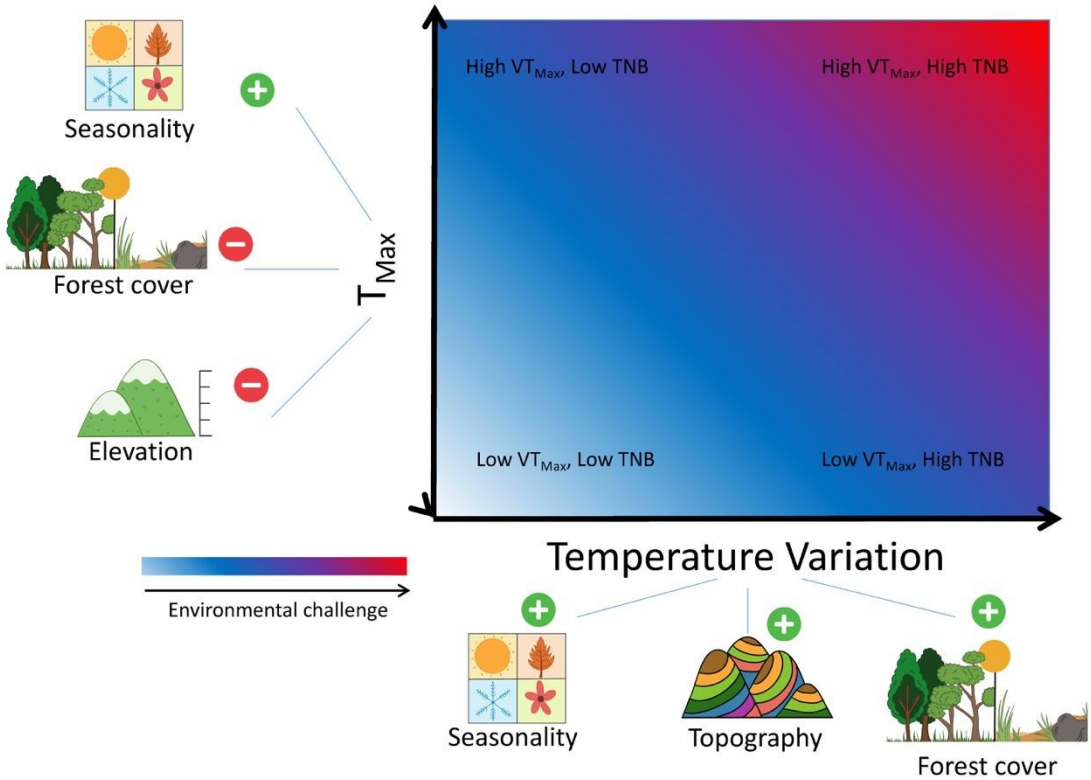
Table S3. Temperature data of *P. nattereri* during experiments. For more details see supplementary material in https://doi.org/10.1371/journal.pone.0239485.s006

Table S4. Geographical records of both species in the Cerrado ecoregion. Data from a distribution database built for another study (VALDUJO et al., 2012). For more details see supplementary material in https://doi.org/10.1371/journal.pone.0239485.s007

S5 Table. Habitat and abundance data for both species in six localities of the Cerrado ecoregion. (MOTTA, 1999; THOMÉ, 2006; OLIVEIRA, 2012; ARAUJO & ALMEIDA-SANTOS, 2013; DULEBA, 2013; RAMALHO et al., 2014).

7pen						**Forest**						**local**	**State**	**lat**	**long**	**Source**
CS		**CC**		**SS**		**CD**		**Mata Ciliar/Semi-decidua**								
P. cuvieri	***P. nattereri***	***P. cuvieri***	***P. nattereri***	***P. cuvieri***	***P. nattereri***	***P. cuvieri***	***P. nattereri***	***P. cuvieri***	***P. nattereri***	***N.cuv***	***N.nat***					
173	32	58	12	338	94	9	2			578	140	EE Santa Barbara	SP	-22.816389	-49.236667	This work
17	1	0	0	14	19	-	-	9	43	40	63	EE Jatai	SP	-21.6175	-47.765278	Oliveira 2012
130	3	181	13	8	16	4	1	-	-	323	33	EE Itirapina	SP	-22.219444	-47.898333	Thomé 2006
1255	130	460	239	493	50	173	164	-	-	2381	583	EE Santa Barbara	SP	-22.816389	-49.236667	This work
1428	162	518	251	831	144	182	166	-	-	2959	723	EE Santa Barbara	SP	-22.816389	-49.236667	This work
-	-	28	4	-	-	60	3	-	-	88	7	EFLEX Silvania	GO	-16.63	-48.66	Motta 2019
2	21	-	-	-	-	0	6	-	-	2	27	RPPN Cabeceira do Prata	MS	-21.4653	-56.442335	Duleba 2013
13	14	-	-	11	22	44	10	38	76	106	122	Rio Apore	GO/MS	-18.777891	-52.384812	Ramalho et al. 2014

4. CHAPTER 3. ON THE BRINK OF CHANGE? BEHAVIORAL THERMAL TOLERANCE AND THERMAL NICHE BREADTH OF SOUTH AMERICAN PITVIPERS



To Submit

DIAZ-RICAURTE JC, SERRANO FC, CAMACHO A, NOGUEIRA C, TRAVAGLIA-CARDOSO SR, MARTINS M. On the brink of change? Behavioral thermal tolerance and thermal niche breadth of South American pitvipers.

Capítulo 3: À beira da mudança? Tolerância térmica comportamental e amplitude de nicho térmico de jararacas Sul-Americanas

Resumo

A distribuição geográfica das espécies está intimamente relacionada à interação de fatores ambientais com a fisiologia e a ecologia dos organismos. Comparar as tolerâncias térmicas com as temperaturas ambientais é uma abordagem simples para entender as restrições térmicas na distribuição das espécies. Aqui exploramos a relação entre os valores do máximo térmico voluntário (VT_{Max}) e características ambientais (por exemplo, complexidade topográfica) em um grupo monofilético de serpentes sul-americanas (jararacas dos gêneros *Bothrops* e *Bothrocophias*). Nossa principal questão é: quão bem as características ambientais predizem o VT_{Max} em jararacas da América do Sul? Devido a um potencial trade-off entre tolerâncias térmicas e plasticidade fisiológica, esperamos VT_{Max} mais baixo para espécies com exposição menos constante a altas temperaturas e maior amplitude de nicho térmico (TNB). Medimos experimentalmente a VT_{Max} de 15 espécies e coletamos dados sobre suas faixas geográficas e características ambientais associadas, como temperatura, precipitação e complexidade topográfica (p. ex., rugosidade e elevação do terreno). Por fim, exploramos a evolução das temperaturas máximas (T_{Max}) e mínimas (T_{Min}) ambientais e da VT_{Max} fazendo uma reconstrução ancestral e testando o sinal filogenético dessas variáveis, bem como realizando regressões PGLS entre a VT_{Max} e cada característica ambiental. Descobrimos que os valores médios de VT_{Max}, bem como o intervalo de temperaturas ambientais, foram semelhantes para quase todas as espécies. A análise de reconstrução de estados ancestrais mostrou diferenças na evolução dos limites térmicos geográficos do TNB e do VT_{Max} dos clados. Descobrimos que o sinal filogenético é significativo apenas para T_{Max}. Além disso, descobrimos que espécies com maior amplitude latitudinal tendem a ter um VT_{Max} menor. Nossos resultados sugerem que as características ambientais remanescentes não são boas preditores da VT_{Max} em jararacas sul-americanas, e que fatores comportamentais e de microhabitat complexos não considerados aqui podem modular diferenças entre as espécies.

Palavras-chave: Fatores ambientais, estudos comparativos evolutivos, ectotermos Neotropicais, ambientes térmicos, máximo térmico voluntário.

Chapter 3: On the brink of change? Behavioral thermal tolerance and thermal niche breadth of South American pitvipers

Abstract

The geographical distribution of species is closely related to the interplay of environmental factors with organismal physiology and ecology. Comparing thermal tolerances with environmental temperatures is a simple approach to understand thermal constraints on species distributions. Herein we explore the relationship between the voluntary thermal maximum (VT_{Max}) values and environmental features (e. g., topographic complexity, precipitation) in a monophyletic group of South American snakes (the pitvipers of the genera *Bothrops* and *Bothrocophias*). Our main question is: How well do environmental features predict VT_{Max} in South American pitvipers? Due to a potential trade-off between thermal tolerances and physiological plasticity, we expect lower VT_{Max} for species with less constant exposure to high temperatures and wider Thermal Niche Breadth (TNB). We experimentally measured VT_{Max} of 15 species and collected data on their geographic ranges and associated environmental features such as temperature, precipitation and topographical complexity (e.g., terrain ruggedness and elevation). Finally, we explored the evolution of environmental maximum (T_{Max}) and minimum (T_{Min}) temperatures and of VT_{Max} by making an ancestral reconstruction and testing for phylogenetic signal of these variables, as well as performing pairwise PGLS regressions between VT_{Max} and each environmental feature. We found that mean VT_{Max} values as well the range of environmental temperatures were similar for almost all species. The ancestral state reconstruction analysis showed differences in the evolution of geographic thermal limits of the TNB and the VT_{Max} of clades. We found that phylogenetic signal is only significant for T_{Max}. Additionally, we found that species with a greater latitudinal range tend to have a lower $VT_{Max.}$ Our results suggest that the remaining environmental features are not good predictors of the VT_{Max} in South American pitvipers, and that complex behavioral and microhabitat factors not considered herein may modulate differences among species.

Keywords: Environmental factors, evolutionary comparative studies, Neotropical ectotherms, thermal environments, voluntary thermal maximum.

4.1. Introduction

For decades, studies in ecology and biogeography have widely accepted that the geographical distribution of species is closely related to the interplay of environmental factors (e. g., temperature and precipitation) with their physiology and ecology (e. g., KEARNEY, 2006; YACKULIC et al., 2013; WEBER et al., 2017; DIAZ-RICAURTE et al., 2020). These studies have focused on understanding the effects of thermal constraints on the behavior and distribution patterns of species (e. g., BESTELMEYER, 1997; WITTMAN et al., 2010; SUNDAY et al., 2014), although few tested the relationship between environmental factors and the combination of behavioral and physiological limits.

The set of climatic attributes of the geographic distribution of a species is known as its climatic envelope (e. g., IBAÑEZ et al., 2006; JIGUET et al., 2007; GREEN et al., 2008). These might arise from the influence of such environmental factors, limiting both altitudinal and latitudinal ranges of these species (e. g., JIGUET et al., 2007). Furthermore, understanding these effects also require the understanding of how the evolutionary history of clades shaped current distribution patterns (WIENS, 2011; HUEY et al., 2012; MACLEAN & BEISSINGER, 2017; SCHUETZ et al., 2018). Still, understanding large-scale biogeographical patterns, such as how lineages are distributed across thermal gradients, remains a major challenge for ecology, biogeography and evolutionary biology (SCHLUTER, 2016). Some studies have identified the processes responsible for differences in species diversity among geographical regions or distinct clades (RABOSKY et al., 2007; LI et al., 2018), and several studies have shown that species diversity can be influenced by both extrinsic (e. g., environmental stability, climate) and intrinsic factors (e. g., dispersal ability; BARNOSKY, 2001; BENTON, 2014). These factors are strongly related to diversification processes and diverse types of events (e. g., speciation or/and extinction; EZARD et al., 2011). Indeed, many historical and ecological factors have been proposed to explain the relationship between latitudinal gradients and environmental factors (e. g., MITTELBACH et al., 2007). For example, some studies suggested that lineages at higher latitudes should experience relatively lower rates of diversification, resulting in overall low species richness compared to the tropics (WEIR & SHULUTER, 2007; BOTERO et al., 2014; CUTTER & GRAY, 2016). These different processes can be more adequately distinguished using macroevolutionary approaches (e. g., LI et al., 2018).

The similarity of niches between closely-related clades might reflect phylogenetic niche conservatism (LOSOS & GLOR, 2003; FITZPATRICK & TURELLI, 2006) because lineages may maintain ancestral ecological characteristics through time (HOLT & GAINES,

1992). Conversely, niche divergence along evolutionary time scales is also able to act both on speciation, generating patterns of diversification, and on species distribution (EVANS et al., 2009; DURAN & PIE, 2015; PYRON et al., 2015; SEXTON et al., 2017). For instance, climatic conditions can affect the phylogenetic structure of species assemblages (CAVALHERI et al., 2015; BURBRINK et al., 2015), leading to higher diversification rates under a higher climatic diversity at lower latitudes and to convergence under a narrower set of conditions at higher latitudes (CADLE & GREENE, 1993; MORINIÈRE et al., 2016). Furthermore, phylogenetic niche conservatism may favor decline in species richness of clades when environments change and important traits to survive in such environments are strongly conserved in the group (e. g., thermal tolerance and TNB; SINERVO et al., 2010; PYRON & BURBRINK, 2011). Species unable to adapt to changing climatic conditions might go locally extinct or the distribution of their populations might gradually change to track suitable environmental conditions (WIENS et al., 2010). Indeed, the physiological performance of individuals can be negatively affected by high environmental temperatures (CURRIE & FRITZ, 1993), which can lead to declining populations and/or local extinctions at the limits of a species' distribution (e. g., KINGSOLVER et al., 2013; GAMLIEL et al., 2020). Thus, climatic niche throughout evolutionary history of a clade can affect species distribution patterns while having important implications for their thermal ecology and conservation. This is corroborated by many taxa that have suffered changes in their distribution due to recent climate change (PARMESAN, 2006; LENOIR et al., 2010; POLOCZANSKA et al., 2013).

Ectothermic organisms use physiological and behavioral mechanisms for thermoregulation (e. g., HUEY & SLATKIN, 1976; LELIÈVRE et al., 2011; MITCHELL & BERGMANN, 2016). When unable to tolerate the increase of environmental temperatures, they can change their behavior to avoid overheating by adjusting the body posture or moving to another place to reduce its body temperature (e. g., SUNDAY et al., 2011; SEEBACHER et al., 2015; DIAZ-RICAURTE et al., 2020, DIAZ-RICAURTE et al., 2022a, b). Thus, the distribution of ectotherms can be shaped by behavioral and physiological thermal tolerances, so identifying thermal tolerance thresholds (i. e., measurable thermal limits) allows for the identification of challenges that directly affect their behavioral and physiological response in face of recent climate change. Among these behavioral thresholds, the Voluntary Thermal Maximum (VT_{Max}) can be experimentally measured as the maximum temperature that an individual will endure before moving to avoid further heating (CAMACHO et al., 2018). Unable to do so, an individual that exceeds its maximum voluntary thermal tolerance might reach critical temperatures, which generally leads to loss of locomotor performance and/or

death (COWLES & BOGERT, 1944; REZENDE et al., 2014). The VT_{Max} can be informative to identify thermal constraints on ecological aspects (e. g., habitat use, life history; DIAZ-RICAURTE et al., 2020) and geographical distribution of species (e. g. RECODER et al., 2018, DIAZ-RICAURTE et al., 2020). The utility of VT_{Max} has recently been demonstrated in studies that aim to understand biogeographic and ecological aspects, as well as the configuration of local and regional distribution patterns of ectothermic animals (CAMACHO et al., 2018; DIAZ-RICAURTE et al., 2020).

South American pitvipers of the genera *Bothrops* and *Bothrocophias* comprise a monophyletic and diverse snake group (CARRASCO et al., 2012; ALENCAR et al., 2016), which are among the best-studied Neotropical snake clades. This clade is widely distributed in the Neotropical region, occupying a wide range of environments, from the tropical lowland forests of Central America and Amazonia to the open colder areas in the Andes and Patagonia (CAMPBELL & LAMAR, 2004; NOGUEIRA et al., 2019). Their taxonomy, systematics, and natural history are well documented (HOGE, 1981; HOGE & ROMANO, 1978; MARTINS et al., 2001; MARTINS et al., 2002; MARQUES et al., 2002; CAMPBELL & LAMAR, 2004; HARVEY et al., 2005; CISNEROS - HEREDIA et al., 2006; CARRASCO et al., 2009, 2010; ALENCAR et al., 2016; ESPÍNDOLA AYALA, 2018), as well as ecophysiological aspects associated with voluntary thermal tolerances (DIAZ-RICAURTE & SERRANO, 2021). Thus, this clade is an interesting model for studies on the relationship of environmental features, environmental thermal limits, behavioral thermal tolerances and Thermal Niche Breadth (TNB). The Thermal Niche Breadth is a widely used metric that represents the difference between maximum and minimum environmental temperature across a species range (e. g. JANZEN 1967; ADDO - BEDIAKO et al., 2000; GASTON et al., 2009; SUNDAY et al., 2011, 2014; LANCASTER et al., 2015). Thereby, the Thermal Niche Breadth (TNB) may help to explain how the distribution of species (especially ectotherms) might reflect their diversification patterns, as well as how their geographical limits are related to thermal tolerances.

Considering that thermal constraints determine the distribution of species, here we explore the relationship between the VT_{Max} and environmental features (e. g., topographic complexity, precipitation, and maximum and minimum environmental temperatures) in South American pitvipers of the genera *Bothrops* and *Bothrocophias*. Our main question is: Can environmental features predict VT_{Max} in South American pitvipers? Due to a potential trade-off between thermal tolerances and physiological plasticity, species that experience more

constant high temperatures will tend to show higher VT_{Max} in order to maintain their biological processes (e. g., feeding and reproduction) (SOMERO, 2010; VAN HEERWAARDEN & KELLERMANN, 2020). On the other hand, species that occur in more seasonal or variable environments are less frequently subjected to high temperatures and thus likely have not been selected for high VT_{Max}. Likewise, lineages that evolved across colder regions and have maintained lower thermal tolerances (BENNETT et al., 2021) could also show lower VT_{Max}. Thus, we expect lower VT_{Max} for species with higher TNB such as those that occur in regions with higher topographic complexity, higher climatic seasonality and lower total rainfall. On the other hand, a higher VT_{Max} would be expected in species from regions with low climatic variability and higher maximum temperatures, as well as areas of low elevation in more tropical zones. We also use a historical approach to explore how environmental thermal limits and VT_{Max} evolved in South American pitvipers. We thus test for phylogenetic niche conservatism and how this may have shaped behavioral thermal tolerances (i. e., VT_{Max}) in the group. Given the large latitudinal range of the group's distribution, involving great heterogeneity of relief and climate, and the presence in different habitats in the Neotropical region, we expect intra-clade (species level) variation of environmental temperatures and, consequently, of behavioral thermal limits, and thus weak phylogenetic niche conservatism.

4.2. Materials and Methods

4.2.1. VT_{Max} Measurements and data collected

For the VT_{Max} measurements, we used an experimental setup already used in snakes in recent studies (DIAZ-RICAURTE & SERRANO, 2021; DIAZ-RICAURTE et al., 2022a). The experimental setup consisted of a transparent plastic box connected to a metal box wrapped with a thermal resistance sheet for heating (the “heating box”). During each experiment, we recorded the body temperature every 10 seconds with the help of a thermocouple that remained in the animal's cloaca throughout the experiment. Before each experiment, each individual was allowed to voluntarily enter the heating box and after a period of five minutes at room temperature, the metal box was gradually heated with the snake inside. The heating of the box occurred slowly to ensure that its temperature did not exceed the individual's body temperature by more than 5–6 °C, to avoid the animal to prematurely exit the box. Additionally, we recorded the box temperature with a different thermocouple attached to the heater sheet surface. The final temperature recorded at the

moment the individual left the experimental box was considered as its VT_{Max} with its heating rate (HR) being calculated as the difference between the initial and final body temperature (i.e., VT_{Max}) divided by the duration of the respective experiment. This study was conducted under a permit by the ethics committee of Instituto Butantan (CEUA permit #2325141019); a permit by Instituto Florestal for fieldwork at Estação Ecológica de Santa Bárbara (permit #260108 - 008.476/2014), and a permit by ICMBio - SISBIO (permit #50658 - 3) to collect snake specimens.

4.2.2. Geographical range data and environmental features

We gathered distribution data in the literature (e. g., QUIÑONES-BETANCOURT et al., 2018; BIRSKIS-BARROS et al., 2019, NOGUEIRA et al., 2019) and mapped species distributions using the occurrence records. To improve the mapping of their range, all species were modeled using alpha hulls, which take into account maximum latitudinal and longitudinal limits, avoiding overestimation and best representing the distribution range of species. Alpha hulls were created with: alpha = sqrt ((longitudinal range + latitudinal range) / 4 + 0.5 degree); cf. ROLL et al., 2017). We gathered a total of 31,763 occurrence records and produced geographical range maps for 15 continental species of South American pitvipers, including the phylogenetically-related South American rattlesnake, *Crotalus durissus*.

To explore the effects of different environmental attributes of South American pitvipers on their behavioral thermal limits (i. e., VT_{Max}), and to characterize their macro-ecological niche, we used topographic (elevation; ~1 km × 1 km resolution; USGS 2019; https://www.usgs.gov/centers/eros/science/) and climatic variables (temperature and precipitation; WorldClim, see FICK & HIJMANS, 2017 at 30 seconds resolution; https://www.worldclim.org). For the species *B. insularis*, environmental temperatures were taken from BOVO et al. (2010) since data was not available for the Queimada Grande island, to which it is endemic. We extracted the maximum (Lat_{Max}) and minimum (Lat_{Min}) latitudes of occurrence, latitudinal range ($Lat_{Ran;}$ Lat_{Max}- Lat_{Min}), and latitude of the centroid (Lat_{Cen}), as well as the maximum (Alt_{Max}) and minimum (Alt_{Min}) elevation, and altitudinal range ($Alt_{Ran;}$ Alt_{Max} - Alt_{Min}) of each species (the 95th percentile of each variable; see Supplementary material). From the elevation raster, we also extracted the maximum (Rug_{Max}), and minimum (Rug_{Min}) ruggedness (i. e., topographic complexity), which is measured in meters of elevation difference for grid points 30 arc-seconds. We also obtained the maximum (T_{Max}; BIO5 in WorldClim) and minimum temperatures of coldest month (T_{Min}; BIO6 in WorldClim) across each species range. From the difference of T_{Max} and T_{Min}, we obtained the Thermal Niche

Breadth (TNB). For precipitation we considered the maximum ($Prec_{Max}$), minimum ($Prec_{Min}$), and precipitation breadth ($Prec_{Bre;}$ $Prec_{Max}$ - $Prec_{Min}$), as well the maximum ($PSeas_{Max}$), minimum ($PSeas_{Min}$), and precipitation seasonality breadth ($PSeas_{Bre;}$ $PSeas_{Max}$ - $PSeas_{Min}$) values. To extract environmental and geographical data, we used the 'Point sampling tool' plugin in QGIS version 3.6 (QGIS, 2019).

4.2.3. Phylogenetic comparative analyses

To explore the evolution of environmental thermal limits in South American pitvipers, we reconstructed ancestral states for T_{Max}, T_{Min}, TNB, and VT_{Max} using an available phylogeny (ALENCAR et al., 2016). The manipulation of trees was performed using the R-packages 'ape' (PARADIS et al., 2004; PARADIS & SCHLIEP, 2018), 'geiger' (HARMON et al., 2007), and 'phytools' (REVELL, 2012) in R (available at http://cran.r-project.org). Also, we calculated the means of the geographic thermal limits (T_{Max}, T_{Min}) and TNB for each species, from the previously used distribution data, and reconstructed these variables in the phylogeny. We also tested for phylogenetic signal for each of these variables using the Blomberg's K metric (BLOMBERG et al., 2003), as implemented in the *phylosig* function of the *phytools* R-package (REVELL, 2012) with 999 simulations. Blomberg's K compares variance of a trait with that resulting from a Brownian Motion model ($K = 1$), with values of $K < 1$ representing less phylogenetic signal than expected (larger intra-clade variance) and values of $K > 1$ representing more phylogenetic signal than expected (larger inter-clade variance).

Furthermore, to test the effects of each environmental feature (predictors) on VT_{Max} (responses), we performed pairwise PGLS regressions between VT_{Max} and each environmental feature (i. e., geographic and climatic range variables). We used 100 trees sampled from the phylogeny to account for phylogenetic uncertainty and obtain the means and confidence intervals for all statistical descriptors and *p*-values.

4.3. Results

4.3.1. VT_{Max} measurements

Overall, the mean VT_{Max} values were similar among South American pitvipers (n = 83 individuals tested, from 15 species and eight clades; Figure 1, Table 1), mostly in the 34 – 36 ºC range. However, several pairs of species were noticeably different, often even within the same clade (e. g., *B. insularis* and *B. jararaca* in clade F and *B. moojeni* and *B. atrox* in clade D). *Bothrops jararacussu* and *B. moojeni* were the species with the highest mean values (39.5 ºC and 37.72 ºC, respectively), while *B. jararaca* and *B. neuwiedi* had the lowest mean values (34.20 ºC and 34.78 ºC, respectively). Furthermore, the VT_{Max} values of South American pitvipers were not very different from those shown by *C. durissus*, the species used as external group. There were also differences in the range and variance of VT_{Max}, as evident for species in clade G, in which the species *B. erythromelas* varies markedly less than *B. neuwiedi* and *B. pauloensis*. As with the experimental parameters of VT_{Max}, the heating rate values varied between 0.10–0.48 ºC/min (Table 1) with species with the highest heating rate (*B. insularis* and *B. pauloensis*) also showing differences in its variance. The detailed experimental values of each individual can be seen in Table S1. The linear mixed-effect models showed that none of the selected variables significantly explained variation of VT_{Max} across South American pitvipers (Table 2, Table S2). However, the other models that were not selected reflect interactive effects of HR, sex, IBT, SVL and WT (see Table S2), which have been observed in other studies (see discussion below).

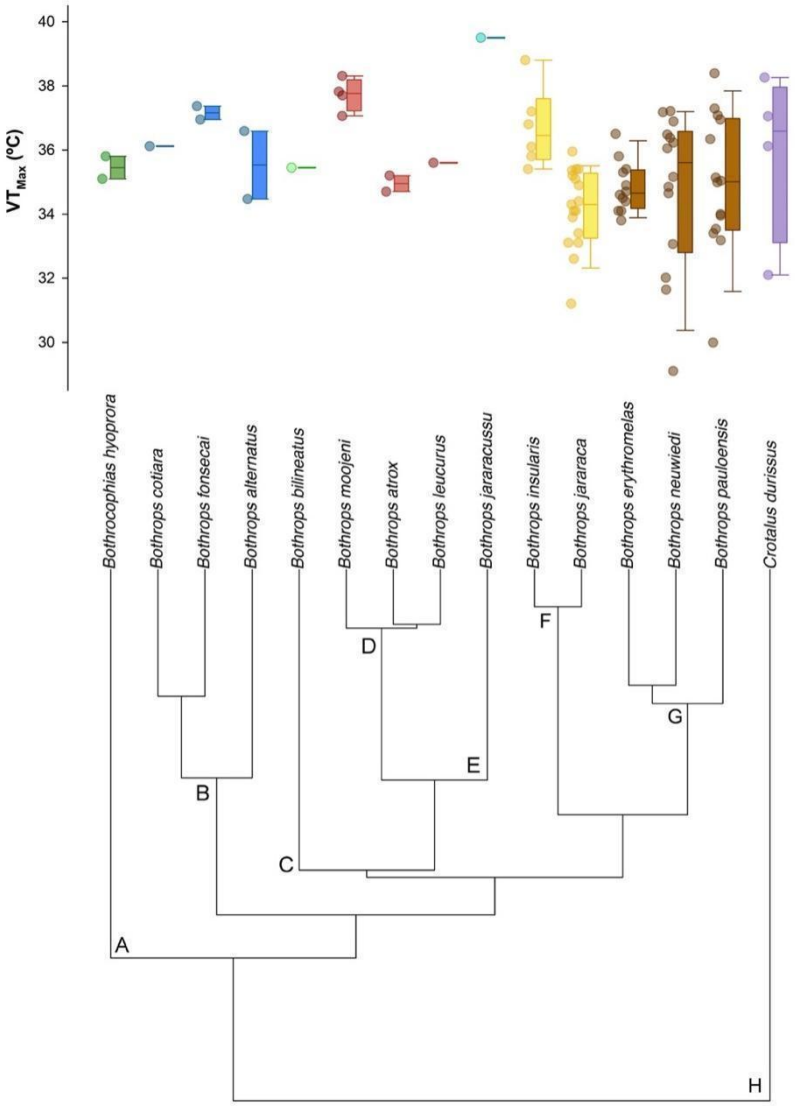


Figure 1. Comparison of Voluntary Thermal Maximum (VT_{Max}) of South American pitvipers. Species are grouped in clades as: A) *Bothrocophias* clade*;* B) *Bothrops alternatus* clade*;* C) *Bothrops taeniatus* clade*;* D) *Bothrops atrox* clade*;* E) *Bothrops jararacussu* clade*;* F) *Bothrops jararaca* clade*;* G) *Bothrops neuwiedi* clade*;* and H) *Crotalus durissus* species as outgroup. Colors and capital letters represent clades within the South American pitvipers.

Table 1. VT_{Max} values of South American pitvipers.

Species	N	Clade	Mean VT_{Max} ± SD	Range	Mean Heating Rate (ºC/min) ± SD	Source
Bothrocophias hyoprora	2	A	35.45 ± 0.49	35.10–35.80	0.16 ± 0.00	This study
Bothrops alternatus	2	B	35.53 ± 1.50	34.47–36.59	0.24 ± 0.07	This study
Bothrops cotiara	1	B	36.12	-	-	This study
Bothrops fonsecai	2	B	37.16 ± 0.30	36.95–37.37	0.13 ± 0.03	This study
Bothrops bilineatus	1	C	35.45	-	-	This study
Bothrops moojeni	4	D	37.72 ± 0.51	37.06–38.31	0.21 ± 0.08	This study
Bothrops atrox	2	D	34.95 ± 0.35	34.70–35.20	0.23 ± 0.00	This study
Bothrops leucurus	1	D	35.6	-	-	This study
Bothrops jararacussu	1	E	39.5	-	-	This study
Bothrops jararaca	17	F	34.20 ± 1.23	31.20–35.95	0.25 ± 0.17	This study
Bothrops insularis	6	F	36.68 ± 1.23	35.40–38.80	0.48 ± 0.16	This study
Bothrops neuwiedi	14	G	34.78 ± 2.45	29.10–37.21	0.23 ± 0.09	This study
Bothrops pauloensis	14	G	34.94 ± 2.18	29.99–38.39	0.27 ± 0.13	Diaz-Ricaurte and Serrano 2021; This study
Bothrops erythromelas	12	G	34.84 ± 0.78	33.80–36.50	0.28 ± 0.10	This study
Crotalus durissus	4	H	35.88 ± 2.66	32.10–38.26	0.15 ± 0.14	Diaz-Ricaurte et al. 2022a

Table 2. Summary of the best explanatory models showing the possible effects of HR and SVL and their interactions on the VT_{Max} of South American pitvipers. The results of the other models can be seen in Table S2 in Supplementary material.

Model	Variable	Estimate	Std. Error	df	t value	p-value	AIC-value	AICwt
Null	(Intercept)	35.599	0.320	4	111.300	**0.000**	343.660	0.44
IV	(Intercept)	34.890	0.870	7	40.111	0.000	342.605	0.55
	HR	0.454	2.912		0.156	0.876		
	SVL	0.000	0.002		0.132	0.895		
	HR:SVL	0.005	0.007		0.751	0.455		

4.3.2. Geographical ranges and environmental features

The South American pitvipers studied here occur from 38.6º S in southern South America (*B. alternatus*) to 10.7º N (*B. atrox*; Table S3, Figure 2, Figure 4); three fourths of the species show a minimum altitudinal limit below 100 m above sea level and the maximum upper altitudinal limit is 934 m above sea level for *B. fonsecai* (Figure 2, Table S3). Among South American pitvipers, the mean T_{Max} varied from 26.60 ºC in *B. cotiara* to 33.17 ºC in *B. erythromelas*, whereas mean T_{Min} varied from 5.50 ºC in *B. cotiara* to 19.53 ºC in *B. atrox* (Figure 2, Table S3–S4).

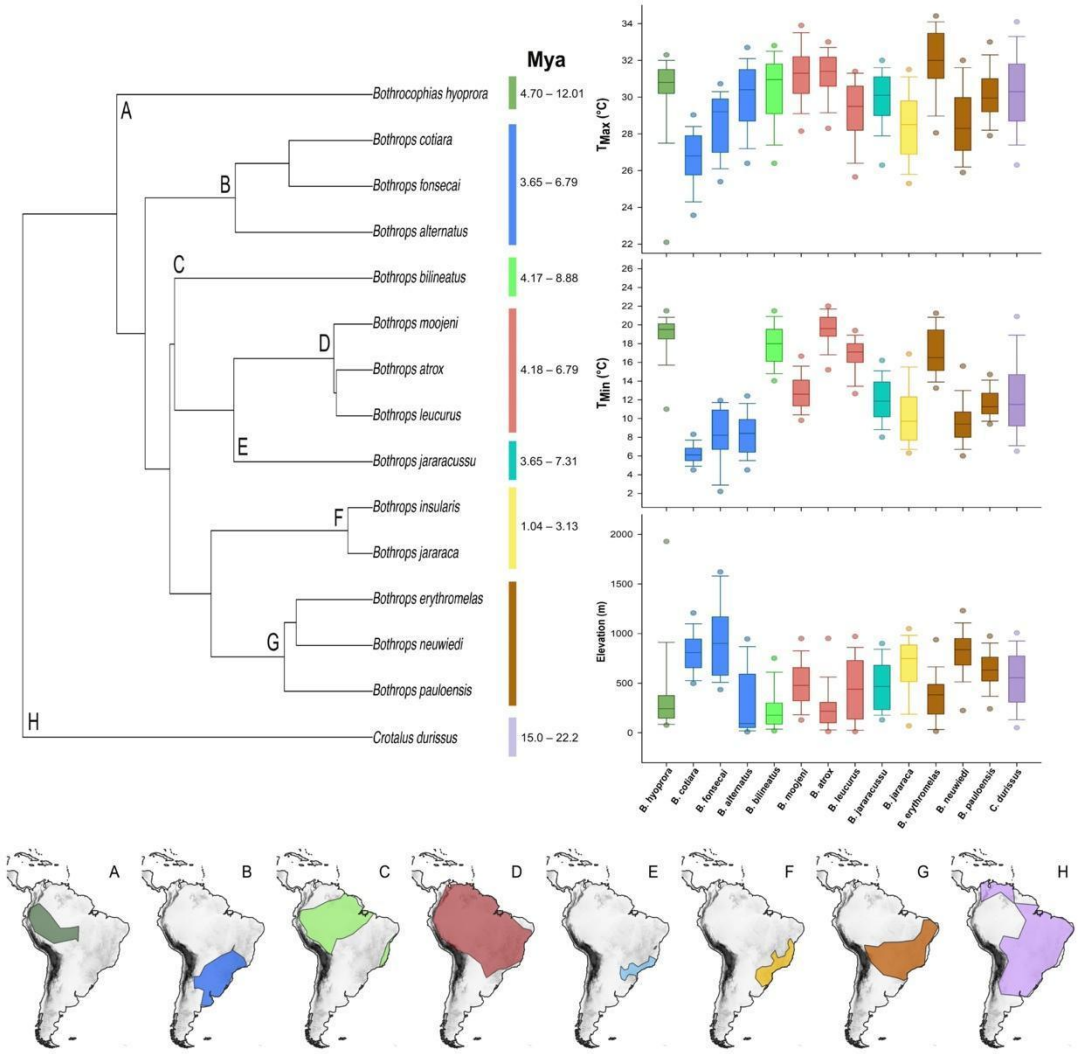


Figure 2. Phylogenetic relationships (ALENCAR et al. 2016) and geographic range of South American pitvipers. High posterior density interval of age estimates was taken from ALENCAR et al. (2016). The boxplots correspond to niche envelope of the South American pitvipers. The whiskers correspond to 5th and 95th percentiles and the dots the maximum and minimum values for the variable. Colors represent clades within the South American pitvipers.

The comparison of the clades within the South American pitvipers showed relatively similar altitudinal and T_{Max} ranges, and those clades with more tropical distributions (*Bothrocophias* group, *B. taeniatus* group and *B. atrox* group; Figure S1) showed higher T_{Min}. Furthermore, the lineage to which the species belongs explained the variation in T_{Min} (T_{Min}, F

= 6.96; df = 7; $p < 0.01$). In contrast, T_{Max} and TNB were not explained by lineage (T_{Max}, F = 2.15; df = 7; p = 0.15 and TNB, F = 2.14; df = 7; p = 0.18).

Nearly all of the VT_{Max} values measured for the studied viper species were lower than the 95th percentile of maximal air temperatures (bio5) measured in the shade across their geographic distribution (Figure 3, Table S5).

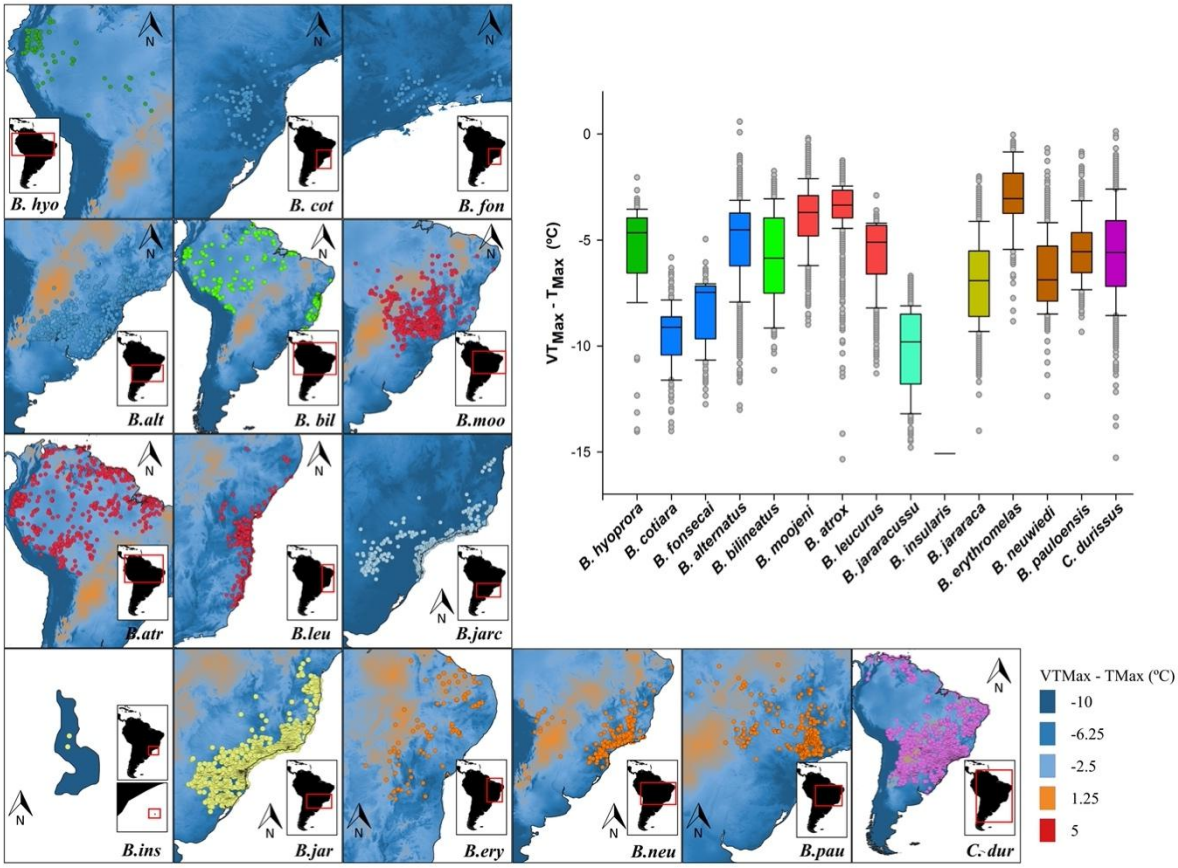


Figure 3. Geographical distribution and $VT_{Max} - T_{Max}$ values throughout the distribution of South American pivipers. $VT_{Max} - T_{Max}$ represents the difference between VT_{Max} and maximum daily temperature (BIO5, WorldClim). Species maps are presented as follows: *B. hyo = Bothrocophias hyoprora; B. cot = Bothrops cotiara; B. fon = Bothrops fonsecai; B. alt = Bothrops alternatus; B. bil = Bothrops bilineatus; B. moo = Bothrops moojeni; B. atr = Bothrops atrox; B. leu = Bothrops leucurus; B. jarc = Bothrops jararacussu; B. ins = Bothrops insularis; B. jar = Bothrops jararaca; B. ery = Bothrops erythromelas; B. neu = Bothrops neuwiedi; B. pau = Bothrops pauloensis; C. dur = Crotalus durissus.* Colors of dots in the maps and boxplots represent clades within the South American pitvipers (See Figure 1).

4.3.3. Phylogenetic comparative analyses

Ancestral state reconstruction of environmental thermal limits (T_{Max}, T_{Min}, TNB and VT_{Max}) for South American pitvipers showed that some geographic thermal limits differ between clades (Figure 4). For instance, some basal clades (e. g., *B. alternatus* clade) are more associated with lower temperatures (see T_{Min} in Figure 3B) and have a mostly cis-Andean distribution in areas of low to moderate latitudes (Figure 2). Species from other basal clades (e. g., *Bothrocophias* clade), which also have a cis-Andean distribution, remained associated not only to high elevation areas but also to areas with higher T_{Min} (Figure 2 and Figure 4B). Overall, reconstructions suggest the following patterns. (1) For T_{Max}, transitions from moderate to low T_{Max} occurred in clade F and species from clades B (i. e., *B. cotiara* and *B. fonsecai*) and G (*B. neuwiedi*), whereas transitions from moderate to high T_{Max} was evident only in *B. atrox* and *C. durissus* clades (Figure 3A). (2) For T_{Min}, transitions from moderate to low T_{Min} occurred in clades B and H, whereas transitions from moderate to high T_{Min} occurred especially in the same clades A and F (Figure 4B). (3) For TNB, transitions from moderate to low TNB occurred in clades A, C and F, but not for the *B. alternatus* and *B. atrox* clade, whereas transitions from moderate to high TNB occurred in clade H (Figure 3C; Supplementary material). And (4) for VT_{Max}, transitions from moderate to low behavioral tolerances occurred in clades A, D, G, and transitions from low to high VT_{Max} occurred only in the clade E (Figure 4D).

Finally, the test for phylogenetic signal indicated that only T_{Max} ($K = 0.87$, $p = 0.02$) had a significant phylogenetic signal, with a K lower than 1, while T_{Min} ($K = 0.31$, $p = 0.72$), TNB ($K = 0.37$, $p = 0.53$) and VT_{Max} ($K = 0.48$, $p = 0.33$) were not conserved traits in South American pitvipers. Pairwise PGLS analyses between VT_{Max} and environmental features identified a weak but significant contribution ($F = 5.84$; $df = 7$; $p = 0.02$) of only one biogeographical feature, Latitudinal Range (Lat_{Ran}), in explaining the macroevolutionary variation of VT_{Max} in South American pitvipers (Figure 5; Supplementary material).

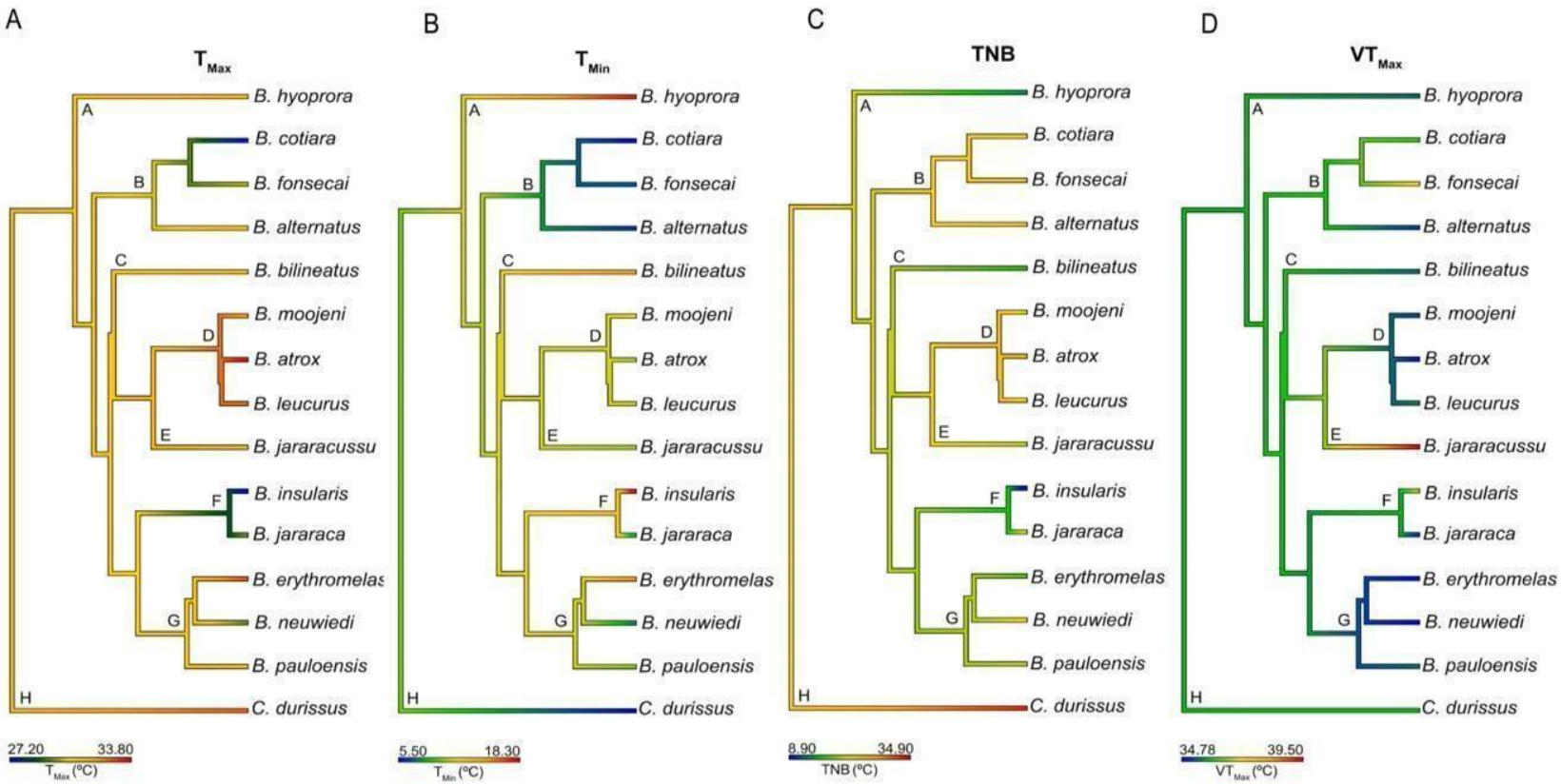


Figure 4. Reconstructed ancestral states for A) Maximum environmental temperature (T_{Max}), B) Minimum environmental temperature (T_{Min}), C) thermal niche breadth (TNB) and D) VT_{Max} of South American pitvipers. Clades (capital letters at nodes) and species are shown as in Figure 1. Mean confidence interval (CI) of reconstructed states for 100 of the most plausible phylogenetic trees with Equal Rates (ER) model for Bio 5 = 0.95, Bio 6 = 0.93, TNB = 0.94, VT_{Max} = 0.96.

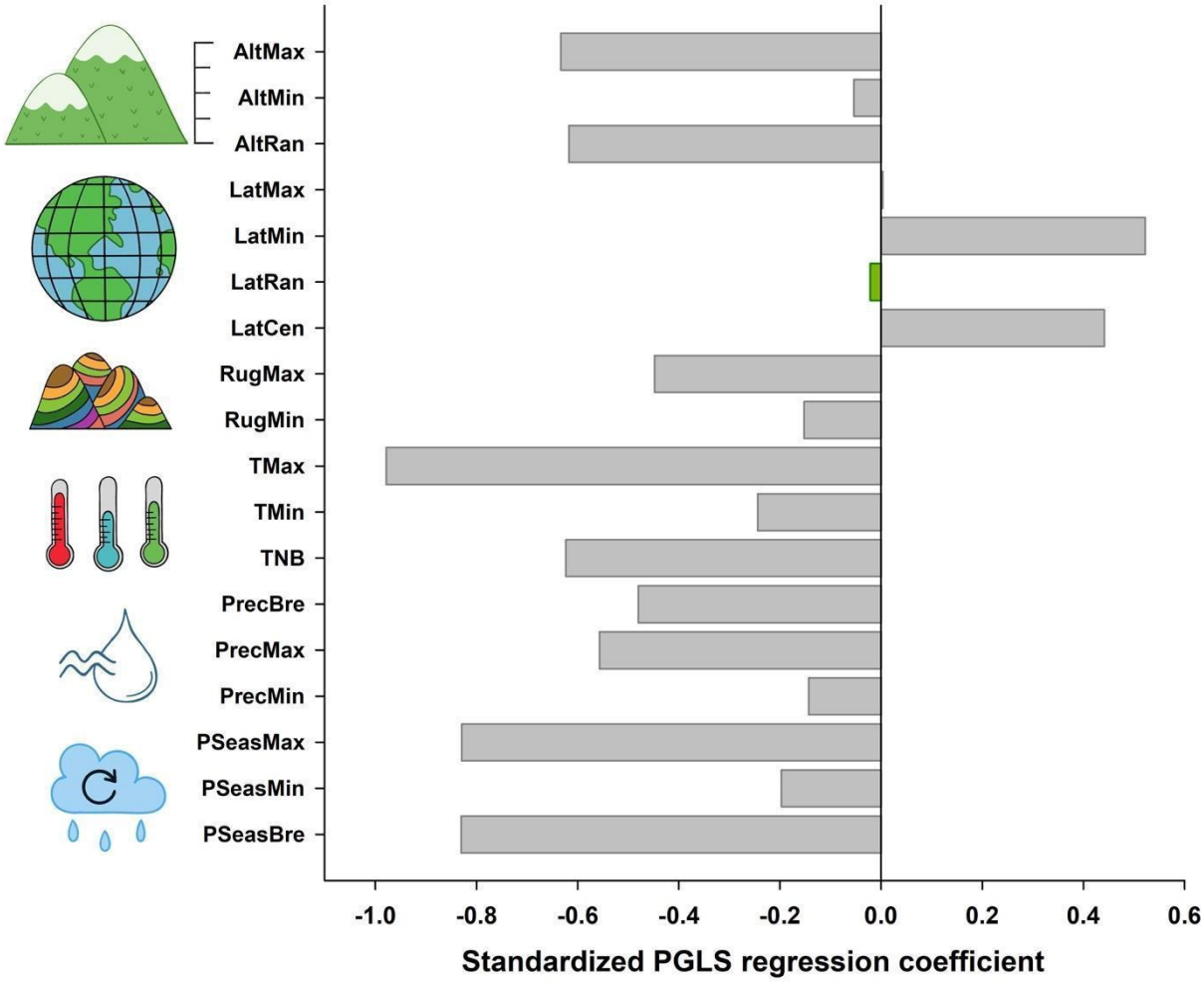


Figure 5. Estimated coefficients of PGLS models fit to examine the evolutionary covariation of the VT_{Max} and environmental features. Significant co-variables are marked in green. See main text for environmental features codes.

4.4. Discussion

Our results show that VT_{Max} does not vary markedly among South American pitvipers measured in this study. Moreover, we found no marked difference in heating rates among species. This similarity was also observed in the maximum environmental temperatures of species (and lineages) but not for minimum temperatures, in which there were clear differences between some clades (e. g., *B. hyoprora* and *B. alternatus* clade). Nevertheless, there were changes along the evolutionary timescale with the ancestral state reconstruction showing differences in mean values between the environmental thermal limits, TNB and VT_{Max} of clades. While we observed no phylogenetic signal for T_{Min}, TNB, and VT_{Max}, we recovered a significant phylogenetic signal for T_{Max} which suggests larger inter-clade than

intra-clade variance. However, the absence of phylogenetic signal may be due to the relatively low number of species used in this study. Our results also suggest that the environmental features used herein, except Latitudinal Range, are not strong predictors of VT_{Max} in South American pitvipers, but also that there may be complex factors at play regarding the tiny differences found among species.

4.4.1. VT_{Max}, distribution patterns and environmental temperatures

South American pitvipers are distributed throughout the entire continent, occurring in several ecoregions and habitats (e. g., Amazonia, Atlantic Forest, Cerrado), with differences in their distribution patterns (NOGUEIRA et al., 2019) and in traits such as body size (e. g., CAMPBELL & LAMAR, 2004). While it may be surprising that species with different distribution patterns from different lineages showed similar behavioral thermal tolerances, the VT_{Max} has already been shown to be conserved across lizard clades separated by thousands to millions of years (WIENS et al., 2019). This conservatism may be related to other ecological aspects. Even though pitviper species have multiple differences in ecological terms, these species have a high degree of similarity in aspects of their natural history (for example by being ambush predators; MARTINS et al., 2002) and of their physiological requirements (HEATWOLE & TAYLOR, 1987; CAMPBELL & LAMAR, 2004). Snakes, and especially pitvipers, tend to have small home ranges and low dispersal abilities (e. g., SAZIMA, 1988, 1992; SECOR, 1994; VALDUJO et al., 2002; SASA et al., 2009; SMANIOTTO et al., 2020; MUSCAT et al., 2021) and are remarkably efficient at behavioral thermoregulation (e. g., HEATWOLE & TAYLOR, 1987; HEATWOLE & JOHNSON, 1979). Behavioral traits have been shown to be more evolutionarily labile than other types of traits such as physiological traits (e. g., HUEY & BENNETT, 1987; GITTLEMAN et al., 1996; BLOMBERG et al., 2003). Thus, there could have been important selective pressures against changes in VT_{Max} along the evolutionary history of South American pitvipers.

In spite of species having similar VT_{Max} but occurring in different regions and ecoregions throughout the continent, the species studied here showed similar patterns of $VT_{Max} - T_{Max}$ values throughout their ranges, with nearly all species having all of their records in localities with temperatures below their VT_{Max}. The occurrence in regions with temperatures lower than their VT_{Max} offers some protection against the probability of being exposed to temperatures outside their tolerated limits (e. g., see DIDHAM & LAWTON, 1999; EWERS & BANKS-LEITE, 2013). Furthermore, the behavioral thermal tolerances presented

here are similar to the ranges found for other Neotropical snake species of different families measured in two large Brazilian ecoregions (DIAZ-RICAURTE et al., 2022a), which shows that behavioral tolerances of Neotropical snakes are likely within the same range, between 29.81 – 39.5 ºC (see DIAZ-RICAURTE et al., 2022a; this study) and thus that these limits might be conserved at a higher taxonomic scale rather than on vipers alone. Furthermore, our results showed that no feature other than Latitudinal Range (with wider latitudinal ranges tending to have a lower VT_{Max}) (Figure 5) sufficiently explains the evolutionary covariation of VT_{Max}. Environmental and geographical features (e. g., minimum temperatures, precipitation, ruggedness, and altitudinal limits) do not seem to be strong drivers of macroevolutionary variation of the VT_{Max} for this group of Neotropical snakes.

4.4.2. VT_{Max} and macroevolutionary patterns

Besides VT_{Max} values being similar among species and differences in climatic and geographical features not explaining the macroevolutionary patterns of thermal tolerance (potentially due to the strong similarity), there was a clear lack of phylogenetic signal for VT_{Max} and other thermal features except T_{Max}. This lack of phylogenetic signal in thermal niche had already been shown for some groups of endotherms (KAMILAR & MULDOON, 2010; SOBERÓN & MARTÍNEZ-GORDILLO, 2012). Even so, the significant phylogenetic signal in T_{Max}, with larger inter-clade variance than within clades, suggests that species in the same clade are subjected to similarly high temperatures. This may have to do with shared ecoregion occurrence (e. g., Atlantic Forest for the *B. jararaca* clade or Atlantic Forest, Pampas and Cerrado for the *B. alternatus* clade; Figure 3). Additionally, there is a lower latitudinal variation for high temperatures than for lower temperatures. Thus, phylogenetically-related species with similar ranges might experience the similar T_{Max} but different T_{Min}, as other factors related to low temperatures (e. g., elevation) vary enough locally to generate intra-clade differences. On the other hand, the lack of phylogenetic signal for VT_{Max} might corroborate a similarly weak selective pressure on different lineages, despite their differences in distribution and body size. Thus, our results indicate that there is no strong climatic niche conservatism in the ranges of South American pitvipers, at least on a large spatial scale. It is however likely that, on smaller scales (e. g., local), environmental niches show less lability and thus a strong phylogenetic signal, as has been observed in other comparative evolutionary studies (e. g., PEIXOTO et al., 2016). We suggest considering different spatial scales in future studies, as well as considering ecological aspects such as

habitat use, which have previously been shown to have a high relationship with behavioral tolerances of other ectotherms (see RECODER et al. 2018; DIAZ-RICAURTE et al., 2020; DIAZ-RICAURTE et al., 2022b).

Shifts in thermal tolerances throughout the evolutionary history of the group may have shaped current species tolerances. This is corroborated by ESPARZA-ESTRADA et al. (2022), who suggest that shifts in niche breadth allowed some species of vipers to tolerate environments with high climatic variability during their dispersal in South America. The ancestral niche of these snake lineages for all the variables used was predominantly moderate temperatures, consistent with the Andean origin suggested by PONTES-NOGUEIRA et al. (2021), with basal lineages such as *B. pictus* and the *Bothrocophias* clades being associated with mountainous environments (which have lower environmental temperatures), as well as lineages in high latitudinal areas such as the *B. alternatus* clade. This may indicate that the ancestor of these clades occurred in regions with little variability in temperature, which may also suggest that both T_{Max}, T_{Min} and thermal tolerances underwent recent changes in current lineages. These changes are probably due to geomorphological and climatic events that also caused the diversification of the group and its journey on the continent, such as glaciations in the Oligocene at 28–25 mya, followed by short cooling periods (e. g., CARVALHO & ALMEIDA, 2016). This could have subjected many species to extreme temperatures, while also allowing them to adapt to low temperatures. Most changes of thermal aspects and tolerances were concurrent with an accelerated formation of new lineages, shortly after their arrival in new areas (e. g., *B. atrox* and *B. jararaca* clades; BURBRINK et al., 2012; ALENCAR et al., 2016). For example, our ancestral state reconstruction suggests that shifts to climatic regions with lower T_{Max} (higher latitude or altitude) are evident only in the *B. alternatus* clade and in species within the *B. neuwiedi* complex. *Bothrops alternatus* is different from the other species of its clade in both T_{Max} and VT_{Max} (see Figure 4A, D). This species separated from the other species of its clade in the late Miocene (10 Mya) and dispersed to the Chaco, Pampas and the Southern Atlantic Forest (see PONTES-NOGUEIRA et al., 2021), thus increasing the T_{Max} in its range while also leading to a higher VT_{Max} likely due to these areas being hotter and less variable. Our reconstruction also suggests that shifts to regions with lower T_{Min} (higher latitude) occurred independently in some clades, especially in some species within clades (evident in species such as *B. cotiara* and *B. alternatus*, and the *B. jararaca* clade).

Our results indicate that, overall, the behavioral thermal tolerance of South American pitvipers shows a consistent clade-wide spatial and environmental pattern, with species having similar values and no major environmental features significantly explaining its variation across species ranges. There is also a likely interplay of environmental temperatures and species distribution, with significant differences in maximum environmental temperatures between clades (and not within clades) but similar patterns of Voluntary Thermal Maximum values, always below the maximum environmental temperatures across their range. This is congruent with similar physiological and natural history aspects that limit species exposure to challenging upper temperatures, which have also been shown for other ectotherms. However, macroecological and ecophysiological studies which aim to understand how environmental temperatures influence the distribution and evolutionary history of species (e. g., CLUSELLA-TRULLAS et al., 2011; SUNDAY et al., 2014; HANGARTNER & HOFFMANN, 2016; THOMAS et al., 2016; ROZEN-RECHELS et al., 2019) rarely take behavioral thermal tolerances into account. Thus, we highlight the importance of considering behavioral thermal tolerances such as VT_{Max} in future comparative studies in order to provide insights into how temperature changes may impact ectotherms across the globe.

Acknowledgments

We thank the Butantan Institute for authorizing the use of snakes under their care for the experiments. We thank Fernando Ribeiro, Ricardo Sawaya, Laura Alencar and Caroline Molina for their valuable comments on previous versions of this manuscript. We thank Pamela Santos and Danusa Camanduchy Maia for their collaboration during the manipulation of some individuals during the experiments. We thank the other members of the herpetological team (João Paulo dos Santos Vieira de Alencar, Gabriella Leal and Carolina Farhat) from the Laboratory of Ecology, Evolution, and Conservation of Amphibians and Reptiles and the mastozoological team (Giovana Felício, Luciana Furtado and Patrícia Marques) led by Ana Paula Carmignotto for their occasional help and support in the field. Finally, we thank the staff of Instituto Florestal at Estação Ecológica de Santa Bárbara, (especially Marcos Pestana) and Companhia Melhoramentos for logistical support during fieldwork.

Funding

This study benefited from grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; #2015/20215-7, #2018/14091-1 and #2020/12658-4); the H2020 MSCA

(897901) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001".

Data Availability Statement

The data that supports the findings of this study are available in the Supporting Information of this article.

Author Contributions

JCDR, AC, CCN and MM planned the study; JCDR collected the data and performed the experiments; JCDR analyzed the data; JCDR and MM led the writing of the manuscript. JCDR wrote the original draft; AC, CCN and MM supervised the manuscript; JCDR and FCS wrote the final version of manuscript. All authors contributed to the manuscript and approved the final version of this manuscript for publication.

Conflict of Interests

The authors declare no conflict of interest.

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Supplementary Material

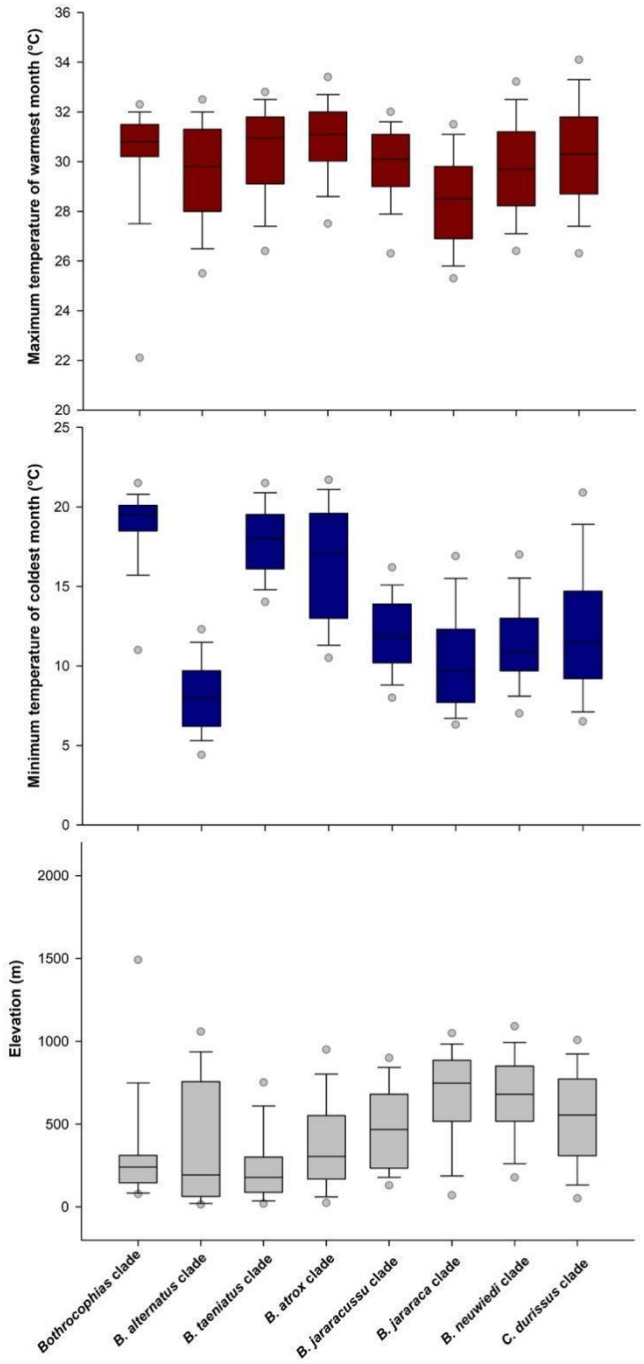


Figure S1. Boxplots to niche envelope of the South American pitvipers lineages. The whiskers correspond to 5th and 95th percentiles and the dots the maximum and minimum values for the variable.

Supplementary Tables

Table S1. Physiological data of species. Data on each individual tested for Voluntary Thermal Maximum (VT_{Max}) in this study.

Experiment data	Voucher/ID	Species	Locality	Lineage	Sex	WT	SVL	TL	ST	FT	time	IBT	Final body temperature	HR
16/05/19	SB779	*Bothrocophias hyoprora*	AC	A	M	9.07	257	33	09:47	11:10	83	22.3	35.1	0.154
16/05/19	SB780	*Bothrocophias hyoprora*	AC	A	M	7.3	247	29	09:47	11:15	88	21.3	35.8	0.165
10/05/19	SB993	*Bothrops alternatus*	SP	B	M	31	385	36	13:01	14:07	66	24.1	36.59	0.189
19/18/2020	-	*Bothrops alternatus*	ASB(SP)	B	M	18.3	271	41	14:49	15:04	15	30.03	34.47	0.296
15/05/19	SB977	*Bothrops cotiara*		B	M	46	430	47	13:32	15:28	118	23.96	36.12	0.103
17/05/19	-	*Bothrops fonsecai*	Serra da Mantiqueira (Campos do Jordao)	B	F	178	671	112	14:10	16:11	121	24.32	37.37	0.108
17/05/19	-	*Bothrops fonsecai*	Serra da Mantiqueira (Campos do Jordao)	B	M	151	553	102	16:15	17:46	91	23.87	36.95	0.144
09/05/19	SB781	*Bothrops bilineatus*	RN	C	M	28	480	85	11:00	11:56	56	26.86	35.45	0.153
15/10/19	MB6455	*Bothrops atrox*	Monte Alegre, Pará	D	M	175	776	155			38	26.1	34.7	0.226

15/10/19		*Bothrops atrox*	Monte Alegre, Pará	D	M						43	25.5	35.2	0.226
09/05/19	SB970	*Bothrops jararacussu*	RJ	E	F	10	275	97	14:41	15:22	41	22.6	39.5	0.412
20/11/18	-	*Bothrops moojeni*	ASB(SP)	D	F	84	527	93	16:38	17:17	39	31.25	37.82	0.168
25/04/19	-	*Bothrops moojeni*	ASB(SP)	D	F	65	648	112	14:07	15:08	61	28.87	37.06	0.134
14/10/19	MB6507	*Bothrops moojeni*	SP	D	F	16.4	340	50	14:53	15:21	28	28.6	37.7	0.325
30/11/19	-	*Bothrops moojeni*	ASB(SP)	D	F	160	660	114	15:22	16:21	59	26.8	38.31	0.195
15/10/19	MB5354	*Bothrops leucurus*	Porto Seguro, Bahia	D	F	984	1340	200			45	24.6	35.6	0.244
14/10/19	MB6349	*Bothrops erythromelas*	SP	G	F	4	185	30	09:45	10:20	35	27.1	34.5	0.172
14/10/19	MB6350	*Bothrops erythromelas*	SP	G	F	4	175	30	09:45	10:31	46	26.8	34.7	0.337
14/10/19	MB6351	*Bothrops erythromelas*	SP	G	F	4	175	30	10:42	11:09	27	26.3	35.4	0.252
14/10/19	MB6352	*Bothrops erythromelas*	SP	G	M	4	185	35	10:42	11:15	33	26.6	34.9	0.221
14/10/19	MB6353	*Bothrops erythromelas*	SP	G	M	4	175	33	13:37	14:10	33	28	35.3	0.34
14/10/19	MB6356	*Bothrops erythromelas*	SP	G	F	3	185	32	13:37	13:57	20	27.8	34.6	0.24
14/10/19	MB6357	*Bothrops erythromelas*	SP	G	F	4	180	30	14:05	14:40	35	27.4	35.8	0.332
14/10/19	MB6358	*Bothrops erythromelas*	SP	G	F	4	175	30	11:16	11:41	25	28.2	36.5	0.153

14/10/19	MB6359	*Bothrops erythromelas*	SP	G	F	4	185	30	09:56	10:41	45	27.2	34.1	0.545
14/10/19	MB6361	*Bothrops erythromelas*	SP	G	M	3	185	33	11:16	11:27	11	27.8	33.8	0.203
14/10/19	MB6362	*Bothrops erythromelas*	SP	G	M	4	175	35	09:56	10:35	39	26.5	34.4	0.284
14/10/19	MB6363	*Bothrops erythromelas*	SP	G	M	3	170	30	14:53	15:12	19	28.7	34.1	0.325
05/11/19	MB5447	*Bothrops insularis*	Captivity	F	F	7.4	262	33	10:10	10:40	30	26.3	35.4	0.303
05/11/19	MB6470	*Bothrops insularis*	Captivity	F	F	8.2	263	34	10:10	10:38	28	25.7	35.8	0.361
05/11/19	MB6472	*Bothrops insularis*	Captivity	F	F	7.4	255	30	13:18	13:37	19	26.1	37.2	0.584
05/11/19	MB6473	*Bothrops insularis*	Captivity	F	M	7.8	269	35	13:18	13:39	21	26.7	36.1	0.448
05/11/19	MB6474	*Bothrops insularis*	Captivity	F	M	7.12	249	35	13:47	13:59	12	27.8	36.8	0.75
05/11/19	MB6475	*Bothrops insularis*	Captivity	F	M	8.15	258	34	13:47	14:12	25	28.3	38.8	0.42
10/05/19	SBBJ03	*Bothrops jararaca*	SP	F	M	8.69	255	45	14:21	14:52	31	23.6	35.1	0.371
10/05/19	SBBJ04	*Bothrops jararaca*	SP	F	M	7.39	235	40	13:55	15:10	65	24.5	35.4	0.168
10/05/19	SBBJ06	*Bothrops jararaca*	SP	F	M	8.38	255	40	14:17	15:28	71	24.7	34.9	0.144
15/05/19	SBBJ07	*Bothrops jararaca*	SP	F	M	7.02	240	35	13:32	15:17	105	21.06	34.4	0.127
16/05/19	SBBJ11	*Bothrops jararaca*	SP	F	F	9.07	265	45	14:14	15:14	60	22.6	34.1	0.192
16/05/19	SBBJ12	*Bothrops jararaca*	SP	F	F	8.49	260	45	14:14	15:13	59	22.9	35.2	0.208

16/05/19	SBBJ13	*Bothrops jararaca*	SP	F	F	7.32	250	45	12:54	14:00	66	20.7	34.3	0.206
16/05/19	SBBJ14	*Bothrops jararaca*	SP	F	F	8.25	260	45	12:54	14:05	71	19.4	33.1	0.193
17/05/19	SBBJ01	*Bothrops jararaca*	SP	F	F	7.79	260	40	13:30	14:04	34	20	31.2	0.329
17/05/19	SBBJ08	*Bothrops jararaca*	SP	F	M	8.12	245	40	14:22	15:23	61	21.8	34.1	0.202
17/05/19	SBBJ10	*Bothrops jararaca*	SP	F	F	8.41	255	40	13:30	14:04	34	20.2	33.1	0.379
21/05/19	SBBJ05	*Bothrops jararaca*	SP	F	F	7.77	255	45	09:28	10:37	69	21	32.6	0.168
21/05/19	SBBJ09	*Bothrops jararaca*	SP	F	M	8.08	260	40	09:28	10:53	85	20.9	33.9	0.153
22/05/19	SBBJ02	*Bothrops jararaca*	SP	F	M	8.23	255	40	09:43	10:54	71	19.7	33.4	0.193
22/10/20	-	*Bothrops jararaca*	Monte verde, MG	F	M	8	276	43	15:09	15:22	12	25.49	35.36	0.823
24/10/20	-	*Bothrops jararaca*	Monte verde, MG	F	F	811	1375	135	08:15	09:23	68	26.4	35.4	0.132
29/10/20	-	*Bothrops jararaca*	Monte verde, MG	F	M	145	611	90	10:06	19:39	33	25.83	35.95	0.307
14/11/19	MB5726	*Bothrops neuwiedi*	Munhoz	G	F	480	975	142	14:06	14:43	37	24.86	36.38	0.311
18/11/19	MB5727	*Bothrops neuwiedi*	Munhoz	G	M	190	815	122	15:57	16:22	25	24.81	35.16	0.414
18/11/19	MB5729	*Bothrops neuwiedi*	Munhoz	G	F	250	847	71	12:38	13:38	60	24.64	36.89	0.204
18/11/19	MB5730	*Bothrops neuwiedi*	Munhoz	G	M	120	728	126	11:00	11:37	37	23.68	36.23	0.339
18/11/19	MB5733	*Bothrops neuwiedi*	Munhoz	G	M	95	680	104	10:04	10:44	40	23.31	37.21	0.348

18/11/19	MB6060	*Bothrops neuwiedi*	Munhoz	G	F	215	735	105	14:45	15:37	52	23.04	36.48	0.258
19/11/19	MB5549	*Bothrops neuwiedi*	Munhoz	G	M	200	696	101	15:12	15:37	25	24.77	31.64	0.275
19/11/19	MB5723	*Bothrops neuwiedi*	Munhoz	G	F	140	780	124	13:47	14:46	59	27.62	37.18	0.162
19/11/19	MB5725	*Bothrops neuwiedi*	Munhoz	G	M	155	815	132	12:44	13:37	53	25.37	32.01	0.125
19/11/19	MB5731	*Bothrops neuwiedi*	Munhoz	G	F	200	850	103	08:39	10:03	81	23.62	34.85	0.139
19/11/19	MB5736	*Bothrops neuwiedi*	Munhoz	G	F	240	860	100	10:16	11:13	57	23.92	33.06	0.16
22/11/19	MB5666	*Bothrops neuwiedi*	Munhoz	G	F	690	1140	160	12:49	14:50	121	25.94	34.65	0.072
25/11/19	MB5669	*Bothrops neuwiedi*	-	G	F	385	1010	126	10:03	11:36	93	21.65	36.05	0.155
29/11/19	MB6287	*Bothrops neuwiedi*	-	G	F	250	835	129	12:50	13:18	28	23.05	29.1	0.216
10/03/18	-	*Bothrops pauloensis*	ASB(SP)	G	F	59	590	110	16:12	17:05	53	25.39	34.99	0.181
10/03/18	-	*Bothrops pauloensis*	ASB(SP)	G	F	78	558	93	20:21	21:36	75	29.26	33.95	0.063
17/03/18	-	*Bothrops pauloensis*	ASB(SP)	G	M	117	139	101	16:30	17:19	49	29.64	35.04	0.11
23/10/18	-	*Bothrops pauloensis*	ASB(SP)	G	F	95	625	88	19:24	20:12	48	26.71	33.18	0.135
25/10/18	-	*Bothrops pauloensis*	ASB(SP)	G	M	67	490	84	22:15	23:11	56	24.6	37.08	0.223
24/04/19	-	*Bothrops pauloensis*	ASB(SP)	G	M	98	580	75	15:23	15:51	26	30.3	38.39	0.311
09/05/19	SB934	*Bothrops pauloensis*	SP	G	M	25	145	111	14:00	14:28	28	26.15	33.4	0.257

29/11/19	MB6496	*Bothrops pauloensis*	-	G	M	125	433	50	13:33	14:28	55	26.59	36.34	0.177
29/11/19	MB6497	*Bothrops pauloensis*	-	G	M	105	527	66	14:55	15:15	20	24.6	29.99	0.27
30/11/19	-	*Bothrops pauloensis*	ASB(SP)	G	M	70	440	56	14:46	15:00	14	28.1	35.14	0.503
30/11/19	-	*Bothrops pauloensis*	ASB(SP)	G	F	120	500	40	14:07	14:29	22	27.2	36.95	0.443
30/11/19	-	*Bothrops pauloensis*	ASB(SP)	G	M	90	510	77	11:39	12:02	23	27.14	33.53	0.278
30/11/19	-	*Bothrops pauloensis*	ASB(SP)	G	F	180	590	50	13:22	13:44	22	26.8	37.29	0.477
30/11/19	-	*Bothrops pauloensis*	ASB(SP)	G	F	100	459	71	12:47	13:11	24	26.7	34	0.304
09/03/18	-	*Crotalus durissus*	SBES	H	M	400	825	93	20:00	22:53	173	26.66	38.26	0.067
15/03/18	-	*Crotalus durissus*	SBES	H	F	193	540	42	21:10	00:49	239	28.52	36.12	0.032
30/09/19	-	*Crotalus durissus*	SBES	H	F	186	550	52	17:00	18:05	65	21.82	32.1	0.158
30/11/19	-	*Crotalus durissus*	MV	H	M	210	700	60	16:37	17:18	41	23.16	37.05	0.339

Table S2. Summary of the fitted linear mixed-effects model showing the effect of different variables (HR, IBT, SVL, WT, Sex and their interactions) on the VT_{Max} of South American pitvipers. * indicates statistical significance

Model	Variable	Estimate	Std. Error	df	t value	p-value	AIC-value	AICwt
Null	(Intercept)	35.599	0.320	4.000	111.300	<2e-16 ***	343.660	0.44
I	(Intercept)	22.370	35.720	35	0.626	0.533	354.520	0
	HR	94.660	123.700		0.765	0.447		
	IBT	0.575	1.365		0.421	0.675		
	SVL	0.055	0.138		0.399	0.691		
	WT	0.898	0.480		1.872	0.06475 .		
	SexF	16.790	50.020		0.336	0.738		
	HR:IBT	-4.408	4.786		-0.921	0.360		
	HR:SVL	-0.545	0.489		-1.116	0.268		
	IBT:SVL	-0.003	0.005		-0.487	0.628		
	HR:WT	-2.396	3.177		-0.754	0.453		
	IBT:WT	-0.029	0.018		-1.676	0.098		
	SVL:WT	-0.002	0.001		-1.960	0.053		
	HR:SexF	-112.200	238.900		-0.469	0.640		
	IBT:SexF	-0.611	1.872		-0.326	0.745		
	SVL:SexF	-0.104	0.192		-0.540	0.590		
	WT:SexF	-1.375	0.615		-2.237	0.028*		
	HR:IBT:SVL	0.025	0.019		1.287	0.202		
	HR:IBT:WT	0.070	0.121		0.578	0.565		
	HR:SVL:WT	0.008	0.005		1.672	0.098		
	IBT:SVL:WT	0.000	0.000		1.895	0.062		
	HR:IBT:SexF	4.295	8.888		0.483	0.630		
	HR:SVL:SexF	0.584	0.950		0.615	0.540		

	IBT:SVL:SexF	0.004	0.007		0.523	0.603		
	HR:WT:SexF	5.779	4.003		1.444	0.153		
	IBT:WT:SexF	0.050	0.022		2.250	**0.027***		
	SVL:WT:SexF	0.003	0.001		2.761	**0.007****		
	HR:IBT:SVL:WT	0.000	0.000		-1.628	0.107		
	HR:IBT:SVL:SexF	-0.022	0.035		-0.617	0.539		
	HR:IBT:WT:SexF	-0.217	0.151		-1.438	0.154		
	HR:SVL:WT:SexF	-0.014	0.005		-2.673	**0.009****		
	IBT:SVL:WT:SexF	0.000	0.000		-2.756	**0.007****		
	HR:IBT:SVL:WT:SexF	0.001	0.000		2.690	**0.008****		
II	(Intercept)	43.350	15.850	19	2.735	0.007**	353.862	0
	HR	-44.870	70.070		-0.640	0.524		
	IBT	-0.279	0.600		-0.465	0.644		
	SVL	-0.036	0.054		-0.652	0.516		
	WT	-0.099	0.243		-0.409	0.684		
	HR:IBT	1.497	2.690		0.557	0.580		
	HR:SVL	0.147	0.264		0.555	0.581		
	IBT:SVL	0.001	0.002		0.596	0.553		
	HR:WT	0.073	1.506		0.048	0.962		
	IBT:WT	0.004	0.009		0.466	0.643		
	SVL:WT	0.000	0.000		0.711	0.479		
	HR:IBT:SVL	-0.005	0.010		-0.443	0.659		
	HR:IBT:WT	-0.005	0.059		-0.085	0.932		
	HR:SVL:WT	-0.001	0.002		-0.420	0.675		
	IBT:SVL:WT	0.000	0.000		-0.720	0.474		
	HR:IBT:SVL:WT	0.000	0.000		0.423	0.673		

III	(Intercept)	35.820	1.251	11	28.625	0.000***	347.952	0.01
	HR	-5.122	4.678		-1.095	0.277		
	SVL	-0.004	0.004		-1.131	0.262		
	WT	0.014	0.014		1.021	0.311		
	HR:SVL	0.029	0.017		1.677	0.098		
	HR:WT	-0.067	0.068		-0.980	0.330		
	SVL:WT	0.000	0.000		-0.500	0.618		
	HR:SVL:WT	0.000	0.000		0.404	0.687		
IV	(Intercept)	34.890	0.870	7	40.111	0.000***	342.605	0.55
	HR	0.454	2.912		0.156	0.876		
	SVL	0.000	0.002		0.132	0.895		
	HR:SVL	0.005	0.007		0.751	0.455		

Table S3. Summary of thermal, climatic and geographical limits of South American pitvipers

Species	Climatic Niche		TNB	Elevation (m)		Latitude (degrees)		Annual precipitation (mm)		Annual precipitation breadth (mm)	Precipitation Seasonality		Precipitation Seasonality breadth	Ruggedness (metres of elevation difference for grid points 30 arc-seconds)		Altitudinal breadth (m asl)	Latitudinal breadth (degrees)
	T_{Max} (ºC)	T_{Min} (ºC)		Alt_{Max}	Alt_{Min}	Lat_{Max}	Lat_{Min}	Prec Max	Prec Min		PSeas Max	PSeas Min		Rug Max	Rug Min		
Bothrocophias hyoprora	31.5	18.05	13.45	1989	63	2.7058	-13.45	4330	1068	3262	80.53616	11.07257	69.46359	334.125	0.5	2040	16.1558
Bothrops alternatus	31.4	6.2	25.2	1349	0	-14.0055	-38.5667	2110	587	1523	88.6556	4.37258	84.28302	137	0	1349	24.5612
Bothrops cotiara	26.6	5.5	21.1	1349	381	-23.3167	-29.45	2204	1218	986	49.07894	9.36956	39.70938	107.625	3.5	968	6.1333
Bothrops fonsecai	30	6.9	23.1	1749	6	-21.529	-23.55	2375	1307	1068	79.24365	41.16722	38.07643	226.5	2.5	1743	2.021
Bothrops bilineatus	31.5	15.2	16.3	1526	2	7.0338	-22.95	4374	728	3646	85.14628	9.77987	75.36641	231.125	0.25	1524	29.9838
Bothrops atrox	32.3	19.53	12.77	1200	0	10.71	-17.8	4332	710	3622	91.2206	12.94861	78.27199	431.625	0.25	1200	28.51
Bothrops moojeni	32.3	11.7	20.6	1258	11	-7.15	-27.2667	2385	442	1943	93.56424	9.81731	83.74693	193.5	0.125	1247	20.1167
Bothrops leucurus	32.7	11.3	21.4	1108	0	-7.3333	-20.362714	2360	855	1505	101.41967	16.13687	85.2828	106.25	0	1108	13.029414
Bothrops jararacussu	31	10.7	20.3	1000	0	-14.3161	-28.5439	3218	610	2608	86.08622	9.85337	76.23285	265.75	0.25	1000	14.2278
Bothrops jararaca	29.6	8.3	21.3	2541	5	-11.4333	-30.875	2983	622	2361	94.80858	5.74571	89.06287	310.75	0.375	2536	19.4417
Bothrops insularis	27.2	18.3	8.9	200	0	-24.5	-24.5	-	-	-	-	-		-	-	200	0
Bothrops neuwiedi	29.6	7.8	21.8	2054	4	-6.8059	-30.583	1935	409	1526	118.2747	25.79074	92.48396	110.25	1	2050	23.7771
Bothrops pauloensis	30.8	10.4	20.4	1223	75	-12.8167	-24.05	2204	478	1726	99.33213	10.46042	88.87171	182.875	0	1148	11.2333
Bothrops erythromelas	33.17	15.3	17.87	1108	1	-3.7853	-14.737	2027	1139	888	90.55186	29.5514	61.00046	155.375	0.125	1107	10.9517

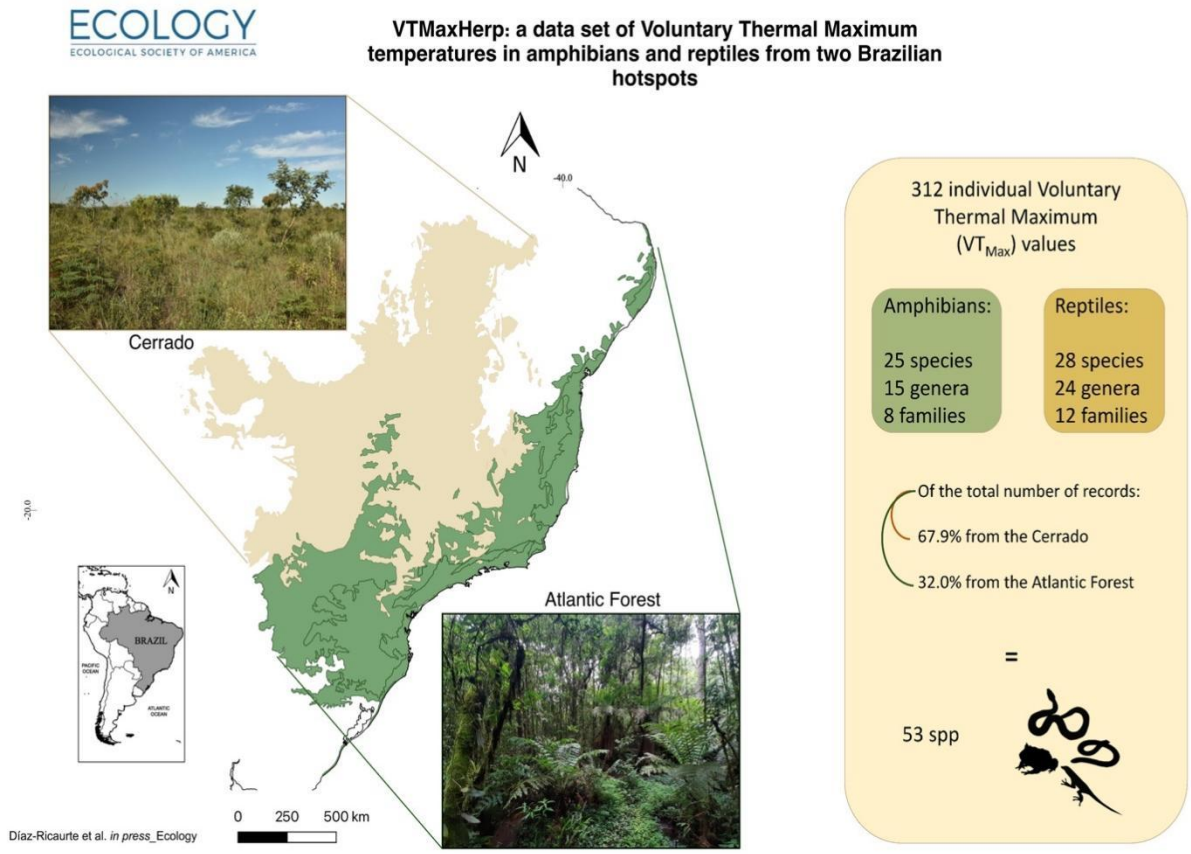
Crotalus durissus	36	5.9	30.1	2321	0	12.512972	-34.866667	3000	186	2814	120.73592	5.74571	114.99021	245.875	0.125	2321	47.37963867

Table S4. Raw data of thermal, climatic and geographical limits of South American pitvipers.

Table S5. Raw data of difference between VT_{Max} and maximum daily temperature (BIO5, WorldClim) for South American pitvipers.

More details in https://bit.ly/3PKYAaM

5. CHAPTER 4: VTMAXHERP: A DATA SET OF VOLUNTARY THERMAL MAXIMUM TEMPERATURES OF AMPHIBIANS AND REPTILES FROM TWO BRAZILIAN HOTSPOTS



Published

DIAZ-RICAURTE JC, SERRANO CF, MARTINS, M. 2022. VTMaxHerp: a data set of Voluntary Thermal Maximum temperatures of amphibians and reptiles from two Brazilian hotspots. ***Ecology***, 103(3): e3602. https://doi.org/10.1002/ECY.3602

Capítulo 4: VTMaxHerp: um conjunto de dados de temperaturas máximas térmicas voluntárias de anfíbios e répteis de dois hotspots brasileiros

Resumo

Anfíbios e répteis são animais ectotérmicos e, portanto, dependem das temperaturas ambientais para manter suas funções fisiológicas. Apesar de pouco documentados, dados sobre limiares comportamentais térmicos para evitar o superaquecimento em seus habitats são essenciais para melhorar o entendimento de sua ecologia e fisiologia térmica. Aqui, fornecemos um conjunto de dados de 312 valores individuais voluntários máximos térmicos (VTMax), a temperatura máxima tolerada pelos indivíduos antes de se moverem ativamente para um local mais frio, para 53 espécies de anfíbios e répteis das savanas da Mata Atlântica e Cerrado do sudeste da América do Sul. Os valores térmicos máximos voluntários foram registrados como a temperatura corporal na qual os indivíduos saíram de uma configuração experimental de caixa de aquecimento. Este conjunto de dados compreende 25 espécies de 15 gêneros e oito famílias de anfíbios e 28 espécies de 24 gêneros e 12 famílias de répteis. Do total de registros individuais, 67,9% são provenientes do Cerrado e 32,0% das ecorregiões da Mata Atlântica. No geral, os lagartos tiveram VT_{Max} mais alto do que anfíbios e cobras, e os indivíduos da Mata Atlântica tiveram valores VT_{Max} mais altos do que os do Cerrado. Este conjunto de dados é o primeiro a compilar valores VT_{Max} para a herpetofauna da América do Sul, junto com informações experimentais detalhadas, como taxas de aquecimento e tempo para atingir VT_{Max}. Este conjunto de dados fornece uma linha de base para a compreensão das tolerâncias térmicas e requisitos de espécies de vertebrados ectotérmicos neotropicais, o que pode ser útil para pesquisas futuras sobre o impacto das mudanças climáticas e para testar novas hipóteses ecológicas.

Palavras-chave: Conservação animal, anuros, aquecimento global, lagartos, herpetofauna Neotropical, serpentes, fisiologia térmica.

Chapter 4: VTMaxHerp: a data set of Voluntary Thermal Maximum Temperatures of amphibians and reptiles from two Brazilian hotspots

Abstract

Amphibians and reptiles are ectothermic animals and thus depend on environmental temperatures to maintain their physiological functions. Despite being poorly documented, data on thermal behavioral thresholds to avoid overheating in their habitats is essential to improve the understanding of their thermal ecology and physiology. Herein we provide a dataset of 312 individual voluntary thermal maximum (VT_{Max}) values, the maximum temperature tolerated by individuals before actively moving to a colder place, for 53 species of amphibians and reptiles of the Atlantic Forest and Cerrado savannas of southeastern South America. Voluntary thermal maximum values were recorded as the body temperature at which the individuals exited a heating box experimental setup. This dataset comprises 25 species of 15 genera and eight families of amphibians and 28 species of 24 genera and 12 families of reptiles. Of the total number of individual records, 67.9% derive from the Cerrado and 32.0% from the Atlantic Forest ecoregions. Overall, lizards had higher VT_{Max} than amphibians and snakes, and individuals in the Atlantic Forest had overall higher VT_{Max} values than the ones in Cerrado. This dataset is the first to compile VT_{Max} values for South American herpetofauna, along with detailed experimental information such as heating rates and time to reach VT_{Max}. This dataset provides a baseline for understanding thermal tolerances and requirements of Neotropical ectothermic vertebrate species, which might be useful for future research on the impact of climate change and to test novel ecological hypotheses.

Keywords: Animal conservation, anurans, global warming, lizards, Neotropical herpetofauna, snakes, thermal physiology.

5.1. Introduction

Environmental temperatures are a major constraint on the abundance and diversity of ectotherms - animals with limited ability to use metabolic heat to maintain their body temperature (ANGILLETTA, 2009) - and imposes limits on ecological aspects such as period of activity and life-history and thus strongly influence their behavior and physiology (HUEY and STEVENSON, 1979). Indeed, ectotherms are especially vulnerable to fluctuations in temperature (BICKFORD et al., 2011, SEEBACHER et al., 2015) and have the ability to detect such changes and modify their behavior or physiology accordingly (HOFFMANN & SGRÒ 2011). Increasing environmental temperatures can place these organisms outside or on the edge of their physiological optimal and thermal tolerance limits (KINGSOLVER et al., 2013, SUNDAY et al., 2014). These thermal tolerances are influenced by behavior - when individuals change their body location or position to maintain a stable core temperature, or physiology - when an individual does not move, but uses other strategies to thermoregulate (e.g. increased respiration rates; CAMACHO & RUSCH 2018). These behavioral thermal tolerances represent the thresholds of preferred body temperature of individuals and can be experimentally measured to identify which temperatures affect their behavior in natural conditions (e.g. DIAZ-RICAURTE et al., 2020, DIAZ-RICAURTE & SERRANO, 2020a, b). One of these thresholds is the Voluntary Thermal Maximum (VT_{Max}), which is defined as the temperature that causes an individual to move to a place with lower temperature (COWLES & BOGERT, 1944) in order to maintain the body temperature within the preferred temperatures. When an individual fails to respond to VT_{Max}, it might be exposed to temperatures near its maximum physiological limits, i.e., critical temperatures, which might lead to loss of locomotion or eventually death (COWLES & BOGERT, 1944, REZENDE et al., 2014). Therefore, the use of behavior to buffer extreme environmental temperatures might allow species to mitigate the detrimental effects of abiotic long-lasting disturbances such as those resulting from climate change (HUEY et al., 2012).

In addition to increasing average environmental temperatures, climate change is also increasing both the intensity and frequency of extreme thermal events (Stillman 2019). This is leading to a disruption in species distribution (TAHERI et al., 2021), abundance (IVERSON & PRASAD 1998), timing of migration and hibernation (INOUYE et al., 2000) and their biotic interactions (SCHWEIGER et al., 2008). The capacity to respond to this disruption is mediated by intrinsic traits such as thermal tolerances and phenotypic plasticity (CHRISTMAS et al., 2016). By being able to tolerate higher temperatures, species might behaviorally or ecologically adapt to rising temperatures, allowing them to persist in their

habitats (BURRACO et al., 2020). While intraspecific variation in thermal tolerances might have important consequences for the susceptibility of populations to climate change (HENN et al., 2018; BENNETT et al., 2019), it is also important to document how these tolerances vary at community level (DIELE-VIEGAS et al., 2018, FRANKEN et al., 2018, NOWAKOWSKI et al., 2018).

In spite of the current global trends of climate change, its effects might differ across regions and taxonomic groups. For example, terrestrial taxa are facing faster warming rates than aquatic taxa (GUNDERSON & STILLMAN, 2015) and species living in hotter environments might be more threatened due to their low plasticity in heat tolerance (STILLMAN, 2003, HUEY et al., 2009, TEWKSBURY, et al., 2008). As a result, tropical ectotherms are more likely to experience temperatures higher than their thermal tolerances. Even though recent studies have assessed thermal tolerances of ectotherms at regional and global scales, South American taxa are still underrepresented (SUNDAY et al., 2011, GUNDERSON & STILLMAN, 2015, LEIVA et al., 2019), despite the high richness in this region (NOGUEIRA et al., 2019, ROLL et al., 2017). It is therefore crucial to understand how Neotropical taxa tolerate high temperatures and how they might be affected by global warming since this data might offer valuable insights to their conservation.

Among ectotherms, amphibians and reptiles are in dire need of conservation (GIBBONS et al., 2000, STUART et al., 2004) as its species are usually abundant and play critical roles in their ecosystems (CORTÉS-GOMEZ et al., 2015). It has been shown that climate change is already impacting their populations, albeit with scarce data for the Neotropics (WINTER et al., 2016) despite having the highest expected vulnerability to climate change (BÖHM et al., 2016). Herein, we present a dataset of Voluntary Thermal Maximum, a metric of behavioral thermal tolerance, for 53 species of amphibians and reptiles in the Cerrado savannas and Atlantic Forest of southeastern South America, two Neotropical biodiversity hotspots (MYERS et al., 2000). The dataset consists of VT_{Max} values with detailed experimental data such as heating rates and time to reach VT_{Max}. Future directions might include testing the phylogenetic patterns in these tolerances and including further ecological information such as time of activity, microhabitat use and morphology to better understand how upper temperatures shape Neotropical herpetofaunal communities.

5.2. Metadata

5.2.1. Class I. Data Set Descriptors

5.2.1.1. Data set identity:

Title: VTMaxHerp: a dataset of Voluntary Thermal Maximum

temperatures of amphibians and reptiles from two Brazilian hotspots

5.2.1.2. Data set and metadata identification codes:
Suggested Data Set Identity Codes: VTMaxHerp_Database.csv

5.2.1.3. Data set description:

1. Originator: *Juan C. Diaz-Ricaurte*, Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, Brazil; Escola Superior de Agricultura Luiz de Queiroz, Programa de Pós-Graduação em Ecologia Aplicada, Universidade de São Paulo, Piracicaba, Brazil, and Semillero de Investigación en Ecofisiología y Biogeografía de Vertebrados, Grupo de investigación en Biodiversidad y Desarrollo Amazónico (BYDA), Centro de investigaciones Amazónicas Macagual – Cesar Augusto Estrada González, Universidad de la Amazonia, Florencia, Caquetá, Colombia.

2. Abstract: Amphibians and reptiles are ectothermic animals and thus depend on external temperatures to maintain their physiological functions. Despite poorly documented, data on thermal behavioral thresholds to avoid overheating in their habitats is essential to improve the understanding of their thermal ecology and physiology. Herein we provide a dataset of 312 individual voluntary thermal maximum (VT_{Max}) values, the maximum temperature tolerated by individuals before actively moving to a colder place, for 53 species of amphibians and reptiles of the Atlantic Forest and Cerrado savannas of southeastern South America. Voluntary thermal maximum values were recorded as the body temperature at which the individuals exited a heating box experimental setu*P*. This dataset comprises 25 species of 15 genera and eight families of amphibians and 28 species of 24 genera and 12 families of reptiles. Of the total number of individual records, 67.9% derive from the Cerrado and 32.0% from the Atlantic Forest ecoregions. Overall, lizards had higher VT_{Max} than amphibians and snakes, and individuals in the Atlantic Forest had overall higher VT_{Max} values than the ones in Cerrado. This dataset is the first to compile VT_{Max} values for South American herpetofauna, along with detailed experimental information such as heating rates and time to reach VT_{Max}. This dataset provides a baseline for understanding thermal tolerances and requirements of Neotropical ectothermic vertebrate species, which might be useful for future research on the impact of climate change and to test novel ecological hypotheses.

D. Keywords: Thermal physiology, Neotropical herpetofauna, VT_{Max}, Anurans, Lizards, Snakes, Animal conservation, Global warming.

E. Summary: The data set has 312 records of voluntary thermal maximum from two different Neotropical hotspots—Atlantic Forest and Cerrado—for 25 species of 15 genera and eight families of amphibians and 28 species of 24 genera and 12 families of reptiles. Each record has 20 fields that detail the taxonomic identity (e.g. ID, Order, Family, Species), body size (e.g., SVL, TL), body mass, data of capture and experimental temperature, heating rate, sex, initial body temperature, time of experiments, VT_{Max} and reference information for each record. This information, mainly body size and body mass, is relevant for functional ecology studies.

F. Description. Fieldwork was carried out at i) Santa Bárbara Ecological Station (SBES; 22˚49’2.43"S, 49˚14’11.29"W; WGS84, 590 m elevation) in the municipality of Águas de Santa Bárbara, São Paulo, and ii) in Monte Verde (MV; 22° 51’S; 46° 02’W, WGS84, 1250 m elevation), in the municipality of Camanducaia, Minas Gerais, located in the extreme southwest of the Serra da Mantiqueira mountain range, as part of two ongoing projects on anthropogenic impact on herpetofauna (Figure 1). Individuals were captured by pitfall trapping in the morning (SBES; e.g., DURIGAN et al., 2020 but see CORN, 1994; CECHIN & MARTINS, 2000), and road cruising (MV; e.g., MARTINS & OLIVEIRA 1998; RODRIGUES et al., 2015) and active search (SBES and MV; see MARTINS & OLIVEIRA 1998; DIAZ-RICAURTE et al., 2020) at night in monthly field trips from 2017 to 2021. For further experimental details of pitfall trapping and active search see DURIGAN et al. (2020).

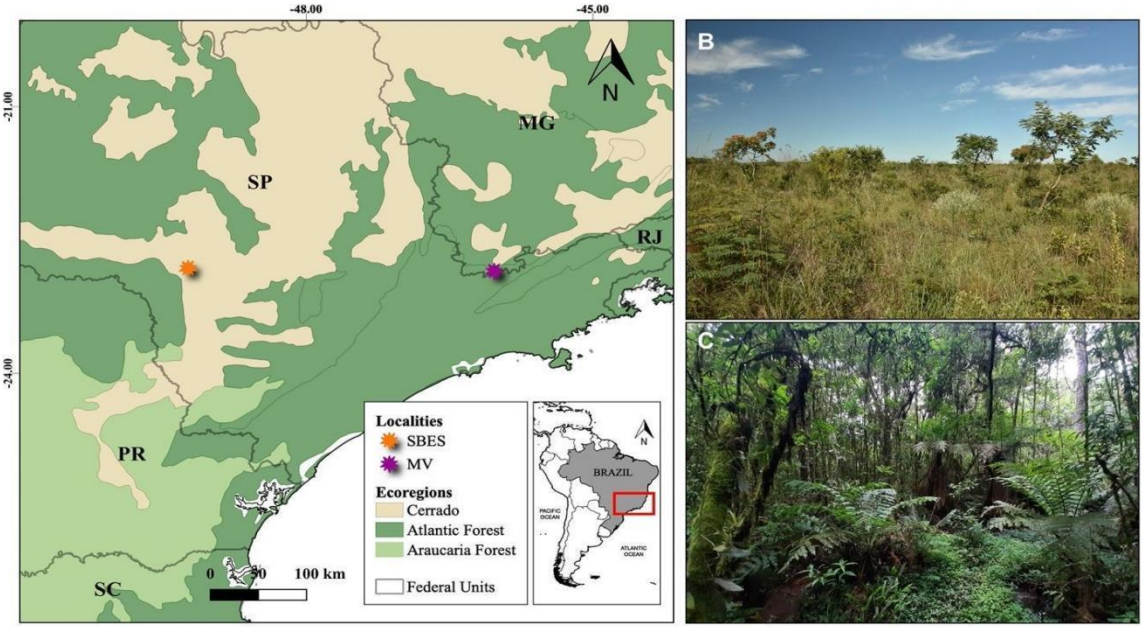


Figure 1. A) Map of southeastern Brazil showing ecoregions (adapted from Dinerstein et al., 2017) and the study sites. B) Brazilian Cerrado in the Águas de Santa Bárbara Ecological Station, São Paulo; C) Brazilian Atlantic forest in Monte Verde, Minas Gerais. Photos by Marcio Martins (B) and Filipe C. Serrano (C).

To collect body temperature data, we considered the methodology used in CAMACHO & RUSCH (2017), DIAZ-RICAURTE & SERRANO (2020a, b) for reptiles, and GUEVARA-MOLINA et al. (2020) and DIAZ-RICAURTE et al. (2020) for amphibians. We used an experimental setup that consisted of a transparent plastic box (500 x 500 x 300 mm) attached to a dark metal box wrapped in thermal resistance for heating (hereafter "heating box"; see methods in CAMACHO & RUSCH 2018; DIAZ-RICAURTE & SERRANO 2020a, b; DIAZ-RICAURTE et al., 2020; Guevara-Molina et al., 2020). Given the difference in size between the groups (e.g., anurans and snakes), two metal boxes were used, one with 350 x 200 x 250 mm for medium and large reptiles and a smaller one (150 x 80 x 80 mm) for anurans and small reptiles. For snakes and amphisbaenians, we recorded body temperature during the experiment by placing a thin thermocouple (type T; Omega®) in the animal's vent for large animals, while for lizards the final body temperature was attained with an Infrared thermometer pistol (Etekcity Lasergrip 1080). We chose to use the Infrared thermometer for lizards because they are usually smaller and more fragile (i.e. tail breakage), a methodology used in other studies on VT_{Max} (VIREN & CREE, 2019) and

confirmed by preliminary observations. For amphibians, we recorded body temperature during the experiment by externally attaching the thermocouple to the ventral region with paper tape. Previous studies have shown that the temperature of the venter in anuran amphibians does not differ from the internal temperature (GUEVARA-MOLINA et al., 2020). We moved each individual inside the metal box and after an adjustment period of five minutes at ambient temperature, the metal box was slowly heated (e.g. see CAMACHO et al., 2018; DIAZ-RICAURTE & SERRANO, 2020b; GUEVARA-MOLINA et al., 2020). We measured each individual separately to avoid thermal interference from other individuals. While heating, the box's warming rate was kept constant to guarantee that box temperature did not exceed the individual's body temperature by more than 5–6°C, in order to allow the animal to thermoregulate until it voluntarily left the box (See DIAZ-RICAURTE et al., 2020, DIAZ-RICAURTE & SERRANO, 2020 a, b, GUEVARA-MOLINA et al., 2020). Constant warming rate was possible by placing a different type T thermocouple on the surface of the heating box to record its temperature. Both thermocouples were calibrated and connected to a FieldLogger PicoLog TC - 08, recording temperature data every ten seconds. The final temperature recorded at the time the individual left the experimental box was considered as the VT_{Max}. Even though exiting the box could be due to exploratory behavior, this is unlikely since individuals would perceive the box as a refuge and thus avoid exposing themselves to potential predators, which validates VT_{Max} as a relevant behavioral, ecological and physiological threshold.

The dataset includes 312 records from 25 species of amphibians and 28 species of reptiles, totaling 53 species (Figure 2; Supplementary material). In spite of SBES having a higher number of measured species, MV had more amphibian species represented (Figure 3). Lizards had the highest VT_{Max} values (38.95 ± 2.28 ºC), followed by snakes (34.66 ± 2.04 ºC) and amphibians (30.83 ± 1.98 ºC) (Figure 4). Overall, the VT_{Max} values were similar between sites (Figure 5).

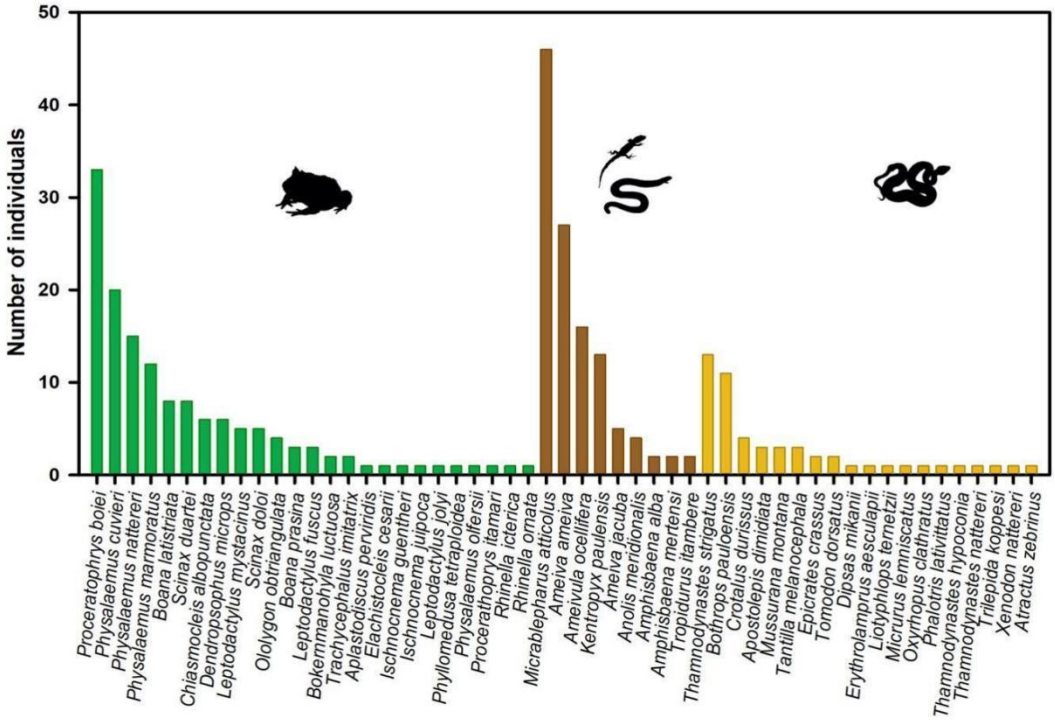


Figure 2. Distribution of the number of individuals of amphibians and reptiles sampled in the study.

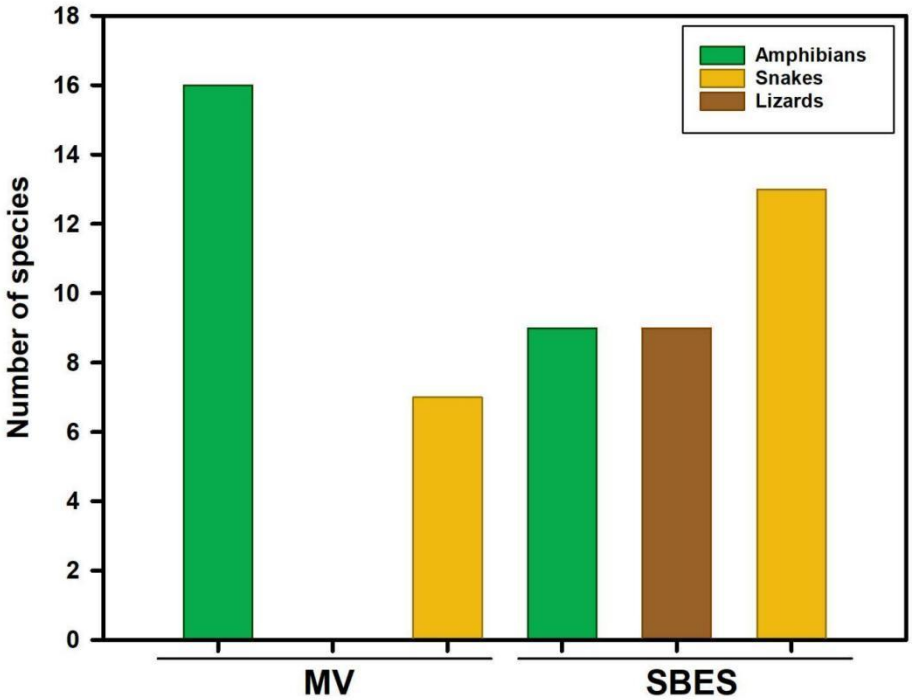


Figure 3. Number of amphibians and reptiles species for localities sampled in the study.

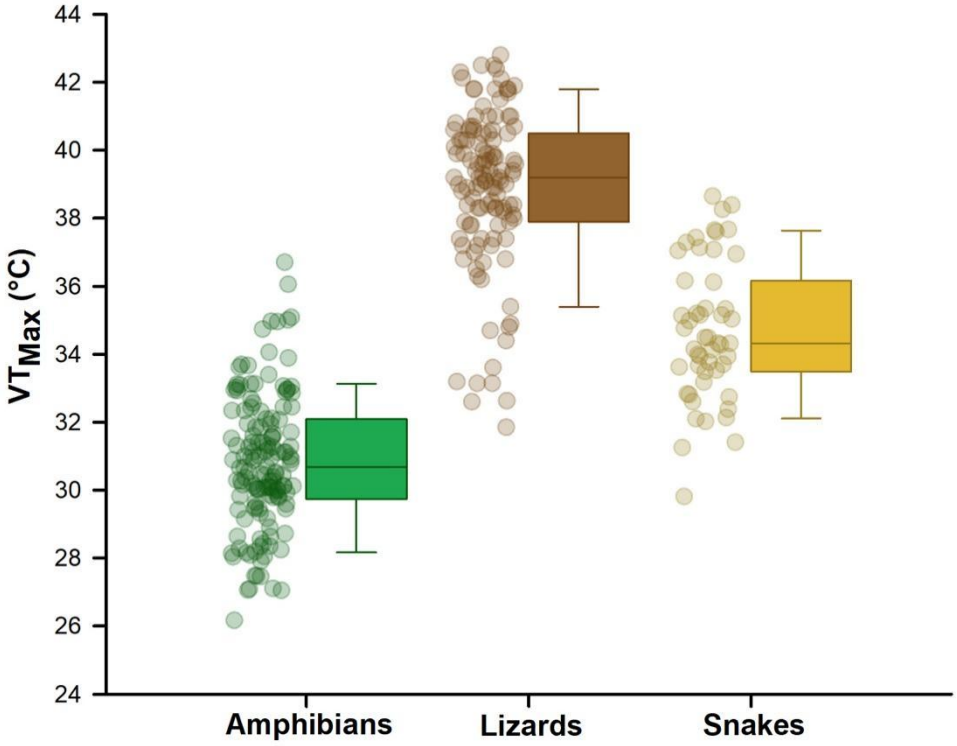


Figure 4. Comparison of VT_{Max} values of amphibians and reptiles (lizards and snakes).

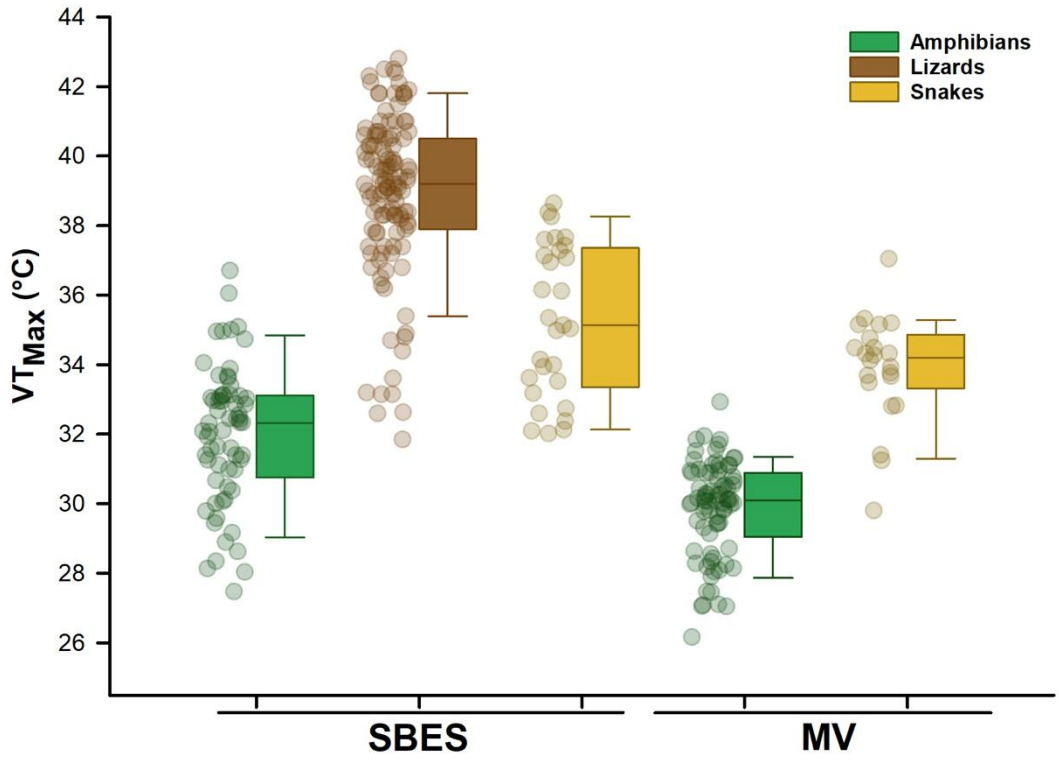


Figure 5. Comparison of VT_{Max} values of amphibians and reptiles (lizards and snakes) between the localities.

5.2.2. Class II. Research Origin Descriptors

5.2.2.1. Overall Project Description

1. **Identity**: Voluntary Thermal Maximum of Neotropical reptiles and amphibians.

2. Originators: The project "Voluntary Thermal Maximum of Neotropical reptiles and amphibians" was coordinated by Juan C. Diaz-Ricaurte. The database was compiled with help from Filipe C. Serrano and Marcio Martins. Fieldwork was carried out with a grant (See Sources of Funding Section) to Marcio Martins.

3. Period of study: **2017-2021.**

4. Objectives: The objective was to assemble a comprehensive and consistent dataset about voluntary thermal maximum (see summary above). The resultant data should facilitate novel, comparative analysis and hypothesis testing regarding behavioral thermal tolerances (i.e. VT_{Max}).

5. Abstract: Same as above.

6. Source (s) of funding: This study was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES Finance Code 001 (Juan C. Diaz-Ricaurte and Filipe C. Serrano) and by Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP (Marcio Martins, grant #2018/14091 - 8).

5.2.2.2. Specific subproject description

Sites description: Individuals were collected at two sites in Southeastern Brazil: Santa Bárbara Ecological Station (SBES) in the municipality of Águas de Santa Bárbara, state of São Paulo, and Monte Verde (MV), municipality of Camanducaia, state of Minas Gerais. Santa Bárbara Ecological Station (SBES) (22°46 'to 22°41' S and 49°16' to 49°10' W, elevation 600–680 m; Figure 1) is one of the last large remnants of Cerrado savannas (3,154 ha) in Southeastern Brazil (MELO & DURIGAN 2011). This area contains several Cerrado vegetation types such as *campo sujo*, *campo cerrado* (open areas) and *cerradão* and typical cerrado woodland (forest areas). It has a Humid subtropical climate according to Köppen's

classification (PEEL et al., 2007), with maximum temperatures of 35.2 ºC and minimum of 3.4 ºC (means of 24˚C and 17˚C in the warmest and coldest months, respectively). Also, this area experiences annual rainfall between 1,010–2,051 mm (mean = 1,454.2 mm) and two marked dry and wet seasons (April–September with a mean 70.2 mm and October–March with a mean 172.1 mm, respectively) (CIIAGRO, 2015). On the other hand, Monte Verde (MV) (22° 51'S; 46° 02'W, WGS84, 1,250–1,650 m elevation) is an Atlantic Forest remnant mixed with eucalyptus plantations in the Serra da Mantiqueira mountain range. Serra da Mantiqueira, locally called "Serra dos Poncianos", corresponds to one of the geomorphological units that make up the Campos do Jordão Plateau in the states of São Paulo and Minas Gerais. The climate of this location is classified as subtropical in altitude, with mild and rainy summers and cold, dry winters (MEIRELES et al., 2014). MV is characterized by an agroforestry mosaic, which consists of fragments of native Atlantic Forest (reaching a canopy of up to 20 m above the ground) and plantations of eucalyptus, pine and *Araucaria angustifolia*, all within the premises of the silviculture company Melhoramentos Florestal S.A. This area has an average annual rainfall of 1,740 mm, ranging from 1,380 mm to 2,360 mm. It has a maximum temperature between 26.3 and 29.7 ºC (in the hot and humid months of October – March) and a minimum temperature between 3 to 9.9 ºC (in the coldest and driest months of April – September) (data measured by Melhoramentos Florestal S. A).

Data acquisition: **See CLASS I, section F.**

Units and unit conversions: We used SI units such as ºC for temperature, g for body mass and mm for lengths such as SVL (Snout-to-Vent Length) and TL (Tail Length).

Taxonomy and systematics: Taxonomic names were used according to standardized updated online databases: The Reptile Database (https://reptile-database.reptarium.cz/) (UETZ et al., 2020) for reptiles and Amphibian Species of the World: Online Reference Vr 6.1 (https://amphibiansoftheworld.amnh.org/index.php) (FROST, 2021) for amphibians.

Legal/organizational requirements: This study was conducted under a permit for experiments by Comissão de Ética no Uso de Animais (CEUA #2325141019) of Instituto Butantan; a permit by Instituto Florestal for our work at Santa Bárbara Ecological Station (permit #260108 - 008.476/2014) and a permit by ICMBio - SISBIO (permit#50658 - 3) to

capture specimens.

1. Project personnel: Juan Diaz-Ricaurte, Filipe C. Serrano, Marcio Martins.

5.2.3. Class III. Data Set Status and Accessibility

A. Status

1. **Latest update:** December 2020. Data compilation is ongoing and will be added as collected and verified.
2. **Latest archive**: December 2020.
3. **Metadata status:** Metadata is complete and up-to-date.
4. **Data verification:** *Juan C. Diaz-Ricaurte*, with assistance from *Filipe C. Serrano*, entered records directly from experiments into a .csv document. All values were checked before inclusion in the joint dataset and once again before publication.

B. Accessibility

1. **Contact person:** Juan C. Diaz-Ricaurte (email: juan.diaz@usp.br / bio.jcd@gmail.com), Filipe C. Serrano (e-mail: filipe.serrano@usp.br), Marcio Martins (e-mail: martinsmrc@usp.br) Departamento de Ecologia, Universidade de São Paulo, São Paulo, SP, 05508-090, Brazil.

2. **Storage location and medium:** The data set is available as Supporting Information to this Data Paper publication in Ecology. A digital version of the data set in csv format is held by Juan C. Diaz-Ricaurte.

3. **Copyright restrictions:** None.

4. **Proprietary restrictions:** None, except this data paper should be cited when data are used for publication. In addition, we would appreciate hearing for which research projects or teaching purposes these data are used.
5. **Costs:** None.

5.2.4. Class IV. Data Structural Descriptors

A. Data set file

1. **Identity:** VTMaxHerp _Database.csv
2. **Size:** 313 records (including header) and 20 fields, tab delimited. 54 KB

3. **Format and storage mode**: comma-separated values (.csv).
4. **Header information:** See column description in section B.
5. **Row information:** Each row represents an individual with its respective biometry, experimental values, capture and experimental date.

Alphanumeric attributes: Mixed.

6. **Special characters/fields:** None.

B. Authentication procedures: None.

C. Variable information

Table 1: Description of the fields related to species information – VTMaxHerp_Database.csv

TYPE OF INFORMATION	FIELD	DESCRIPTION	EXAMPLE
Biological	*ID*	Unique identifier code for each row	1
	Order	The taxonomic order of the species	Squamata
	Family	The taxonomic family of the species	Viperidae
	Genus	The taxonomic genus of the species	*Bothrops*
	Species	Taxonomic name of the species	*Bothrops pauloensis*
	Weight	The mass of the individual in grams	5.5
	Sex	Sex	F
	SVL	Snout-to-vent length in millimeters	580
	TL	Tail length in millimeters	110
Site	*Locality*	Specific location of the study	ASB
Capture	*Capture_date*	Data of collection from the wild, in dd/mm/yyyy	15/03/21
Experimental	*Experiment_date*	Data of experimental procedures, in dd/mm/yyyy	16/03/21
	Start_time	Time at which the experiment started, in hh:mm	15:00

	Final_time	Time at which the experiment ended, in hh:mm	15:15
	Duration_of_experiments	Total time elapsed since Start_time and Final_time, in minutes	15
	Initial_body_temperature	Body temperature of individual prior to the experiments, in ºC	23.5
	Final_body_temperature	Body temperature of individual at the end of the experiments, in ºC	35.6
	VT_{Max}	Maximum temperature tolerated by individual, before exiting the heating box (Same as the previous field), in ºC	35.6
	Heating_rate	The rate at which each individual raised its body temperature, in degrees per minute	0.146
Reference	*Source*	Extended information of the reference	Diaz-Ricaurte, J. C., F. Serrano., E. C. Guevara-Molina., C. Araujo, and M. Martins. 2020. Behavioral thermal tolerance predicts distribution pattern but not habitat use in sympatric Neotropical frogs. PLoS ONE 15:e0239485.

5.2.5. Class V. Supplemental Descriptors

A. Data acquisition

1. Data request history: None

2. Data set updates history: None

3. Data entry/verification procedures

G. History of data set usage:

5.3. Additional publications about Chapter 4

5.3.1 Behavioral Thermoregulation of Amphibians and Reptiles From Two Brazilian Hotspots

Published

DIAZ-RICAURTE JC, SERRANO CF, MARTINS, M. 2022. Behavioral Thermoregulation of Amphibians and Reptiles from Two Brazilian Hotspots. ***The Bulletin of the Ecological Society of America***, 103(2): e01965. https://doi.org/10.1002/bes2.1965

Study description

We present novel data on the behavioral thermal tolerances of 53 species of amphibians and reptiles of the Atlantic Forest and Cerrado savannas of South America, to improve the understanding of their thermal ecology and physiology. Our results indicate that lizards have higher voluntary thermal maximum values (VT_{Max}, temperatures tolerated by individuals before actively moving to a colder place) than amphibians and snakes, and individuals in the Atlantic Forest had overall higher VT_{Max} values than those in Cerrado. This information is useful for understanding species vulnerabilities to recent climate change.

Photo 1: Brazilian Atlantic forest at Monte Verde, Minas Gerais, Brazil. Araucaria trees and ferns are common in this region of Atlantic Forest. Photo credits: Marcio Martins (top left), Filipe C. Serrano (bottom left), Juan C. Diaz-Ricaurte (right).

Photo 2: Brazilian Cerrado at the Santa Bárbara Ecological Station, São Paulo, Brazil. In this vegetation type of the Cerrado, scattered trees, sandy soils and high thermal variability predominate and can impose both behavioral and thermal challenges for the species that inhabit it. Photo credit: Marcio Martins.

Photo 3: Ectotherms regularly require external sources of heat to carry out both their biological and physiological processes. A snake (*Bothrops jararacussu*) can be seen apparently thermoregulating on a rock. Thus, in some environments with less temperature variability, such as forests, the availability of thermally favorable microhabitats facilitates the thermoregulation of ectothermic animals. Photo credit: Marcio Martins.

Photo 4: Usually, in open areas of the Cerrado, ectothermic animals (e.g., amphibians and reptiles) avoid predators, natural fires, and/or temperatures that may expose them to their upper thermal limits. Thus, they commonly use burrows either to thermoregulate or to avoid being exposed to high temperatures and predators. For example, in the left two images, two individuals are taking refuge in burrows (the lizard, *Ameiva ameiva*, and the snake, *Bothrops pauloensis*, respectively). The lizard shows a behavioral thermoregulation (reorientation) mechanism, while the snake is evidently taking refuge in the burrow. The right image shows the entrance to a burrow after the natural fire has passed in the Cerrado. Thus, taking refuge is vital for the persistence of species that inhabit the more open Cerrado vegetation types. Photo credit: Marcio Martins.

Photo 5: As a result of the challenges that environments impose on ectothermic animals, various behavioral strategies have been observed in nature. For example, in the Cerrado, many species of amphibians and reptiles burrow in the sand to avoid both predators and temperatures that may exceed their maximum tolerated temperatures (like the lizard, *Micrablepharus atticolus*, in the upper left panel, and the frog, *Physalaemus marmoratus*, in the lower left). On the other hand, in the Atlantic Forest, species can use herbaceous vegetation to protect themselves during the day from predators and challenging temperatures (like the treefrog, *Aplastodiscus perviridis*, in the right panel). Photo credits: Carolina Farhat (left two images), Juan C. Diaz-Ricaurte (right image).

These photographs illustrate the paper "VTMaxHerp: a data set of Voluntary Thermal Maximum temperatures of amphibians and reptiles from two Brazilian hotspots" by Juan C. Diaz-Ricaurte, Filipe C. Serrano, and Marcio Martins published in *Ecology*. https://doi.org/10.1002/ecy.3602

Acknowledgments

We thank the other members of the herpetological team (João Paulo dos Santos Vieira de Alencar, Gabriella Leal and Carolina Farhat) and the mastozoological team (Giovana Felício, Luciana Furtado and Patrícia Marques) led by Ana Paula Carmignotto for their occasional help and support in the field; and the technical staff from Instituto Florestal at Estação Ecológica de Santa Bárbara and Companhia Melhoramentos for logistical support. Furthermore, we thank Luisa Maria Diele-Viegas and an anonymous reviewer for their helpful comments and suggestions which improved the manuscript.

Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article.

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Marcio Martins, https://orcid.org/0000-0001-8108-6309

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Supplementary Material

Supplementary material S1. VTMaxHerp_Database. https://doi.org/10.1002/ecy.3602

ID	Order	Family	Genus	Species	Locality	Cap_date	Exp_date	SVL	TL	ST	FT	Time	IBT	FBT	VT_{Max}	Weight	Sex	HR	Source
1	Squamata	Viperidae	*Crotalus*	*Crotalus durissus*	SBES	09/03/18	09/03/18	825	93	0.833	0.953	173	26.66	38.26	38.26	400	M	0.067	This work
2	Squamata	Viperidae	*Bothrops*	*Bothrops pauloensis*	SBES	10/03/18	10/03/18	590	110	0.675	0.712	53	25.39	34.99	34.99	59	F	0.181	Diaz-Ricaurte & Serrano. 2020b.
3	Squamata	Viperidae	*Bothrops*	*Bothrops pauloensis*	SBES	10/03/18	10/03/18	558	93	0.848	0.900	75	29.26	33.95	33.95	78	F	0.063	Diaz-Ricaurte & Serrano. 2020b.
4	Squamata	Viperidae	*Crotalus*	*Crotalus durissus*	SBES	15/03/18	15/03/18	540	42	0.882	0.034	239	28.52	36.12	36.12	193	F	0.032	This work
5	Squamata	Viperidae	*Bothrops*	*Bothrops pauloensis*	SBES	17/03/18	17/03/18	139	101	0.688	0.722	49	29.64	35.04	35.04	117	M	0.11	Diaz-Ricaurte & Serrano. 2020b.
6	Squamata	Viperidae	*Bothrops*	*Bothrops pauloensis*	SBES	23/10/18	23/10/18	625	88	0.808	0.842	48	26.71	33.18	33.18	95	F	0.135	Diaz-Ricaurte &

ID	Order	Family	Genus	Species	Locality	Cap_date	Exp_date	SVL	TL	ST	FT	Time	IBT	FBT	VT_{Max}	Weight	Sex	HR	Source
7	Squamata	Viperidae	*Bothrops*	*Bothrops pauloensis*	SBES	25/10/18	25/10/18	490	84	0.927	0.966	56	24.6	37.08	37.08	67	M	0.223	Diaz-Ricaurte & Serrano. 2020b.
8	Squamata	Amphisbaenidae	*Amphisbaena*	*Amphisbaena alba*	SBES	15/03/19	15/03/19	420	41	-	-	36	30.6	34.7	34.7	110	F	0.114	Diaz-Ricaurte & Serrano. 2020a.
9	Squamata	Amphisbaenidae	*Amphisbaena*	*Amphisbaena alba*	SBES	15/03/19	15/03/19	213	32	-	-	50	30.8	34.4	34.4	12	M	0.072	Diaz-Ricaurte & Serrano. 2020a.
10	Squamata	Amphisbaenidae	*Amphisbaena*	*Amphisbaena mertensii*	SBES	30/03/19	30/03/19	33.4	5.6	0.479	0.494	21	26.65	32.63	32.63	18.83	M	0.285	This work
11	Squamata	Elapidae	*Micrurus*	*Micrurus lemniscatus*	SBES	30/03/19	30/03/19	317	25	0.638	0.660	31	25.9	36.16	36.16	6.34	M	0.331	This work
12	Squamata	Viperidae	*Bothrops*	*Bothrops pauloensis*	SBES	24/04/19	24/04/19	580	75	0.641	0.660	26	30.3	38.39	38.39	98	M	0.311	Diaz-Ricaurte & Serrano. 2020b.
13	Squamata	Dipsadidae	*Erythrolamprus*	*Erythrolamprus aesculapii*	SBES	10/09/19	10/09/19	468	63	0.560	0.571	15	25.37	32.13	32.13	20.78	F	0.451	This work
14	Squamata	Dipsadidae	*Dipsas*	*Dipsas mikanii*	SBES	10/09/19	10/09/19	221	44	0.560	0.567	10	25.06	32.02	32.02	3.41	M	0.696	This work

ID	Order	Family	Genus	Species	Locality	Cap_date	Exp_date	SVL	TL	ST	FT	Time	IBT	FBT	VT_{Max}	Weight	Sex	HR	Source
15	Squamata	Viperidae	*Crotalus*	*Crotalus durissus*	SBES	30/09/19	30/09/19	550	52	0.708	0.753	65	21.82	32.1	32.1	186	F	0.158	This work
16	Squamata	Dipsadidae	*Apostolepis*	*Apostolepis dimidiata*	SBES	30/09/19	30/09/19	435	39	0.442	0.476	49	23.6	34.15	34.15	8.57	F	0.215	This work
17	Squamata	Dipsadidae	*Apostolepis*	*Apostolepis dimidiata*	SBES	30/09/19	30/09/19	390	40	0.631	0.674	62	23.8	33.63	33.63	8.84	F	0.159	This work
18	Squamata	Viperidae	*Bothrops*	*Bothrops pauloensis*	SBES	30/11/19	30/11/19	440	56	0.615	0.625	14	28.1	35.14	35.14	70	M	0.503	Diaz-Ricaurte & Serrano. 2020b.
19	Squamata	Viperidae	*Bothrops*	*Bothrops pauloensis*	SBES	30/11/19	30/11/19	500	39.5	0.588	0.603	22	27.2	36.95	36.95	120	F	0.443	Diaz-Ricaurte & Serrano. 2020b.
20	Squamata	Viperidae	*Bothrops*	*Bothrops pauloensis*	SBES	30/11/19	30/11/19	510	77	0.485	0.501	23	27.14	33.53	33.53	90	M	0.278	Diaz-Ricaurte & Serrano. 2020b.
21	Squamata	Viperidae	*Bothrops*	*Bothrops pauloensis*	SBES	30/11/19	30/11/19	590	50	0.557	0.572	22	26.8	37.29	37.29	180	F	0.477	Diaz-Ricaurte & Serrano. 2020b.
22	Squamata	Viperidae	*Bothrops*	*Bothrops pauloensis*	SBES	30/11/19	30/11/19	459	71	0.533	0.549	24	26.7	34	34	100	F	0.304	Diaz-Ricaurte & Serrano. 2020b.

ID	Order	Family	Genus	Species	Locality	Cap_date	Exp_date	SVL	TL	ST	FT	Time	IBT	FBT	VT_{Max}	Weight	Sex	HR	Source
23	Squamata	Leptotyphlopidae	*Trilepida*	*Trilepida koppesi*	SBES	28/01/20	28/01/20	222	20	0.814	0.822	22	31.78	33.61	33.61	6	M	0.083	This work
24	Squamata	Colubridae	*Tantilla*	*Tantilla melanocephala*	SBES	28/01/20	28/01/20	230	78	0.830	0.838	12	33.49	37.67	37.67	5	M	0.348	This work
25	Anura	Leptodactylidae	*Leptodactylus*	*Leptodactylus jolyi*	SBES	28/01/20	28/01/20	48	-	0.846	0.862	23	30.91	35.09	35.09	11	-	0.182	This work
26	Anura	Leptodactylidae	*Leptodactylus*	*Leptodactylus fuscus*	SBES	28/01/20	28/01/20	45	-	0.867	0.890	33	32.07	34.06	34.06	13	-	0.06	This work
27	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus marmoratus*	SBES	28/01/20	28/01/20	20	-	0.896	0.900	6	30.56	31.12	31.12	0.5	-	0.093	This work
28	Squamata	Dipsadidae	*Thamnodynastes*	*Thamnodynastes hypoconia*	SBES	29/01/20	29/01/20	393	124	0.799	0.810	16	30.95	37.6	37.6	23	F	0.416	This work
29	Anura	Phyllomedusidae	*Phyllomedusa*	*Phyllomedusa tetraploidea*	SBES	30/01/20	30/01/20	74	-	0.631	0.642	15	29.77	32.68	32.68	18	-	0.194	This work
30	Squamata	Dipsadidae	*Phalotris*	*Phalotris lativittatus*	SBES	30/01/20	30/01/20	551	78	0.649	0.663	24	31.36	35.35	35.35	53	F	0.166	This work
31	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	16/03/20	16/03/20	30	47	0.636	0.652	23	28	40.7	40.7	1	-	0.552	This work
32	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	16/03/20	16/03/20	95	130	0.636	0.665	42	28.5	39.6	39.6	18	F	0.264	This work
33	Squamata	Teiidae	*Ameivula*	*Ameivula ocellifera*	SBES	16/03/20	16/03/20	75	106	0.636	0.703	37	25.8	39	39	7	F	0.357	This work
34	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	16/03/20	16/03/20	43	64	0.636	0.656	28	27.4	41.3	41.3	1.3	M	0.496	This work
35	Squamata	Colubridae	*Tantilla*	*Tantilla melanocephala*	SBES	16/03/20	16/03/20	111	35	0.697	0.699	3	33.82	37.65	37.65	0.56	M	1.277	This work
36	Squamata	Boidae	*Epicrates*	*Epicrates crassus*	SBES	17/03/20	17/03/20	1090	98	0.614	0.662	69	29.32	32.38	32.38	900	M	0.044	This work
37	Anura	Leptodactylidae	*Leptodactylus*	*Leptodactylus fuscus*	SBES	17/03/20	17/03/20	41	-	0.884	0.891	10	28.73	32.97	32.97	5.16	-	0.424	This work

38	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus marmoratus*	SBES	17/03/20	17/03/20	28	-	0.896	0.899	5	29.48	31.4	31.4	1.78	-	0.384	This work
ID	**Order**	**Family**	**Genus**	**Species**	**Locality**	**Cap_date**	**Exp_date**	**SVL**	**TL**	**ST**	**FT**	**Time**	**IBT**	**FBT**	**VT_{Max}**	**Weight**	**Sex**	**HR**	**Source**
39	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus marmoratus*	SBES	17/03/20	17/03/20	28	-	0.906	0.909	5	30.24	32.95	32.95	1.88	-	0.542	This work
40	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus marmoratus*	SBES	17/03/20	17/03/20	33	-	0.917	0.924	10	29.35	31	31	2.2	-	0.165	This work
41	Squamata	Teiidae	*Kentropyx*	*Kentropyx paulensis*	SBES	18/03/20	18/03/20	66	133	0.543	0.570	40	27.6	42.8	42.8	11.87	M	0.38	This work
42	Anura	Leptodactylidae	*Leptodactylus*	*Leptodactylus fuscus*	SBES	18/03/20	18/03/20	41	-	0.755	0.761	9	28.19	33.12	33.12	3.11	-	0.548	This work
43	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus marmoratus*	SBES	18/03/20	18/03/20	32.5	-	0.572	0.575	4	30.7	32.34	32.34	4.2	-	0.41	This work
44	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus marmoratus*	SBES	18/03/20	18/03/20	36.5	-	0.578	0.587	15	27.97	31.4	31.4	4.9	-	0.229	This work
45	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus marmoratus*	SBES	18/03/20	18/03/20	34.2	-	0.589	0.594	8	26.22	32.86	32.86	3.19	-	0.83	This work
46	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus marmoratus*	SBES	18/03/20	18/03/20	42	-	0.776	0.783	10	30.46	31.6	31.6	4.6	-	0.114	This work
47	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus marmoratus*	SBES	18/03/20	18/03/20	38	-	0.619	0.624	6	26.5	32.32	32.32	4.1	-	0.97	This work
48	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus marmoratus*	SBES	18/03/20	18/03/20	32	-	0.626	0.629	4	26.95	31.64	31.64	3.8	-	1.173	This work
49	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus marmoratus*	SBES	18/03/20	18/03/20	37	-	0.635	0.639	6	25.25	32.88	32.88	4	-	1.272	This work
50	Anura	Leptodactylidae	*Leptodactylus*	*Leptodactylus mystacinus*	SBES	18/03/20	18/03/20	69	-	0.558	0.567	14	28.29	32.11	32.11	18.48	-	0.273	This work
51	Anura	Leptodactylidae	*Leptodactylus*	*Leptodactylus mystacinus*	SBES	18/03/20	18/03/20	49	-	0.696	0.701	8	28.38	33.7	33.7	16.15	-	0.665	This work
52	Anura	Leptodactylidae	*Leptodactylus*	*Leptodactylus mystacinus*	SBES	18/03/20	18/03/20	33	-	0.703	0.708	6	27.36	32.56	32.56	3.04	-	0.867	This work

53	Anura	Leptodactylidae	*Leptodactylus*	*Leptodactylus mystacinus*	SBES	18/03/20	18/03/20	37	-	0.713	0.724	15	27.51	34.96	34.96	2.63	-	0.497	This work
54	Anura	Leptodactylidae	*Leptodactylus*	*Leptodactylus mystacinus*	SBES	18/03/20	18/03/20	44	-	0.732	0.738	11	25.94	33.4	33.4	3.7	-	0.678	This work
ID	**Order**	**Family**	**Genus**	**Species**	**Locality**	**Cap_date**	**Exp_date**	**SVL**	**TL**	**ST**	**FT**	**Time**	**IBT**	**FBT**	**VT_{Max}**	**Weight**	**Sex**	**HR**	**Source**
55	Anura	Microhylidae	*Chiasmocleis*	*Chiasmocleis albopunctata*	SBES	18/03/20	18/03/20	29	-	0.645	0.652	10	27.8	33.63	33.63	1.44	-	0.583	This work
56	Anura	Microhylidae	*Chiasmocleis*	*Chiasmocleis albopunctata*	SBES	18/03/20	18/03/20	31	-	0.656	0.659	5	26.09	32.43	32.43	1.49	-	1.268	This work
57	Anura	Microhylidae	*Chiasmocleis*	*Chiasmocleis albopunctata*	SBES	18/03/20	18/03/20	29.3	-	0.662	0.667	8	25.84	33.1	33.1	1.6	-	0.908	This work
58	Anura	Microhylidae	*Chiasmocleis*	*Chiasmocleis albopunctata*	SBES	18/03/20	18/03/20	27.3	-	0.669	0.674	7	26.09	32.34	32.34	1.2	-	0.893	This work
59	Anura	Microhylidae	*Chiasmocleis*	*Chiasmocleis albopunctata*	SBES	18/03/20	18/03/20	30.4	-	0.677	0.684	10	26.49	33.13	33.13	1.7	-	0.664	This work
60	Anura	Microhylidae	*Chiasmocleis*	*Chiasmocleis albopunctata*	SBES	18/03/20	18/03/20	33.2	-	0.688	0.692	6	26.19	32.95	32.95	2.1	-	1.127	This work
61	Anura	Microhylidae	*Elachistocleis*	*Elachistocleis cesarii*	SBES	18/03/20	18/03/20	29	-	0.770	0.774	6	28.06	29.45	29.45	2.93	-	0.232	This work
62	Squamata	Colubridae	*Tantilla*	*Tantilla melanocephala*	SBES	19/03/20	19/03/20	105	23	0.582	0.588	9	27.92	37.14	37.14	2.3	M	1.024	This work
63	Squamata	Dipsadidae	*Xenodon*	*Xenodon nattereri*	SBES	19/03/20	19/03/20	134	18	0.598	0.605	10	30.54	37.43	37.43	3.4	F	0.689	This work
64	Squamata	Dactyloidae	*Anolis*	*Anolis meridionalis*	SBES	19/03/20	19/03/20	40	93	0.509	0.524	12	25.6	33.15	33.15	1.45	M	0.629	This work
65	Squamata	Dactyloidae	*Anolis*	*Anolis meridionalis*	SBES	20/03/20	20/03/20	40	93	0.509	0.524	22	25.6	33.15	33.15	1.7	M	0.343	This work
66	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	22/04/19	24/04/19	32	80	0.633	0.645	18	29	38.2	38.2	0.8	-	0.511	This work
67	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	22/04/19	24/04/19	30	86	0.633	0.643	15	28.6	36.7	36.7	0.58	F	0.54	This work

68	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	22/07/19	22/07/19	35.4	136	0.653	0.680	39	23.9	36.3	36.3	1.1	M	0.318	This work
69	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	22/07/19	22/07/19	35	140	0.653	0.680	39	23.3	36.8	36.8	1	M	0.346	This work
70	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	22/07/19	22/07/19	36.5	141	0.653	0.680	39	23.6	37	37	1.2	M	0.344	This work
71	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	22/07/19	22/07/19	39.4	99.4	0.683	0.707	34	21.4	37.4	37.4	1	M	0.471	This work
72	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	22/07/19	22/07/19	35	80	0.683	0.697	19	22	36.5	36.5	0.6	M	0.763	This work
73	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	24.4	-	0.339	0.379	30	27.18	28.04	28.04	1.29	F	0.029	Diaz-Ricaurte et al. 2020.
74	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	31	-	0.382	0.403	29	27.29	31.94	31.94	2.27	M	0.16	Diaz-Ricaurte et al. 2020.
75	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	30	-	0.407	0.447	56	25.98	31.28	31.28	2.66	F	0.095	Diaz-Ricaurte et al. 2020.
76	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	29	-	0.456	0.478	32	24.38	30.07	30.07	2.01	M	0.178	Diaz-Ricaurte et al. 2020.
77	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	31	-	0.482	0.520	56	28.11	28.63	28.63	2.1	-	0.009	Diaz-Ricaurte et al. 2020.

78	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	26.6	-	0.522	0.558	47	26.62	30.12	30.12	1.53	-	0.074	Diaz-Ricaurte et al. 2020.
79	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	30	-	0.561	0.622	86	25.37	29.17	29.17	1.99	-	0.044	Diaz-Ricaurte et al. 2020.
81	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	34	-	0.660	0.685	37	26.1	30.48	30.48	2.23	-	0.118	Diaz-Ricaurte et al. 2020.
82	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	19.9	-	0.040	0.053	17	28.99	28.9	28.9	1.9	M	-0.005	Diaz-Ricaurte et al. 2020.
83	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	29.4	-	0.056	0.077	31	27.38	31.4	31.4	2.09	F	0.13	Diaz-Ricaurte et al. 2020.
84	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	30	-	0.081	0.098	24	27.64	29.79	29.79	3.19	F	0.09	Diaz-Ricaurte et al. 2020.
85	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	28	-	0.101	0.113	18	26.58	33.13	33.13	1.78	-	0.364	Diaz-Ricaurte et al. 2020.
86	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	25.5	-	0.768	0.808	54	22.95	32.07	32.07	3.29	M	0.169	Diaz-Ricaurte et al. 2020.

87	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	29	-	0.854	0.889	49	25.78	30.98	30.98	3.39	F	0.106	Diaz-Ricaurte et al. 2020.
88	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	31.4	-	0.894	0.926	46	27.54	31.58	31.58	3.39	F	0.088	Diaz-Ricaurte et al. 2020.
89	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	24	-	0.930	0.934	6	27.75	28.14	28.14	1.19	M	0.065	Diaz-Ricaurte et al. 2020.
90	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	29.4	-	0.941	0.958	24	25.93	28.35	28.35	2.43	F	0.101	Diaz-Ricaurte et al. 2020.
91	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	28.3	-	0.965	0.992	39	27.12	31.26	31.26	2.53	F	0.106	Diaz-Ricaurte et al. 2020.
92	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus cuvieri*	SBES	22/10/18	22/10/18	22.1	-	0.999	0.038	55	29.43	33.05	33.05	2.41	F	0.066	Diaz-Ricaurte et al. 2020.
93	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus nattereri*	SBES	22/10/18	22/10/18	40.4	-	0.002	0.026	34	26.76	30.67	30.67	7.9	-	0.115	Diaz-Ricaurte et al. 2020.
94	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus nattereri*	SBES	22/10/18	22/10/18	40.4	-	0.481	0.515	48	25.79	32.09	32.09	7.51	-	0.131	Diaz-Ricaurte et al. 2020.

95	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus nattereri*	SBES	22/10/18	22/10/18	37.7	-	0.656	0.683	41	30.83	36.71	36.71	6.28	-	0.143	Diaz-Ricaurte et al. 2020.
96	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus nattereri*	SBES	22/10/18	22/10/18	38.1	-	0.687	0.722	44	31.11	34.74	34.74	6.4	-	0.083	Diaz-Ricaurte et al. 2020.
97	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus nattereri*	SBES	22/10/18	22/10/18	37.4	-	0.699	0.744	62	25.28	35.01	35.01	4.94	-	0.157	Diaz-Ricaurte et al. 2020.
98	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus nattereri*	SBES	22/10/18	22/10/18	27.9	-	0.727	0.735	12	31.13	33.89	33.89	7.24	-	0.23	Diaz-Ricaurte et al. 2020.
99	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus nattereri*	SBES	22/10/18	22/10/18	38	-	0.742	0.747	6	30.36	33.03	33.03	10.65	-	0.445	Diaz-Ricaurte et al. 2020.
100	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus nattereri*	SBES	22/10/18	22/10/18	42	-	0.750	0.772	32	22.73	33.67	33.67	7.41	-	0.342	Diaz-Ricaurte et al. 2020.
101	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus nattereri*	SBES	22/10/18	22/10/18	37.4	-	0.756	0.813	81	26.94	33.07	33.07	5.18	-	0.076	Diaz-Ricaurte et al. 2020.
102	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus nattereri*	SBES	22/10/18	22/10/18	49	-	0.821	0.846	36	26.33	34.97	34.97	19.27	-	0.24	Diaz-Ricaurte et al. 2020.

103	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus nattereri*	SBES	22/10/18	22/10/18	37	-	0.852	0.881	41	29.34	32.45	32.45	32.45	-	0.076	Diaz-Ricaurte et al. 2020.
104	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus nattereri*	SBES	22/10/18	22/10/18	34	-	0.884	0.933	71	25.14	29.59	29.59	4.86	-	0.063	Diaz-Ricaurte et al. 2020.
105	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus nattereri*	SBES	22/10/18	22/10/18	36	-	0.937	0.967	44	28.45	30.38	30.38	5.94	-	0.044	Diaz-Ricaurte et al. 2020.
106	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus nattereri*	SBES	22/10/18	22/10/18	40.4	-	0.972	0.997	36	26.49	30.01	30.01	7.63	-	0.098	Diaz-Ricaurte et al. 2020.
107	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus nattereri*	SBES	22/10/18	22/10/18	43.1	-	0.481	0.026	44	24.97	32.45	32.45	11.98	-	0.17	Diaz-Ricaurte et al. 2020.
108	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	23/04/19	24/04/19	68	144	0.735	0.757	32	30.5	39.7	39.7	10.09	F	0.288	This work
109	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	23/04/19	24/04/19	70	170	0.735	0.751	24	29.2	39.2	39.2	10.89	F	0.417	This work
110	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	23/04/19	24/04/19	61.5	136	0.771	0.785	20	28.7	39.6	39.6	7.5	M	0.545	This work
111	Squamata	Teiidae	*Kentropyx*	*Kentropyx paulensis*	SBES	23/04/19	24/04/19	67.5	135	0.735	0.747	17	28.7	39.6	39.6	9.8	M	0.641	This work
112	Squamata	Tropiduridae	*Tropidurus*	*Tropidurus itambere*	SBES	23/04/19	24/04/19	36	-	0.721	0.728	11	28.2	38.6	38.6	2.63	M	0.945	This work
113	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	23/07/19	23/07/19	35.7	80.7	0.717	0.747	43	21	38.1	38.1	1	F	0.398	This work

114	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	23/07/19	23/07/19	39.2	66	0.717	0.744	39	21.1	40.3	40.3	1	M	0.492	This work
115	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	23/07/19	23/07/19	34.8	79.8	0.717	0.742	36	21.4	40.5	40.5	1.4	F	0.531	This work
116	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	23/07/19	23/07/19	41.3	67	0.717	0.736	28	22	39.3	39.3	2	F	0.618	This work
117	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	23/07/19	23/07/19	39.2	69	0.752	0.782	43	24.3	38.3	38.3	1.5	M	0.326	This work
118	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	23/07/19	23/07/19	33.4	90.4	0.752	0.780	40	23.7	39.1	39.1	1.23	M	0.385	This work
119	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	23/07/19	23/07/19	36.5	59	0.752	0.776	35	25.3	38.3	38.3	1.2	-	0.371	This work
120	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	24/04/19	24/04/19	46.5	955	0.771	0.783	17	28.3	38.9	38.9	6.2	F	0.624	This work
121	Squamata	Teiidae	*Ameivula*	*Ameivula ocellifera*	SBES	24/04/19	24/04/19	38.5	-	0.721	0.728	10	27.9	39.7	39.7	2.2		1.18	This work
122	Squamata	Teiidae	*Ameivula*	*Ameivula ocellifera*	SBES	24/04/19	24/04/19	51	96	0.771	0.790	27	28.1	40.6	40.6	4.8	M	0.463	This work
123	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	24/04/19	24/04/19	31	-	0.721	0.726	8	29	37.8	37.8	1.2	-	1.1	This work
124	Squamata	Teiidae	*Ameivula*	*Ameivula ocellifera*	SBES	24/07/19	24/07/19	50.5	133	0.556	0.580	35	21.3	37.4	37.4	4.35	M	0.46	This work
125	Squamata	Teiidae	*Kentropyx*	*Kentropyx paulensis*	SBES	24/07/19	24/07/19	48.7	139.7	0.556	0.573	25	21.2	34.8	34.8	3	F	0.544	This work
126	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	24/07/19	24/07/19	33.6	66.3	0.556	0.561	8	20.8	39.4	39.4	1.5	-	2.325	This work
127	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	24/07/19	24/07/19	35.1	92.1	0.556	0.566	15	21	39.9	39.9	1.8	M	1.26	This work
128	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	24/07/19	24/07/19	36	92	0.569	0.590	30	22.3	39.1	39.1	0.8	-	0.56	This work
129	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	24/07/19	24/07/19	40	101	0.569	0.592	33	23	40.3	40.3	1	M	0.524	This work

130	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	24/07/19	24/07/19	32	88	0.594	0.608	20	25.2	40.5	40.5	1.5	-	0.765	This work
131	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	24/07/19	24/07/19	38.8	103.8	0.594	0.612	25	24.5	37.9	37.9	1.3	-	0.536	This work
132	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	24/07/19	24/07/19	36.9	90.9	0.594	0.610	22	23.4	39.2	39.2	1	-	0.718	This work
133	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	24/07/19	24/07/19	34.7	81.7	0.594	0.614	28	24.9	38.9	38.9	1	-	0.5	This work
134	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	24/07/19	24/07/19	41.3	95.3	0.616	0.641	36	24.1	38.8	38.8	2.1	F	0.408	This work
135	Squamata	Teiidae	*Ameivula*	*Ameivula ocellifera*	SBES	24/08/19	24/08/19	64.3	172.3	0.642	0.670	41	17.7	39.8	39.8	7.5	M	0.539	This work
136	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	24/08/19	24/08/19	41	94	0.642	0.674	46	19.2	40.6	40.6	2.1	F	0.465	This work
137	Squamata	Teiidae	*Ameivula*	*Ameivula ocellifera*	SBES	25/02/20	25/02/20	40	105	0.680	0.702	32	24.6	39	39	7.5	-	0.45	This work
138	Squamata	Teiidae	*Kentropyx*	*Kentropyx paulensis*	SBES	25/02/20	25/02/20	72	167	0.568	0.590	31	24.9	40.3	40.3	9	M	0.497	This work
139	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	25/04/19	25/04/19	166	300	0.676	0.724	68	26.5	41.8	41.8	183	M	0.225	This work
140	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	25/04/19	25/04/19	43	100	0.897	0.912	22	27.2	38.3	38.3	2.85	M	0.505	This work
141	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	25/04/19	25/04/19	56.5	128	0.911	0.927	23	26.6	40.7	40.7	5.83	M	0.613	This work
142	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	25/04/19	25/04/19	51.5	110	0.911	0.919	11	26.7	39.4	39.4	4.6	F	1.155	This work
143	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	25/04/19	25/04/19	64	149	0.911	0.917	8	27.2	39.3	39.3	8.34	M	1.513	This work
144	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	25/04/19	25/04/19	41	91	0.932	0.944	17	27.6	38.9	38.9	2.7	M	0.665	This work
145	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	25/04/19	25/04/19	44	97	0.932	0.940	12	26.9	39.2	39.2	3.03	M	1.025	This work

146	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	25/04/19	25/04/19	70	-	0.932	0.948	23	26.4	40.6	40.6	10.34	F	0.617	This work
147	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	25/04/19	25/04/19	42	59	0.871	0.883	18	26.2	37.2	37.2	1.24	-	0.611	This work
148	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	25/04/19	25/04/19	42	-	0.871	0.888	24	27.2	38.4	38.4	1.14	F	0.467	This work
149	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	25/04/19	25/04/19	31	-	0.871	0.885	20	26.6	37.2	37.2	0.62	-	0.53	This work
150	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	25/04/19	25/04/19	36	83	0.897	0.901	6	28.3	37.9	37.9	0.94	-	1.6	This work
151	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	25/04/19	25/04/19	39	90	0.897	0.903	10	27.4	37.4	37.4	0.93	-	1	This work
152	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	25/07/19	25/07/19	89.2	304.2	0.466	0.512	66	19.5	41.8	41.8	23.5	M	0.338	This work
153	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	25/07/19	25/07/19	63	-	0.489	0.513	36	21.7	38	38	6.5	F	0.453	This work
154	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	25/07/19	25/07/19	39.5	100.5	0.466	0.488	31	19.9	38.5	38.5	1.8	M	0.6	This work
155	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	25/07/19	25/07/19	32	83	0.466	0.499	47	20.4	38.4	38.4	1.5	-	0.383	This work
156	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	25/07/19	25/07/19	34	-	0.466	0.495	42	21.3	37.4	37.4	1.3	-	0.383	This work
157	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	25/08/19	25/08/19	83	266	0.771	0.806	51	22.7	41.7	41.7	17.5	M	0.373	This work
158	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	25/08/19	25/08/19	33.5	83.5	0.771	0.802	45	23.1	39.7	39.7	1.5	-	0.369	This work
159	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	25/08/19	25/08/19	34	75	0.771	0.788	25	22.9	39.2	39.2	1.3	-	0.652	This work
160	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	25/08/19	25/08/19	36.4	77.4	0.771	0.787	23	22.7	39.9	39.9	1.2	-	0.748	This work
161	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	26/02/20	26/02/20	85	228	0.635	0.655	28	27	39.1	39.1	11.3	F	0.432	This work

162	Squamata	Teiidae	*Ameivula*	*Ameivula ocellifera*	SBES	26/02/20	26/02/20	47	119	0.625	0.642	24	25.3	37.8	37.8	9	F	0.521	This work
163	Squamata	Teiidae	*Ameivula*	*Ameivula ocellifera*	SBES	26/02/20	26/02/20	49	148	0.625	0.638	19	25	38.3	38.3	11	M	0.7	This work
164	Squamata	Teiidae	*Ameivula*	*Ameivula ocellifera*	SBES	26/02/20	26/02/20	71	159	0.625	0.649	34	24.7	38.4	38.4	8.5	F	0.403	This work
165	Squamata	Teiidae	*Kentropyx*	*Kentropyx paulensis*	SBES	26/02/20	26/02/20	97	232	0.674	0.699	35	25.1	41	41	9.4	M	0.454	This work
166	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	26/02/20	26/02/20	29	71	0.653	0.672	27	27	41	41	1	-	0.519	This work
167	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	26/02/20	26/02/20	35	79	0.653	0.675	32	27.3	41.5	41.5	1.2	-	0.444	This work
168	Squamata	Teiidae	*Ameivula*	*Ameivula ocellifera*	SBES	26/04/19	29/04/19	37	77	0.458	0.467	14	25.1	36.8	36.8	1.4	M	0.836	This work
169	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	26/08/19	26/08/19	71.6	151	0.679	0.695	23	26.3	39.9	39.9	10.5	M	0.591	This work
170	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	26/08/19	26/08/19	67.2	51	0.679	0.708	42	25.3	42.5	42.5	7.5	F	0.409	This work
171	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	26/08/19	26/08/19	95.8	210	0.679	0.700	30	24.5	40.1	40.1	25	F	0.52	This work
172	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	26/08/19	26/08/19	61	128	0.691	0.704	19	25.4	39	39	4.5	M	0.715	This work
173	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	26/08/19	26/08/19	98	165	0.635	0.671	51	24.7	41.9	41.9	27.4	F	0.337	This work
174	Squamata	Teiidae	*Ameivula*	*Ameivula ocellifera*	SBES	26/08/19	26/08/19	57.3	102	0.679	0.706	38	26.2	38.9	38.9	5.5	M	0.334	This work
175	Squamata	Teiidae	*Ameivula*	*Ameivula ocellifera*	SBES	26/08/19	26/08/19	39	63	0.635	0.656	30	24.9	42.13	42.13	3.3	M	0.574	This work
176	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	26/08/19	26/08/19	37	32	0.635	0.649	19	25.2	37.2	37.2	1.5	-	0.631	This work
177	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	26/08/19	26/08/19	33	47	0.635	0.658	32	25.1	41.8	41.8	2	-	0.521	This work

178	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	27/02/20	27/02/20	56.5	142.5	0.604	0.633	42	28.3	39.4	39.4	8.8	F	0.264	This work
179	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	27/02/20	27/02/20	54	121	0.604	0.623	27	27	40	40	7.6	F	0.481	This work
180	Squamata	Teiidae	*Kentropyx*	*Kentropyx paulensis*	SBES	27/02/20	27/02/20	66	199	0.649	0.665	24	28.7	41	41	10	F	0.513	This work
181	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	27/02/20	27/02/20	32	32	0.639	0.659	29	26.2	40.8	40.8	1	-	0.503	This work
182	Squamata	Amphisbaenidae	*Amphisbaena*	*Amphisbaena mertensii*	SBES	27/02/20	27/02/20	347	23	0.594	0.608	20	27.29	31.85	31.85	15	M	0.228	This work
183	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	27/08/19	27/08/19	57.1	187.1	0.584	0.622	55	24.7	42.4	42.4	7.5	F	0.321	This work
184	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	27/08/19	27/08/19	64.9	205.9	0.629	0.649	29	28.6	41.8	41.8	8.5	F	0.455	This work
185	Squamata	Teiidae	*Ameivula*	*Ameivula ocellifera*	SBES	27/08/19	27/08/19	51	150	0.584	0.608	35	25.3	40.5	40.5	3.7	M	0.434	This work
186	Squamata	Teiidae	*Ameiva*	*Ameiva jacuba*	SBES	27/08/19	27/08/19	55.8	162.8	0.629	0.643	20	28.3	40.3	40.3	6.5	M	0.6	This work
187	Squamata	Teiidae	*Ameiva*	*Ameiva jacuba*	SBES	27/08/19	27/08/19	43.5	119.5	0.629	0.647	26	27.2	41	41	3.5	M	0.53	This work
188	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	27/08/19	27/08/19	35.5	93.5	0.584	0.605	30	26.6	39.9	39.9	1.4	M	0.443	This work
189	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	27/08/19	27/08/19	35.2	81.2	0.584	0.600	23	22.9	39.4	39.4	1.4	F	0.717	This work
190	Squamata	Gymnophtalmidae	*Micrablepharus*	*Micrablepharus atticolus*	SBES	27/08/19	27/08/19	32	66	0.629	0.653	34	29.5	42.5	42.5	1.4	-	0.382	This work
191	Squamata	Dactyloidae	*Anolis*	*Anolis meridionalis*	SBES	27/09/19	30/09/19	51	113	0.403	0.422	25	25.9	32.6	32.6	1.75	M	0.268	This work
192	Squamata	Teiidae	*Ameivula*	*Ameivula ocellifera*	SBES	28/01/20	28/01/20	52.7	63.4	0.743	0.717	22	30.3	38.7	38.7	7	F	0.382	This work
193	Squamata	Teiidae	*Ameiva*	*Ameiva jacuba*	SBES	28/01/20	28/01/20	91	161	0.680	0.698	26	30.1	41.8	41.8	29	F	0.446	This work

194	Squamata	Teiidae	*Kentropyx*	*Kentropyx paulensis*	SBES	28/01/20	28/01/20	65	112	0.680	0.685	10	29.2	36.2	36.2	12	F	0.7	This work
195	Squamata	Teiidae	*Ameiva*	*Ameiva jacuba*	SBES	28/01/20	28/01/20	67.4	182	0.743	0.754	16	29.7	39.7	39.7	11	M	0.625	This work
196	Squamata	Teiidae	*Ameiva*	*Ameiva jacuba*	SBES	28/01/20	28/01/20	46.5	83.9	0.764	0.776	18	29	35.4	35.4	2	M	0.356	This work
197	Squamata	Teiidae	*Kentropyx*	*Kentropyx paulensis*	SBES	28/01/20	28/01/20	66	86	0.764	0.778	20	30.2	40.7	40.7	14	M	0.525	This work
198	Squamata	Teiidae	*Ameiva*	*Ameiva ameiva*	SBES	28/02/20	28/02/20	197	428	0.656	0.685	41	29	41	41	165	M	0.293	This work
199	Squamata	Teiidae	*Kentropyx*	*Kentropyx paulensis*	SBES	28/02/20	28/02/20	65	112	0.510	0.529	28	31	42.3	42.3	13.6	F	0.404	This work
200	Squamata	Teiidae	*Kentropyx*	*Kentropyx paulensis*	SBES	28/02/20	28/02/20	69	207	0.510	0.526	23	31	42.1	42.1	14.3	F	0.483	This work
201	Squamata	Dactyloidae	*Anolis*	*Anolis meridionalis*	SBES	28/09/19	30/09/19	49	115	0.403	0.419	23	24.6	33.2	33.2	1.7	M	0.373	This work
202	Squamata	Teiidae	*Kentropyx*	*Kentropyx paulensis*	SBES	29/01/20	29/01/20	63	69	0.729	0.742	19	29.4	40.2	40.2	13	F	0.568	This work
203	Squamata	Teiidae	*Ameiva*	*Ameiva jacuba*	SBES	29/01/20	29/01/20	56.5	102	0.729	0.738	13	30.2	34.9	34.9	6	M	0.362	This work
204	Squamata	Tropiduridae	*Tropidurus*	*Tropidurus itambere*	SBES	29/01/20	29/01/20	40	52	0.747	0.763	23	31	38.4	38.4	5	M	0.322	This work
205	Squamata	Dipsadidae	*Apostolepis*	*Apostolepis dimidiata*	SBES	29/02/20	29/02/20	183	21	0.766	0.776	14	26.44	32.6	32.6	1.84	M	0.44	This work
206	Squamata	Teiidae	*Ameivula*	*Ameivula ocellifera*	SBES	30/01/20	30/01/20	29.5	43.8	0.605	0.617	19	29.1	37.8	37.8	8	M	0.458	This work
207	Squamata	Teiidae	*Ameivula*	*Ameivula ocellifera*	SBES	30/01/20	30/01/20	54.5	111.5	0.605	0.622	24	28.9	38.3	38.3	12	F	0.392	This work
208	Squamata	Teiidae	*Kentropyx*	*Kentropyx paulensis*	SBES	31/01/20	31/01/20	54	105	0.684	0.709	36	29.5	39.8	39.8	12	F	0.286	This work
209	Squamata	Teiidae	*Kentropyx*	*Kentropyx paulensis*	SBES	31/01/20	31/01/20	67.7	111	0.684	0.701	24	27.3	40.6	40.6	10	M	0.554	This work

210	Squamata	Viperidae	*Crotalus*	*Crotalus durissus*	MV	30/11/19	30/11/19	700	60	0.692	0.721	41	23.16	37.05	37.05	210	M	0.339	This work
211	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	22/10/20	22/10/20	68	-	0.656	0.674	25	25.81	31.2	31.2	31.2	M	0.216	This work
212	Anura	Brachycephalidae	*Ischnocnema*	*Ischnocnema juipoca*	MV	22/10/20	22/10/20	25	-	0.706	0.710	6	28.52	31.7	31.7	1.6		0.53	This work
213	Anura	Hylidae	*Trachycephalus*	*Trachycephalus imitatrix*	MV	22/10/20	22/10/20	75	-	0.682	0.696	20	24.19	30.65	30.65	47	-	0.323	This work
214	Anura	Craugastoridae	*Ischnocnema*	*Ischnocnema guentheri*	MV	23/10/20	23/10/20	40	-	0.383	0.403	28	22.6	28.09	28.09	6.04	F	0.196	This work
215	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	24/10/20	24/10/20	40	-	0.394	0.413	27	23.99	30.11	30.11	4.73	M	0.227	This work
216	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	24/10/20	24/10/20	73	-	0.417	0.433	23	25.75	31.11	31.11	45.4	F	0.233	This work
217	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	24/10/20	24/10/20	68	-	0.438	0.454	24	23.1	30.13	30.13	43.23	F	0.293	This work
218	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	24/10/20	24/10/20	66	-	0.459	0.469	15	25.37	30.25	30.25	46.15	F	0.325	This work
219	Anura	Hylidae	*Ololygon*	*Ololygon obtriangulata*	MV	24/10/20	24/10/20	32	-	0.372	0.380	12	26.01	31.32	31.32	1.4	-	0.443	This work
220	anura	Hylidae	*Boana*	*Boana prasina*	MV	25/10/20	25/10/20	43	-	0.432	0.438	9	24.87	31.26	31.26	5.17	M	0.71	This work
221	anura	Hylidae	*Boana*	*Boana prasina*	MV	25/10/20	25/10/20	41	-	0.447	0.456	13	24.93	30.03	30.03	3.35	M	0.392	This work
222	anura	Hylidae	*Bokernmanohyla*	*Bokernmanohyla luctuosa*	MV	25/10/20	25/10/20	55	-	0.403	0.414	15	23.9	29.51	29.51	9.91	M	0.374	This work
223	anura	Hylidae	*Bokernmanohyla*	*Bokernmanohyla luctuosa*	MV	25/10/20	25/10/20	79	-	0.414	0.423	13	22.61	29.93	29.93	13.6	-	0.563	This work
224	Anura	Bufonidae	*Rhinella*	*Rhinella ornata*	MV	25/10/20	25/10/20	46	-	0.386	0.403	25	24.23	29.32	29.32	8.4	F	0.204	This work
225	squamata	Dipsadidae	*Thamnodynastes*	*Thamnodynastes nattereri*	MV	25/10/20	25/10/20	443	123	0.465	0.473	11	29.87	34.77	34.77	39.3	M	0.445	This work

226	squamata	Dipsadidae	*Thamnodynastes*	*Thamnodynastes strigatus*	MV	25/10/20	25/10/20	230	64	0.420	0.425	7	28.45	34.32	34.32	23.6	M	0.839	This work
227	squamata	Dipsadidae	*Thamnodynastes*	*Thamnodynastes strigatus*	MV	25/10/20	25/10/20	483	131	0.483	0.495	17	28.94	34.49	34.49	38.7	M	0.326	This work
228	squamata	Dipsadidae	*Tomodon*	*Tomodon dorsatus*	MV	25/10/20	25/10/20	490	130	0.442	0.453	17	25.64	32.81	32.81	53.4	F	0.422	This work
229	Anura	Hylidae	*Scinax*	*Scinax duartei*	MV	28/10/20	28/10/20	40	-	0.374	0.384	14	22.77	32.93	32.93	3.22	M	0.726	This work
230	anura	hylidae	*Scinax*	*Scinax doloi*	MV	29/10/20	29/10/20	28	-	0.371	0.377	9	21.34	30.53	30.53	0.87	-	1.021	This work
231	Anura	Hylidae	*Trachycephalus*	*Trachycephalus imitatrix*	MV	29/10/20	29/10/20	50	-	0.388	0.399	16	24.95	30.29	30.29	7.85	-	0.334	This work
232	Anura	Hylidae	*Aplastodiscus*	*Aplastodiscus perviridis*	MV	30/10/20	30/10/20	40	-	0.456	0.465	12	26.33	31.52	31.52	4.62	F	0.433	This work
233	Anura	Hylidae	*Boana*	*Boana prasina*	MV	30/10/20	30/10/20	48	-	0.445	0.451	8	26.72	31.11	31.11	6.95	F	0.549	This work
234	Squamata	Dipsadidae	*Oxyrophus*	*Oxyrophus clathratus*	MV	30/10/20	30/10/20	311	65	0.433	0.436	5	27.49	31.41	31.41	23	F	0.784	This work
235	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus olfersii*	MV	30/10/20	30/10/20	28	-	0.472	0.478	8	27.34	29.64	29.64	2.03	-	0.288	This work
236	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys itamari*	MV	30/10/20	30/10/20	30	-	0.513	0.521	11	23.7	30.09	30.09	2.28	-	0.581	This work
237	Anura	Hylidae	*Scinax*	*Scinax doloi*	MV	30/10/20	30/10/20	25	-	0.485	0.487	3	27.09	30.54	30.54	1.2	M	1.15	This work
238	Anura	Hylidae	*Scinax*	*Scinax doloi*	MV	30/10/20	30/10/20	28	-	0.491	0.494	4	24.98	29.46	29.46	0.97	M	1.12	This work
239	Anura	Hylidae	*Scinax*	*Scinax duartei*	MV	30/10/20	30/10/20	29	-	0.501	0.506	7	25.46	31.31	31.31	1.7	M	0.836	This work
240	Squamata	Dipsadidae	*Thamnodynastes*	*Thamnodynastes strigatus*	MV	30/10/20	30/10/20	457	122	0.399	0.416	25	25.51	35.33	35.33	89	M	0.393	This work
241	Anura	Hylidae	*Scinax*	*Scinax duartei*	MV	11/11/20	11/11/20	40	-	0.417	0.433	22	24.48	31.12	31.12	4.67	-	0.302	This work

242	Anura	Bufonidae	*Rhinella*	*Rhinella icterica*	MV	11/11/20	11/11/20	58	-	0.445	0.463	25	26.71	31.84	31.84	22.43	-	0.205	This work
243	Squamata	Dipsadidae	*Thamnodynastes*	*Thamnodynastes strigatus*	MV	11/11/20	11/11/20	330	79	0.472	0.488	22	28.17	34.49	34.49	47.3	F	0.287	This work
244	Squamata	Dipsadidae	*Thamnodynastes*	*Thamnodynastes strigatus*	MV	11/11/20	11/11/20	483	131	0.493	0.513	27	23.32	34.33	34.33	111	F	0.408	This work
245	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	12/11/20	12/11/20	73	-	0.000	0.021	30	22.3	27.48	27.48	37.64	-	0.173	This work
246	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	12/11/20	12/11/20	66	-	0.025	0.040	22	24.04	27.09	27.09	41.32	-	0.139	This work
247	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	12/11/20	12/11/20	66	-	0.044	0.053	14	24.45	26.17	26.17	30.64	-	0.123	This work
248	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	12/11/20	12/11/20	44	-	0.049	0.061	18	24.82	27.06	27.06	27.06	-	0.124	This work
249	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	12/11/20	12/11/20	72	-	0.065	0.074	14	23.86	27.46	27.46	57.14	-	0.257	This work
250	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	12/11/20	12/11/20	70	-	0.078	0.088	14	24.6	27.05	27.05	31.56	-	0.175	This work
251	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	12/11/20	12/11/20	66	-	0.094	0.107	19	25.19	27.91	27.91	34.8	-	0.143	This work
252	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	12/11/20	12/11/20	56	-	0.344	0.358	20	25.81	28.19	28.19	31.23	-	0.119	This work
253	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	12/11/20	12/11/20	38	-	0.362	0.376	19	25.79	30.45	30.45	17.42	-	0.245	This work
254	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	12/11/20	12/11/20	37	-	0.377	0.385	11	24.78	30.2	30.2	4.82	-	0.493	This work
255	Anura	Hylidae	*Scinax*	*Scinax duartei*	MV	12/11/20	12/11/20	38	-	0.388	0.392	5	26.46	31.13	31.13	4.43	-	0.934	This work
256	Anura	Hylidae	*Scinax*	*Scinax duartei*	MV	12/11/20	12/11/20	42	-	0.394	0.398	5	24.64	30.89	30.89	4.05	-	1.25	This work
257	Anura	Hylidae	*Scinax*	*Scinax duartei*	MV	12/11/20	12/11/20	28	-	0.407	0.410	4	23.86	30.9	30.9	0.82	-	1.76	This work

258	Anura	Hylidae	*Scinax*	*Scinax duartei*	MV	12/11/20	12/11/20	33	-	0.412	0.415	4	24.12	30.95	30.95	0.8	-	1.708	This work
259	Anura	Hylidae	*Scinax*	*Scinax duartei*	MV	12/11/20	12/11/20	28	-	0.419	0.422	4	24.64	29.15	29.15	0.89	-	1.128	This work
260	Anura	Hylidae	*Scinax*	*Scinax doloi*	MV	12/11/20	12/11/20	31	-	0.427	0.430	4	27.27	29.99	29.99	0.9	-	0.68	This work
261	Anura	Hylidae	*Scinax*	*Scinax doloi*	MV	12/11/20	12/11/20	29	-	0.432	0.434	5	24.66	29.45	29.45	0.85	-	0.958	This work
262	Anura	Hylidae	*Ololygon*	*Ololygon obtriangulata*	MV	12/11/20	12/11/20	36	-	0.438	0.442	6	26.42	31.56	31.56	1.11	-	0.857	This work
263	Anura	Hylidae	*Ololygon*	*Ololygon obtriangulata*	MV	12/11/20	12/11/20	31	-	0.447	0.450	5	27.54	30.07	30.07	0.98	-	0.506	This work
264	Anura	Hylidae	*Ololygon*	*Ololygon obtriangulata*	MV	12/11/20	12/11/20	33	-	0.452	0.456	5	24.16	30.37	30.37	1.03	-	1.242	This work
265	Anura	Hylidae	*Dendropsophus*	*Dendropsophus microps*	MV	12/11/20	12/11/20	25	-	0.464	0.466	3	25.21	29.85	29.85	0.56	M	1.547	This work
266	Anura	Hylidae	*Dendropsophus*	*Dendropsophus microps*	MV	12/11/20	12/11/20	24	-	0.467	0.469	3	27.06	29.42	29.42	0.45	M	0.787	This work
267	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	13/11/20	13/11/20	67	-	0.651	0.667	23	25.21	30.96	30.96	39.5	F	0.25	This work
268	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	13/11/20	13/11/20	70	-	0.670	0.692	32	24.89	30.88	30.88	38.45	F	0.187	This work
269	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	13/11/20	13/11/20	76	-	0.694	0.713	25.4	25.72	30.78	30.78	40.46	F	0.199	This work
270	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	13/11/20	13/11/20	56	-	0.972	0.980	12	24.87	28.29	28.29	18.34	M	0.285	This work
271	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	13/11/20	13/11/20	77	-	0.985	0.991	8	24.21	28.72	28.72	42.15	F	0.564	This work
272	Anura	Hylidae	*Dendropsophus*	*Dendropsophus microps*	MV	13/11/20	13/11/20	27	-	0.687	0.690	5	26.19	29.78	29.78	0.83	M	0.718	This work
273	Anura	Hylidae	*Dendropsophus*	*Dendropsophus microps*	MV	13/11/20	13/11/20	26	-	0.692	0.697	7	25.81	30.03	30.03	0.79	M	0.603	This work

274	Anura	Hylidae	*Dendropsophus*	*Dendropsophus microps*	MV	13/11/20	13/11/20	24	-	0.988	0.992	6	23.49	27.11	27.11	0.47	M	0.603	This work
275	Anura	Hylidae	*Dendropsophus*	*Dendropsophus microps*	MV	13/11/20	13/11/20	25	-	0.993	0.997	6	24.37	28.15	28.15	0.57	M	0.63	This work
276	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	14/11/20	14/11/20	48	-	0.008	0.014	9	24.23	28.34	28.34	15.97	M	0.457	This work
277	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	14/11/20	14/11/20	76	-	0.014	0.018	6	25.03	28.25	28.25	47.05	F	0.537	This work
278	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	14/11/20	14/11/20	66	-	0.020	0.024	5	25.82	28.43	28.43	33.92	F	0.522	This work
279	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	14/11/20	14/11/20	78	-	0.361	0.369	12	24.53	30.27	30.27	52.11	F	0.478	This work
280	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	14/11/20	14/11/20	81	-	0.372	0.384	18	25.15	30.49	30.49	50.36	F	0.297	This work
281	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	14/11/20	14/11/20	52	-	0.389	0.400	16	24.79	30.09	30.09	22.7	M	0.331	This work
282	Squamata	Dipsadidae	*Thamnodynastes*	*Thamnodynastes strigatus*	MV	14/11/20	14/11/20	483	131	0.406	0.421	21	28.19	34.28	34.28	41.5	F	0.29	This work
283	Anura	Hylidae	*Boana*	*Boana latistriata*	MV	14/11/20	14/11/20	34	-	0.422	0.431	13	24.61	31.95	31.95	1.33	F	0.565	This work
284	Anura	Hylidae	*Boana*	*Boana latistriata*	MV	14/11/20	14/11/20	30	-	0.424	0.426	4	24.07	31.85	31.85	1.12	M	1.945	This work
285	Anura	Hylidae	*Boana*	*Boana latistriata*	MV	14/11/20	14/11/20	28	-	0.427	0.432	7	24.93	30.45	30.45	0.9	M	0.789	This work
286	Anura	Hylidae	*Boana*	*Boana latistriata*	MV	14/11/20	14/11/20	27	-	0.433	0.435	3	26.2	30.27	30.27	0.87	M	1.357	This work
287	Anura	Hylidae	*Boana*	*Boana latistriata*	MV	14/11/20	14/11/20	29	-	0.441	0.445	6	24.32	30.12	30.12	0.91	M	0.967	This work
288	Anura	Hylidae	*Boana*	*Boana latistriata*	MV	14/11/20	14/11/20	28	-	0.458	0.462	5	25.32	29.82	29.82	0.65	M	0.9	This work
289	Anura	Hylidae	*Boana*	*Boana latistriata*	MV	14/11/20	14/11/20	35	-	0.463	0.468	7	25.04	30.7	30.7	1.21	F	0.809	This work

290	Anura	Hylidae	*Boana*	*Boana latistriata*	MV	14/11/20	14/11/20	33	-	0.472	0.476	5	24.91	29.97	29.97	1.3	F	1.012	This work
291	Squamata	Dipsadidae	*Tomodon*	*Tomodon dorsatus*	MV	15/11/20	15/11/20	516	148	0.588	0.599	16	24.79	29.81	29.81	112	F	0.314	This work
292	Squamata	Dipsadidae	*Thamnodynastes*	*Thamnodynastes strigatus*	MV	15/11/20	15/11/20	305	89	0.601	0.607	9	27.89	34.13	34.13	4.67	M	0.693	This work
293	Squamata	Dipsadidae	*Mussurana*	*Mussurana montana*	MV	16/11/20	16/11/20	710	147	0.701	0.710	13	24.29	35.2	35.2	117	F	0.839	This work
294	Squamata	Dipsadidae	*Mussurana*	*Mussurana montana*	MV	16/11/20	16/11/20	275	45	0.715	0.721	8	26.52	33.67	33.67	4.76	M	0.894	This work
295	Squamata	Dipsadidae	*Atractus*	*Atractus zebrinus*	MV	16/11/20	16/11/20	191	13	0.978	0.984	8	30.52	35.16	35.16	3.5	F	0.58	This work
296	Squamata	Dipsadidae	*Thamnodynastes*	*Thamnodynastes strigatus*	MV	16/11/20	16/11/20	137	47	0.985	0.998	19	25.03	33.77	33.77	2.5	M	0.46	This work
297	Squamata	Dipsadidae	*Thamnodynastes*	*Thamnodynastes strigatus*	MV	16/11/20	16/11/20	428	102	0.993	0.010	24	24.65	33.7	33.7	51	F	0.377	This work
298	Squamata	Dipsadidae	*Thamnodynastes*	*Thamnodynastes strigatus*	MV	17/11/20	17/11/20	331	81	0.001	0.008	9	24.35	31.25	31.25	17	M	0.767	This work
299	Squamata	Dipsadidae	*Thamnodynastes*	*Thamnodynastes strigatus*	MV	17/11/20	17/11/20	439	112	0.010	0.035	35	22.77	33.49	33.49	55	F	0.306	This work
300	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	17/11/20	17/11/20	71	-	0.033	0.037	7	25.38	28.05	28.05	53.6	F	0.381	This work
301	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	17/11/20	17/11/20	53	-	0.038	0.042	5	24.48	29.82	29.82	31.06	M	1.068	This work
302	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	17/11/20	17/11/20	74	-	0.045	0.052	10	25.55	28.64	28.64	47.83	F	0.309	This work
303	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	17/11/20	17/11/20	70	-	0.052	0.060	10	26.18	28.56	28.56	50.06	F	0.238	This work
304	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	17/11/20	17/11/20	55	-	0.438	0.451	19	25.23	30.16	30.16	29.79	M	0.259	This work
305	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	17/11/20	17/11/20	51	-	0.451	0.458	9	24.93	30.02	30.02	30.11	M	0.566	This work

306	Anura	Odontophrynidae	*Procerathophrys*	*Proceratophrys boiei*	MV	17/11/20	17/11/20	48	-	0.462	0.467	8	25.85	30.99	30.99	28.7	M	0.643	This work
307	Squamata	Dipsadidae	*Thamnodynastes*	*Thamnodynastes strigatus*	MV	20/11/20	20/11/20	170	43	0.917	0.931	21	22.4	32.83	32.83	3.89	M	0.497	This work
308	Squamata	Dipsadidae	*Thamnodynastes*	*Thamnodynastes strigatus*	MV	20/11/20	20/11/20	246	46	0.934	0.951	24	22.18	35.16	35.16	9.07	F	0.541	This work
309	Squamata	Dipsadidae	*Mussurana*	*Mussurana montana*	MV	20/11/20	20/11/20	523	77	0.941	0.949	12	21.54	33.94	33.94	37	F	1.033	This work
310	Anura	Leptodactylidae	*Physalaemus*	*Physalaemus marmoratus*	SBES	20/01/21	22/11/21	45	-	0.819	0.829	14	27.74	36.06	36.06	8.02	F	0.594	This work
311	Squamata	Boidae	*Epicrates*	*Epicrates crassus*	SBES	20/01/21	22/11/21	380	42	0.847	0.863	22	26.44	32.74	32.74	29.33	F	0.286	This work
312	Squamata	Anomalepididae	*Liotyphlops*	*Liotyphlops ternetzii*	SBES	20/01/21	22/11/21	200	5	0.837	0.842	7	26.31	38.65	38.65	1.75	M	1.763	This work

6. FINAL REMARKS

Understanding the impacts of recent climate change on ectothermic organisms is necessary for multiple reasonable grounds. First, the assessment of impacts on various scales is necessary to understand how aspects of the biology and ecology of ectotherms are related to their climatic vulnerability. In this sense, and in order to understand these aspects, we show that behavioral thermal tolerances are a useful tool to understand not only the processes, but also the mechanisms that ectothermic animals use when they change their behavior to avoid overheating and thus persist in their habitats. Additionally, the methods used here integrate the thermal physiology of amphibians and reptiles with biogeographic, ecological, and natural history aspects to complement the life history information known for some species. The aim of this thesis was to present novel data of voluntary thermal tolerances of South American amphibians and reptiles from multiple approaches: (i) experimentally estimating behavioral thermal tolerances in amphibians and reptiles and evaluating the impact of captivity on these tolerances; ii) association of tolerance data with natural history information and distribution records of species as a way to understand biogeographic patterns and the relationship of natural history with physiological requirements; iii) relationship of the behavioral thermal tolerances and distribution patterns with the evolutionary history of lineages to understand how the physiological requirements and the distribution of the species evolve; iv) description of thermal tolerances of several species from two conservation hotspots to document variation in behavioral thermal tolerances in South American amphibians and reptiles. We highlight the importance of measuring behavioral tolerances and integrating them with aspects of natural history, as well as considering the evolutionary relationships of species to improve predictions of vulnerability to recent climate change. Below, I emphasize the main outcomes of the thesis which can also be used as recommendations in conservation plans as well as can be used as baseline for future scientific development.

6.1. Chapter 1: Short-term captivity and thermal tolerance of a pitviper

6.1.1. *Outcomes and recommendations*

- The invariant voluntary thermal maximum implies a hardwired thermal tolerance, not affected by capture and short-term captivity.
- Measurements taken during a short captivity period might be a good proxy for behavioral thermal tolerance of individuals in the wild.

- There was low phenotypic plasticity but a significant interindividual variation, which might be important for individual survival (and thus population dynamics) and thermal ecology.

6.1.2. Next steps:

- VT_{Max} measurements over longer periods for *B. pauloensis* and other species of the same lineage (*B. neuwiedi* complex).

- VT_{Max} measurements with a higher number of individuals to increase the effect size and improve our findings.

6.2. Chapter 2: Behavioral thermal tolerance, distribution and habitat use in Neotropical frogs

6.2.1. *Outcomes and recommendations*

- The species with lower VT_{Max} is more abundant in open habitats than in non-open habitats.
- Species widespread in the Cerrado showed different patterns of $VT_{Max} – ET_{Max}$ values throughout their ranges.
- Small-scale habitat use might reveal a link between behavioral thermal tolerance and natural history strategies.

6.2.2. Next steps

- Future mechanistic studies on amphibian thermal ecology and on the impact of global warming on species distribution.
- Other VT_{Max} measurements using additional sympatric species of the genus *Physalaemus* such as *P. centralis*, from the same clade of *P. cuvieri*, and *P. marmoratus*, from the same clade of *P. nattereri*, to elucidate if those differences are due to body size variation or if tolerances are phylogenetically conserved.

6.3. Chapter 3: Geographical distribution, environmental thermal limits and thermal niche breadth of pitvipers

6.3.1. *Outcomes and recommendations*

- Behavioral thermal limits such as VT_{Max} does not vary markedly among South American pitviper species.
- South American pitvipers did not show significant differences in their heating rates.
- In spite of species being widespread, the localities where they are distributed do not reach their VT_{Max}.
- Species with a greater latitudinal range tend to have a lower $VT_{Max.}$
- Environmental features such as temperature, precipitation, ruggedness, and altitudinal limits do not seem to be the main driver of the macroevolutionary variation of VT_{Max} in this group of Neotropical snakes, whereas latitudinal range (Lat_{Ran}) was the most relevant geographical feature influencing behavioral thermal tolerances.

6.3.2. Next steps:

- Include behavioral thermal tolerances such as VT_{Max} in futures evolutionary comparative studies.
- Include other clades of vipers in an evolutionary context to better understand the evolution of voluntary thermal tolerances and their relationship with ecological aspects.
- Estimate and compare critical thermal limits with the VT_{Max} and to i) test if behavioral thermal limits are related to the critical thermal limits; and ii) evaluate how behavioral thermal limits evolve in relation to critical thermal limits.

6.4. Chapter 4: Thermal tolerances of Neotropical amphibians and reptiles

6.4.1. Outcomes and recommendations

- Although poorly documented, data on thermal behavioral thresholds to avoid overheating in their habitats is essential to improve the understanding of the thermal ecology and physiology of amphibians and reptiles.
- Among the species studied, lizards showed higher VT_{Max} than amphibians and snakes, and individuals from the Atlantic Forest showed overall higher VT_{Max} than those from the Cerrado.

6.4.2. Next steps:

- Obtain VT_{Max} measurements of other amphibians and reptiles from other ecoregions or conservation hotpots to understand the impact of recent climate change in the South American herpetofauna.

- Use phylogenetic approaches to understand how behavioral thermal tolerances can be related to the evolutionary history of lineages.

- Obtain measurements of other behavioral limits (voluntary thermal minimum, VT_{Min}) of Neotropical species to explore how these behavioral thresholds are related to physiological tolerances.

6.5. Next steps of research

- Estimate behavioral tolerances in long-term captivity environments to test whether behavioral tolerances such as VT_{Max} are affected by long-term captivity.
- Document and describe other behavioral tolerance limits in other ectothermic animals.
- Integrate other spatial scales into future studies that integrate behavioral thermal tolerances, as well as add species with different types of ecological strategies such as habitat use.
- Integrate mechanistic analyzes with the tolerance data obtained here to make more realistic climate change predictions, focusing mainly on tolerances that cause behavior to change and as a consequence their distribution patterns and/or other ecological aspects such as habitat use.

6.6. Next steps in my career

- First, I will look for an employment, either as a professor or as a postdoctoral researcher.
- Second, submit the third chapter for publication on the second semester of 2022.
- Finally, continue my research on the thermal ecology, biogeography and behavioral ecology of South American ectotherms, focusing primarily on describing the relationship of behavioral thermal thresholds, thermal landscapes, evolutionary history,

and the natural history of organisms to improve predictions about their vulnerability to recent climate change.

7. CONSIDERAÇÕES FINAIS

Compreender os impactos das mudanças climáticas recentes sobre os organismos ectotérmicos é necessário por vários motivos plausíveis. Primeiro, os impactos em várias escalas são necessários para entender como os aspectos da biologia e ecologia dos ectotérmicos estão relacionados à sua vulnerabilidade climática. Nesse sentido, e com o intuito de entender esses aspectos, mostramos que as tolerâncias térmicas comportamentais são uma ferramenta útil para entender não só os processos, mas também os mecanismos que os animais ectotérmicos utilizam quando mudam seu comportamento para evitar o superaquecimento e assim persistir em seus habitats. Além disso, os métodos aqui utilizados integram a fisiologia térmica de anfíbios e répteis com aspectos biogeográficos, ecológicos e de história natural para complementar as informações de história de vida conhecidas para algumas espécies. O objetivo desta tese foi apresentar novos dados de tolerâncias térmicas voluntárias de anfíbios e répteis sul-americanos a partir de múltiplas abordagens: (i) estimar experimentalmente as tolerâncias térmicas comportamentais em anfíbios e répteis e avaliar o impacto do cativeiro sobre essas tolerâncias; ii) associação de dados de tolerância com informações de história natural e registros de distribuição de espécies como forma de entender os padrões biogeográficos e a relação da história natural com as exigências fisiológicas; iii) relação das tolerâncias térmicas comportamentais e padrões de distribuição com a história evolutiva das linhagens para entender como as exigências fisiológicas e a distribuição das espécies evoluem; iv) descrição das tolerâncias térmicas de várias espécies de dois hotspots de conservação para documentar a variação nas tolerâncias térmicas comportamentais em anfíbios e répteis da América do Sul. Destacamos a importância de medir tolerâncias comportamentais e integrá-las com aspectos da história natural, bem como considerar as relações evolutivas das espécies para melhorar as previsões de vulnerabilidade às mudanças climáticas recentes. Abaixo, enfatizo os principais resultados da tese que também podem ser usados como recomendações em planos de conservação, bem como podem ser usados como base para o desenvolvimento científico futuro.

7.1. Capítulo 1: Cativeiro de curto prazo e tolerância térmica de um pitviper

7.1.1. *Resultados e recomendações*

- O máximo térmico voluntário invariante implica uma tolerância térmica relacionada, não afetada pela captura e cativeiro de curto prazo.
- Medidas feitas durante um curto período de cativeiro podem ser um bom substituto para a tolerância térmica comportamental de indivíduos na natureza.
- Houve baixa plasticidade fenotípica, mas uma variação interindividual significativa, que pode ser importante para a dinâmica populacional e ecologia térmica.

7.1.2. Próximos passos:

- Medições de VT_{Max} em períodos de longo prazo, incluindo *B. pauloensis* e outras espécies da mesma linhagem (complexo *B. neuwiedi*).
- Medições VT_{Max} com um número maior de indivíduos para aumentar o tamanho do efeito e melhorar nossos achados.

7.2. Capítulo 2: Tolerância térmica comportamental, distribuição e uso de habitat em sapos Neotropicais

7.2.1. *Resultados e recomendações*

- As espécies com VT_{Max} mais baixo são mais abundantes em habitats abertos do que em habitats não abertos.
- Espécies amplamente distribuídas no Cerrado mostraram diferentes padrões de valores VT_{Max} – ET_{Max} em todos os seus ranges.
- O uso de habitat em pequena escala pode revelar uma ligação entre a tolerância térmica comportamental e as estratégias de história natural.

7.2.2. Próximos passos:

- Futuros estudos mecanísticos sobre a ecologia térmica dos anfíbios e o impacto do aquecimento global na distribuição das espécies.
- Outras medições VT_{Max} usando espécies simpátricas adicionais do gênero *Physalaemus*, como *P. centralis*, do mesmo clado de *P. cuvieri* e *P. marmoratus*, do mesmo clado de *P. nattereri* para elucidar se essas diferenças são devido à variação do tamanho do corpo ou se as tolerâncias são conservadas filogeneticamente.

7.3. Capítulo 3: Distribuição geográfica, limites térmicos ambientais e amplitude de nicho térmico de pitvipers

7.3.1. *Resultados e recomendações*

- Os limites térmicos comportamentais, como VT_{Max}, não variam entre as espécies de Jararacas da América do Sul.
- Jararacas da América do Sul não mostraram diferenças significativas em suas taxas de aquecimento.
- Apesar de as espécies estarem amplamente distribuídas, as localidades onde estão distribuídas não ultrapassam o seu VT_{Max}.
- Espécies com uma faixa latitudinal maior tendem a ter um VT_{Max} mais baixo.
- Características ambientais como temperatura, precipitação, rugosidade e limites altitudinais, não parecem ser o principal impulsionador da variação macroevolutiva de VT_{Max} neste grupo de jararacas Neotropicais, apesar de a amplitude latitudinal (Lat_{Ran}) ter sido destacada como a característica geográfica mais relevante influenciando tolerâncias térmicas comportamentais.

7.3.2. Próximos passos:

- Incluir as tolerâncias térmicas comportamentais, como VT_{Max}, em estudos comparativos evolutivos futuros.
- Incluir outros clados de serpentes em um contexto evolutivo para entender a evolução das tolerâncias térmicas voluntárias e sua relação com os aspectos ecológicos.
- Estimar e comparar os limites térmicos críticos com o VT_{Max} para i) testar se os limites térmicos comportamentais estão relacionados com os limites térmicos críticos; e ii) avaliar como os limites térmicos comportamentais evoluem em relação aos limites térmicos críticos.

7.4. Capítulo 4: Tolerâncias térmicas de anfíbios e repteis Neotropicais

7.4.1. *Resultados e recomendações*

- Apesar dos dados pouco documentados sobre os limites térmicos comportamentais, para evitar o superaquecimento em seus habitats, é essencial melhorar a compreensão de sua ecologia e fisiologia térmica.

- Lagartos têm VT_{Max} mais alto do que anfíbios e cobras, e os indivíduos da Mata Atlântica têm valores VT_{Max} mais altos do que os do Cerrado.
- Os valores de VT_{Max} foram semelhantes entre as espécies do Cerrado e da Mata Atlântica.

7.4.2. Próximos passos:

- Medições VT_{Max} de outros anfíbios e repteis de outras ecorregiões ou hotspots para entender o impacto das mudanças climáticas recentes na herpetofauna Sul-Americana.
- Abordagens filogenéticas para entender como as tolerâncias comportamentais podem estar relacionadas à história evolutiva das linhagens.
- Medições de outros limites comportamentais (mínimo térmico voluntário, VT_{Min}) de espécies Neotropicais para explorar como relacionam estes limites comportamentais com tolerâncias fisiológicas.

7.5. Próximos passos na pesquisa

- Estimar as tolerâncias comportamentais em ambientes de cativeiro de longo prazo, para testar se as tolerâncias comportamentais, como VT_{Max}, não são realmente afetadas pelo cativeiro.
- Documentar e descrever outros limites de tolerância comportamental em outros animais ectotérmicos.
- Integrar outras escalas espaciais em estudos futuros que integrem tolerâncias comportamentais, bem como adicionar espécies com diferentes tipos de estratégias ecológicas como o uso do habitat.
- Integrar análises mecanicistas com os dados de tolerância obtidos aqui para fazer previsões de mudanças climáticas mais realistas, focando principalmente nas tolerâncias que fazem que o comportamento mude e, como consequência, seus padrões de distribuição e / ou outros aspectos ecológicos, como uso de habitat.

7.6. Meus próximos passos

- Primeiramente, eu vou procurar um emprego, seja como professor ou como pesquisador.

- Em segundo lugar, enviar o terceiro capítulo para publicação no segundo semestre de 2022.
- Finalmente, continuar a minha pesquisa sobre Ecologia térmica, Biogeografia e Ecologia Comportamental de animais ectotérmicos da América do Sul, focando principalmente em descrever a relação dos limites térmicos comportamentais, paisagens termais, história evolutiva e história natural dos organismos para melhorar as previsões sobre a vulnerabilidade dos organismos diante das recentes mudanças climáticas.

8. SCIENTIFIC PUBLICATIONS DURING THE Ph.D. PROGRAM

1. **DIAZ-RICAURTE, JC**; SERRANO F; MARTINS, M. **2022.** VTMaxHerp: a data set of Voluntary Thermal Maximum temperatures of amphibians and reptiles from two Brazilian hotspots. ***Ecology,*** 103(3): e3602. https://doi.org/10.1002/ECY.3602

2. SERRANO, F; **DIAZ-RICAURTE, JC;** MARTINS, M. **2022**. Finding love in a hopeless place: a global database of misdirected amplexus in anurans. ***Ecology.*** https://doi.org/10.1002/ecy.3737
3. **DIAZ-RICAURTE, JC;** GUEVARA-MOLINA, EC; ALVES-NUNES, JM; SERRANO, F; HRNCIR, M. **2022.** Linking body condition and thermal physiology in limping crickets: does limb autotomy incur costs concerning behavioral thermal tolerance? ***Journal of Experimental Zoology Part-A Ecological and Integrative Physiology,*** 337: 393–402. https://doi.org/10.1002/jez.2577

4. **DIAZ-RICAURTE, JC**; SERRANO F; MARTINS, M. **2022.** Behavioral Thermoregulation of Amphibians and Reptiles from Two Brazilian Hotspots. ***Bulletin of the Ecological Society of America,*** 103(2): e01965. https://doi.org/10.1002/bes2.1965

5. SERRANO, F; **DIAZ-RICAURTE, JC**; BUSSCHAU, T; PING, T. **2022**. Mix and match: New records of interspecific amplexus among South African frogs, with a review of sub-Saharan Africa. ***African Journal of Ecology***, 00: 1–6. https://doi.org/10.1111/aje.12981

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7. ANTÚNEZ-FONSECA, CA; ELVIR-VALLE, FA; **DIAZ-RICAURTE, JC**. **2022**. Male-male Aggression in the Introduced Cuban Brown Anole, *Anolis sagrei* (Reptilia: Dactyloidae), in Honduras. ***Reptile & Amphibians***, 229: 30–32. https://doi.org/10.17161/randa.v29i1.15981

8. **DIAZ-RICAURTE, JC**; ARRIAGA-VILLEGAS, NC; LÓPEZ-CORONADO, JD; MACÍAS-GARZÓN GX; FIORILLO, BF. **2022**. Effects of agricultural systems on the anuran diversity in the Colombian amazon. ***Studies on Neotropical Fauna and Environment***, 57(1): 18–28. https://doi.org/10.1080/01650521.2020.1809334

9. **DIAZ-RICAURTE, JC**; GUEVARA-MOLINA, EC. **2022**. Morphological and molecular data reveal new country records and distribution extensions of some Glassfrogs (Anura: Centrolenidae) for Colombia. ***Studies on Neotropical Fauna and Environment***, 57(1): 51–65. https://doi.org/10.1080/01650521.2020.1809333

10. **DIAZ-RICAURTE, JC;** SERRANO, F. **2022**. *Rhinella Icterica* (Yellow Cururu Toad) Amplexus displacement attempt. ***Herpetological review***.

11. **DIAZ-RICAURTE, JC**; ANTÚNEZ-FONSECA, CA; GARAVITO-DAVID, Y; SERRANO, F. **2022.** *Phyllomedusa vaillantii* (White-lined Leaf Frog) and *Engystomops pustulosus* (Tungara Frog). Defensive behaviors. ***Herpetological Review***.

12. **DIAZ-RICAURTE, JC**; ANTÚNEZ-FONSECA, CA; GARAVITO-DAVID, Y; SERRANO, F. **2022**. *Phyllomedusa vaillantii* (White-lined Leaf Frog). Geographical distribution note. ***Herpetological review.***

13. **DIAZ-RICAURTE, JC**; MARQUES, P. **2021**. *Leptodactylus labyrinthicus* (Pepper Frog). Batracophagy. *Natural history notes.* ***Herpetological review*** 52(1):117. Link: https://bit.ly/3GahYZ2

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21. GARAVITO-DAVID, Y; GUEVARA-MOLINA, EC; **DIAZ-RICAURTE, JC. 2020**. Egg attendance behaviour of the Amazonian poison frog *Ameerega hahneli* (Anura: Dendrobatidae). **Herpetological bulletin,** 153: 31–34. https://doi.org/10.33256/hb153.3134

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