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VARIAÇÕES POPULACIONAIS DO TAMANHO DO CORPO DE BESOUROS ESCARABEÍNEOS RELACIONADAS À TEMPERATURA

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Variações populacionais do tamanho do corpo de besouros escarabeíneos relacionadas à temperatura

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Carolina Oliveira de Alcântara

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“Prezo insetos mais que aviões.
Prezo a velocidade
das tartarugas mais que a dos mísseis.”
(DE BARROS, Manoel em *O apanhador de desperdícios*, 2011)

RESUMO

O tamanho corporal é uma característica importante para os organismos pois pode estar relacionado com fecundidade, capacidade de aquisição de recursos, dispersão e até taxa respiratória. A temperatura é um fator abiótico que afeta o crescimento dos animais e, aliada à genética, é importante tanto na seleção natural como na seleção sexual. Para insetos, as temperaturas do ambiente afetam efetivamente as reações enzimáticas até seus comportamentos, pois não possuem mecanismos complexos de geração de calor. A quantidade de gordura armazenada pelos insetos, por exemplo, além do fator genético, também é influenciada pela temperatura, constituindo um tecido que apresenta atividades biossintéticas e metabólicas e ainda atua como reservatório de energia. Em coleópteros, a temperatura afeta o desenvolvimento larval, influenciando o tamanho do corpo dos adultos, por serem holometábolos. *Canthon rutilans cyanescens* e *Dichotomius sericeus* são espécies de besouros da subfamília Scarabaeinae abundantes nas florestas nativas do sul do Brasil, mas com hábitos diferentes: enquanto *C. rutilans cyanescens* tem comportamento rolador diurno, *D. sericeus* é uma espécie tuneleira noturna. Buscamos comparar o tamanho corporal e a proporção de gordura e músculos de populações destas duas espécies, sob a hipótese de que em locais mais frios sejam encontrados indivíduos menores, mas com maiores proporções de gordura no corpo. Para testar se a condição corporal varia nas populações dependendo da distribuição espacial e temporal foi comparado o peso seco de indivíduos de ambas espécies provenientes de quatro populações geograficamente separadas no estado de Santa Catarina, mas sob condições climáticas semelhantes, coletadas no verão de 2012 e de 2013. Posteriormente, foi comparado o comprimento do corpo de indivíduos coletados mensalmente, ao longo de um ano, em locais de diferentes temperaturas devido ao gradiente altitudinal (200 e 800 m de elevação); por fim, foi realizada uma coleta durante o verão de 2020 nestes mesmos locais para a avaliação e comparação do tamanho do corpo por meio de medidas de comprimento, além de medidas de peso durante o protocolo de extração de gordura e músculos realizado em laboratório. Os resultados mostram que o tamanho corporal de *C. rutilans cyanescens* é diferente entre localidades e temporalmente. Além disso, existe flutuação anual dentro das populações, com maior tamanho corporal nos meses do verão e na localidade de menor elevação e maior temperatura ambiental. Existe uma relação linear positiva entre o tamanho médio mensal e as temperaturas médias do solo. Além disso, os indivíduos de *C. rutilans cyanescens* coletados em locais de maior elevação e, portanto, mais frios, apresentam um tamanho corporal menor e maior proporção de gordura do que aqueles de locais mais baixos. As populações geograficamente separadas de *D. sericeus* também apresentam variação no tamanho corporal e ao longo do tempo, com variação sazonal de tamanho, sendo maiores nos meses de verão; no entanto, a análise de regressão, não mostrou relação entre o tamanho do corpo e a temperatura, assim como não houve diferença nas proporções de músculos e gordura das populações coletadas em locais de diferentes temperaturas. Portanto, existe variabilidade no tamanho corporal de escarabeíneos, o qual é afetado por fatores geográficos e ambientais, sendo a espécie diurna fortemente afetada pela temperatura.

Palavras-chave: Coleoptera. Ecofisiologia. Ecologia de populações. Insecta. Scarabaeinae.*Canthon rutilans cyanescens*. *Dichotomius sericeus*.

ABSTRACT

Body size is an important trait for organisms because it can be related to fertility, ability to acquire resources, dispersion and even respiration. Temperature is an abiotic factor that affects the animal growth, and, with genetics, it is important in both natural and sexual selection. For insects, external temperatures affect from enzymatic reactions to their behavior, as they do not have complexes heat generator mechanisms. The amount of fat stored by insects, for example, in addition to the genetic factor, is also influenced by the external temperature, constituting a tissue that presents biosynthetic and metabolic activities, also acting as an energy reservoir. In Coleoptera, temperature affects larval development, influencing adult body size. *Canthon rutilans cyanescens* and *Dichotomius sericeus* are species of dung beetles of the subfamily Scarabaeinae, abundant in native forests of southern Brazil, with different habits: while *C. rutilans cyanescens* is a diurnal roller, *D. sericeus* is a nocturnal tunneler species. We aimed to compare the body size and amounts of fat and muscle of individuals from populations of these two species. We hypothesized that in colder places individuals with smaller body size but and with higher proportions of body fat are found. To test whether body size varies in populations depending on spatial and temporal distribution, through analysis of variance, the dry weight of individuals of both species from four geographically separated populations in the state of Santa Catarina, but under similar climatic conditions, collected in the summers of 2012 and 2012 was compared. Subsequently, the body length of individuals collected over a year at different temperature locations due to the altitudinal gradient (200 and 800 m elevation) was compared; ultimately, a collection was carried out during the summer of 2020 in the same places to compare body size, body mass, and fat and muscle mass extraction protocol carried out in the laboratory. The results show that the body size of *C. rutilans cyanescens* varies between locations and temporally: there is annual fluctuation within populations, with greater body size in the summer months and in the location with the lowest elevation and highest environmental temperature; presenting is a positive linear relationship between average body size and average soil temperatures. In addition, individuals of *C. rutilans cyanescens* collected at higher elevations and, therefore, cooler, have a smaller body size and higher amounts of fat than those from lower locations. Populations of *D. sericeus* also vary in body size and over time, with seasonal variation in size, being larger in the summer months; however, regression analysis did not show a relation between body size and temperature, as well as the proportions of body fat and muscles are not different within the populations of warmer and colder sites. Therefore, there is variability in the body size of dung beetles, which is affected by geographic and environmental factors, with the diurnal species being strongly affected by temperature.

Keywords: Coleoptera. Dung beetles. Ecophysiology. Insecta. Population ecology. *Canthon rutilans cyanescens*. *Dichotomius sericeus*.

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1 APRESENTAÇÃO

Esta dissertação de mestrado foi elaborada em meio à pandemia do vírus SARS-CoV-2. Nesse momento, vivenciamos isolamento social, uso de máscaras e outras medidas preventivas, superlotações em hospitais e intercorrências de planos de ações governamentais; desse modo, houve mudanças do projeto ao longo do curso do mestrado. Adaptações foram feitas para que fossem utilizados bancos de dados previamente coletados, embora conseguimos também incluir novos dados. É importante salientar que mudanças não ocorreram apenas no âmbito acadêmico, uma vez que a disseminação do vírus mudou a visão que temos sobre relações interpessoais, saúde mental e trabalho em casa.

1.1 INTRODUÇÃO GERAL

Neste trabalho, estudamos o tamanho corporal de besouros escarabeíneos (Coleoptera: Scarabaeidae: Scarabaeinae), por ser uma característica importante para os organismos, pois está relacionado com a fecundidade, capacidade de aquisição de recursos, dispersão e até mesmo respiração (Peters, 1986). Para os insetos, o tamanho do corpo está diretamente relacionado com o “fitness” ou aptidão reprodutiva, assim como com a longevidade dos indivíduos (Beukeboom, 2014), sendo um fator importante tanto na seleção natural quanto na seleção sexual (Mousseau & Roff, 1987). O tamanho do corpo desses invertebrados pode ser afetado por diversas causas, incluindo o tamanho dos ovos, nutrição dos estágios imaturos, condições ambientais, além de ter bases genéticas (Meister et al., 2018; Najafpour et al., 2018; Shelly, 2018; Tran et al., 2018).

Esse grupo de insetos provê importantes serviços ecossistêmicos. Os escarabeíneos atuam na ciclagem de nutrientes, uma vez que se alimentam de matéria orgânica, como fezes ou matéria orgânica em decomposição, dissipando esses recursos e enterrando-os, além de exercer dispersão secundária de sementes como consequência (Halffter & Edmonds, 1982; Galbiati et al., 1995; Bertone, 2004; Lastro, 2006; Yamada et al., 2007; Nichols et al., 2008). O tamanho corporal regula a quantidade de recurso que os indivíduos são capazes de remover, tendo um papel fundamental sobre o serviço ecossistêmico promovido por esses besouros (Andresen, 2002; Slade et al., 2007; Kaartinen et al., 2013; Nervo et al., 2014).

A temperatura do ambiente é um fator que influencia a presença e o desenvolvimento dos seres vivos, assim como seu tamanho corporal. Ainda que existam organismos adaptados para a sobrevivência em locais gelados ou locais muito quentes, os animais estão adaptados a uma temperatura onde o funcionamento do seu metabolismo será ótimo para seu desenvolvimento (Goldsmith & Sladen, 1961; Verschelde et al., 1998). O mesmo acontece dentro do grande grupo dos insetos: as temperaturas externas afetam diretamente desde as reações enzimáticas até seus comportamentos, uma vez que não possuem mecanismos sofisticados para a geração de calor endógeno (Lee, 1991; Régnière et al., 2012).

Para os escarabeíneos, que são holometábolos, a temperatura influencia o desenvolvimento larval, o que reflete no tamanho dos adultos, uma vez que é no estágio larval quando ocorre o desenvolvimento das características fenotípicas exibidas pelos adultos (Emlen & Nijhout, 2000; Kellermann et al., 2017, Slotsbo et al., 2016, Telemeco et al., 2017). Ademais, estudos mostram que os besouros escarabeíneos apresentam plasticidade fenotípica passiva, que permite sua adaptação às flutuações de temperatura, existindo variações no tamanho corporal dos adultos de uma mesma população que se desenvolveram em períodos de diferentes temperaturas (Whitman & Agrawal, 2009; Havird et al., 2020). Apesar de existir a plasticidade, temperaturas extremas ou muitas flutuações de temperatura podem não ser compensadas e interferirem negativamente na aptidão e sobrevivência (Williams et al., 2012).

O acúmulo de gordura é considerado um fator que influencia positivamente a aptidão de insetos, sendo um indicador de boa saúde e sistema imunológico (Köskimaki et al., 2004; Salomão et al., 2019). Um dos fatores ambientais que exercem influência sobre o acúmulo de gordura é a temperatura, assim como sobre outras condições corporais dos escarabeíneos (Stevenson & Woods, 2006; Salomão et al., 2018). Ainda, a gordura corporal têm papel crucial na tolerância térmica; esse tecido é uma medida de aclimação e resistência às temperaturas baixas (Storey & Storey, 1992, 1996; Duman, 2001; Duman et al., 2004). Sabe-se que espécies de escarabeíneos inclusive apresentam hábitos alimentares diferentes em períodos mais frios, preferindo recursos ricos em gordura (Verdu et al., 2010).

Assim, o presente trabalho teve dois objetivos: o primeiro foi investigar se há diferenças no tamanho do corpo entre populações de escarabeíneos geograficamente separadas e o segundo foi investigar se a temperatura ambiental influencia o tamanho corporal dessas populações. Para o primeiro objetivo, dispomos da hipótese de que fatores biogeográficos (dispersão, deriva, eventos estocásticos e seleção) influenciam o tamanho do corpo de populações geograficamente separadas, assim, esperamos que populações em áreas distintas apresentem tamanhos corporais diferentes. Para o segundo objetivo, temos a hipótese de que as populações são influenciadas pelos fatores ambientais das áreas em que estão inseridas, sendo a temperatura o foco do estudo.

Dada a influência da temperatura sobre o desenvolvimento dos besouros e, consequentemente, o tamanho corporal e as condições corporais dos adultos, espera-se que indivíduos maiores sejam encontrados em locais mais quentes. A menor proporção de superfície corporal em contato com o meio em relação ao volume, previne a perda de água por evaporação, além disso, depósitos de gordura não seriam necessários para a manutenção de temperatura desses organismos. Nos locais mais frios, além de serem menores de tamanho, os insetos devem acumular mais gordura, tanto como reserva energética como para isolamento térmico.

Para tal, utilizamos como modelo de estudo duas espécies de coleópteros escarabeíneos que apresentam diferentes hábitos e comportamentos: *Canthon rutilans cyanescens* e *Dichotomius sericeus*. *Canthon rutilans cyanescens* é uma espécie diurna e roladora, ou seja, rola o alimento em pequenas esferas, das quais se alimentam tanto os adultos como as larvas. Os adultos, de cor azul, medem em média 9 mm de comprimento e são muito comuns nas áreas litorâneas do estado de Santa Catarina, mas sua distribuição também abrange os estados do Mato Grosso do Sul, São Paulo, Paraná e Rio Grande do Sul, além da região norte da Argentina e no Uruguai (Vaz-de-Mello et al., 2014; Hensen et al., 2018; Hensen et al., 2021). *Dichotomius sericeus* tem maior tamanho, com cerca de 13 mm de comprimento, é de cor preta e tem comportamento noturno e tuneleiro, de modo que enterram seu alimento logo abaixo do recurso e usam-no como alimento e depósito de ovos para o desenvolvimento da prole. Essa espécie é encontrada nos estados brasileiros Rio de Janeiro, Espírito Santo, São Paulo, Santa Catarina, Paraná, Rio Grande do Sul e Minas Gerais, além de províncias do Paraguai e Argentina (Valois et al., 2017).

Devido à elevada diversidade taxonômica, comportamental e ecológica dos besouros escarabeíneos (Fleming et al., 2021), relativamente pouco se sabe sobre como a temperatura age nas diferentes espécies de besouros. Ainda, em um cenário de mudanças climáticas, pesquisas como essa se fazem necessárias a fim de entender a história natural das espécies e prever os impactos sobre os seres. As adaptações das populações e das diferentes espécies podem oferecer pistas do caminho evolutivo e adaptações ecológicas a serem seguidas em conjunturas de alterações ambientais (i.e., Emlen & Nijhout, 2000; Whitman & Agrawal, 2009; Slotsbo et al., 2016; Kellermann et al., 2017; Telemeco et al., 2017; Havird et al., 2020).

1.2 OBJETIVOS

1.2.1 Objetivo Geral

O presente trabalho tem como objetivo geral averiguar se existem diferenças no tamanho corporal e proporção de gordura de diferentes populações de duas espécies de besouros da subfamília Scarabaeinae relacionadas com fatores geográficos e ambientais.

1.2.2 Objetivos Específicos

1. Investigar se há variação no tamanho do corpo de populações de escarabeíneos geograficamente separadas (fator espacial) e ao longo do ano (fator temporal);
2. Averiguar se o tamanho corporal e a proporção de gordura e músculos de populações de escarabeíneos que se desenvolveram em locais de diferentes temperaturas ambientais são influenciados por esse fator abiótico.

2 CAPÍTULO 1

Artigo a ser submetido para a revista Entomologia Experimentalis et Applicata, seguindo as regras de formatação da mesma.

Geographical and temporal factors influence body size and body condition in two dung beetles species

Alcântara, Carolina Oliveira de; da Silva, Pedro Giovâni; Hernández, Malva Isabel Medina

Abstract

Temperature affects the growth of animals and their body size, which is an important trait in natural and sexual selection. For insects, temperatures affect from enzymatic reactions to their behavior, as they do not have complexes temperature control mechanisms. The amount of fat stored by insects is also influenced by the external temperature and constitutes a tissue that presents biosynthetic and metabolic activities, acting as an energy reservoir. We assessed the effect of geographical separation and environment temperature due elevation above sea level on beetle body size, body mass and body conditions. For this study, we analyzed individuals of two species: *Canthon rutilans cyanescens* and *Dichotomius sericeus* are dung beetles of the subfamily Scarabaeinae, abundant in southern Brazilian forests, with different habits: the first one is a diurnal roller and the second one is a nocturnal tunneler. Summer months and sites with lower elevations and higher temperatures presented larger *C. rutilans cyanescens* individuals. There was a positive linear relationship between average monthly body size and average soil temperatures. Furthermore, *C. rutilans cyanescens* from cooler sites had higher amounts of body fat. Similar to the observed in *C. rutilans cyanescens*, *D. sericeus* individuals were larger in summer months. However, regression analysis did not show relation between body size and temperature as well as the amounts of body fat and muscles which shown no difference between the populations collected under different temperatures.

Thus, there is variability in the body size of the studied dung beetles, which is related to geographical and temporal factors but also to their activity time patterns.

Key words: Coleoptera, Ecophysiology, Population ecology, Scarabaeinae, *Canthon rutilans cyanescens*, *Dichotomius sericeus.*

2.1 INTRODUCTION

Temperature has widely been proven to influence insect distribution spatially and temporally (Wolda, 1978). Insects, in general, if exposed to lower temperatures than those considered optimal for their survival can enter a quiescent or diapause state, when their metabolism and development are slower and there is a chance of survival when the temperature is normalized; in extreme cases, insects may fall into a coma or die (Lee, 1991; Bale, 1993). On the other hand, when exposed to temperatures higher than the optimal, insects may present accelerate development and metabolism, but very high temperature can also lead them to death (Huey & Kingsolver, 1993; Gilbert & Raworth, 1996). Studies show that most flying insects cannot start a flight when their thorax temperature is below of 25°C and suffer thermal shock at temperatures between 45-47°C, being unable to survive in temperatures of 50–53°C (Machin et al., 1962; May, 1976, 1978; Heinrich, 1980; Christian & Morton, 1992; Wu et al., 2002; Chown & Nicholson, 2004; Verdú et al., 2006; Verdú & Lobo, 2008).

Among insects, body surface plays a key role in the balance between the individual condition and the surrounding environment, being related to several factors including water exchange and thermal regulation (Le Lagadec et al., 1998; Kühsel & Blüthgen, 2015). The volume-surface ratio, mediated by insect length, predicts that larger individuals have a smaller volume-surface ratio compared to smaller individuals. Insects with a smaller volume-surface ratio are known to lose less water so they have a better heat retention capacity (Kühsel et al., 2017). Besides the volume-surface ratio, the exoesqueleton thickness and color can affect the heating capacity. The thickness of the exoskeleton influences heat maintenance, so that thicker exoskeletons cause individuals to heat up more slowly (Amore et al., 2017). The color is also related to the period of activity in dung beetles: a variety of colors, such as blue, green and red, can be found in diurnal species while black-colored species are expected to be nocturnal (Crowson, 1981; Hernández, 2002). The thermal melanism hypothesis (TMH) proposes that dark colored insets can acquire and preserve higher temperatures when exposed to the sun so they can survive in colder environments and be active in nocturnal periods (Watt, 1968; Kingsolver, 1987; Stanbrook et al. 2021). Body color also works as interspecific communication as it presents aposematic signals to predators but also favor camouflage to the nocturnal species (Otronen 1988; Endler 1990; Guilford 1990).

Animal fat levels, in addition to being a genetic factor, are also influenced by external temperature (Pelleymounter et al., 1995; Sun Yoo et al., 2014). For insects, body fat is a tissue that has biosynthetic, metabolic activities and acts as an energy reservoir (Law & Wells, 1989). In this sense, organisms inhabiting colder and less resourceful environments should present higher fat accumulation when compared to individuals living in warmer and more resourceful sites, for the purpose of thermoregulation and for energy reserve (Colinet et al., 2006; Salomão et al., 2019). Fat accumulation provides a rise in the proteins presented in the hemolymph and consequently significantly decreases the super-cooling point of the insects, so that insects can survive colder temperatures (Verdú et al. 2010; Ben-Hamo et al., 2011).

Included in the class Insecta, Coleoptera is the group with the highest species diversity among all living things, being present in all continents, except the Antarctic (Rafael et al., 2012). Some species of this group were studied and responses in color or body size were observed when they developed under different temperatures: an example is the *Agasicles hygrophila* beetle, whose adult weight changes as a result of the temperature at which their larvae grow (Stewart et al., 1998). Furthermore, it has been shown that the thickness of the exoskeleton influences heat maintenance so that thicker exoskeletons cause individuals to heat up more slowly (Amore et al., 2017).

Due to geographical and environmental effects on the development and morphology of beetles, the aim of the study was to investigate if there are differences in body size and body condition of different populations of two species of Scarabaeinae beetles related to geographic and temporal factors. We used the dung beetles species *Canthon rutilans cyanescens* Harold, 1868 and *Dichotomius sericeus* (Harold, 1867) as study models. Included in the subfamily Scarabaeinae, the first one is a blue iridescent diurnal roller dung beetle, while the second one is a black nocturnal tunneler species (Hernández et al., 2020). Both species are Neotropical, widely distributed in tropical and subtropical forests in southern Brazil (Vaz-de-Mello et al., 2014; Valois et al., 2017). Differences in body size and proportion of fat of individuals inhabiting locations with different temperatures are expected, with larger individuals found in warmer sites as the lower proportion of body surface to body volume minimizes evaporative water loss. In addition to the influence of temperature on body size, it is expected that in colder places insects accumulate more fat as a reserve of energy and for thermal maintenance.

2.2 MATERIALS AND METHODS

The study was carried out in an Atlantic Forest environment of Dense Ombrophilous Forest in Santa Catarina state, southern Brazil. This forest is characterized by complex vegetation, involving lianas and epiphytes associated with woody trees, integrating an environment with heterogeneous structures and microclimates, therefore being a biodiversity hotspot for fauna and flora. All the study areas are located in a Cfa climatic region (Köppen, 1936; Leite & Klein, 1990; IBGE, 2012).

2.2.1 Body size – geographical and temporal factor

To understand if there are differences in the body size of populations influenced by the geographic factor we used a database of dry weight of all individuals of the two studied species collected in Itapema, Anhatomirim, Ratones and Peri in 2012 and 2013 (da Silva & Hernández, 2014, 2015).

Four areas of Atlantic Forest located on the coast of Santa Catarina state were selected to sampling the beetles: two on the mainland of the state and two on the Island of Santa Catarina. The two areas sampled on the continent are located in the municipality of Itapema (Conservation Unit, 27º05’13”S, 48º35’54”W) and at Anhatomirim Environmental Conservation Unit (municipality of Governador Celso Ramos, 27º25’10”S, 48º34’25”W). The two areas on the Island of Santa Catarina were located in the municipality of Florianópolis: Ratones (Conservation Unit, including the Conservation Unit of Desterro, 27º31’52”S, 48º30’45”W) in the north of the island and Lagoa do Peri Natural Monument in the south of the island (27º43’30”S, 48º32’18”W). The altitude of the areas varies between 38 and 240 meters above sea level, whereas the distance between the sites ranges from 13.5 to 71 km.

Collection of beetles in fild were carried out in the summer of two consequtive years, 2012 and 2013. Baited pitfall traps were used, which consist of plastic containers (15 cm diameter × 20 cm depth) buried with the opening at the ground level. We covered the traps with a plastic lid, positioned with bamboo sticks to support the bait and to prevent flooding by rain. The bait was human feces wrapped in fabric and placed hanging below the lid. In each trap, we added approximately 300 ml of a solution of water with detergent to capture the attracted insects. Traps were left in the field for 48 hours and these collected beetles were dried in an oven at 60ºC for 72 h, weighed and deposited at Mitia Heusi Silveira Entomology Collection, at the Biological Sciences Center of the Universidade Federal de Santa Catarina.

To estimate individual body size and dry weight, all individuals of *C. rutilans cyanescens* and *D. sericeus* were properly identified and classified by the location at which they were captured. All measurements were performed manually using a digital caliper (0.01 mm) and a precision balance (0.0001 g). The weight of individuals from each species collected in Itapema, Anhatomirim, Ratones and Peri in 2012 and 2013 were compared by

Two-Way ANOVA tests. All statistical analyzes were performed with R software, using the *vegan* package (Oksanen et al., 2017; R Core Team, 2020).

2.2.2 Body size – seasonal factor and temperature

To verify whether geographical separation of populations, sampling months and temperatures influence body size, we compared collected individuals from two distinct locations of different altitudes: Santo Amaro da Imperatriz, at 200 m a.s.l. (27º44’05”S, 48º48’33”W) and Rancho Queimado, at 800 m a.s.l. (27º41’22”S, 49º00’52”W), both in Santa Catarina state and approximately 30.5 km apart. Populations of *C. rutilans cyanescens* and *D. sericeus* were also sampled with baited pitfall traps, once a month, between June 2015 and July 2016. Traps were left in the field for 48 hours and these collected beetles were deposited in alcohol at Mitia Heusi Silveira Entomology Collection, at the Biological Sciences Center of the Universidade Federal de Santa Catarina. These insects were measured from the clypeus to the pygidium in order to obtain total length. We also analyzed databases of daily temperatures of each location acquired by the installation of dataloggers in the field.

For individuals of *D. sericeus* collected monthly throughout June of 2015 and July in the locations of 200 and 800 m a.s.l., interpopulational and intrapopulational body size variation was also compared by Two-way ANOVA. Due to lack of individuals of *C. rutilans cyanescens* throughout the year in the 800 m study area (n = 8), only the populations collected in January 2016 were compared between areas using ANOVA. In order to understand if temperatures are crucial in defining the adult length of dung beetles, we tested relation between the body size of both species and the mean soil temperatures of each site collection over a year applying regression analysis.

2.2.3 Body condition

In 2020, alive individuals of *C. rutilans cyanescens* and *D. sericeus* were collected in the two above mentioned locations during the summer of 2020 for the extraction of muscle and body fat in order to understand the difference of these physiological factors between separated populations. Twelve baited pitfall traps were placed in Santo Amaro da Imperatriz and in Rancho Queimado simultaneously until at least 60 individuals of each species were acquired (about six days). In order to attract live insects, the traps were left in the field for up to 24 hours; the buried containers were filled with 5 cm of local soil and 20 g of dog feces as bait, obtained from the Central Bioterium of the Universidade Federal de Santa Catarina, Brazil. We placed a lid with a triangle cutout that allows the entry of the beetles, but makes their exit difficult, in addition to an uncut lid kept just above the trap by bamboo sticks, functioning as an “umbrella”.

For these individuals was measured the total length and weight. Also, to quantify the muscle mass and body fat mass of each individual, we followed the methodology of González-Tokman et al. (2011). Therefore, alive insects were individually sacrificed by freezing in eppendorf tubes and then kept in an oven at 50ºC for 48 h; after this time, they were weighed, and this measurement was recorded as the dry weight or initial weight (P1). Then 1 mL of chloroform was added in each tube, and after 48 h at room temperature, excess chloroform was drained and the beetles were placed in the oven at 50°C for 48 h then weighed to obtain the weight 2 (P2). The amount of body fat was calculated by subtracting P2 from P1. Afterwards, 1 mL of 0.8 M KOH was added to each tube, submerging the beetles, and they were kept at room temperature for 24 h. After this period, KOH was removed and distilled water was added to each tube, followed by 24 h conditioning at room temperature. Then the distilled water was removed and 1 mL of the same liquid added, which after 2 h of immersion was removed and the insects were returned to the oven for 48 h at 50ºC. At the end of this period, the last weighing was carried out, recording weight 3 (P3). Subtracting P3 from P2, we obtained the muscle weight of each individual. The weight ratio of each individual that sets up for fat and muscle was calculated.

Finally, we verified if there were differences between the length, weight and fat mass and muscle mass of dung beetles from the two different locations using T-tests for parametric data or Wilcoxon rank-sum test for non-parametric data.

2.3 RESULTS

2.3.1 Canthon rutilans cyanescens

There was a significant difference of individual dry body weight among the four different studied locations (Two-way ANOVA; $F_3 = 26.31$, $p < 0.01$; Figure 1A and 1B). Furthermore, individual weight also varied between the years of sampling (Two-way ANOVA; $F_3 = 3.502$, $p = 0.01$).

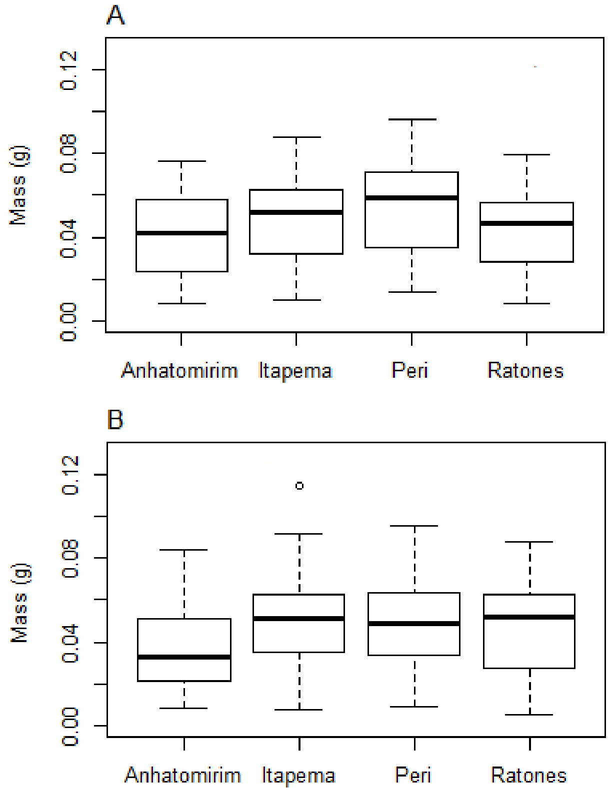

Figura 1 Variations of dry weight of individuals of the species *Canthon rutilans cyanescens* collected in four geographically separated locations (Anhatomirim, Itapema, Peri and Ratones) in the coastal area of Santa Catarina state, South of Brazil, during the summer of 2012 (A) and 2013 (B).

Individuals from Anhatomirim sampled in 2013 were about 13% lighter than all others, weighing an average of 0.04 g, while individuals of 2012 were not different, showing that there is geographic variation in body mass between populations and a temporal variation between the same population within different years.

Regarding the temporal variation throughout the year, the fluctuation of body lenght in the 200 m location showed that in December, February and April (i.e., during the summer period in the southern hemisphere) individuals were significantly larger than in other months whenever the species is present (ANOVA; $F_9 = 8{,}286$, $p < 0.001$; Figure 2A). The body length was 11.17, 10.99 and 10.75 mm, respectively, compared to an average of 10.27 mm in the other months. There was no significant difference in the body lenght of individuals living at the elevation of 800 m (ANOVA; $F_1 = 0.242$, $p = 0.623$; Figure 2B) sampled in December and January.

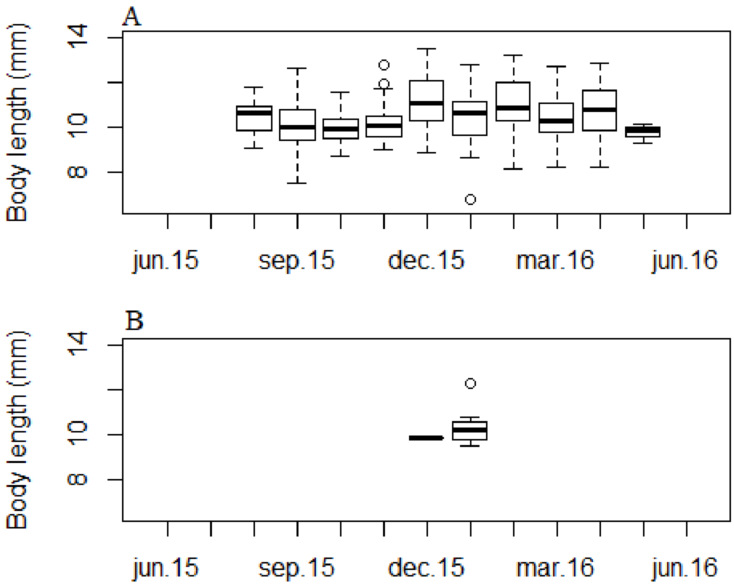

Figura 2 Measures of body length in millimeters of individuals of Canthon rutilans cyanescens collected between August/2015 and May/2016 in (A) Santo Amaro da Imperatriz (200 m a.s.l.) and (B) Rancho Queimado (800 m a.s.l.) in Santa Catarina state, south of Brazil.

There was a positive relation between the average soil temperatures and *C. rutilans cyanecens* body length at the 200 m location (Figure 7, Supplementary material; $r = 0.76$, $t_9 = 3.474$, $p < 0.01$). Also, by linear regression, for every 1ºC of temperature, the body length of the species increases by 0.16 mm (Size = 7.3 + 0.16 * Temperature; $p < 0.01$).

The 29 individuals collected during the summer of 2020 at the 200 m location and the 36 individuals from the 800 m location showed significant differences in size between both populations, with larger ones found in the lower-altitude location. Body size, measured as length and weight was different in the insects that have developed in different places. The insects from the lowest location were 39% larger (T-test, $t_{48} = 7.755$, $p < 0.001$) and 13% heavier ($t_{54} = 7.434$, $p < 0.001$).

There was a significant difference in the amount of body fat between beetles sampled from the different locations. Individuals collected in the highest (and coldest) location showed 56% more body fat than those from the lowest and warmer location (Figure 3; Wilcoxon test, $W = 23$, $p < 0.05$). There was no difference in the amounts of muscles of individuals from different locations (Figure 3; Wilcoxon test, $W = 47$, $p = 0.85$).

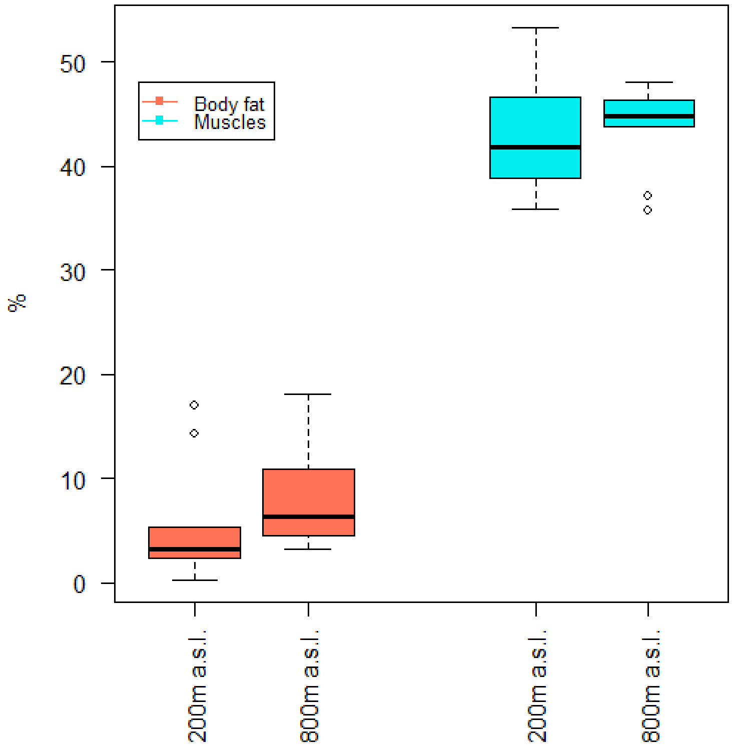

Figura 3 Body fat mass (red boxes) and muscle mass (blue boxes) of individuals of C. rutilans cyanescens collected at two locations: Santo Amaro da Imperatriz (200 m a.s.l.) and Rancho Queimado (800 m a.s.l.) in Santa Catarina state, south of Brazil.

2.3.2 Dichotomius sericeus

For the species *D. sericeus*, their body mass varied significantly between the four collection sites (Two-way ANOVA; $F_3 = 25.38$, $p < 0.01$; Figure 4A and 4B) and between the two years of sampling (Two-way ANOVA; $F_1 = 176.41$, $p < 0.01$).

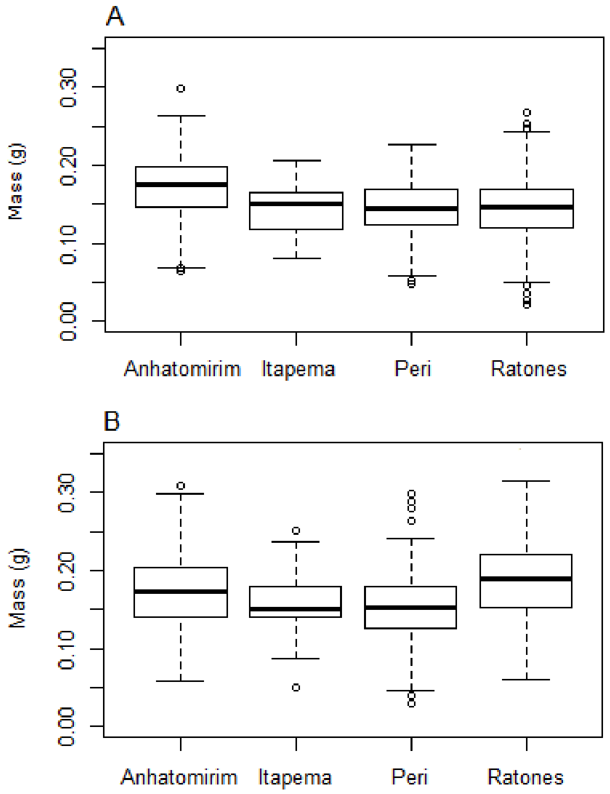

Figura 4 Variations of body dry weight, in mg, of individuals of the species Dichotomius sericeus collected in four geographically separated locations (Anhatomirim, Itapema, Peri and Ratones) in the coastal area of Santa Catarina state, South of Brazil, during the summer of 2012 (A) and 2013 (B).

Individuals collected at Ratones in 2013 weigh an average of 0.18 g, being heavier than all others collected in whichever year, whose average weigh were 0.15 g, including the individuals collected at Ratones in 2012, with the average weigh of 0.14 g. This result attests that there is geographic variation in body size between populations and a temporal variation between the same population within different years (Table 2).

Comparing the populations from the sites of 200 and 800 meters and throughout the year, the occurrence of the species was observed between the months of November and March at 200 m location (Figure 5A) and between December and April at 800 m location (Figure 5B). We found that the length of *D. sericeus* varies locally (Two-way ANOVA; $F_1 = 6.835$, p

< 0.05), presenting a mean size of 16.33 mm in 800 m (Rancho Queimado) while the individuals of 200 m (Santo Amaro da Imperatriz) presented a mean size of 15.69 mm. Also, the body length varies monthly (Two-way ANOVA, $F_5 = 3.145$, $p < 0.05$), with larger individuals found in January and February in Rancho Queimado, whose body length was 16.69 and 16.73 mm, respectively.

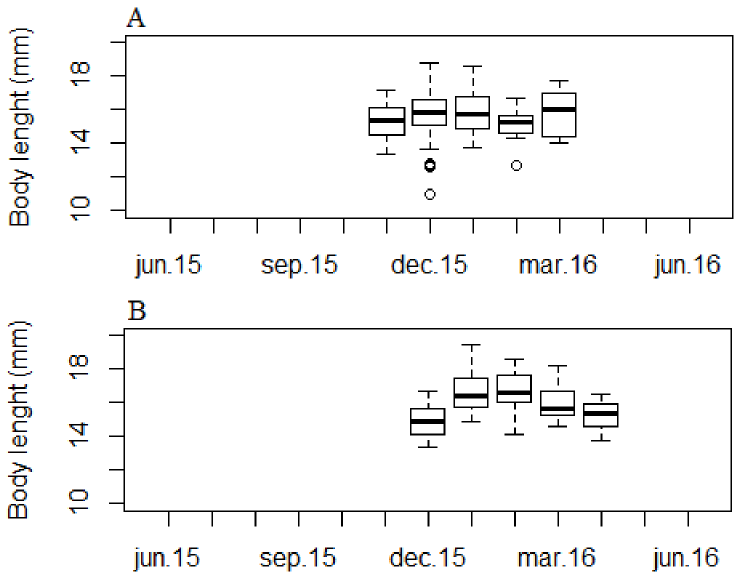

Figura 5 Measures of body length in millimeters of individuals of Dichotomius sericeus collected between November/2015 and April/2016 in two locations: (A) Santo Amaro da Imperatriz (200 m a.s.l.) and (B) Rancho Queimado (800 m a.s.l.) in Santa Catarina state.

The body length of *D. sericeus* is not related to temperature, with no correlation between the body length of individuals collected at the 200 m site and the mean soil temperatures ($r = 0.04$, $t_3 = 0.07$, $p = 0.95$). Likewise, there was no correlation between the body length of individuals collected at the 800 m site and soil temperatures ($r = 0.614$, $t_3 = 1.32$, $p = 0.27$). Nonetheless, we believe that this result is due to the low sample in both areas: there were only 5 months of occurrence of the specie in each area.

Furthermore, during the collection in the summer of 2020, when 60 individuals were collected, 29 in 200 m site and 31 in 800 m site, we found that individuals have a length 5.7% greater ($t_{51} = 3.104$, $p < 0.05$) at the 800 m site; however, there was no significant difference in relation to the weight of individuals (t_{56} = 1.123, p = 0.266). Comparing the fat of individuals collected in the 200 and 800 m locations, there is no significant difference (T-test, t_{18} = 0.92, p = 0.369). Also, comparing the proportions of muscles, there is no significant difference (T-test, t_{19} =0.37, p = 0.711).

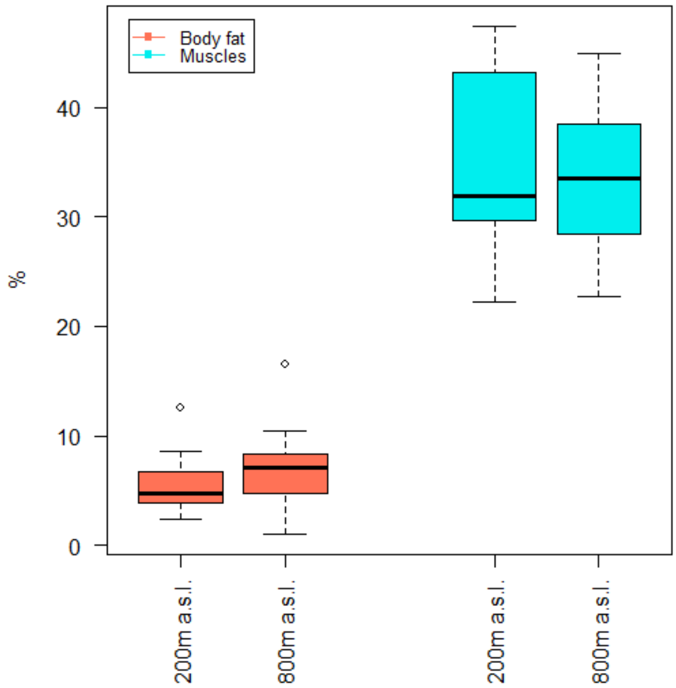

Figura 6 Results of the body fat (red boxes) and muscle (blue boxes) extractions experiment of individuals of D. sericeus collected at two locations: Santo Amaro da Imperatriz (200 m a.s.l.) and Rancho Queimado (800 m a.s.l.) in Santa Catarina state, south of Brazil.

2.4 DISCUSSION

Our results show that geographically separated dung beetle populations present different body sizes and physiological condition. The environmental temperature can act in an intraspecific level, so that species with a complex development such as the holometabolous may respond differently to the temperature accordingly to ontogenetic stage (Dahlgaard & Loeschcke, 1997; Folguera et al., 2010). The developmental rate of insects is positively affected by the temperature (Gillooly et al., 2002; Jarosik et al., 2002; Jarosik et al., 2004) and this can result in different body sizes within the same species, as we have shown. In that regard, evidencing distinct responses of populations when confronted to distinct environmental conditions, indicating that immature stages of insects may have influenced where to invest, in terms of physiological traits, depending on the conditions during their development (McNamara & Houston, 1996; Rombough, 2003; Folguera et al., 2010).

The body size of *C. rutilans cyanescens* varies with temperature, with larger individuals being found at periods and sites of higher temperatures. Furthermore, this species accumulates more fat when it lives in colder places. *D. sericeus* also presents differences between separated populations and are bigger in the warmer months, however, the body size is not correlated to the environment temperature and the proportions of muscles and body fat are not different between populations. Given that our study species present different habits and colors, it was expected to observe different responses to the environmental temperatures (Basset & Springate, 1992; Springate & Basset, 1996). Larger and black insects are predicted to be found at night (Hernández, 2002), once they are less visible to predators and the cool temperatures will not affect them due to their smaller surface area to volume ratio maintaining the internal heat, while smaller-body insects will be found during day-time, once they will heat up faster due to their larger surface area to volume ratio (Lima & Dill, 1990; Guevara & Avilés, 2013). These species presents distinc behavior activity patterns and body sizes: the diurnal roller species *C. rutilans cyanescens* has a blue iridescent color and an average body length of 0.9 cm long while the nocturnal tuneller species *D. sericeus* is black and present an average body length of 1,2 cm (Silva et al. 2010; Vaz-de-Mello et al., 2014; Valois et al. 2017; Hensen et al., 2018). The smaller body sizes of *C. rutilans cyanescens* and it's thinner exoskeleton exposed to sunlight during the day allow greater effectiveness in behaviors promoting heating, such as perching, in which the individuals sit in a leaf during day time for thermoregulation. Also an energy reservoir may be necessary so the insect can be active in days without sun, so they accumulate more fat in colder sites (Young, 1984; Davis, 1999; Feer, 2016; Noriega & Vulinec, 2020). *D. sericeus*, being a nocturnal and bigger species, is used to be active in colder temperatures, so that passive heating maintenance is expected in this species, regardeless the sites that the inhabit (Verdú & Lobo, 2008; Verdú et al., 2012; Amore et al., 2017; Gallego et al., 2018). In this sense, the results support and coincide with the thermal melanism hypothesis.

Fat levels are a directly measurement of body condition in insects, while body size is an indirect measurement, as it also depends on the environmental characteristics where the individuals are found. Studies demonstrated that insects tend to increase their fat levels when in stressful conditions, including anthropogenically disturbed scenarios (Contreras-Garduño et al., 2008; Moya-Laraño et al., 2008; González-Tokman et al., 2011; González-Tokman & Martínez-Garza, 2015; França et al., 2016). For one species evaluated in this study, we found that the populations with smaller body sizes present more fat proportion, both being indicators of stress, which shows that the allocation of resources to fat is different between populations without affecting the allocation of resources to the muscular structure of the animal (Salomão et al., 2018, 2019). In this context, we found out that the body conditions of dung beetles species can be different in populations even if the environmental conditions are similar and according to the seasons. It is important that future studies focus on temperature itself or other isolated factors as explanatory variable for the changes in morphology and physiology of insects for these variations to be elucidated, eliminating other natural variables that may also influence the developmental conditions of the beetles.

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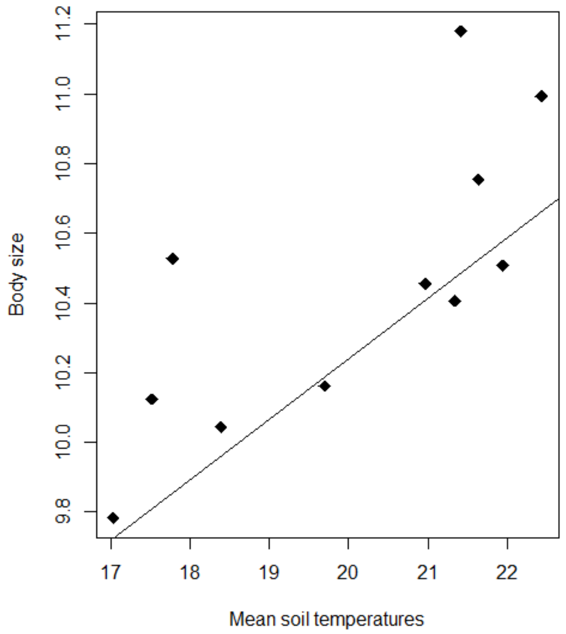
Supplementary material

Supplementary Table 1 Posteriori Tukey test of dry weight of individuals of the species *Canthon rutilans cyanescens* collected in four different locations (Anhatomirim, Itapema, Peri and Ratones), in two years (2012 and 2013), in Santa Catarina state, South of Brazil.

	Ratones 2013	**Ratones 2012**	**Peri 2013**	**Peri 2012**	**Itapema 2013**	**Itapema 2012**	**Anhat. 2013**	**Anhat. 2012**
Anhat. 2012	p=0.45	p=0.91	$p<0.05$ ***	$p<0.05$ ***	$p<0.05$ ***	p=0.06	p=0.38	-
Anhat. 2013	$p<0.05$ ***	$p<0.05$ ***	$p<0.05$ ***	$p<0.05$ ***	$p<0.05$ ***	$p<0.05$ ***	-	-
Itapema 2012	p=0.99	p=0.61	p=0.99	p=0.08	p=0.99	-	-	-
Itapema 2013	p=0.87	p=0.09	p=0.99	p=0.14	-	-	-	-
Peri 2012	$p<0.05$ ***	$p<0.05$ ***	p=0.12	-	-	-	-	-
Peri 2013	p=0.18	p=0.18	-	-	-	-	-	-
Ratones 2012	p=0.98	-	-	-	-	-	-	-
Ratones 2013	-	-	-	-	-	-	-	-



Supplementary Figure 1 Positive correlation between mean soil temperatures and body lenght of the species *Canthon rutilans cyanescens* collected at the site of 200 m a.s.l. in the years of 2015 and 2016.

Supplementary Table 2 Posteriori Tukey test of body dry weight of individuals of the species *Dichotomius sericeus* collected in four different locations (Anhatomirim, Itapema, Peri and Ratones), in two years (2012 and 2013), in Santa Catarina state, South of Brazil.

	Ratones 2013	**Ratones 2012**	**Peri 2012**	**Peri 2013**	**Itapema 2013**	**Itapema 2012**	**Anhat. 2013**	**Anhat. 2012**
Anhat. 2012	p<0.05 ***	p<0.05 ***	p<0.05 ***	p<0.05 ***	p=0.10	p<0.05 ***	p=1.0	-
Anhat. 2013	p<0.05 ***	p<0.05 ***	p<0.05 ***	p<0.05 ***	p=0.13	p<0.05 ***	-	-
Itapema 2012	p<0.05 ***	p=0.99	p=0.98	p=0.99	p=0.81	-	-	-
Itapema 2013	p<0.05 ***	p=0.35	p=0.96	p=0.47	-	-	-	-
Peri 2012	p<0.05 ***	p=1.0	p=0.82	-	-	-	-	-
Peri 2013	p<0.05 ***	p=0.58	-	-	-	-	-	-
Ratones 2012	p<0.05 ***	-	-	-	-	-	-	-
Ratones 2013	-	-	-	-	-	-	-	-

3 CONCLUSÕES GERAIS

Neste estudo foi possível observar que populações geograficamente separadas de duas espécies de besouros escarabeíneos apresentam diferenças no tamanho corporal, apresentando maiores tamanho corporais em áreas e períodos de maior temperatura ambiental. Uma das espécies estudadas, *Canthon rutilans cyanescens*, teve seu tamanho corporal relacionado positivamente com a temperatura média do solo ao longo do ano. Além disso, embora menores em tamanho, a população de um local de maior altitude e menor temperatura apresentou uma maior porcentagem de gordura corporal, o que se entende como uma adaptação evolutiva às condições ambientais, sendo esta espécie diurna fortemente afetada pela temperatura. A outra espécie, *Dichotomius sericeus,* não teve o tamanho corporal correlacionado com a temperatura ao longo do ano, nem maior acúmulo de gordura em locais mais frios, indicando uma adaptação evolutiva distinta às condições ambientais. Tais resultados se explicam pela diferença de hábitos entre ambas espécies. *C. rutilans cyanescens* tem comportamento diuno e coloração azul iridescente, de modo que seu aquecimento é favorecido com a radiação solar através do seu comportamento, ao ficar pousado sobre as folhas ("perching"); em situações de menor temperatura, quando este comportamento não é suficiente, o acúmulo de gordura seria a alternativa para que os indivíduos consigam iniciar e manter sua atividade. Já *Dichotomius sericeus,* é uma espécie noturna, de coloração preta, estando adaptada para aquecer-se sem influencia da incidência direta dos raios solares , o que permite sua atividade no período da noite, sendo menos influenciada pelas variações ambientais de temperatura.

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APÊNDICE A – Peso médio e quantidade de indivíduos das espécies *Canthon rutilans cyanescens* e *Dichotomius sericeus* coletados nos anos de 2012 e 2013, em 4 localidades no estado de Santa Catarina, no sul do Brasil.

Tabela 1 Peso médio, desvio padrão e quantidade de indivíduos das espécies *Canthon rutilans cyanescens* e *Dichotomius sericeus* coletados nos anos de 2012 e 2013, em 4 localidades no estado de Santa Catarina, no sul do Brasil.

Espécie	Ano	Local	Quantidade	Peso médio e d.p. (mg)
Canthon rutilans cyanescens	2012	Peri	146	0.0574 ± 0.0177
		Ratones	159	0.0438 ± 0.0177
		Itapema	134	0.0443 ± 0.0168
		Anhatomirim	139	0.0409 ± 0.0189
	2013	Peri	216	0.0489 ± 0.0202
		Ratones	98	0.0462 ± 0.0211
		Itapema	268	0.0492 ± 0.0188
		Anhatomirim	158	0.0361 ± 0.0180
Dichotomius sericeus	2012	Peri	21	0.1452 ± 0.0373
		Ratones	744	0.1453 ± 0.0381
		Itapema	33	0.1428 ± 0.0325
		Anhatomirim	194	0.1729 ± 0.0413
	2013	Peri	264	0.1508 ± 0.0455
		Ratones	410	0.1888 ± 0.0468
		Itapema	70	0.1568 ± 0.0374
		Anhatomirim	185	0.1725 ± 0.0477

APÊNDICE B – Quantidade de indivíduos medidos da Coleção Entomológica Mitia Heusi Silveira, do Centro de Ciências Biológicas - UFSC

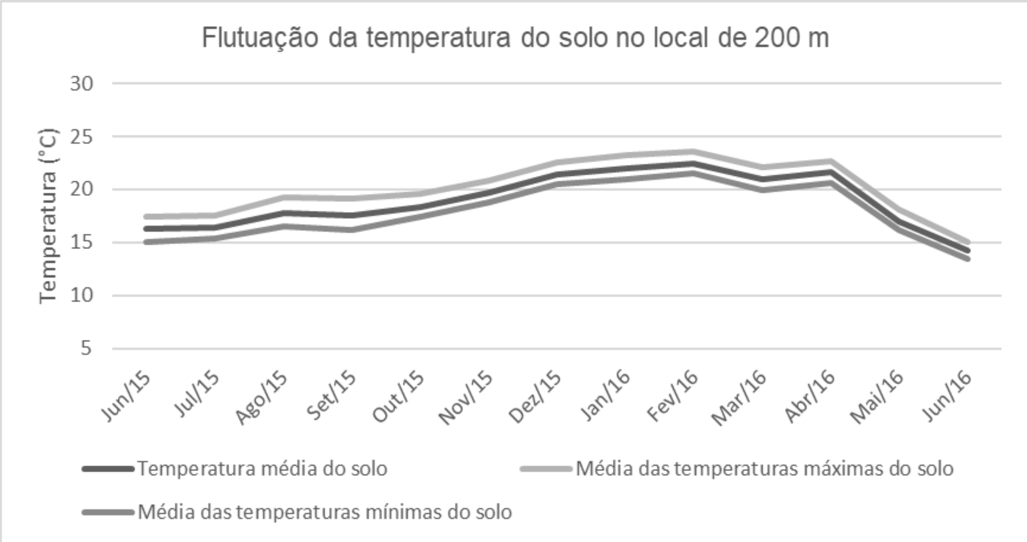
Tabela 2 Quantidade de indivíduos medidos da espécie *Dichotomius sericeus*, coletados em áreas de 200 m de altitude (Santo Amaro da Imperatriz – SC) e 800 m de altitude (Rancho Queimado – SC), entre novembro de 2015 e abril de 2016.

	200 m	**800 m**
Nov/2015	10	0
Dez/2015	56	8
Jan/2016	30	30
Fev/2016	10	29
Mar/2016	10	5
Abr/2016	0	9

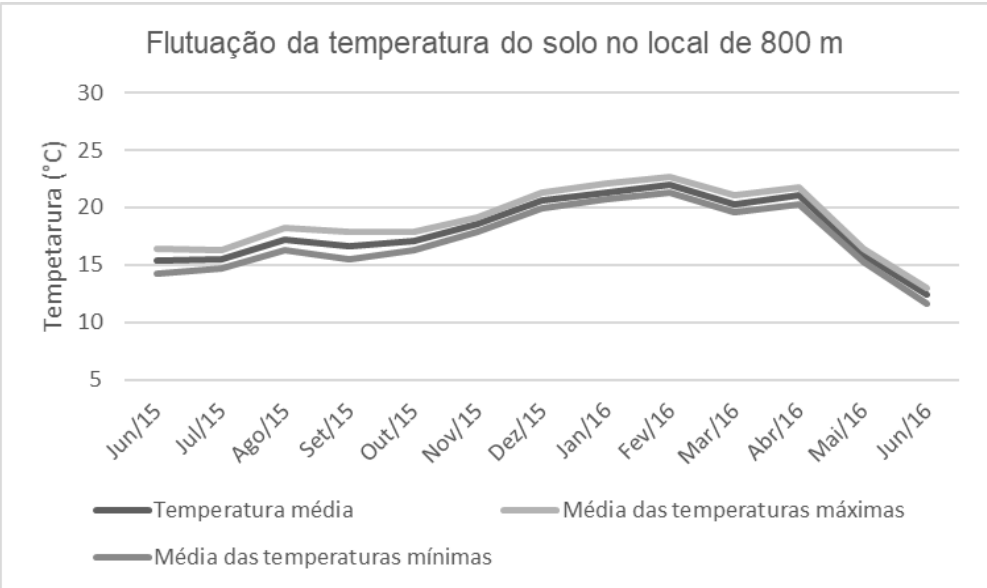
Tabela 3 Quantidade de indivíduos medidos da espécie *Canthon rutilans cyanescens* coletados em áreas de 200 m de altitude (Santo Amaro da Imperatriz – SC) e 800 m de altitude (Rancho Queimado – SC), entre agosto de 2015 e maio de 2016.

	200 m	**800 m**
Ago/2015	17	0
Set/2015	59	0
Out/2015	36	0
Nov/2015	43	0
Dez/2015	110	1
Jan/2016	32	7
Fev/2016	31	0
Mar/2016	60	0
Abr/2016	85	0
Mai/2016	3	0

APÊNDICE C – Temperaturas do solo registradas nos locais de estudo.



Apêndice Figura 1 Flutuação das temperaturas do solo registradas entre junho de 2015 e junho de 2016, em Santo Amaro da Imperatriz – SC. Foram observadas a média das temperaturas, a média das temperaturas máximas e média das temperaturas mínimas.



Apêndice Figura 2 Flutuação das temperaturas do solo registradas entre junho de 2015 e junho de 2016, em Rancho Queimado – SC. Foram observadas a média das temperaturas, a média das temperaturas máximas e média das temperaturas mínimas.