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EFEITO DA TEMPERATURA NO COMPORTAMENTO E NA DISTRIBUIÇÃO DE DUAS SUBESPÉCIES DE *CANTHON RUTILANS* CASTELNAU, 1840 (COLEOPTERA: SCARABAEINAE)

FLORIANÓPOLIS

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Tese submetida ao Programa de Pós-Graduação em Ecologia da Universidade Federal de Santa Catarina para a obtenção do título de Doutor em Ecologia.
Orientadora: Prof.ª Dr.ª Malva Isabel Medina Hernández
Coorientador: Prof. Dr. Jorge Miguel Lobo

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O presente trabalho em nível de doutorado foi avaliado e aprovado por banca examinadora composta pelos seguintes membros:

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Certificamos que esta é a **versão original e final** do trabalho de conclusão que foi julgado adequado para obtenção do título de doutor em Ecologia.

———————————————
Prof.ª Dr.ª Andrea Santarosa Freire
Coordenadora do Programa

———————————————
Prof.ª Dr.ª Malva Isabel Medina Hernández
Orientadora

Florianópolis, 26 de setembro de 2019.

Este trabalho é dedicado aos meus pais,
à minha irmã e à minha sobrinha.

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RESUMO

Este trabalho teve como objetivo geral estudar o efeito da temperatura em duas subespécies de *Canthon rutilans* (Coleoptera: Scarabaeidae: Scarabaeinae) com a finalidade de observar diferenças na distribuição e no comportamento que justifiquem serem consideradas ecologicamente como duas espécies. Assim, esta tese foi dividida em três capítulos, sendo o primeiro sobre a distribuição geográfica das duas subespécies em um gradiente de elevação no estado de Santa Catarina, o segundo sobre o comportamento dos adultos e desenvolvimento larval em diferentes condições de temperatura e o terceiro sobre a distribuição real e provável em escala local e regional. O primeiro capítulo examinou o grau de segregação espacial e temporal das duas subespécies, ao longo de um gradiente de elevação com dados de um ano e verificou diferenças nos seus nichos térmicos. Os resultados mostraram que *C. rutilans cyanescens* e *C. rutilans rutilans* não se sobrepõem espacial nem temporalmente. Os limites de temperatura média dos dias de coleta para *C. rutilans cyanescens* foram de 15.3 e 24.0°C e no caso de *C. rutilans rutilans* foram de 14.4 e 18.6°C, com uma sobreposição do nicho térmico limitada a 5.6%. Esses resultados sugerem que ambas subespécies poderiam ser consideradas espécies distintas com diferentes requisitos fisiológicos e ecológicos. No segundo capítulo, comparamos em laboratório a influência da temperatura no comportamento e no desenvolvimento larval das subespécies para confirmar adaptações às diferentes condições térmicas das áreas de distribuição. Em cinco condições de temperatura (15, 20, 25, 30 e 35°C), o comportamento alimentar não diferiu entre as duas subespécies nem nas temperaturas, mas sim houve diferença no comportamento reprodutivo, sendo o tamanho das bolas-ninho dependente da temperatura e da subespécie, uma vez que as bolas-ninho de *C. rutilans rutilans* são maiores e mais pesadas a baixas temperaturas. O número de descendentes e o tempo de emergência dos adultos também foram dependentes da temperatura e o tempo de vida dos adultos de *C. rutilans rutilans* diminui quando a temperatura aumenta. Estes resultados confirmam que *C. rutilans rutilans* tem comportamento reprodutivo com adaptações para viver em regiões frias, sendo mais dependente da temperatura, em comparação com *C. rutilans cyanescens* que habita lugares mais quentes, reforçando que ambas as subespécies são ecologicamente diferentes. No terceiro capítulo criamos mapas da distribuição real e provável das duas subespécies baseados em dados provenientes de sete coleções entomológicas no Brasil, somando 46 localidades para *C. rutilans rutilans* e 120 localidades para *C. rutilans cyanescens*. As análises das médias de 23 variáveis ambientais mostraram diferenças evidentes entre as duas subespécies, principalmente nas variáveis relacionadas à temperatura e elevação. A área de sobreposição foi de 70% em âmbito regional, mas na escala local elas não ocorrem juntas. Dependendo da escala espacial, as duas subespécies poder ser simpátricas (escala regional) ou alopátricas (escala local) uma vez que *C. rutilans rutilans* e *C. rutilans cyanescens* têm requerimentos ambientais diferentes, com adaptações locais específicas.

Palavras-chave: Escarabeíneos. Gradiente de elevação. Nicho térmico. Ecofisiologia. Etologia. Adaptação térmica. Especiação. Distribuição geográfica.

ABSTRACT

The aim of this work was to study the temperature effect of two subspecies of *Canthon rutilans* (Coleoptera: Scarabaeidae: Scarabaeinae) in order to observe differences in distribution and behavior that justify that they can be considered ecologically two different species. Therefore, it was divided into three chapters, first one on the geographic distribution of the two subspecies in an elevational gradient of Santa Catarina State, second one on the behaviour of adults and larval development under different temperature conditions and the last one chapter of the actual and probable distribution on a local and regional scale. The first chapter examined the degree of spatial and temporal segregation of the two subspecies along an elevational gradient with one-year data and found differences in their thermal niches. The results showed that *C. rutilans cyanescens* and *C. rutilans rutilans* have not spatially or temporally overlap. The mean temperature limits of the collection days for *C. rutilans cyanescens* were 15.3 and 24.0°C and for *C. rutilans rutilans* were 14.4 and 18.6°C, with an overlap of the niche limited to 5.6%. These results suggest that both subspecies could be considered two species with different physiological and ecological requirements. At the second chapter we compare in laboratory the influence of temperature on behaviour and larval development of subspecies to confirm adaptations to different thermal conditions of distribution areas. Under five temperature conditions (15, 20, 25, 30 and 35°C), feeding behaviour did not differ between the two subspecies or temperature, but there was a difference in reproductive behaviour, with the size of the broad balls dependent on temperature and subspecies, as brood balls of *C. rutilans rutilans* are larger and heavier at low temperatures. The number of offspring and emergence time of new adults were also temperature dependent and the adult survival of *C. rutilans rutilans* decreased as temperature increased. These results confirm that *C. rutilans rutilans* has reproductive behaviour with adaptations to live in cold regions, being more dependent on temperature, compared to *C. rutilans cyanescens* that live in warmer regions, reinforcing that both subspecies are ecologically different. At the third chapter we create maps of the real and probable distribution of the two subspecies based on data from seven entomological collections in Brazil, totaling 46 localities for *C. rutilans rutilans* and 120 localities for *C. rutilans cyanescens*. Analysis of the mean of 23 environmental variables showed evident differences between the two subspecies, mainly in the variables related to temperature and elevation. The overlapping area was 70% at the regional scale, but at the local scale they do not occur together. Depending on the spatial scale, the two subspecies may be sympatric (regional scale) or allopatric (local scale) since *C. rutilans rutilans* and *C. rutilans cyanescens* have different environmental requirements, with specific local adaptations.

Keywords: Dung beetles. Elevational gradient. Thermal niche. Ecophysiology. Ethology. Thermal adaptation. Speciation. Geographical distribution.

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1 INTRODUÇÃO

As condições ambientais necessárias para a sobrevivência de uma espécie definem seu o nicho Grinnelliano (Grinnell 1917). Deste ponto de vista, o nicho envolve subconjuntos das variáveis ambientais, como por exemplo, temperatura, precipitação e radiação solar, que definem o nicho Grinnelliano da espécie e que podem ser equivalentes às áreas de distribuição ou às áreas nas quais uma determinada espécie pode viver (Soberón 2007). Já o conceito de nicho térmico envolve os limites fisiológicos próprios de cada espécie relacionados com a temperatura corporal do indivíduo, sendo esses limites de temperatura os que vão limitar a distribuição espacial (Verdú et al. 2007; Verdú & Lobo 2008).

Os insetos, como organismos ectotérmicos, são fortemente dependentes da temperatura, afetando tanto a demografia como a sua aptidão ou *fitness* (Huey & Berrigan, 2001). A temperatura ambiental tem uma alta capacidade explicativa tanto da abundância como da riqueza de espécies, estando correlacionada positivamente com ambas em diferentes habitats, embora não possa usada como padrão ou regra geral já que a abundância depende de características ecológicas e biológicas de cada espécie (Lobo et al. 2019). Os insetos podem modificar a sua resposta ao efeito da temperatura pois têm a capacidade de se adaptarem ao longo do intervalo de temperatura em que eles são ativos (Chown & Nicholson 2004), mostrando uma maior variedade de respostas fisiológicas a temperaturas potencialmente letais e subletais baixas do que em altas temperaturas (Chown & Nicholson 2004).

Devido à limitada capacidade endotérmica de alguns besouros coprófagos, a temperatura atua como uma limitação na distribuição real e potencial das espécies (Verdú et al. 2007). Algumas espécies possuem capacidade de termorregulação (Verdú et al. 2006) e inclusive existem espécies, como *Thorectes lusitanicus*, que incluem na sua dieta, principalmente coprófaga, frutos do gênero *Quercus* que proporcionam maior resistência às baixas temperaturas, aumentando o conteúdo de gordura no corpo (Pérez-Ramos et al., 2007; Verdú et al., 2010). Estas adaptações geram um benefício competitivo na aquisição dos recursos, sendo resultado da adaptação à temperatura. Outro mecanismo de adaptação de besouros escarabeíneos é o polimorfismo cromático, que pode estar relacionado com fatores ambientais como elevação, precipitação e temperatura (Davis et al. 2008). A cor do exoesqueleto estaria relacionada com a termorregulação, uma vez que as cores têm diferentes

propriedades, podendo refletir, dispersar e desviar a luz diferencialmente, dependendo do seu comprimento de onda (Davis et al. 2008; Amore et al. 2017; Alves et al. 2018).

O gradiente de elevação afeta a distribuição espacial e temporal da maioria dos organismos já que inclui variações climáticas (temperatura, umidade, pressão atmosférica, ventos e precipitações) que implicam mudanças na produtividade, processos de competição e predação dentro do sistema (Rahbek 1995; Lieberman et al. 1996; Lomolino 2001). Os processos históricos e os mecanismos de especiação locais são a origem da distribuição atual de espécies, incluindo os sistemas montanhosos, por isso cada montanha é particular quanto à distribuição, riqueza e taxa de endemismo das espécies (Jay-Robert et al. 1997). Assim, a elevação afeta os padrões de diversidade de espécies de escarabeíneos em regiões tropicais, existindo uma relação curvilínea entre a elevação e a riqueza de espécies, onde há maior riqueza nas elevações intermediárias e um declínio geral com a elevação. A maior riqueza de espécies em elevações intermediárias pode ser a consequência de menores variações ambientais e climáticas do que em áreas de alta e baixa elevação (Escobar et al. 2005; Nunes et al. 2016; da Silva et al. 2018).

Scarabaeinae (Coleoptera: Scarabaeidae) é um grupo amplamente estudado e sensível diante de mudanças de habitats (Halffter & Favila 1993; Gardner et al. 2008; Hernández et al. 2014) e por este motivo tem sido apontado como um bom indicador ecológico. Distribuídas principalmente nas regiões tropicais do planeta, se calcula a existência de 7.000 espécies, desconhecendo-se para muitas delas tanto a biologia como a ecologia (Hanski 1991). A principal característica desta subfamília é sua base alimentar, constituída por excrementos de vertebrados, principalmente mamíferos, e em alguns casos de carcaças, frutos e vegetais em processo de decomposição, tanto no estágio larval como adulto (Halffter & Edmonds 1982). Nos ecossistemas tropicais é um grupo importante, já que participam nos processos de decomposição da matéria orgânica, sendo ativos no serviço ecossistêmico de ciclagem de nutrientes (Nichols et al. 2008). Dependendo do hábito alimentar, podem-se classificar as espécies em saprófagas, alimentando-se de material vegetal em decomposição; coprófagas, alimentando-se de fezes; necrófagas, alimentando-se de carcaças, ou generalistas (Halffter & Matthews 1966).

Canthon (Francmonrosia) rutilans Castelnau, 1840 é uma espécie de escarabeíneo abundante na porção sul da Mata Atlântica (Vaz-de-Mello et al. IUCN 2014) e possui duas subespécies: *Canthon rutilans rutilans* Castelnau, 1840 e *Canthon rutilans cyanescens* Harold, 1868, que apresentam coloração diferente, sendo a primeira subespécie avermelhada e

a segunda azul. Ambas as subespécies são diurnas e têm comportamento coprófago rolador (telecoprídeas), sendo as fezes de vertebrados o recurso alimentar mais frequentemente usado para completar seu ciclo de vida, tanto no estádio larval como ao longo da vida adulta (Halffter & Edmonds, 1982; Hernández et al 2019). Os adultos constroem uma bola-alimento com as fezes e a rolam longe da fonte para se alimentar ou para nidificar. O comportamento de nidificação é do tipo V: a fêmea transforma a bola-alimento em uma bola-ninho e coloca um ovo no interior da bola e, posteriormente, macho e fêmea colaboram conjuntamente no cuidado parental das bolas ninho (Halffter & Edmonds, 1982). Assim, com este trabalho queremos ver a influência que a temperatura exerce na adaptação destas duas subespécies, influenciando sua distribuição.

1.1 OBJETIVO

1.1.1 Objetivo Geral

Avaliar o efeito da temperatura em duas subespécies de *Canthon rutilans* (Coleoptera: Scarabaeidae: Scarabaeinae) com a finalidade de testar diferenças na distribuição e no comportamento de ambas que justifiquem a elevação da posição taxonômica de subespécies para espécies distintas.

1.1.2 Objetivos Específicos

Artigo I: **Distribution of *Canthon rutilans rutilans* and *Canthon rutilans cyanescens* along spatial-temporal and temperature gradients**

Objetivos: (i) examinar o grau de segregação espacial e temporal dessas duas subespécies, ao longo de um gradiente de elevação com dados de um ano; (ii) estimar o nicho térmico e o grau de sobreposição de nicho térmico entre as duas subespécies; (iii) reforçar o atual status de subespécies ou considerar ambas como duas espécies.

Artigo II: Differences in the reproductive behaviour and larval development of two *Canthon rutilans* subspecies reinforce their thermal regional segregation

Objetivo: comparar em laboratório a influência da temperatura no comportamento, desenvolvimento larval e longevidade de *C. rutilans rutilans* e *C. rutilans cyanescens* para confirmar adaptações das subespécies às diferentes condições térmicas das áreas de distribuição.

Artigo III: Regional sympatry but local allopatry in the distribution of the two subspecies of the dung beetle *Canthon rutilans* Castelnau, 1840

Objetivo: a partir de dados de distribuição geográfica, testar se *C. rutilans rutilans* e *C. rutilans cyanescens* mostram diferentes preferências ambientais e gerar mapas de distribuição provável com a finalidade de examinar a distribuição em escala local e regional.

2 ARTIGO I: DISTRIBUTION OF *CANTHON RUTILANS RUTILAN*S AND *CANTHON RUTILANS CYANESCENS* ALONG SPATIO-TEMPORAL AND TEMPERATURE GRADIENTS

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Distribution of *Canthon rutilans rutilans* and *Canthon rutilans cyanescens* Along Spatio-Temporal and Temperature Gradients

ABSTRACT

Subspecies is a debated taxonomic rank that in some cases could indicate that a speciation process is taking place. Studying the degree of co-occurrence among subspecies along environmental gradients may help to determine its taxonomic status. In this study, we explore the distribution of two subspecies of *Canthon rutilans* along spatial-temporal and temperature gradients in the Atlantic Forest of southern Brazil in order to reinforce their current sub-specific status or to support their consideration as two different species. A yearly survey conducted along an elevational transect (from 250 m to 1630 m) shows that there is no spatiotemporal overlap between the two taxa. We collected 899 individuals of *Canthon rutilans cyanescens* and 29 individuals of *Canthon rutilans rutilans*. *C. rutilans cyanescens* can be found at 250 m (all year except in Jun), 430 m (August to April), and 840 m (September to April) in elevation, and when the air temperature oscillates from 15.3°C to 24.0°C. *C. rutilans rutilans* can be found at 1360 m (October to February), 1630 m (January) in elevation, and when the air temperature oscillates from 14.4°C to 18.6°C. Furthermore, local temperature data taken during the survey indicates that both subspecies also have a limited overlap in their thermal response curves. All these results suggest that these two taxa could be considered as two different species with dissimilar physiological and ecological requirements probably as a consequence of temperature-mediated divergent adaptation. Further molecular data can confirm or reject this supposition in the near future.

Key-words: Speciation; Dung beetles; *Canthon rutilans*; elevation, gradient; temperature; spatio-temporal distribution

INTRODUCTION

The lack of spatial and temporal co-occurrence in taxonomically related taxa, as well as their differential distribution across environmental gradients, has traditionally been considered key evidence, which identifies specific characteristics of related taxa with a recent common ancestor [1-4]. Thus, in order to establish the species status of morphologically similar taxa, it is important to understand the environmental or ecological factors that allow or hinder their coexistence. Although the mechanisms of speciation are considered complex, varied, and interlinked, isolation and lack of hybridization seem to be a necessary requisite since these generate divergent natural selection forces on different environmental conditions [5]. Thus, the role played by different potential isolating barriers on species pairs recently achieving their specific status may shed light on possible speciation processes.

The elevational gradient is a limiting factor for the spatial and temporal distribution of most organisms exposed to climatic variations (e.g. temperature, humidity, atmospheric pressure, insolation, wind, and precipitation) [6, 7]. Tropical insects are highly affected by temperature due to their general narrow physiological tolerances [8] and this limitation may restrict their realized and potential distributions. As consequence, the environmental temperature may be a divergent selection pressure promoting speciation because populations living at different environmental temperatures may become isolated [9, 10].

Subspecies is a controversial taxonomical rank subordinate to species not always representative of distinct phylogenetic lineages [11] in which individuals do not have reproductive isolation, even though they have some different characteristics [12]. In some cases, subspecies are identifiable taxa experiencing a process of incipient speciation. Consequently, the degree of co-occurrence between related subspecies across environmental gradients would support or refute their taxonomic status. Data on the seasonal and spatial distribution of two subspecies of dung beetles are used in this study to provide additional evidence on their possible taxonomic status. Since environmental temperature may affect the distribution and abundance of ectothermic groups, such as insects, due to its influence on metabolic functions and development or growth rates [13], we also used local temperature measurements to examine the degree of segregation between these two subspecies.

Canthon (*Francmonrosia*) *rutilans* Castelnau, 1840 is a Neotropical dung beetle species (Coleoptera: Scarabaeidae: Scarabaeinae) whose distribution extends from the province of Misiones in Argentina to the states of Mato Grosso do Sul, São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul in Brazil, and lastly to Uruguay [14]. The climate in this large region corresponds to group C in Köppen climate classification (including Cf and Cw), that is, midlatitude climate, in which the average temperature of the coldest month is between -3 and 18°C and the warmest above 10°C [15]. Like other species of the genus *Canthon*, they are diurnal, and larvae and adults feed mainly on faeces or carrion [16]. Adults carry food in the form of spherical balls in order to save food resources, as well as to make nesting balls where larvae develop [17]. Due to this behaviour, the dung beetles are very active in the ecosystem service of organic matter removal [18].

This species is separated into two subspecies: the originally described red coloured (on the back) *Canthon rutilans rutilans* Castelnau, 1840 and the blue coloured *Canthon rutilans cyanescens* Harold, 1868. Colour polymorphism in Scarabaeinae species has been associated with the control of body temperature in response to environmental conditions [19, 20]. Different coloration may be an evolutionary response linked to the thermal adaptation towards environmental conditions. Black dung beetles are usually nocturnal, whereas diurnal dung beetles show varied colours, thus, the diurnal species colouring may be important in sexual or natural selection [21]. Colour in dung beetles is a property of the exoskeleton because it can reflect, disperse, and deflect light differentially depending on its wavelength. Beetles with darker colorations should be heated more at higher solar radiation levels but species of beetles with different colours may have a similar thermal response to visible light but not to infrared radiation. For example, *Canthon rutilans rutilans* (light red) compared to *Homocopris* sp. (deep black) have similar adjusted heating rates under simulated sun radiation [22].

Despite the different coloration in both subspecies, there is no knowledge of differential behaviour. In regards to feeding habits, both *C. rutilans cyanescens* [23-25] and *C. rutilans rutilans* [26-30] have a preferentially coprophagous diet. However, a revision of the literature suggests that both subspecies do not seem to inhabit together, in the same locality: *Canthon rutilans cyanescens* is found in rainforest habitats with high temperatures located at elevations under 1000 m [23, 31-39], while *Canthon rutilans rutilans* tends to be collected in grasslands or eucalyptus plantations in cold locations or in forests situated at more than 1000

m in elevation [26-29, 40, 41]. This divergence may be a result of a physiological adaptation to temperature for biogeographic reasons, which may have led to speciation.

Using data from a comprehensive survey conducted during a complete calendar year in several locations along an elevation gradient, this study aims to examine the degree of spatial and temporal segregation of these two subspecies in order to corroborate the speciation supposition. However, even if both subspecies are spatial-temporally segregated they could still share thermal niche preferences [42]; therefore, their degree of co-occurrence throughout a temperature gradient was also estimated. Accordingly, using local temperature information of each surveyed site and time, temperature response curves were also compared in order to estimate the degree of thermal niche overlap between the two taxa. The evidence found would then be used to reinforce their current sub-specific status or to support their consideration as two different species.

MATERIAL AND METHODS

Study area

Six sites were chosen along an elevation gradient in the Atlantic Forest of the state of Santa Catarina, southern Brazil. These sites were located along a transect extended northeast-southwest (27°44' to 28°9'S; 48°48' to 49°37'W) from 200 to 1600 m a.s.l. Two areas were located in the municipality of Santo Amaro da Imperatriz, one at 250 m and the second at 430 m; one area in Rancho Queimado municipality at 840 m; three areas in the municipality of Urubici, one area in RPPN-Reserva Leão da Montanha at 1060 m, and the other two areas in Parque Nacional de São Joaquim at 1360 m and 1630 m. The study areas are classified into two climatic regions, Cfa and Cfb, according to Köppen climatic classification. Both regions present a uniform rainfall throughout the year and humid mesothermal climate. Cfb is located in elevations below 800 m, with warm summer temperatures compared to Cfa (i.e. temperatures above 22°C). Cfa is located above 800 m in elevation, with cooler summer temperatures (i.e. the temperature does not reach 22°C), and frost frequently occurs during colder months [15]. The vegetation in the study areas included Dense Ombrophilous Forest, an evergreen forest whose canopy reaches 30 m and has dense shrub vegetation, consisting of

ferns, bromeliads and palms, as well as a Mixed Ombrophilous Forest characterized by the presence of *Araucaria angustifolia*, which appears at high elevations, above 800 m a.s.l. [43].

Beetle sampling

Dung beetles were sampled monthly at each sampling site from June 2015 to June 2016 using traps that remained in the field for 48 hours. Along the elevation gradient, at each sampling site, we established five sampling points separated 100 m from each other. At each sampling point three different traps were placed, totalling 15 traps per sampling site, which included five traps that allowed both immigration and emigration (TIE), five traps that prevented emigration and were baited with human dung (TE$_D$), and five traps that also prevented emigration, but were baited with rotting pork flesh (TE$_F$). All the traps were plastic containers (15 cm diameter and 20 cm depth) buried into the ground up to the rim and protected from rain by a plastic cover approximately 10 cm above the trap. TIE containers were filled with local soil and a piece of approximately 10 gr of human dung was placed in the centre. TE$_D$ and TE$_F$ traps were similar, however, 200 ml of water and a few drops of detergent were added to each container to catch the attracted insects and avoid their escape. Human faeces or rotting flesh (also ca. 10 g) were used to attract dung beetles (i.e., coprophagous and necrophagous species, respectively). The bait was wrapped in a thin cloth and tied to the central part of the plastic cap. The total number of individuals collected within the 15 traps placed at each site and period were considered as a sampling unit (the final number of used sampling units was 75 because on three occasions it was not possible to collect it).

All the *Canthon rutilans* individuals collected were identified and voucher specimens (dried and mounted in entomological pins) were deposited in institutional collections (Coleção Entomológica do Centro de Ciências Biológicas da Universidade Federal de Santa Catarina and Coleção Zoológica da Universidade Federal de Mato Grosso, Brazil). The taxonomist Dr. Fernando Vaz-de-Mello confirmed the species (Universidade Federal de Mato Grosso). The permission to collect dung beetles was issued by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/MMA, permit #49486-1).

Temperature measurements

The local air temperature of each sampling site was measured every 15 minutes during the complete period of study using a datalogger (HOBO Pendant®) placed on a tree trunk one meter from the ground.

Data analysis

Generalized Linear Models (GLMs) were used to quantify the relevance of local temperature measurements on either the presence–absence of each species or the variation in their number of individuals. Separate models were run in order to compare the effect of temperature in delimiting both the occurrence of each species and the variation in abundance within the sampling units with positive occurrences. In the first case, we used a binomial error distribution linked to the set of predictor variables via a logit link function. In the second case, a Poisson distribution was assumed and a logarithmic link function used. The significance of linear and quadratic functions was assessed to consider possible curvilinear relationships. Our aim with these models was simply to estimate the individual explanatory capacity of air temperature, and how this variability is measured as a change in deviance from a null model [44]. All these analyses were performed using StatSoft's STATISTICA v12.0 (StatSoft Inc, 204 Tulsa, Oklahoma, USA).

A logistic fitting curve was used to visualize the relationship between air temperature experienced at each sampling unit during the sample time (48 hours) and the relative frequency of both taxa. The adjusted curves are considered an estimation of the variation in the maximum attained abundance according to temperature. Taking into account the minimum (5°C) and maximum (25°C) mean temperatures registered during the surveys, the area under the adjusted curves of the two species was computed in order to measure the extent of their favourable thermal conditions. These calculations were done using the software CurveExpert 1.4 (www.curveexpert.net). The value of the area under the curve for *C. rutilans cyanescens* (A) and *C. rutilans rutilans* (B) were used to estimate the relative shared thermal range between the two subspecies (C), calculated as: *max* (A,B)-A = B-C; *max* (A,B)-B = A-C; and then C = *max* (A,B)-[(B-C) + (A-C)].

RESULTS

Spatial and temporal segregation

A total of 899 individuals of *C. rutilans cyanescens* and 29 individuals of *C. rutilans rutilans* were collected. Most of the individuals of *C. rutilans cyanescens* (N=825, 92%) were collected at the 250 m site throughout the year except in June, 52 individuals were sampled at

the site located at 430 m from August to April, and 22 individuals at the 840 m site from September to April (Fig. 1). In the case of *C. rutilans rutilans* most of the individuals (N=28, 96%) were collected at the site located at 1360 m during the hottest months of the year, from October to February; only one individual was sampled in January at the 1630 m site. Neither of these two subspecies occur in the site located at 1060 m, since their absence emphasizes their spatial isolation as none of them seem to be able to maintain stable populations at intermediate elevations; the blue morphotype occurs at lower elevations (up to 860 m) while the red one appears at the highest elevations (above 1360 m). Consequently, and according to the obtained data, there is no spatial-temporal overlap between the two taxa (Fig. 1).
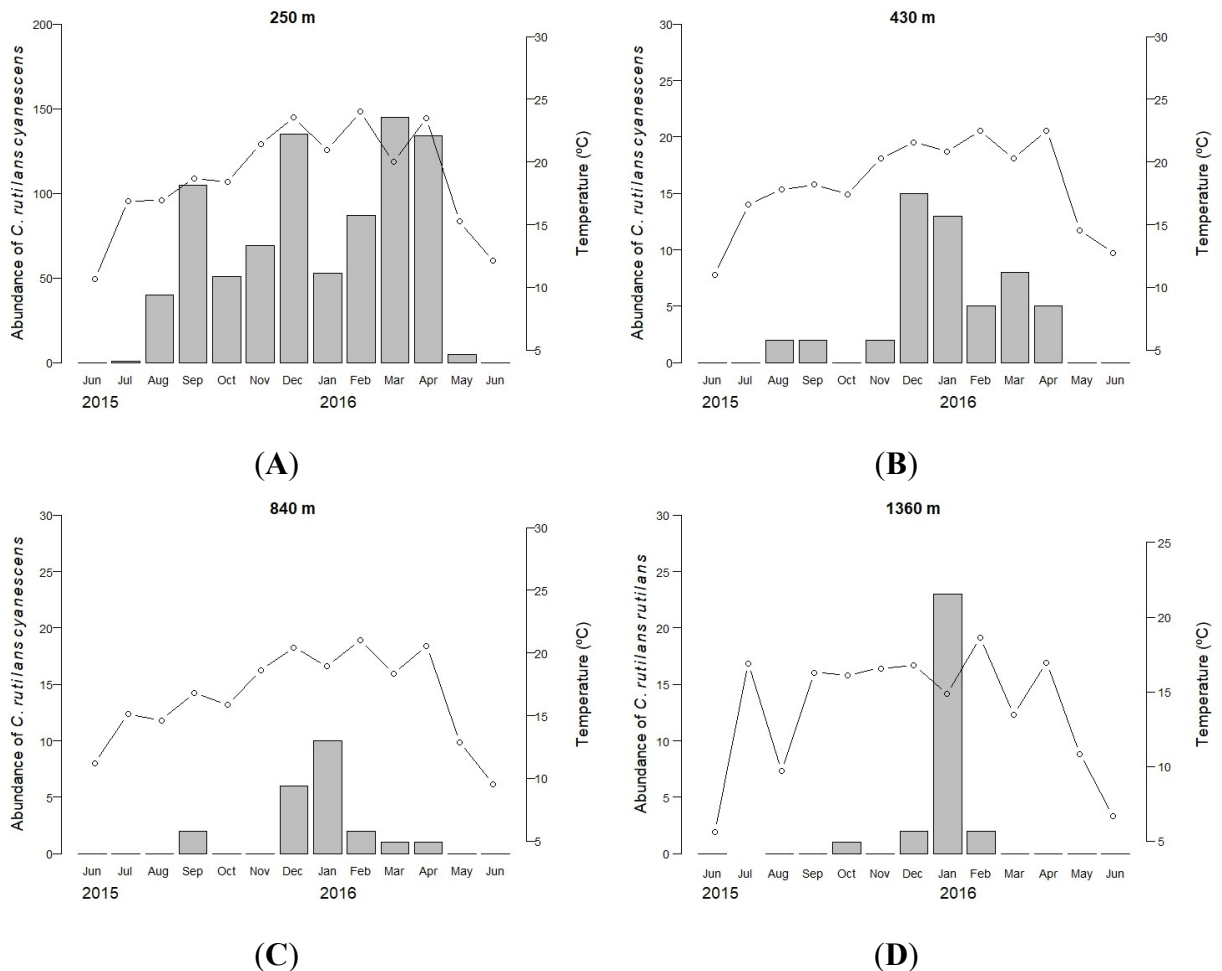

Figure 1. Spatial and temporal distribution of *Canthon rutilans cyanescens* at A) 250 m, B) 430 m, and C) 840 m; and *Canthon rutilans rutilans* at D) at 1360 m, taking into account the number of collected individuals (bars), and variation in mean air temperature (circles) during each of the 48 hour surveys.

The explanatory capacity of temperature

A quadratic function of temperature (curvilinear) was able to account for 59.9% of total deviance in the presence-absence variation of *C. rutilans cyanescens*, while this explanatory capacity only reached 15.9% for *C. rutilans rutilans.* However*,* abundance variation at the sites with positive occurrences shows a linear and positive relationship with air temperature, accounting for 23.7% and 20.5% of the total variability, respectively.

Derived thermal responses

The air temperature of the sampling units at which both taxa were recorded oscillates from 15.3°C to 24.0°C in the case of C. *rutilans cyanescens*, and from 14.4°C to 18.6°C for *C. rutilans rutilans* (Fig. 2). This overlap across the thermal gradient when thermal limits derived from samples are considered is significantly less pronounced if relative abundance frequencies are considered (Fig. 2). In this case, only 5.6% of the area under the response curves would correspond to the overlapped thermal range. The maximum abundance of *C. rutilans rutilans* would appear at air temperatures from 14°C to 16°C, while *C. rutilans cyanescens* would prefer higher temperatures (at least equal to or greater than 20°C) even higher than those experienced in the studied sites. Data on the presence of adults at different temperatures can be seen in Table 1 (absence data were omitted from the table, but not from the analyses).
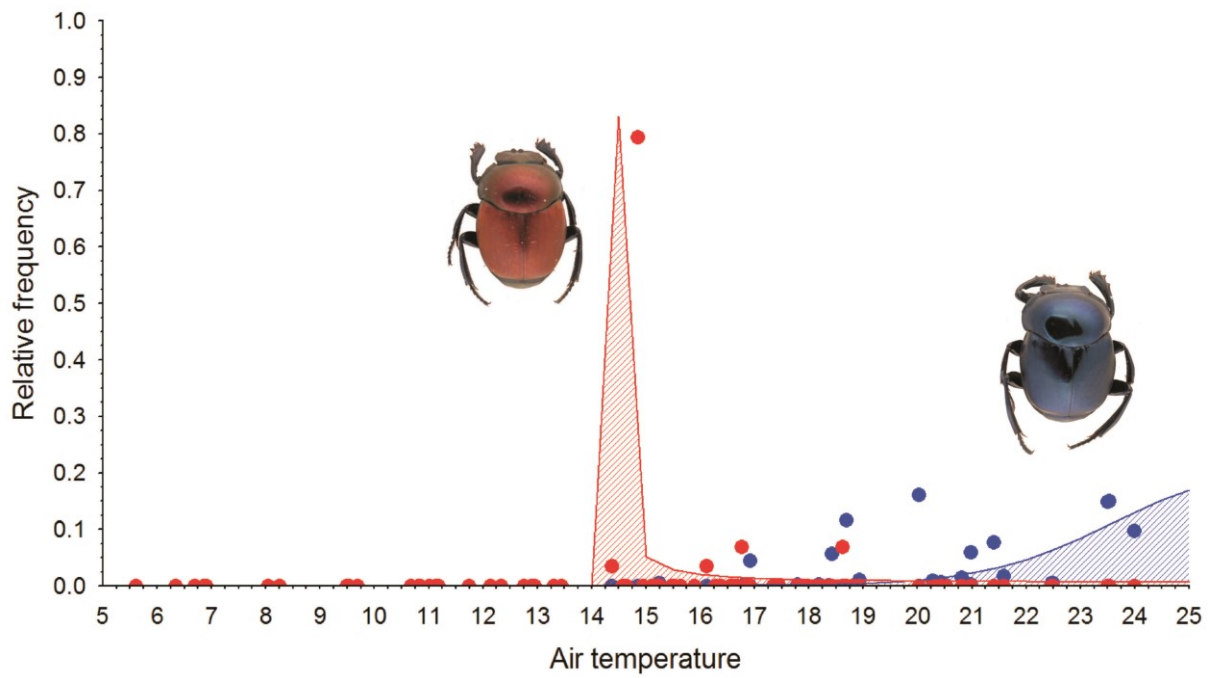
Figure 2. Temperature response curves for *C. rutilans rutilans* (in red) and *C. rutilans cyanescens* (in blue) considering the relative abundance of each sampling unit and its associated mean air temperature values. Points are observed values and lines represent adjusted curves.

Table 1. Presence of *Canthon rutilans cyanescens* and *Canthon rutilans rutilans* adults during the sampled year (June 2015 at June 2016). Mean temperature and mean minimum temperature during the three days of sampling and also minimum temperature during the month.

Subspecies	Area	Data	Mean Temperature (°C) (3 d)	Mean Minimum Temperature (°C) (3 d)	Minimum Temperature (°C) (Month)
Canthon rutilans cyanescens	250m	VII/2015	16.91	15.47	6.67
Canthon rutilans cyanescens	250m	VIII/2015	16.91	13.23	8.88
Canthon rutilans cyanescens	250m	IX/2015	18.69	14.86	6.98
Canthon rutilans cyanescens	250m	X/2015	18.42	15.85	10.26
Canthon rutilans cyanescens	250m	XI/2015	21.40	18.62	12.98
Canthon rutilans cyanescens	250m	XII/2015	23.54	19.76	15.57
Canthon rutilans cyanescens	250m	I/2016	20.98	17.44	16.14
Canthon rutilans cyanescens	250m	II/2016	24.00	20.49	14.80
Canthon rutilans cyanescens	250m	III/2016	20.03	17.70	14.71
Canthon rutilans cyanescens	250m	IV/2016	23.50	20.52	5.84
Canthon rutilans cyanescens	250m	V/2016	15.25	13.36	7.06
Canthon rutilans cyanescens	430m	VIII/2015	17.79	13.64	6.06
Canthon rutilans cyanescens	430m	IX/2015	18.19	15.56	6.06
Canthon rutilans cyanescens	430m	XI/2015	20.31	18.20	13.46
Canthon rutilans cyanescens	430m	XII/2015	21.59	19.09	14.61
Canthon rutilans cyanescens	430m	I/2016	20.82	17.60	16.24
Canthon rutilans cyanescens	430m	II/2016	22.48	20.20	15.86
Canthon rutilans cyanescens	430m	III/2016	20.29	17.57	14.13
Canthon rutilans cyanescens	430m	IV/2016	22.48	20.42	5.76
Canthon rutilans cyanescens	840m	IX/2015	16.82	14.38	2.20
Canthon rutilans cyanescens	840m	XII/2015	20.44	17.47	12.88
Canthon rutilans cyanescens	840m	I/2016	18.93	14.80	13.17
Canthon rutilans cyanescens	840m	II/2016	20.99	18.65	13.17
Canthon rutilans cyanescens	840m	III/2016	18.36	14.99	12.01
Canthon rutilans cyanescens	840m	IV/2016	20.53	18.14	3.89
Canthon rutilans rutilans	1360m	X/2015	16.12	12.82	2.73
Canthon rutilans rutilans	1360m	XII/2015	16.77	13.46	6.67
Canthon rutilans rutilans	1360m	I/2016	14.85	8.81	7.18
Canthon rutilans rutilans	1360m	II/2016	18.62	12.91	4.83
Canthon rutilans rutilans	1630m	I/2016	14.38	8.61	7.68

DISCUSSION

The obtained results indicate that *C. rutilans cyanescens* and *C. rutilans rutilans* do not overlap spatial-temporally as suggested by the previous available faunistic and ecological studies [23, 31-39]. Some examples of speciation through habitat isolation [1, 3, 45, 46] show that the previous isolation is followed by a subsequent adaption to the specific environmental conditions of each occurrence area [5]. Consequently, it is difficult to distinguish the ecological or geographical nature of this possible speciation process.

The different explanatory capacity of temperature on presence-absence data in both subspecies shows a higher dependence of *Canthon rutilans cyanescens* (59.9%) adults to temperature than *C. rutilans rutilans* (15.9%). *C. rutilans cyanescens* occurs in rainforest habitats with high temperatures and lower elevations while *C. rutilans rutilans* lives at higher elevations and lower temperatures. Dung beetle species adapted to low temperatures generally have a shorter time to complete their reproductive activity. Therefore, they copulate and search for food to nest during a short period of time, mainly in the summer, possibly as an adaptation to cold environments [47]. In our study, there were months along the year (Fig. 1) where the mean temperature could allow the activity of *Canthon rutilans rutilans* adults, however, they are absent. This could be because the life cycle only allows adult emergence when summer conditions arrive. After this short period of adult reproductive activity (winged phase), adults may go into hibernation during cold periods or perhaps go into a diapause phase in larvae or egg stages. All these possible adaptations would lead to a long life cycle waiting for a favourable period of time.

CONCLUSIONS

The spatial-temporal segregation of *C. rutilans* is associated with a differential capacity of environmental temperature to explain the occurrence of these two subspecies, especially with a limited overlap in their thermal response curves. These data suggest that each subspecies would also inhabit relatively different temperature conditions. Changes in the climatic conditions along an elevational gradient can become a barrier to dispersion, which facilitates allopatric speciation but also promotes changes in species richness and compositional patterns [48-50]. Considering that a thermal barrier may reflect the range of thermal conditions at which an organism can have a net positive demographic growth rate [51], the available results

suggest that *C. rutilans rutilans* and *C. rutilans cyanescens* could be considered two different species with different physiological and ecological requirements. Thus, temperature-mediated divergent adaptation could well have generated the appearance of these subspecies as seems to have happened in other groups [9].

The process that could have caused separation in two different species can be attributed to the vertical colonization, in which species living in low and warm regions expand their distribution to higher and cooler regions, adapting to lower temperatures [52, 53]. According to Sobel and collaborators [5], the problem posed by ecogeographic isolation is that a difference in the niche of two taxa only shows that they are found in places with different environmental conditions, but this does not guarantee that there actually are genetic differences. Additional molecular, morphological and thermal physiological studies under controlled laboratory conditions would be necessary to corroborate the probable specific status of these two taxa.

Author Contributions

Conceptualization, M.C.H., M.I.M.H. and J.M.L.; Methodology, M.C.H., M.I.M.H., P.G.d.S., V.A. and J.M.L.; Formal analysis, M.C.H. and J.M.L.; Investigation, M.C.H., M.I.M.H., P.G.d.S., V.A. and J.M.L.; Data curation, M.I.M.H.; Writing – review & editing, M.C.H., M.I.M.H., P.G.d.S. and J.M.L.; Supervision, J.M.L. and M.I.M.H.; Project administration, M.I.M.H.; Funding acquisition, M.I.M.H.

Conflicts of Interest

The authors declare no conflict of interest.

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3 ARTIGO II: DIFFERENCES IN THE REPRODUCTIVE BEHAVIOUR AND LARVAL DEVELOPMENT OF TWO *CANTHON RUTILANS* SUBSPECIES REINFORCE THEIR THERMAL REGIONAL SEGREGATION

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Differences in the reproductive behaviour and larval development of two *Canthon rutilans* subspecies reinforce their thermal regional segregation

ABSTRACT

Two dung beetle subspecies of *Canthon rutilans* Castelnau (Coleoptera: Scarabaeinae), *C. rutilans rutilans* and *C. rutilans cyanescens*, inhabit under different environmental temperature conditions in southern Brazil. We developed a laboratory breeding experiment of 60 couples of both subspecies at five temperature conditions (from 15°C to 35°C) to compare their influence on the behaviour of adults and the development of larvae. Behavioural patterns of the adults of both subspecies differ according to temperature. The size of food balls was smaller and lighter in *C. rutilans cyanescens*. Although temperature did not influence the alimentary behaviour (measured as the number of food balls constructed), reproductive behaviour (measured as the number of brood balls constructed by couple) was significantly lower at 15°C in both subspecies. Besides that, brood balls from *C. rutilans rutilans* were bigger and heavier at lower temperatures. The number of offspring and the time of emergence depend on temperature too. However, the weight of offspring and the longevity of adults depend on the subspecies and temperature treatment. These results demonstrate that both subspecies have different thermal adaptations, where *C. rutilans rutilans* has a reproductive behaviour with adaptations to live under colder conditions than *C. rutilans cyanescens*. Thus, both subspecies can be considered two different species from the ecological point of view.

Key-words: Dung beetles, life cycle, ecophysiology, ethology, thermal adaptation

INTRODUCTION

Insects are able to modify their response to temperature due to their capacity to adapt to the range of temperatures where they are active (Chown & Nicholson, 2004). Individuals living at different temperatures may have distinct physiological temperature limits, which restricts their actual and potential distribution. The elevational gradient affects the spatial and temporal distribution of most organisms. As consequence, insects are exposed to climatic variations (temperature, humidity, atmospheric pressure, wind, and precipitation) that may involve changes in productivity, competition processes, and predation (Rahbek, 1995; Lieberman et al., 1996; Lomolino, 2001). Therefore, temperature conditions during development can affect the adults of many ectotherms (Atkinson, 1994; Atkinson & Sibly, 1997).

Insects have response mechanisms at non-freezing low temperatures (Chown & Nicholson, 2004). The endothermic capacity for flight in some insects modifies their geographical distribution, such as habitat selection (Blouin-Demers & Weatherhead, 2002; Chown & Nicholson, 2004). Depending on their physiological limits, each insect species would have their own thermal niche, which can be used to explain their distribution and limit the actual and potential range (Verdú et al., 2006). Dung beetle species (Coleoptera: Scarabaeinae) are preferentially diversified in tropical regions (Halffter, 1991) since temperature is a limiting factor for the development of dung beetles inhabiting cold-temperate areas (Hanski, 1991). Similarly, the richness of dung beetle species decreases with elevation (Escobar et al., 2007; da Silva et al., 2018). At higher elevations, the annual and daily thermal range is greater, and consequently, dung beetle species inhabiting under these conditions have a larger range of thermal tolerance than species at lower elevations (Gaston & Chown, 1999).

Dung beetle species with the capacity of thermoregulate their body temperatures have a higher resistance to low temperatures (Verdú et al., 2006). Other adaptations to low temperatures include the use of alternative trophic resources as in the case of *Thorectes lusitanicus*, which is able to eat acorns from the *Quercus* genus, making this behaviour more resistant to low temperatures (Pérez-Ramos et al., 2007; Verdú et al., 2010). However, the most common dung beetle trophic resource is the vertebrate faeces, which are used to complete their life cycle, during the larval stage, as well as during their adult life (Halffter & Edmonds, 1982).

Recently, it was proposed that two subspecies from the Neotropical species *Canthon (Francmonrosia) rutilans* Castelnau, 1840 (the red coloured *Canthon rutilans rutilans* Castelnau, 1840 and the blue coloured *Canthon rutilans cyanescens* Harold, 1868) may be in process of speciation considering their differing thermal preferences found during a regional study (Hensen at al., 2018). In southern Brazil, *C. rutilans rutilans* occurs in cool and subtropical montane regions at more than 1000 m in elevation, with adults active from 14.4°C to 18.6°C in environmental temperature. Meanwhile, *C. rutilans cyanescens* occurs in warmer and lowland regions under 1000 m, with adults that are active from 15.3°C to 24.0°C in environmental temperature (Hensen at al., 2018). As several South American Deltochilini, these two subspecies have a nesting behaviour pattern V (Halffter & Edmonds, 1982); these are ball-rolling species (telecoprid), making a food ball from faeces and rolling it to the place where they will build their nest to use these balls for feeding or nesting. When nesting, females transform a food ball into a brood ball and put one egg into it. Males and females cooperate in the parental care of brood balls (Halffter & Edmonds, 1982).

In this study, we analysed the behaviour and the developmental biology of these two subspecies under laboratory conditions. Our hypothesis is that since these two subspecies have different thermal conditions along their regional occurrences, they may have also different behavioural responses to environmental temperatures, as a consequence of different temperature dependent fitness. Thus, the main objective of this study was to compare the influence of temperature on the behaviour, larval development, and longevity of *C. rutilans rutilans* and *C. rutilans cyanescens*. The two subspecies were bred in the laboratory at different temperatures to answer the following questions: 1) Is the behaviour of adults (measured from the activity in ethograms and by the size and number of balls produced) different in both subspecies according to temperature? 2) Is the life cycle (measured from the time of larval development and adult longevity) different for both subspecies according to the environmental temperature?

MATERIALS AND METHODS

Dung beetle capture for experiments

Dung beetles were captured in two areas of the Santa Catarina State, southern Brazil. The specimens of *C. rutilans rutilans* were collected during the summer (end of December 2016) at 1360 m a.s.l., in the São Joaquim National Park (28°8'28"S - 49°38'13"W) located in the

municipality of Urubici. The individuals of *C. rutilans cyanescens,* were also collected during the summer (December 2017 and January 2018) at 250 m a.s.l. in the Serra do Tabuleiro State Park (27°44'01"S - 48°48'33"W) located in the municipality Santo Amaro da Imperatriz. Although the specimens of the two subspecies were collected in different years by logistic reasons, we assume that the effect of this difference is negligible compared with the one studied in the experimental design. Dung beetles were sampled several times until we collected enough individuals to conduct laboratory experiments. All unnecessary specimens (from other species) were released in the same place location where they were collected. We used pitfall traps baited with dog faeces to collect the attracted beetles twice a day (morning and afternoon). These traps were plastic containers (15 cm in diameter and 20 cm deep) buried in the ground up to the rim and closed with a plastic lid containing a hole to permit the entry but prevent the escape of individuals. All of the traps were protected from the rain by a plastic cover approximately 10 cm above the container. The bottom of the traps was filled with local soil and a piece of approximately 30 g of dog faeces was placed in the centre. The faeces were previously obtained from dogs not submitted to any medical treatment and maintained under refrigeration conditions at the Biotério Central (Bioterium) of the Universidade Federal de Santa Catarina.

Rearing and maintenance under different temperatures

The beetles were transported to the Laboratório de Ecologia Terrestre Animal (LECOTA/UFSC) and maintained at 26±1°C for a photoperiod of L12:D12. They were stored in five litres plastic pots with soil from the location and were fed weekly with dog faeces until the beginning of the experiments. The individuals of *C. rutilans rutilans* (Figure 1A) and *C. rutilans cyanescens* (Figure 1B) were individually identified, weighed, sexed, and classified into five age categories: young, young-mature, mature, mature-old, and old. Only those specimens identified as young-mature or mature were separated to be subsequently used in the experiments in order to guarantee the sexual maturity of the individuals. We used the tibia degree and clypeus erosion to identify these age categories (Tyndale-Biscoe, 1984).
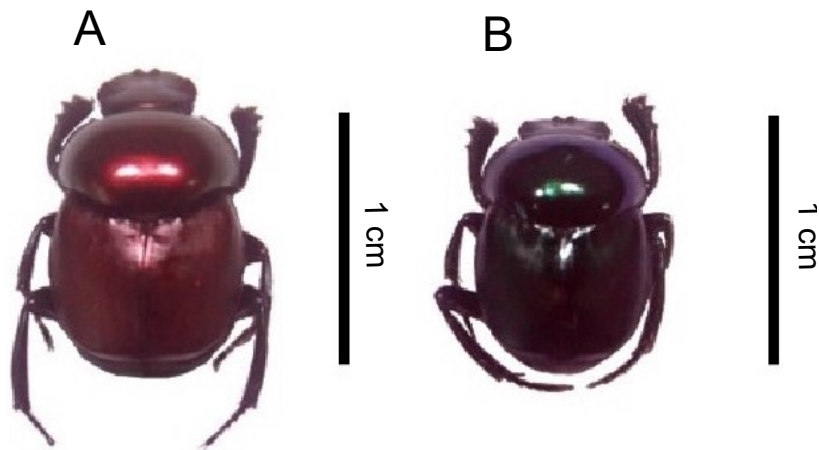
Figure 1. Adults of *Canthon rutilans* subspecies: *Canthon rutilans rutilans* with characteristically red colour (A) and *Canthon rutilans cyanescens* with blue colour (B).

Thirty couples of each subspecies were selected taking special care to ensure that the male and female from each couple had a similar weight. These couples represent a weight gradient from light to heavy couples distributed in each treatment to eliminate bias due to body size. Each couple was kept in a transparent plastic container (13 cm in diameter and 11 cm deep) with 500 g of soil from the location where each subspecies was collected, as suggested by Villalobos et al. (1998) to avoid submitting each subspecies to different soil conditions than those experienced under natural situations. The soil was previously sifted and heated for 90 seconds in a microwave at maximum power in order to remove possible pathogenic bacteria and fungi. The containers were covered with a plastic lid with a hole in the middle covered by a tissue that allowed the entry of air. During the experiment, the soil in the containers was kept constantly moist by periodically spraying it with water. All couples were fed weekly with 10 g of dog faeces from Biotério Central (Bioterium).

Couples included in the containers were maintained at five different thermal conditions or treatments (15, 20, 25, 30, and 35°C) and each treatment was repeated six times; thus, we used a total of 120 individuals (2 subspecies × 2 individuals × 6 repetitions × 5 thermal treatments) and 30 experimental units per subspecies (6 repetitions × 5 thermal treatments). We selected the minimum temperature (15°C) considering the lowest environmental temperature experienced by active adults of these two subspecies in the collection areas (Hensen et al., 2018). Beginning with this value, treatments increased by 5 degrees to a hypothetical maximum of 35°C. The temperature of 15°C was maintained in the laboratory by air conditioning, while the other four warmer temperatures were maintained by placing the containers in four independent electric warmer showcases Titã® ACL-5810D (77

× 36.5 × 44.5 cm). Although the experiments of each subspecies were carried out in different years, they started at the same period, during the summer: for *C. rutilans rutilans* the experiment started in January 2017 and for *C. rutilans cyanescens* in February 2018. After the experiment (7 weeks of data), we maintained the couples under the experimental conditions until 90% of the individuals of each subspecies died. Therefore, the experiment of *C. rutilans rutilans* ended in November 2017 and for *C. rutilans cyanescens* in June 2018.

Behaviour activity

Photographs of each couple/container were taken the day the couple was fed. The transparent plastic container lids were removed and replaced with glass lids. A camera (Canon® T3) connected to a laptop was attached to a tripod to take pictures from above. The camera was taking pictures every five minutes for three hours (time lapse), beginning this process at 3 p.m. and finishing at 6 p.m. Each couple was photographed only one day in the experiment, with a total of 40 photos *per* couple and 1200 pictures (40 photos per couple × 6 couples per temperature × 5 temperature treatment). Since there were two individuals per container, for each photo we registered what each individual was doing; thus, we have two behavioural activities per photo. In total we had 80 responses per couple. The photographs were taken in time sequence (time lapse each 5 minutes). Therefore, it was possible to observe the "movement" of the individuals and identify different behavioural patterns. Based on these observations we described the behaviour and classified the behavioural patterns into four behavioural categories: sexual, locomotion, alimentation and others (Table 1). The behavioural data of each beetle was analysed from the five behavioural patterns: copulation (COP), parental care (PCARE), move on the surface (MOVE), feed (FEED), stay on the food ball (STAY), and buried (BURY). Thus, we created a matrix showing the frequency of the different behavioural patterns and performed a Principal Components Analysis (PCA) in order to obtain new uncorrelated variables that represent the behaviour variability. This method was used in order to analyse all the variables together at the same time. Afterwards, the values of each couple in the first and second PCA components were submitted to a two-way Analysis of Variance and *post hoc* Tukey test for multiple comparisons in order to quantify the behavioural differences observed at each temperature treatment *per* subspecies. These

calculations were done using the software R (version 3.5.3), histogram tools ggplot2 (Wickham & Chang, 2016) and the vegan package (Oksanen, 2011).

Table 1. Ethogram of behavioural categories observed in *Canthon rutilans ex situ* (laboratory conditions).

Behavioural Categories	Behavioural Patterns	Description of the behaviour
Sexual	Copulation (COP)	The male adheres to the female in the dorsal part
	Parental care (PCARE)	The individual is above the brood ball
		The individual dislocates/rolls the brood ball
		The individual is burying the brood ball
Locomotion	Move on the surface (MOVE)	The individual walks along the side of the case
		The individual tries to scale by the wall of the case
		The individual is on the surface of the soil
Alimentation	Feed (FEED)	The individual feeds on faeces or food balls
	Stay on the food ball (STAY)	The individual is on the food ball
Others	Buried (BURY)	The individual is hidden in the soil

Dung ball production

The 30 experimental units per subspecies were followed for 8 weeks (7 weeks of data) with weekly counting the number of food and brood dung balls made by each beetle couple. Additionally, the weight and size (diameter) of the balls were also measured. Food and brood dung balls can be clearly distinguished due to the "drop" shape of brood balls. The mean weight and diameter of the balls made by each couple were used in the statistical analyses since individual identification of each ball is not possible (balls can be destroyed by a couple and new balls can be built between observation periods). We consider that the data obtained of each couple are statistically independent, because their own behavioural do not influence the behavioural of the other couples (Schank & Koehnle, 2009).

Emerged individuals and longevity

The number of new specimens emerged in each container was counted and individuals were weighed in order to assess the possible differences according to temperature treatments and subspecies identity. Lastly, we calculated the number of weeks that passed until the death of

90% of the specimens of each subspecies (longevity) in order to examine whether the average lifetime of the two subspecies differs according to temperature treatments.

Statistical treatment

A full factorial design in General Linear Models was used to estimate the statistical significance of the different dependent variables including temperature treatments (five levels) and subspecies identity (two levels) as explanatory factors. We examined the statistical significance (P<0.05) of the two main effects (temperature treatment and subspecies identity) and the interaction between both (temperature × subspecies), but if the interaction was statistically significant main effects were ignored in the interpretation of the results. We used Tukey HSD post hoc tests after Bonferroni corrections to examine the statistically significant differences in mean values among the levels of the factors. These calculations were done using the software R (version 3.2.3) and histogram tools ggplot2 (Wickham & Chang 2016).

RESULTS

Body weight differences

The subspecies identity factor is the only statistically significant factor in accounting for body weight differences ($F_{1,116} = 6.98$, $P = 0.009$), where the individuals of *C. rutilans rutilans* (mean ± SE= 0.171 ± 0.039 g, N = 60) are heavier than those of *C. rutilans cyanescens* (0.153 ± 0.035 g, N = 60). The interaction subspecies × sex ($F_{1,116} = 0.01$, $P = 0.92$) and the sex factor ($F_{1,116} = 0.61$, $P = 0.44$) do not appear as statistically significant in explaining body weight differences.

Ethogram

We analysed a total of 1200 photographs of *C. rutilans rutilans* (240 photographs for each temperature treatment) and only a total of 1000 photographs of *C. rutilans cyanescens* because five couples of this last subspecies died before they could be photographed. Thus, the final analysed data came from 30 couples of *C. rutilans rutilans* and 25 couples of *C. rutilans cyanescens*. The predominant behaviour observed at all temperature treatments in the two subspecies is to stay buried under the ground (BURY), which was approximately observed in

more than half of total occasions. Individuals moving on the ground (MOVE) or feeding in the dung (FEED) were observed in 20% and 10% of the occasions, respectively. The other behavioural patterns were observed much less frequently (Table 2).

Table 2. Percent of behavioural patterns observed in photographs of Canthon rutilans couples in the laboratory, in five temperatures. Temperature (T), Copulation (COP), Parental care (PCARE), Move on the surface (MOVE), Feeding (FEED), Stay on the food ball (STAY), Bury in the soil (BURY).

Subspecies	T (°C)	COP (%)	PCARE (%)	MOVE (%)	FEED (%)	STAY (%)	BURY (%)
C. rutilans rutilans	35	6.67	3.33	19.17	19.58	4.37	46.88
	30	0.00	4.37	10.00	12.92	14.38	58.33
	25	0.00	12.92	8.54	3.54	6.25	68.75
	20	0.00	0.00	11.67	5.21	0.00	83.12
	15	0.83	0.21	21.88	2.92	10.83	63.33
C. rutilans cyanescens	35	0.00	0.25	21.50	15.00	8.25	55.00
	30	3.5	15.00	31.50	5.25	18.00	26.75
	25	0.00	14.25	32.25	5.75	0.00	47.75
	20	0.00	0.00	13.12	0.63	0.31	85.94
	15	0.83	0.00	42.71	5.83	0.00	50.63

The PCA analysis generated two main components that jointly accounted for 76.5% of total variability in behavioural categories, 57.3% for the first component and 19.2% for the second component. The first axis represents a behavioural pattern positively related with BURY and negatively related with all the other behaviours, thus, discriminating above ground active from inactive behaviours (Table 3). The second PCA component is positively related with the MOVE behaviour, therefore, reflecting a gradient of movement behaviour on the soil to other active behaviours including parental care or to stay on the food ball (Table 3).

Table 3. Factor loadings of the six behavioural patterns for the two main PCA components estimated by considering the frequency of these types of behaviour in 30 couples of *C. rutilans rutilans* and 25 couples of *C. rutilans cyanescens*.

Behavioural Patterns	PCA 1	PCA 2
Copulation (COP)	-0.400	-0.175
Parental care (PCARE)	-1.385	-1.717
Move on the surface (MOVE)	-2.650	6.627
Feed (FEED)	-1.232	-0.258
Stay on the food ball (STAY)	-1.563	-1.492
Buried (BURY)	11.714	1.064

For the behaviour of being buried (BURY, Figure 2A) and movement (MOVE, Figure 2B), ANOVA results showed that there were no statistically significant differences in the values of these two behavioural PCA components among temperature treatments (Table 4). However, the values of the first component seem to differ between the two subspecies; *C. rutilans rutilans* couples tend to have positive values while *C. rutilans cyanescens* have negative values (Figure 2A). This result suggests that the couples of the first subspecies remain buried in the soil much more frequently than those of *C. rutilans cyanescens* independently of temperature.

Table 4. Results from a GLM full factorial design including the temperature treatments (T; five levels) and subspecies identity (Ss; two levels) as explanatory factors, as well as the percentage of total explained variability ($R^2 \times 100$) of the eight dependent variables considered. Statistically significant relationships ($P<0.05$) are in bold.

PCA Factor	T	Ss	T × Ss	$R^2 \times 100$
PCA 1	$F_{4,50} = 2.17$ $P = 0.08$	$\mathbf{F_{1,50} = 7.89}$ $\mathbf{P = 0.007}$	$F_{4,50} = 0.74$ $P = 0.57$	57.3
PCA 2	$F_{4,50} = 2.42$ $P = 0.06$	$F_{1,50} = 2.35$ $P = 0.13$	$F_{4,50} = 1.11$ $P = 0.36$	19.2
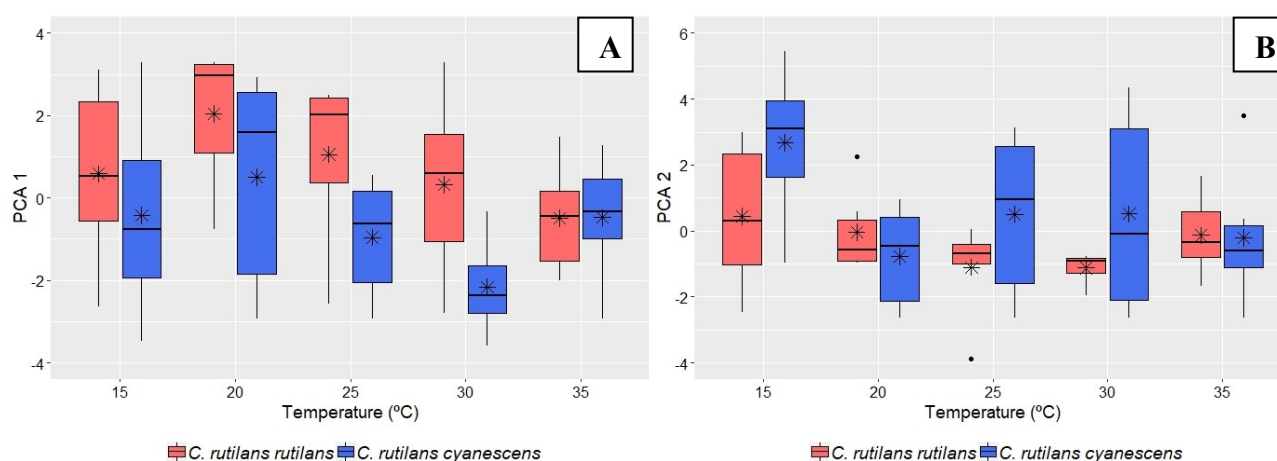
Figure 2. Values of A: PCA 1 ("BURY") and B: PCA 2 ("MOVE") of behaviour activity of *C. rutilans rutilans* (red colour) and *C. rutilans cyanescens* (blue colour) at different temperatures (°C). The mean and the median *per* couple for each temperature are represented by the star and by the central line of each box, respectively. Boxes show 75th percentile and 25th, lines are upper and lower limits, and points are outliers.

Food and brood ball production

The number of food balls per couple oscillates from 0 to 37 (mean ± SE = 12.5 ± 2.0), while the number of brood balls is lower and oscillates from 0 to 24 (6.2 ± 4.4). The number of food balls did not statistically differ between the two subspecies or between temperature treatments (Table 5). However, the number of brood balls differed between temperature treatments and the probability associated with the temperature × subspecies interaction is low but non-significant (P = 0.06) (Table 5). Post hoc Tukey HSD tests suggest that the number of brood balls at 15°C is significantly lower (P = 0.02) than those appearing at higher temperature treatments, and that a higher sample size may allow the observation of a lower number of brood balls at 20°C (Figure 3) for *C. rutilans cyanescens*.

Table 5. Results from a GLM full factorial design accounting for the number of food balls and brood balls including temperature treatments (T; five levels) and subspecies identity (Ss; two levels) as explanatory factors. $R^2 \times 100$ is the percentage of total variability explained by the complete model. Statistically significant relationships (P<0.05) are in bold.

	T	Ss	T × Ss	$R^2 \times 100$
Number of food balls	$F_{4,50} = 1.58$ P = 0.19	$F_{1,50} = 0.27$ P = 0.61	$F_{4,50} = 1.04$ P = 0.40	17.7
Number of brood balls	**$F_{4,50} = 2.92$** **P = 0.03**	$F_{1,50} = 1.93$ P = 0.17	$F_{4,50} = 2.48$ P = 0.06	32.0
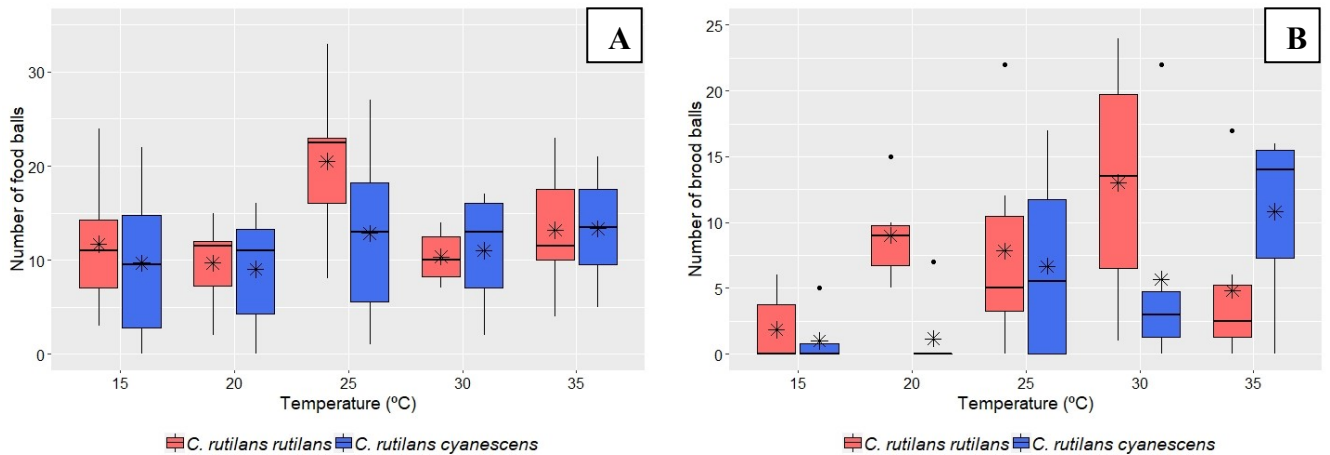
Figure 3. Number of food (A) and brood balls (B) made by couples of *C. rutilans rutilans* (red colour) and *C. rutilans cyanescens* (blue colour), during seven weeks of laboratory experiments at five different temperatures. The mean and the median *per* couple for each temperature are represented by the star and by the central line of each box, respectively. Boxes show 75th percentile and 25th, lines are upper and lower limits, and points are outliers.

Weight and size of food and brood balls

The correlation between the weight and diameter of food or brood balls is very high ($r = 0.95$ in both cases, P<0.001). The weight and diameter of food balls differ between temperatures and subspecies (Table 6). Food balls are smaller and lighter for *C. rutilans cyanescens* and in the extreme temperature treatments (15°C and 35°C; P-values of Tukey HSD tests oscillated from 0.02 to 0.002; see Figure 4A and B). For brood balls the temperature × subspecies interaction is always statistically significant (Table 6), indicating that the responses of weight and diameter of the dung balls to temperature treatments depends on the subspecies (Figure 4C, D). Interestingly, *C. rutilans rutilans* brood balls seem to be bigger and heavier at lower temperatures, while those of *C. rutilans cyanescens* remain practically unchanged along the different temperature treatments.

Table 6. Results from a GLM full factorial design for the weight and diameter of food balls and brood balls including the treatments of temperature (T; levels) and subspecies identity (Ss; two levels) as explanatory factors. $R^2 \times 100$ is the percentage of total variability explained by the complete model. Statistically significant relationships (P<0.05) are in bold.

	T	Ss	T × Ss	$R^2 \times 100$
Weight food balls	$F_{4,48} = 7.90$ **P < 0.001**	$F_{1,48} = 27.73$ **P < 0.001**	$F_{4,48} = 1.59$ P = 0.19	57.8
Weight brood balls	$F_{4,30} = 15.14$ **P < 0.001**	$F_{1,30} = 30.78$ **P < 0.001**	$F_{4,30} = 3.01$ **P = 0.03**	77.5
Diameter food balls	$F_{4,48} = 5.95$ **P < 0.001**	$F_{1,48} = 38.50$ **P < 0.001**	$F_{4,48} = 2.36$ P = 0.07	59.9
Diameter brood balls	$F_{4,30} = 15.35$ **P < 0.001**	$F_{1,30} = 33.33$ **P < 0.001**	$F_{4,30} = 2.80$ **P = 0.04**	77.9
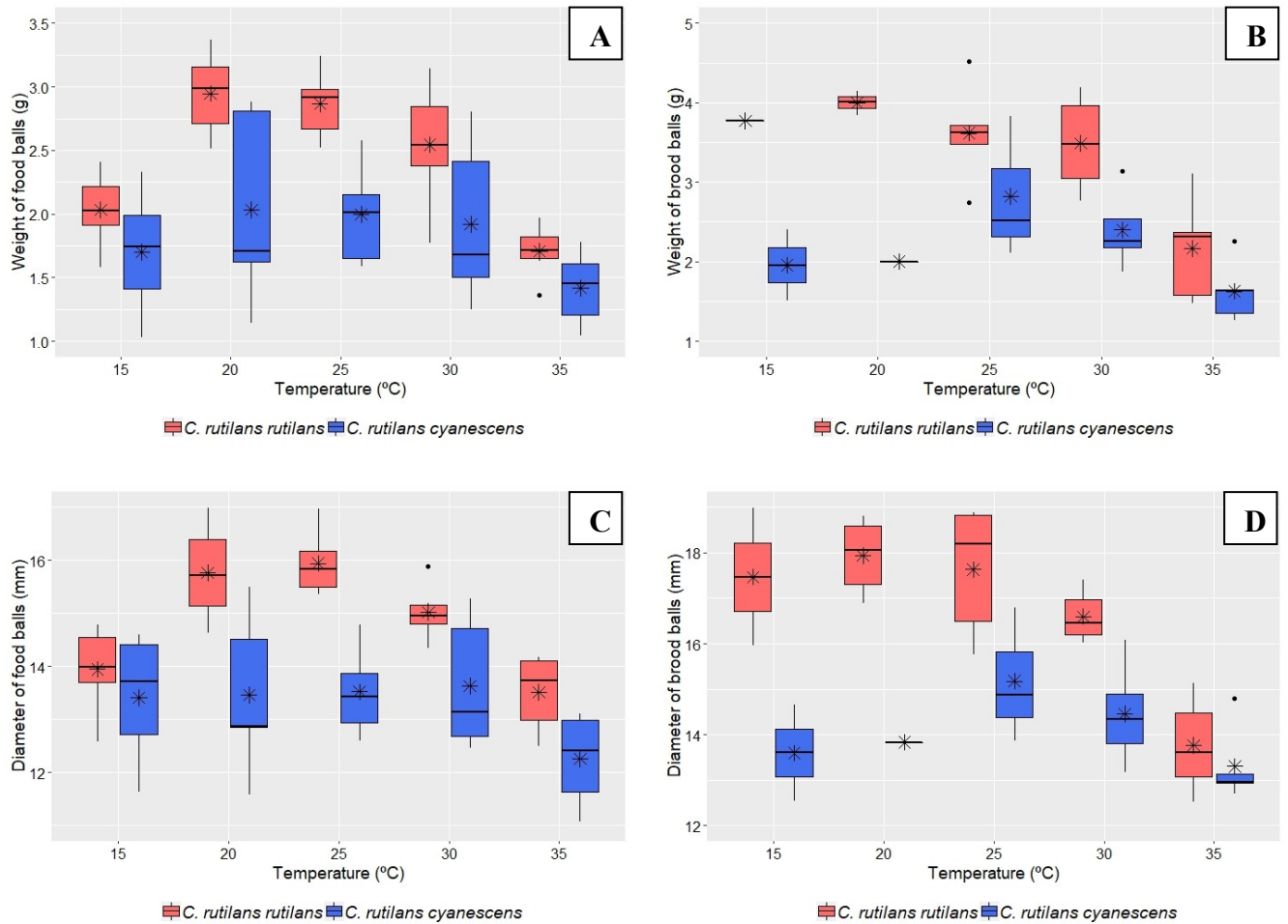
Figure 4. Weight (A, B) and diameter (C, D) of food and brood balls made by couples of *C. rutilans rutilans* (red colour) and *C. rutilans cyanescens* (blue colour), during seven weeks of laboratory experiments at five different temperatures. The mean and the median *per* couple for each temperature are represented by the star and by the central line of each box, respectively. Boxes show 75th percentile and 25th, lines are upper and lower limits, and points are outliers.

Emerged adults and longevity

A total of 28 new adults emerged from the brood balls of *C. rutilans rutilans* and 15 from *C. rutilans cyanescens*. The first individual of *C. rutilans rutilans* emerged during the seventh week at 30°C treatment while the last one emerged on the 16th week at 15°C. In the case of *C. rutilans cyanescens* the first individual emerged during the fifth week at 30°C and the last one emerged during the 12th week at the 25°C treatment. The number of new emerged individuals did not differ between the two subspecies but it is highly dependent on the temperature treatment (Table 7). In general, there is an increase in the emergence of new individuals in temperatures up to 30°C, followed by a drastic decrease at 35°C, regardless of the subspecies (Figure 5A).

The weight of the newly emerged individuals differed between temperature treatments and it was highly dependent on subspecies (Table 7), where new adults of *C. rutilans rutilans* were heavier than those of *C. rutilans cyanescens*. Tukey HSD tests indicate that the weight of the emerged individuals was significantly heavier at 20°C than at higher temperatures (Figure 5B).

The time required for the emergence of new adults differed between temperature treatments (Table 7) but not between subspecies. Thus, new adults need more time to emerge under low temperature treatments (15°C and 20°C; P-values of Tukey HSD tests P<0.001; see Figure 5C). Finally, the average longevity of the two subspecies is 11.0 ± 1.3 weeks (mean ± SE), oscillating from 1 to 30 weeks. Interestingly the temperature × subspecies interaction is highly significant in explaining the longevity of adults (Table 7), where *C. rutilans rutilans* individuals seem to live longer compared to *C. rutilans cyanescens* at all the temperatures, except for 35°C (Figure 5D). Thus, while the longevity of the individuals of *C. rutilans rutilans* decreases with the increase in temperature this pattern is not observed for *C. rutilans cyanescens* (Figure 5D).

Table 7. Results from a GLM full factorial design for the number, weight, and emergence time of F1 and for adult longevity including the temperature treatments (T; five levels) and subspecies identity (Ss; two levels) as explanatory factors. $R^2 \times 100$ is the percentage of total variability explained by the complete model. Statistically significant relationships (P<0.05) are in bold.

Parameters	T	Ss	T × Ss	$R^2 \times 100$
Number of F1	$F_{4,50} = 4.62$ **P = 0.003**	$F_{1,50} = 2.39$ P = 0.13	$F_{4,50} = 1.01$ P = 0.41	33.3
Weight of F1	$F_{3,35} = 4.49$ **P = 0.009**	$F_{1,35} = 30.09$ **P < 0.001**	$F_{2,35} = 1.33$ P = 0.28	56.9
Time emergence of F1	$F_{3,36} = 17.70$ **P < 0.001**	$F_{1,36} = 3.32$ P = 0.08	$F_{2,36} = 1.22$ P = 0.31	62.0
Longevity	$F_{4,101} = 7.33$ **P < 0.001**	$F_{1,101} = 181.72$ **P < 0.001**	$F_{4,101} = 7.01$ **P < 0.001**	70.3
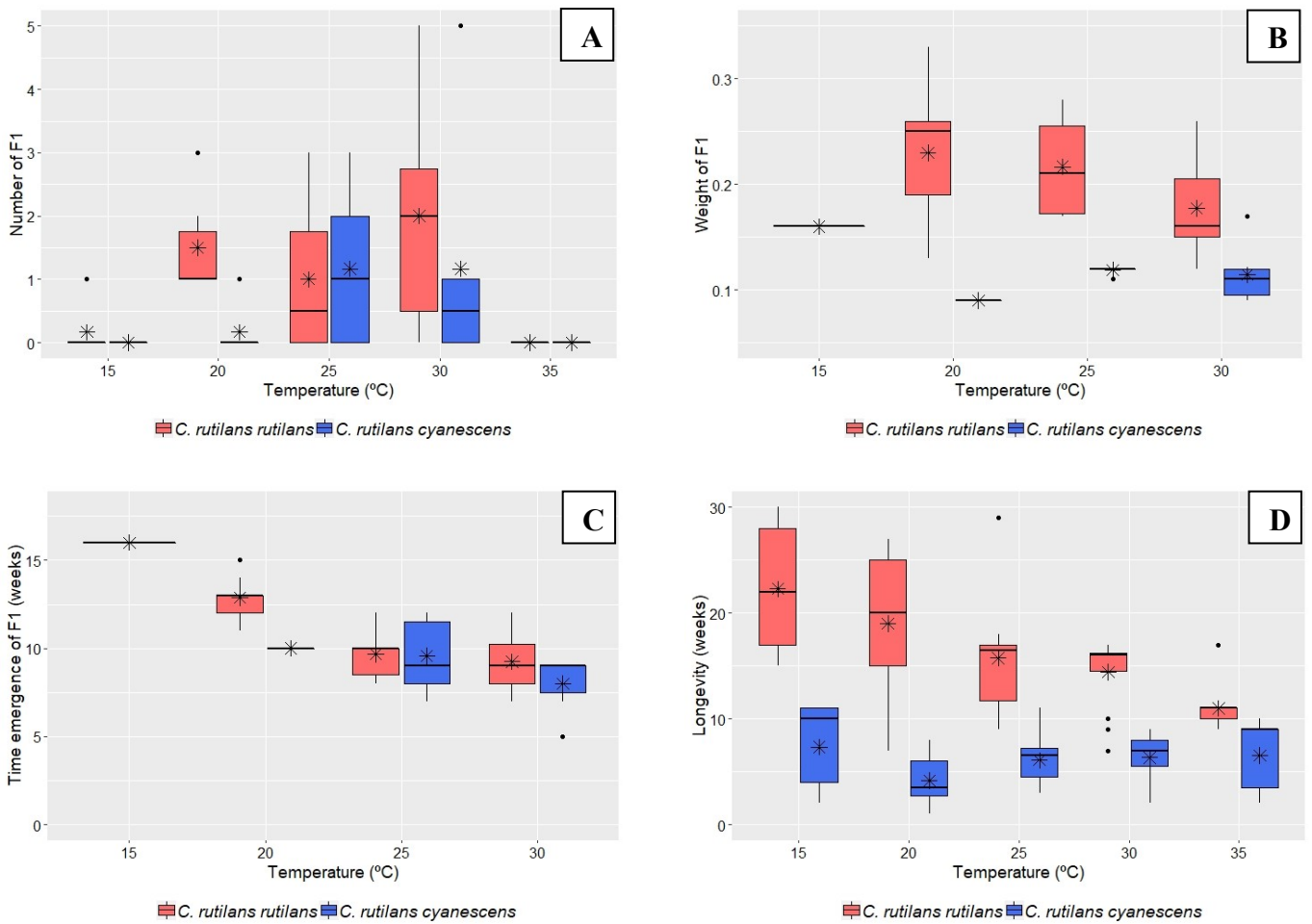
Figure 5. Number (A), weight (B), time of emergence of F1 individuals (C) and adult longevity in weeks (D) of *C. rutilans rutilans* (red colour) and *C. rutilans cyanescens* (blue colour) *per* temperature. The mean and the median of F1 individuals for each temperature are represented by the star and by the central line of each, box respectively. Boxes show 75th percentile and 25th, lines are upper and lower limits, and points are outliers. In Figures B and C, the boxplot at 15°C belongs to *C. rutilans rutilans*.

DISCUSSION

Considering all the precautions necessary when dealing with laboratory data, our results suggest that the two subspecies differ in their reproductive strategy depending on the environmental temperature. Although the number of produced food balls does not differ between subspecies, the dung balls for breeding in *C. rutilans cyanescens* are few, if any, below 25°C. This is in agreement with the distribution of this subspecies, which occur under warmer conditions and localities (Hensen et al., 2018). Furthermore, at low temperatures both the weight and the diameter of the brood balls are significantly higher in *C. rutilans rutilans*, the subspecies generally distributed under colder climates. The probable thermal segregation

between the two studied subspecies, manifested in differences in brood dung balls, is reinforced when the body weight of new specimens and the longevity of parental beetles are considered. Thus, although the number of newly emerged individuals does not significantly differ between the two subspecies, both the weight of these new emerged adults and the longevity of parental specimens are higher in the cold-subspecies (*C. rutilans rutilans*) at the lowest considered temperature treatments. All these results support the existence of differential biological and developmental strategies linked with their vital thermal requirements.

Our results also corroborate the important role of temperature in determining body size, developmental time, or brood ball weight. In ectotherms, the temperature can determinate the final adult body size because temperature affects the rate of development and may also affect the developmental time or the age which the individual reaches its body size (Atkinson & Sibly, 1997; Angilletta & Dunham, 2003). Our results show that adults of *C. rutilans rutilans* are larger than those of *C. rutilans cyanescens*. In a revision, Atkinson (1994) showed that in 83.5% of cases, body size of ectotherms is significantly reduced with an increase in temperature. Simple explanations trying to link body size and temperature in ectotherms are insufficient to stablish a general temperature-size rule, since in many special cases the mechanisms that are involved may be complex (Atkinson & Sibly, 1997; Angilletta & Dunham, 2003).

In addition, the effect of the temperature may influence the behaviour of the two subspecies unequally. Adults of *C. rutilans rutilans* remain buried in the ground for significantly more time than those of *C. rutilans cyanescens*, which moved on the ground more. This behavioural difference can be explained by the adaptation of the characteristically cold-subspecies *C. rutilans rutilans*, since it has an underground habit to avoid cool temperatures, and the soil has a temperature buffering capacity (Lobo et al., 2019). Temperature variations did not influence the alimentary patterns in the two subspecies in opposition to the influence of temperature on the reproductive behaviour. The production of brood balls depends on temperature in both subspecies. However, *C. rutilans rutilans* produce larger and bigger brood balls (more quantity of faeces inside) at lower temperatures. This may be another adaptation directed to increase fitness at lower temperatures, since larvae may need more time for development under these conditions. Dung beetles can adjust the brood ball size

according to dung quality (Kishi, 2014). In addition to the genetic factor, the maternal manufacture effect (indirect genetic effect) defines the size of the brood balls, with a positive correlation between the brood mass weight and body size of the new adult (Hunt & Simmons, 2000, 2002). In the case of *C. rutilans rutilans*, females produced fewer brood balls at lower temperatures. However, the brood balls were bigger to provide the larvae with more food resources. Therefore, the weight of new adults was dependent on temperature, where adults at lower temperatures were heavier. It is known that temperature influences larval development time, and thus, at lower temperatures the larvae take more time to develop.

In the case of both subspecies, the first individual emerged at the same temperature. However, for *C. rutilans rutilans* the new adult need seven weeks to complete its life cycle and *C. rutilans cyanescens* only need five weeks. On the contrary, the last individual to emerge for *C. rutilans rutilans* was after four months at the lowest temperature, and for *C. rutilans cyanescens* it requires three months at 25°C, reinforcing the adaptation of *C. rutilans rutilans* towards cold conditions. Both subspecies produced brood balls at the lowest temperature, although fewer, but only the larvae of *C. rutilans rutilans* were able to complete their development in a considerable time. Thus, the females from the cold-adapted subspecies made heavy brood balls at low temperatures and in compensation the time for larvae development was greater.

Some studies with *C. rutilans cyanescens* showed that adults, feeding on dog faeces at 25°C, had a F1 with a larval development cycle that lasts 33 ± 4 days in average (Hernández, Niero, Schumacher and Wuerges; unpublished data). The most studied species of this genus is *Canthon cyanellus cyanellus* LeConte, in which the ideal breeding temperature under laboratory conditions has been found to be 27 ± 1°C in Mexico (Favila et al., 2005). The development time increases considerably at low temperatures in most insects (Ratte, 1984) and there are no differences between the number of larval stages at different temperatures, only the time that they need to develop and to reach the same size changes (Sehnal, 1991). The survival of males from *C. rutilans cyanescens* reaches more than three months (Wuerges & Hernandez, in press), but we observed that temperature did not affect its survival. Nevertheless, the survival of *C. rutilans rutilans* was almost three times less at high temperatures. This result demonstrates the relationship between temperature and mortality due to aging; with an increase in temperature there is greater molecular damage, and therefore, mortality will increase (Calow, 1978).

Differential adaptations to the climatic conditions existing along elevational gradients can produce allopatric speciation (Janzen, 1967; Ghalambor et al., 2006; Mittelbach et al., 2007). Currently, *C. rutilans rutilans* and *C. rutilans cyanescens* are considered two subspecies, but they are spatially and temporally segregated also inhabiting under relatively different environmental temperature conditions (Hensen et al., 2018). In spite of their spatial and temporal segregation, we know that hybridization is possible between them under laboratory conditions (Alves and Hernández, personal communication, 2018). In this study, we showed that environmental temperature influenced both subspecies differently: cold adapted subspecies *C. rutilans rutilans*, occurring at high elevations, has a behavioural pattern that depends strongly on temperature, in contrast to *C. rutilans cyanescens*. Thus, our results supports that they can be considered ecologically two different species.

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4 ARTIGO III: REGIONAL SYMPATRY BUT LOCAL ALLOPATRY IN THE DISTRIBUTION OF THE TWO SUBSPECIES OF THE DUNG BEETLE *CANTHON RUTILANS* CASTELNAU, 1840

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Regional sympatry but local allopatry in the distribution of the two subspecies of the dung beetle *Canthon rutilans* Castelnau, 1840

ABSTRACT

Studying the environmental requirements among subspecies from distributional data may help to estimate probable niche differences and so, to assess the taxonomic s*tatus* of these subspecies. Distribution data of the two subspecies of *Canthon rutilans* were used here to examine if they show differential environmental preferences. To do so, seven Brazilian entomological collections were visited with the aim of achieving a database able to include most part of the available distributional information about these subspecies. A total of 192 specimens of *Canthon rutilans rutilans* belonging to 46 different localities, and 523 specimens of *Canthon rutilans cyanescens* from 120 different localities were finally compiled (avoiding duplications). The data derived from 23 environmental variables (2 topographic and 21 bioclimatic variables) in the occurrence cells were used to generate distributional maps reflecting the probable distribution of the two subspecies by means of a recently proposed procedure (García–Roselló *et al.* 2019). These data were also used to estimate those variables with different mean values among the two subspecies. Twelve variables had statistically significant differences between both subspecies, strongly related with temperature. The subspecies *C. rutilans rutilans,* would be present in colder places, living in the high elevation localities of the Atlantic Forest biome or Pampas biome. On the contrary; *C. rutilans cyanescens* seems to be restricted to lowland forested areas of the Atlantic Forest biome. Probable distribution maps show geographical sympatry with large areas of overlap between the two subspecies (70%), but they are in allopatry at a local scale, because they do not occur together in the same place. These results suggest that coarse-grain data as those used in biogeographical analyses should be used with caution when we aim is to estimate niche differences between related taxa.

Key words: Niche differences, geographical distribution, Species distribution models, Scarabaeinae.

INTRODUCTION

The multidimensional environmental space defined by the values of non-interactive variables in the occurrence localities of a species may help to delimit their Grinnellian niche (Grinnell 1917; Soberón 2007). However, the *a priori* favourable environmental conditions thus obtained do not necessarily reflect the full set of conditions at which a species can have a positive demographic rate (Soberón & Peterson 2005). Physiological data related, for example, with thermal responses may add supplementary valuable information about the environmental requirements of species and so to infer more accurately the niche of a species (Verdú & Lobo 2008; Strangas *et al.* 2019). According to Thompson (1998), despite the tendency to think that ecological and evolutionary processes act at different timescales, a deep connection between these two types of processes could be the norm. The geographical and temporal patterns of species can thus be understood considering physiological, ecological and evolutionary processes jointly, because all of them can help to understand the causes of species distribution limits (Gaston *et al.* 2009). However, prolonged exposures outside the limits of physiological tolerances may be an important selective force (Calosi *et al.* 2007), so that individuals may change their environmental tolerances by acclimation in order to maintain their homeostasis under varied local environmental conditions (Bozinovic *et al.* 2011).

Canthon (Francmonrosia) rutilans Castelnau, 1840 is a species of dung beetle with two recognized subspecies: *Canthon rutilans rutilans* Castelnau, 1840 and *Canthon rutilans cyanescens* Harold, 1868. Its geographical distribution extends from Brazil in the states of Mato Grosso do Sul, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul, to Argentina (Misiones), Uruguay and probably Paraguay (Vaz-de-Mello *et al.* 2014). The subspecies *C. rutilans cyanescens* occurs in forest habitats at elevations under 1000 m in the Atlantic Forest biome (Campos & Hernández 2013, 2015; Korasaki *et al.* 2013; Bogoni & Hernández 2014; Costa-Silva *et al.* 2014; da Silva & Hernández 2014, 2015, 2016; Hernández *et al.* 2019). However, *C. rutilans rutilans* inhabits the high-altitude grasslands or forests of the Atlantic Forest Biome situated at more than 1000 m in elevation or in the Pampas biome or *Eucalyptus* plantations under cold environmental conditions (da Silva *et al.* 2008, 2009, 2012; Hernández & Vaz-de-Mello 2009; Audino *et al.* 2011; da Silva 2017; Hensen *et al.* 2018). In a study that

explore the yearly distribution of these two subspecies along an elevational gradient in southern Brazil it was shown that both subspecies are spatially segregated and that both had a limited overlap in the their thermal niche according to microclimatic measurements of air temperature (5.6%; see Hensen *et al.* 2018).

The so called Species Distribution Models (SDMs) are increasingly used to derive spatial predictions about the present and future distribution of species considering the available occurrence data and varied environmental information (Guisan *et al.* 2017). In this study we use a simple recently proposed SDM approach directed to estimate the probable distribution of the species from partial data (García–Roselló *et al.* 2019) to generate spatial predictions about the distribution of each one of these two subspecies. Additionally, the environmental information of the occurrence localities was used to assess if the detected environmental segregation detected at regional scale is corroborated when distribution data is used. Thus, the main aim of this study is to examine if exist congruence between the environmental preferences derived from distributional and regional data. If that was so, low resolution distributional information could potentially be used to estimate niche differences between closely related species, at least in some situations.

MATERIALS AND METHODS

Origin of distribution data

Seven Brazilian Entomological Collections were visited during the year 2017 to compile distributional data of *Canthon rutilans*: one in Santa Catarina State (Coleção Entomológica Mitia Heusi Silveira da CCB-UFSC, Florianópolis), one in Paraná State (Coleção de Entomologia Pe. J. S. Moure da UFPR, Curitiba), two in São Paulo State (Laboratório de Entomologia Geral- Instituto Biológico and Coleção de Scarabaeinae do Museu de Zoologia da USP), two in Rio Grande do Sul State (Coleção Entomológica do Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul and Museu Colégio Anchieta, Porto Alegre) and one in Mato Grosso do Sul State (Coleção Zoológica da Universidade Federal de Mato Grosso, Cuiabá). From the specimens identified and verified one by one as *C. rutilans* we gathered the information of the entomological label and also we taken three pictures of each specimen using a camera (Canon® T3). A maximum of 10 individuals per place were included. If the entomological label only had written the name of the place, but not the

geographical coordinate, we obtained it through Google Earth Pro. All the occurrences located at less than 1km of distance from each other were considered as single occurrences.

Origin of environmental data

A total of 23 environmental variables at a resolution of 2.5' (approximately 25 km^2) were used as predictors: 2 topographic (elevation and elevation range) and 21 bioclimatic variables. Topographic variables were extracted from a digital elevation model downloaded from the USGS EROS Data Center (http://eros.usgs.gov/). Bioclimatic variables come from WorldClim (see http://www.worldclim.org; Hijmans *et al.* 2005) adding aridity and continentality as calculated by Valencia-Barrera *et al.* (2002).

Modelling procedure

The modelling procedure is directed to provide a geographical representation of the suitable and accessible areas with environmental conditions similar to those existing in the observed occurrence areas at a resolution of 2.5 minutes (a cell of approximately 5×5 km). A simple protocol has been followed for this purpose (Lobo 2016), avoiding the use of complex modelling techniques and the so-called background absences. The complete procedure, called NOO (Niche of Occurrence), is fully described in García–Roselló *et al.* (2019) but can be summarized in three steps. Firstly, the distributional extent or accessible area of each taxa was delimited as the one composed by the set of river basins of lower level with presence observations that, in turn, enable the connection of all the available occurrences. Watershed information provided by the WaterBase project (www.waterbase.org) is used for this purpose. Subsequently, the most relevant predictor variables within this area are selected eliminating sequentially those with a Variance Inflaction Factor (VIF) lower than 5, and submitting the remaining predictors to an Instability Index (InsInd) able to select the variables with a high capacity of discriminating the occurrence cells in the selected region (Guisande *et al.* 2017). In this case, this standard process directed to select the most relevant environmental predictors is complemented with another in which the selected environmental variables are those having statistically significant different mean values according to a Student t-test ($P \leq 0.05$). The values of the so-selected explanatory variables in the occurrence cells of the target taxa are handled to obtain maximum and minimum values for each variable, which are subsequently

used to obtain a geographical projection. Thus, those cells with environmental conditions similar to those existing in the occurrence localities are discriminated and a binary distribution map is carried out within the accessible area.

RESULTS

Data coming from a total of 192 individuals of *C. rutilans rutilans* belonging to 46 different locations and 523 individuals of *C. rutilans cyanescens* from 120 different locations were compiled during the visits to the entomological collections (Appendix S1 for collections and Appendix S2 for localities). Mean values of 12 of the 23 considered environmental variables significantly differed between the two taxa (Table 1) showing that *C. rutilans cyanescens* specimens inhabit in lowland localities with higher temperature values and lower aridity, opposite to *C. rutilans rutilans*; annual mean temperature of the occurrence cells of *C. rutilans rutilans* is 17.13 ± 2.34 ºC (mean $\pm$ SD) and in the case of *C. rutilans cyanescens* is 19.20 ± 1.57 ºC. All precipitation related variables do not differ between the two subspecies (Table 1).

Table 1. Environmental values and results of t-tests comparing the mean values of the used environmental *variables* between the localities with occurrences of *C. rutilans cyanescens* (*Crc*; *n* = 120) and *C. rutilans rutilans* (Crr; *n* = 46). Statistically significant relationships (*P* < 0.05) are in bold.

Environmental variables	*Crc*	*Crr*	*t-value*	*df*	*P*
Elevation	**349.89**	**777.61**	**-6.59**	**164**	**<0.001**
Annual Mean Temperature (in ºC)	**19.20**	**17.13**	**6.56**	**164**	**<0.001**
Annual Precipitation	1554.91	1590.22	-0.90	164	0.370
Aridity	**53.33**	**59.52**	**-3.75**	**164**	**<0.001**
Continentality	9.60	9.11	0.49	164	0.622
Elevation range	**3.47**	**3.68**	**-2.85**	**164**	**0.005**
Isothermality	**52.32**	**55.72**	**-3.17**	**164**	**0.002**
Max. Temperatureof Warmest Month	**28.16**	**26.64**	**4.17**	**164**	**<0.001**
Mean Diurnal Range	**9.59**	**10.68**	**-3.74**	**164**	**<0.001**
Mean Temperature of Coldest Quarter	**15.45**	**13.42**	**6.29**	**164**	**<0.001**
Mean Temperature of Driest Quarter	16.73	15.81	1.86	164	0.065
Mean Temperature of Warmest Quarter	**22.71**	**20.51**	**6.75**	**164**	**<0.001**
Mean Temperature of Wettest Quarter	**21.37**	**18.24**	**5.95**	**164**	**<0.001**
Min. Temperature of Coldest Month	**10.07**	**7.58**	**7.50**	**164**	**<0.001**
Precipitation of Coldest Quarter	283.60	300.20	-0.88	164	0.380
Precipitation of Driest Month	79.49	83.07	-0.64	164	0.526
Precipitation of Driest Quarter	267.48	276.35	-0.50	164	0.618
Precipitation of Warmest Quarter	533.22	507.26	1.24	164	0.218
Precipitation of Wettest Month	199.45	196.63	0.36	164	0.720
Precipitation of Wettest Quarter	546.10	543.41	0.13	164	0.899
Precipitation Seasonality	29.89	29.91	-0.01	164	0.994
Temperature Annual Range	**18.09**	**19.06**	**-2.86**	**164**	**0.005**
Temperature Seasonality	2882.35	2844.87	0.47	164	0.640

A visual inspection of the occurrences belonging to the two subspecies shows that both share a large part of their geographical distribution (Fig. 1).
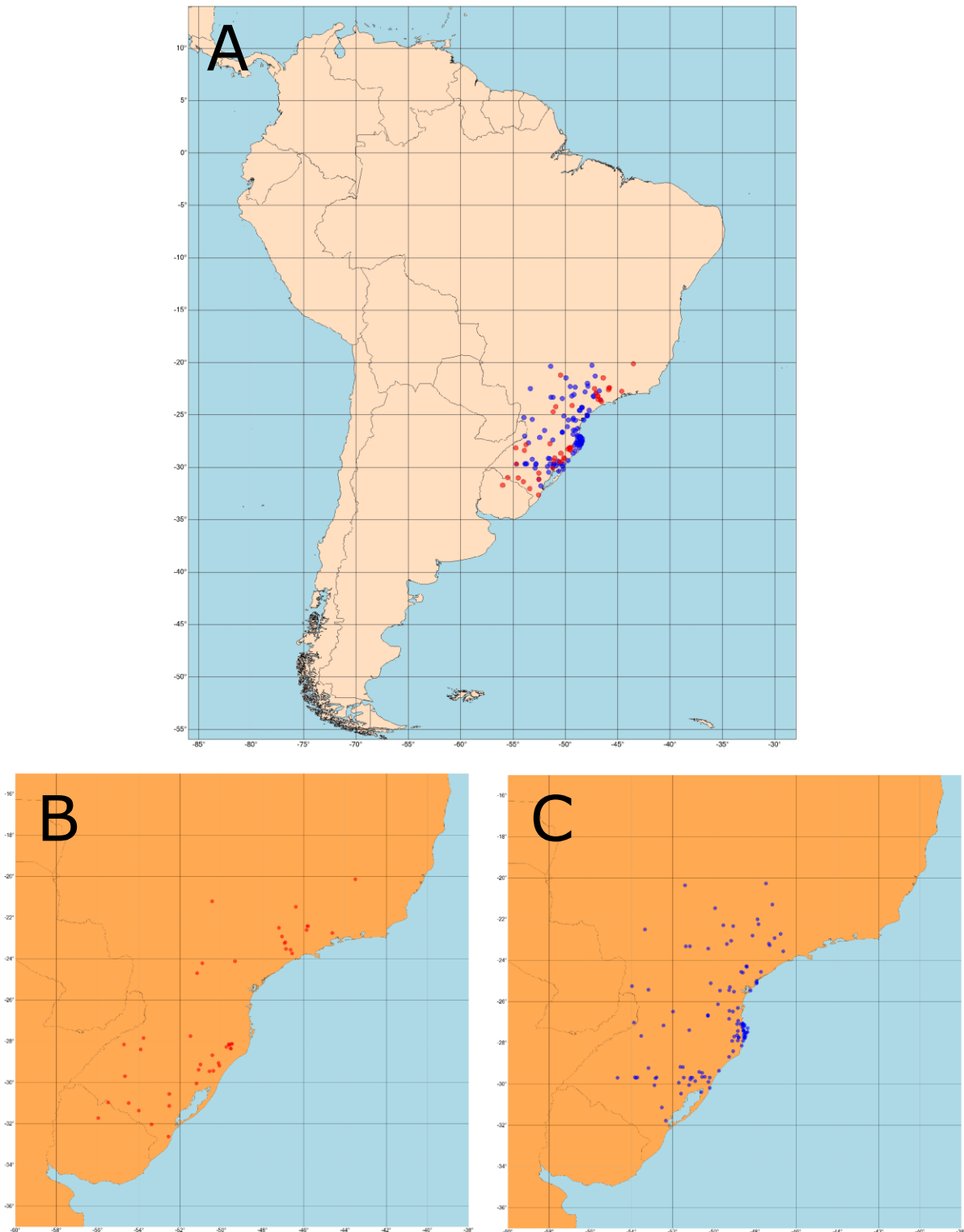
Figure 1. Occurrence localities of *Canthon rutilans* in South America (A), as well as those corresponding to *C. rutilans rutilans* (B) and *C. rutilans cyanescens* (C).

The NOO process of variable selection (VIF plus InsInd) allowed us to obtain as relevant variables: elevation, aridity, continentality, elevation range, isothermality, mean diurnal range, mean temperature of driest quarter, mean temperature of wettest quarter,

precipitation of warmest quarter and precipitation seasonality. These variables generate distributional predictions (Fig. 2 A, C and E) in which the sympatric area represents a high percentage (71.7%) of the total area covered by the two taxa. The predicted distributional area of *C. rutilans rutilans* is 1,068,418 km^2 comprising the states of Espírito Santo, Rio de Janeiro, Minas Gerais, São Paulo, Mato Grosso do Sul, Paraná, Santa Catarina and Rio Grande do Sul, and some regions of Uruguay, Paraguay and Argentina. The predicted distributional area of *C. rutilans cyanescens* is 831,066 km^2 comprising the states of Rio de Janeiro, Minas Gerais, São Paulo, Mato Grosso do Sul, Goiás, Paraná, Santa Catarina and Rio Grande do Sul, and regions of Paraguay and Argentina. The use of the statistically significant variables showing differences between the two taxa (Table 1) generates relatively similar distributions (Fig. 2 B, D and F), although in this case the presence of *C. rutilans rutilans* (962,211 km^2) would be reduced in the north-western boundary, while *C. rutilans cyanescens* (812,301 km^2) would be also present in Uruguay. The area overlapped by the two taxa is 68.1% of the total area covered by the two taxa.
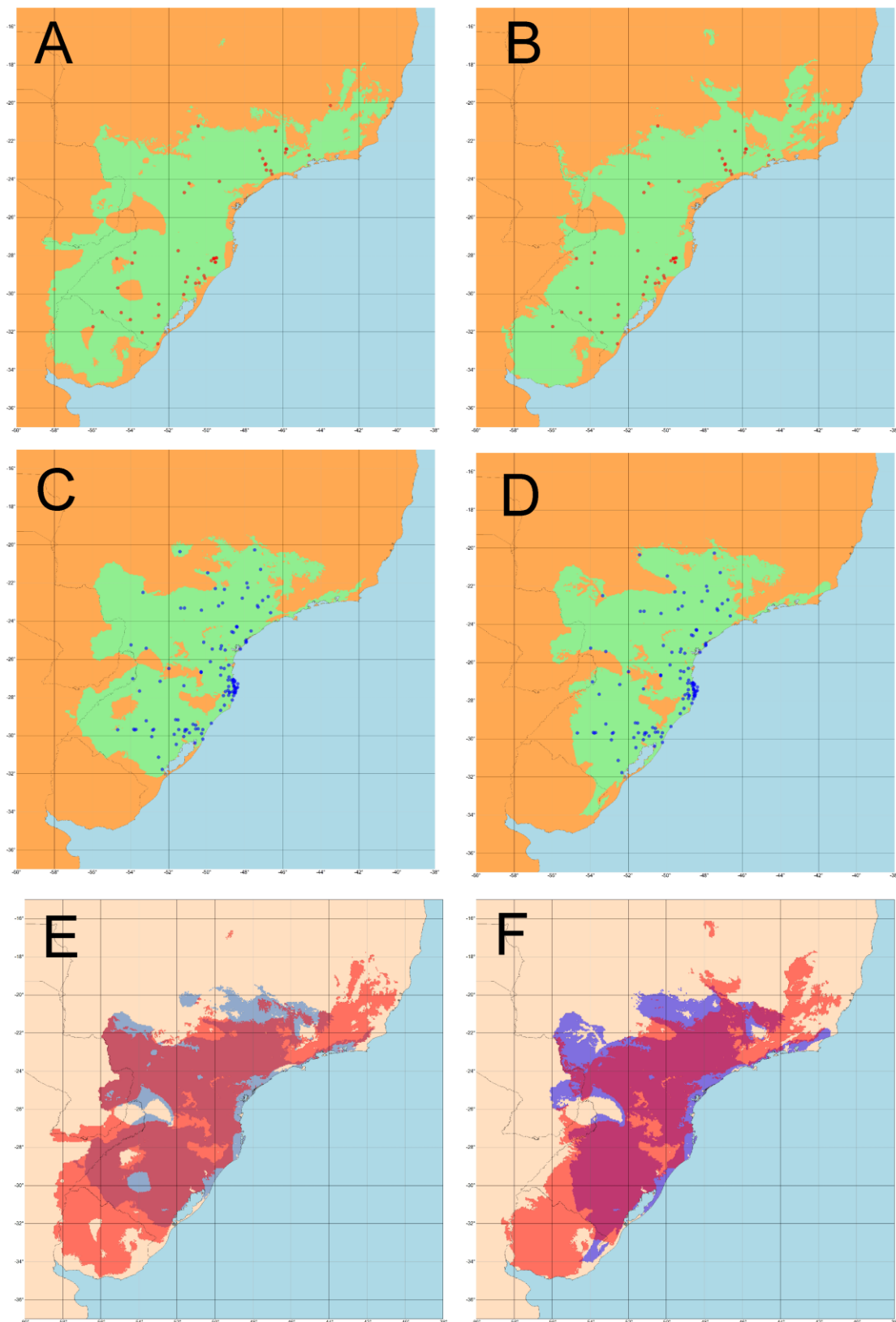
Figure 2. Probable distribution of *C. rutilans rutilans* (A and B). and *C. rutilans cyanescens* (C and D) using the NOO procedure for variable selection (VIF plus InsInd; A and C)), and using those variables that significantly differ between the two taxa (B and D, see Table 1).The probable distributions of both subspecies were also jointly mapped using (E) or not (F) the NOO approach for the selection of the variables: *C. rutilans rutilans* in light red and *C. rutilans cyanescens* in blue, the overlapping area in darker magenta.

DISCUSSION

Based on our results, we show that both subspecies of *C. rutilans* differ in their environmental requirements, especially those related to temperature and elevation. In spite of the proximity of some occurrence localities between both subspecies, these could probably differ in elevation, being *C. rutilans rutilans* the subspecies more related to high elevation in warmer regions than *C. rutilans cyanescens*. In elevational gradients in the tropics the species that occur at low and high elevations have a greater difference in their climatic range than in temperate zones (Janzen 1967). Hence, these differences in environmental requirements are a clear reflection that they are living in different places on a local scale. However, their real and probable distribution maps show that the area of overlap is very high between both subspecies, which means that they can be sympatric regionally, but not locally. This result also questions the capacity of coarse-grain geographical data to identify niche requirements (Rebaudo *et al.* 2016). Therefore, *C. rutilans rutilans* and *C. rutilans cyanescens* would be found in a geographical sympatry because they share a high percentage of the area that they occur (72 % or 68%, depending on the modelling procedure), but at the local scale they do not occur together, demonstrating that this allopatry is, probably, the result of different environmental adaptations (Anacker & Strauss 2014).

Although much of the regional distribution is the same, occurrence maps of these two subspecies show that *C. rutilans rutilans* extends its distribution further south and that *C. rutilans cyanescens* further north-western of southern South of America. Most part of the distributional area of both subspecies was found in areas of Atlantic Forest and the particular orography of this region allows different types of environments (IBGE 1992). The distribution of *C. rutilans cyanescens* is related to the rainforest which explains its limitation of southward displacement (Campos & Hernández 2013, 2015; Korasaki *et al.* 2013; Bogoni & Hernández 2014; Costa-Silva *et al.* 2014; da Silva & Hernández 2014, 2015, 2016; da Silva *et al.* 2018; Hernández *et al.* 2019). Although *C. rutilans rutilans* may occurs at higher elevation within the Atlantic Forest biome (Hernández & Vaz-de-Mello 2009; Hensen *et al.* 2018; da Silva *et al.* 2019), its distribution is related with the presence of high-altitude grasslands or Pampas biome (da Silva *et al.* 2008, 2009, 2012, 2019; Audino *et al.* 2011; da Silva 2017). Species of dung beetles than inhabit high-altitude grasslands and Pampas in southern Brazil share 90% of species. This result could be as a consequence of the shared biogeographic origin of these two

habitats in South Brazil, which have expanded and contracted their ranges during the Pleistocene glacial/interglacial periods (Leite *et al.* 2016).

In conclusion, this study shows that environmental variables are important factors determining the distribution of these two subspecies. Although other factors may help to understand species distributions (Costa *et al.* 2008), the environmental requirements of the two studied subspecies show that *C. rutilans rutilans* would be distributed in colder (~17ºC) and higher elevation areas (~800 m), while *C. rutilans cyanescens* is distributed in warmer localities (~19ºC) at lower elevations (~350 m) ones. In our case, two subspecies with differing environmental preferences at regional scale (Hensen *et al.* 2018), may appear as highly sympatric geographically thus supporting that allopatry and sympatry are scale-dependent concepts (Anacker & Strauss 2014).However, this high distributional overlap need not be a serious obstacle to estimate environmental differences between closely related taxa.

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Supporting Information

Appendix S1. Number of records used for analysis after visiting seven Entomological Collections in five states of Brazil.

Entomological Collections	*C. rutilans cyanescens*	*C. rutilans rutilans*
Coleção Zoológica da Universidade Federal de Mato Grosso (Mato Grosso do Sul State)	161	59
Coleção de Entomologia Pe. J. S. Moure da UFPR (Paraná State)	24	4
Coleção Entomológica do Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul (Rio Grande do Sul State)	63	18
Museu Colégio Anchieta (Rio Grande do Sul State)	14	19
Coleção Entomológica Mitia Heusi Silveira da CCB-UFSC (Santa Catarina State)	224	64
Coleção de Scarabaeinae do Museu de Zoologia da USP (São Paulo State)	34	23
Laboratório de Entomologia Geral- Instituto Biológico (São Paulo State)	3	5

Appendix S2. Description of localities with occurrences of *C. rutilans cyanescen*s (*n* = 120) and *C. rutilans rutilans* (*n* = 46), country, state, town and geographical UTM coordinates. Abbreviations for the seven states: Mato Grosso do Sul (MS), Paraná (PR), Rio Grande do Sul (RS), Santa Catarina (SC), São Paulo (SP), Minas Gerais (MG), Uruguay (UY).

Subspecies	Country	State	Town	Latitude	Longitude
C. rutilans cyanescens	Brasil	MS	Taquarussú	-22.490	-53.353
C. rutilans cyanescens	Brasil	MS	Selvíria	-20.348	-51.414
C. rutilans cyanescens	Brasil	PR	Balsa Nova	-25.462	-49.710
C. rutilans cyanescens	Brasil	PR	Colombo	-25.293	-49.226
C. rutilans cyanescens	Brasil	PR	Curitiba	-25.435	-49.270
C. rutilans cyanescens	Brasil	PR	Guaraqueçaba	-25.461	-48.231
C. rutilans cyanescens	Brasil	PR	Jundiaí do Sul	-23.424	-50.282
C. rutilans cyanescens	Brasil	PR	Londrina	-23.308	-51.169
C. rutilans cyanescens	Brasil	PR	Matelândia	-25.243	-53.979
C. rutilans cyanescens	Brasil	PR	Palmas	-26.480	-51.991
C. rutilans cyanescens	Brasil	PR	Piraquara	-25.508	-49.026
C. rutilans cyanescens	Brasil	PR	Ponta Grossa	-25.099	-50.158

C. rutilans cyanescens	Brasil	PR	Rolandia	-23.311	-51.367
C. rutilans cyanescens	Brasil	PR	Tres Barras do Paraná	-25.422	-53.184
C. rutilans cyanescens	Brasil	RS	Bento Gonçalves	-29.169	-51.510
C. rutilans cyanescens	Brasil	RS	Cachoeira do Sul	-30.049	-52.889
C. rutilans cyanescens	Brasil	RS	Campo Bom	-29.676	-51.061
C. rutilans cyanescens	Brasil	RS	Candelaria	-29.707	-52.840
C. rutilans cyanescens	Brasil	RS	Candelaria	-29.669	-52.788
C. rutilans cyanescens	Brasil	RS	Canela	-29.402	-50.724
C. rutilans cyanescens	Brasil	RS	Canguçu	-31.133	-52.533
C. rutilans cyanescens	Brasil	RS	Cidreira	-30.177	-50.209
C. rutilans cyanescens	Brasil	RS	Derrubadas	-27.012	-53.886
C. rutilans cyanescens	Brasil	RS	Dois Irmãos das Missões	-27.659	-53.531
C. rutilans cyanescens	Brasil	RS	Estrela Velha	-29.215	-53.183
C. rutilans cyanescens	Brasil	RS	Gravataí	-29.853	-50.908
C. rutilans cyanescens	Brasil	RS	Maquiné	-29.680	-50.208
C. rutilans cyanescens	Brasil	RS	Monte Belo do Sul	-29.162	-51.634
C. rutilans cyanescens	Brasil	RS	Montenegro	-29.683	-51.467
C. rutilans cyanescens	Brasil	RS	Novo Hamburgo	-29.682	-51.129
C. rutilans cyanescens	Brasil	RS	Osorio	-29.889	-50.270
C. rutilans cyanescens	Brasil	RS	Palmares do Sul	-30.383	-50.617
C. rutilans cyanescens	Brasil	RS	Pelotas	-31.766	-52.338
C. rutilans cyanescens	Brasil	RS	Porto Alegre	-30.037	-51.210
C. rutilans cyanescens	Brasil	RS	Riozinho	-29.642	-50.454
C. rutilans cyanescens	Brasil	RS	Rolante	-29.652	-50.577
C. rutilans cyanescens	Brasil	RS	Santa Maria	-29.655	-53.835
C. rutilans cyanescens	Brasil	RS	Santa Maria	-29.701	-53.790
C. rutilans cyanescens	Brasil	RS	Santa Maria	-29.674	-53.720
C. rutilans cyanescens	Brasil	RS	São Francisco de Paula	-29.446	-50.579
C. rutilans cyanescens	Brasil	RS	São Leopoldo	-29.770	-51.148
C. rutilans cyanescens	Brasil	RS	São Vicente do Sul	-29.693	-54.677
C. rutilans cyanescens	Brasil	RS	Sertão Santana	-30.462	-51.604
C. rutilans cyanescens	Brasil	RS	Torres	-29.354	-49.755

C. rutilans cyanescens	Brasil	RS	Triunfo	-29.930	-51.709
C. rutilans cyanescens	Brasil	SC	Anitápolis	-27.900	-49.117
C. rutilans cyanescens	Brasil	SC	Antonio Carlos	-27.417	-48.850
C. rutilans cyanescens	Brasil	SC	Bombinhas	-27.196	-48.561
C. rutilans cyanescens	Brasil	SC	Bombinhas	-27.177	-48.529
C. rutilans cyanescens	Brasil	SC	Brusque	-27.083	-48.883
C. rutilans cyanescens	Brasil	SC	Brusque	-27.083	-48.717
C. rutilans cyanescens	Brasil	SC	Campos Novos	-27.383	-51.200
C. rutilans cyanescens	Brasil	SC	Corupá	-26.435	-49.245
C. rutilans cyanescens	Brasil	SC	Florianópolis	-27.596	-48.549
C. rutilans cyanescens	Brasil	SC	Florianópolis	-27.750	-48.533
C. rutilans cyanescens	Brasil	SC	Florianópolis	-27.733	-48.533
C. rutilans cyanescens	Brasil	SC	Florianópolis	-27.717	-48.533
C. rutilans cyanescens	Brasil	SC	Florianópolis	-27.700	-48.517
C. rutilans cyanescens	Brasil	SC	Florianópolis	-27.767	-48.500
C. rutilans cyanescens	Brasil	SC	Florianópolis	-27.517	-48.500
C. rutilans cyanescens	Brasil	SC	Florianópolis	-27.533	-48.483
C. rutilans cyanescens	Brasil	SC	Florianópolis	-27.650	-48.467
C. rutilans cyanescens	Brasil	SC	Florianópolis	-27.467	-48.378
C. rutilans cyanescens	Brasil	SC	Governador Celso Ramos	-27.417	-48.567
C. rutilans cyanescens	Brasil	SC	Governador Celso Ramos	-27.400	-48.567
C. rutilans cyanescens	Brasil	SC	Governador Celso Ramos	-27.383	-48.567
C. rutilans cyanescens	Brasil	SC	Governador Celso Ramos	-27.378	-48.560
C. rutilans cyanescens	Brasil	SC	Guarda do Embaú	-27.903	-48.591
C. rutilans cyanescens	Brasil	SC	Içara	-28.667	-49.267
C. rutilans cyanescens	Brasil	SC	Ilha do Arvoredo	-27.282	-48.365
C. rutilans cyanescens	Brasil	SC	Ilhota	-26.924	-48.826
C. rutilans cyanescens	Brasil	SC	Imbituba	-28.130	-48.642
C. rutilans cyanescens	Brasil	SC	Itapema	-27.096	-48.618
C. rutilans cyanescens	Brasil	SC	Itapema	-27.090	-48.607

C. rutilans cyanescens	Brasil	SC	Itapema	-27.067	-48.600
C. rutilans cyanescens	Brasil	SC	Itapema	-27.050	-48.600
C. rutilans cyanescens	Brasil	SC	Itapema	-27.087	-48.598
C. rutilans cyanescens	Brasil	SC	Itapema	-27.079	-48.594
C. rutilans cyanescens	Brasil	SC	Jaraguá do Sul	-26.482	-49.076
C. rutilans cyanescens	Brasil	SC	Joinville	-26.300	-48.847
C. rutilans cyanescens	Brasil	SC	Mafra	-26.121	-49.807
C. rutilans cyanescens	Brasil	SC	Monte Castelo	-26.683	-50.300
C. rutilans cyanescens	Brasil	SC	Monte Castelo	-26.667	-50.300
C. rutilans cyanescens	Brasil	SC	Monte Castelo	-26.650	-50.300
C. rutilans cyanescens	Brasil	SC	Morro das Pedras	-27.720	-48.505
C. rutilans cyanescens	Brasil	SC	Pinhal	-27.147	-52.451
C. rutilans cyanescens	Brasil	SC	Porto Belo	-27.120	-48.521
C. rutilans cyanescens	Brasil	SC	Rancho Quimado	-27.688	-49.018
C. rutilans cyanescens	Brasil	SC	Rio Vermelho	-27.490	-48.416
C. rutilans cyanescens	Brasil	SC	Santo Amaro da Imperatriz	-27.735	-48.809
C. rutilans cyanescens	Brasil	SC	Santo Amaro da Imperatriz	-27.733	-48.800
C. rutilans cyanescens	Brasil	SC	São Bonifácio	-27.883	-48.850
C. rutilans cyanescens	Brasil	SC	São Pedro de Alcântara	-27.617	-48.883
C. rutilans cyanescens	Brasil	SC	Tijucas	-27.238	-48.624
C. rutilans cyanescens	Brasil	SC	Timbó	-26.825	-49.270
C. rutilans cyanescens	Brasil	SC	Tubarão	-28.400	-49.067
C. rutilans cyanescens	Brasil	SP	Amparo	-22.709	-46.773
C. rutilans cyanescens	Brasil	SP	Anhembi	-22.789	-48.132
C. rutilans cyanescens	Brasil	SP	Avanhandava	-21.461	-49.948
C. rutilans cyanescens	Brasil	SP	Bauru	-22.325	-49.070
C. rutilans cyanescens	Brasil	SP	Campinas	-22.912	-47.059
C. rutilans cyanescens	Brasil	SP	Cananéia	-25.097	-47.930
C. rutilans cyanescens	Brasil	SP	Cananéia	-24.987	-47.928
C. rutilans cyanescens	Brasil	SP	Cananéia	-25.077	-47.922
C. rutilans cyanescens	Brasil	SP	Cássia dos Coqueiros	-21.282	-47.169

C. rutilans cyanescens	Brasil	SP	Cerqueira César	-23.038	-49.166
C. rutilans cyanescens	Brasil	SP	Descalvado	-23.178	-47.342
C. rutilans cyanescens	Brasil	SP	Gália	-22.293	-49.551
C. rutilans cyanescens	Brasil	SP	Iporanga	-24.586	-48.596
C. rutilans cyanescens	Brasil	SP	Itirapina	-22.240	-47.837
C. rutilans cyanescens	Brasil	SP	Itu	-23.264	-47.301
C. rutilans cyanescens	Brasil	SP	Pedregulho	-20.257	-47.479
C. rutilans cyanescens	Brasil	SP	Piraju	-23.196	-49.383
C. rutilans cyanescens	Brasil	SP	Porto Cabral	-24.516	-47.701
C. rutilans cyanescens	Brasil	SP	Ribeirão Grande	-24.277	-48.433
C. rutilans cyanescens	Brasil	SP	Ribeirão Grande	-24.283	-48.417
C. rutilans cyanescens	Brasil	SP	Ribeirão Grande	-24.311	-48.406
C. rutilans cyanescens	Brasil	SP	São Carlos	-22.004	-47.887
C. rutilans cyanescens	Brasil	SP	São Paulo	-23.547	-46.635
C. rutilans cyanescens	Brasil	SP	Vale do Ribeira	-24.551	-48.681
C. rutilans rutilans	Brasil	MG	Cabo Verde	-21.470	-46.392
C. rutilans rutilans	Brasil	MG	Conceição dos Ouros	-22.405	-45.832
C. rutilans rutilans	Brasil	MG	Conceição dos Ouros	-22.413	-45.798
C. rutilans rutilans	Brasil	MG	Paraisópolis	-22.600	-45.867
C. rutilans rutilans	Brasil	MG	Serra do Caraça	-20.133	-43.500
C. rutilans rutilans	Brasil	PR	Ortigueira	-24.210	-50.927
C. rutilans rutilans	Brasil	PR	Três Bicos	-24.693	-51.182
C. rutilans rutilans	Brasil	RS	Bagé	-30.986	-54.496
C. rutilans rutilans	Brasil	RS	Bagé	-31.355	-54.013
C. rutilans rutilans	Brasil	RS	Bom Jesus	-28.672	-50.435
C. rutilans rutilans	Brasil	RS	Cambará do Sul	-29.048	-50.144
C. rutilans rutilans	Brasil	RS	Cambará do Sul	-29.183	-50.099
C. rutilans rutilans	Brasil	RS	Canguçu	-31.133	-52.533
C. rutilans rutilans	Brasil	RS	Caxias do Sul	-29.122	-51.019
C. rutilans rutilans	Brasil	RS	Cerro Azul	-27.742	-51.500

C. rutilans rutilans	Brasil	RS	Cerro Largo	-28.147	-54.730
C. rutilans rutilans	Brasil	RS	Encruzilhada do Sul	-30.546	-52.526
C. rutilans rutilans	Brasil	RS	Herval	-32.029	-53.394
C. rutilans rutilans	Brasil	RS	Ijuí	-28.389	-53.921
C. rutilans rutilans	Brasil	RS	Nova Petrópolis	-29.378	-51.112
C. rutilans rutilans	Brasil	RS	Porto Alegre	-30.037	-51.210
C. rutilans rutilans	Brasil	RS	Rio Grande	-32.620	-52.573
C. rutilans rutilans	Brasil	RS	Santo Augusto	-27.844	-53.776
C. rutilans rutilans	Brasil	RS	São Francisco de Paula	-29.446	-50.579
C. rutilans rutilans	Brasil	RS	São Francisco de Paula	-29.432	-50.394
C. rutilans rutilans	Brasil	RS	São Vicente do Sul	-29.693	-54.677
C. rutilans rutilans	Brasil	SC	Bom Jardim da Serra	-28.266	-49.762
C. rutilans rutilans	Brasil	SC	Bom Jardim da Serra	-28.340	-49.554
C. rutilans rutilans	Brasil	SC	Bom Jardim da Serra	-28.349	-49.537
C. rutilans rutilans	Brasil	SC	Urubici	-28.141	-49.637
C. rutilans rutilans	Brasil	SC	Urubici	-28.158	-49.627
C. rutilans rutilans	Brasil	SC	Urubici	-28.164	-49.612
C. rutilans rutilans	Brasil	SC	Urubici	-28.117	-49.501
C. rutilans rutilans	Brasil	SC	Urubici	-28.120	-49.485
C. rutilans rutilans	Brasil	SP	Araçatuba	-21.204	-50.452
C. rutilans rutilans	Brasil	SP	Barueri	-23.514	-46.867
C. rutilans rutilans	Brasil	SP	Campinas	-22.912	-47.059
C. rutilans rutilans	Brasil	SP	Engenheiro Coelho	-22.490	-47.213
C. rutilans rutilans	Brasil	SP	Itararé	-24.109	-49.335
C. rutilans rutilans	Brasil	SP	Jundiaí	-23.186	-46.897
C. rutilans rutilans	Brasil	SP	Jundiaí	-23.233	-46.933
C. rutilans rutilans	Brasil	SP	São Bernardo	-23.728	-46.573
C. rutilans rutilans	Brasil	SP	São Paulo	-23.547	-46.635
C. rutilans rutilans	Brasil	SP	Serra da Bocaina	-22.735	-44.617
C. rutilans rutilans	Uruguay	UY	Rivera	-30.960	-55.498
C. rutilans rutilans	Uruguay	UY	Tacuarembó	-31.723	-55.982

5 CONCLUSÕES GERAIS

Nossos resultados de campo mostraram, em um levantamento anual conduzido ao longo de um gradiente de elevação (de 250 até 1630 m), que as duas subespécies de *Canthon rutilans* não apresentam sobreposição espaço-temporal entre elas. A subespécie *C. rutilans cyanescens* encontra-se mensalmente, exceto em junho, a 250 m de elevação, de agosto até abril a 430 m e de setembro até abril em 840 m, com temperaturas médias do ar entre 15.3°C e 24.0°C. A subespécie *C. rutilans rutilans* encontra-se de outubro a fevereiro em 1360 m de elevação e somente no mês de janeiro a 1630 m, com temperaturas médias do ar entre 14.4°C e 18.6°C. Assim, ambas as subespécies têm uma sobreposição limitada a apenas 5.6% de suas curvas de resposta térmica. Estes resultados sugerem que elas podem ser consideradas como duas espécies diferentes, com requisitos fisiológicos e ecológicos diferentes, provavelmente como consequência de adaptação à temperatura. Dados moleculares podem vir confirmar ou rejeitar esta suposição no futuro próximo.

Posteriormente, experimentos realizados em laboratório em cinco condições de temperatura (15, 20, 25, 30 e 35°C) sugerem que as duas subespécies diferem em estratégias comportamentais e reprodutivas, dependendo da temperatura ambiente. Os adultos de *C. rutilans rutilans* permanecem enterrados no solo por tempo significativamente maior que os adultos de *C. rutilans cyanescens*, que são mais ativos na superfície do solo. No entanto, o comportamento alimentar (medido como o número e o tamanho de bolas alimento realizado por casal) não diferiu entre as temperaturas e as subespécies. Já o comportamento reprodutivo (medido como o número e o tamanho de bolas ninho realizado pelo casal) foi dependente da temperatura e diferente entre as subespécies: a baixas temperaturas, tanto o peso como o diâmetro das bolas-ninho são significativamente maiores em *C. rutilans rutilans*, a subespécie geralmente distribuída em locais com clima mais frio. Assim, embora o número de indivíduos recém-emergidos não tenha diferido significativamente entre as duas subespécies, tanto o peso desses novos adultos, como o tempo de emergência foi dependente da temperatura e da subespécie. Quanto à longevidade dos indivíduos parentais, *C. rutilans rutilans* aumenta o tempo de sobrevivência conforme diminui a temperatura. Esses resultados apoiam a existência de estratégias biológicas e de desenvolvimento diferenciadas, ligadas ao nicho

térmico, onde *C. rutilans rutilans* tem comportamento reprodutivo com adaptações para viver em regiões frias. Isso reforça a ideia de que as duas subespécies podem ser consideradas espécies ecologicamente diferentes.

Finalmente, ao visitar sete diferentes coleções entomológicas do Brasil, conseguimos dados de distribuição de 46 locais diferentes para *C. rutilans rutilans* e 120 para *C. rutilans cyanescens*. Os valores médios de 12 das 23 variáveis ambientais (duas variáveis topográficas e 21 bioclimáticas) nas áreas de ocorrência diferiram entre as duas subespécies, especialmente aqueles relacionados à temperatura e elevação. Posterioremente, a partir dos dados derivados das variáveis ambientais foram gerados mapas de distribuição provável das duas subespécies. Os resultados mostraram que *C. rutilans rutilans* está presente em locais mais frios, vivendo nas localidades de alta elevação do bioma Mata Atlântica ou nos Pampas. Por sua parte, *C. rutilans cyanescens* parece estar restrito a áreas de floresta do bioma Mata Atlântica com temperaturas mais elevadas. Na escala regional, as duas subespécies têm simpatria geográfica, uma vez que a área de sobreposição da distribuição foi de 70% entre elas. Já na escala local elas não ocorrem juntas, encontrando-se em alopatria, uma vez que as duas subespécies têm diferentes requerimentos ambientais produto de adaptações fisiológicas e comportamentais.

Assim, nossos resultados sustentam que as duas subespécies de *Canthon rutilans* podem ser consideradas duas espécies ecologicamente diferentes.

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