



**GABRIEL AUGUSTO SILVA VAZ**

**EXPLORING THE FACTORS SHAPING CAVE  
INVERTEBRATE COMMUNITIES AND HABITAT  
SELECTION IN A NEW HOTSPOT OF SUBTERRANEAN  
BIODIVERSITY IN SOUTH AMERICA**

**LAVRAS-MG  
2023**

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Trabalho de Conclusão de Curso  
apresentado à Universidade Federal de  
Lavras, como parte as exigências do Curso  
de Ciências Biológicas, para a obtenção do  
título de Bacharel.

Prof. Dr. Marconi Souza Silva

Orientador

Prof. Dr. Rodrigo Lopes Ferreira

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**EXPLORANDO OS FATORES QUE MOLDAM AS COMUNIDADES DE  
INVERTEBRADOS EM CAVERNAS E A SELEÇÃO DE HABITAT EM UM  
NOVO HOTSPOT DE BIODIVERSIDADE SUBTERRÂNEA NA AMÉRICA DO  
SUL**

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**PRIMEIRA PARTE**  
**INTRODUÇÃO GERAL**

## 1 INTRODUÇÃO GERAL

Entender a distribuição das espécies no espaço e no tempo são informações importantes para delimitar áreas com alta biodiversidade e alto endemismo (MYERS et al., 2000), assim como compreender os processos que mantêm a biodiversidade. Diversas teorias surgiram ao longo do tempo visando explicar a distribuição e riqueza das espécies. Importantes trabalhos de MacArthur e colaboradores em 1967, como “A Similaridade, Convergência e Divergência Limitantes de Espécies Coexistentes” (MACARTHUR; LEVINS, 1967) e “A Teoria de Biogeografia de Ilhas” (MACARTHUR; WILSON, 1967), impulsionaram duas vertentes em ecologia de comunidades. Uma explicava a organização da biodiversidade a partir das dinâmicas de nicho ecológico a outra explicava que o número de espécies era determinado através de processos estocásticos de imigração e extinção.

Ambos os conceitos foram aprimorados por trabalhos subsequentes. Chesson (2000) definiu os mecanismos que levam a coexistência como equalizadores, que atuam na diminuição da diferença competitiva entre competidores, e estabilizadores, que atuam na diminuição da sobreposição de nicho. Assim a coexistência seria mantida por usos diferenciais dos recursos e por respostas aos a fatores limitantes, além da relação de dependência entre nível de competitividade e a sobreposição de nicho. Baseada nos processos estocásticos Hubbell (2001) propôs a “Teoria Neutra da Ecologia”, sua hipótese de equivalência funcional não considera a identidade das espécies e atribui a manutenção da riqueza por processos de chegada (imigração/especiação) e saída (migração/extinção) de indivíduos.

Outras explicações se basearam na estabilidade climática histórica, sugerindo que as áreas tropicais teriam mais espécies devido à falta de eventos climáticos extremos no passado. Nesses locais “intocados” as linhagens tiveram mais tempo para divergir (JASSON; DYNESIUS, 2002). A teoria da Heterogeneidade de habitat também de MacArthur (1961), propõem que em ambientes com maior disponibilidade de habitats diferentes a sobreposição de nicho seria menor até mesmo em espécies com características similares, o que geraria maior coexistência. Também seriam locais que poderiam propiciar especiação. Já a teoria da produtividade, relaciona a riqueza de espécies com áreas com maior produtividade primária/biomassa (GRACE; ANDERSON; SEABLOOM, 2016)

Porém esses processos ecológicos não geram padrões gerais, existem exceções dependendo da escala sob análise e de acordo com o grupo taxonômico (GRACE; ANDERSON; SEABLOOM, 2016; STORCH; BOHDALKOVÁ; OKIE; 2018; THOMSEN et al., 2022). Os padrões fundamentais em ecologia se baseiam na relação espécie área (CONOR; MCCOY, 2001) e na distribuição de abundância de espécies (BALDRIDGE et al., 2016). Apesar de que a relação espécie área também parece depender de outros parâmetros que afetam a inclinação da relação (DRAKARE; LENNON; HILLEBRAND, 2005).

Este pequeno histórico demonstra a complexidade de se entender os processos que moldam as comunidades. Porém como esses processos operam em ambientes mais simples como as cavernas? Este ambiente subterrâneo apresenta características marcantes como ausência total de luz em áreas mais profundas, estabilidade climática (temperatura e umidade) e oligotrofia (ausência de produtores primários, sendo a entrada de energia por meio alóctone). O ambiente cavernícola então pode ser considerado mais simples que o meio epígio, tanto em relação ao habitat como em relação a cadeia trófica. Poulson (1977) e Culver (1973, 1975) propuseram que a competição e as dinâmicas de nicho poderiam ser processos importantes na montagem das comunidades em cavernas (MAMMOLA; ISAIA, 2018), mas até o trabalho de Fišer e colaboradores em 2012 o principal pensamento era esperar que nessas condições a evolução favoreceria a convergência morfológica e funcional, tornando as espécies da comunidade equivalentes. O resultado deste trabalho demonstrou que espécies de um gênero de anfípodes subterrâneos chamado *Niphargus* poderiam coexistir devido à diferenciação de nicho, ocupando espaços subterrâneos diferentes (águas subterrâneas intersticiais e cavernas) de acordo com sua morfologia. Trabalhos subsequentes também mostraram a importância do nicho ecológico e das interações entre espécies nos ambientes subterrâneos (FIŠER et al., 2015; MAMMOLA; PIANO; ISAIA, 2016).

Todas essas características dos ambientes subterrâneos os fazem bons modelos para estudos em ecologia de comunidades, biogeografia e evolução (MAMMOLA, 2018). A vida subterrânea fornece serviços ecossistêmicos importantes como a polinização, decomposição de matéria orgânica, controle de insetos (pragas) e manutenção da qualidade das águas subterrâneas (CULVER; PIPAN, 2019; MAMMOLA et al., 2019). Também é composta por espécies estritamente cavernícolas que costumam ser endêmicas, demonstrando a importância e o desafio em torno da conservação de um dos ecossistemas menos conhecidos (MAMMOLA et al., 2019).



No Brasil esses desafios são eminentes, visto que, muitas espécies restritas de cavernas ainda não foram descritas, assim não é possível entender o status de conservação dessas espécies de acordo com o critério estabelecido pela Union for Conservation of Nature (IUCN) (SOUZA-SILVA et al., 2021). Além disso dos quatro Hotspots de Biodiversidade Subterrânea descrito no país, termo utilizado para definir cavernas que possuem 20 ou mais espécies exclusivamente cavernícolas (troglóbias (terrestres) e stigóbias (aquáticas)) (CULVER; SKET, 2000), apenas dois estão protegidos por áreas de preservação ambiental (Sistema de Cavernas Areias que fica no Parque Estadual Turístico do Alto Ribeira (PETAR) e o Sistema de Cavernas de Igatu que fica no Parque Nacional da Chapada Diamantina) (SOUZA-SILVA; FERREIRA, 2016; GALLÃO et al., 2023).

Para tomar decisões acertadas para conservação do mundo subterrâneo, há primeiro uma necessidade urgente de acelerar a investigação científica, destinada a explorar a biodiversidade subterrânea juntamente com os fatores abióticos e bióticos que impulsionam os seus padrões de distribuição no espaço e no tempo (MAMMOLA et al., 2019). Desta forma, nesta pesquisa buscou-se compreender quais são os fatores ambientais, relacionados ao chão de uma caverna, que influenciam na variação de composição e riqueza de espécies de invertebrados cavernícolas. Nos concentramos em uma única caverna da região semiárida da Bahia para desenvolver essa pesquisa, em uma área que tem se mostrado prioritária para conservação do patrimônio espeleológico do Brasil (FERREIRA; BERBERT-BORN; SOUZA-SILVA, 2023).

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**SEGUNDA PARTE**

**ARTIGO**

# Exploring the factors shaping cave invertebrate communities and habitat selection in a new Hotspot of Subterranean Biodiversity in South America

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## Abstract

Caves serve as exceptional ecological models for studying the environmental factors that shape biological communities. However, there has been limited exploration into understanding how these environmental factors affects communities within the same cave system. To address this gap, we employed standardized sampling methodologies across two scales within a limestone cave located in the Brazilian semi-arid region (Pedro Cassiano cave). Our objective was to uncover how substrate characteristics and conditions may contribute to variations in the composition and richness of invertebrates and niche overlapping. Furthermore, we aimed to comprehend the habitat selection and distribution patterns of some troglobitic species. This particular cave hosts a rich restricted fauna, with 20 obligate cave species (19 invertebrates and 1 fish), which positions it as new Hotspot of Subterranean Biodiversity in South America. The diversity of substrates, acting as a proxy for habitat heterogeneity, emerged as a pivotal

36 environmental factor shaping invertebrate communities. Our observations emphasized  
37 that the factors structuring invertebrate communities depend on the analytical scale.  
38 Furthermore, troglobiotic and non-troglobiotic invertebrates are influenced by distinct  
39 environmental determinants. Troglobitic species are more prevalent in the cave's deeper  
40 areas than non-troglobitic species, and they exhibit minimal niche overlap. Finally, we  
41 deliberated on the urgency of conservation efforts for this important cave.

42

43 Key Words: Cave conservation; Habitat heterogeneity; Troglobites; Non-troglobites,  
44 Niche.

45

## 46 **1. Introduction**

47 Understanding the factors that shape biological communities and unraveling  
48 species distribution patterns at regional and local scales pose significant challenges in the  
49 field of community ecology. These challenges are compounded by the multifactorial  
50 nature of responses and the inherent difficulty in controlling variables in natural  
51 environments. In comparison to surface habitats, caves offer a simpler yet fascinating  
52 ecological setting, both in terms of their biological communities and their environmental  
53 conditions and characteristics (Poulson & White, 1969; Sánchez-Fernández et al., 2018a).

54 Despite their simplicity, caves exhibit a diverse array of microhabitats and organic  
55 resources (Souza-Silva et al., 2011b; Lunghi et al., 2017; Lunghi and Manenti, 2020;  
56 Mammola et al., 2020), which are distributed along gradients from the entrance to the  
57 deeper areas (Tobin et al., 2013; Prous et al., 2015; Mammola and Isaia, 2018; Lunghi  
58 and Manenti, 2020). These unique characteristics make caves an ideal "natural  
59 laboratory" for conducting ecological, biogeographical, and evolutionary studies  
60 (Poulson and White, 1969; Sánchez-Fernández et al., 2018; Mammola et al., 2019).

61 The richness and composition of cave communities are intricately shaped by  
62 various factors, including the presence of distinct zones with differing light conditions,  
63 availability of organic resources, temperature, and humidity (Tobin et al., 2013; Prous et  
64 al., 2004, 2015; Kozel et al., 2019; Mammola et al., 2017; Lunghi and Manenti, 2020).  
65 Cave entrances harbor communities that exhibit remarkable resilience to fluctuations in  
66 daily and seasonal environmental conditions. In contrast, deeper areas of caves  
67 experience relatively stable temperature and humidity, yet suffer from a scarcity of  
68 organic resources (Tobin et al., 2013; Mammola et al., 2019). Consequently, these deeper

69 regions are typically inhabited by highly specialized species, which present adaptations  
70 to cope with nutrient limitations and challenging conditions (Novak et al., 2012; Kozel et  
71 al., 2019).

72         Pioneering researchers, including Schiner in 1854 and Racovitza in 1907, laid the  
73 groundwork for understanding the intricate relationships between species inhabiting  
74 caves and their environment. These early scholars classified organisms based on their  
75 ecological-evolutionary characteristics. Troglloxenes, for instance, utilize caves for  
76 specific purposes such as shelter, but depend of the aboveground environment to  
77 complete their life cycle. In contrast, Trogllophiles are capable of establishing viable  
78 populations both inside and outside caves. Finally, Trogllobites, highly specialized for life  
79 in perpetually dark and often nutrient-poor environments, are exclusively restricted to  
80 cave habitats.

81         Troglloxenes and trogllophiles are occasionally grouped as non-trogllobites due to  
82 the limited availability of practical criteria for distinguishing between these categories  
83 (Novak et al., 2012). In contrast, trogllobites can be identified by their troglomorphic  
84 traits, characterized by features such as reduced or absent eyes, depigmentation, and the  
85 presence of elongated appendages (Cristiansen 1962). However, it is noteworthy that  
86 certain groups, such as Palpigradi, consistently exhibit depigmentation and blindness,  
87 necessitating the examination of additional morphological characteristics for accurate  
88 classification (Souza and Ferreira, 2010).

89         The distribution patterns of non-trogllobites and trogllobites within cave systems  
90 are governed by spatial and temporal dynamics that are influenced by microclimatic,  
91 physical, and trophic variations occurring along the cave passages (Novak et al., 2012;  
92 Tobin et al., 2013; Bento et al., 2016; Lunghi et al., 2017; Kozel et al., 2019; Souza-Silva  
93 et al., 2021). Additionally, the occurrence of interspecific competition within this  
94 environment is closely linked to the eco-evolutionary categories, as trogllobites and non-  
95 trogllobites often display limited spatial overlap along the cave's extent. This is primarily  
96 due to the trogllobitic species' preference for more stable and specialized environments,  
97 resulting in distinct ecological niches (Sket, 1999; Novak et al., 2012; Souza-Silva et al.,  
98 2021).

99         However, our understanding of subterranean biodiversity remains incomplete.  
100 The ecological factors that govern subterranean habitats and the characterization of  
101 species' ecological niches are still inadequately understood (Mammola and Isaia, 2016).  
102 Furthermore, patterns of subterranean diversity are subject to geographical biases

103 (Sánchez-Fernández et al., 2018). For instance, in Neotropical regions, the diversity  
104 patterns and factors influencing the distribution of subterranean species are less explored  
105 compared to temperate regions (Niemiller et al., 2018; Mendes-Rabelo et al., 2018).

106 With the increase in the number of studies in the ecology of subterranean  
107 ecosystems in recent years, some ecological factors influencing the fauna have been  
108 described, such as cave extent, seasonal variation, landscape structure, cave lithology,  
109 habitat heterogeneity, among others (Souza-Silva et al., 2011a; Pellegrini et al., 2016;  
110 Jaffé et al., 2018; Bento et al., 2016; Pacheco et al., 2020a). Some of these studies have  
111 focused on factors that affect troglobitic species, partly due to their importance for  
112 conservation, as well as on variations that occur between caves and regions.

113 The term "hotspots of subterranean biodiversity" (HSB) was initially introduced  
114 by Culver and Sket (2000) to designate subterranean habitats that harbor a minimum of  
115 twenty or more cave-obligate species. Subsequently, Culver and Pipan (2013) refined this  
116 threshold by suggesting that an HSB should encompass a minimum of 25 species  
117 restricted to cave environments. However, in a recent study, Ferreira et al., (2023)  
118 deliberated on the limitations of maintaining a fixed cutoff value, considering various  
119 factors such as geographic location, lithology, and biome, which directly influence the  
120 species richness of cave-restricted organisms within a specific cave. Consequently, they  
121 proposed a more flexible approach, suggesting that the determination of an HSB cutoff  
122 should be adaptable depending on the contextual factors associated with the cave's  
123 environment.

124 It is crucial to acknowledge that relying solely on species richness as a criterion  
125 for identifying hotspots may not fully capture the ecological significance of these habitats  
126 or their susceptibility to anthropogenic disturbances (Ferreira et al., 2023). Thus, it  
127 becomes essential to consider not only the biodiversity value but also the potential threats  
128 to the habitat when identifying and prioritizing conservation areas. Accordingly, it is  
129 paramount to comprehend the potential patterns of subterranean biodiversity at different  
130 scales to effectively implement protective measures for this ecosystem amidst  
131 anthropogenic changes. Additionally, the detection of new hotspots of subterranean  
132 biodiversity is of significant importance as these areas often receive greater attention in  
133 conservation efforts. Thus, the primary objectives of this study were to investigate the  
134 environmental factors that govern the variation in richness and composition of  
135 invertebrate communities within a cave situated in the Brazilian semi-arid region, while  
136 considering two distinct sampling scales. Additionally, we aimed to elucidate the



137 distribution patterns of troglobitic species and their habitat preferences within this cave.  
138 Notably, this study unveils a novel hotspot of subterranean biodiversity in South America.

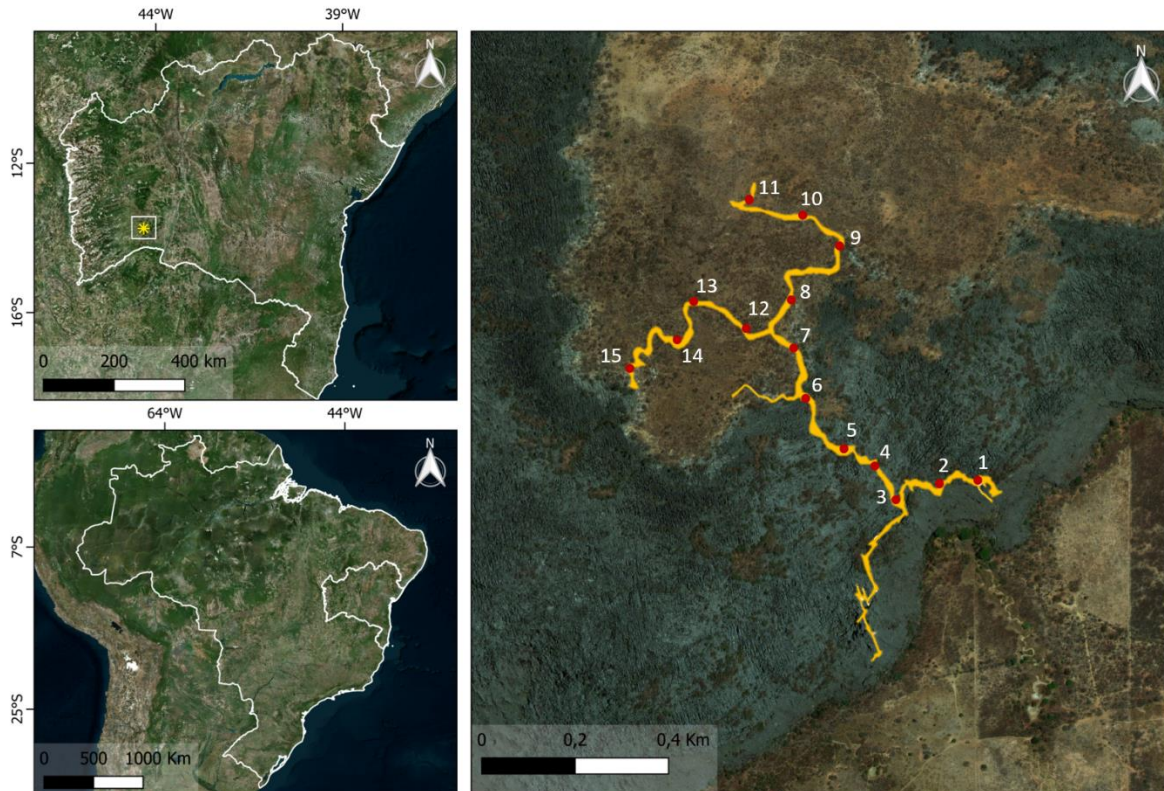
139 We hypothesized that the diversity of substrates on the cave floor (a proxy for  
140 habitat heterogeneity), the trophic resource availability, the distance from the cave  
141 entrance, and microclimatic characteristics (temperature and humidity) would serve as  
142 pivotal factors influencing the variations in invertebrate composition and richness within  
143 the cave ecosystem. Furthermore, we anticipated that ecological-evolutionary categories  
144 would exhibit differential responses to these habitat components. Moreover, we expected  
145 that the influence of habitat components on invertebrates would exhibit variations  
146 contingent on the chosen sampling scale. Finally, we postulated that troglobitic species  
147 would demonstrate a greater occurrence in areas distanced from the cave entrance and  
148 that widely distributed troglobites would exhibit minimal niche overlap.

149

## 150 **2. Material and Methods**

### 151 *2.1. Study site*

152 The study was carried out in Pedro Cassiano Cave (UTM 23L: 617427 - 8474330),  
153 situated in the municipality of Carinhanha, within the western region of Bahia state in  
154 northeastern Brazil. The study area is located within the Serra do Ramalho karst region,  
155 which encompasses a remarkable expanse of karst terrain housing numerous known  
156 caves. This region is particularly notable for the presence of extensive subterranean  
157 hydrological systems that serve as habitats for numerous species that are restricted to cave  
158 environments (Ferreira et al., 2023). The local climate is classified as "Aw" according to  
159 the Köppen-Geiger climate classification system, characterized as tropical with a dry  
160 winter season spanning from March to October (Alvares et al., 2013). The region is part  
161 of the Caatinga biome, which represents the only semi-arid biome in Brazil (Fig. 1)



162

163 Figure 1. Location of the study site in the municipality of Carinhanha (yellow star, within  
 164 the white square), Bahia state, Brazil. The cave map was modified from the original  
 165 survey produced by the team from the Bambui Speleological group  
 166 ([http://www.gsbm.fr/publications/gsbm/2001\\_bahia99/2001\\_OCaste13\\_94\\_Rubbioli.p](http://www.gsbm.fr/publications/gsbm/2001_bahia99/2001_OCaste13_94_Rubbioli.pdf)  
 167 [df](http://www.gsbm.fr/publications/gsbm/2001_bahia99/2001_OCaste13_94_Rubbioli.pdf)), and the red dots represent the sampling areas (quadrants and sectors) within the cave.

168 The Pedro Cassiano cave is situated within carbonate rock formations and spans  
 169 a total length of 2,660 meters (Fig. 2A). It is characterized by the presence of two  
 170 autogenic drainages that traverse the cave, each flowing through separate tributary  
 171 branches in its final section (upstream) (Fig. 2C). These tributaries subsequently converge  
 172 to form a unified drainage system, which emerges at the main cave entrance, which  
 173 represent an intermittent resurgence (Fig. 2B).



174

175 Figure 2. Pedro Cassiano cave and its surroundings: A) Limestone outcrop where the cave  
 176 is located (the yellow arrow indicates the main entrance of the cave); B) Main cave  
 177 entrance; C) Cave inner conduit.

178

## 179 **2.2. Sampling design**

180 The sampling of biotic and abiotic data was conducted at both the mesoscale  
 181 (sectors) and microscale (quadrants) on the cave floor during a single visit to the cave  
 182 (September 21th, 2021). The sampling design involved dividing the cave floor into



183 distinct sectors, each measuring 10x3 meters. Within each sector, three 1m<sup>2</sup> quadrants  
184 were established: two located at the extremities and one positioned in the center. A total  
185 of 15 sectors were sampled along the entire length of the cave, encompassing areas  
186 ranging from proximity to the cave entrance to deeper regions. More information about  
187 sampling design can be found in Souza-Silva et al., (2021).

### 188 ***2.3. Invertebrate sampling and identification***

189 The invertebrate survey was conducted using active visual search and manual  
190 collection techniques, as described by Wynne et al., (2019), using tweezers and brushes.  
191 The sampling of invertebrates followed a sequential approach, beginning with the  
192 quadrats and subsequently extending to the corresponding transects. Three collectors  
193 participated in the sampling process, ensuring thorough coverage, and the sampling effort  
194 continued until all invertebrates were accounted for. Due to the structural differences  
195 across various sampling areas within the cave (e.g., the presence or absence of crevices,  
196 rocks, and ledges), the time required for searching varied among each sampling unit  
197 (Souza-Silva et al., 2021). Additionally, to enhance the detection of cave-restricted  
198 species, direct and intuitive search techniques were employed beyond the designated  
199 sampling units. This approach aimed to ensure comprehensive coverage of all  
200 microhabitats within the cave (Wynne et al., 2019).

201 The collected specimens were carefully preserved in labeled vials filled with a  
202 70% ethanol solution. Subsequently, they underwent sorting procedures under a  
203 stereomicroscope at the Center for Studies in Subterranean Biology (CEBS) of the  
204 Federal University of Lavras (UFLA). The specimens were then identified to the lowest  
205 taxonomic level achievable, employing identification keys, and subsequently categorized  
206 into distinct morphotypes (Oliver and Beattie, 1996). The identification of potentially  
207 troglotic species was conducted by assessing the presence of troglomorphic traits, such  
208 as reduced or absent eyes, depigmentation, and elongated appendages (Christiansen  
209 1962). Furthermore, the expertise of taxonomic specialists in various taxa was sought to  
210 aid in the recognition of specific troglomorphic characteristics (the contributions of these  
211 specialists are acknowledged). Finally, the specimens were deposited in the Collection of  
212 Subterranean Invertebrates of Lavras (ISLA), which is affiliated with the Center for  
213 Studies in Subterranean Biology at the Federal University of Lavras  
214 ([www.biologiasubterranea.com.br](http://www.biologiasubterranea.com.br)).

### 215 ***2.4. Measuring habitat structure***

216 Temperature and air humidity measurements were conducted in each sector using  
217 a thermo-hygrometer positioned near the cave floor. To ensure accurate readings, the  
218 device was allowed to stabilize for approximately 15 minutes within each sector. Each  
219 sector was further divided into 10 one-meter sections, and the percentage of different  
220 substrates and resources (such as guano, animal and plant debris, fungi, fine sediment,  
221 gravel, blocks, speleothems, matrix rock, among others) was visually estimated in each  
222 section, following the methodology employed by Souza-Silva et al, (2021). It is  
223 noteworthy that the same researcher conducted the characterization of all transects to  
224 minimize observer error.

225 In the laboratory, the proportions of each substrate were calculated by summing  
226 the direct measurements from each session, and the arithmetic average of substrate  
227 coverage was determined for the entire sector. At the microscale, digital photographs of  
228 each quadrant (4000 x 3000 pixels) were captured in the field, taken at the researcher's  
229 chest height, using a Canon Powershot SX60HS camera positioned as close as possible  
230 to a 90° angle. These photographs were later analyzed in the laboratory using ImageJ  
231 1.53K software (Ferreira and Rasband, 2012) to characterize and measure the substrates  
232 present.

233 The distances from each transect to the cave's entrance were obtained by plotting  
234 the position of each transect on the cave map, providing a quantitative assessment of their  
235 spatial relationship to the entrance.

### 236 ***2.5. Habitat traits surveyed in each sampling scale***

237 The environmental parameters analyzed at the mesoscale (sectors) were: guano  
238 (GU), plant debris (PD), water (W) - which is a grouping of watercourses (WC) and water  
239 pools (WP), dripping (DP), actinomycetes (ACT), basidiomycetes (BAM), blocks (64-  
240 250 mm) (CB), coarse gravel (17-63 mm) (GRAC), fine gravel (2-16 mm) (GRAF), sand  
241 (SAN), silt (0.2 - 0.05 mm) (SIL), hardpan (HRP), speleothems (SPL), temperature (°C)  
242 (Temp), humidity (HUM), and distance from the cave entrance (m) (DE).

243 At the microscale, the analyzed parameters were guano, roots (ROO), water -  
244 which in this case refers to water pools (WP) - rough rocks (RR), small boulders (SB),  
245 blocks, coarse gravel, fine gravel, sand, silt, hardpan, speleothems, and distance from the  
246 cave entrance.

247 Habitat heterogeneity was assessed in both scales using the Shannon diversity  
248 index (H'). The calculations encompassed measures of substrate diversity (Subs H'),  
249 shelter diversity (Shelter H'), and resource diversity (Resource H') (Pellegrini et al., 2016;

250 Pacheco et al., 2020; Furtado Oliveira et al., 2022; Cardoso et al., 2022). However,  
251 resource diversity was not considered in the microscale analyses as it consistently yielded  
252 a value of zero across almost all the quadrants.

253 To analyze the environmental parameters influencing variations in invertebrate  
254 richness, certain variables were grouped together to reduce the number of predictors and  
255 quantify resource and shelter availability at different scales. At the mesoscale, the variable  
256 "resource" was computed as the sum of proportions of guano, plant debris, actinomycetes,  
257 and basidiomycetes. The variable "shelter" was derived by summing the proportions of  
258 dripstones holes, boulders, coarse gravel, fine gravel, and speleothems. On the other hand,  
259 at the microscale, the variable "resource" was calculated as the sum of guano and roots,  
260 while the variable "shelter" was determined by summing rough rocks, small boulders,  
261 blocks, coarse gravel, fine gravel, and speleothems (Supplementary material I)

262 Lastly, we investigated the potential linear relationship between the  
263 environmental parameters and the distance from the cave entrance. This analysis aimed  
264 to provide insights into the arrangement of the physical, microclimatic, and trophic  
265 components within the Pedro Cassiano Cave along the surface-to-deeper areas gradient.

## 266 **2.6. Data analysis**

267 The richness and abundance of invertebrates were determined by quantifying the  
268 number of individuals and morphotypes in each sample unit, comprising 15 sectors and  
269 45 quadrants. It is important to note that the sectors' richness encompassed the species  
270 found in both the sectors *per se* and their respective quadrants.

271 To investigate the key components of habitat structure influencing variations in  
272 overall species composition, troglobitic species composition, and non-troglobitic species  
273 composition, we employed a DistLM (distance-based linear model) analysis at both  
274 sampling scales. Prior to the analysis, the species abundance values were standardized  
275 through a square root transformation. Subsequently, a similarity analysis was conducted  
276 using the Bray-Curtis index to assess the resemblance between sampling points. Model  
277 selection was performed using the AICc (Akaike Information Criterion corrected for  
278 small sample size) with the Forward procedure, which identifies the best models based  
279 on the lowest criterion value (Anderson et al., 2008). The software Primer 7 &  
280 Permanova® was utilized for conducting these analyses.

281 We employed Generalized Linear Models (GLM) to investigate the components  
282 of habitat structure that contribute to variations in overall species richness, troglobitic  
283 species richness, and non-troglobitic species richness. Due to limited troglobitic species

284 at the microscale, our focus was solely on overall species richness at this scale. To ensure  
285 the reliability of our analysis, we first examined collinearity among all predictor variables  
286 using the Spearman correlation index, via 'CHART.CORRELATION' function of the  
287 'PERFORMANCEANALYTICS' package (Peterson and Carl, 2018). Predictor variables  
288 exhibiting correlation values exceeding 70% ( $r^2 \geq 0.7$ ) were assessed separately (Schober  
289 et al., 2018).

290 For each type of richness, we initially generated six GLM models with the Poisson  
291 distribution family at the mesoscale (whose models do not present variance inflation  
292 factors). Utilizing the forward stepwise procedure, we successively eliminated predictors  
293 with the highest p-value until only significant predictors remained within each of the six  
294 models. Finally, for each type of richness, we selected the model with the lowest Akaike  
295 information criterion (AIC). In each model, we assessed the presence of overdispersion  
296 using the 'SIMULATIONOUTPUT' function from the 'DHARMA' package (Hartig, 2022). In  
297 these cases, a change was made to the distribution for a better fit, which was the negative  
298 binomial distribution. At the microscale, none of the predictors exhibited collinearity,  
299 allowing the construction of a Poisson Generalized Linear Model (GLM) that  
300 incorporated all the measured environmental variables at this scale. However, the initial  
301 model displayed multicollinearity issues indicated by elevated variance inflation factors  
302 (VIF). To tackle this challenge, predictors surpassing a VIF threshold of 10 were  
303 eliminated from the model, and a fresh set of variables was introduced for reanalysis. As  
304 a result, three alternative Poisson GLM models were generated, effectively alleviating  
305 concerns of collinearity and multicollinearity. These refined models underwent the same  
306 procedure outlined for the mesoscale analysis.

307 To assess whether troglobitic species exhibit a greater prevalence in the deeper  
308 regions of the cave, a simple linear regression analysis was conducted. Prior to this  
309 analysis, we performed a Shapiro-Wilk test using the 'SHAPIRO.TEST' function from the  
310 'STATS' package to confirm that the residuals followed a normal distribution.

311 In the regression analysis, the distance from the cave entrance was chosen as the  
312 predictor variable, while the proportion of troglobitic richness in relation to the  
313 invertebrate richness within each sector was considered as the response variable. It is  
314 important to highlight that, since certain sectors exclusively contained troglobitic species,  
315 we had to employ an alternative ratio instead of the troglobitic richness/non-troglobitic  
316 richness ratio utilized in the study by Souza-Silva et al., (2021).

317 The ecological niches of selected troglobitic species (those occurring in at least  
318 three sectors) were assessed using the Outlying Mean Index (OMI) analysis (Dolédec et  
319 al., 2000) conducted with the 'ade4' package (Dray and Dufour, 2007). The OMI analysis  
320 allowed us to position each species in a two-dimensional Euclidean space, decomposing  
321 their distribution patterns into marginality, tolerance, and residual tolerance. Species  
322 marginality represents the distance between the average environmental conditions used  
323 by the species and the overall average conditions. Species are positioned based on their  
324 deviation from a reference species (ubiquitous species occurring under all available  
325 habitat conditions). Lower values of marginality indicate a broader occurrence of the  
326 species within the studied environment. Tolerance reflects the variation in environmental  
327 conditions used, with higher values suggesting generalist species and lower values  
328 indicating specialist species. Residual tolerance provides insight into the reliability of the  
329 identified niche (Dolédec et al., 2000).

330 To execute the OMI analysis, we initially conducted a principal component  
331 analysis (PCA) using the mesoscale habitat structure components. Subsequently, we  
332 calculated and plotted the niche of each of the six selected species within the  
333 environmental niche. Finally, we employed the Monte Carlo test, employing 999  
334 permutations, to evaluate the significance of the difference between the observed species'  
335 marginality and simulated values (Dolédec et al., 2000).

336

### 337 **3. Results**

#### 338 ***3.1. Richness and composition of cave fauna***

339 Through all sampling methods employed, a total of 976 invertebrate specimens  
340 and one vertebrate were documented within Pedro Cassiano cave (excluding bats, which  
341 were not included in the count). These organisms were identified as belonging to 56  
342 species, spanning at least 40 families (Supplementary material II). Among these species,  
343 20 exhibited troglomorphic traits, and were considered as cave-restricted. The troglobitic  
344 species encompassed various taxa, including Amblypygi (*Charinus troglobius* - Baptista  
345 and Giupponi, 2002), Araneae (Ochyroceratidae, Oonopidae and Pholcidae), Opiliones  
346 (*Giupponia chagasi* - Pérez-González and Kury, 2002), Palpigradi (*Eukoenia* sp.),  
347 Pseudoscorpiones (*Pseudochthonius koinopolitea* - Prado and Ferreira, 2023), Blattodea,  
348 Coleoptera (Carabidae: Clivinina - 2 species), Entomobryomorpha (*Trogolaphysa* sp. - 2  
349 species), Neelipleona, Orthoptera (*Endecous infernalis* - Carvalho et al., 2023),



350 Symphypleona, Isopoda (*Xangoniscus* sp. and *Pectenoniscus* sp.), Polydesmida  
 351 (Oniscodesmidae), Mollusca: (*Spiripockia* sp.) and Siluriformes (*Trichomycterus*  
 352 sp.)(Figure 3).



353

354 Figure 3. Some troglobites species from the Gruna do Pedro Cassiano cave: A) *Giupponia*  
 355 *chagasi* (Opiliones: Gonyleptidae); B) *Charinus troglobius* (Amblypygi: Charinidae); C)  
 356 Araneae sp4 (Araneae: Oonopidae); D) *Eukoenenia* sp. (Palpigradi: Eukoeneniidae); E)  
 357 *Pseudochthonius koinopoliteia* (Pseudoscorpiones: Chthoniidae); F) Ochyroceratidae  
 358 (Araneae); G) *Metagonia* sp. (Araneae: Pholcidae); H) *Clivinina* sp1 (Coleoptera); I)  
 359 *Clivinina* sp2 (Coleoptera); J) *Endecous infernalis* (Ensifera, Phalangopsidae); K)

360 Blattidae (Blattodea); L) *Trogolaphysa* sp1 (Collembola: Paronellidae); M) Neelipleona  
 361 (Collembola); N) Oniscodesmidae sp1 (Polydesmida); O) *Xangoniscus* sp. (Isopoda:  
 362 Styloniscidae); P) *Pectenoniscus* sp. (Isopoda: Styloniscidae); Q) *Trychomycterus* sp.  
 363 (Silurifomes: Trichomycteridae).

364 Within the 15 sampled sectors, we documented a total of 41 invertebrate species,  
 365 including 13 troglobitic species and 28 non-troglobitic species. In the quadrants, we  
 366 recorded 15 invertebrate species, with 5 being troglobitic and 10 non-troglobitic.  
 367 Considering the significant number of cave-restricted species discovered in Pedro  
 368 Cassiano cave (20 species), we propose that this cave be recognized as a new hotspot of  
 369 subterranean biodiversity in South America (see discussion for further details).

### 370 **3.2. Richness, composition, and distribution of cave invertebrates**

371 At the mesoscale, substrate diversity emerged as a crucial environmental  
 372 parameter influencing the variation in overall invertebrate composition (AICc = 121.48;  
 373  $R^2 = 0.1554$ ;  $p = 0.014$ ), troglobitic species composition (AICc = 118.15;  $R^2 = 0.1548$ ;  $p$   
 374  $= 0.032$ ), and non-troglobitic species composition (AICc = 100.94;  $R^2 = 0.2012$ ;  $p =$   
 375  $0.004$ ). Additionally, at this sampling scale, sand was identified as the variable that best  
 376 explained the variation in both overall invertebrate species richness and troglobitic  
 377 richness (Table 1) (Fig. 4 A, B). Conversely, resource availability and shelter availability  
 378 were found to be more effective in explaining the variation in non-troglobitic richness  
 379 (Table 1) (Fig. 4 C, D).

380

381 Table 1. GLM models selected to explain the variation of richness in the mesoescale. S  
 382 (invertebrate richness), S-t (troglobite richness), S-nt (non-troglobite richness), SAN (%  
 383 sand), subs H' (diversity of substrate), HRP (% hardpan), shelter H' (diversity of shelter),  
 384 W (% of areas with bodies of water), HUM (humidity), PseudoR<sup>2</sup> (percentage of  
 385 explanation of models)

386

Model: S ~ SAN + subs H' + shelter H', family = negative binomial				
PseudoR <sup>2</sup> = 0,6630				
Variables	Estimate	Std.Error	Zvalue	Pr(> z )
Intercept	1,258443	0,528028	2,383	0,017159
SAN	-0,02088	0,6143	-3,399	0,000676*
subs H'	0,822354	0,48044	1,712	0,086957
shelter H'	0,438641	0,25902	1,693	0,090368

Model: S-t ~ HRP + SAN + shelter H', family = negative binomial				
PseudoR <sup>2</sup> = 0,6695				

Variables	Estimate	Std.Error	Zvalue	Pr(> z )
Intercept	1,14254	0,303031	3,77	0,000163
HRP	0,006742	0,004127	1,634	0,102339
SAN	-0,02203	0,008909	-2,473	0,013408*
shelter H'	0,05683	0,308535	1,842	0,065485

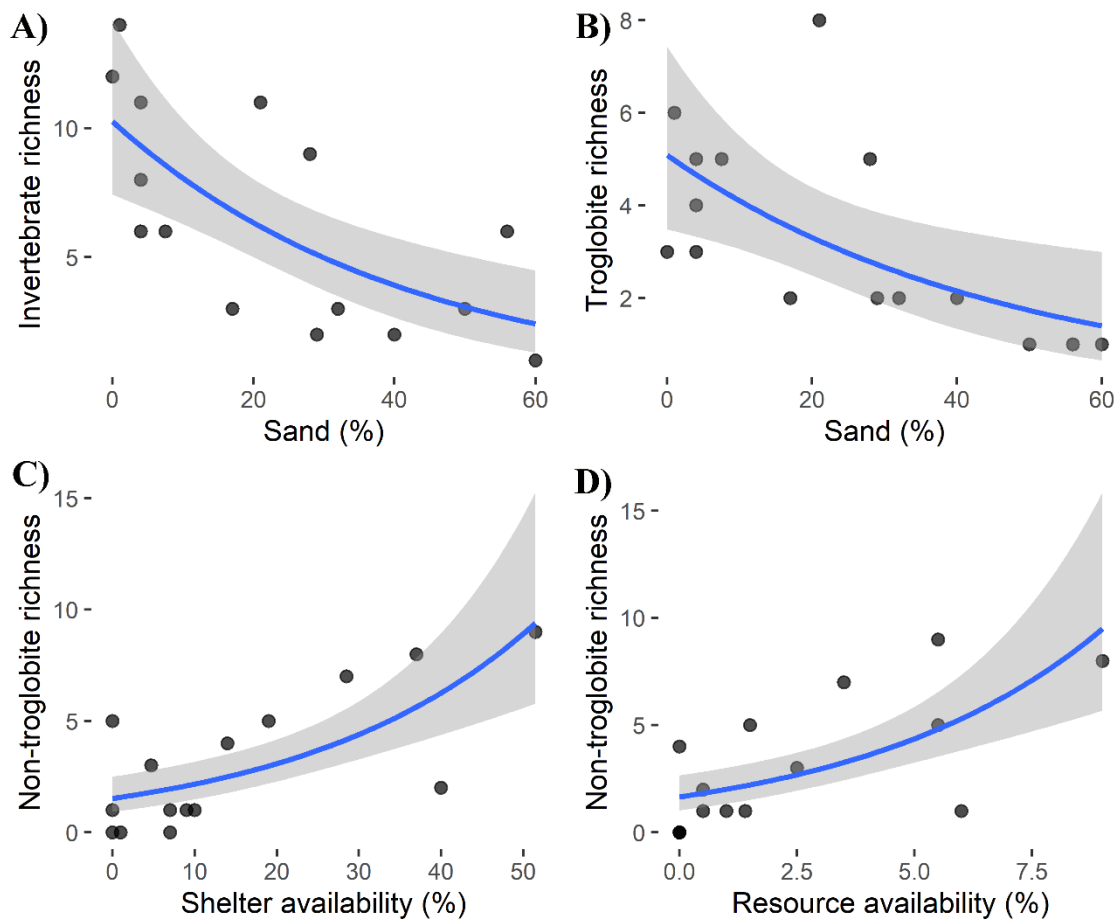
Model: S-nt ~ resource + HUM + shelter + W, family = poisson

PseudoR<sup>2</sup> = 0,6428

Variables	Estimate	Std.Error	Zvalue	Pr(> z )
Intercept	-9,21993	5,71600	-1,613	0,10674
resource	0,19486	0,07464	2,611	0,00904*
HUM'	0,09686	0,05763	1,681	0,09285
Shelter	0,04177	0,01368	3,053	0,00226*
W	-0,15305	0,09342	-1,638	0,10139

387

388



389

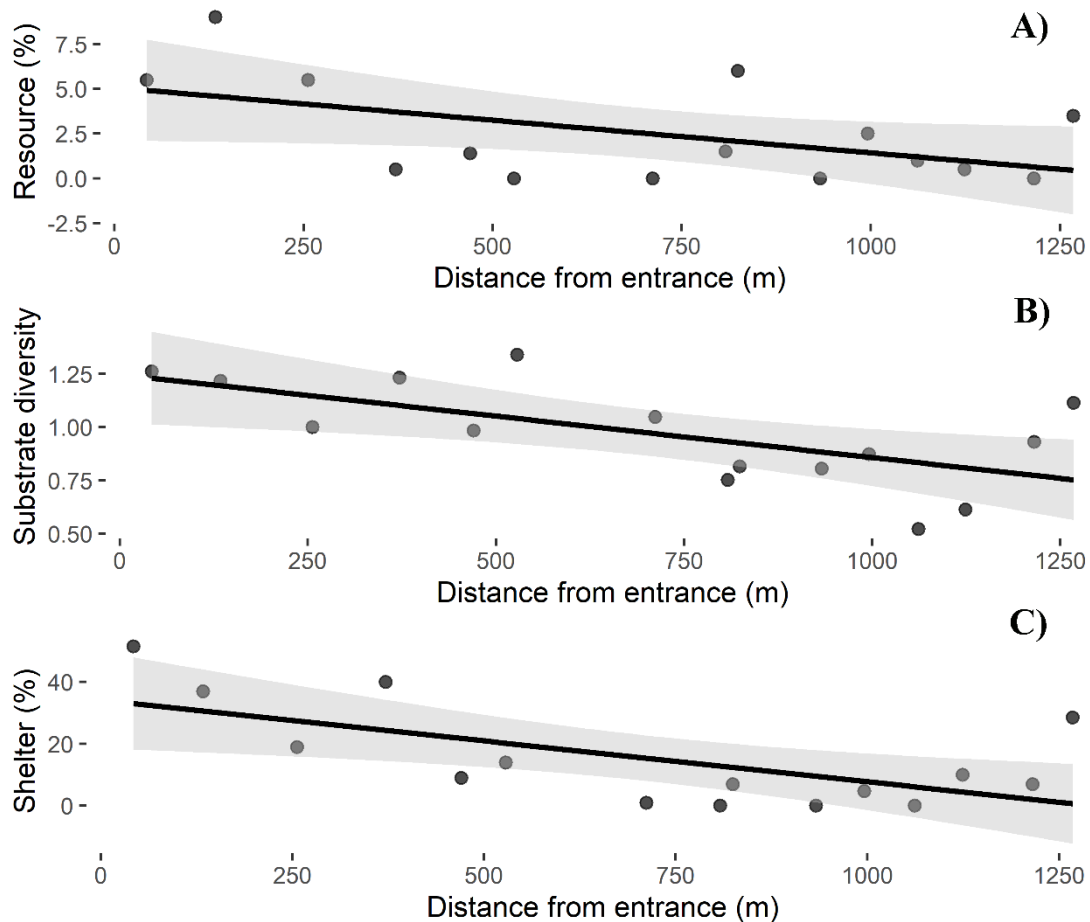
390 Figure 4. Relationship between the invertebrate richness with the percentage of sand on the  
 391 mesoscale (A), the relationship between the troglobitic species richness with the  
 392 percentage of sand on the mesoscale (B), the relationship between the non-troglobitic

393 species richness with the shelter availability (C) and percentage of food resource at the  
 394 mesoscale (D).

395

396 The Pedro Cassiano Cave displayed gradients along its length. Notably, resource  
 397 availability ( $F_{1,13}= 4.664$ ;  $R= 0.455$ ;  $p= 0.05$ ), shelter availability ( $F_{1,13}= 48.846$ ;  $R=$   
 398  $0.599$ ;  $p= 0.01$ ), and substrate diversity ( $F_{1,13}= 9.003$ ;  $R= 0.603$ ;  $p= 0.01$ ) were observed  
 399 to decrease as the cave extended into deeper zone (Fig. 5).

400



401

402 Figure 5. Relationship between percentage of resource availability (A), Substrate  
 403 diversity (B), and percentage of Shelter (C), with distance from the cave entrance (m).

404

405 At the microscale, however, none of the predictor variables employed in this study  
 406 were able to account for the variation in composition. It is plausible that other unexamined  
 407 variables play a significant role in community structuring at this sampling scale.  
 408 Nonetheless, water (puddles), sand, substrate diversity, and shelter were identified as  
 409 effective in explaining the variation in total richness (Table 2) (Fig. 6).

410

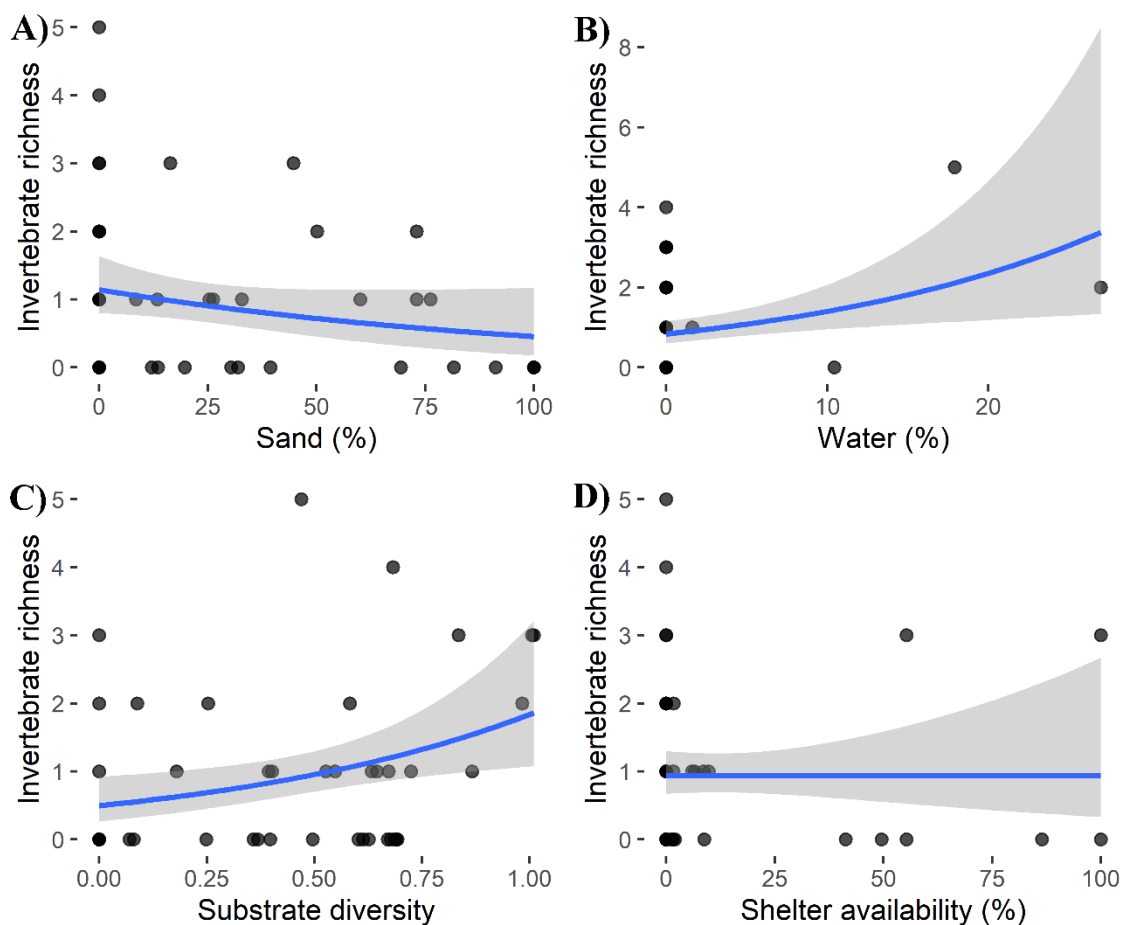
411

412

413 Table 2. GLM model selected to explain the variation of invertebrate richness in  
 414 microscale. SAN (% sand), subs H' (diversity of substrate), HRP (% hardpan), shelter H'  
 415 (diversity of shelter), W (% of areas with bodies of water), PseudoR<sup>2</sup> (percentage of  
 416 explanation of models).  
 417

Model: $S \sim W + SAN + \text{subs } H' + \text{shelter} + \text{shelter } H'$ , family = poisson				
PseudoR <sup>2</sup> = 0,3728				
Variables	Estimate	Std.Error	Zvalue	Pr(> z )
Intercept	-0,52589	0,330073	-1,593	0,1111
W	0,065152	0,020709	3,146	0,00166*
SAN	-0,02322	0,007063	-3,288	0,00101*
subs H'	1,945431	0,60503	3,215	0,0013*
shelter	-0,02601	0,012641	-2,058	0,03962*
shelter H'	2,326601	1,19245	1,951	0,05104

418

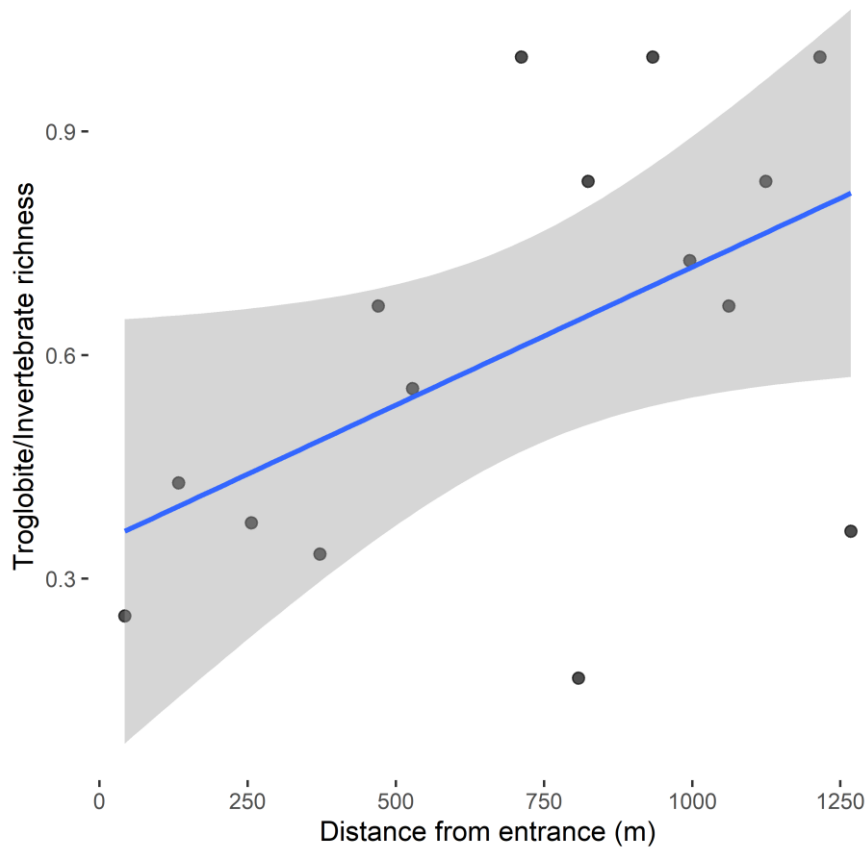


419

420 Figure 6. Relationship between the invertebrate richness with the percentage of sand (A),  
 421 percentage of area with water bodies (B), diversity of substrates (C), and percentage of  
 422 shelter (D) at the microscale.

423

424 The linear regression analysis revealed a clear trend indicating an increase in  
 425 troglobitic species richness as non-troglobitic species richness decreased towards the  
 426 deeper regions of Pedro Cassiano cave ( $F_{1,13} = 4.746$ ;  $R = 0.517$ ;  $p = 0.0483$ ) (Fig. 7).



427

428 Figure 7. The relationship between cave entrance distance and troglobitic species  
 429 richness/total invertebrate richness ratio.

430

### 431 3.3. Habitat selection of some cave-restricted species

432 The OMI analysis encompassed six troglobitic species: *Xangoniscus* sp.1  
 433 (isopod), *Giupponia chagasi* (harvestman), Ochyroceratidae sp.1 (spider), *Trogolaphysa*  
 434 sp.1 (springtail), *Eukoenenia* sp.1 (palpigrade), and *Charinus troglobius* (whip spider).  
 435 Among these species, only *Xangoniscus* sp.1 exhibited a significant result in the  
 436 permutation test (Table 3), indicating that it deviates from the average habitat conditions  
 437 compared to the other species. Thus, it can be considered the most specialized species,  
 438 showing a strong association with the presence of streams and water pools, high  
 439 temperatures, and locations featuring a greater diversity of shelters (Fig. 8).

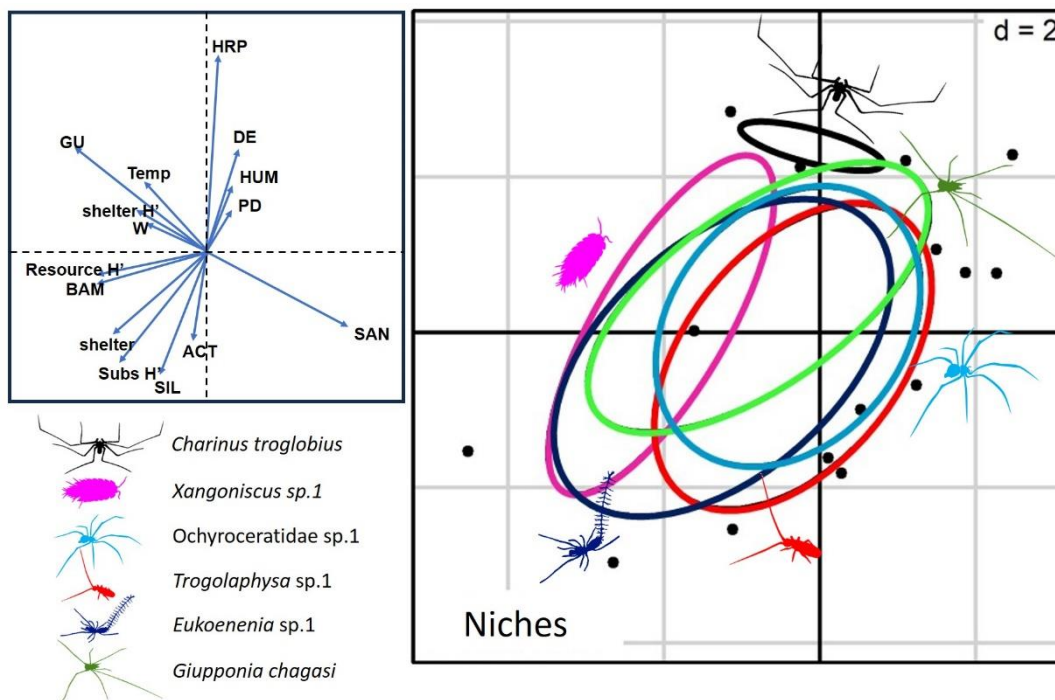
440



441 Table 3. Results of Outlying Mean Index (OMI) analysis for the most widespread  
 442 troglobitic species in cave Pedro Cassiano.  
 443

Troglobitic species	Inertia	OMI	Tol	Rtol	P value
<i>Charinus troglobius</i>	14,71	5,93	0,35	8,41	0,15
<i>Giupponia chagasi</i>	16,08	2,28	2,45	11,34	0,657
Ochyroceratidae sp.1	14,48	0,41	1,22	12,83	0,678
<i>Eukoenenia</i> sp.1	18,56	2,16	4,14	12,26	0,406
<i>Trogolaphysa</i> sp.1	16,35	0,32	4,78	11,24	0,175
<i>Xangoniscus</i> sp.1	21,52	4,38	1,29	15,84	0,02*
OMI mean		2,58			0,109

444



445

446 Figure 8. Outlying Marginality Index (OMI) showing species habitat selecting according  
 447 to habitat components of cave floor. Hardpan (HRP), distance for entrance (DE), humidity  
 448 (HUM), plant debris (PD), sand (SAN), Actinomycetes (ACT), Silt (SIL), substrate  
 449 diversity (Subs H'), Basidiomycetes (BAM), resource diversity (Resource H'), water  
 450 body (W), shelter diversity (shelter H'), guano (GU), temperature (temp).

451

452

453 The remaining species, while utilizing average habitat conditions, displayed  
 454 varying degrees of generalism. The species *Giupponia chagasi*, *Ochyroceratidae* sp.1,  
 455 *Trogolaphysa* sp.1, and *Eukoenenia* sp.1 were more generalist than *Charinus troglobius*.  
 456 The latter species exhibited a high marginality value and a low tolerance value, suggesting

457 a potential preference for deeper locations characterized by high humidity and the  
458 presence of hardpan clay.

459 It is important to acknowledge that the interpretation of these results should  
460 consider the limited occurrence of *Giupponia chagasi* (found in four sectors: 2, 6, 9, and  
461 10) and *Charinus troglobius* (found in three sectors: 8, 9, and 10), which may introduce  
462 some degree of uncertainty and susceptibility to random fluctuations. However,  
463 considering the overall conditions of the cave environment, where troglobitic species are  
464 typically scarce, these findings provide valuable insights into the habitat preferences of  
465 these species.

466

#### 467 **4. Discussion**

468 The results of our study revealed that both habitat heterogeneity and the presence  
469 of specific substrates significantly influenced the community structure of invertebrates  
470 inhabiting the Pedro Cassiano cave. These findings are consistent with previous studies  
471 that have emphasized the importance of habitat heterogeneity in shaping the structure of  
472 invertebrate communities across different caves (Bregović and Zigmajster, 2016;  
473 Pacheco et al., 2020; Reis-Venâncio et al., 2022; Cardoso et al., 2022) and even within  
474 the same cave (Prous et al., 2015; Kozel et al., 2019; Furtado Oliveira et al., 2022).

475 Additionally, our study demonstrated that substrate diversity played a prominent  
476 role in explaining variations in invertebrate composition at the mesoscale within the Pedro  
477 Cassiano cave. Interestingly, even though the distance from the entrance did not  
478 significantly influence the invertebrate communities, sectors characterized by higher  
479 substrate diversity, which were found closer to the cave entrance displayed greater  
480 dissimilarity in comparison to sectors with lower substrate diversity, particularly in the  
481 deeper regions of the cave.

##### 482 ***4.1. Habitat traits determining the richness and composition of cave invertebrates.***

483 Caves exhibit various environmental gradients, including the widely recognized  
484 light availability zones such as the photic, disphotic, and aphotic zones. In the case of  
485 Pedro Cassiano cave, additional gradients are observed, with a decline in resource  
486 availability and habitat heterogeneity as one moves towards deeper areas. Concurrently,  
487 the microclimatic conditions, including temperature and humidity, demonstrate relative  
488 stability in the deeper regions compared to the entrance areas. Interestingly, these  
489 conditions appear to contribute to variations in the composition and richness of



490 invertebrate communities, as already observed for other cave systems, in both tropical  
491 and temperate zones (Novak et al., 2012; Prous et al., 2015).

492         It is important to highlight that the distance from the entrance did not have a  
493 significant impact on the structuring of invertebrate communities in the Pedro Cassiano  
494 cave, which contradicts initial expectations. This can be attributed to two main factors.  
495 Firstly, the first sector sampled was located approximately 40 meters from the cave  
496 entrance, resulting in a reduced influence of epigeal species and limited variation in  
497 species composition and richness along the distance gradient. Additionally, the cave's  
498 drainages are autogenic, originating from the end of the cave and flowing towards the  
499 entrance. Consequently, the availability of organic resources brought by these drainages  
500 is limited. Although there is a gradual decline in resource availability towards deeper  
501 areas, it is not as pronounced as in caves with allogenic drainages, where there is a  
502 substantial reduction in organic resources from the entrance to deeper regions. Therefore,  
503 the relatively modest decrease in resource availability along the surface-to-deeper  
504 gradient may have contributed to the observed pattern in this cave. While these findings  
505 contradict some previous studies (Novak et al., 2012; Furtado-Oliveira et al., 2022), it is  
506 evident that the distance from the entrance still influences species distribution in most  
507 tropical caves, as trophic resource availability and habitat heterogeneity decrease from  
508 the entrance to deeper areas (Souza-Silva et al., 2021).

509         The influence of factors on species distribution and diversity is known to vary  
510 across different spatiotemporal scales (Bregovic & Zigmajster, 2016). However, there  
511 are certain factors that cannot be adequately assessed at specific scales, posing challenges  
512 in such studies (González-Magias et al., 2007). In subterranean environments, limited  
513 research has been conducted on the influence of spatial scale on terrestrial community  
514 structure (Pellegrini et al., 2016; Pacheco et al., 2020b; Furtado-Oliveira et al., 2022).

515         In our study, we examined variations in the structure of invertebrate communities  
516 using two sampling scales. Interestingly, substrate diversity was found to be a significant  
517 variable only at the mesoscale. This may be attributed to the low number of species  
518 encountered at the microscale, particularly troglobitic species. It is important to note that  
519 sampling bias can play a determinant role in results when investigating different scales,  
520 especially when dealing with highly spatially restricted scales. In cave environments,  
521 which are known to impose restrictive conditions on most species, understanding the  
522 effects of habitat structure on invertebrate communities at micro spatial scales can be  
523 challenging, primarily due to the limited availability of suitable specimens for analysis.

524           Nevertheless, certain variables proved to be influential in determining invertebrate  
525 richness at the microscale. Specifically, the percentage of areas occupied by water bodies  
526 (W) exhibited a significant positive relationship with richness. This finding aligns with  
527 the preferences of subterranean species for microhabitats characterized by high humidity  
528 (Tobin et al., 2013; Mammola et al., 2015, 2016). Water presence plays a crucial role in  
529 maintaining adequate humidity levels at this scale, thereby providing favorable conditions  
530 for a greater number of subterranean species, particularly troglobitic species with thinner  
531 cuticles that rely on moisture to prevent desiccation (Tobin et al., 2013; Kozel et al., 2019;  
532 Souza-Silva et al., 2021).

533           Few studies have investigated how habitat structure can affect troglobitic and non-  
534 troglobitic species separately (Pacheco et al., 2020b; Mammola et al. 2020b; Furtado  
535 Oliveira et al., 2022). Our analyses revealed that substrate diversity had a stronger  
536 influence on species composition variation for non-troglobitic species compared to  
537 troglobitic species, although the differences observed were subtle. Furthermore, only the  
538 species richness of non-troglobitic species was affected by shelter availability, an  
539 environmental parameter related to habitat heterogeneity (Reis-Venâncio et al., 2022).  
540 This suggests that the pronounced specializations of troglobitic species obviates the  
541 necessity for diverse sheltering strategies observed in non-troglobitic species. This  
542 divergence is potentially attributable to the heightened physiological adaptations of  
543 troglobitic species, affording them to thrive in several distinct cave microclimatic  
544 conditions. Conversely, the physiological constraints of non-troglobitic species likely  
545 prompt them to seek out a range of sheltering options. This selection process appears to  
546 be contingent upon the prevailing microclimatic conditions specific to each distinct cave  
547 area.

548           Caves are recognized as oligotrophic environments, primarily relying on external  
549 inputs of resources (Souza-Silva et al., 2012). Guano deposits and plant debris carried by  
550 water are considered the primary energy sources in tropical caves (Ferreira et al., 2007;  
551 Souza-Silva et al., 2012). Furthermore, these organic inputs can also provide suitable  
552 habitats for certain species (Ferreira et al., 2007). Consequently, intense intra- and  
553 interspecific competition for these limited organic resources becomes a crucial factor in  
554 shaping cave communities (Culver et al., 1991; Schneider et al., 2011; Culver and Pipan,  
555 2015).

556           In our study, we found a positive and significant relationship between resource  
557 availability and the richness only for the non-troglobitic species. Such species are

558 generally less specialized compared to the cave-restricted species, and therefore, they  
559 have higher energy demands. Consequently, areas with greater resource availability are  
560 more likely to support a higher diversity of non-troglobitic species. On the other hand,  
561 cave-restricted species exhibit high specialization, including an enhanced tolerance to  
562 starvation (Kozel et al., 2023). This adaptability enables them to persist in highly  
563 oligotrophic areas, which may explain why we did not identify resource availability as a  
564 significant factor influencing the richness of troglobitic species. Furthermore, non-  
565 troglobitic species have a competitive advantage over troglobitic species (Sket, 1999),  
566 leading the latter to avoid resource-rich areas within the caves. Deharveng and Bedos,  
567 (2000) also observed that troglobitic invertebrates prefer areas farther away from trophic  
568 resources to minimize competition with non-troglobitic species. Studies focusing on  
569 guano communities in Neotropical caves have also demonstrated that the establishment  
570 of large populations of non-troglobitic species in guano piles can displace troglobitic  
571 species (Ferreira et al., 2007).

572 Substrate diversity exhibited a significant and positive correlation with  
573 invertebrate richness. Heterogeneous sites offer a variety of organic resources, refuges,  
574 shelters, and microclimatic conditions that promote niche differentiation and support a  
575 greater number of coexisting species (Poulson and Culver 1969; Bregovic and  
576 Zagnajster, 2016). Conversely, more homogeneous habitats tend to have the opposite  
577 effect on communities. For instance, the presence of a higher percentage of sand in the  
578 sectors had a negative impact on invertebrate richness, emphasizing the importance of  
579 habitat heterogeneity for the cave fauna in the Pedro Cassiano cave.

580 At both sampling scales, an increase in sand content led to a decrease in species  
581 richness, indicating a scale-invariant relationship. Specifically, we observed a decline in  
582 species richness with each 7.5% rise in sand content within the sectors. Fine substrates  
583 like sand create a more homogeneous environment that does not provide favorable  
584 conditions for supporting a diverse invertebrate community (Reis-Venâncio et al., 2022).  
585 Additionally, we found that troglobitic species richness also declined with increasing sand  
586 content in the sectors. This suggests that areas with diverse microhabitats, characterized  
587 by the overlapping of rocks and sediments, can provide suitable microclimatic conditions  
588 for these specialized species (Mammola et al., 2016).

589 Finally, at the microscale, the availability of shelters exhibited an unexpected  
590 negative relationship with richness, contradicting previous studies that proposed a  
591 positive association between shelter availability and habitat heterogeneity (Reis-

592 Venâncio et al., 2022). It is important to note, however, that despite the significant  
593 relationship observed in the model, the estimate value is quite low, thus indicating a very  
594 weak relation between such variables. It was observed that many quadrants within the  
595 Pedro Cassiano Cave lacked shelters entirely, and those that did contain shelters typically  
596 had a maximum of three distinct substrates. In this particular case, the limited diversity  
597 of available shelters represents a less varied microhabitat, incapable of supporting a  
598 greater number of species. This interpretation is reinforced by the relationship between  
599 shelter diversity and species richness, which was almost statistically significant, and  
600 displayed a positive estimate, suggesting a potential increase in species numbers within  
601 quadrants with higher shelter diversity index values.

#### 602 **4.2. Invertebrates' distribution**

603 Areas closer to cave entrances typically harbor ecotonal fauna, which relies on an  
604 environment with diverse substrate types and a greater abundance of shelters and organic  
605 resources (Prous et al., 2015). These communities are typically composed of epigean  
606 edaphic, troglone, and troglophilic species, collectively referred to as "para-epigean"  
607 communities (Prous et al., 2004, 2015). However, as one moves away from the entrance,  
608 these characteristics and species composition change and only those species with a  
609 stronger affinity for subterranean habitats are able to establish themselves. Consequently,  
610 in the deeper areas of the cave, troglophilic and troglotic species prevail (Kozel et al.,  
611 2019).

612 These characteristics of strictly subterranean species described so far limit their  
613 distribution to more stable areas within caves. Just as the scarcity of organic resources  
614 and the reduction of substrate diversity influence the distribution of non-troglotic  
615 species (Sket, 1999), which decreases richness towards deeper zones (Souza-Silva et al.,  
616 2021). Thus, in many caves, there is a low spatial interspecific overlap between the eco-  
617 evolutionary categories (Sket, 1999; Novak et al., 2012; Souza-Silva et al., 2021). In areas  
618 closer to the entrance, when climatic stability occurs, troglotic and non-troglotic  
619 species can even coexist (Kozel et al., 2019), but in deeper zones, troglotes prevail.  
620 Through our analyses, we reinforce this hypothesis of invertebrate distribution in  
621 Neotropical caves.

622

#### 623 **4.3. Habitat selection**

624 Souza-Silva et al. (2021) conducted a niche analysis of ten troglotic species  
625 within the Água Clara cave system (ACCS), which is situated in the same geographical

626 area as our present study. Many of the species investigated by them also inhabit the Pedro  
627 Cassiano cave, allowing for meaningful comparisons. Several predatory species,  
628 including Ochyroceratidae sp.1, *Eukoenenia* sp.1, and *Giupponia chagasi*, demonstrated  
629 a notable tolerance for the diverse environmental conditions present on the cave floor.  
630 This adaptability likely arises from their necessity to hunt for prey in oligotrophic  
631 environments. The whip spider species *Charinus troglobius* exhibited a lower degree of  
632 generalism compared to the other predators, showing a subtle preference for deeper  
633 locations (Baptista and Giupponi, 2002), with high humidity and the presence of hardpan.

634         However, it is important to highlight that certain distinctions emerged between the  
635 two cave systems. For instance, *Giupponia chagasi* displayed a niche that was more  
636 closely linked to factors such as moisture content and distance from the cave entrance in  
637 the ACCS. In contrast, in the Pedro Cassiano cave, this species' niche appeared to be more  
638 influenced by variables like shelter availability and resource availability, including guano.  
639 The paligrade *Eukoenenia* sp.1 exhibited a niche determined by moisture content and  
640 distance from the cave entrance in the ACCS. Conversely, in the Pedro Cassiano cave,  
641 the species' niche leaned more towards shelter availability and substrate diversity. The  
642 springtail *Trogolaphysa* sp. 1 (referred to as Entomobryomorpha sp. 1 in the ACCS)  
643 displayed a niche defined by shelter availability and substrate diversity in the former cave.  
644 In the Pedro Cassiano cave, the species appeared to adopt a more generalized niche. The  
645 isopod *Xangoniscus* sp.1 had its niche largely determined by temperature in both cave  
646 systems. However, in the Pedro Cassiano cave, additional factors like guano, water and  
647 the diversity of shelters also played significant roles in shaping its niche. Lastly, only the  
648 Ochyroceratidae sp.1 (the same species in both caves) presented similar niches, being  
649 generalist in both cave systems.

650         These disparities observed between the two cave systems underscore the fact that  
651 the same species can occupy distinct niches contingent upon the specific cave  
652 environment. It is important to highlight that this information not only represents an  
653 innovative contribution to the field but also holds significant implications for  
654 conservation efforts, particularly given the scarcity of studies pertaining to the niches of  
655 cave-restricted species.

656

#### 657 **4.4. Why is Pedro Cassiano cave so rich in cave restricted species?**

658         Ferreira et al. (2023) proposed that caves experiencing flood pulses during rainy  
659 periods could maintain a high diversity of species. The possible explanation for this

660 pattern is based on the intermediate disturbance hypothesis (IDH) (Dial and  
661 Roughgarden, 1998). In this context, flood pulses seasonally alter the cave floor  
662 substrates, creating a type of disturbance that can be classified as intermediate. This  
663 disturbance prevents dominant species from establishing themselves by temporarily and  
664 partially modifying the microhabitats, allowing different stages of ecological succession  
665 to coexist after the flood pulse. This process fosters long-term coexistence and,  
666 consequently, biodiversity.

667 Pedro Cassiano Cave exhibits seasonal flood pulses and presents a high richness  
668 of troglobitic species, as observed in the Água Clara cave system. As argued by Ferreira  
669 et al. (2023), the theory of intermediate disturbance could also provide a potential  
670 explanation for the maintenance of a substantial number of troglobites in this particular  
671 cave. Furthermore, through OMI analysis, we revealed the presence of specialist  
672 troglobitic species and troglobitic species with varying degrees of generalism, which  
673 aligns with the findings of Souza-Silva et al., (2021), supporting our hypothesis that there  
674 is not a high niche overlap among them.

675 Lastly, it is worth emphasizing the significant overlap of cave-restricted species  
676 between the Pedro Cassiano cave and the Água Clara cave system (ACCS). Out of the 20  
677 cave-restricted species identified in the Pedro Cassiano Cave, a remarkable 15 are also  
678 present in the ACCS. This finding underscores the likelihood that both systems have been  
679 subjected to similar ecological and evolutionary pressures and conditions, ultimately  
680 resulting in a notable abundance of cave-restricted taxa.

#### 681 ***4.5. Conservation issues***

682 It is crucial to underscore the impressive number of troglobites discovered within  
683 Pedro Cassiano Cave, especially when considering that this abundance was determined  
684 from a single collection effort. The number of species exclusive to the cave environment  
685 observed here aligns with the originally established threshold for designating a cave (or  
686 cave system) as a Hotspot of Subterranean Biodiversity (HSB) (20  
687 troglobites/stygiobites) (Culver and Sket, 2000). However, it is worth noting that some  
688 researchers have questioned the arbitrariness of the 20-species criterion (Souza-Silva and  
689 Ferreira, 2016; Zeppelini et al., 2022; Ferreira et al., 2023). Ferreira et al., (2023)  
690 emphasizes the importance of incorporating additional parameters in defining an HSB.  
691 These parameters might encompass factors such as scale, latitude, lithology associated  
692 with caves, levels of endemism, and the degree of threat that the subterranean habitat  
693 faces, as proposed by Myers et al., (2000).

694 Therefore, especially when considering parameters as degree of threats to enhance  
695 the comprehension of subterranean hotspots, Pedro Cassiano Cave is certainly a new HSB  
696 in South America. Conducting additional samplings utilizing supplementary  
697 methodologies, like micro invertebrate sampling, undoubtedly holds the promise of  
698 revealing further species residing within this cave. It is equally crucial to highlight that  
699 endeavors directed towards species description play a pivotal role in conserving this  
700 potential priority area, currently facing escalated anthropogenic interventions, including  
701 deforestation (Ferreira et al., 2023).

702 It is important to note that this cave is threatened, especially when considering the  
703 strong anthropic alterations occurring in its surroundings. The deforestation around cave  
704 entrances disrupts the availability of organic resources and microhabitats crucial for  
705 hypogean fauna (Pellegrini et al., 2016; Cardoso et al., 2022). Furthermore, it exposes the  
706 soil to erosive processes capable of conveying fine sediments into the cavities,  
707 culminating in watercourse sedimentation and the uniformity of cave substrates. Within  
708 this context, Pedro Cassiano Cave could experience a substantial decline in its  
709 invertebrate diversity, as our study's findings emphasize the critical role of substrate  
710 diversity in sustaining this diversity. Another consequence of deforestation in karst  
711 landscapes is the reduction of preserved vegetation corridors, which are essential for  
712 species movement between caves, particularly troglone and troglophile species  
713 (Cardoso et al., 2022). This population isolation contributes to reduced genetic diversity,  
714 heightening the vulnerability of species to extinction risks (Campbell Grant, 2011).

715 Lastly, it is important to highlight that the Pedro Cassiano Cave is located  
716 approximately 4 km in a straight line from the Água Clara Cave System (ACCS),  
717 recognized as the most diverse Hotspot of Subterranean Biodiversity (HSB) in South  
718 America (Souza-Silva et al., 2021; Ferreira et al., 2023). The ACCS comprises four  
719 functionally interconnected caves, all intersected by the same intermittent drainage.  
720 Ferreira et al. (2023) initially reported 31 cave-restricted species within this system.  
721 However, recent sampling efforts conducted in September 2023 revealed ten new and  
722 previously undocumented cave-restricted species. This discovery brings the total count  
723 of cave-restricted species within the system to 41, further emphasizing its significance in  
724 South America (Ferreira, unpublished data).

725 Moreover, the ACCS and the Pedro Cassiano Cave share a considerable number  
726 of species, as previously mentioned. This underscores the urgent need for collaborative  
727 conservation strategies that encompass both cave systems. Establishing comprehensive

728 conservation measures, such as the creation of a protected area encompassing the  
729 hydrological basins that contribute to these cave networks, becomes imperative to  
730 safeguard the unique subterranean fauna of these remarkable Neotropical HSBs.

## 731 **5. Conclusions**

732 We have delved into the environmental factors that play a crucial role in shaping  
733 the richness and composition of invertebrates within a cave located in Brazil's semi-arid  
734 region. We want emphasize the pressing need for conservation efforts to safeguard this  
735 new Hotspot of Subterranean Biodiversity. We summarized the following points:

- 736 1) The Substrate heterogeneity turned out to be a key factor influencing the  
737 structure of invertebrate communities, confirming what previous literature has  
738 suggested;
- 739 2) Areas with a higher abundance of trophic resources housed a greater number  
740 of non-troglobitic species. However, this pattern might not hold true for  
741 troglobitic species;
- 742 3) The distance from the cave entrance was not a determining variable in  
743 community structuring, as initially expected. The reasons for this observation  
744 include the location of the first sampling sector (approximately 40 meters from  
745 the entrance), which minimized the contribution of epigean species, and the  
746 presence of autogenic drainage, which seems to prevent a significant decrease  
747 in resources towards the deeper areas of the cave;
- 748 4) As anticipated, environmental factors influencing the richness and  
749 composition of troglobitic and non-troglobitic invertebrates differed due to the  
750 specialized adaptations of these organisms to the cave environment;
- 751 5) It is worth noting that the environmental variables driving the variations in  
752 composition and richness of cave invertebrates are dependent on the scale of  
753 sampling;
- 754 6) Our study also supports the hypothesis that troglobitic species in tropical  
755 regions tend to be more prevalent in deep cave areas compared to non-  
756 troglobitic species. This preference is likely due to the climatic stability  
757 offered by these deeper areas, creating an ideal habitat for troglobitic species;
- 758 7) Our findings reveal low niche overlap among widely distributed troglobitic  
759 species, suggesting that they select different habitat conditions;



760 8) Urgent collaborative conservation strategies are imperative to encompass both  
761 the Pedro Cassiano and Água Clara cave systems. A primary objective should  
762 be the establishment of a protected area that covers the hydrological basins  
763 contributing to these cave networks. This step is critical for safeguarding the  
764 exceptional subterranean fauna found in these remarkable Neotropical HSBs.

765

#### 766 **Author contributions**

767 Rodrigo Lopes Ferreira and Marconi Souza-Silva formulated the idea, built the  
768 sample design and contributed to the literature review. Alicia Helena Souza Rodrigues  
769 Ferreira collected the data and sorted the invertebrates. Gabriel Augusto Silva Vaz  
770 performed the statistical analysis. Gabriel Augusto Silva Vaz wrote the manuscript with  
771 collaboration from Rodrigo Lopes Ferreira and Marconi Souza-Silva. Rodrigo Lopes  
772 Ferreira finalized the manuscript and contributed to the discussion of the results.

773

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