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ELEVATIONAL SPECIALIZATION AND THE MONITORING OF THE EFFECTS OF CLIMATE CHANGE IN INSECTS: BEETLES IN A BRAZILIAN RAINFOREST MOUNTAIN

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Abstract

Mountains have provided important insights on the impacts of climate change on species distribution. Organisms from tropical mountains are expected to be specialized to certain temperature limits (demonstrating low thermal tolerance), often with narrow elevational distributions relative to temperate species, and may shift their elevational range in response to climate change. Importantly, insects are sensitive, and respond rapidly, to temperature variation, making them suitable bioindicators to monitor the effects of climate change. However, to monitor the effects of climate change in mountains it is important to understand present elevational distribution and other ecological characteristics of local insect populations. In this context, we suggest a method to identify beetle taxa that can be used to monitor climate change effects in tropical mountainous insect species. We illustrate the method by describing the elevational distribution of different beetle groups, associating

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this distribution with species' thermal distribution in a tropical mountain forest in Southeast Brazil. Sampling was conducted at Serra dos Órgãos National Park, RJ, Brazil, in the Atlantic Rainforest, one of the main global biodiversity hotspots. In order to systematically sample beetle diversity across elevations, we used flight interception 'Malaise' traps at fifteen different sites, from 130 m to 2170 m a.s.l., over three consecutive months during the rainy season. To investigate species' climatic niches, we recorded climatic variables for this period. We collected 2963 individuals of 272 species, belonging to six Coleoptera groups over a temperature gradient that decreased about 0.5 °C for each 100 m in elevation. Considering the thermal tolerance of species from tropical mountains and their narrow elevational range and abundance, five Coleoptera species belonging to Cerambycidae, Eumolpinae (Chrysomelidae), Lampyridae and Phengodidae were considered suitable bioindicators, and the Eumolpinae and Lampyridae were the ones with the narrowest elevational range. We suggest that the use of abundant species or groups with narrow elevational range as bioindicators can be valuable to monitor the effects of climate change on the biota, allowing us to evaluate how species are responding to changes over time.

Keywords: climate change; elevational gradients; range shifts; thermal tolerance.

1 Introduction

The effect of climate change on living organisms is one of the most important current issues in ecology and such effects are widespread and often pernicious (*e.g.* Hughes, 2000; Walther *et al.*, 2002; Root *et al.*, 2003; Menéndez, 2007; Wilson *et al.*, 2007; Bellard *et al.*, 2012; Grimm *et al.*, 2013; Menéndez *et al.*, 2014; Sheldon, 2019). These and other studies have shown that climate can affect the structure and dynamics of ecosystems, biodiversity and the composition of biological communities, species' distribution, range and abundance, phenology and interactions with other organisms, both directly and indirectly.

Mountain environments provide vivid insights into the impact of climate change on species' distributions, since these environments can enclose both the lower and upper

distributional limits of many of the species inhabiting them (Menéndez *et al.*, 2014). Given the wide variation of environmental parameters observed along mountains, such as temperature and precipitation, this can greatly affect the occurrence, abundance and distribution of organisms along elevational gradients (Hodkinson, 2005; Hodkinson and Jackson, 2005; Martinelli, 2007). Tropical mountains are considered hotspots of biodiversity (Mittermeier *et al*., 2011), with many endemic species, strongly threatened by environmental changes in developing countries such as Brazil (Martinelli, 2007). Montane organisms are potentially more vulnerable to temperature changes than those in some other environments, as they show low physiological tolerance to temperature variations (Janzen, 1967; García-Robledo *et al*., 2016) and enhanced warming rates are expected with increasing elevation because of elevation-dependent warming (Pepin *et al.*, 2015). Mechanisms contributing to these differential warming rates include changes in albedo, clouds and cloud properties, water vapour and radiative fluxes, aerosols and different combinations of these mechanisms.

It is widely reported that species may shift range distribution in elevation due to climate change, moving up in elevation according to their physiological tolerances to temperature (*e.g.* Parmesan, 1996; Root *et al.*, 2003; Menendez, 2007; Menendez *et al.*, 2014; Sheldon, 2019). Furthermore, it is likely that organisms from rainforest mountains show narrow thermal tolerance (being specialized to certain temperature limits), especially those from higher elevations (but see Sunday *et al.*, 2019) and, consequently, present narrow elevational range distribution, which makes them particularly vulnerable to environmental changes (Laurance *et al.*, 2011; García-Robledo *et al.*, 2016; Macedo *et al.*, 2018). The situation is even worse on mountaintops, since these environments present smaller areas, with many endemic species and with little possibility of expanding their distribution (García-Robledo *et al.*, 2016; Macedo *et al.*, 2016). However, for the majority of insect groups, which comprise a large fraction of global biodiversity, little is known about species' elevational range in tropical mountains and how climate change could affect the distribution of these organisms.

Ecological indicators are frequently used to monitor the effects of environmental change on biotic systems (McGeoch, 1998). Climate change is affecting ecological processes and species' biology and ecology in different ways. This makes the selection of bioindicators a fundamental task to monitor these effects, although it is very difficult to select appropriate species. In this sense, some studies have proposed species selection criteria, such as that conducted by Groot *et al.* (1995) and that reviewed by McGeoch (1998). Abundance and ready identification of species are criteria common to any bioindicator considered in the literature (McGeoch, 1998).

Bioindicators are "species, or group of species, that readily reflects the biotic or abiotic state of an environment; that represents the impacts of environmental changes in a habitat, community or ecosystem; or indicates the diversity of a set of taxa or the entire diversity of an area" (McGeoch, 1998). These organisms should play an important role in monitoring the effects of environmental changes in mountains, enabling biodiversity management and conservation actions.

Insects are highly suitable as bioindicators because of their high sensitivity and rapid response to anthropogenic disturbances, allowing scientists to understand how organisms respond to changes in both biotic and abiotic factors (Brown, 1996; McGeoch, 1998; Hughes, 2000; Hodkinson and Jackson, 2005; Menéndez, 2007; Uehara-Prado *et al.*, 2009; Menéndez *et al.*, 2014). They have also been reported as good bioindicators of climate change (Menéndez, 2007; Gerlach *et al.*, 2013), since climate directly influences their survival, reproduction and development (Bale *et al.*, 2002). Insects generally have short life cycles compared to many other living organisms and play important roles in ecosystem function, having representatives at many different trophic levels. However, to monitor the effects of climate change in mountains it is important to understand present elevational distribution and other ecological characteristics of local insect populations. Among insects, beetles (Coleoptera) stand out as the most diverse known living organisms, with 300,000 to 450,000 existing species (Nielsen and Mound, 1999; Bouchard *et al.*, 2017), being easily sampled using different techniques, and have already been suggested as

bioindicators in other contexts (Rainio and Niemelä, 2003; Hodkinson and Jackson, 2005; Gerlach *et al.*, 2013; Oliveira *et al*., 2014).

The aim of the present study is to suggest a method to identify Coleoptera taxa that can be used to monitor climate change effects in tropical mountainous insect species. We illustrate the method by describing the elevational distribution of different beetle groups, associating this distribution with species' thermal distribution in a tropical mountain forest in Southeast Brazil.

2 **Methods**

2.2 Study area

The study was conducted at Serra dos Órgãos National Park (22°32'S; 43°07'W) (Fig. A.1), a protected area forming part of the Serra do Mar mountain range in southeastern Brazil, Rio de Janeiro State. The Park covers an area of 20024 ha of Atlantic Rainforest vegetation from 80 m up to 2263 m a.s.l. (Castro, 2008) and is the third oldest National Park in Brazil, founded in 1939, and aims to conserve biodiversity in the Serra do Mar area and especially the high elevation grasslands ('Campos de Altitude'), containing many endemic species (Vasconcelos, 2011). The mountains are characterized by decreasing temperature with increasing elevation, though the rate of decrease has never been precisely quantified in this region. There is a season of higher precipitation and temperatures (the rainy season), from October to March, and relatively drier and cooler season (the dry season), from April to September (Castro, 2008).

The Park encloses four different vegetation belts: lower montane forest (below ~800 m), montane forest (from ~800 to ~1500 m), upper montane forest (from ~1500 to ~2000 m) and high elevation grasslands (above ~2000) (see Rizzini, 1954 for flora identification; Veloso *et al.*, 1991 for vegetation classification and; see also Macedo *et al.*, 2018 for more details on the study location and vegetation).

The Atlantic Rainforest biome has been designated one of the most important global biodiversity hotspots (Myers *et al.*, 2000; Mittermeier *et al.*, 2011). This biome shows a great diversity of animal and plant species, many of these being endemic (Ribeiro *et al.*, 2009). Despite this, the Atlantic Rainforest biome has been strongly affected by anthropogenic activities, especially land use change, which not only generates large amounts of greenhouse gases, but also affects biodiversity and soil composition. Only about 11.73% of the original biome vegetation remains at present (Ribeiro *et al.*, 2009; Scarano and Ceotto, 2015) and due to the strong change suffered by the biome in recent and past decades, hundreds of animal and plant species are now at risk of extinction (Scarano and Ceotto, 2015). This pattern of vulnerability is general to many other biodiversity hotspots, underlining the importance of monitoring the biotic responses in these environments to future change (Bellard *et al.*, 2014).

2.2 Sampling

The sampling was conducted using 30 'Malaise' flight interception traps (Fig. A.2), which have already been reported as one of the most efficient methods for sampling Coleoptera (Hosking, 1979; Ganho and Marinoni, 2003; Skvarla and Dowling, 2017), installed at ground level with the collecting head at a height of 1.5 meters above the ground. Trap sites followed along the road from Guapimirim (130 m a.s.l.) up to Teresópolis (880 m), and from Serra dos Órgãos National Park entrance (940 m) at Teresópolis up to 2170 m along Pedra do Sino trail (Macedo *et al.*, 2018), very close to the maximum elevation in the region, at 2263 m. Sites below 1250 m elevation are more under the influence of human activity and the road from Guapimirim up to Teresópolis is paved and with high traffic.

Malaise traps were placed in replicated pairs at least 50 m from the road at along the elevational gradient in 15 sites, from 130 m up to 2170 m (Table A.1), roughly spaced at 100 to 200 m elevation intervals. In each elevational site, the two traps were placed at least 50 m apart from each other to ensure that neither trap affected the catch of the other and to sample sufficiently different environmental space. Trap collecting bottles (1-litre capacity)

contained 98° alcohol for preservation of the sampled material and were replaced monthly (30 days of collecting). The samples were collected during the rainy season, from December 2014 to February 2015, totaling 90 samples. The rainy season was used because beetles are more active and abundant, being sampled in greater quantity (even at high elevations) in this season than during the dry season, when it would be too cold for some species (*e.g.* Bouzan *et al.*, 2015; Flinte *et al.*, 2015; but see Silveira and Mermudes, 2017). Four collecting bottles were lost during the sampling, as follows: one from 2170 m in December 2014, one from 550 m and 1680 m in January 2015 and one from 2170 m in February 2015. For these lost samples, no estimation was made.

In order to investigate species' climatic niches, temperature (ºC) data was collected every hour for all sampling elevations using Data Loggers (MicroLite II USB Temperature Data Logger, Fourtec - Fourier Technologies Ltd.) for December 2014, January 2015 and February 2015. From these data we obtained the mean, maximum and minimum daily temperature, from which we calculated the monthly means and the rainy season means temperature for each elevational site.

2.3 Sorting and identification

Insects were preserved in 98% alcohol and stored in plastic bottles. Sample sorting was performed in the laboratory using a stereoscopic microscope. All Coleoptera were sorted and six groups (families or subfamilies, identified using taxonomic keys) of beetles, belonging to different trophic groups, were counted and identified, when possible, to species level. The trophic groups were represented by predators (Carabidae, Lampyridae and Phengodidae), herbivores (Cerambycidae and Eumolpinae) and fungivores (Anthribidae). As we do not know *a priori* which taxonomic groups might be more suitable as bioindicators, we chose these beetle groups because species in different trophic positions in the ecosystem can respond differently to environmental changes (Voight *et al.*, 2003) and show different degrees of elevational specialization (Macedo *et al.*, 2018).

Expert taxonomists of the related groups identified the different taxonomic groups, being them: José Ricardo Miras Mermudes and André Silva Roza – Instituto de Biologia –

Universidade Federal do Rio de Janeiro (UFRJ) – Brazil (Anthribidae, Carabidae and Phengodidae); Luiz Felipe Lima da Silveira – Western Carolina University – United States of America (Lampyridae); and Marcela Laura Monné Freire – Museu Nacional do Rio de Janeiro – UFRJ – Brazil (Cerambycidae). The Eumolpinae were sorted into morphospecies and were later checked by Jéssica Herzog Viana (Universidade do Estado do Pará - UEPA - Brazil). Most of the specimens were identified to genus and then to morphospecies, nonetheless all of them will be referred to as species.

The use of morphospecies (*i.e.* individuals sorted based on phenotypic characters) as surrogates for species is widely discussed, as well as its use in the estimation of species richness for comparisons over time and space (Oliver and Beattie, 1996; Derraik, 2002). Although morphospecification can lead to the split of a single species into many different morphospecies ('splitting') or aggregation of different species into a single one ('lumping'), it is often the only way to assess species diversity in groups with poor taxonomic standards (Oliver and Beattie, 1996; Derraik, 2002).

The sampled material is deposited at the following Brazilian entomological collections (acronyms shown before collection names): Anthribidae, Carabidae and Phengodidae specimens – DZRJ – Coleção Entomológica Professor José Alfredo Pinheiro Dutra, Universidade Federal do Rio de Janeiro; Eumolpinae and Lampyridae specimens – CLEI – Coleção do Laboratório de Ecologia de Insetos, Universidade Federal do Rio de Janeiro; and Cerambycidae specimens – MNRJ – Coleção Entomológica, Museu Nacional, Universidade Federal do Rio de Janeiro, state of Rio de Janeiro, Brazil.

2.4 Data analyses

2.4.1 Bioindicators selection criteria

McGeoch (1998) has pointed out that many authors have discussed biological and practical criteria for the selection of bioindicators. In this work, the selection of potential bioindicator species considered a main criterion, based on species' biology and ecology, combining elevation range and abundance. Tropical species are expected to be more

sensitive to temperature variations, with species living in conditions very tight to their temperature tolerance (Janzen, 1967; Deutsch *et al.*, 2008). In this sense, the most specialized species in terms of elevational range are supposed to present narrower thermal tolerances than the species with broader elevational ranges, which means that they would probably respond faster to temperature variations (Laurance *et al.*, 2011; García-Robledo *et al.*, 2016; Macedo *et al.*, 2018). In this context, for selection purposes, species elevational range should be narrow. Considering the distances between the 15 sample sites, 610 m elevation range guarantees a minimum sampling of four sites, which is important to enable the monitoring of elevation distribution variation of species over time and to reduce the effects of interannual natural distribution variations. Furthermore, species with elevational range broader than 610 m, in theory, are subject to a greater temperature variation (>3 °C), among other factors, and thus would be less responsive to potential environmental changes, reducing their potential as bioindicators. In addition, species abundance should be simultaneously considered, as the higher the abundance, the higher the probability of species resampling, leaving out those species which may be difficult to monitor. Many studies suggest the use of species abundance as a criterion for selecting bioindicators (*e.g.* McGeoch, 1998; Uehara-Prado *et al.*, 2009). However, no specific number of individuals is suggested and the studies usually refer to 'adequate', 'abundant' or any other subjective term. In this study, species were considered suitable if their total abundance was at least 25 individuals for those with up to 610 m elevation range, or at least 15 individuals if sampled in a single elevation.

For the species selected as potential bioindicators considering the combination of elevational range and abundance, we used an additional criterion to select the most suitable indicator species, being that: Species recognition should be easy, as it allows bioindicator species to be readily recognized by the monitoring team (Groot *et al.*, 1995). So, larger species and/or with easily distinguishable traits, when compared with other species of the same group sampled in this region, were given priority.

2.4.2 Composition, abundance, species richness and sampling completeness

The number of individuals was counted for all the species within the six beetle groups, and the total abundance considered across the three sampling months. To assess species richness along the elevational gradient, species were considered present at all elevation sites between its lowest and highest sites of occurrence (interpolation), as in Grytnes and Veetas (2002) and Almeida-Neto *et al.* (2006).

In order to assess sampling completeness of each beetle group (Anthribidae, Carabidae, Cerambycidae, Eumolpinae, Lampyridae and Phengodidae), we have plotted, using individual-based (abundance) data, "Sample-size-based" and "Coverage-based" rarefaction and extrapolation sampling curves (q = 0), along with the "Sample Completeness" curve, all considering 99% confidence intervals for each of the six studied beetle groups. The analysis were performed using the package 'iNEXT' (Hsieh *et al*., 2016) of the R program, Version 4.0.0 (2020-04-24, Arbor Day).

2.4.3 Species elevation range

Elevation range was calculated as the highest minus the lowest elevations where each species was collected. Similarly, each species temperature range was obtained as the difference between the mean temperature of the lowest and the highest elevations it was collected. Using species' elevation range data we could assess the degree of specialization of different species on the mountain in order to calculate the mean elevational range for each beetle group and to suggest the taxa with the narrowest elevational ranges as those most suitable for monitoring environmental changes. To assess differences on mean elevational range across taxonomic groups, we used an analysis of variance (ANOVA) and further Tukey's *post hoc* analysis to identify pairs that significantly differ in mean elevational distribution. We considered only species with abundance higher than three and the dependent variable was squared-root to meet normality and homoscedasticity.

3 Results

3.1 Temperature

During the rainy season, mean, maximum and minimum temperature reduced about 0.5 ºC each 100 m in elevation (Fig. 1) at Serra dos Órgãos National Park. Mean temperature reduced 0.47 ºC / 100 m, maximum temperature reduced 0.41 ºC / 100 m and minimum temperature 0.51 ºC / 100 m, but all three variables had highly significant linear regression coefficients ($R^2 > 0.96$; $P < 0.0001$; $n = 15$).

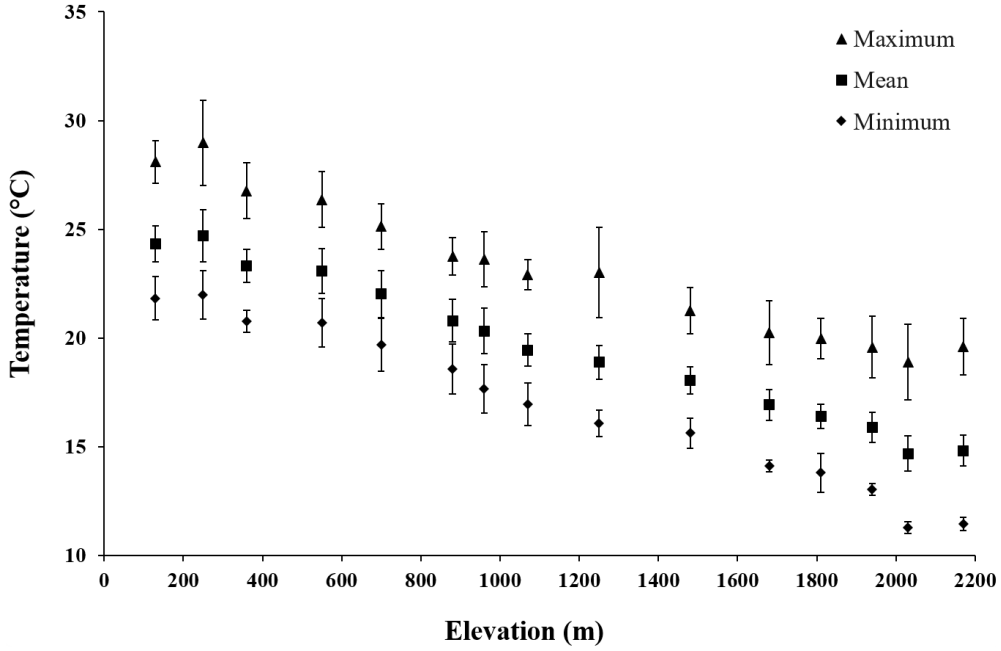

Figure 1. Average maximum, mean and minimum temperature (with standard deviation bars) for the rainy season (December / 2014 and January and February / 2015) along the sampled elevation gradient at Serra dos Órgãos National Park, Rio de Janeiro, Brazil.

3.2 Composition, abundance, species richness and sampling completeness

A total of 2,963 beetles, belonging to the six groups were sampled along the entire study. From this total, 115 species were predators (41 species of Carabidae, 51 of Lampyridae and 23 of Phengodidae), 111 species were herbivores (48 species of Cerambycidae and 63 of Eumolpinae) and 46 species were fungivores (all Anthribidae) (Table 1; Table A.2). The Coleoptera sampled were largely represented by singletons and

doubletons (n = 126), corresponding to 46.32% of total species sampled. From those, fungivores represented the highest number of species with up to two individuals (Anthribidae – 56.52%), followed by herbivores (Total: 52.25%; Cerambycidae – 58.33%; Eumolpinae – 47.62%) and predators (Total: 36.52%; Carabidae – 41.46%; Lampyridae – 35.29%; Phengodidae – 30.43%).

Table 1. Total and relative abundance and number of species of the studied beetle groups, from 130 m up to 2170 m elevation, during the rainy season (December / 2014, January and February / 2015), at Serra dos Órgãos National Park.

Groups	**Abundance**	**% Abundance**	**Richness**	**% Richness**
Anthribidae	173	5.8	46	16.9
Carabidae	406	13.7	41	15.1
Cerambycidae	256	8.6	48	17.6
Eumolpinae	912	30.8	63	23.2
Lampyridae	597	20.1	51	18.7
Phengodidae	619	20.9	23	8.5
Total	2963	100.0	272	100.0

The rarefaction curves of Lampyridae and Phengodidae seem to have nearly asymptoted (Fig. 2) and had their observed number of species within the confidence interval limits (p = 0.01; Table A.3). Furthermore, Carabidae and Eumolpinae had just one or two species below the lower confidence interval limit. Cerambycidae and Anthribidae, though having about three or four species below the lower confidence interval limit, cannot be considered poorly sampled.

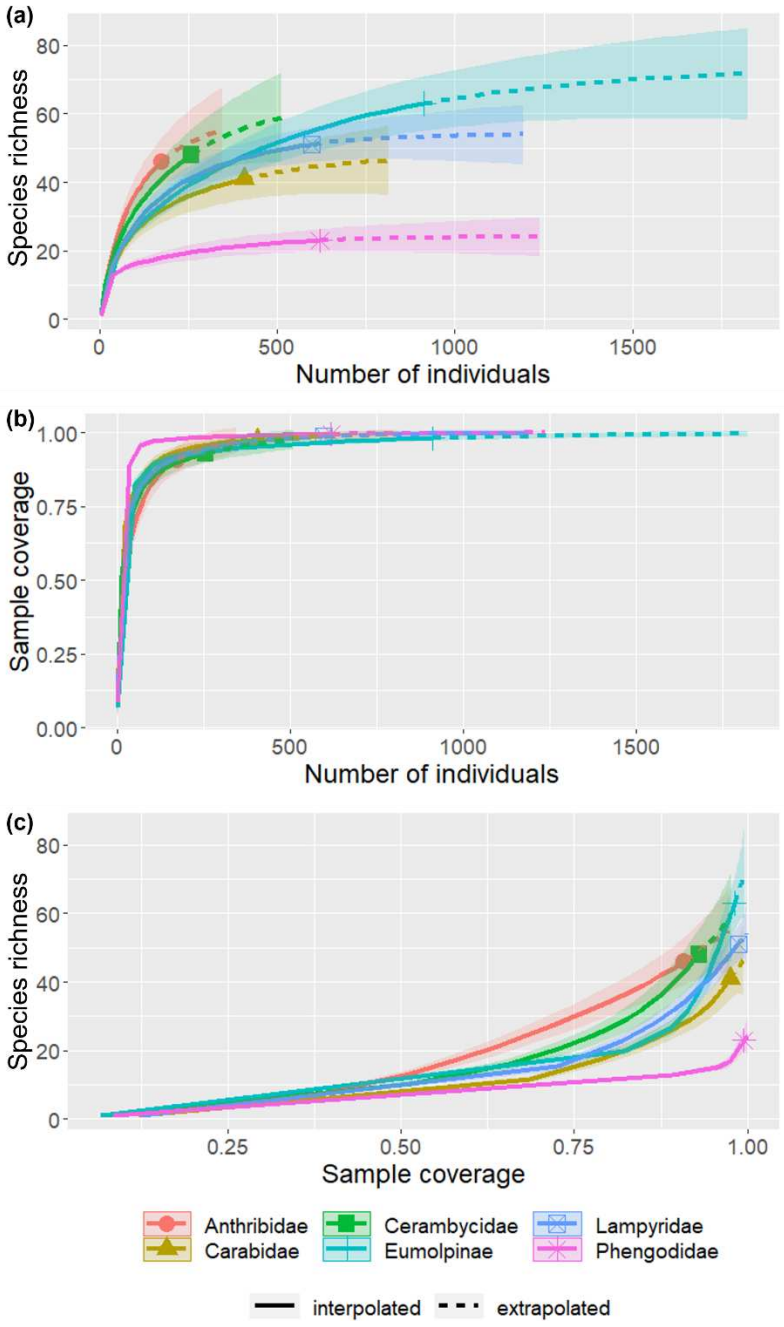

Figure 2. (a) Sample-size-based and (c) coverage-based rarefaction (solid line segment) and extrapolation (dotted line segments) sampling curves for species richness (q = 0) with 99% confidence intervals (shaded areas) for the six beetle groups (Anthribidae, Carabidae, Cerambycidae, Eumolpinae, Lampyridae and Phengodidae) considering all 15 studied elevation sites of the Serra dos Órgãos National Park, using individual-based abundance

data. The solid dots and the other four symbols represent the six beetle groups. (b) Sample completeness curves linking curves in (a) and (c).

3.3 Beetle elevational range

There were significant differences in the mean squared-root elevational range among the six studied beetle groups considering all sampled species with abundance ≥ 3 individuals (df = 5; F = 7.11; P < 0.001; Fig. 3). The groups with the narrowest mean elevational ranges were Eumolpinae (about 317 m) and Lampyridae (about 450 m). Anthribidae, Carabidae and Cerambycidae presented the broadest mean elevational ranges (~684 m, ~765 m and 835 m, respectively), while Phengodidae was the species group with intermediate mean elevational range (about 573 m).

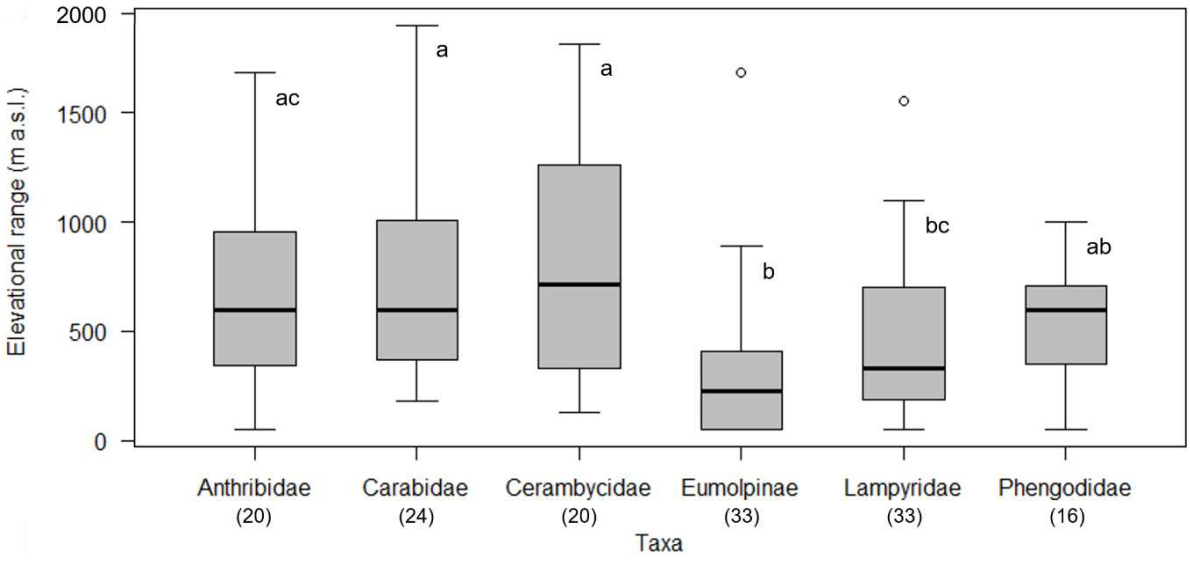

Figure 3. Box plot of median elevational range for each of the six beetle groups (number of species) considering the species with n ≥ 3 individuals, during the rainy season (December/2014, January and February/2015) at Serra dos Órgãos National Park, Rio de Janeiro, Brazil, collected from 130 m up to 2170 m a.s.l.. The top of the box represents the 75th percentile, the bottom of the box represents the 25th percentile, and the line in the middle represents the 50th percentile (median). The whiskers represents the highest (1.5 times interquartile range above 75th percentile) and lowest (1.5 times interquartile range below 25th percentile) values that are not outliers or extreme values. Circles beyond the

whiskers represent outliers and extreme values beyond either end of the box. Different letters above bars mean significant difference in mean elevational range between groups (Tukey's test; $P < 0.05$).

3.4 Potential bioindicators

From all the six studied Coleoptera groups, only Cerambycidae, Eumolpinae, Lampyridae and Phengodidae, had at least one species in accordance with the combined elevation range and abundance criterion (with abundance ≥ 15 if sampled in a single elevation or ≥ 25 with up to 610 m elevation range distribution). No Anthribidae or Carabidae species met either criteria (Table 2).

Table 2. Abundance, total number of species, number of species with abundance ≥ 15 (total and sampled in a single elevation), and number of species with abundance ≥ 25 (total and with up to 610 m elevation range distribution limit).

Groups	Abundance	Number of species				
		Total	Ab. ≥ 15		Ab. ≥ 25	
			Total	Single elevation	Total	up to 610 m
Anthribidae	173	46	2	0	1	0
Carabidae	406	41	5	0	3	0
Cerambycidae	256	48	4	0	2	1
Eumolpinae	912	63	15	1	13	8
Lampyridae	597	51	7	1	5	0
Phengodidae	619	23	13	0	11	4
Total	2963	272	46	2	35	13

Only one species of Cerambycidae, *Onocephala obliquata*, met this criterion, with a 460 m elevation range. Nine species of Eumolpinae met the combined elevation range and

abundance criterion. From those, one species had an elevation range of 520 m, seven species 230 m and one species was only found at a single elevation (Eumolpinae sp. 14). The only species of Lampyridae that met this criterion was *Photuris elliptica*. This firefly species was only found at a single elevation, with an abundance of 15 individuals. Four species of Phengodidae met the combined elevation range and abundance main criterion, with an abundance of 40 individuals or more and with elevation range distribution of up to 610 m, namely *Stenophrixothrix* sp. 4, *Stenophrixothrix* sp. 2, *Howdenia* sp. 4 and *Taximastinocerus* sp. 1 (Fig. 4; Table 2).

Therefore, in total, two species with abundance ≥ 15, sampled in a single elevation, and 13 species with abundance ≥ 25 with up to 610 m elevation range distribution met the main selection criterion, which combines elevation range and abundance (Table 2), and thus were selected as potential bioindicators. It is important to emphasize here that from those 15 species, none of them presented a temperature range higher than 2.58 °C (Table 3). Those species were then evaluated concerning their ease of recognition for the final selection.

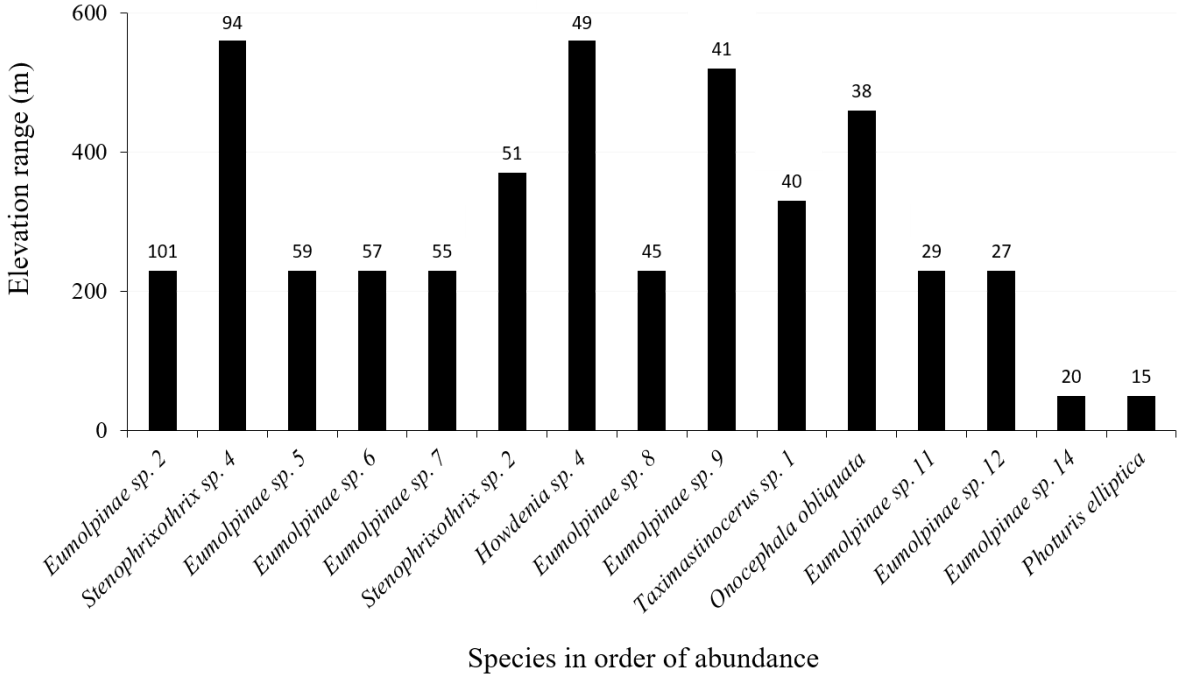

Figure 4. Elevational range distribution of the 15 potential bioindicator species, in abundance order, at Serra dos Órgãos National Park, state of Rio de Janeiro, Brazil. Species' abundance are shown above each bar.

Considering the additional criterion of ease of recognition for the 15 pre-selected species, five of them would be suitable as bioindicators (Table 3). For these species, we present their pictures in different views along with the description of the main traits used to recognize them (Appendix B). From those, three species were herbivores: two Eumolpinae species (Chrysomelidae - Figs. B.1 and B.2) and *Onocephala obliquata* (Cerambycidae - Fig. B.3), and two species were predators: *Photuris elliptica* (Lampyridae - Fig. B.4) and *Howdenia* sp. 4 (Phengodidae - Fig. B.5).

Table 3. Fifteen species selected as potential bioindicators, at Serra dos Órgãos National Park, state of Rio de Janeiro, Brazil. Information on their abundance, elevational range, size, ease of recognition and temperature range are provided. The first five species (in bold) are the suggested bioindicators.

Species	Abundance	Elevational range (m)	Mean Size* (mm)	Ease of recognition	Temperature range (°C)
***Howdenia* sp. 4**	**49**	**560** (1250 m to 1810 m)	**5.11**	**Easy to recognize**	**2.5**
Onocephala obliquata	**38**	**460** (1480 m to 1940 m)	**16.39**	**Easy to recognize**	**2.15**
Eumolpinae sp. 11	**29**	**230** (1940 m to 2170 m)	**4.37**	**Easy to recognize**	**1.07**
Eumolpinae sp. 12	**27**	**230** (1940 m to 2170 m)	**5.03**	**Easy to recognize**	**1.07**
Photuris elliptica	**15**	**50** (sampled only in 1250 m)	**13.39**	**Easy to recognize**	**0.5****
Eumolpinae sp. 2	101	**230** (1940 m to 2170 m)	2.49	Difficult to recognize	1.07
Stenophrixothrix sp. 4	94	**560** (1250 m to 1810 m)	5.94	Difficult to recognize	2.5
Eumolpinae sp. 5	59	**230** (1940 m to 2170 m)	2.47	Difficult to recognize	1.07

Eumolpinae sp. 6	57	**230** (1250 m to 1480 m)	2.76	Difficult to recognize	0.83
Eumolpinae sp. 7	55	**230** (1940 m to 2170 m)	2.96	Difficult to recognize	1.07
Stenophrixothrix sp. 2	51	**370** (700 m to 1070 m)	6.48	Difficult to recognize	2.58
Eumolpinae sp. 8	45	**230** (1940 m to 2170 m)	3.04	Difficult to recognize	1.07
Eumolpinae sp. 9	41	**520** (360 m to 880 m)	5.32	Difficult to recognize	2.52
Taximastinocerus sp. 1	40	**330** (550 m to 880 m)	4.75	Difficult to recognize	2.29
Eumolpinae sp. 14	20	**50** (sampled only in 1250 m)	2.77	Difficult to recognize	0.5**

* Mean size considering 5 individuals of the same species.
** For the species collected in a single elevation we arbitrarily considered 0.5 °C of temperature range.

4 Discussion

In this study, we suggested a method to identify Coleoptera taxa that can be used to monitor climate change effects in tropical mountainous insect species. The description of the elevational distribution of different beetle groups was used to illustrate the method, associating this distribution with species' thermal distribution in a tropical mountain forest in Southeast Brazil. We observed a temperature decrease of about 0.5 ºC for each 100 m in elevation; ours is the first study to report data on temperature variation along 2100 m elevation in the Brazilian Rainforest. Our guidelines for the selection of bioindicators insect species to monitor climate change effects in mountainous biota are: (i) narrow elevational range combined with good abundance as main criterion for species selection, and (ii) an elevational range of 100 m is a good distance in sampling design to detect species variation in elevational range or abundance along time. Besides that, Eumolpinae (Chrysomelidae) and Lampyridae can be considered adequate taxa to search for bioindicators, as both were well represented in Malaise sampling and exhibited narrow elevational ranges. The findings of this work are discussed under possible direct and indirect effects of climate change on

tropical insect species in mountain systems and their implications for conservation and the potential use of the studied groups to monitor environmental changes. This approach can provide insight into future responses to climate change.

Many of the 272 Coleoptera species collected were only found as singletons and doubletons (46.32%), and few were highly abundant, a very usual general pattern observed for several insect groups in species-rich communities, mainly in the tropics (Novotny and Basset, 2000). Many explanations have been suggested for this uneven abundance (McGill *et al.*, 2007) and insufficient sampling is one of the hypotheses (Novotny and Basset, 2000). However, in the present work two out of the six Coleoptera groups (Lampyridae and Phengodidae) had their observed number of species within the confidence interval limits of the richness estimators (Appendix A - Table A.3; $p = 0.01$) and the other four groups, though cannot be considered badly sampled, may have still more species to be sampled. Despite that, we highlight that 'Malaise' traps have already been reported as a good method to sample many Coleoptera families (*e.g.* Cerambycidae (Ganho and Marinoni, 2003, 2005, 2006; Skvarla and Dowling, 2017), Chrysomelidae (Marinoni and Dutra 1993, 1997; Ganho and Marinoni 2003, 2005, 2006; Furth *et al.*, 2003; Linzmeier *et al.* 2006; Linzmeier and Ribeiro-Costa, 2012), Lampyridae (Ganho and Marinoni, 2003, 2006; Silveira *et al.*, in press) and Phengodidae (Ganho and Marinoni, 2003; Roza *et al.* 2017, 2018; Roza and Mermudes 2019, 2020)). However, according to Skvarla and Dowling (2017), the combined use of pitfall and Malaise traps is considered the best sampling method for Carabidae and Cerambycidae.

It is important to highlight that the lack of taxonomic and biogeographical information, known as Linnean and Wallacean shortfalls, respectively, is certainly an important limitation to the selection and use of insects as bioindicators, as well as for conservation issues (Whittaker *et al.*, 2005). In this sense, our study stands as an important step towards monitoring and conserving the diverse tropical insect fauna by overcoming the aforementioned shortfalls, providing a list of species along with their elevational distribution.

Five species of Coleoptera were suggested as the best potential bioindicators for Serra dos Órgãos National Park, based on combined elevation range and abundance, and ease of recognition criteria (see section 2.4.1 of Methods). Although three out of these five species are not identified to species level, they can be recognized using the diagnosis (see Appendix B) and the figures (Figs. B.1; B.2; B.3; B.4 e B.5) provided in Supplementary Material. Out of the suggested bioindicators, three species were herbivores (Eumolpinae sp. 11; Eumolpinae sp. 12 and *Onocephala obliquata*) and two were predators (*Photuris elliptica* and *Howdenia* sp. 4). Moreover, the most specialized groups were leaf beetles (Eumolpinae: Chrysomelidae) and fireflies (Lampyridae), herbivores and predators, respectively. Voight *et al.* (2003) found that the sensitivity to climate change is greater at higher trophic levels when analyzing the mean temporal variation in arthropod species' abundance that is explained by climate. On the other hand, Macedo *et al.* (2016) observed that, when considering species elevational ranges, insect herbivores tended to show narrower ranges along two elevational gradients in the Atlantic Rainforest biome, while predators showed the broadest, suggesting that herbivores may be more vulnerable to climate change than predators. However, we believe that depending on the variables considered, different groups can respond differently to the effects of changing environment.

The 5th Assessment Report of the Intergovernmental Panel on Climate Change (Collins *et al.*, 2013) projects a mean surface air temperature increase in the tropics from 0.9 to 2.3 °C (RCP 4.5) and from 2.2 to 4.4 °C (RCP 8.5) above the 1986-2005 reference period by the end of this century (Collins *et al.*, 2013). Considering the observed temperature variation of 0.5 °C each 100 m elevation at Serra dos Órgãos National Park, an increase of 2.3 °C (RCP 4.5) and 4.4 °C (RCP 8.5) would mean a shift in bioclimatic zones of about 460 m and about 880 m in elevation, respectively. Groot *et al.* (1995), points out a similar pattern, suggesting that an increase of 3 °C in temperature would mean a latitudinal shift of 600 km or an elevational shift of 600 m in bioclimatic zones in Europe.

Given these scenarios and assuming the narrow thermal tolerance of tropical mountain organisms (Janzen, 1967; Polato *et al.*, 2018), an alteration of bioclimatic zones could result in an imbalance between species distribution and, consequently, in species

interactions (Groot *et al.*, 1995; Hughes, 2000; Menendez, 2007; Sheldon, 2019). In such scenarios, the suggested narrowly distributed abundant bioindicators could rapidly respond to an increase in temperature, and the observed response, if monitored, could help decision making on what to do to mitigate increasing temperature impacts.

Beyond the relevance of suggesting species as bioindicators and describing beetle groups' elevational ranges, which is useful to track the biota response to temperature variations, the description of temperature throughout the gradient *per se* enables us to quantify the increase in temperature locally. Although a single transect was sampled in the present study, there were two traps at each elevation, increasing the chances of sampling different microhabitats. However, the possibility of underestimating species' range distribution cannot be ruled out. We highlight that, in the present study, we have only considered the distribution of species and its climatic niche, regardless of organisms' interactions, which certainly affect species distribution (Groot *et al.*, 1995; Hughes, 2000; Walther *et al.*, 2002; Menendez, 2007; Sheldon, 2019). This is the most common approach (Sheldon, 2019), but we expect that with increasing information concerning species' biology, such variables can be included in the models to make more accurate projections.

Within this approach, one of the main points is whether species will be able to follow range shifts of bioclimatic zones and, if so, some considerations can be made. Taking as an example the suggested bioindicator species Eumolpinae sp. 12, a further increase in temperature of 2.3 ºC (RCP 4.5) by the end of the century, would mean an upward shift of 460 m in its distribution. Considering that Eumolpinae sp. 12 is restricted to high elevation grasslands vegetation, the species would be considered locally extinct because there would be no habitat of the necessary elevation at its temperature optimum. In this scenario, the high elevation grasslands and the species restricted to this vegetation belt seem particularly threatened by climate change, especially if their elevational range is narrow, as pointed out by Macedo *et al.* (2016).

Taking another suggested bioindicator species as an example, *Howdenia* sp. 4, which ranges from 1250 m to 1810 m elevation, a further increase of 2.3 °C (RCP 4.5)

would mean that the species would probably have a new lower limit of its distribution at about 1710 m. The species could also have its upper limit increased by 460 m. However, if we take the high elevation grasslands as a vegetation belt not only limited by climatic conditions, but also by other variables, as the soil, for example (Vasconcelos, 2011), species could not easily go up above ~2000 m, resulting in a reduced elevational range. The situation is even worse if we consider an increase of 4.4 °C by the end of the century, as projected by RCP 8.5. In this scenario, *Howdenia* sp. 4 would have its lower limit distribution shifted to 2130 m, meaning that it would become locally extinct if not able to colonize the high elevation grasslands. The projected examples provided here are only estimates based in mean temperature, what clearly oversimplifies the array of variables directly and indirectly affecting species distribution in space and time. Even if only considering the direct effects of temperature, species responses to climate change are more tightly related to their heat and cold thermal tolerance limits (*e.g.* Sunday *et al.*, 2014; García-Robledo *et al.*, 2016; Sunday *et al.*, 2019; González-Tokman *et al.*, 2020) than with mean temperatures.

Some studies suggest that insect species' tolerance to varying temperature is a trait of limited phenotypic plasticity and evolvability (*e.g.* García-Robledo *et al.*, 2016). García-Robledo *et al.* (2016) observed that species from warmer parts of the mountain (mountain base – lower montane forest vegetation), show greater tolerance to increasing temperature than those from upper elevations. In this sense, the importance of mountaintop species adaptive capacity becomes evident. However, we emphasize that shifts in species' ranges are not the only possible consequences of changing climate on the biota in mountains. Changes in species interactions, abundance, phenology, life cycle, community changes, local and global extinction, and evolutionary responses can be also expected (Groot *et al.*, 1995; Hughes, 2000; Walther *et al.*, 2002; Menendez, 2007; Sheldon, 2019).

In contrast, recent study on species' tolerance to temperature variations has shown that upper thermal limits of species from elevational gradients does not decline with increasing elevation as does lower thermal limits (Sunday *et al.*, 2019). However, the authors discuss that this trend may be related to the broad ranges of the terrestrial

ectotherms data-set, which may consequently not be locally adapted to the elevation site they were collected. This is different from our work, as many species in the studied mountain range have restricted distribution, what makes it more reasonable to predict lower tolerance to increasing temperature of species from upper sites, as observed by García-Robledo *et al.* (2016).

When designing a monitoring sampling scheme for bioindicators, the sampling sites should be rearranged with 100 m distance in elevation, given that a decrease of 0.5 °C in temperature can be observed at each 100 m in elevation. Importantly, we also emphasize that the monitoring of any part of an elevational gradient must include at least two sampling sites 100 m apart in elevation from the lower and upper limits of distribution of each studied species. This would represent the best possibility to record a possible change in species' distribution for an increase of up to 1°C in temperature. Nonetheless, interannual variation in species' distribution need to be considered carefully. We emphasize that the use of 'Malaise' traps must be maintained as the abundance pattern is clearly influenced by sampling technique. Besides that, 'Malaise' traps are effective in sampling the two most elevational specialized groups, Eumolpinae and Lampyridae, and this passive method is of low cost and require little maintenance. In this context, we recommend their use for monitoring temperature variation effects on the biota, particularly in mountainous regions, where access can be difficult. The use of different sampling techniques can show diverse patterns of species richness, abundance and distribution. Therefore, considering using another sampling method or a combination of methods requires a preliminary study on the elevational specialization of the focal group, being it Coleoptera or any other insect group for monitoring purposes.

We have presented the findings of this work from species to higher taxonomic levels (*e.g.* genera, subfamily) when discussing the suggested bioindicators below. Despite the geographical distribution of some studied species potentially being restricted to Southern Brazilian Atlantic Rainforest or even to Serra do Mar Mountain Range, some general considerations can be discussed in relation to higher taxonomic levels. The Eumolpinae (Chrysomelidae) was the group with the smallest average elevational range

(Fig. 3). In this context, this group can be considered suitable for monitoring the effects of climate change, particularly in Atlantic Forest mountaintops.

The Lampyridae also showed a relatively small mean elevational range, suggesting that this group can also be included as a group of relevant interest when designing a monitoring scheme. As some species in this family have also been suggested as potential bioindicators of light pollution and environmental impacts (*e.g.* Viviani *et al.*, 2010), other environmental changes can also be studied when sampling the Lampyridae. This soft-bodied group is particularly interesting because many species have limited dispersal ability (Cicero, 1998), therefore making them more sensitive to temperature and humidity changes.

5 Conclusions

Our approach provides important information on regional temperature variation in an elevational gradient and guidelines for selecting beetle bioindicators and monitoring the responses of insect biota to climate change in tropical mountains using 'Malaise' traps. Most research on the effects of climate change on the biota relates to temperate and polar environments (Sheldon, 2019). Despite that, tropical species are expected to be more sensitive to climate change because of their evolutionary history, given the smaller seasonal variations in temperature, with species living in conditions very tight to their temperature tolerance (Janzen, 1967; Deutsch *et al.*, 2008; Sunday *et al.*, 2014). Based on that, we suggest that the use of abundant species or groups with narrow elevational range as bioindicators can be valuable to monitor the effects of climate change on the biota, allowing us to evaluate how species are responding to changes over time.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Appendix B. Diagnosis of the suggested bioindicators

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Appendix A. Supplementary data

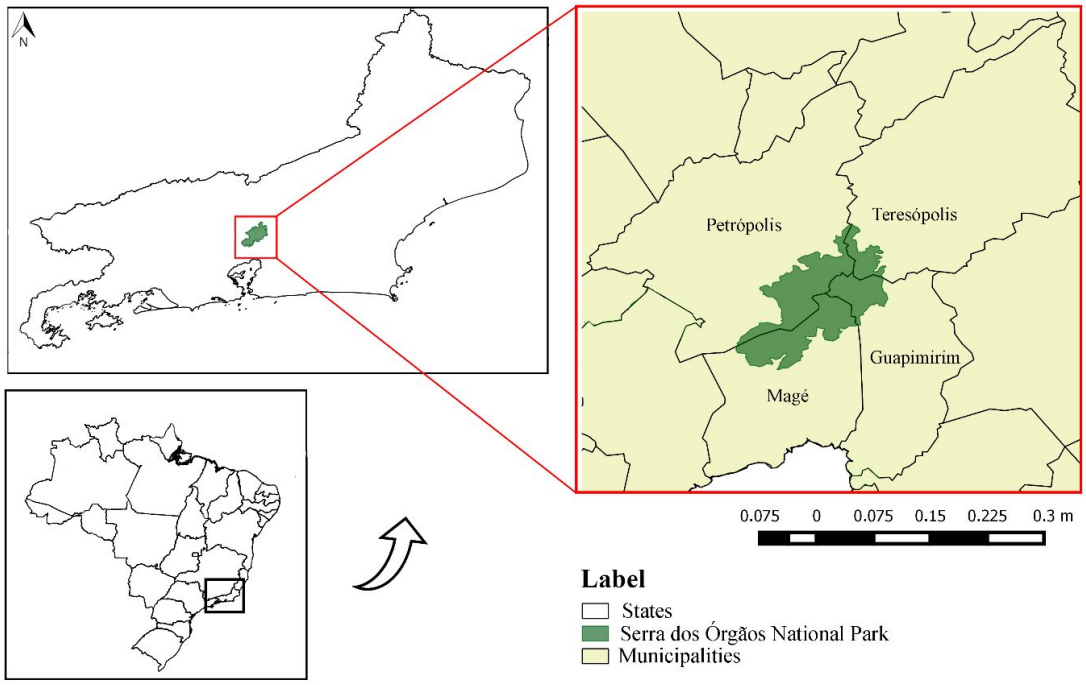

Figure A.1. Location of Serra dos Órgãos National Park in Rio de Janeiro State, Brazil. The Park land falls in the four municipalities of Guapimirim, Magé, Petrópolis and Teresópolis. The National Park Services headquarters office in Teresópolis city is located at about 1000 m a.s.l. (22°32'S; 43°07'W).

Figure A.2. 'Malaise' flight interception trap used for insect sampling in the present study. The red arrow indicates the collecting bottle. Adapted from Vivian Flinte.

Table A.1. Elevations and geographic coordinates of the sampling sites at Serra dos Órgãos National Park, Rio de Janeiro, Brazil.

Elevations	Coordinates
130 m	22°31'55"S; 43°00'07"W
250 m	22°31'00"S; 43°00'24"W
360 m	22°29'41"S; 42°59'54"W
550 m	22°28'36"S; 42°59'31"W
700 m	22°28'37"S; 42°59'45"W
880 m	22°28'11"S; 43°00'06"W
960 m	22°27'29"S; 42°59'11"W
1070 m	22°27'11"S; 42°59'34"W
1250 m	22°26'55"S; 43°00'16"W
1480 m	22°26'54"S; 43°00'49"W
1680 m	22°27'8"S; 43°00'54"W
1810 m	22°27'18"S; 43°00'59"W
1940 m	22°27'18"S; 43°01'12"W
2030 m	22°27'35"S; 43°01'36"W
2170 m	22°27'39"S; 43°01'46"W

Table A.2. List of the sampled species of the six studied groups of Coleoptera from Serra dos Órgãos National Park. Species are arranged in alphabetic order, considering the family and subfamily that they belong. Species' range distribution is also provided.

TAXON	Range (m)
ANTHRIBIDAE	

Subfamily Anthribinae

Anthrenosoma sp. 1	420
Anthrenosoma sp. 2	50
Dasyrhopala tarsalis	50
Discotenes sp. 1	50
Euparius pardalis	50
Euparius sp. 1	600
Euparius sp. 2	200
Euparius sp. 3	720
Euparius sp. 4	50
Euparius sp. 5	50
Eusphyrus sp. 1	50
Gymnognathus sp. 1	1680
Gymnognathus sp. 2	750
Gymnognathus sp. 3	50
Homocloeus sp. 1	1240
Homocloeus sp. 2	300
Homocloeus sp. 4	50
Hylotribus plaumanni	360
Hylotribus sp. 1	50
Hypselotropis prasinata	50
Monocloeus sp. 1	420
Monocloeus sp. 2	1130
Monocloeus sp. 6	50
Ormiscus sp. 1	720
Ormiscus sp. 3	780

Phaenithon cryptocephaloides	180
Phaenithon semigriseus	230
Phaenithon sp. 1	330
Phaenithon sp. 2	120
Phaenithon sp. 3	50
Phaenithon sp. 4	50
Piesocorynus aspis	600
Piesocorynus dispar	750
Piesocorynus tristis	180
Ptychoderes antiquus	300
Scymnopis sp. 1	780
Stenocerus sigillatus	50
Strabus sp. 1	1230
Toxonotus farinatus	1350
Zygaenodini gen. 1 sp. 1	560
Subfamily Choraginae	
Araecerini gen. 1 sp. 1	50
Araecerini gen. 1 sp. 3	50
Choraginae gen. 1 sp. 1	50
Choragini gen. 1 sp. 1	600
Choragini gen. 1 sp. 2	330
Choragini gen. 1 sp. 3	550
CARABIDAE	
Subfamily Anthiinae	
Helluomorphoides sp. 1	290
Subfamily Cicindelinae	

Ctenostoma (Euctenostoma) rugosum	570
Ctenostoma (Euctenostoma) sahlberg	330
Ctenostoma (Myrmecilla) coracinum	330
Ctenostoma (Myrmecilla) ichneumoneum	410
Ctenostoma (Myrmecilla) pygmaeum	50
Ctenostoma (Myrmecilla) unifasciatum	150
Euprosopus quadrinotatus	50
Iresia binotata	1350
Odontocheila nodicornis	940
Opisthencentrus dentipennis	50
Pentacomia (Mesochila) procera	50
Pentacomia (Mesochila) smaragdula	850
Phyllodroma luteomaculata	450
Subfamily Harpalinae	
Morion brasiliensis	50
Stenolophus sp. 1	890
Stenolophus sp. 2	1900
Subfamily Lebiinae	
Agra rutilipennis	50
Calleida sp. 1	50
Cryptobatida sp. 1	50
Lebia (Chelonodema) sp. 1	50
Lebia (Lebia) sp. 1	1060
Lebia (Lebia) sp. 10	410
Lebia (Lebia) sp. 11	600
Lebia (Lebia) sp. 12	50

Lebia (Lebia) sp. 13	50
Lebia (Lebia) sp. 2	600
Lebia (Lebia) sp. 3	630
Lebia (Lebia) sp. 5	520
Lebia (Lebia) sp. 6	1130
Lebia (Lebia) sp. 7	690
Lebia (Lebia) sp. 8	1690
Lebia (Lebia) sp. 9	960
Lebia (Loxopeza) sp. 1	50
Lebiini (Calleidina) sp. 1	50
Pentagonica sp. 1	1900
Pentagonica sp. 2	330
Pentagonica sp. 3	180
Pentagonica sp. 4	230
Subfamily Trechinae	
Elaphropus sp. 1	570
Elaphropus sp. 2	50
CERAMBYCIDAE	
Subfamily Lamiinae	
Adetus analis	50
Alcidion ludicrum	300
Colobothea poecila	150
Colobothea subcincta	180
Esthlogena (Esthlogena) glaucipennis	1810
Estola sp. 1	1060
Eutrypanus sp. 1	150

Eutrypanus sp. 2	50
Hippopsis sp. 1	50
Hypsioma affinis	1260
Macropophora accentifer	260
Nealcidion bicristatum	1210
Nealcidion simillimum	1670
Nealcidion sp. 1	330
Nyssodrysina lignaria	1680
Nyssodrysternum sp. 1	50
Obereoides sp. 1	50
Onocephala obliquata	460
Onocephala vittipennis	330
Oreodera aerumnosa	330
Oreodera candida	50
Ozineus sp. 1	50
Phacellocera sp. 1	600
Psapharochrus juno	410
Rosalba sp. 1	50
Sangaris duplex	50
Sciadosoma sp. 1	50
Scleronotus scabrosus	520
Trestonia capreola	50
Subfamily Cerambycinae	
Allopeba quadripunctata	330
Allopeba signaticornis	1620
Batus hirticornis	800

Chariergodes flava	50
Chlorida costata	1260
Chydarteres dimidiatus	50
Compsa albopicta	930
Compsibidion divisum	80
Compsibidion sp. 1	50
Compsibidion vanum	50
Eburodacrys alini	50
Eurysthea obliqua	50
Pantomallus morosus	330
Poeciloxestia dorsalis	130
Stizocera sp. 1	50
Xestiodion pictipes	980
Subfamily Prioninae	
Meroscelisus servillei	50
Myzomorphus quadripunctatus	410
Polyoza lacordairei	630
CHRYSOMELIDAE	
Subfamily Eumolpinae	
Eumolpinae sp. 1	870
Eumolpinae sp. 2	230
Eumolpinae sp. 3	1680
Eumolpinae sp. 4	890
Eumolpinae sp. 5	230
Eumolpinae sp. 6	230
Eumolpinae sp. 7	230

Eumolpinae sp. 8	230
Eumolpinae sp. 9	520
Eumolpinae sp. 10	780
Eumolpinae sp. 11	230
Eumolpinae sp. 12	230
Eumolpinae sp. 13	800
Eumolpinae sp. 14	50
Eumolpinae sp. 15	410
Eumolpinae sp. 16	750
Eumolpinae sp. 17	290
Eumolpinae sp. 18	50
Eumolpinae sp. 19	50
Eumolpinae sp. 20	290
Eumolpinae sp. 21	50
Eumolpinae sp. 22	50
Eumolpinae sp. 23	260
Eumolpinae sp. 24	50
Eumolpinae sp. 25	50
Eumolpinae sp. 26	430
Eumolpinae sp. 27	150
Eumolpinae sp. 28	50
Eumolpinae sp. 29	50
Eumolpinae sp. 30	50
Eumolpinae sp. 31	50
Eumolpinae sp. 32	130
Eumolpinae sp. 33	50

Eumolpinae sp. 34	180
Eumolpinae sp. 35	50
Eumolpinae sp. 36	130
Eumolpinae sp. 37	50
Eumolpinae sp. 38	370
Eumolpinae sp. 39	330
Eumolpinae sp. 40	50
Eumolpinae sp. 41	230
Eumolpinae sp. 42	50
Eumolpinae sp. 43	50
Eumolpinae sp. 44	450
Eumolpinae sp. 45	50
Eumolpinae sp. 46	50
Eumolpinae sp. 47	50
Eumolpinae sp. 48	50
Eumolpinae sp. 49	50
Eumolpinae sp. 50	50
Eumolpinae sp. 51	50
Eumolpinae sp. 52	50
Eumolpinae sp. 53	50
Eumolpinae sp. 54	50
Eumolpinae sp. 55	50
Eumolpinae sp. 56	50
Eumolpinae sp. 57	50
Eumolpinae sp. 58	50
Eumolpinae sp. 59	50

Eumolpinae sp. 60	50
Eumolpinae sp. 61	50
Eumolpinae sp. 62	50
Eumolpinae sp. 63	50
LAMPYRIDAE	
Subfamily Amydetinae	
Amydetes apicalis	750
Amydetes fastigiata	50
Amydetes sp. 1	50
Amydetes sp. 2	110
Cladodes illigeri	520
Cladodes sp. 1	330
Cladodes sp. 2	50
Ethra axillaris	780
Ethra cf. addicta	50
Ethra inculta	190
Ethra marginata	260
Magnoculus sp. 1	290
Magnoculus sp. 2	50
Psilocladus sigillatus	50
Psilocladus sp. 2	560
Sissicauda disjuncta	610
Subfamily Photurinae	
Bicellonycha aff. Tenuicornis	50
Bicellonycha sp. 1	130
Bicellonycha tenuicornis	780

Photuris elliptica	50
Photuris fulvipes	50
Photuris sp. 1	50
Photuris sp. 2	50
Photuris sp. 3	180
Pyrogaster angustatus	700
Pyrogaster atrocinctus	290
Pyrogaster aureus	50
Pyrogaster coxalis	50
Pyrogaster lunifer	50
Pyrogaster sp. 7	50
Subfamily Lampyrinae	
Dilychnia succensa	50
Gen. nov. 1 sp. nov. 1	290
Lucidota flabellicornis	800
Lucidota sp. 1	1100
Lucidota sp. 12	220
Lucidota sp. 13	550
Lucidota sp. 2	930
Lucidota sp. 3	550
Lucidota sp. 4	200
Lucidota sp. 5	140
Lucidota sp. 6	410
Lucidota sp. 8	360
Luciuranus jameshooki	50
Luciuranus sp. 2	50

Macrolampis frater	1000
Phaenolis basalis	1550
Phaenolis sp. 1	50
Photinus sp. 2	50
Photinus sp. 3	110
Photinus sp. 4	230
Ybytyramoan monteirorum	930
PHENGODIDAE	
Subfamily Mastinocerinae	
Akamboja cleidae	520
Akamboja minimum	630
Gen. nov. 1 sp. nov. 1	50
Gen. nov. 1 sp. nov. 2	50
Gen. nov. 1 sp. nov. 3	50
Gen. nov. 2 sp. nov. 1	50
Gen. nov. 2 sp. nov. 2	50
Howdenia sp. 1	630
Howdenia sp. 2	920
Howdenia sp. 3	720
Howdenia sp. 4	560
Mastinocerus sp. 1	700
Mastinocerus sp. 2	50
Mastinocerus sp. 3	180
Mastinomorphus sp. 1	50
Mastinomorphus sp. 2	920
Oxymastinocerus aff. peruanus sp. 1	570

Stenophrixothrix sp. 1	1000
Stenophrixothrix sp. 2	370
Stenophrixothrix sp. 3	690
Stenophrixothrix sp. 4	560
Taximastinocerus sp. 1	330
Subfamily Phengodinae	
Pseudophengodes aff. brasiliensis sp. 1	330

Table A.3. Observed (S_{obs}) and asymptotic estimates of species richness (S_{est}) for the different beetle groups within the entire elevational gradient. Standard error (SE), lower confidence limit (LCL) and upper confidence limit (UCL) are also indicated (CI = 0.99; p=0.01).

TAXA	S_{obs}	S_{est}	SE	LCL	UCL
Anthribidae	46.0	58.7	8.3	48.7	105.3
Carabidae	41.0	48.1	5.9	42.1	86.7
Cerambycidae	48.0	64.1	10.0	51.7	118.1
Eumolpinae	63.0	74.1	7.0	65.5	112.6
Lampyridae	51.0	54.2	3.1	51.4	76.4
Phengodidae	23.0	24.1	1.8	23.1	42.9

Appendix B. Diagnosis of the suggested bioindicators

An objective diagnosis is provided for each of the five suggested bioindicators in order to help monitoring team from Serra dos Órgãos National Park recognizing the species of the region. For complete diagnosis, consider looking the original description of the species or genus.

Eumolpinae sp. 11 (Figure B.1)

Diagnosis. Color: Antennae brownish-yellow; scape dark brown on the basis and light brown on the apex; pedicel and antennomeres III-IV light brown and darkening towards the apex. Integument iridescent, metallic dark green (Fig. B.1 A; B). Ventral segments and legs with short and sparse pale yellow pubescence (Fig. B.1 B). Morphology: Mean size – 4.37 mm. Antennae filiform. Head, pronotum and elytra strongly punctuated (Fig. B.1 A; B). Two dents at each lateral margin of pronotum. Elytral humeri tuberculate. Elytral epipleura folded on the apical third of the elytra, creating an intumescence (Fig. B.1 B; highlighted in C).

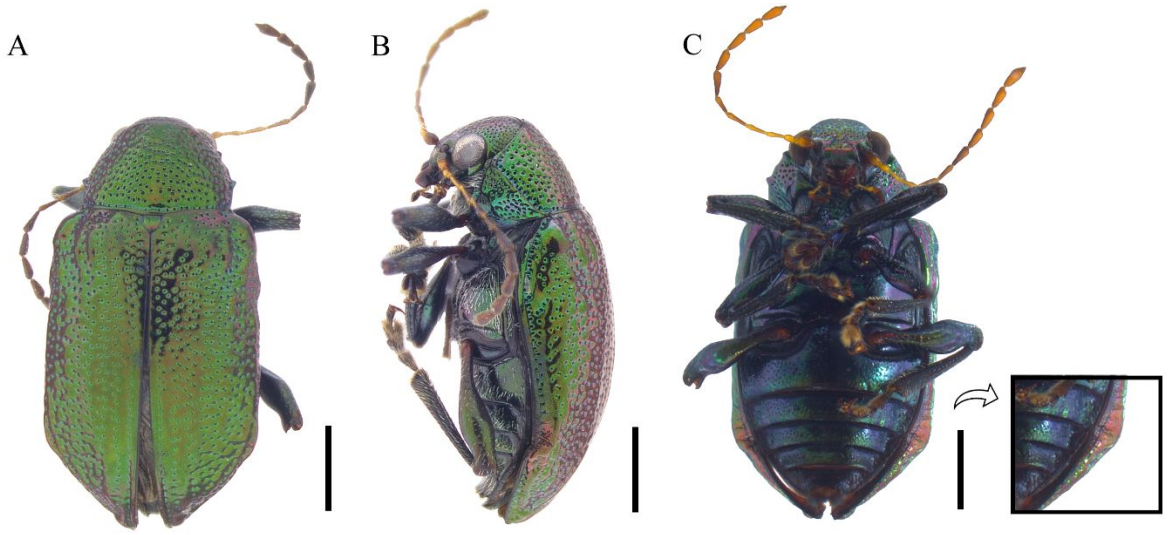

Figure B.1. Dorsal (A), lateral (B) and ventral (C) views of Eumolpinae sp. 11 (Chrysomelidae). Elytral intumescence highlighted in C. Scale bar = 1 mm.

Eumolpinae sp. 12 (Figure B.2)

Diagnosis. Color: Antennae yellowish light brown. Color varies from a small metallic stain in the elytra, with an overall light brown color to completely metallic green, except for the dark brown legs (some of the variations are presented in Fig. B.2 A; A1 and A2). Morphology: Mean size – 5.03 mm. Antennae filiform. Head, pronotum and elytra strongly punctate. One dent on the lateral margin of pronotum. Humeri tuberculate. Elytra lateral margins obliquely truncate at apical third; elytral apex truncate, abruptly depressed in lateral view (Fig. B.2, highlighted in B).

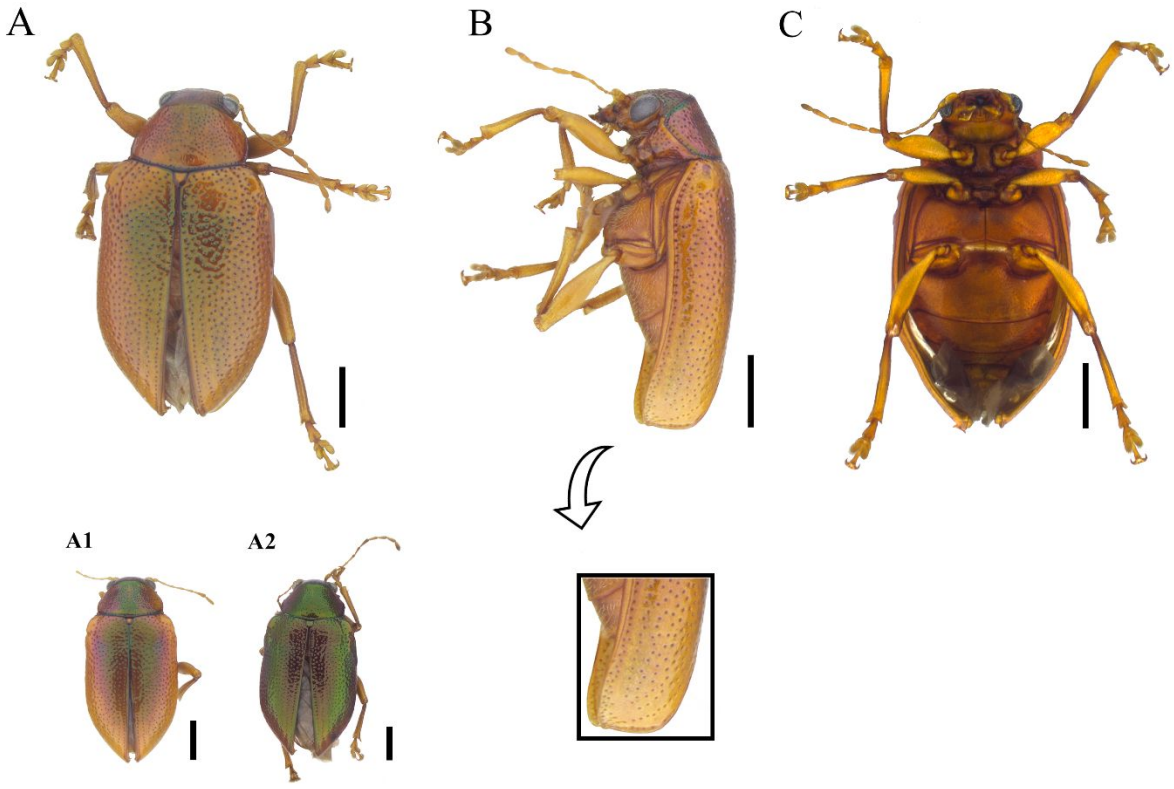

Figure B.2. Dorsal (A), lateral (B) and ventral (C) views of Eumolpinae sp. 12 (Chrysomelidae). Color variation is shown in A, A1 and A2. Elytral apex truncate in lateral view highlighted in B. Scale bar = 1 mm.

Onocephala obliquata (Figure B.3)

Diagnosis. Color: Antennae brownish-yellow; antennal tubercles, scape, pedicel and antennomeres III–IV covered with dense pale yellowish pubescence. Integument brown, entirely covered with short, dense pale yellowish pubescence. Morphology: Mean size – 16.39 mm. Antennae filiform and size varies from 1 to 2.5x longer than body length. Pronotum cylindrical, with a longitudinal median stripe and pubescent scutellum. Humeri pronounced, acute and tuberculate. Elytral base tuberculated and large anterior tubercles organized in longitudinal sinuous lines. Elytra with a premedian pale yellowish lateral macula (Fig. B.3, highlighted in A), elongated, narrow and oblique, not reaching the sternal margin (Fig. B.3, highlighted in B); elytra with two yellowish longitudinal stripes of pubescence, dorsolateral, post median originated and punctate along the length (Fig. B.3, highlighted in A).

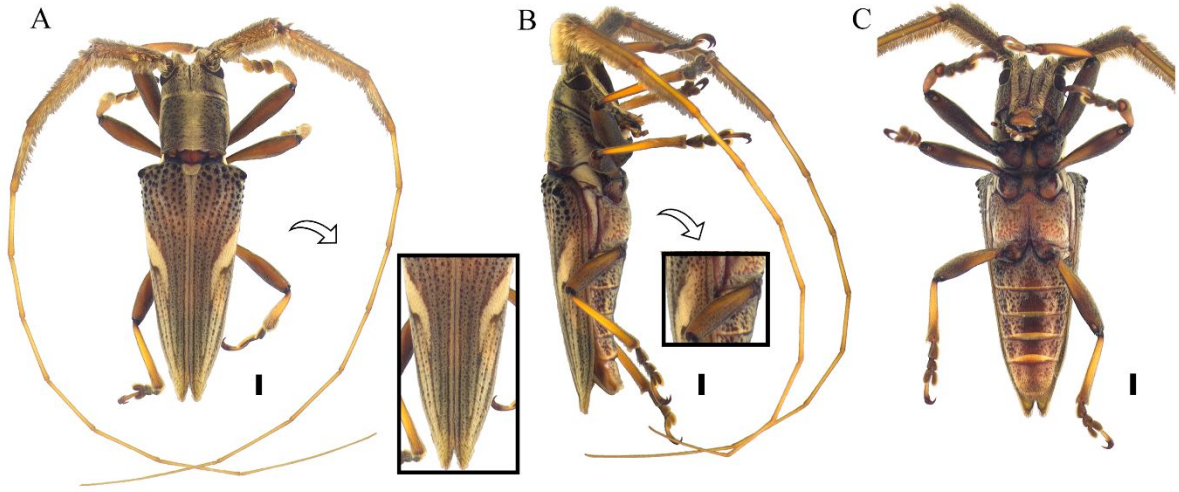

Figure B.3. Dorsal (A), lateral (B) and ventral (C) views of *Onocephala obliquata* (Cerambycidae). Elytral macula highlighted in A and detailing sternal margin in B. Scale bar = 1 mm.

Photuris elliptica (Figure B.4)

Diagnosis. Color: Head and antennae dark brown to black. Pronotum yellowish. Elytron dark brown to black. Pro and mesosternum yellow. Pro and mesocoxae yellow. Legs dark brown/black, except for the pro and mesofemora with basal 2/3 yellow. First four ventral abdominal segments dark brown to black. Morphology: Mean size – 13.39 mm. Antennae filiform. Eyes exposed in front of pronotum. Pronotum semicircular, almost entirely covering the head. Elytron rounded, covered with fine and short pubescence (Fig. B.4 A). Lanterns occupying almost entirely sterna V and VI, with anterior margin straight and posterior margin somewhat rounded; anterior lantern larger than posterior (Fig. B.4 B).

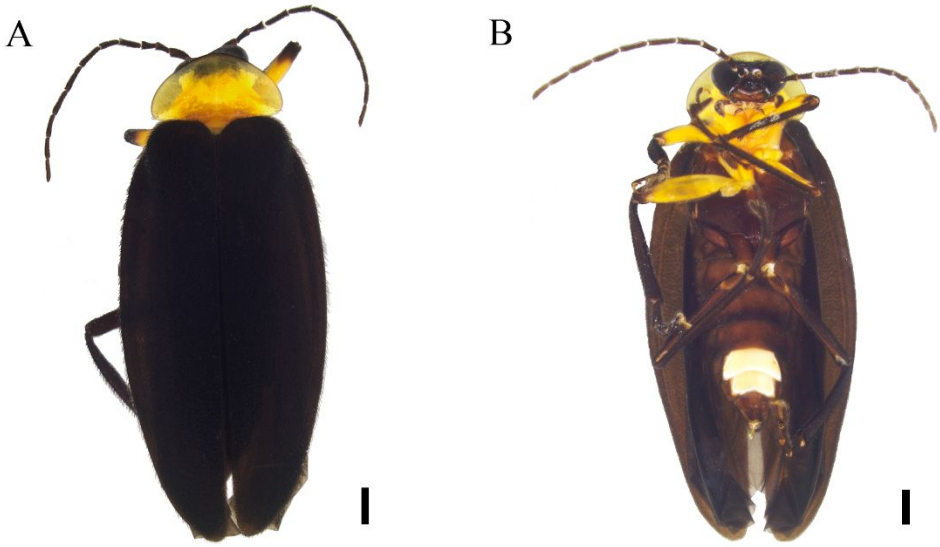

Figure B.4. Dorsal (A) and ventral (B) views of the firefly *Photuris elliptica* (Lampyridae). Scale bar = 1 mm.

***Howdenia* sp. 4** (Figure B.5)

Diagnosis. Color: Head dark brown. Antennae, with XII antennomere, scape and antennomere II yellowish brown, antennomere III-XII brown. Pronotum dark brown, entirely covered with dense black pubescence. Elytron dark brown, with the humeri yellowish brown, much lighter than the elytra (Fig. B.5, highlighted in A). Tergites and

sternites black, except for the last two yellow segments. Legs yellow, except for yellowish brown tibia and tarsus, which are highly pubescent (Fig. B.5 A; B). Morphology: Mean size – 5.11 mm. Antennae biflabellate. Pronotum trapezoidal and narrower than humeral distance. Elytron short, 3.5x longer than wide, thickened apically.

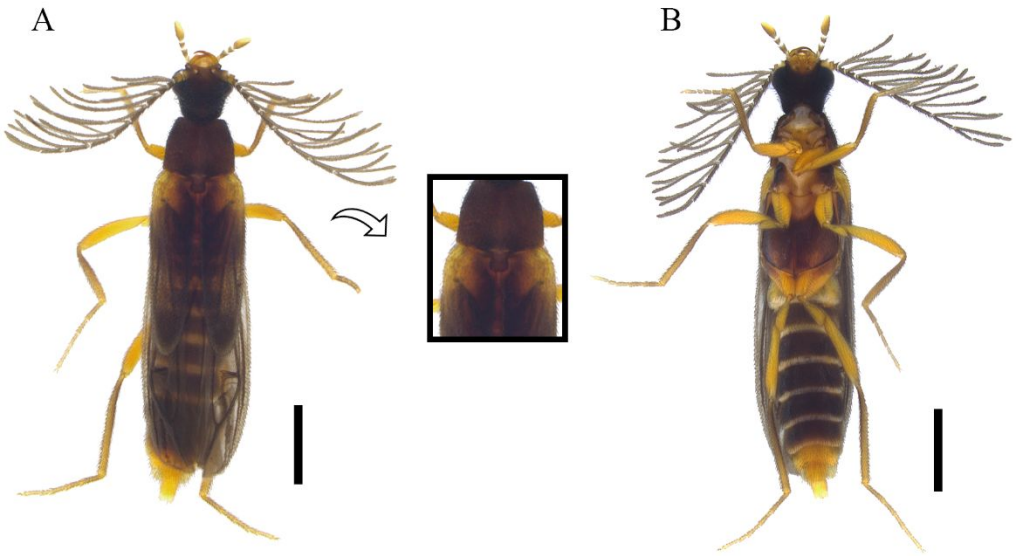

Figure B.5. Dorsal (A) and ventral (B) views of *Howdenia* sp. 4 (Phengodidae). Humeri much lighter than the elytra highlighted in A. Scale bar = 1 mm.