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ALICIA HELENA SOUZA RODRIGUES FERREIRA

RESPONSE OF CAVE INVERTEBRATE COMMUNITIES TO HABITAT HETEROGENEITY AT DIFFERENT SCALES

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RESUMO

Essa dissertação foi desenvolvida com o intuito de avaliar a influência da heterogeneidade de habitat, medida através de atributos físicos, tróficos e microclimáticos do piso de cavernas sobre a composição e riqueza de invertebrados cavernícolas em distintas escalas amostrais. Local de estudo foi a Serra do Ramalho, no Oeste da Bahia e englobou 26 cavernas distribuídas em uma área de aproximadamente 2.300 km^2. Os resultados aqui gerados, podem contribuir não somente com a ecologia de comunidades, mas também com a conservação da fauna de invertebrados cavernícolas no Brasil. A dissertação é composta de dois manuscritos redigidos conforme as normas de dois periódicos científicos distintos. O primeiro foi construído de acordo com as normas do periódico "*Biodiversity and conservation*", cujo objetivo foi avaliar atributos locais e regionais determinantes da composição e riqueza de invertebrados cavernícolas. Localmente, coletamos em 26 cavernas e avaliamos a influência de atributos tróficos, fiscos e microclimáticos sobre a fauna troglóbia e não troglóbia. Regionalmente, avaliamos a influência das bacias de drenagem e zonas de descarga e recarga hídrica sobre a composição da fauna troglóbia e não troglóbia. Os resultados mostram que variáveis relacionadas aos atributos tróficos, de microclima e de atributos físicos do substrato das cavernas juntos e/ou isoladamente exercem influência na estruturação das comunidades cavernícola. Além disto, a escala de amostragem se mostrou um importante fator na detecção das respostas das comunidades. Ao nível de paisagem a única variável que influenciou a composição da fauna cavernícola foi a zona de recarga e descarga hídrica. O segundo manuscrito foi redigido de acordo com as normas do periódico "*Acta Oecologica*", e teve como objetivo principal avaliar o papel da distância das entradas na determinação de variações nas características dos micros habitats, na composição e riqueza da fauna de troglóbios e não-troglóbios. Para este estudo utilizamos a Gruna das Três Cobras que possui sete entradas e mais de cinco mil metros de desenvolvimento linear. Os resultados mostram que a distância da entrada afeta as condições e recursos ao longo da caverna e consequentemente, cria distintos micro habitats, o que determina variações na composição e riqueza da fauna troglóbia.

Palavras-chave: Cavernas. Invertebrados. Escalas amostrais. Riqueza.

ABSTRACT

This dissertation was developed with the aim of evaluating the influence of habitat heterogeneity, measured through physical, trophic and microclimatic attributes of the cave floor on the composition and richness of cave invertebrates at different sample scales. The study site was Serra do Ramalho, in Western Bahia, and comprises 26 caves spread over an area of approximately 2.300 km2. The results showed here can contribute not only to the Ecology of communities, but also to the conservation of cave invertebrate fauna in Brazil. The dissertation is composed of two manuscripts written according to the norms of two different scientific journals. The first was written according to the rules of the journal "*Biodiversity and conservation*", whose objective was to evaluate local and regional attributes that determine the composition and richness of cave invertebrates. Locally, we collected in 26 caves and evaluated the influence of trophic, physical and microclimatic attributes on the troglobitic and non-troglobitic fauna. Regionally, we evaluated the influence of drainage basins and water discharge and recharge zones on the composition of troglobitic and non-troglobitic fauna. The results show that variables related to trophic attributes, microclimate and physical attributes of the cave substrate together and/or separately influence the structure of cave communities. In addition, the scale proved to be an important factor in detecting community responses. At the landscape level, the only variable that influences the composition of the cave fauna was the water recharge and discharge zone. The second manuscript was written according to the norms of the journal "*Acta Oecologica*", and had as main objective to evaluate the role of the distance of the entrances in the determination of variations in the characteristics of the microhabitats, in the composition and richness of the fauna of troglobitic and non- troglobitic. For this work, we used Três Cobras Cave, which has seven entrances and more than five thousand meters of linear development. The results showed that distance from the entrance affects the conditions and resources throughout the cave and consequently, creates distinct microhabitats and determines variations in the composition and richness of the troglobitic fauna.

Keywords: Caves. Invertebrates. Sample scale. Richness**.**

SUMÁRIO

PRIMEIRA PARTE

INTRODUÇÃO GERAL

A maior parte das cavidades naturais subterrâneas do mundo são encontradas em relevos cársticos compostos de rochas carbonáticas, como calcário e dolomita. Essas cavernas são consideradas partes ou subunidades do carste, formando um sistema de espaços vazios que controla a infiltração de água e os processos de armazenamento de materiais aluviais. Isso contribui para o transporte e armazenamento de recursos dentro das cavidades subterrâneas (Gibert et al. 1994).

Além da sua relevância hidro-geológica, os sistemas cársticos e cavernas são também importantes ecossistemas subterrâneos, servindo como locais complexos de nidificação e abrigo para uma grande variedade de espécies de invertebrados e vertebrados. Muitas vezes, essas cavidades também apresentam centros de endemismo, destacando ainda mais sua importância para a conservação da biodiversidade.

Durante anos, os padrões de distribuição de espécies cavernícolas tem sido foco de diversos trabalhos que buscam entender os fatores determinantes dessas comunidades (Dunson & Travis 1991; Kolasa & Pickett 1991; Cushman & McGarigal 2004; Steinitz et al. 2006; Talley 2007; Pacheco et al. 2020). Dessa forma, fatores bióticos e abióticos são levados em consideração, onde alguns dos fatores bióticos são: interações intra e interespecíficas; e os fatores abióticos são: temperatura, umidade, quantidade de abrigos, tipos de substratos, entre outros (Dunson & Travis 1991; Pacheco et al. 2020). De forma que o conjunto dos fatores bióticos e abióticos formam a heterogeneidade de habitat, e este também é um fator estudado para se entender padrões de distribuição e a estrutura das comunidades (Souza-Silva et al. 2021). Então, os ambientes subterrâneos (hipógeos) são considerados bons modelos para análise em diferentes escalas, uma vez que apresentam características mais estáveis aos ambientes superficiais, ausência permanente de luz, isolamento em relação aos ambientes de superfície e tendência à oligotrofia (Poulson & White 1969; Culver e Pipan 2009).

Por outro lado, tais ambientes abrigam uma fauna relativamente simples se considerado o número de espécies, teias tróficas limitadas e estabilidade nas condições ambientais, essa última contribuindo para a ocorrência de micro habitats estáveis e, consequentemente, a ocorrência de espécies com distintos graus de especializações morfológicas, comportamentais, reprodutivas e fisiológicas (Culver 1982, Gilbert e Deharveng 2002, Tobin et al. 2013, Sánchez-Fernández et al. 2018 Poulson & White 1969, Peck 1974, Sket 2008, Culver e Pipan 2009, Romero 2009).

Entretanto, a estabilidade ambiental não é homogenia por toda a extensão da caverna, e isso pode ser percebido com a medição de diversos parâmetros capazes de detectar variabilidade ambiental (Romero 2009). Essa estabilidade também varia de acordo com a região climática, onde nas regiões temperadas costumam apresentar maior sazonalidade quando comparadas com as cavernas encontradas em regiões tropicais (Mitchell 1969). Além disso, a escala a ser usada também é essencial para compreender melhor os padrões de distribuição e a estruturada das comunidades, já que criam micro habitats distintos ao longo de uma mesma caverna.

Como dito anteriormente, os ambientes subterrâneos apresentam escassez em recursos, que podem chegar até a caverna através da transferência de nutrientes e detritos do meio externo (epígeo) para o meio hipógeo por agentes físicos ou biológicos (Souza-Silva 2011). Esse carreamento pode ser feito através das chuvas e cursos d'água ou através do crescimento de raízes, carcaças de animais acidentais ou fezes de animais como quirópteros, dessa forma, aumentando as possibilidades de manutenção de um maior número de espécies com diferentes exigências de habitat (Gilbert et al. 1994, Souza-Silva et al. 2012, Souza-Silva et al. 2020).

Os organismos encontrados nas cavernas são classificados em uma perspectiva ecológico-evolutiva pelo sistema Schinner-Racovitza, modificado por Sket (2008), onde os organismos podem ser: trogloxenos, troglófilos e troglóbios. Os trogloxenos são aqueles que precisam do meio epígeo para completar seu ciclo de vida, como por exemplo, para buscar alimentos, como é o caso dos morcegos; os troglófilos são aqueles que conseguem estabelecer populações viáveis tanto no meio epígeo quanto no meio hipógeo, e os troglóbios são restritos ao meio subterrâneo. Já os organismos acidentais, categoria criada por Barr (1968), são aqueles que entraram na caverna e não conseguem manter uma população viável, sendo provável que fiquem aprisionados na caverna até morrerem (Barr 1968).

Os organismos troglóbios podem apresentar troglomorfismos, que são adaptações morfológicas (redução ocular, alongamento de apêndices e despigmentação), fisiológicas (diminuição da taxa metabólica e baixa tolerância às variações ambientais) e comportamentais, o que permite que estejam nesse ambiente durante todo o seu ciclo de vida (Romero & Green 2005). Troglóbios são raros e endêmicos, apresentando baixa densidade populacional, baixa tolerância às flutuações ambientais e são K estrategistas. Essas especializações evoluíram em resposta às pressões seletivas do meio hipógeo e/ou ausência de pressões seletivas comuns aos ambientes epígeos (Culver 1982).

Portanto, entender a estrutura de habitat das espécies nos ambientes subterrâneos é essencial, uma vez que, os levantamentos taxonômicos podem não ser suficientes para a conservação desses ambientes (Trajano et al. 2010). Então, entender como são os padrões de distribuição de espécies e a estrutura dessas comunidades no espaço e tempo é imprescindível para a conservação das espécies cavernícolas (Legendre et al. 2005; Jost et al., 2010). Para tal, a distância da entrada, heterogeneidade de habitat, disponibilidade de recursos e a escala amostral agem como disponibilizadores de distintos micros habitats para a fauna cavernícola.

Esta dissertação foi desenvolvida com a intenção de avaliar a influência da temperatura, umidade, componentes do substrato, atributos físicos e tróficos (aqui atribuídos como heterogeneidade de habitat) e distância da entrada sobre a composição e riqueza de invertebrados de caverna em distintas escalas amostrais (quadrantes, setores e paisagem). Esse estudo foi desenvolvido em 26 cavernas localizadas na Serra do Ramalho/BA.

A dissertação é composta por dois manuscritos, escritos conforme as regras de diferentes periódicos científicos. O primeiro artigo foi redigido de acordo com as normas do periódico “Biodiversity Conservation” e investiga a influência dos atributos físicos, tróficos e microclimáticos sobre a composição e riqueza de invertebrados nas 26 cavernas, usando micro e meso-escala (quadrante e setor), além de avaliar a influência de dois atributos da paisagem sobre a composição (microbacias e zonas hídricas). Nas unidades amostrais de micro e meso-escala, os componentes de substrato foram usados pra criar variáveis de diversidade de substrato, diversidade trófica e de diversidade de abrigo. Os resultados apontaram que as diversidades dos substratos são importantes elementos determinantes das variações na composição e riqueza da fauna, bem como atributos microclimáticos e componentes de habitat. Em relação aos elementos de paisagem, apenas as zonas de recarga e descarga influenciam a composição da fauna.

O segundo manuscrito foi redigido de acordo com as normas do periódico “Acta Carsologica”, e avaliou a influência da localização e distância da entrada sobre os atributos físicos, tróficos, microclimáticos e como estes afetam a fauna cavernícola, e se troglóbios respondem de forma diferente dos não-troglóbios, em distintas escalas, usando apenas uma caverna (com sete entradas). A metodologia foi a mesma citada acima, exceto que nesse manuscrito não foram usados elementos da paisagem. Os resultados mostraram que os troglóbios preferem locais úmidos, mais estáveis e com escassez de recursos alimentares, o que configura os locais mais fundos, e isso leva a não competição com as espécies não-troglóbias. Já estas mostraram que preferem ambientes com mais recursos que o fundo e com maior heterogeneidade de habitat, que são por sua vez, locais mais próximos à entrada. A posição das entradas e distância em relação às entradas da caverna também mostraram-se fatores importantes na estruturação dessas comunidades.

REFERÊNCIAS

BARR, T. C. **Cave Ecology and the Evolution of Troglobites.** In: DOBZHANSKY, T.; HECHT, M. K.; STEERE, W. C. (Eds.). Evolutionary Biology. New York/London: Plenun press, 1968. p. 35-102.

CULVER, D. C. **Cave life: Evolution and Ecology.** Massachusetts and London: Harvard University Press, 1982.

CULVER, D. C.; PIPAN, T. (Eds.). **The subterranean domain.** In: CULVER, D. C.; PIPAN, T. (Eds.). **The biology of caves and other subterranean habitats.** Oxford: Oxford University Press, 2009. p. 1-22.

CUSHMAN, S.; MCGARIGAL, K. **Patterns in the species-environment relationship depend on both scale and choice of response variables.** Oikos, v. 105, p. 117-124, 2004. Disponível em: https://doi.org/10.1111/j.0030-1299.2004.12524.x.

DUNSON, W. A.; TRAVIS, J. **The role of abiotic factors in community organization.** The American Naturalist, v. 138, p. 1067-1091, 1991. Disponível em: https://doi.org/10.1086/285270.

GIBERT, J.; DEHARVENG, L. **Subterranean Ecosystems: A Truncated Functional Biodiversity.** BioScience, v. 52, p. 473-481, 2002.

GILBERT, J.; DANIELPOL, D. L.; STANFORD, J. A. **Groundwater Ecology.** Academic Press Limited, 1994.

JOST, L. et al. **Partitioning diversity for conservation analyses.** Diversity and Distributions, v. 16, n. 1, p. 65-76, 2010.

KOLASA, J.; PICKETT, T. A. (Eds.). **Ecological heterogeneity. Ecological studies.** Springer, New York, 1991. Disponível em: https://doi.org/10.1007/978-1-4612-3062-5.

LEGENDRE, P.; BORCARD, D.; PERES-NETO, P. R. **Partitioning the Spatial Variation of Community Composition Data.** Ecological Monographs, v. 75, p. 435-450, 2005.

MITCHELL, R. W. **A comparison of temperate and tropical cave communities.** The Southwestern Naturalist, Memphis, v. 14, n. 1, p. 73-89, 1969.

PACHECO, G. S. M. et al. **The role of microhabitats in structuring cave invertebrate communities in Guatemala.** International Journal of Speleology, v. 49, n. 2, p. 161-169, 2020. Disponível em: https://doi.org/10.5038/1827-806X.49.2.2333.

PECK, S. B. **The Invertebrate Fauna of Tropical American Caves, Part II: Puerto Rico, An Ecological and Zoogeographic Analysis.** Biotropica, v. 6, n. 1, p. 14-31, 1974. Disponível em: https://www.jstor.org/stable/2989693.

POULSON, L. T.; WHITE, B. W. **The Cave Environment.** Science, v. 165, n. 3897, p. 971-981, 1969.

ROMERO, A.; GREEN, M. **The end of regressive evolution: examining and interpreting the evidence from cave fishes.** Journal of Fish Biology, v. 67, n. 1, p. 3-32, 2005.

ROMERO, A. **The evolutionary biology of cave organisms.** In: CAVE BIOLOGY: LIFE IN DARKNESS. 1st ed. New York: Cambridge University Press, 2009. p. 130-158.

SÁNCHEZ-FERNÁNDEZ, D. et al. **The deep subterranean environment as a potential model system in ecological, biogeographical and evolutionary research.** Subterranean Biology, v. 25, p. 1-7, 2018. Disponível em: https://doi.org/10.3897/subtbiol.25.23530.

SKET, B. **Can we agree on an ecological classification of subterranean animals?** Journal of Natural History, v. 42, n. 21-22, p. 1549-1563, 2008.

SOUZA SILVA, M.; INIESTA, L. F. M.; FERREIRA, R. L. **Invertebrates diversity in mountain Neotropical quartzite caves: which factors can influence the composition, richness, and distribution of the cave communities?** Subterranean Biology, v. 33, p. 23-43, 2020. Disponível em: https://doi.org/10.3897/subtbiol.33.46444.

SOUZA-SILVA, M.; MARTINS, R. P.; FERREIRA, R. L. **Trophic Dynamics in a Neotropical Limestone Cave.** Subterranean Biology, v. 9, p. 127-138, 2011.

SOUZA-SILVA, M.; BERNARDI, L. F. O.; MARTINS, R. P.; FERREIRA, R. L. **Transport and Consumption of Organic Detritus in a Neotropical Limestone Cave.** Acta Carstologica, v. 41, p. 139-150, 2012.

SOUZA-SILVA, M.; CERQUEIRA, R. F. V.; PELLEGRINI, T. G.; FERREIRA, R. L. **Habitat selection of cave-restricted fauna in a new hotspot of subterranean biodiversity in Neotropics.** Biodiversity and Conservation. Disponível em: https://doi.org/10.1007/s10531-021-02302-8.

STEINITZ, O. et al. **Environment, dispersal and patterns of species similarity.** Journal of Biogeography, v. 33, p. 1044-1054, 2006. Disponível em: https://doi.org/10.1111/j.1365-2699.2006.01473.x.

TALLEY, T. S. **Which spatial heterogeneity framework? Consequences for conclusions about patchy population distributions.** Ecology, v. 88, p. 1476-1489, 2007. Disponível em: https://doi.org/10.1890/06-0555.

TOBIN, B. W.; HUTCHINS, B. T.; SCHWARTZ, B. F. **Spatial and temporal changes in invertebrate assemblage structure from the entrance to deep-cave zone of a temperate marble cave**. International Journal of Speleology, v. 42, n. 3, p. 203-214, 2013. Disponível em: https://doi.org/10.5038/1827-806X.42.3.4.

TRAJANO, E.; BICHUETTE, M. **Relevância de cavernas: porque estudos ambientais espeleobiológicos não funcionam.** Espeleo-Tema, v. 21, p. 105-112, 2010.

SEGUNDA PARTE - MANUSCRITOS DE ARTIGOS

Artigo 1: Local and regional influences of the habitat characteristics on cave invertebrates' assemblage: from micro-habitat heterogeneity to landscape features

Este capítulo foi escrito em formato de manuscrito de artigo (versão preliminar), redigido conforme as normas para a publicação da revista "Biodiversity and Conservation"

LOCAL AND REGIONAL INFLUENCES OF THE HABITAT CHARACTERISTICS ON CAVE INVERTEBRATES' ASSEMBLAGE: FROM MICRO-HABITATS HETEROGENEITY TO LANDSCAPE FEATURES

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ABSTRACT

The Serra do Ramalho karst in Brazil remains poorly understood, but previous research has shown that the area is location to an endemic fauna that is threatened with extinction. This study was conducted to contribute to the understanding of the ecology and conservation of subterranean fauna in the region. Specifically, the study aimed to evaluate the influence of habitat heterogeneity, including physical, trophic, microclimatic, and substrate features, on species richness and composition at different sampling scales. Additionally, the study aimed to determine whether the response to these variables differed between troglobitic and non-troglobitic taxa. Sampling was conducted in 26 caves located across three municipalities in Serra do Ramalho, with two sampling events conducted in 2021 and 2022, however, each cave was only sampled once. Invertebrate fauna in the caves was sampled using sectors measuring 10x3m and quadrats measuring 1x1m, which were placed along the cave floor. Two landscape features, micro basins (São Francisco River basin and Corrente River basin) and water zones (discharge and recharge), were also evaluated to assess their effects on subterranean composition. The study concluded that physical, trophic, microclimatic, and substrate features are important for structuring the cave fauna, either in isolation or together, at different scales. These features also have distinct effects on non-troglobitic and troglobitic species. However, the only landscape variable that was found to have a significant influence on composition was the recharge and discharge zones.

Keywords: Serra do Ramalho, fauna, troglobitic, scales, richness.

INTRODUCTION

For a long time, subterranean environments were thought to be unable to sustain a diverse fauna due to the absence of light, low productivity, and oligotrophic characteristics. However, recent research has shown that caves can support high invertebrate diversity due to the high temporal and spatial heterogeneity of resources and conditions (Gilbert and Deharveng 2002; Simon et al. 2007; Culver and Pipan 2009). These environments present spatial variations and gradients of resources and conditions, which in turn support the existence of different microhabitats between and along caves. This can potentially reduce competition and niche overlapping (Culver and Pipan 2010; Pacheco et al. 2020; Souza-Silva et al. 2021; Simões et al. 2022).

To fully understand the local and regional distribution patterns of subterranean communities, it is necessary to take into account not only the abiotic and biotic factors but also the spatial scales of analysis. Local scale abiotic factors such as temperature, humidity, type of substrate, resource availability, and number of shelters are important (Dunson and Travis 1991; Pacheco et al. 2020), as are regional scale factors like rock type, altitude, productivity, vegetation types, drainage basins, and water recharge zones. Additionally, resource availability and intra and interspecific interactions like predation and competition may have significant impacts on subterranean communities (Danks 1991; Dunson and Travis 1991). Temperature and humidity are among the main factors that influence the distribution of obligate cave fauna (troglobitic), as they tend to select microhabitats with higher and more stable levels of temperature and humidity. These species are highly sensitive to even small variations in these factors (Pacheco et al. 2020; Howarth 1980; Pallarés et al. 2019, 2020).

On the other hand, non-obligate cave fauna (troglophiles) living near the entrance may experience greater fluctuations in microclimate, allowing them to tolerate these changes more than troglobitic species living in the deepest parts of the cave (Prous et al. 2004; Lunghi et al. 2014; Prous et al. 2015; Mammola et al. 2017; Mammola and Isaia 2017). Therefore, it is equally important to understand microhabitat selection as it is to understand patterns of distribution at the local and regional scale. Recently, it has been suggested that microhabitats play a fundamental role in driving subterranean species diversity. The presence of different types of microhabitats can attract various species, including those seeking refuge, which may have different morphological, behavioral, reproductive, and physiological specializations (Souza-Silva et al. 2021; Gibert and Deharveng 2002; Tobin et al. 2013). Therefore, microhabitat selection is an essential factor for understanding the coexistence of different species. However, it is important to note that studies regarding microhabitat selection should also consider biotic factors (Dunson and Travis 1991). In contrast, the landscape context can also have a significant influence on biodiversity (MacDonald et al. 2000). Since caves are devoid of light or have limited light, photoautotrophs are absent, and the subterranean fauna rely heavily on allochthonous resources and roots (Howarth 1983; Ferreira et al. 2007; Schneider et al. 2011). As a result, alterations in the surrounding landscapes can affect nutrient input and microhabitat availability within the cave (Pellegrini et al. 2016a).

Habitat heterogeneity is a significant predictor of animal diversity and abundance and has been studied by many authors in search of answers regarding species distribution patterns (Amarasekare and Nisbet 2001; Cornell 2010; Yang et al. 2015; Stein et al. 2015; Vargas-Mena et al. 2020; Souza-Silva et al. 2021). However, the relationship between habitat heterogeneity and diversity depends on spatial and temporal scales (González-Megías et al. 2007; Mehrabi et al. 2014). In addition, historical factors, geographic position, and regional hydrology, combined with the factors mentioned above, such as habitat heterogeneity and availability, can also explain the distribution of subterranean species (Christman et al. 2016). For example, environmental differences between micro basins and hydrological zones, such as recharge and discharge zones, explain the faunistic identities of subterranean fauna on a broad scale (Alvarenga et al. 2020; Stein et al. 2012; Iannella et al. 2020).

There are few studies evaluating the space-scale dependence on communities for cave invertebrates (Pellegrini et al. 2016a; Bento et al. 2021; Oliveira Furtado et al. 2022). Such studies are essential to identify and understand the characteristics and patterns for future management and conservation of cave environments, since models get more accurate at larger scales once it is possible to incorporate variables that are not present in smaller scales (Pellegrini et al. 2016a). However, combining different scales can provide a more precise understanding of invertebrate fauna and its structure. Therefore, this study aims to investigate the responses of cave invertebrate fauna (composition and richness) to physical, trophic, and microclimatic variations, substrate heterogeneity/diversity, and substrate features within caves at multiple scales, including microscale (1 m2 quadrats), mesoscale (30 m2 sectors), and landscape features (micro basins and water zones). For this purpose, (i) we hypothesized that higher substrate diversity will support higher invertebrate species richness in the sectors and quadrats; (ii) troglobitic species tend to respond differently to the sampled variables compared to non-troglobitic species due to their preference for more stable environments combined to higher levels of specialization to subterranean habitats; (iii) we hypothesized that the water zone and micro basins are going to function as geographical barriers, limiting the dispersion of cave fauna.

MATERIALS AND METHODS

Studied caves and surroundings areas

The study was conducted in 26 caves in the municipalities of Carinhanha, Coribe, and Serra do Ramanho, Bahia-Brazil (Figure 1, Table 1). The Serra do Ramalho karst is in Rio São Francisco basin and holds a variety of limestone outcrops formed by the Bambuí Group. The region comprehends important cave systems, some with several kilometers of extension, therefore, potentially having a high subterranean diversity. This region is in the Caatinga biome and experiences a tropical dry climate, the “Aw” type characterized by a dry winter following the classification of Köeppen (Alvares et al. 2013). Due to tropical rains which occur in the region, it is safe to access these caves only during the dry periods (March to October). The first sampling was made in September of 2021 and the second in August 2022. Caves extension varies from 12m to 8400m (sd= 708.3), and caves altitudes varies from 473.5 to 742.19 (sd= 102.2).

Field procedures

Invertebrate quantitative sampling on the cave floor

To evaluate the composition and richness of invertebrate communities, 145 sectors were established in the 26 sampled caves, where each sector (3 x 10m) contained 3 quadrats (1 x 1m), here considered “mesoscale’ and “microscale”, respectively. All transects were placed on the cave floor at least 100m apart from each other (when possible).

The invertebrate fauna was recorded into the sectors and quadrats through detailed visual search and hand sampling (Souza-Silva et al. 2021; Oliveira Furtado et al. 2022). The sampling method was exhaustive, and the invertebrates were collected with aid of tweezers and brushes and placed in vials with alcohol 70% in order to preserve the material for further identification in the laboratory. To minimize the impact of the sampling on the invertebrate communities some specimens were collected and their abundance were accounted.

All specimens were identified at the lowest taxonomic level possible with the assistance of taxonomic keys and other literature. The troglobitic species were separated by traits that indicate their evolution and adaptability in subterranean environments. The troglomorphisms observed were a lack or reduction in ocular structure and pigmentation, and elongation of appendices and sensorial structures (Culver and Pipan 2009). Experts reviewed species from the following groups: Isopoda, Pseudoscorpiones, Orthoptera, Acari, Entomobryomorpha, Symplypleona and Diplopoda. All the collected specimens are deposited in the Lavras Subterranean Invertebrates Collection (ISLA), affined to the Center of Studies on Subterranean Biology at the Federal University of Lavras, Minas Gerais, Brazil.

Abiotic attributes of the cave floor

The temperature and humidity were measured with the aid of a thermohydrometer device in each sector. The device was set on the cave floor for approximately 15 minutes and its values were taken after its stabilization. The presence of people was avoided to not interfere with or alter such measures (Souza-Silva et al. 2021; Oliveira-Furtado et al. 2022).

The measurement of abiotic attributes in mesoscale (sectors) was made using the methodology proposed by Pellegrini et al. (2016a), and Souza-Silva et al. (2021). The sectors were divided into 10 sections of 1 meter each and it was made a visual estimate of the percentual of substrate and resources available on the cave floor. In the laboratory we calculated the sum of these proportions and the average of every substrate and resource proportion in the sector, as well as the Shannon-diversity Index (H') for each sector. On the other hand, the measurement of abiotic attributes on a microscale (quadrats) was made using photographs. Digital photographs (4000 x 3000 pixels) were taken in the field in a vertical position (camera positioned at 90º relative to the cave floor) using the Canon Powershot SX50, HS. In the laboratory, the photos were analyzed through ImageJ software (Rasband 1997), and the arithmetic average of every abiotic attribute was calculated (Souza-Silva et al 2021, Oliveira-Furtado et al. 2022).

The definition of hydrological zone-recharge and discharge was made based on terrain elevation after getting caves entrance geographic position and plotted in a map. Altitudes values higher than 550 meters were considered recharge zones and altitudes lower than 550 meters were considered discharge zones (Figure 1). The altitudes were taken in QGis software based on a Digital Elevation Model (DEM) of the area. To define the micro basins were used the function CHANNEL NETWORK AND DRAINAGE BASINS from the 'Saga Next Generation' plugin based on a Digital Elevation Model (DEM) of the area, using QGis software version 3.22.11.

Data analysis

Biotic attributes of the caves

The abundance and richness of the total sampled fauna, non-troglobitic and troglobitic, were obtained by counting individuals and morphotypes of each sample unit (26 caves, 145 transects, and 435 quadrats).

Abiotic attributes on the cave floor regarding the sectors

All the *physical*, *trophic* and *microclimatic* characteristics of the sectors were evaluated and classified in the following classes: guano—GU, insectivorous guano —GI, hematophagous guano—GH, bird feces—FZA, others mammals feces—FZM, mocó feces—FZMO, tamanduá feces—FZT, feces—FZ, carcass—CRC, roots—RZ, litter—SER, plants debris—DTV (> 10mm), fine branch —GALF (11-30 mm), medium branch —GALM (31-50 mm), coarse branch—GALG (65-250 mm), trunk—TRO (>250mm of width), river—R, river pond—PR, water pond—WP, stalactite—EL, drip water—DP, hyphae—HI, actinomycetes biofilms—ACT, basidiomycetes—BAM, shell—CO, seedlings—PL, another organic substrate—OTO, smooth rock—RL, rough rock—RR, concrete like floor—RC, wide rock—XB (1000-4000mm), medium rock —MB(500-1000mm), small rock —SB(250-500mm), cobbles—CB (64-250mm), coarse gravel—CAG (16-64mm), fine gravel—CAF (2-16mm), sand—ARE (0.06-2mm), silt—SEF (≤ 0.05 mm), hardpan—HP, travertine—TV, flowstone—EC, cauliflower-like speleothem—CF, rough stalagmite—ER, stalagmite—EG, calcite rafts—JS, *megalobolimus* shell—CM, gastropod shell—CG, speleothems—ES, worm acorns —BM, retraction cracks—GR, cave-wall—PA, water pipe—CA, hardpan pinnacle—PHP, another inorganic substrate—OTI.

Based on such classes we obtained the *physical features* which included distance from the entrance, the substrate diversity (calculated considering all classes above), the shelter diversity (calculated considering RR, RC, XB, MB, SB, CB, CAG, CAF, TV, CF, ES, EG, CM, CG, BM, GR and CA) and trophic resources diversity (calculated considering GU, GI,

GH, FZM, FZMO, FZT, FZ, CRC, RZ, SER, DTV, GALF, GALM, GALG, TRO, CR, FG, AL, HI, ACT, BAM and PL). All diversities were calculated using Shannon-Weaver Index (Buttigieg and Ramette 2014).

The availabilities of each *Abiotic attribute* were also included as *physical*, *trophic* and *microclimatic* characteristics. The shelter availability was calculated by the sum of RR, RC, XB, MB, SB, CB, CAG, CAF, TV, CF, ES, EG, CM, CG, BM, GR, CO and CA in each sector. The trophic resources availability was calculated by the sum of GU, GI, GH, FZM, FZMO, FZT, FZ, CRC, RZ, SER, DTV, GALF, GALM, GALG, TRO, CR, HI, ACT, BAM and PL in each transect, and were also the same for the *trophic resources.* The *microclimatic variables* considered were temperature and humidity.

When using the analysis with individual classes, some classes were set to minimized variables. GH, GU, GI, and GU were grouped into GU. The classes ES, CF, EL, EG, ER, TV, and JS were grouped into ES. Classes CO, CM, and CG were grouped into CO. Lastly, R, WP, and PR were grouped into W.

Abiotic attributes on the cave floor regarding the quadrats

On the other hand, *physical* and *trophic* characteristics of the quadrats were evaluated and classified into the following classes: guano—GU, feces—FZ, carcass—CRC, roots—RZ, plants debris—DTV (> 10mm), fine branch —GALF (11-30 mm), medium branch —GALM (31-50 mm), coarse branch—GALG (65-250 mm), trunk—TRO (>250mm of width), water pond—WP, drip water—DP, another organic substrate—OTO, shell—CO, cow bone—CN, smooth rock—RL, rough rock—RR, concrete floor—RC, wide rock—XB (1000-4000mm), medium rock—MB(500-1000mm), a small rock—SB (250-500mm), cobbles—CB (64-250mm), coarse gravel—CAG (16-64mm), fine gravel—CAF (2-16mm), sand—ARE (0.06-

2mm), silt—SEF (0.2 < diameter ≤ 0.05 mm), hardpan—HP, speleothems—ES, worm acorns —BM, flowstone—EC, water pipe—CA, card burn—CU and retraction cracks—GR.

Based on such classes we obtained the *physical features* that included the distance from the entrance, the substrate diversity (calculated considering all classes above), the shelter diversity (calculated based on DTV, GALF, GALM, GALG, TRO, CO, RR, RC, XB, MB, SB, CB, CAG, CAF, TV, EG, BM, CA, CU and GR), and trophic resources diversity (calculated based on GU, FZ, CRC, RZ, DTV, GALF, GALM, GALG, TRO and OTO). All diversities were calculated using Shannon-Weaver Index (Buttigieg and Ramette 2014).

The availabilities were also included in *physical*, *trophic,* and *microclimatic* characteristics. The shelter availability was calculated by the sum of CO, RR, RC, XB, MB, SB, CB, CAG, CAF, TV, EG, BM, CA, CU and GR in each quadrat; while the trophic resources availability was calculated by the sum of GU, FZ, CRC, RZ, DTV, GALF, GALM, GALG, TRO and OTO in each quadrat, and were also the same for the *trophic resources*.

For the quadrats, some classes were set to minimized variables, where the same groups were formed when using the analysis with individual classes.

Spatial variations on habitat Structure

To compare the existence of differences in the average in temperature, humidity, substrate diversity, resource diversity, shelter diversity, availability of resources, and shelter among all 26 sampled caves, it was performed the Kruskal-Wallis test, using a KRUSKAL.TEST function from the ‘Stats’ package in RStudio. About the sectors, it was tested if the trophic, microclimatic and physical attributes varied depending on the distance from the entrance using a *Linear Regression*. It was also tested if the quadrats trophic, microclimatic, and physical attributes varied depending on the distance from the entrance using a *Linear*

Regression. All regressions were calculated using the function LM from 'Stats' package in RStudio.

Relationship between habitat structure of the sectors and quadrats with cave fauna

Linear models (GLM e GLMM) were performed to predict the influence of local abiotic variables on total species richness, species richness of non-troglobitic and species richness of troglobitic (response variables), using transects and quadrats as sample units, performed in RStudio. We used two different models to evaluate the influence of the substrate on the response variables. The variables included in the first model were temperature (^{0}C), humidity (%), distance from the cave entrance, diversity of shelter, diversity of substrate, and diversity of resources, and availability of shelter and availability of resources. The variables used in the second model were temperature (^{0}C), humidity (%), distance from cave entrance, diversity of shelter, diversity of substrate, and diversity of resource and the components of the substrate in their single version (for the sectors: guano, insectivorous guano, hematophagous guano, bird feces, others mammals feces, mocó feces, tamanduá feces, other feces, carcass, roots, litter, plants debris, fine branch, medium branch, coarse branch, trunk, river, river pond, water pond, stalactite, drip water, hyphae, actinomycetes biofilms, basidiomycetes, shell, seedlings, another organic substrate, smooth rock, rough rock, concrete floor, wide rock, medium rock, small rock, cobbles, coarse gravel, fine gravel, sand, silt, hardpan, travertine, flowstone, cauliflower-like speleothem, rough stalagmite, stalagmite, calcite rafts, *megalobolimus* shell, gastropod shell, speleothems, word acorn, retraction cracks, cave-wall, water pipe, hardpan and pinnacles, another inorganic substrate; inside quadrats: guano, feces, carcass, roots, plants debris, fine, medium branch, coarse branch, trunk, water pond, drip water, another organic substrate, shell, cow bone, smooth rock, rough rock, concrete floor, wide rock, medium rock, small rock, cobbles, coarse gravel, fine gravel, sand, silt, hardpan, speleothems, word acorn, flowstone, pipe, card burn and retraction crack). For that, the Poisson family was adopted, since is often used in models that count of occurrences given in time or space. To compare the model results with the null models, the ANOVA function from 'Vegan' package was made. To evaluate the overdispersion it was used the function CHECK_OVERDISPERSION from the package 'Performance'. To obtain r^2 values of the GLMMs, we used the function r.squaredGLMM from the 'MuMIn' package, while to obtain r^2 values of the GLM's, we used the function r.squaredLR from the 'piecewiseSEM' package. Before running the GLM's and GLMM's, we tested the correlation of all variables included in their respective model through the function CHART. CORRELATION from the 'PerformanceAnalytics' package. Variables with high correlation values (>0.65) were excluded from the model (Zuur et al. 2010). The multicollinearity was also tested before running the models through function VIF from 'Car' package. The variables that presented multicollinearity higher than 10 were excluded from the model, one by one (Zuur et al. 2010).

To explain the possible relationship, strength, and direction (- or +) between overall fauna, non-troglobitic and troglobitic species composition with the *physical*, *trophic*, and *microclimatic* variables, it was performed a Distance-based redundancy analysis (dbRDA) based on Bray-Curtis similarity matrix (Clarke et al. 2014). To perform this analysis, we used the two models as explained before.

Relationship between landscape features, cave habitat structure and cave fauna

To access the relationship between landscape features and invertebrates' composition (overall fauna, TB and nTB) a similarity analysis (ANOSIM) was performed using micro basins (São Francisco River and Corrente River), and hydro zones (water zone of recharge and water zone of discharge) as factors. The similarity was based on a Bray-Curtis similarity matrix, using transects as sampling units. Species abundance was square root transformed before running a resemblance analysis (Clarke 1993). This analysis was performed using Primer-e7 software (https://www.primer-e.com/our-software/primer-version-7/).

To access the relationship between landscape features and abiotic attributes of the caves we also performed an ANOSIM using micro basins (São Francisco River and Corrente River), and hydro zones (water zone of recharge and water zone of discharge) as factors. For that it was performed a Euclidean-distance matrix, using the transects as sampling units. Before running the ANOSIM was performed a square root transformation of the data was and created a resemblance matrix (Clarke 1993). This analysis was performed using Primer-e7 software (https://www.primer-e.com/our-software/primer-version-7/).

RESULTS

Biotic attributes of the cave

A total of 9178 individuals were recorded, distributed in 43 orders and 123 families, and 416 morphospecies, of which 72 were considered troglobitic (17,3 %). The richest order considering overall fauna was Araneae (116 ssp.), followed by Coleoptera (73 ssp.) and Diptera (62 ssp.), (Figure 2a). The richest order of non-troglobitic species was Araneae (104 ssp.), followed by Coleoptera (73 ssp.) and Diptera (61 spp.) (Figure 2b). Lastly, the richest orders of troglobitic species were Araneae (12 ssp.), Polydesmida (9 ssp.) and Isopoda, Palpigradi and Pseudoscorpiones (6 ssp. Each) (Figure 2c). Some of the troglobitic found among the 26 caves are shown in Figure 3.

Relationship between habitat structure of the sectors with cave fauna

For the total fauna (troglobitic and non-troglobitic combined), in the first model showed that the variation in morphospecies richness was better explained by temperature and resource availability (R^2= 0.22), while in the second model the variation on morphospecies richness was better explained by temperature, DTV, GALF, GALM, TRO, DP, OTO, SEF, FZA, FZMO, BAM and PHP (R^2: 0.80). For the non-troglobitic (nTB), the first model showed that the variation in morphospecies richness was better explained by temperature, distance from the entrance and resource availability (R^2= 0.31), while the second model showed that the variation in morphospecies richness was better explained by temperature, distance from the entrance, humidity, FZMO, DTV, GALF, GALM, TRO, ACT, OTO, CAF and SEF (R^2= 87). For the troglobitic fauna (TB), the first model showed that the variation in morphospecies richness was better explained by temperature and humidity (R^2=0.20), while on the second model showed that the variation in morphospecies richness was better explained by temperature, humidity, FZA, RZ, DP, ACT and BAM (R^2= 0.43). The p-values are in Table 2.

For the overall fauna, the first dbRDA model showed that distance, resource availability, humidity and temperature were the best predictors, explaining 9.99% of the variation in species composition (p-value: 0.005 for all) (Figure 4a), while the second model showed that distance, humidity, temperature, DTV, SEF, GU, GALM, TRO, FZMO, RZ were the best predictors, explaining 19% of the variation in species composition), (Figure 4b). For the non-troglobitic (nTB), the first dbRDA model showed that distance from the entrance, resource availability, temperature and humidity were the best predictors, explaining 9% of the variation in species composition (Figure 4c), while the second model showed that distance, temperature, humidity, resource div., DTV, SEF, GU, FZT, TRO, ARE and RZ were the best predictors, explaining 18% of the variation in species composition (Figure 4d). For the troglobitic fauna (TB), the first dbRDA model showed that distance from the entrance and humidity were the best predictors, explaining 6% of the variation in species composition (p-value: 0.005 for both) (Figure 4e), while the second model showed that distance, temperature, humidity, GALM, RZ, FZMO, ES, SEF, TRO and OTO were the best predictors, explaining 20% of the variation in species composition (Figure 4f). The p-values are shown in Table 3.

Relationship between habitat structure of the quadrats with cave fauna

For the overall fauna (troglobitic and non-troglobitic), the first model showed that the variation in morphospecies richness was best explained by distance and resource availability (R^2: 0.12), while in the second model, the variation in morphospecies richness was best explained by distance, resource div., substrate div., shelter div., GU, DTV, WP, OTO, SEF and BM (R^2: 0.12). For the non-troglobitic (nTB), the first model showed that the variation in morphospecies richness was best explained by distance from the entrance and substrate diversity (R^2= 0.25), while in the second model, the variation in morphospecies richness was best explained by distance, substrate div., GU, DTV, GALF, OTO, MB, SB, CAG, ARE, SEF, and HP (R^2= 0.85). Finally, for the troglobitic fauna (TB), the first model showed that the variation in morphospecies richness was best explained by distance from the entrance and resource availability (R^2 = 0.12), while in the second model the variation on morphospecies richness was best explained by distance, resource div., shelter div., substrate div., GU, DTV, OTO, SEF and BM (R^2= 0.79). The p-values are in Table 4.

For the overall fauna, the first dbRDA model showed that resource availability and substrate diversity were the best predictors, explaining 11% of the variation in species composition (p-value: 0.005 for all) (Figure 5a), while the second model showed that distance, resource diversity, substrate diversity, GU, DTV, SEF, BM, OTO, TRO were best predictors, explaining 17% of the variation in species composition (Figure 5b). For the non-troglobitic (nTB), the first dbRDA model showed that distance from the entrance and substrate diversity were the best predictors, explaining 13% of the variation in species composition (p-value: 0.005, for all) (Figure 5c), while the second model showed that distance, substrate div., OTO, GR, WP, GU, SEF, and RC were best predictors, explaining 18% of the variation in species composition (Figure 5d). Finally, for the troglobitic (TB), the first dbRDA model showed that distance, resource availability, and resource diversity were the best predictors, explaining 14% of the variation in species composition (Figure 5e), while the second model showed that distance, GR, SEF and OTO were best predictors, explaining 16% of the variation in species composition (Figure 5f). The p-values are shown in Table 3.

Spatial variations on habitat structure

The values of temperature, humidity, substrate diversity, resource diversity, shelter diversity, availability of resources, and shelter of the sectors and quadrats are presented in Material Supplementary I and II. Temperature, humidity, shelter and resources diversity, and resource availability showed differences in the averages among all 26 caves. Regarding the sectors, the humidity indicated a positive relationship with the distance from the entrance ($R^2 = 0.08$, $p = 0.0003$), while the substrate diversity ($R^2 = 0.07$, $p = 0.001$), shelter diversity ($R^2 = 0.05$, $p = 0.006$), resource availability ($R^2 = 0.06$, $p = 0.003$) and shelter availability ($R^2 = 0.04$, $p = 0.01$), showed a negative relationship with the distance from the entrance ($R^2 = 0.07$, $p = 0.001$). Additionally, regarding the quadrats, the resource diversity ($R^2 = 0.02$, $p = 0.001$), shelter diversity ($R^2 = 0.01$, $p = 0.006$), and resource availability ($R^2 = 0.01$, $p = 0.003$), indicated a negative relationship with the distance from the entrance.

Relationship between landscape features and cave fauna

The ANOSIM revealed significant differences in overall fauna, the non-troglobitic and troglobitic composition in the sectors among hydro zones (Global R= 0.14, p= 0.001; Global R- 0.082, p= 0.001; Global R= 0.099, p= 0.001, respectively). It also revealed significant differences in substrates composition among hydro zones (Global R= 0.14, p= 0.021). Lastly, the fauna also varied among caves (Global R: 0.39, p= 0.001).

DISCUSSION

Our study highlights the importance of different environmental factors, including spatial scales to explain local and regional variations in the composition of substrate components and in the number of species and composition of the invertebrate fauna on the cave floor. Both biotic and abiotic attributes of the caves were extremely heterogeneous in micro (between quadrats of 1m2), meso (sectors of 30m2), and macro-scale (caves and recharge zones). That spatial habitat and microhabitats differentiation driven by changes in substrate components on the cave floor might be promoting replacement in species richness and similarity at the local and regional scale and permitting the occurrence of distinct faunistic identities in a narrow geographic area. Distance from the entrance showed a strong influence in reducing substrate diversity, shelter diversity, resource availability and shelter availability, but increasing moisture content in the microhabitats. Then we can assign high heterogeneity in microhabitats spatial distribution even inside a single cave.

Relationship between habitat structure of the sectors and quadrats with cave fauna

The results showed that substrate heterogeneity was an important factor in determining the richness and composition of cave invertebrate species, but that this influence differed between troglobitic and non-troglobitic species. Non-troglobitic species richness and composition responded to a variety of factors, including temperature, humidity, distance from the entrance, resource availability, substrate diversity, mocó feces, plants debris, medium and fine branch, trunk, actinomycetes, fine gravel, silt, and other organic substrates. In contrast, troglobitic species richness and composition were mainly influenced by temperature, humidity, distance from the entrance, resource availability, and resource diversity.

These findings are consistent with previous studies that have shown that substrate heterogeneity is an important driver of cave invertebrate diversity. For example, a study by

Moldovan and Zagmajster (2017) found that substrate type and availability were key factors influencing the distribution of aquatic cave invertebrates in Slovenia. Similarly, a study by Trajano and Gnaspini (2010) found that the availability of suitable substrates was a key factor influencing the distribution of subterranean insects in Brazilian caves.

The influence of temperature and humidity on cave fauna is known, once temperature at deepest parts of the cave is almost the same as the annual average of the external surrounding of the cave where its located, while for the humidity, it tends to saturation as far from the entrance of the cave. When compared to temperate areas, tropical areas possess less differentiation between inside the cave and external areas (Tobin et al. 2013). The zone connecting these two environments (internal and external) are called ecotones (Prous et al. 2015). These ecotones possess both hypogean and epigean fauna transitioning, seeking for shelter and/or refuge, as a result the patterns of distribution and composition of species tend to be different when compared to the fauna at deepest parts of the cave.

Temperature also produces distinct types of habitats, one near the entrance and one at the deepest parts of the cave, therefore influencing on species richness, which decreases as far from the entrance of the cave. However, the humidity increases as far as the entrance and consequently increases the richness, but in this case, the richness of troglobitic species, since the distance from the entrance affect species composition and limits species richness of non-troglobitic, therefore, remaining only the species more adapted to even more oligotrophic and humid environments. This same pattern was observed by Souza-Silva et al. (2021) and Deharveng and Bedos (2000), where troglobitic species were found in cave areas far from entrance, since they manage to keep away from drier areas and competitors, in this case being the non-troglobitic species.

The resource diversity and resource availability, and the number of classes of organic matter such as plants debris, branches and trunk were expected since the subterranean invertebrates are on dependent of organic resources input (Culver and White 2005), which can occur from surface runoff and percolation (Ferreira et al. 2010) and through rivers importation (Souza-Siva et al. 2011), that act as distribution agents, supporting a regular food supply (Hawes 1939). One of the possible interpretations is that forests in limestone areas produce more leaf during dry periods (Brina 1998), which during rainfall periods can be imported, including fallen trunks, however it only moves inside the caves in rainy periods with high velocity and high-water flux. That creates an almost dependence on organic matter importation (Bento et al. 2016). Although, the distinction between the use of microhabitats and food resources not only depends on their availability but also on the competition for it between species (Souza-Siva et al 2021).

About the resources such guano and feces, they are provided to the caves by bats and by other mammals there enter seeking for shelter or food, therefore, playing an important role in community structure. Concerning the types of guano, here are three mains: frugivorous, hematophagous, and insectivorous. They are related to their feeding habitats, which varies from seeds, blood of mammals and insects or others arthropods. Their feeding habits are also influenced by variations in seasonality where in beginning of the rainy season there is an thrive in flowering and fructification, leading to increase food availability to bats and consequently intensifying guano deposition inside caves, and if they are deposited where is not accessible to water, being available for longer time (Faria 1996; Wolda 1988; Souza-Silva et al. 2011). Since most caves are an oligotrophic environment, places having such resources can present higher diversity of invertebrate fauna and act as the main food font, as shown for some authors (Decu 1986; Gnaspini 1989; Ferreira and Martins 1998; Ferreira and Martins

1999; Ferreira et al. 2000). Although these variables work together, they can behave in different ways and level over the subterranean fauna (Simões et al. 2015).

The troglobitic fauna are more specialized to live in these oligotrophic environments. One of the specializations is the resistance to starvation. In contrast, the non-troglobitic fauna showed that is needed a wider range of food resources. These corroborate strongly to our findings once the TB species were found mostly at the deepest parts of the caves and nTB species reduces as far from the entrance. These indicate that there is no or low competition for food resources and shelter. A similar pattern was found in Águas Claras Cave System (ACCS), where the microclimatic variables were the most important influencing the structure and distribution of troglobitic and not the organic resources and habitat physical structure (Souza- Silva 2021). On the opposite, non-troglobitic-fauna can support variations in microclimatic features.

Variations in Habitat Structure

The observed differences in temperature, humidity, shelter and resource diversity, and availability among the studied caves were expected, given the variability in cave features and surface interactions that can facilitate or impede environmental dynamics. These features include the distance between caves, altitude, number, distribution and size of entrances, and cave extension. Additionally, cave relief morphology, position in recharge or discharge areas, the presence of water courses, and vegetation near entrances can all affect internal cave dynamics (Souza-Silva et al. 2020; Cardoso et al. 2022). Recharge areas with different external features play a vital role in the movement, transport and deposition of organic and inorganic sediment inside caves (Bonacci et al. 2009; Souza-Silva et al. 2011). Different openings and water courses, such as skylights, horizontal entrances, rivers, runoffs and waters that percolate through rock pores or fractures, represent various forms of transport for particulate or dissolved organic matter into the caves (Bonacci et al. 2009; Culver and Pipan 2019).

These findings are consistent with previous research that has highlighted the importance of external factors in shaping internal cave environments and their associated biodiversity. For instance, a study by Culver and Pipan (2019) found that the presence and distribution of recharge areas, water courses, and other surface features were critical in determining the abundance and distribution of cave-dwelling fauna. Similarly, Bonacci et al. (2009) emphasized the role of external factors in shaping sediment transport and deposition inside caves, which in turn can influence nutrient availability and other environmental factors that impact cave biodiversity.

Taken together, these findings underscore the importance of considering external factors in understanding the dynamics of cave environments and their associated biodiversity. Such knowledge is critical for effective cave management and conservation efforts, as it can help identify areas of high biodiversity value and inform strategies to mitigate threats to cave ecosystems from human activities and other stressors.

In addition, the variation in physical and microclimatic features within caves can occur at different scales, such as meso and micro scales, and can be related to the distance from the entrance. Previous studies have shown that humidity, substrate diversity, resource diversity, shelter availability, and resource availability varied along different sectors and quadrats within caves (Pellegrini et al. 2016b). Humidity showed a positive relationship with distance from the entrance, indicating higher environmental stability further inside the cave. However, the other variables showed a negative effect with distance from the entrance, suggesting a decrease in their presence further inside the cave. This could be attributed to the importation of organic matter into the cave, which is influenced by hydrological factors and determines resource availability. The width of cave entrances is another determinant of resource availability since larger entrances allow for more importation of organic matter, leading to an increase in species richness from hypogean environments to the cave (Simões et al. 2015, Cardoso et al. 2022).

These findings highlight the importance of considering both macro and micro environmental factors when studying cave ecosystems, as they can have significant effects on species richness and community composition. Future studies could further investigate the relationship between these environmental factors and the functioning of cave ecosystems, as well as the potential impacts of anthropogenic disturbances on these delicate and unique environments.

Relationship between landscape features and cave fauna

Our study showed that there were significant differences in the overall fauna between caves and hydro zones, while the non-troglobitic and troglobitic fauna, as well as the substrate features, only varied between hydro zones. This indicates that the presence of water bodies, such as rivers and aquifers, can have a significant impact on the diversity and distribution of invertebrate fauna in caves. This finding is consistent with previous studies that have demonstrated the importance of water bodies in providing suitable conditions for subterranean fauna (Culver and Piper 2009).

The presence of water bodies can also contribute to the transport of rocky substrates, which in turn can provide shelter and increase the richness of invertebrate communities. In semiarid conditions, the existence of groundwater is particularly important, as it can provide the necessary conditions for the colonization of stygobites and also benefit terrestrial troglobites by carrying organic matter and maintaining humidity levels (Bento et al. 2016).

Overall, our findings highlight the importance of water bodies and substrate features in shaping the diversity and distribution of cave invertebrate fauna. Further studies are needed to better understand the mechanisms driving these patterns and to develop effective conservation strategies for these unique and often fragile ecosystems.

Several studies have reported a positive correlation between invertebrate population size and rainfall (Vasconcellos et al. 2010; Araújo et al. 2010; Hernández 2007). This suggests that the availability of water bodies and rivers inputting organic matter increases the availability of resources and shelter, leading to a higher number of species and microhabitats (Ferreira et al. 2016). However, the amount of organic matter entering the cave is also influenced by the season, as during the dry season leaves fall and form a little that is later carried into the cave during the rainy season, along with substrates of different sizes (Souza-Silva et al. 2011, 2012). On the other hand, intensive floods can also have a negative impact on the fauna, especially in small caves with strict fauna, but this may not apply to large caves with extensive linear extensions, where some species may be able to escape from the river and flood areas (Simões et al. 2015).

That is an interesting finding, and it highlights the importance of considering different scales when studying subterranean fauna Eberle et al. (2018). Microhabitats can have a significant impact on species diversity, and larger landscape features such as recharge and discharge zones can also play a role in determining the identity of cave fauna. It is also worth noting that the presence of rivers and water bodies can carry species and organic matter, contributing to the richness of subterranean fauna. By examining these factors at different scales, researchers can gain a more complete understanding of the complexity of subterranean environments and the factors that influence species composition (Pellegrini et al. 2016a).

CONCLUSION

In summary, the study highlights the importance of habitat heterogeneity and different scales of sampling in understanding subterranean fauna. While non-troglobitic fauna may have a wider range of resources and tolerance to microclimatic features, troglobitic fauna require more specific conditions. Hydro zones were found to be important to the cave fauna community, but further studies are needed to understand this relationship specifically. Deforestation around cave entrances can negatively impact epigean and cave fauna by reducing movement and affecting temperature and humidity conditions. Biological research is crucial for understanding cave biodiversity and can inform management and conservation efforts.

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REFERENCES

Alvarenga D, Silva M, & Ferreira R (2020) Trogloregions: Delimiting subterranean faunistic identities in Afrotropics and Neotropics.

Alvares CA, Stape JL, Sentelhas PC, Moraes-Gonçalves JL, Sparovek G (2013) Köppen's climate classifcation map for Brazil. Meteorol Z 22:711–728. https://doi.org/10.1127/0941-2948/2013/0507

Amarasekare P, Nisbet R (2001) Spatial heterogeneity, source-sink dynamics and the local coexistence of competing species. Am Nat 158:572–584. https://doi.org/10.1086/323586

Araújo CS, Candido DM, Araújo HFP, Dias SC, Vasconcellos A (2010a), Seasonal variations in scorpion activities (Arachnida: Scorpiones) in an area of Caatinga vegetation in northeastern Brazil: Zoologia, 3:372–376.

Bento DDM, Ferreira RL, Prous X, Souza-Silva M, Bellini BC, Vasconcellos A (2016) Seasonal Variations in Cave Invertebrate Communities in the Semiarid Caatinga, Brazil. J Caves Karst Stud 78:61–71. https://doi.org/10.4311/2015LSC0111

Bento DM et al (2016) Seasonal variations in cave invertebrate communities in the semiarid Caatinga, Brazil. *Journal of Cave and Karst Studies* 78.2:61-71.

Bento DM, Diego et al (2021) Subterranean "oasis" in the Brazilian semiarid region: neglected sources of biodiversity. *Biodiversity and Conservation* 30:3837-3857.

Bonacci O, Pipan T, Culver D C (2009). A framework for karst ecohydrology. Environmental Geology 56:891-900.

Brina AE, (1998) Aspectos da dinâmica da vegetação associada a afloramentos calcários na APA Carste de Lagoa Santa. Dissertation. Universidade Federal de Minas Gerais.

Buttigieg PL, Ramette A (2014) A Guide to Statistical Analysis in Microbial Ecology: a communityfocused, living review of multivariate data analyses. FEMS Microbiol Ecol 90:543–550. https://doi. org/10.1111/1574-6941.12437.

Cardoso RC, Ferreira RL, Souza-Silva M (2022) Multi-spatial analysis on cave ecosystems to predict the diversity of subterranean invertebrates. *Basic and Applied Ecology* 65:111-122.

Caruso D (1994) La fauna della grotta Monello. Atti e Memorie dell'Ente Fauna Siciliana 2: 87-21

Christman MC et al (2005) Patterns of endemism of the eastern North American cave fauna. *Journal of Biogeography* 32.8:1441-1452.

Christman MC, Doctor DH, Niemiller ML, Weary DJ, Young JA, Zigler KS, Culver DC (2016) Predicting the occurrence of cave-inhabiting fauna based on features of the earth surface environment. PLoS ONE, 11:e0160408.

Cornell HW (2010) Niche overlaping. In: Hastings A, Gross LJ (ed) Encyclopedia of theoretical ecology. University of California Press, Oakland

Culver DC, Pipan T (2009) Biology of Caves and Other Subterranean Habitats, 1st edn. Oxford, New York

Culver DC, Pipan T (2010) Climate, abiotic factors, and the evolution of subterranean life. Acta carsologica

Culver DC, Pipan T (2019) The biology of caves and other subterranean habitats. Oxford University Press.

Danks HV (1991) Winter habitats and ecological adaptations for winter survival." *Insects at low temperature* 231-259.

Decu V (1986) Some considerations on the bat guano synusia. Travaux de L'Institut de Spéologie "Emile Racovitza" 25:41-51. doi:10.1590/S1984-46702010000300008.

Dunson WA, Travis J (1991) The role of abiotic factors in community organization. The American Naturalist, 138:1067-1091. https://doi.org/10.1086/285270

Eberle J, Dimitrov D, Valdez-Mondragón A, Huber, BA (2018). Microhabitat change drives diversification in pholcid spiders. BMC Evolutionary Biology 18:141.

Ferreira R.L, Prous X, Martins RP (2007) Structure of bat guano communities in a dry Brazilian cave. Tropical Zoology, 20:55-74.

Ferreira RL, Cardoso RC, Souza-Silva M (2016) Composição, riqueza e diversidade de invertebrados em cavernas de Dianópolis (TO). Revista Brasileira de Espeleologia 01-18

Ferreira RL, Martins RP (1998) Diversity and distribution of spiders associated with bat guano piles in Morrinho cave (Bahia State, Brazil). Diversity and Distributions 4:235-241. https://www.jstor.org/stable/2999829

Ferreira RL, Martins RP (1999) Trophic structure and natural history of bat guano invertebrate communities with special reference to Brazilian caves. Tropical Zoology, 12:231-259. https://doi.org/10.1080/03946975.1999.10539391

Ferreira RL, Martins RP, Yanega D (2000) Ecology of bat guano arthropod communities in a Brazilian dry cave. Ecotropica, 6:105-116.

Ferreira RL, Prous X, Bernardi LFO, Souza-Silva M (2010) Fauna subterrânea do Estado do Rio Grande do Norte: Caracterização e impactos. Rev Bras Espeleol 1:25–51.

Gibert J, Deharveng L (2002) Subterranean ecosystems: a truncated functional biodiversity. American Institute of Biological Sciences Bulletin, 52:473-481.

Gnaspini P (1989) Análise comparativa da fauna associada a depósitos de guano de morcegos cavernícolas no Brasil: primeira aproximação. Revista Brasileira de Entomologia, 33:183-192.

Goldsbrough CL, Hochuli DF, Shine R (2003) Invertebrate biodiversity under hot rocks: habitat use by the fauna of sandstone outcrops in the Sydney region. *Biological Conservation* 109.1:85-93.

González-Megías A, Gómez JM, Sánchez-Piñero F (2007) Diversity-habitat heterogeneity relationship at diferent spatial and temporal scales. Ecography 30:31–41. https://doi.org/10.1111/j.0906-7590.2007. 04867.x

Hawes RS (1939) The flood factor in the ecology of caves. *The Journal of Animal Ecology* 1-5.

Hernández MIM (2007) Besouros escarabeíneos (Coleoptera: Scarabaeidae) da caatinga paraibana, Brasil: Oecologia Brasiliensis 356–364.

Howarth FG (1980) The zoogeography of specialized cave animals: a bioclimatic model. *Evolution* 394-406.

Howarth FG (1983) Ecology of cave arthropods. Annual Review of Entomology 28:365-389. https://doi.org/10.1111/j.2041-210X.2009.00001.x

Hüppop K (2005) Adaptation to low food. In: Culver D, White WB (ed) Encyclopediaof caves. Ensevier, Amsterdam, pp 4–10.

Iannella M, Fiasca B, Di Lorenzo T, Biondi M, Di Cicco M, Galassi DM (2020) Spatial distribution of stygobitic crustacean harpacticoids at the boundaries of groundwater habitat types in Europe. Scientific Reports, 10: 1-10.

Lobo HAS, Boggiani PC, Perinotto JAJ (2015) Speleoclimate dynamics in Santana Cave (Petar, São Paulo State, Brazil): general characterization and implications for tourist management. Int J Speleol 44(1):61–73. https://doi.org/10.5038/1827-806X.44.1.6

Lunghi E, Raoul M, Ficetola GF (2014) Do cave features affect underground habitat exploitation by non-troglobite species?.*Acta Oecologica* 55:29-35.

MacArthur RH, Levins R (1967) The limiting similarity, convergence, and divergence of coexisting species. Am Nat 101:377–385. https://doi.org/10.1086/282505

Mammola S, Leroy B 2017) Applying species distribution models to caves and other subterranean habitats. Ecography 41:1194-1208. https://doi.org/10.1111/ecog.03464

Mammola S, Marco I (2017) Spiders in caves. *Proceedings of the Royal Society B: Biological Sciences* 284.1853: 20170193.

Mehrabi Z, Slade EM, Solis A, Mann DJ (2014) The Importance of microhabitat for biodiversity sampling. PLoS ONE 9(12):E114015. https://doi.org/10.1371/journal.pone.0114015

Novak T et al (2014) Cold tolerance in terrestrial invertebrates inhabiting subterranean habitats. *Int. J. Speleol*. 43: 265– 272.

Oliveira, LF et al (2022) Recreational caving impacts of visitors in a high-altitude cave in Bolivian Andes: main effects on microhabitat structure and faunal distribution." *International Journal of Speleology* 51.2:2.

Pacheco GSM, Souza-Silva M, Cano E, Ferreira RL (2020) The role of microhabitats in structuring cave invertebrate communities in Guatemala. International Journal of Speleology 49:161-169.

Pallarés S et al (2019) Heat tolerance and acclimation capacity in subterranean arthropods living under common and stable thermal conditions. *Ecology and Evolution* 9.24: 13731-13739.

Pallarés S et al (2020) Beyond survival experiments: using biomarkers of oxidative stress and neurotoxicity to assess vulnerability of subterranean fauna to climate change. *Conservation Physiology* 8.1: coaa067.

Pellegrini TG, et al. (2016a) Linking spatial scale dependence of land-use descriptors and invertebrate cave community composition. Subterranean Biology 18:17-38.

Pellegrini TG, Ferreira RL (2012) Sampling effort in mite communities associated with cave bat guano. Speleobiology Notes 4:10-16.

Pellegrini TG, Ferreira RL (2013) Structure and interactions in a cave guano–soil continuum community. European Journal of Soil Biology 57:19-26. https://doi.org/10.1016/j.ejsobi.2013.03.003

Pellegrini TG, Ferreira RL (2016b) Are inner cave communities more stable than entrance communities in Lapa Nova show cave?. Subterranean Biology 20:15-37.

Poulson TL, Culver DC (1969) Diversity in terrestrial cave communities. Ecology 50:153-158.

Prous X, Ferreira RL, Jacobi CM (2015) The entrance as a complex ecotone in a Neotropical cave. International Journal of Speleology 44: 177-189. https://doi.org/10.5038/1827-806X.44.2.7

Prous X, Ferreira RL, Martins RP (2004) Ecotone delimitation: epigean–hypogean transition in cave ecosystems. Austral Ecology 29:374-382.

Rasband WS (1997) Image, J., US National Institutes of Health. "ImageJ64," US National Institutes of Health, Bethesda, MD.

Romero A (2009) Cave biology: life in darkness. Cambridge University Press, New York.

Schneider K, Christman MC, Fagan WF (2011) The influence of resource subsidies on cave invertebrates: results from an ecosystem-level manipulation experiment. Ecology 92:765-776. https://doi.org/10.1890/10-0157.1

Simões MH, Souza-Silva M, Ferreira RL (2015) Cave physical attributes influencing the structure of terrestrial invertebrate communities in Neotropics. Subterranean Biology 16:103-121. https://doi.org/10.3897/subtbiol.16.5470

Simões MH, Souza-Silva M, Lopes RF (2002) Species-area relationship and richness persistence as a proxy of environmental carrying capacity: A case study in a neotropical show cave. *Acta Oecologica* 116: 103848.

Simon KS, Pipan T, Culver DC (2007) A conceptual model of the flow and distribution of organic carbon in caves. *Journal of Cave and Karst Studies* 69.2: 279-284.

Souza-Silva M, Bernardi LFO, Martins RP, Ferreira RL (2012) Transport and consumption of organic detritus in a neotropical limestone cave. Acta Carsologica 41: 139–150. doi: 10.3986/ac.v41i1.54

Souza-Silva M, Cerqueira RFV, Pellegrini TG et al (2021) Habitat selection of cave-restricted fauna in a new hotspot of subterranean biodiversity in Neotropics. Biodivers Conserv 30:4223–4250 (2021). https://doi.org/10.1007/s10531-021-02302-8

Souza-Silva M, Iniesta LFM, Ferreira R (2020) Cave lithology effect on subterranean biodiversity: A case study in quartzite and granitoid caves. *Acta Oecologica* 108: 103-645.

Souza-Silva M, Martins RP, Ferreira RL (2011) Trophic Dynamics in a Neotropical Limestone Cave. Subterranean Biology 9:127–138. doi: 10.3897/subtbiol.9.2515

Stein A, Beck J, Meyer C, Waldmann E, Weigelt P, Kreft H (2015) Diferential efects of environmental heterogeneity on global mammal species richness. Global Ecol Biogeogr 24(9):1072–1083. https:// doi.org/10.1111/geb.12337

Stein H, Griebler C, Berkhoff S, Matzke D, Fuchs A, Hahn HJ, (2012) Stygoregions–a promising approach to a bioregional classification of groundwater systems. Scientific reports.

Tobin BW, Hutchins BT, Schwartz BF (2013). Spatial and temporal changes in invertebrate assemblage structure from the entrance to deep-cave zone of a temperate marble cave. International Journal of Speleology 42:203-214. https://doi.org/10.5038/1827-806X.42.3.4

Vargas-Mena JC, Cordero-Schmidt E, Rodriguez-Herrera B et al (2020) Inside or out? Cave size and landscape effects on cave-roosting bat assemblages in Brazilian Caatinga caves. J Mammal 101(2):464– 475. https://doi.org/10.1093/jmammal/gyz206

Vasconcellos A, Andreazze R, Almeida AM, Araujo HFP, Oliveira ES, Oliveira U (2010) Seasonality of insects in the semi-arid Caatinga of northeastern Brazil: Revista Brasileira de Entomologia 54: 471–476. doi:10.1590/S0085-56262010000300019.

Wolda H (1988) Insect seasonality: why? Ann. Review. Ecol. Syst. 19:1-18.

Yang Z, Liu X, Zhou M, Ai D, Wang G et al (2015) The efect of environmental heterogeneity on species richness depends on community position along the environmental gradient. Sci Rep 5(1):1–7. https:// doi.org/10.1038/srep15723

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1:3-14.

FIGURES AND TABLES

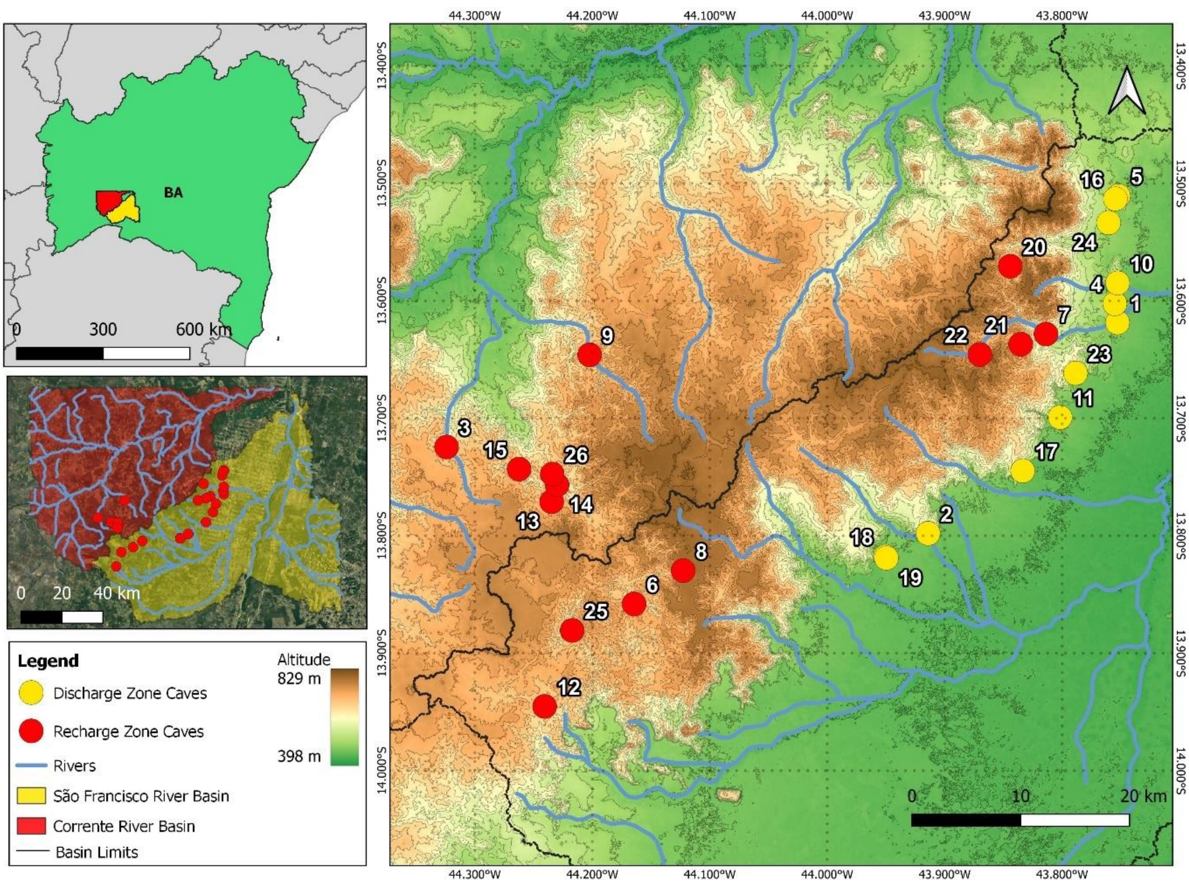

Figure 1: Map of caves location, micro basins associated to the caves and if the cave are at discharge or recharge zone. The numbers represent the caves:1- Três Cobras Cave; 2- Pedro Cassiano Cave; 3- Serra Verde Cave; 4- Grande Cave; 5- Serra Solta II Cave; 6- Baixão da Canoa Cave; 7- Google Cave; 8- Pingueira do João Nonato; 9- Enfurnado Cave; 10- Riacho do Floriano Cave; 11- Três Bocas Cave; 12- Govi Cave; 13- Zeferini Cave; 14- Lagoa do Meio Cave; 15- Ventilador Cave; 16- Serra Solta III Cave; 17- Domingão Cave; 18- Água Escura I Cave; 19- Água Escura II Cave; 20- Zoológico Cave; 21- Vandecir Cave; 22- Tocas II Cave; 23- Pé de Serra Cave; 24- Mandiaçu Cave; 25- Dedê Cave; 26- Quatro Cabras Cave. The yellow dots are representing discharge zones, while the red dots are representing recharge zones. The fade yellow represents the São Francisco River Basin, while the fade red represents the Corrent River Basin. Lastly, the rivers are represented by the lines in blue.

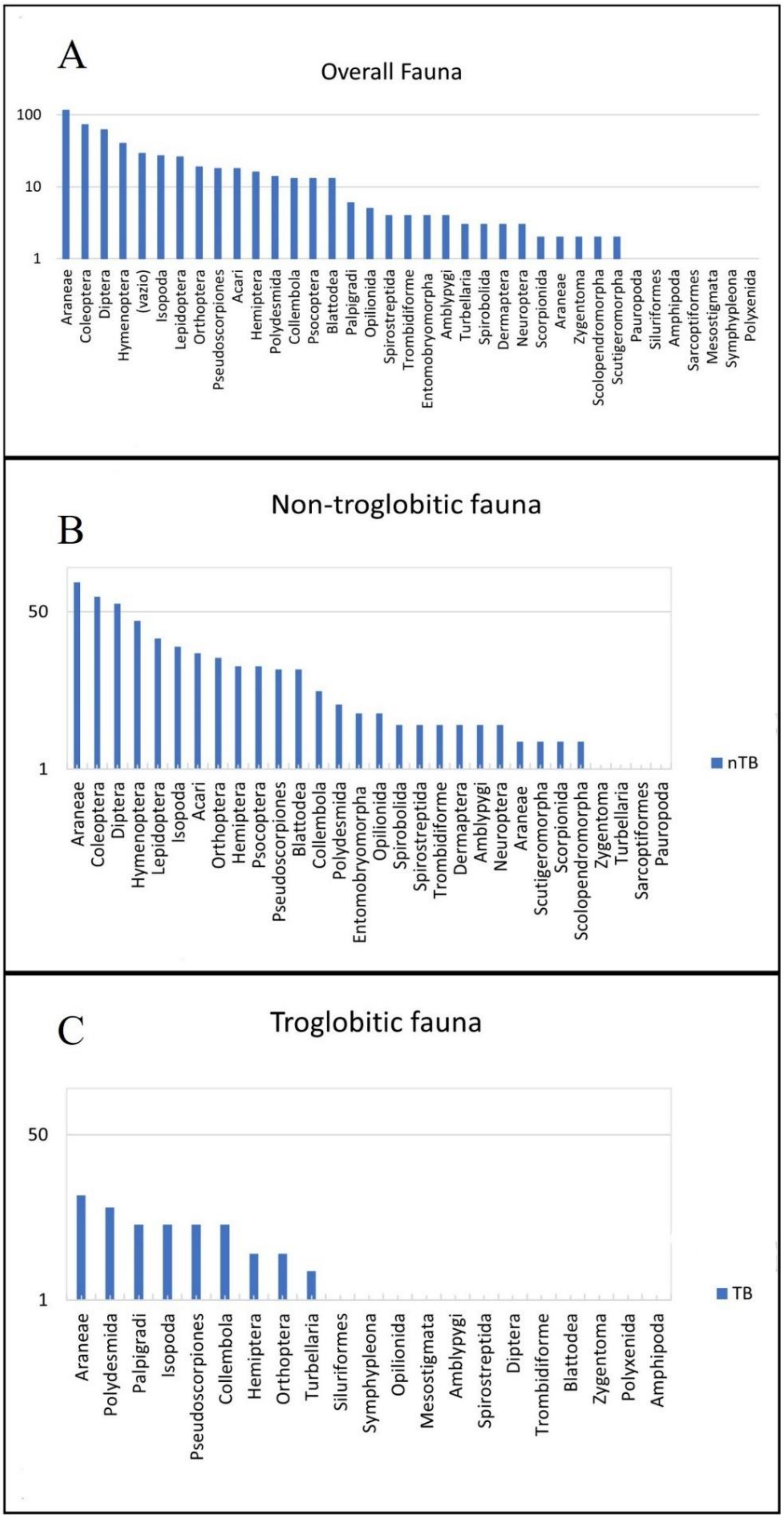

Figure 2. Richness of Overall Fauna (A); The richness of Non-troglobitic Fauna (B); The richness of Troglobitic Fauna (C); nTB means non-troglobitic, while TB means troglobitic.

Figure 3. Some of the troglomorphic species found in Serra do Ramalho: *Xangoniscus uai* (A); *Pectenoniscus carinhanhensis* (B); *Chaimowiczia monviridis* (C); Styloniscidae (D); *Speleaeogammarus ginnae* (F); Diplopoda (G, H, I); *Pseudonannolene* (J); *Phaneromerium* (K); Araneae (L); *Eukoenenia* (M); *Pseudochthonius* (N); Acari (O); *Giupponia chagasi* (P);

Charinus troglobius (Q); Ochyroceratidae (R); Ideoroncidae (S); *Troglobentosminthurus luridos* (T); *Endecous infernalis* (U); Blattodea (V); Carabidae (W, X); Gastropoda (Y,Z*);* *Girardia* (A'); *Trichomycterus rubbioli* (B'); Siluriformes (C',D').

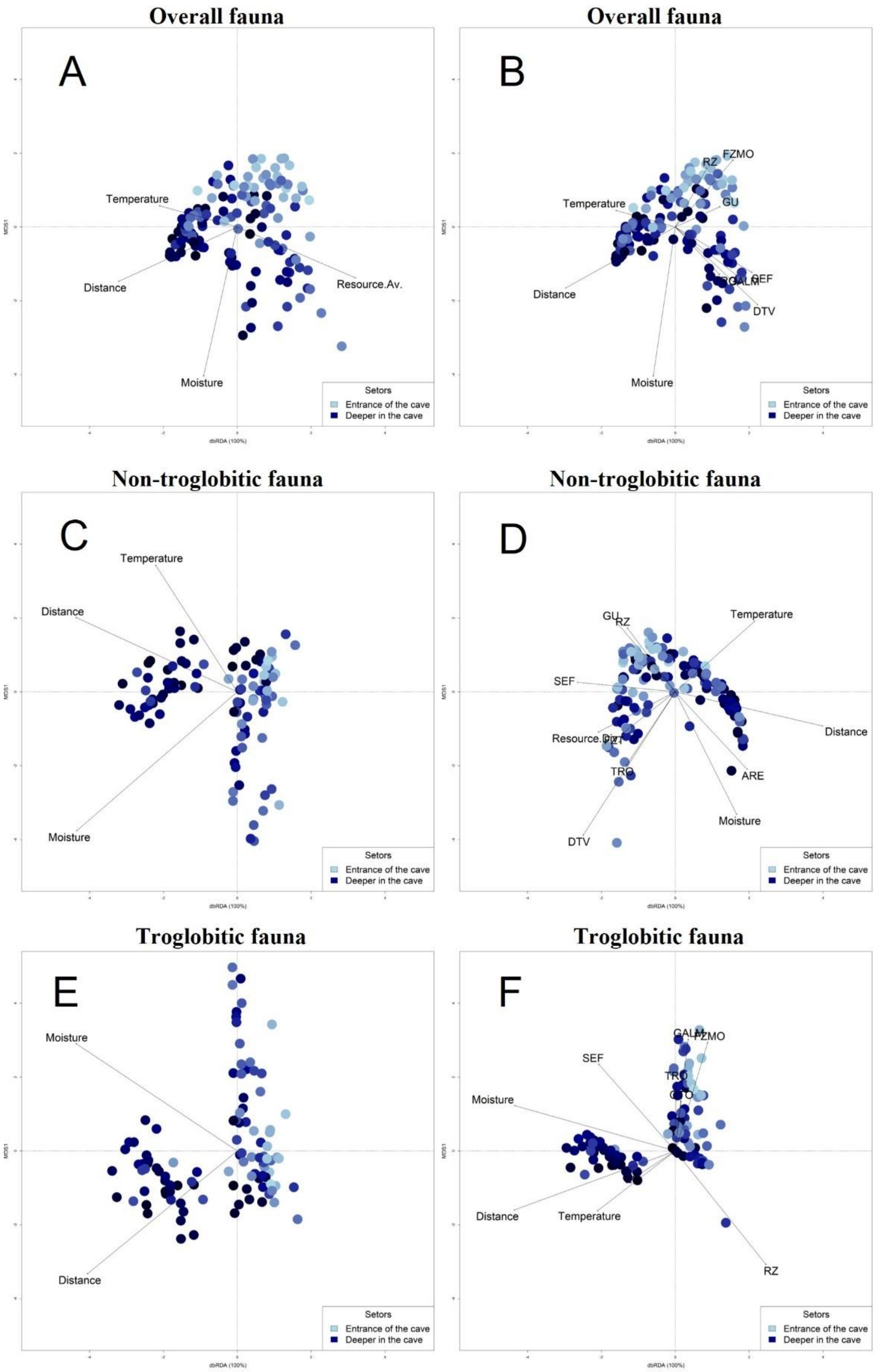

Figure 4. Metric multidimensional scaling (MDS) to show variations of the first model on temperature, moisture, distance from the entrance and resource availability for the overall fauna in the sectors (A); MDS showing variations of the second model on temperature, moisture, distance from the entrance, roots (RZ), Mocó feces (FZMO), guano (GU), trunks (TRO), silt (SEF), medium branch (GALM) and plants debris (DTV) for the overall fauna in

the sectors (B); MDS showing variations of the first model on temperature, moisture and distance from the entrance for the non-troglobitic fauna in the sectors (C); MDS showing variations of the second model on temperature, moisture, distance from the entrance, resource diversity, roots (RZ), Tamanduá feces (FZT), guano (GU), trunks (TRO), silt (SEF), sand (ARE) and plants debris (DTV) for the non-troglobitic fauna in the sectors (D); MDS showing variations of the first model on moisture and distance from the entrance for the troglobitic fauna in the sectors (E); MDS showing variations of the second model on temperature, moisture, distance from the entrance, roots (RZ), Mocó feces (FZMO), medium branch (GALM), trunk (TRO), other organic substrate (OTO) and silt (SEF) for the troglobitic fauna in the sectors (F). The colors of the dots represent the distance from the cave entrance to the bottom of the cave, so as lighter the dot the closer from the entrance, and the darker the dot the deeper is the sector.

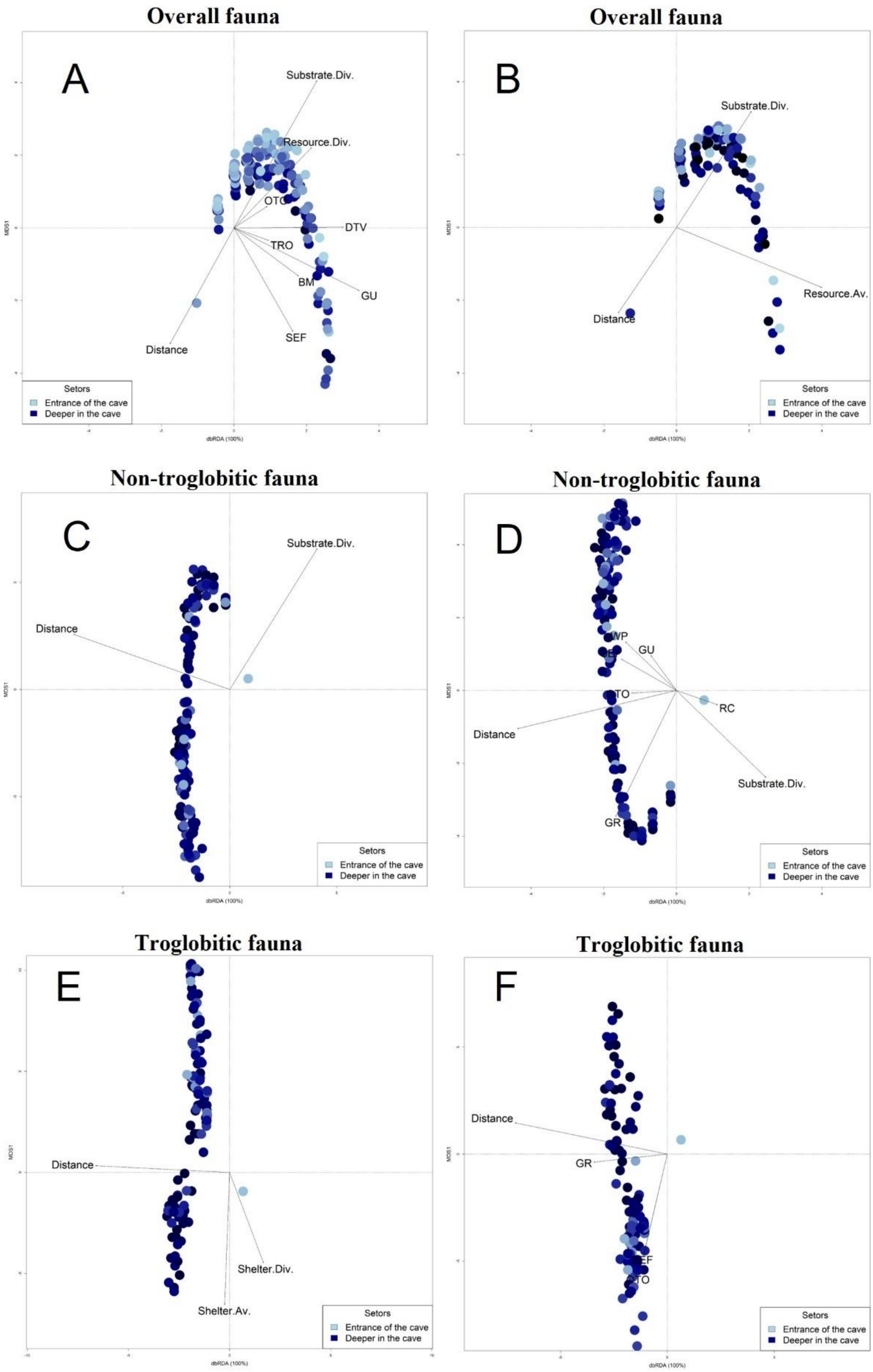

Figure 5. Metric multidimensional scaling (MDS) to show variations of the first model on substrate diversity, resource diversity, distance from the entrance, guano (GU), trunks (TRO), silt (SEF), plants debris (DTV), word acorn (BM) and other organic substrate (OTO) for the overall fauna in the quadrats (A); MDS showing variations of the second model on substrate

diversity, resource availability and distance from the entrance for the overall fauna in the quadrats (B); MDS showing variations of the first model on substrate diversity and distance from the entrance for the non-troglobitic fauna in the quadrats (C); MDS showing variations of the second model on substrate diversity, distance from the entrance, guano (GU), water pond (WP), silt (SEF), concrete like floor (RC), retraction cracks (GR) and other organic substrate (OTO) for the non-troglobitic fauna in the quadrats (D); MDS showing variations of the first model on distance from the entrance, shelter availability and diversity for the troglobitic fauna in the sectors (E); MDS showing variations of the second model on distance from the entrance, retraction cracks (GR), silt (SEF) and other organic substrate (OTO) for the troglobitic fauna in the sectors (F). The colors one the dots represent the distance from the cave entrance to bottom of the cave, so as lighter the dot the closer from the entrance, and the darker the dot the deeper is the quadrat.

TABLES

Table 1. Information regarding the caves coordinates (UTM), municipality, altitudes, the number of sectors that were collected, total richness and troglobitic richness of the cave, the microbasin and hydro zone that the cave is insert. TB: troglobitic; SF: São Francisco River Basin; CR: Corrente River Basin; R: Recharge zone; D: Water zone;

Caves	Municipality	nº sectors	Lat	Long	Total Richness	TB Richness	Altitude	Micro basins	Hydro zones (R/D)
Três Cobras Cave	Serra do Ramalho	22	-13.618507	-43.7527	67	14	486.475	SF	D
Pedro Cassiano Cave	Carinhanha	15	-13.798	-43.914	39	5	473.538	SF	D
Serra Verde Cave	Coribe	10	-13.724455	-44.3241	29	2	663.924	CR	D
Grande Cave	Serra do Ramalho	9	-13.602472	-43.7551	41	3	486.443	SF	D
Serra Solta 2 Cave	Serra do Ramalho	9	-13.510601	-43.7521	20	3	500.781	SF	D
Baixão da Canoa Cave	Coribe	8	-13.858135	-44.1644	27	3	654.786	SF	R
Google Cave	Serra do Ramalho	7	-13.628315	-43.8136	32	3	565.711	SF	R
Pingueira do João Nogueira Cave	Coribe	6	-13.829726	-44.1226	15	4	742.194	SF	R
Enfurnado Cave	Coribe	6	-13.646025	-44.2022	12	3	637.067	CR	D
Riacho do Floriano Cave	Serra do Ramalho	6	-13.584813	-43.7527	19	2	486.809	SF	D
Três Bocas Cave	Serra do Ramalho	5	-13.699167	-43.8016	12	1	486.513	SF	D
Govi Cave	Coribe	5	-13.945361	-44.2405	42	12	682.335	SF	R
Zeferini Cave	Coribe	5	-13.771024	-44.2346	26	2	698.921	CR	D
Lagoa do Meio Cave	Coribe	4	-13.756661	-44.23	39	3	691.703	CR	D
Ventilador Cave	Coribe	4	-13.743252	-44.2624	16	1	629.643	CR	D
Serra Solta 3 Cave	Serra do Ramalho	4	-13.513154	-43.7545	19	1	497.387	SF	D
Domingão Cave	Carinhanha	3	-13.744734	-43.8335	8	1	473.96	SF	D
Água Escura 1 Cave	Carinhanha	3	-13.817694	-43.9504	11	1	477.839	SF	D
Água Escura 2 Cave	Carinhanha	3	-13.818957	-43.9496	12	2	476.769	SF	D
Zoológico Cave	Serra do Ramalho	2	-13.57058	-43.844	31	5	698.2	SF	R
Vandecir Cave	Serra do Ramalho	2	-13.636943	-43.8352	48	8	738.202	SF	R
Tocas 2 Cave	Serra do Ramalho	2	-13.645741	-43.8699	37	8	734.591	SF	R
Pé de Serra Cave	Serra do Ramalho	2	-13.661617	-43.7885	50	7	526.655	SF	D
Mandiaçu Cave	Serra do Ramalho	1	-13.533514	-43.7603	59	9	499.485	SF	D
Dedê Cave	Coribe	1	-13.880596	-44.2171	39	5	681.085	SF	R
4 Cabras Cave	Coribe	1	-13.747524	-44.2341	43	3	669.033	CR	D

Table 2. Relationship between habitat structure of the sectors with species richness. P-value (P); Estimate value (Est.); Overall is regarding all the fauna; T is for troglobitic fauna and n-T is for the non-troglobitic fauna; plants debris (DTV), fine branch (GALF), medium branch (GALM), trunk (TRO), other organic substrate (OTO), silt (SEF), bird feces (FZA), Mocó feces (FZMO), basidiomycetes (BAM), hardpan pinnacles (PHP), actinomycetes (ACT), fine gravel (CAF) and roots (RZ); R^2: refers to square R value of GLM models; R^2M: refers to square R value of fixed variables of GLMM models, and R^2C: refers to square R value of random variables of GLMM models.

	Mesoscale/Sector											
	Model 1						**Model 2**					
Variables	**Overall**		**T**		**n-T**		**Overall**		**T**		**n-T**	
	P	**Est.**	**P**	**Est.**	**P**	**Est.**	**P**	**Est.**	**P**	**Est.**	**P**	**Est.**
Distance					1.0E¯1	-0.180					3.0E¯1	-0.150
Temperature	0.020	-0.1100	0.0300	0.150	0.006	-0.140	0.003		0.030	0.220	8.37 E¯5	-0.160
Humidity			0.0004	0.300					0.010	0.270	0.010	-0.090
Resource Av.	2.00E-04	0.1600			5.00E-04	0.160						
DTV							1.09E¯8	-0.110			1.33E-08	0.130
GALF							2.00E-04	0.080			0.001	0.070
GALM							0.010	0.070			0.020	0.070
TRO							7.75E¯6	0.090			2.33E-06	0.110
DP							0.004	0.070	0.010	0.090		
OTO							0.001	0.060			7.00E-04	0.070
SEF							1.02E¯5	0.160			4.11E-05	0.170
FZA							0.020	0.040	0.010	0.120		
FZMO							0.009	0.060			0.012	0.060
BAM							0.040	0.040	0.002	0.120		
PHP							0.030	0.050				
ACT									0.004	0.200	0.040	
CAF											0.020	0.080
RZ									0.020	-0.390		
R^2/R^2M	**22.48%**		**20.00%**		**31.62%**		**80.07%**		**43.69%**		**87.14%**	
R^2C	**68.99%**				**72.92%**		**80.13%**					

Table 3. Relationship between habitat structure of the sectors and quadrats with species composition. dbRDA 1: refers to model number one; dbRDA 2: refers to model number two. Relationship between habitat structure of the sectors with species richness. Overall is regarding all the fauna; T is for troglobitic fauna and n-T is for the non-troglobitic fauna; plants debris (DTV), silt (SEF), guano (GU), medium branch (GALM), trunk (TRO), Mocó feces (FZMO), roots (RZ), Tamanduá feces (FZT), sand (ARE), speleothems (ES), other organic substrate (OTO), basidiomycetes (BAM), retraction crack (GR), water pond (WP) and concrete like floor (RC).

Variables	Mesoscales/Sector						Microscale/Quadrat					
	dbRDA 1			dbRDA 2			dbRDA 1			dbRDA 2		
	Overalll	T	n-T	Overall	T	n-T	Overall	T	n-T	Overall	T	n-T
Distance	0.005	0.005	0.005	0.005	0.005	0.005		0.005	0.005	0.015	0.005	0.005
Humidity	0.005	0.005	0.005	0.010	0.005	0.005						
Temperature	0.005	0.005		0.005	0.04	0.005						
Resource av.	0.005	0.005					0.005					
Resource div.						0.050		0.045		0.010		
Substrate div.							0.005		0.005	0.005		0.005
DTV				0.005		0.005				0.005		
SEF				0.005	0.05	0.010				0.005	0.030	0.010
GU				0.005		0.010				0.005		0.010
GALM				0.005	0.005							
TRO				0.010	0.02	0.005				0.050		
FZMO				0.010	0.005							
RZ				0.040	0.005	0.030						
FZT						0.020						
ARE						0.040						
ES					0.010							
OTO					0.040					0.020	0.040	0.005
BAM										0.005		
GR											0.005	0.010
WP												0.005
RC												0.030
Explanation	**9.99%**	**6.00%**	**9.00%**	**19.00%**	**20.00%**	**18.00%**	**11.00%**	**14.00%**	**13.00%**	**17.00%**	**16.00%**	**18.00%**

Table 4. Relationship between habitat structure of the quadrats with species richness. Relationship between habitat structure of the sectors with species richness. P-value (P); Estimate value (Est.); Overall is regarding all the fauna; T is for troglobitic fauna and n-T is for the non-troglobitic fauna. and R^2: refers to square R value of GLM models; R^2M: refers to square R value of fixed variables of GLMM models, and R^2C: refers to square R value of random variables of GLMM models. Plants debris (DTV), fine branch (GALF), other organic substrate (OTO), silt (SEF), basidiomycetes (BAM), medium block (MB), small block (SB), coarse gravel (CAG), hardpan (HP), sand (ARE), guano (GU), worm acorns (BM) and water ponds (WP).

	Microscale/Quadrat											
	Model 1						Model 2					
Variables	**Overall**		**T**		**n-T**		**Overall**		**T**		**n-T**	
	P	**Est.**	**P**	**Est.**	**P**	**Est.**	**P**	**Est.**	**P**	**Est.**	**P**	**Est.**
Distance	1.00E-02	-0.140	1.00E-02	-0.140	1.06E¯10	1.120	1.00E-03	-0.170	1.00E-03	-0.170	3.17E¯15	0.190
Resource Av.	7.84E¯9	0.240	7.85E¯9	0.240								
Resource div.							0.020	0.080	0.020	0.080		
Substrate div.					0.008	-0.58	0.040	0.110	0.040	0.110	2.20E¯15	-3.43E-01
Shelter div.							0.010	0.140	0.010	0.140		
DTV							9.49E¯11	0.180	6.55E¯10	0.150	1.00E-04	-4.210
GALF											1.00E-02	-9.56E-10
OTO							3.41E¯7	0.110	2.65E¯7	0.110	1.03E¯9	1.04E-01
SEF							4.00E-04	0.210	4.00E-04	0.140		
BAM									8.06E¯10	0.090		
MB											0.003	-2.98E-01
SB											0.030	1.06E-01
CAG											2.00E-04	-2.73E-01
HP											0.010	1.14E-01
ARE											1.54E¯5	1.66E-01
GU							3.71E¯6	0.120	1.75E¯5	0.110	0.020	6.34E-02
BM							2.37E¯10	0.100				
WP							0.020	0.060				
R^2/R^2M	**12.08%**		**12.08%**		**25.49%**		**41.89%**		**79.47%**		**85.00%**	
R^2C	**47.92%**		**47.92%**		**99.79%**							

SUPPLEMENTARY MATERIAL

Supplementary material I. The values of temperature (ºC), humidity (%), distance from the entrance (m), substrate diversity, resource diversity, shelter diversity, availability of resources, and shelter of the sectors. Div: diversity and Avail: availability.

Sector	Temperature (ºC)	Humidity (%)	Distance (m)	Substrate Div.	Shelter Div.	Resource Div.	Shelter Avail.	Resource Avail.
c1s1	21.6	81.5	375	0.918860516	0.9188605	0	100	3
c1s2	23.1	85.7	800	1.569947524	1.0079469	0	25	0
c1s3	20.2	93.3	1175	1.075195712	0.818742	0	89	0
c1s4	22.6	90.3	1612.5	1.464031526	0.7982043	0	28	0
c1s5	21	95.8	2112.5	1.717874015	1.3823662	0	56	0
c1s6	22.6	92.9	2737.5	0.809572547	0.7589368	0	14	0
c1s7	22.7	91.6	3050	0.965730993	1.0889	0	10	0
c1s8	23.6	96	3612.5	0.656423546	0.6931472	0	6	0
c1s9	21.7	95.7	2487.5	1.140926681	0.6931472	0	14	0
c1s10	22.8	89.9	2925	1.02369299	1.3760956	0	30	0

c1s11	23	83.2	3487.5	0.838234932	0.6662784	0	26	2
c1s12	22.8	84.6	1157.5	1.034265558	0.5625654	0	46	0
c1s13	23	83	1687.5	1.248351406	1.4241473	0	31.5	4.5
c1s14	22.4	92.8	1250	1.524279516	1.4118256	0	56	0
c1s15	22.8	84.9	562.5	1.39985641	1.1766972	0	37	0
c1s16	22.8	91.6	2500	0.618946497	0.9069848	0	12	2.2
c1s17	24.5	67	375	1.291890086	1.3173309	0.63651417	46.4	0.6
c1s18	24.5	60.6	312.5	0.948502761	0.6539296	0.66156324	38	8.5
c1s19	24.5	56.6	750	1.323456608	1.0162851	0	76	0
c1s20	21.5	75	250	0.958118172	0.5917104	0	30.5	8.5
c1s21	21.3	86.8	562.5	0.784167587	0.6829081	0	28	16
c1s22	23.6	80.8	1062.5	0.346515337	0	0	11	0
c2s1	23.1	88.3	42.54	1.261702884	0.4780984	0	51.5	5.5

c2s2	25.8	83.3	133.58	1.217850594	0.5547896	0	37	9
c2s3	23.1	94.1	256.06	1.001192531	0.6917615	0	19	5.5
c2s4	24.4	91.5	371.72	1.233311198	0.6615632	0	40	0.5
c2s5	23.4	95.5	470.33	0.962637863	0.2145592	0	9	1
c2s6	24.3	93.1	528.19	1.340989399	0	0	14	0
c2s7	23.6	69.8	711.5	1.048244314	0	0	1	0
c2s8	24.3	95.6	824.13	0.815890209	0	0	7	6
c2s9	24	97.1	995.72	0.901355158	1.2567831	0	5	3.5
c2s10	25	94.9	1123.98	0.613749903	0	0	10	0.5
c2s11	24.8	95.3	1267.57	1.114627243	0.6057975	0	8.5	3.5
c2s12	23.8	97.1	807.99	0.6859298	0	0	0	0
c2s13	25	97.2	933.03	0.805122359	0	0	0	0
c2s14	23.6	96.9	1061.69	0.5222723	0	0	1	1

c2s15	24.4	97.3	1215.51	0.931028851	0.5982696	0	7	0
c3s1	22.2	70.8	50	1.846098494	1.2992659	0	25	6
c3s2	23.4	76.7	119.2	1.809080907	1.1386319	0	87	5.5
c3s3	23	87.6	196.1	1.416789785	0.9605236	0.3488321	47	9
c3s4	23.5	90.5	263.4	1.667721031	1.3249317	0	68	2
c3s5	23.8	92.6	334.6	1.605174004	1.1661146	0	75	0
c3s6	24	94.8	411.5	1.452222165	1.0906913	0	39	2.5
c3s7	25.5	95.3	488.4	0.705142324	0.6931472	0	15	4.5
c3s8	25.5	93.7	559.6	0.936441264	0.6869616	0	9	4
c3s9	25.6	93.7	636.5	0.427128978	0	0	0	3
c3s10	25.3	95.4	684.6	0.460053422	0	0	0	1
c4s1	25.2	71.5	30.7	0.782474324	0.6901857	0.16794415	26	12.5
c4s2	24.4	72.2	130.7	1.413009579	0.9012263	0	26	7

c4s3	25.3	71.5	200	1.458035711	0.1988216	0	49	3
c4s4	24.9	76.1	269	1.757227108	0.8142549	0	43	0
c4s5	24.7	91.5	338	0.95411228	0.7801308	0	42	4
c4s6	25.5	94.6	399.5	0.897945725	0	0	10	0
c4s7	25.8	96.2	468.5	0.864903375	0.6365142	0.69314718	30	1.2
c4s8	26.2	96.2	530	0.610864302	0	0	30	0
c4s9	26.6	96.3	591.5	0.70540882	0	0	29	2.5
c5s1	22	88.8	170	0.879095858	0.3046361	0	44	6.5
c5s2	25.4	74	270	1.211831453	1.0844317	0	21	0
c5s3	23.3	86.8	390	1.496255828	1.2370621	0	45	4
c5s4	24.7	92.7	540	0.659214618	0.4505612	0	24	0
c5s5	24.8	96	740	0.61520864	0	0	9	8
c5s6	26.7	94.2	990	1.742258202	1.0027183	0	54	0

c5s7	27.8	96.3	1135	1.071116074	0.3275448	0	89	0
c5s8	28.5	96.8	1260	0.613064279	0	0	1	9
c5s9	28.6	69.9	1405	1.06066399	0.4825776	0	16	3
c6s1	19	84.1	10.8	1.807557894	1.2911034	0	77	7
c6s2	20.4	91.8	115	0.587375175	0	0	10.5	3
c6s3	21.4	90.3	147	0.424940907	0.6774944	0.54020414	8.5	6.5
c6s4	21.7	96.1	198	0.957770951	0.3078447	0	32.5	1
c6s5	22.6	90.2	260	0.925799513	0.3261798	0	9.9	1.1
c6s6	22.7	96.4	392	0.841446265	0.2711894	0	32.5	3
c6s7	23	91	459	0.892482511	0	0	0	1
c6s8	25.1	97	550	1.149439133	0.5670609	0	52.5	2.5
c7s1	21	84.3	71.5	1.219335245	0.9578369	0	47	1.2
c7s2	22.4	95.7	85.8	1.744831581	1.3326377	0	75	7

c7s3	23.2	96.1	171.5	0.834871407	0	0	27	27
c7s4	24	96.4	328.64	0.500402424	0	0	20	0
c7s5	25.2	96.8	428.64	0.63903186	0	0	10	0
c7s6	25.9	97.7	528.64	1.730491062	1.3563621	0	50	9
c7s7	23.8	95.6	271.5	1.054920168	0.6365142	0	60	20
c8s1	21.2	73.5	17.7	1.243782303	0.6909233	0	7.5	3.9
c8s2	20.2	82.9	24.3	1.504534457	1.0008048	0	62.5	0
c8s3	20.5	86.7	41	1.617620663	0.8382061	0	30	1.8
c8s4	21.8	85.6	97	1.759016598	1.3535984	0	68	0
c8s5	21.1	93.2	110	1.400954708	1.0758865	0	34.5	2
c8s6	21.3	81.8	35	0.850641053	0	0.56233515	0	4
c9s1	22.4	95.8	100	0.689072102	0	0	0	0
c9s2	22.5	91.3	150	1.25963562	0.676526	0	11	0.5

c9s3	19.1	89.5	150	1.545788681	1.0969511	0.39830711	43	22
c9s4	18.4	94.6	250	1.940214432	1.4527131	0	63.5	2
c9s5	19.3	92	84	1.625856673	1.1924073	0.50040242	50	2.5
c9s6	19.7	92.1	66	0.696538931	0.4195559	0.41955589	27	27
c10s1	24	64.5	10	1.50055424	1.0795296	0.61188169	19	16.6
c10s2	24	63	6	1.062910889	0.9238407	0.68900924	17	11
c10s3	24.4	65.6	20	1.362821178	1.0716836	0	78	0
c10s4	27.3	73.2	18	1.441405329	0.8262243	0	39	24
c10s5	23.1	81	100	1.810140704	1.3600547	0	46	10
c10s6	22.2	95.3	108	1.296343293	0	0.69314718	10	20
c11s1	26.6	63.3	20	1.002698356	0.589401	0	28	6
c11s2	25.7	68.5	55	1.010467845	0.6252195	0.42270909	35	12
c11s3	27.3	61	6	1.066999865	0.5088451	0.56233515	28.5	12

c11s4	25.4	66.7	70	1.401772755	0.661601	0	71	10
c11s5	25.1	67.6	150	1.914968919	1.4973687	0	59.5	6.5
c12s1	21.6	77.5	20	1.232867014	1.0311997	0	34	3
c12s2	23	88.8	80	0.723701132	0.6210864	0	16	5
c12s3	22.9	91.2	110	0.821960488	0.9632493	0	13	6.5
c12s4	23	95	190	1.145679349	0.6662784	0.3488321	13	9
c12s5	24	90	215	0.696738085	0.6846163	0	23	0
c13s1	23.6	81.5	13	1.373321666	1.2397966	0	17.2	0
c13s2	25.3	85.3	70	1.900250392	1.0601189	0	38	24
c13s3	26.1	87.9	98	0.945699365	0.1662679	0	76	1
c13s4	26.1	91	125	0.981333553	1.0220831	0	10.5	0
c13s5	27.1	91	150	1.823330674	1.2303844	0	64	0
c14s1	18.1	86.9	45	1.475318905	1.229813	0	71	0.6

c14s2	18.2	89.2	75	1.691504563	1.27098	0	70	4.5
c14s3	19.3	87	145	1.54916733	1.3108206	0	80	16
c14s4	20.5	90.4	207	0.773212075	1.0844459	0	23	2
c15s1	22.5	74.5	19	1.868761889	1.6786784	0.3571521	66	16.5
c15s2	20.8	68.1	23.5	1.838856615	1.5773992	0.63651417	23.5	3.5
c15s3	21.4	76.2	46	1.373631405	0.6953626	0	25.5	0
c15s4	20.7	76.6	60	1.093645411	0.9642722	0	96	0
c16s1	23.5	84	1328	1.264464306	0.6791933	0	72	0
c16s2	23.8	74.6	1079	0.79098735	0	0	30	5
c16s3	23.1	87.6	1204	0.718717575	0	0	30	2.5
c16s4	24.2	90.9	1370	0.783806339	0.6931472	0	2	1.5
c17s1	25.2	73.5	80	0.660362216	0	0	30	0
c17s2	24.9	73.3	176	1.651044846	0.9666413	0	55	0

c17s3	23.4	80.7	304	0.812378725	0	0	0	4
c18s1	22.4	77	11.3	1.079969282	0.9095587	0	24.5	4
c18s2	23.3	72.8	50.7	1.753347507	1.3885424	0	66	0
c18s3	22.5	78.7	36.7	0.845028742	0.5623351	0.63651417	4	1.5
c19s1	23.9	79.3	45.3	1.037392358	0.8231892	0	20.6	1.7
c19s2	24.3	73.7	69.3	0.968073801	0.942675	0	19	0
c19s3	23.3	86.5	96	1.16800607	1.0986123	0	30	0
c20s1	22.1	91.4	15	1.388238689	1.4659832	0.32508297	47.5	5
c20s2	21.5	94.4	50	0.600482908	0.1914441	0	84	80
c21s1	20.8	77.6	0	2.122064371	1.8128126	0.95739426	77.5	24.5
c21s2	21.6	75.8	16	1.596871483	1.4803754	0.12045603	40.3	8.2
c22s1	20.1	21.5	21.5	1.411417766	1.1500326	0	51	2
c22s2	20	89.5	49	0.679484191	0.5982696	0.68696158	14	9

c23s1	20.6	84.7	60	1.35728305	1.1866408	0	14	6
c23s2	20.8	83.2	191	1.291642083	1.3337227	0	16.5	2
c24s1	25.5	68.7	33	1.246263204	0.9634423	0	59.4	0.2
c25s1	24.2	77.7	12	0.936388442	1.0549202	0	2.5	11
c26s1	26.2	80.8	13	1.702516038	1.0955105	0	44.5	9.5

Supplementary material II. The values of distance from the entrance (m), substrate diversity, resource diversity, shelter diversity, availability of resources, and shelter of the quadrats. Div: diversity and Avail: availability.

Quadrats	Distance (m)	Substrate Div.	Shelter Div.	Resource Div.	Shelter Avail.	Resource Avail.
c1s1q1	370	0.143599179	0.1435992	0	10000	0
c1s1q2	375	0	0	0	10000	0
c1s1q3	380	0	0	0	10000	0
c1s2q1	795	0.945564724	0	0	3489.99	0
c1s2q2	800	0.795023829	0.5374896	0	3170.4	0
c1s2q3	805	1.295254009	0	0	993.296	0
c1s3q1	1170	0.67994101	0.679941	0	10000	0
c1s3q2	1175	0.32349147	0.3234915	0	10000	0
c1s3q3	1180	0	0	0	10000	0
c1s4q1	1607.5	0.619915136	0	0	1680.95	0
c1s4q2	1612.5	0.351409779	0.0719829	0	9289.76	0
c1s4q3	1617.5	1.3059572	0.3209671	0	5697.93	0
c1s5q1	2107.5	0.958130092	0.7439304	0	3893.696	0

c1s5q2	2112.5	0.329309568	0	0	8980.67	0
c1s5q3	2117.5	0.949633396	0.4878573	0	3388.48	0
c1s6q1	2732.5	0.730988562	0	0	90.346	0
c1s6q2	2737.5	0.574964446	0	0	0	0
c1s6q3	2742.5	1.183057815	0.6750314	0	3550.27	0
c1s7q1	3045	0.489634047	0.1397707	0	8801.56	0
c1s7q2	3050	1.001158901	0.5433256	0	6255.48	0
c1s7q3	3055	0.972143929	0.6613892	0	8613.92	0
c1s8q1	3607.5	0	0	0	0	0
c1s8q2	3612.5	0.605680023	0.60568	0	10000	0
c1s8q3	3627.5	0.278114334	0	0	0	0
c1s9q1	2482.5	0.678767525	0	0	5845.89	0
c1s9q2	2487.5	0.715583291	0	0	253.59	170.62
c1s9q3	2492.5	0.708999066	0	0	1072.49	252.29
c1s10q1	2920	0.78173716	0.2024968	0	1430.33	1174.05
c1s10q2	2925	1.098076471	0.6931348	0	6512.94	0
c1s10q3	2930	0.818817665	0.6734602	0	208.52	56.33
c1s11q1	3482.5	0.132405413	0	0	0	0
c1s11q2	3487.5	0	0	0	0	0
c1s11q3	3492.5	0.045210608	0	0	0	77.14
c1s12q1	1152.5	0	0	0	10000	0
c1s12q2	1157.5	0.672956891	0	0	6001.35	0
c1s12q3	1162.5	0.137912292	0.0571752	0	9840.94	0
c1s13q1	1682.5	0.405186703	0.4051867	0	10000	0
c1s13q2	1687.5	0.381521652	0.3815217	0	10000	0
c1s13q3	1692.5	0.958877498	0.3637763	0	7560.36	0

c1s14q1	1245	1.32624956	1.0181299	0	7453.47	0
c1s14q2	1250	0.906120107	0.5051058	0	4372.65	0
c1s14q3	1255	0.232071966	0.232072	0	10000	18.24
c1s15q1	557.5	0.862972444	0.5660014	0	3651.26	0
c1s15q2	562.5	1.65358279	1.449162	0	7639.92	0
c1s15q3	567.5	0	0	0	10000	0
c1s16q1	2495	0	0	0	10000	0
c1s16q2	2500	0.228256047	0	0	9395.31	0
c1s16q3	2505	0.156038634	0.1560386	0	10000	0
c1s17q1	370	0.323685885	0.1955187	0	9702.16	297.84
c1s17q2	375	0	0	0	10000	0
c1s17q3	380	0.363305705	0.3633057	0	10000	0
c1s18q1	307.5	0.106012133	0	0	9779.24	220.76
c1s18q2	312.5	0.165764814	0	0.350716172	9645.15	354.85
c1s18q3	317.5	0.826416154	0	0	6018.02	519.15
c1s19q1	745	0.659011052	0.6480785	0	2145.72	0
c1s19q2	750	0.372046163	0.3720462	0	10000	0
c1s19q3	755	0.660362505	0.4756991	0	2370.15	0
c1s20q1	245	0.685978556	0.1083585	0	8084.23	498.99
c1s20q2	250	0.323471622	0	0	9007.32	992.68
c1s20q3	255	0	0	0	10000	0
c1s21q1	557.5	0.653366394	0	0	6400.9	0
c1s21q2	562.5	0.691129444	0	0	4682.48	0
c1s21q3	567.5	0.222514376	0.2225144	0	10000	583.9
c1s22q1	1057.5	0.19909783	0	0	501.98	0
c1s22q2	1062.5	0	0	0	0	0
c1s22q3	1067.5	0	0	0	0	0
c2s1q1	37.54	0.866811892	0	0	863.99	57.22

c2s1q2	42.54	1.010139447	1.0101394	0	10000	0
c2s1q3	47.54	0.83551306	0.2676416	0	5527.94	0
c2s2q1	128.58	0.683040456	0	0	0	5709.67
c2s2q2	133.58	0.401598739	0	0	0	0
c2s2q3	138.58	0	0	0	0	0
c2s3q1	261.06	0	0	0	0	0
c2s3q2	256.06	0.687471699	0	0	5532.2	0
c2s3q3	261.06	0.633109121	0	0	0	0
c2s4q1	366.72	0.496319273	0	0	0	0
c2s4q2	371.72	0.678026629	0	0	4132.7	0
c2s4q3	376.72	0	0	0	0	0
c2s5q1	465.33	0	0	0	0	0
c2s5q2	470.33	0.397172847	0	0	8642.5	0
c2s5q3	475.33	0.248874144	0	0	0	681.68
c2s6q1	523.19	0.98332293	0	0	0	0
c2s6q2	528.19	1.006075493	0	0	0	126.52
c2s6q3	533.19	0	0	0	10000	0
c2s7q1	706.5	0	0	0	0	0
c2s7q2	711.5	0.69311518	0	0	4960	0
c2s7q3	716.5	0.670563982	0	0	0	0
c2s8q1	819.13	0.582960225	0	0	0	0
c2s8q2	824.13	0	0	0	0	0
c2s8q3	829.13	0.672767638	0	0	0	0
c2s9q1	990.72	0	0	0	0	0
c2s9q2	995.72	0	0	0	0	0
c2s9q3	1000.72	0.180313099	0	0	0	195.44
c2s10q1	1118.98	0	0	0	0	0
c2s10q2	1123.98	0.394106976	0	0	0	0
c2s10q3	1128.98	0.724471174	0	0	590.7	0
c2s11q1	1262.57	0.46984328	0	0	0	0

		3				
c2s11q2	1267.57	0.252901116	0	0	0	697.15
c2s11q3	1272.57	0.088247882	0	0	0	0
c2s12q1	802.99	0	0	0	0	0
c2s12q2	807.99	0.61371765	0	0	0	0
c2s12q3	812.99	0.645274413	0	0	0	141.7
c2s13q1	928.03	0.602401522	0	0	0	0
c2s13q2	933.03	0.548367615	0	0	0	0
c2s13q3	938.03	0.690944949	0	0	204.5	0
c2s14q1	1056.69	0.527246355	0	0	658.3	0
c2s14q2	1061.69	0.368972183	0	0	0	0
c2s14q3	1066.69	0.626717102	0	0	0	0
c2s15q1	1210.51	0.358306627	0.6916959	0	878.52	0
c2s15q2	1215.51	0.070496686	0	0	103.84	0
c2s15q3	1220.51	0.080613243	0	0	0	0
c3s1q1	45	0.8809239	0.6331595	0	3596.43	0
c3s1q2	50	1.182036312	1.485636	0	3569.96	0
c3s1q3	55	0.195818145	0	0	490.87	0
c3s2q1	114.2	1.095704186	0.6898028	0	6484.59	0
c3s2q2	119.2	1.068205641	0.6703802	0	5774.3	0
c3s2q3	124.2	0.465588348	0	0	0	0
c3s3q1	191.1	0.325082973	0	0	1000	0
c3s3q2	196.1	0.801818553	0	0	2000	0
c3s3q3	201.1	0.610864302	0	0	3000	0
c3s4q1	258.4	0.863261385	0.6086003	0	3522.94	0
c3s4q2	263.4	0.650062544	0	0	0	0

c3s4q3	268.4	0.682085853	0	0	0	0
c3s5q1	329.6	0.455414969	0	0	0	0
c3s5q2	334.6	0.693147181	0	0	0	0
c3s5q3	339.6	1.201645147	0.8762213	0	6063.4	0
c3s6q1	406.5	1.108522121	0.5527897	0	7338.07	2196.06
c3s6q2	411.5	1.025071524	0.8331386	0	4155.89	0
c3s6q3	416.5	1.086703187	0.8813707	0	4518.06	0
c3s7q1	483.4	0.440022913	0	0	1602.13	0
c3s7q2	488.4	1.167033995	0.9641417	0	4916.56	0
c3s7q3	493.4	0.693117535	0	0	0	4961.5
c3s8q1	554.6	0.163025115	0	0	0	384.62
c3s8q2	559.6	0.716767523	0	0	761.96	1213.47
c3s8q3	564.6	0	0	0	0	0
c3s9q1	631.5	0	0	0	0	0
c3s9q2	636.5	0	0	0	0	0
c3s9q3	641.5	0.05491887	0	0	0	97.65
c3s10q1	679.6	0.733574738	0	0	0	1390.75
c3s10q2	684.6	0.557051772	0	0	0	923.31
c3s10q3	689.6	0.369170904	0.2187297	0	177.26	0
c4s1q1	25.7	0.179128381	0	0	435.7	0
c4s1q2	30.7	0.198515243	0	0	500	0
c4s1q3	35.7	0.167944148	0	0	400	0
c4s2q1	125.7	0.815437893	0	0	1724.43	0
c4s2q2	130.7	0.443956233	0	0	1626	0
c4s2q3	135.7	1.342419126	0.3035134	0.130927208	989.47	3094.94
c4s3q1	195	1.606235262	0	0.62575748	585.03	2531.87
c4s3q2	200	0.81467128	0	0	1139.84	0

		3				
c4s3q3	205	0.130890145	0.1308901	0	10000	0
c4s4q1	264	0.651306896	0	0	6436.2	0
c4s4q2	269	0.744455768	0.4803219	0	2921.97	0
c4s4q3	274	0.551217058	0	0	0	0
c4s5q1	333	0.415794013	0	0	1460.49	0
c4s5q2	338	0.661672457	0	0	3752.14	0
c4s5q3	343	0.680710995	0	0	4213.09	0
c4s6q1	394.5	0.673011667	0	0	4000	0
c4s6q2	399.5	0.647446639	0	0	3500	0
c4s6q3	404.5	0.653418195	0	0	3600	0
c4s7q1	463.5	0.866425466	0	0	3519.08	0
c4s7q2	468.5	0.612038291	0	0	3013.91	0
c4s7q3	473.5	0.610864302	0	0	3000	0
c4s8q1	525	0.592953317	0	0	2800	0
c4s8q2	530	0.619100664	0	0	3100	0
c4s8q3	535	0.664064127	0	0	3800	0
c4s9q1	586.5	0.590314257	0	0	2772.26	0
c4s9q2	591.5	0.836429517	0.3607404	0	4269.12	0
c4s9q3	596.5	0.661563238	0	0	3750	0
c5s1q1	165	0.604520665	0	0	2926.65	0
c5s1q2	170	0.542172014	0	0	2324.1	0
c5s1q3	175	0.581884595	0	0	139	0
c5s2q1	265	0.067951588	0	0	126.73	0
c5s2q2	270	1.334468207	0.6292295	0	3967.5	146.86

c5s2q3	275	0.688645004	0	0	4525.9	0
c5s3q1	385	0.97347247	0.5003541	0	6421.62	0
c5s3q2	390	0.167944148	0	0	400	0
c5s3q3	395	0.167944148	0	0	400	0
c5s4q1	535	0.357947949	0.3286498	0	1000	0
c5s4q2	540	0.265621059	0.4341622	0	640	0
c5s4q3	545	0.264907251	0	0	744.13	0
c5s5q1	735	0.435400328	0	0	1574.4	0
c5s5q2	740	0.813528314	0.6755467	0	3000	0
c5s5q3	745	0.939633131	0.6665537	0	4000	0
c5s6q1	985	1.31610601	0.898705	0	8316.15	0
c5s6q2	990	1.097735055	0.9535276	0	9532.73	0
c5s6q3	995	0.908910424	0	0	0	0
c5s7q1	1130	0	0	0	10000	0
c5s7q2	1135	0.956634989	0.4868389	0	5526.16	0
c5s7q3	1140	0.437892975	0	0	8410.69	0
c5s8q1	1255	0.215010653	0	0	0	557.15
c5s8q2	1260	0.314809304	0	0	0	953.79
c5s8q3	1265	0	0	0	0	0
c5s9q1	1400	0.815856236	0.6125246	0	3149.05	0
c5s9q2	1405	0.655057264	0	0	6371.2	0
c5s9q3	1410	0.37290122	0	0	0	1230.21
c6s1q1	5.8	0.646779963	0.1896342	0	8044.03	0
c6s1q2	10.8	0.891176807	0.3794866	0	5251.78	0
c6s1q3	15.8	0.873865338	0.6345799	0	9136.09	0
c6s2q1	110	0.610864302	0	0	3000	0
c6s2q2	115	0.50040242	0	0	2000	0

		4				
c6s2q3	120	0.500402424	0	0	2000	0
c6s3q1	142	0.728787527	0	0	1740.35	0
c6s3q2	147	0	0	0	0	0
c6s3q3	152	0	0	0	0	0
c6s4q1	193	0.99816936	0	0	1750.98	2890.57
c6s4q2	198	0.645977859	0	0	3476.47	0
c6s4q3	203	0	0	0	0	0
c6s5q1	255	0.028887373	0	0	45.15	45.15
c6s5q2	260	0.581887845	0.6823725	0	1744.39	744.39
c6s5q3	265	0.198515243	0	0	500	0
c6s6q1	387	0.526397424	0	0	0	2195.97
c6s6q2	392	0.703600943	0.6187496	0	2422.78	0
c6s6q3	397	0.735573887	0.1142111	0	6181.18	0
c6s7q1	454	0.729154704	0	0	1435.9	1045.29
c6s7q2	459	0	0	0	0	0
c6s7q3	464	0.619884359	0	0	50	0
c6s8q1	454	0	0	0	10000	0
c6s8q2	550	0.720059189	0	0	225.18	225.18
c6s8q3	555	0.325082973	0	0	1000	0
c7s1q1	66.5	0.820474247	0.8204742	0	10000	0
c7s1q2	71.5	0.693953201	0.5264722	0	2500	0
c7s1q3	76.5	1.047908595	0.6781598	0	5249.62	0
c7s2q1	80.8	1.086551494	0.6833585	0	6142.02	2642.02
c7s2q2	85.8	0.532735291	0.1616643	0	2000	76.08
c7s2q3	90.8	0.325082973	0	0	0	0
c7s3q1	166.5	0.387525692	0	0	1306	1306
c7s3q2	171.5	0.64986964	0	0	3539.7	3539.7

		5				
c7s3q3	176.5	0.441712129	0	0	1612.35	1612.35
c7s4q1	323.64	0.644507695	0	0	3453.3	0
c7s4q2	328.64	0.673011667	0	0	4000	0
c7s4q3	333.64	0.500402424	0	0	2000	0
c7s5q1	423.64	0	0	0	0	0
c7s5q2	428.64	0	0	0	0	0
c7s5q3	433.64	0.310402573	0	0	934.3	934.3
c7s6q1	523.64	1.265186193	0.6241039	0	4233.57	0
c7s6q2	528.64	1.306469483	0.6082386	0	5025.87	1492.32
c7s6q3	533.64	0.861068154	0.6444162	0	1180.23	772.83
c7s7q1	266.5	0.272741154	0.6858764	0	613.49	343.69
c7s7q2	271.5	0.68501928	0	0	366.35	366.35
c7s7q3	276.5	0.975954327	0	0	1947.07	1947.07
c8s1q1	12.7	0.441962702	0	0	1613.87	0
c8s1q2	17.7	1.097267023	0.9237305	0	4442.34	0
c8s1q3	22.7	0.183521137	0	0	9550	450
c8s2q1	19.3	0.972851866	0.9728519	0	10000	0
c8s2q2	24.3	0	0	0	10000	0
c8s2q3	29.3	1.239251414	0.8973283	0	7091.55	0
c8s3q1	36	0.830737955	0.3373712	0	7281.64	0
c8s3q2	41	0.941800821	0.3770554	0	7800.37	0
c8s3q3	46	1.046171657	0.6661852	0	7806	0
c8s4q1	92	0	0	0	10000	0
c8s4q2	97	0.610864302	0	0	7000	0
c8s4q3	102	0.758445998	0.6273338	0	9644.95	0
c8s5q1	105	0.537943779	0	0	2289	0

c8s5q2	110	0.889172718	0.5221716	0	8301.09	0
c8s5q3	115	0.935970446	0.6316574	0	4101.33	0
c8s6q1	30	0.837359763	0.4861733	0	2915.21	203
c8s6q2	35	0.632768989	0	0	6719.78	0
c8s6q3	40	1.293503059	0.6880646	0	5894.67	861.09
c9s1q1	95	0.681979703	0	0	0	0
c9s1q2	100	1.075730215	0	0	3822.69	0
c9s1q3	105	0.528365924	0	0	0	0
c9s2q1	145	0.648925772	0.6489258	0	10000	0
c9s2q2	150	0.570636084	0	0	151.47	0
c9s2q3	155	1.003638079	0	0	727.25	0
c9s3q1	145	0.582933327	0.5829333	0	10000	2696.73
c9s3q2	150	0.784854121	0.2624084	0.262408374	4106.53	4106.53
c9s3q3	155	0.946316268	0.475213	0.475212981	1107.47	1107.47
c9s4q1	245	1.061063125	1.0610631	0	10000	2343.78
c9s4q2	250	0.844538467	0.8445385	0	10000	0
c9s4q3	255	0.514483514	0.5144835	0	10000	0
c9s5q1	79	0.846679464	0.313577	0	6636.79	0
c9s5q2	84	0.907921879	0.4400073	0	4886.97	0
c9s5q3	89	0.671364714	0.3865873	0	9009.07	0
c9s6q1	61	0.917243619	0.8479424	0.847942417	3320.91	3320.91
c9s6q2	66	0.758493471	0	0	143.91	143.91
c9s6q3	71	0.693147181	0	0	5000	5000
c10s1q1	5	0.325082973	0	0	1000	0
c10s1q2	10	0.90089979	0	0	1027.34	0

		7				
c10s1q3	15	0.572309571	0	0	125.05	125.05
c10s2q1	1	0.356698308	0.6017832	0	900	0
c10s2q2	6	0.334119093	0.6918715	0	800	420.2
c10s2q3	11	1.21592225	0.6876365	0	2248.54	0
c10s3q1	15	0.749543093	0.3492103	0	8268.89	0
c10s3q2	20	0.996948937	1.0927974	0	3311.94	0
c10s3q3	25	1.27644904	0.974426	0	8079.94	0
c10s4q1	13	1.043904043	0	0	0	2203.62
c10s4q2	18	1.146510755	1.3092969	0	3716.95	552.62
c10s4q3	23	1.380708792	0.4795426	0	5213.39	2775.01
c10s5q1	95	1.03919488	1.0391949	0	10000	0
c10s5q2	100	0.795695849	0.5215519	0.541975444	1424.27	1323.47
c10s5q3	105	0.70802805	0	0	628.64	967.49
c10s6q1	103	0.6182914	0.646452	0	1944.39	677.43
c10s6q2	108	0.658932415	0.6419507	0	1779.07	154.03
c10s6q3	113	0.578223118	0	0	213.66	118.8
c11s1q1	20	0.500402424	0	0	2000	0
c11s1q2	25	0.760754592	0	0	1500	1156.3
c11s1q3	30	0.500402424	0	0	2000	0
c11s2q1	50	0.897945725	0	0	1000	3000
c11s2q2	55	0.673011667	0	0	4000	0
c11s2q3	60	0.809945912	0.6636054	0	3000	1862.8
c11s3q1	1	0.842893757	0.573808	0.287833378	2810.11	2267.35
c11s3q2	6	0.598410975	0	0	1614.7	385.3
c11s3q3	11	0.394385986	0	0	492.35	507.65
c11s4q1	65	0.98876016	0.287337	0	2265.91	1557.76

		4	5			
c11s4q2	70	0.938553568	0.596858	0	4284.01	0
c11s4q3	75	0.793665307	0.2156184	0	4729.67	0
c11s5q1	145	0.325082973	0	0	1000	0
c11s5q2	150	0.682261927	0.6822619	0	10000	0
c11s5q3	155	1.047962934	0.6249833	0	7017.21	0
c12s1q1	15	0.931937789	0.7025778	0.581891248	3807.51	848.64
c12s1q2	20	0.836456347	0.4891251	0	3666.21	0
c12s1q3	25	0.91250323	0.412033	0	6618.56	79.02
c12s2q1	75	0.887183315	0.621462	0	3685.19	0
c12s2q2	80	0.64209649	0.3856633	0	2389.99	0
c12s2q3	85	0.447688757	0.6878348	0	1193.38	658.14
c12s3q1	105	0.443685697	0	0	0	0
c12s3q2	110	1.015913778	0	0	1539.27	0
c12s3q3	115	0.693147181	0	0	0	0
c12s4q1	185	0.477778635	0	0	1842.51	0
c12s4q2	190	0.669057294	0	0	57.71	0
c12s4q3	195	0.403822797	0	0	599.91	0
c12s5q1	210	0.744250159	0.5243506	0	2830.65	0
c12s5q2	215	0.993251039	0.5764527	0	5223.36	0
c12s5q3	220	0.096388277	0	0	195.77	0
c13s1q1	8	0.980067414	0.8876102	0	2045.38	0
c13s1q2	13	1.117314837	0.322537	0	2590.63	0
c13s1q3	18	0.838100164	0.5900496	0	3371.8	0
c13s2q1	65	0.484441261	0.9362037	0	1217.71	0
c13s2q2	70	1.05345954	0.896649	0	9484.61	0

			9			
c13s2q3	75	0.647381692	0.650959	0	2083.46	0
c13s3q1	93	1.11385281	0	0	709.24	1134.45
c13s3q2	98	0.600338516	0	0	0	0
c13s3q3	103	0.456567378	0	0	1704.3	0
c13s4q1	120	0.882468399	0	0	42.38	0
c13s4q2	125	0.833065573	0.0632217	0	4887.065	0
c13s4q3	130	0.37390564	0	0	1235.33	0
c13s5q1	145	1.135152163	0.821861	0	8337.05	0
c13s5q2	150	1.478939758	1.2308563	0	7192.02	0
c13s5q3	155	1.344910922	0.6018633	0	3775.5	0
c14s1q1	40	1.399678872	1.3996789	0.422465139	10000	601.03
c14s1q2	45	1.284411624	1.1003548	0	9248.59	0
c14s1q3	50	0.929246758	0.4487626	0	5301.72	4424.01
c14s2q1	70	0.857758017	0.857758	0	10000	0
c14s2q2	75	1.144064037	1.144064	0	10000	0
c14s2q3	80	0.875448857	0.8754489	0	10000	53.61
c14s3q1	140	0.616126813	0	0	7808.65	0
c14s3q2	145	0.833062615	0.8330626	0	10000	0
c14s3q3	150	0.930287571	0.9302876	0	10000	0
c14s4q1	202	0.693147181	0	0	5000	0
c14s4q2	207	0.651578935	0	0	6431.59	0
c14s4q3	212	0.693147181	0	0	5000	0
c15s1q1	14	1.139320949	0.9033127	0	4940.1	0
c15s1q2	19	0.820799893	0.8207999	0	10000	5885.97
c15s1q3	24	0.748767045	0.748767	0	10000	3465.79

c15s2q1	18.5	1.351467162	1.0833688	0	8368.62	0
c15s2q2	23.5	0.452681834	1.0491815	0	563.14	0
c15s2q3	28.5	0.758003945	0	0	236.69	0
c15s3q1	41	0.738462479	0.3592589	0	881.68	0
c15s3q2	46	0	0	0	0	0
c15s3q3	51	0.590528859	0.5200183	0	9848.83	0
c15s4q1	55	1.400588177	1.1249567	0	7991.48	0
c15s4q2	60	0.555091039	0	0	2435.09	0
c15s4q3	65	0.610864302	0	0	0	0
c16s1q1	1323	0	0	0	10000	0
c16s1q2	1328	0.017535403	0.017533	0	10000	25.096
c16s1q3	1333	1.131604021	0.7488503	0	7225.827	57.03
c16s2q1	1074	0.330552692	0	0	0	24.403
c16s2q2	1079	0.256521768	0	0	320.656	0
c16s2q3	1084	0.056341251	0	0	0	0
c16s3q1	1199	0.21325796	0	0	0	0
c16s3q2	1204	0.356906039	0	0	0	0
c16s3q3	1209	0.339625645	0	0	0	0
c16s4q1	1365	0.455663973	0	0	0	257.34
c16s4q2	1370	0.333018888	0	0	0	71.226
c16s4q3	1375	0.420489219	0	0	0	148.985
c17s1q1	75	0.610864302	0	0	3000	0
c17s1q2	80	0.610864302	0	0	3000	0
c17s1q3	85	0.610864302	0	0	3000	0
c17s2q1	171	0.610864302	0	0	3000	0
c17s2q2	176	0.562094421	0	0	0	0

c17s2q3	181	0.485652609	0	0	1896.07	0
c17s3q1	299	0.673011667	0	0	4000	0
c17s3q2	304	0.673011667	0	0	4000	0
c17s3q3	309	0.673011667	0	0	4000	0
c18s1q1	6.3	0.092694006	0	0	9813.61	186.39
c18s1q2	11.3	0.611492763	0.6114928	0.683199353	10000	466.49
c18s1q3	16.3	0.301326119	0.3013261	0	10000	0
c18s2q1	45.7	0.673011667	0	0	4000	0
c18s2q2	50.7	1.138639926	1.1386399	0	10000	0
c18s2q3	55.7	0.700713621	0.7007136	0	10000	0
c18s3q1	31.7	0.690279648	0.6902796	0	10000	0
c18s3q2	36.7	0.180839833	0.1686614	0	9983.12	16.88
c18s3q3	41.7	0.184808441	0.0848191	0	9790.36	0
c19s1q1	40.3	1.203312007	0.3689591	0	5318.65	0
c19s1q2	45.3	0.961086419	0.5473847	0	7695.09	63.13
c19s1q3	50.3	0.843015319	0	0	448.72	0
c19s2q1	64.3	0.641535655	0	0	0	0
c19s2q2	69.3	0.66848434	0	0	0	0
c19s2q3	74.3	0	0	0	0	0
c19s3q1	91	0.906687379	0.0008838	0	3637.35	0
c19s3q2	96	0.968956838	0	0	5604.71	0
c19s3q3	101	1.292251122	1.3091881	0	4600.55	0
c20s1q1	10	0.492872894	0.2024449	0	1714.42	88.02
c20s1q2	15	0.903193031	0	0	4862.96	0
c20s1q3	20	0.325082973	0	0	1000	0
c20s2q1	45	0.49754416	0.179017	0.17901709	8942.9	8942.9

		9	1	5		
c20s2q2	50	0.500402424	0	0	8000	8000
c20s2q3	55	0.500402424	0	0	2000	2000
c21s1q1	0	1.441774292	1.4417743	0.839588695	10000	1371.4
c21s1q2	0	1.360878965	1.360879	0.645581376	10000	7597.8
c21s1q3	0	1.33345277	1.4478579	0.392037781	4097.019	998.395
c21s2q1	11	1.220647859	1.1877976	0.094418389	4384.283	1148.498
c21s2q2	16	1.462029381	1.0759964	0.688886335	8250.645	1262.529
c21s2q3	21	1.651416458	1.5114849	0	9369.913	145.188
c22s1q1	16.5	0.909736322	0.7187021	0.69061965	9520.677	479.323
c22s1q2	21.5	1.029587346	1.003328	0.678453549	9957.136	42.864
c22s1q3	26.5	0.09705632	0	0.296292212	9816.33	183.67
c22s2q1	44	0.703262566	0	0	0	18.137
c22s2q2	49	0.358565904	0	0	1158.46	1158.46
c22s2q3	54	0.320872547	0	0	980.933	980.933
c23s1q1	55	1.063496465	0.1894949	0	5894.57	81.95
c23s1q2	60	0.91534606	0	0	845.45	0
c23s1q3	65	0.993469896	0.6401306	0	8231.91	0
c23s2q1	156	0.884413134	0.8844131	0	10000	113.21
c23s2q2	191	0.689068741	0	0	5451.27	0
c23s2q3	196	1.051804077	0.1050813	0	4592.13	0
c24s1q1	27	1.146693705	0.8368897	0	5472.97	0
c24s1q2	33	0.401437121	0	0	1380.66	0
c24s1q3	38	0.763713856	0	0	824.99	1982.83
c25s1q1	7	1.179224852	0.8347258	0	6134.03	0
c25s1q2	12	0.72224911	0.163410	0.58109642	3500.76	37

		6	3	5		
c25s1q3	17	0.224462505	0.4251206	0	384.69	77.46
c26s1q1	8	0.97034075	0.6898686	0	8827.88	0
c26s1q2	13	1.229898719	0.9563307	0	5722.02	0
c26s1q3	18	0.27981589	0	0	0	0

Artigo 2: Determining factors of microhabitats and invertebrate community structure throughout a single cave: the role of spatial location and distance of the main entrances

Esse capítulo foi escrito em formato de artigo (versão preliminar), redigido conforme as normas para a publicação da revista "Acta Carsologica"

DETERMING FACTORS OF MICROHABITAT AND INVERTEBRATE COMMUNITY STRUCTURE THROUGHOUT A SINGLE CAVE: THE ROLE OF SPATIAL LOCATION AND DISTANCE OF THE MAIN ENTRANCES

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Abstract:

Even though the effects of location and distance from the entrance are known for a few decades, however, the effects of these variations along a single cave and on cave fauna are still quite unknown. So, this work has the objective to evaluate the effects of spatial location and distance from the entrance in the microhabitats traits and how these variations affect the troglobitic and non-troglobitic fauna. Other variables were also collected such as temperature, humidity, trophic and physical attributes to assess whether they also influence the fauna indirectly or directly. The invertebrate fauna was sampled using sectors (10x3m) and quadrats (1x1m), displayed on the cave floor. The distance from the entrance had an effect in decreasing humidity and shelter availability, while for resource availability had an effect in increasing it as far from the entrance. The study calls attention to the usage of different sampling scales and different abiotic and biotic features in only one cave. These features showed to be more influential over troglobitic species than non-troglobitic species, in both mesoscale (sector) and microscale (quadrat).

Keywords: Troglobitic, Serra do Ramalho, Savanah.

INTRODUCTION

It is known that distance from the surface is an important environmental factor that influences cave features such as temperature, humidity, substrate heterogeneity, shelter and resource diversity and availability, among others (Souza-Silva et al., 2021; Pacheco et al., 2020). Based on that, it is expected to observe a gradient of resources and conditions that consequently can promote distinct microhabitats and allows different species to co-exist (Culver & Pipan 2010; Pacheco et al., 2020; Souza-Silva et al., 2021). However, such gradient can also work filtering and limiting species distribution for non-troglobitic species, once as far from the entrance, more homogeneous is the habitat, are less resource available when compared to the amount of resource near the entrance, less shelter availability and stabler values of temperature and humidity (Souza-Silva et al., 2021), which creates an unsuited environment for them. Therefore, the richness of non-troglobitic reduces as it enters in the cave.

On the other hand, the richness of troglobitic species increase as it enters the cave, once at deepest parts of the cave possess stabler conditions of temperature and humidity. So, these microclimatic features are one of the main determinants of their distribution since they are not able to tolerate microclimatic fluctuations. Another peculiarity of deeper zones of the cave that allows the presence of troglobitic species, is the scarcity of food resources, since they are resistant to starvation and also reduces the competition with other invertebrates (Novak et al., 2012; Tobin et al., 2013, Souza-Silva et al., 2021).

The maintenance of specific or representative habitats inside the caves can be an important strategy to protect populations and/or cave communities, mainly because the habitat requirements are different at different scales, ranging from macro to microhabitats and habitat specialization is primarily driven by those environmental filtering (Pellegrini & Ferreira, 2012; Mammola et al., 2020a; Nicolosi et al., 2021). For example, Ferreira & Martins (2001) argued that the spatial distribution of terrestrial invertebrate communities in caves is strongly influenced by the distribution of food resources and habitat requirements (resource-space-dependent, resource-space independent, and para-epigean communities). The resource-space-dependent communities are those formed by generally small organisms (smaller than 5 mm) with low mobility, with populations preferentially observed within the limits of the organic resources (e.g. guano communities). The resource-space-independent communities are those in which organisms are not spatially restricted to the piles of organic resources, thus actively moving among distinct organic patches inside caves. Para-epigean communities are those occurring near the cave entrance, being composed of epigean and hypogean organisms with low or moderate mobility (Ferreira & Martins, 2001; Prous et al., 2004). In addition, low-adapted species of spiders have a distribution primarily associated with external or cave entrance microhabitats, intermediate-adapted species live in shallow cave microhabitats, and highly adapted species use deep cave microhabitats (Mammola et al., 2019; Cuff et al., 2021). Furthermore, micro and meso habitats can be defined not only regarding the different zonation of the cave (photic, twilight zone, transition zone and deep zone) but also concerning different compartments within these zones (terrestrial, aquatic habitats, streams, puddles and water table) (Eberhard, 2001; Prous et al., 2004, 2015). Cave species, in turn, can be restricted to a certain type of habitat (Nicolosi et al., 2021, Souza-Silva et al., 2021) or they can present a wide distributional inside the cave, occurring in several habitat types (Peck, 1975; Eberhard, 2001; Ferreira & Martins, 2001).

So, studies regarding space-scale-dependence over cave invertebrates are indispensable in order to identify and understand the patterns for conservation (Pellegrini et al., 2016a). Thus, the main purpose of this study is to assess the response of the invertebrate species richness and composition against variations in some physical, trophic, microclimate features inside a cave. It was also tested if the responses are different at distinct spatial scales. More specifically, it was evaluated the role of the spatial distribution of entrances and distance from the cave entrance on the microhabitats characteristics and how such variations can affect troglobitic and non-troglobitic distribution. It was hypothesized that troglobitic species will respond to variations in cave environmental features choosing stable and oligotrophic places far from the entrance. On the other hand, non-troglobitic distribution inside the cave will be limited by the high humidity and oligotrophy.

MATERIALS AND METHODS

Study area

This study was carried out at “Três Cobras” limestone Cave (13°37'6.63"S 43°45'9.87"O), located in the karst area of Serra do Ramalho, southwestern Bahia, Brazil (Figure 1). This region is formed by large limestone areas, belonging to the Jacaré formation, Bambuí group. It is insert in Caatinga domain and in the middle of São Francisco River basin. According to Köppen’s climate classification system, the local climate type is “Aw”, with a dry winter and an average annual precipitation rate between 800 and 1000 mm (Gonçalves et al., 2018)

The Três Cobras Cave has 5620m of horizontal projection and slope of 32m. The cave is composed of labyrinthine conduits, presenting seven entrances (Figure 1). This region differs from the rest by presenting lower outcrops and isolated outcrops eminences. Entrance A, B and C of the cave is faced to pasture, an area with a few/ almost none trees near, while entrance D faced an area with a dry forest with scattered trees. Finally, the entrances E, F and G, are faced to a densest dry forest.

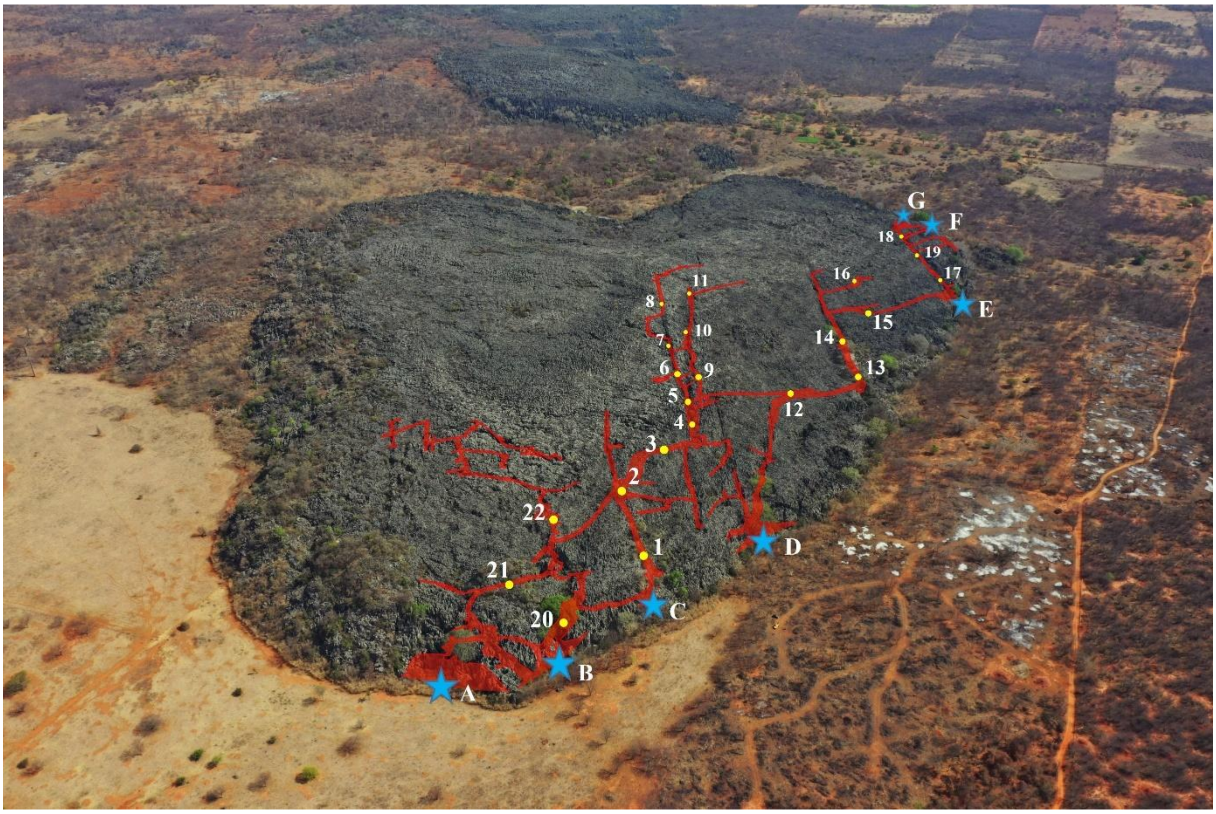

Figure 1. Map showing the outcrop where Three Cobras Cave ais inserted, as showing the mapped area of the cave, the seven main entrances (marked with a blue star and white letters A-F) and the places were the sectors where placed (number from 1 to 22).

Fields procedures

Sampling invertebrates along the Três Cobras cave floor

To evaluate variations on invertebrate species composition and richness along the cave sectors (3 x 10m) containing 3 quadrats (1 x 1m) were placed on the cave floor at least 100m apart from each other. Sectors and quadrants were considered "mesoscale' and "microscale", respectively. Distance from the entrances and sample unit positions were taken using a laser tape measure.

The invertebrate fauna was recorded into the sectors and quadrats through detailed visual search and hand sampling using tweezers and brushes (Souza-Silva et al., 2021; Oliveira Furtado et al., 2022). Sampled invertebrates were stored in plastic tubes with alcohol 70% aiming the preservation of the material for identification in the laboratory. In order to minimize the impact on cave fauna, some specimens were collected and their abundance where accounted (Pacheco et al., 2020; Souza-Silva et al., 2021; Oliveira Furtado et al., 2022).

The specimen's identification was made through taxonomic keys at the lowest taxonomic level accessible. Identify troglobitic species were considered troglomorphisms, which are traits that represent their evolution and adaptability to subterranean environments. The troglomorphisms observed were a lack or reduction in ocular structure and pigmentation, and elongation of appendices and sensorial structures (Culver & Pipan, 2009). When specialists were available, they were consulted for the following groups: Isopoda, Pseudoscorpiones, Orthoptera, Acari, Entomobryomorpha and Diplopoda. All the collected specimens are deposited in the Lavras Subterranean Invertebrates Collection (ISLA), affined to the Center of Studies on Subterranean Biology at the Federal University of Lavras, Minas Gerais, Brazil (http://www.biologiasubterranea.com.br/en/about-the-group/).

Abiotic attributes of the cave floor

The temperature and humidity were taken with a thermohygrometer device inside every sampling sector (accuracy ± 1°C for temperature and ±5% for relative humidity). The device was placed on the cave floor and the values were obtain after its stabilization. To avoid the interference/alter the values, the presence of people prevents (Souza-Silva et al., 2021; Oliveira Furtado et al., 2022).

To measure of abiotic attributes in mesoscale, the sectors were fractionated into 10 sections of 1 meter each and were made a visual estimate of the percentual of substrate and resources available on the cave floor, as proposed by Pellegrini et al., (2016a), and Souza-Silva et al., (2021).

To measure of the abiotic in a microscale/quadrat, photographs were taken before the collect occurred. To take the photographs were used Canon Powershot SX50, HS (4000 x 3000 pixels). Posteriorly, the photos were analyzed with the aid of ImageJ software (Rasband, 1997), and finally, the arithmetic average of every abiotic attribute was made (Souza-Silva et al., 2021; Oliveira Furtado et al., 2022).

Data analysis

Biotic attributes of the caves

The abundance and richness of non-troglobitic and troglobitic were obtained by counting individuals and morphotypes of each sample unit (22 sectors and 66 quadrats).

Abiotic attributes on the cave floor regarding the sectors

All the *physical*, *trophic* and *microclimatic* characteristics of the sectors were classified in the following classes: guano GU, hematophagus guano-GH, others mammals feces-FZM, carcass-CRC, litter-SER, plants debris-DTV (> 10mm), rock-RR, concrete like floor-RC, medium rock -MB(500-1000mm), small rock -SB(250-500mm), cobbles-CB (64-250mm), coarse gravel-CAG (16-64mm), fine gravel-CAF (2-16mm), sand-ARE (0.06-2mm), silt-SEF (≤ 0.05 mm), hardpan-HP, stalagmite-EG, speleothems-ES, retraction cracks-GR, cave-wall-PA, hardpan pinnacle-PHP, speleothems- ES, another inorganic substrate-OTI.

Based on such classes we obtained the *physical features* that included distance from the entrance, the substrate diversity (calculated considering all classes above), the shelter diversity (calculated considering RR, RC, MB, SB, CB, CAG, CAF, ES, EG, GR, PA, and

PHP) and trophic resources diversity (calculated considering GU, GH, FZM, CRC, SER and DTV). All diversities were calculated using Shannon-Weaver Index (Buttigieg & Ramette, 2014).

The availabilities of each *abiotic attribute* were also included as *physical*, *trophic* and *microclimatic* characteristics. The shelter availability was calculated by the sum of SER, DTV, GALF, GALM, GALG, TRO, RR, RC, XB, MB, RR, RC, MB, SB, CB, CAG, CAF, ES, EG, GR, PA, and PHP in each sector. On the other side, the trophic resource availability was calculated by the sum of considering GU, GH, FZM, CRC, SER, and DTV in each sector, and are also the same for the *trophic resources.* Finally, the *microclimatic variables* considered were temperature and humidity.

Abiotic attributes on the cave floor regarding the quadrats

On the other hand, *physical* and *trophic* characteristics of the quadrats were evaluated and classified into the following classes: guano—GU, roots—RZ, plants debris-DTV (> 10mm), fine branch -GRAF (11-30 mm), drip water-DP, another organic substrate**—**OTO, smooth rock**—**RL, rough rock**—**RR, wide rock**—**XB (1000-4000mm), medium rock-MB(500-1000mm), a small rock-SB(250-500mm), cobbles-CB (64-250mm), coarse gravel-CAG (16-64mm), fine gravel-CAF (2-16mm), sand-ARE (0.06-2mm), silt-SEF ($0.2 < \text{diameter} \leq 0.05$ mm), hardpan-HP, speleothems-ES and retraction cracks-GR.

Based on such classes we obtained the *physical features* that included distance from the entrance, the substrate diversity (calculated considering all classes above), the shelter diversity (calculated considering RR, XB, MB, SB, CB, CAG, CAF, and GR), and trophic resources diversity (calculated considering GU, RZ, DTV, GALF, and OTO). All diversities were calculated using Shannon-Weaver Index (Buttigieg & Ramette, 2014).

The availabilities are also included in *physical*, *trophic*, and *microclimatic* characteristics. The shelter availability was calculated by the sum of DTV, GALF, RR, XB, MB, SB, CB, CAG, CAF, and GR in each quadrat. On the other side, the trophic resource availability was calculated by the sum of GU, RZ, DTV, GALF, and OTO in each quadrat, and are also the same for the *trophic resources*.

Relationship between habitat structure of the sectors and quadrats with cave fauna

To predict the influence of local abiotic variables on total species richness, species richness of non-troglobitic and species richness of troglobitic (response variables), using sectors as sample units was performed a *General Linear Models* (GLM) and temperature (ºC), humidity (%), distance from the cave entrance, diversity of shelter, diversity of substrate, diversity of resources, availability of shelter and availability of resources as response variables. The same was made using the quadrats as sample units, however, temperature and humidity were not included in the model, since their values were not taken in each quadrat.

The Poisson family was chosen to be part of the GLM's, since it is used when models depend on the counting of events in space or time. To compare the model results with the null models, the ANOVA function from 'Vegan' package was performed. To assess the overdispersion the function CHECK_OVERDISPERSION from the package 'Performance' was applied. Were used function r.squaredLR from the 'piecewiseSEM' package in order to obtain r² values of the GLM's. The correlation of all variables was tested before running the models, for that, CHART. CORRELATION from the 'PerformanceAnalytics' package. Variables with high correlation values (>0.65) were excluded from the model as proposed by Zuur et al., (2010). To test if exist multicollinearity, the function VIF from 'Car' package was used, however, none of them were presented.

It was performed a Distance-based redundancy analysis (dbRDA) based on the Bray-Curtis similarity matrix in order to explain the possible relationships, s strength, and direction (- or +) between +) between overall fauna, non-troglobitic and troglobitic species composition with the *physical*, *trophic*, and *microclimatic* variables (Clarke et al., 2014).

Variations on habitat structure

The possible differences between temperature, humidity, substrate diversity, shelter diversity, resource diversity, availability of resource and availability of shelter among the seven entrances, sectors, and quadrats (here temperature and humidity were not used since it was not measure in this scale) using KRUSKAL.TEST function from the 'Stats' package in RStudio. To compare all-pairs for Kruskall-Wallis ranked data, where is the difference were used the function KWALLPAIRSNEMENYITEST from the 'PCMCRplus' package. To test if *trophic*, *microclimatic* and *physical* attributes varied depending on the distance from the entrance using the sectors, it was made a *Linear Regression.* The same analysis was performed using the quadrats as sample unit. All regressions were performed using the function LM from 'Stats' package in RStudio.

RESULTS

Biotic attributes of the cave

A total of 571 individuals were recorded, distributed in 26 orders and 55 families, and 126 morphospecies. From the total 15 morphospecies were considered troglobitic (11.9 %). The richest order considering overall fauna was Araneae (31 ssp.), followed by Coleoptera (19 ssp.) and Diptera (12 ssp.). The richest order of non-troglobitic species was Araneae (28 ssp.), followed by Coleoptera (19 ssp.) and Diptera (12 spp.). Lastly, the richest orders of troglobitic species were Isopoda (5 ssp.), Collembola (3 ssp.) and Polydesmida, and Araneae (2 ssp., each) (Figure 2A, B). The relationship between non-troglobitic and troglobitic fauna are shown in Figure 2C. Some of the troglobitic species found in Three Cobras Caves are showing in Figure 3.

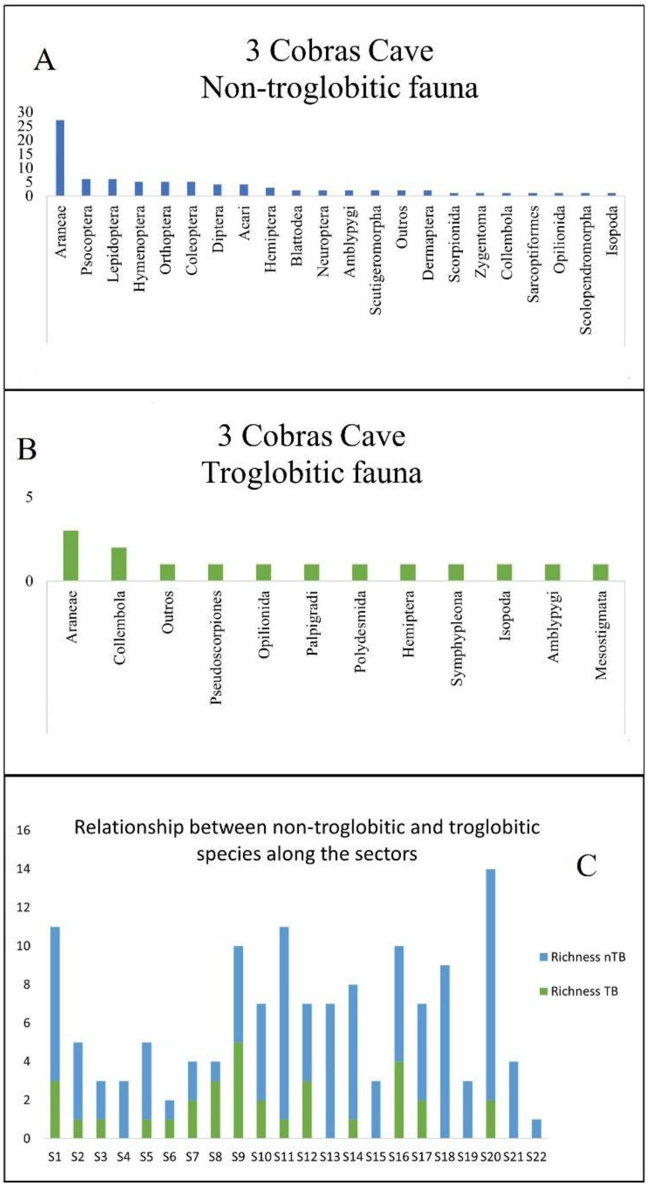

Figure 2. The richness of non-troglobitic species in different taxa observed in Three Cobras Cave (A); The richness of troglobitic species in different taxa observed in Three Cobras Cave (B); The relationship of non-troglobitic and troglobitic species along the sectors. nTB means non-troglobitic and TB means troglobitic.

Figure 3. Some of the troglobitic species found in Three Cobras Cave: *Charinus* (A); Lyniphiidae (B); *Pseudochthonius* (C); Ochyroceratidae (D); *Eukoenenia* (E); Prodidomidae (F); Styloniscidade (G); *Pectenoniscus* (H); *Phaneromerium* (I); Blattodea (J); *Troglobentosminthurus luridos* (K); and Kinnaridae (L).

Relationship between habitat structure inside the sectors with cave fauna

For the overall fauna, the non-troglobitic (nTB) and the troglobitic (TB), none of the variables here used explained its species richness variations. While regarding the dbRDA, for the overall fauna and troglobitic only distance from the entrance was the best predictor, explaining 8.0% and 11% of the variation in species composition (p=0.03 and 0.05, respectively), (Figure 4). None of the used variables explained the variation in the composition of the troglobitic species.

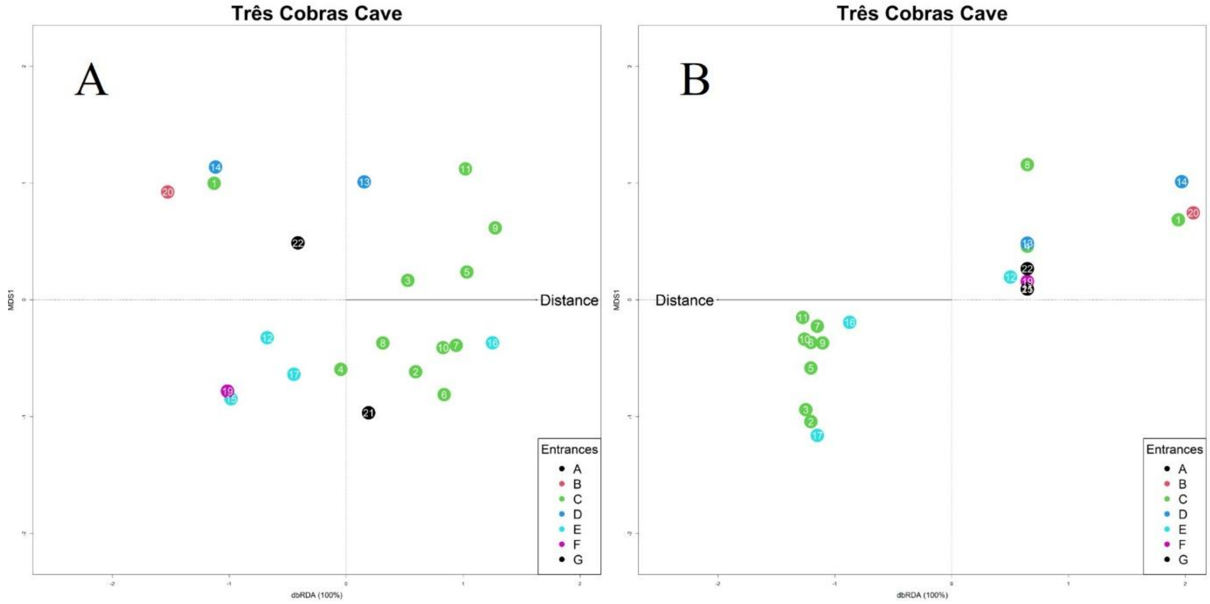

Figure 4. dbRDA shows the spatial variation in overall and troglobitic composition. Distance from the entrance explained 8.0% and 11% of spatial variation in species composition (A and B, respectively). The colored dots are representing the closest entrance from the sector.

Relationship between habitat structure of the quadrats with cave fauna

For the troglobitic species, variation in species richness was best explained only by resource availability (R^2= 0.19, p=0.0007). Regarding the overall fauna and non-troglobitic none of the variables here used explained species richness variations. While regarding the dbRDA, for the troglobitic only resource availability was the best predictor, explaining 12% of the variation in species composition (p=0.005). Regarding the overall fauna and non-troglobitic none of the variables here collected explained variation in species composition (Figure 5).

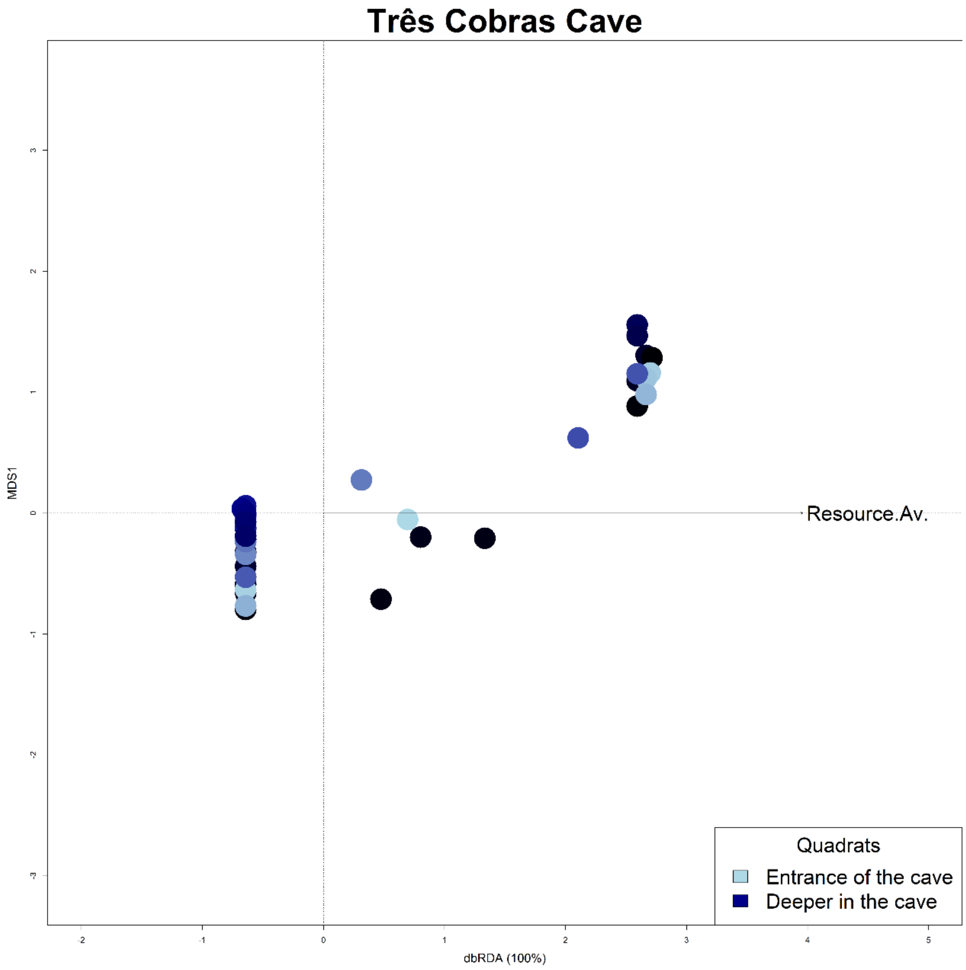

Figure 5. dbRDA showing the spatial variation in troglobitic composition. Resource Availability explained 12% spatial variation in species composition. The dots are representing the closest entrance from the quadrat, the darker the dot the deeper in the cave the quadrat is located.

Variations in habitat structure

The values of temperature, humidity, substrate diversity, resource diversity, shelter diversity, availability of resources, and availability of shelter of the sectors and quadrats are shown in Material Supplementary I and II. When using the data of the sector, none of the variables showed any differences in the averages among the entrances, while using the quadrats data, the distance from the entrance (B≠C, p=0.004; C≠G, P= 0.01), resource availability (A≠ C, p=0.052; B ≠ C, p=0.056; C≠G, P= 0.04) and shelter availability (C≠E, p=0.03) showed differences in the averages among the entrances. Lastly, the remaining predictor variables did not show differences in averages when using sectors and quadrats as variables response.

Regarding the sectors, only the humidity indicated a negative relationship with the distance from the entrance (R^2 = 0.36, p = 0.002), While regarding the quadrats, only the resource availability (R^2 = 0.77, p = 2^{-16}) showed a positive relationship with the distance from the entrance and shelter availability ($R^2 = 0.17$, $p = 0.0003$) showed a negative relationship with the distance from the entrance.

DISCUSSION

The study calls attention to the usage of different sampling scales and different abiotic and biotic features in only one cave. These features showed to be more influential over troglobitic species than non-troglobitic species, in both mesoscale (sector) and microscale (quadrat). Besides that, distance from the entrance had an effect in decreasing humidity and shelter availability, while for resource availability had an effect in increasing it as far from the entrance. So, the distance from the entrance is an important factor in structuring the subterranean cave communities.

Relationship between habitat structure of the sectors and quadrats with cave fauna

Our findings support our hypothesis that troglobitic species presented distinct responses to environmental variables compared to non-troglobitic species, and that their distribution along the cave is also different.

It appears that the troglobitic species showed a relationship between their richness and composition with the availability of resources and their distance from the entrance of the cave. While, the non-troglobitic species did not show any significant response to the variables that were explored. However, it seems that the overall fauna did show a response to the distance from the entrance.

Plant debris and litter are important food resources for the invertebrate communities living in Três Cobras Cave (3CC). During the dry season, leaves and plant debris accumulate in the soil of the Caatinga limestone outcrops. These resources are then transported into the cave by rain or river input during the rainy season. The accumulated resources can then be stored in the sediment bank of the cave, providing a consistent food source for the invertebrate communities. Resource input can also occur through percolation and surface runoff, as described by Ferreira et al. (2010).

In contrast, the other resource available in Três Cobras Cave is guano which is supplied by bats as they enter the cave. This resource is essential in the structure of cavernicolous invertebrate communities once the guano can maintain many species. On the other hand, guano deposited in places far from the water bodies cannot be exported, therefore being available for longer periods (Faria, 1996; Souza-Silva et al., 2011 trophic). The low guano availability can be due to a part of the 3CC surroundings being covered by pasture and the other part by vegetation, meaning that caves surrounded by vegetation can potentially be colonized by a bigger diversity of bats, therefore, preserving caves' surroundings can affect its invertebrate communities (Cardoso et. al. 2022).

The species richness decreased further from the entrances of the cave which can be related to the habitat homogeneity and scarcity of food resource availability, found in the deepest parts. These environments can be suitable for troglobitic species since they present morphological adaptations to live under extreme conditions, such as eye size reduction, appendage elongation, thin coticule, and resistance to starvation, among others (Romero & Green, 2005). These adaptations/specializations were developed through selective pressures or the lack of them at the parts of the cave (Culver, 1982). As a result, troglobitic species are rare and endemic, having a low population rate, and low tolerance to environmental changes.

Variations in habitat structure

The abiotic data set sampled inside the quadrats indicated that resource availability from the entrances A and C, B and C, C and G are different (p=0.052, p=0.056 and 0.04, respectively). The differences noted between these features can be related to the vegetation near the entrances, the distribution, size, and inclination of entrances of Três Cobras Cave, and the type of substrate available. However, is not possible to affirm for sure since not all entrances were accessed, and size and inclination were not measured. On the other hand, some studies showed the importance of the land cover and land use surrounding the cave entrances over cave fauna (Souza-Silva et al., 2015; Jaffé et al., 2018; Canedoli et al., 2022, Cardoso et al 2022), since these underground environments are connected to external one. So, in areas bordering caves with deforestation, soil erosion is common, which might be carried inside the cave and silt up the water bodies present (Bárány-Kevei, 1999).

Furthermore, when considering the physical and microclimatic characteristics within subterranean environments at distinct sampling scales, they may increase/decrease as you move farther from the entrance. About the sectors, only the humidity varied, while for the quadrats only resource availability and shelter availability varied. Resource availability was the only variable positively influenced by the distance from the entrance, which means as far from the entrance higher its value, this can be probably explained due to that half of the sectors were collected at least 1 km from the entrance. On the other hand, the humidity and shelter availability showed a negative relationship with the distance from the entrance, which means as far from the entrance its values reduce. Regarding the humidity, is the opposite of expected, since it is expected the humidity gets stabler as far from the entrance, however in 3CC, eleven sectors were sampled at least one kilometer from the entrance, so probably is due to the places the sectors were placed. The shelter, availability was expected since the cave was extremely dry and presented a heterogeneity of substrates that can be used as shelters along the cave. An elevated number of substrates can provide different types of shelter and consequently attend to different species, increasing both species richness and composition (Poulson & Kane, 1977; Pacheco et al., 2020). These results are supported by Holsinger & Culver (1988), that geological structure affects the subterranean fauna and its ecological complexity.

CONCLUSION

In conclusion, the distance from the entrance can creates filters over conditions and resources that further will affect the subterranean invertebrate fauna by the formation of microhabitats along the caves, permitting the co-existence of several species without niche overlapping. The presence of several entrances is another factor that can influence conditions and resources; however, it was not evaluated in this study, therefore needing more studies to check the relationship between several entrances and their external environment such as forest/pasture, and the distance from the entrance.

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REFERENCES

Bàràny-Kevei, I.,1999. Impact of agricultural land use on some Hungarian karst regions. International Journal of Speleology, 28(1):6.

Buttigieg, P.L., Ramette A., 2014. A Guide to Statistical Analysis in Microbial Ecology: a communityfocused, living review of multivariate data analyses. FEMS Microbiol Ecol 90:543–550. https://doi. org/10.1111/1574-6941.12437.

Canedoli, C., Ficetola, G. F., Corengia, D., Tognini, P., Ferrario, A., Padoa-Schioppa, E., 2022. Integrating landscape ecology and the assessment of ecosystem services in the study of karst areas. Landscape Ecology, 37(1):347–365. doi:10.1007/s10980-021-01351-2.

Cardoso, R. C., Ferreira, R. L., & Souza-Silva, M. (2022). Multi-spatial analysis on cave ecosystems to predict the diversity of subterranean invertebrates. Basic and Applied Ecology, 65, 111-122.

Cuff, J.P., Aharon, S., Armiach Steinpress, I., Seifan, M., Lubin, Y., Gavish-Regev, E., 2021. It's All about the Zone: Spider Assemblages in Different Ecological Zones of Levantine Caves. Diversity, 13:576.

Culver D.C., Pipan, T., 2010. Climate, abiotic factors, and the evolution of subterranean life. Acta carsologica, 39.3.

Culver, D., 1982. Cave life. Harvard University Press.

Culver, D.C., Pipan, T., 2009. Biology of Caves and Other Subterranean Habitats, 1st edn. Oxford, New York.

Eberhard, S., 2001. Cave fauna monitoring and management at Ida Bay, Tasmania. Records of the Western Australia Museum Supplement, 64:97-104.

Ferreira R.L., Prous X., Bernardi L.F.O., Souza-Silva M., 2010. Fauna subterrânea do Estado do Rio Grande do Norte: Caracterização e impactos. Rev Bras Espeleol 1:25–51.

Ferreira, R.L. & Martins, R.P. (2001) Cavernas em risco de 'extinção'. Ciência Hoje, 29, 20-28.

Holsinger, J.R., Culver D.C., 1988. The invertebrate cave fauna of Virginia and a part of eastern Tennessee: Zoogeography and Ecology. Brimleyana 14: 1-162.

Jaffe, R., Prous, X., Calux, A., Gastauer, M., Nicacio, G., Zampaulo, R., Souza-Filho, P. W. M., Oliveira, G., Brandi, I. V., Siqueira, J. O., 2018. Conserving relics from ancient underground worlds: Assessing the influence of cave and landscape features on obligate iron cave dwellers from the Eastern Amazon. PeerJ, 6: e4531. doi:10.7717/peerj.4531.

Juberthie, C., Delay, B., Bouillon, M., 1980. Exten- sion du Milieu Souterrain en zone non-Calcaire. Mem. Biospeol. 7, 19-52.

Mammola, S., Arnedo, M.A., Fišer, C., Cardoso, P., Dejanaz, A.J., Isaia, M., 2020. Environmental filtering and convergent evolution determine the ecological specialization of subterranean spiders. Functional Ecology, 34:1064–1077.

Mammola, S., Piano, E., Malard, F., Vernon, P., Isaia, M., 2019. Extending Janzen's hypothesis to temperate regions: A test using subterranean ecosystems. Functional Ecology, 33:1638–1650.

Nicolosi, G., Mammola, S., Costanzo, S., Sabella, G., Cirrincione, R., Signorello, G., Isaia M., 2021. Microhabitat selection of a Sicilian subterranean woodlouse and its implications for cave management. International Journal of Speleology, 50 (1):53-63.

Oliveira, L.F., et al., 2022. Recreational caving impacts of visitors in a high-altitude cave in Bolivian Andes: main effects on microhabitat structure and faunal distribution. International Journal of Speleology 51.2:2.

Pacheco, G.S.M., Souza-Silva, M., Cano, E., Ferreira R.L., 2020. The role of microhabitats in structuring cave invertebrate communities in Guatemala. International Journal of Speleology, 49 (2):161-169. Tampa, FL (USA) ISSN 0392-6672.

Peck, S.B., 1975 A population study of the cave beetle Ptomaphagus loedingi (Coleoptera; Leiodidae; Catopinae). International Journal of Speleology, 7(1):3.

Pellegrini, T.G., & Ferreira, R.L., 2016. Are inner cave communities more stable than entrance communities in Lapa Nova show cave? Subterranean Biology, 20:15–37.

Poulson, T.L., Kane, T.C., 1977. Ecological diversity and stability: Principles and management. In Proceedings 1976 National Cave Management Symposium: Albuquerque, NM, Speleobooks (pp. 18-21).

Prous, X., Ferreira R.L., Jacobi, C.M., 2015. The entrance as a complex ecotone in a Neotropical cave. International Journal of Speleology, 44 (2):177-189.

Prous, X., Ferreira, R.L., Martins, R.P., 2004. Ecotone delimitation: Epigean–hypogean transition in cave ecosystems. Austral Ecology, 29(4):374-382.

Rasband, W.S., 1997. Image, J., US National Institutes of Health. “ImageJ64,” US National Institutes of Health, Bethesda, MD.

Romero, A., Green, S.M., 2005. The end of regressive evolution: examining and interpreting the evidence from cave fishes. Journal of fish biology, 67(1): 3-32.

Souza-Silva, M., Cerqueira, R.F.V., Pellegrini T.G. et al., 2021. Habitat selection of cave-restricted fauna in a new hotspot of subterranean biodiversity in Neotropics. Biodivers Conserv 30:4223–4250. https://doi.org/10.1007/s10531-021-02302-8.

Souza-Silva, M., Martins, R. P., Ferreira, R.L., 2015. Cave conservation priority index to adopt a rapid protection strategy: A case study in Brazilian Atlantic rain forest. Environmental Management, 55(2):279–295. doi:10.1007/s00267-014-0414-8.

Souza-Silva, M., Martins, R.P., Ferreira, R.L., 2011. Trophic Dynamics in a Neotropical Limestone Cave. Subterranean Biology 9: 127–138. doi: 10.3897/subtbiol.9.2515.

Tobin, B.W., Hutchins, B.T., Schwartz, B.F., 2013. Spatial and temporal changes in invertebrate assemblage structure from the entrance to deep-cave zone of a temperate marble cave. International Journal of Speleology, 42 (3):203-214. https://doi.org/10.5038/1827-806X.42.3.4.

SUPPLEMENTARY MATERIAL

Supplementary material I. The values of temperature (ºC), humidity (%), distance from the entrance (m), substrate diversity, resource diversity, shelter diversity, availability of resources, and shelter of the sectors of Três Cobras Caves. Div: diversity and Avail: availability.

Sectors	Humidity (%)	Distance (m)	Temperatura (ºc)	Substrate Div.	Shelter Div.	Resource Div.	Resource Avail.	Shelter Avail.
S1	81.5	375	21.6	0.918861	0.808369	0	3	97
S2	85.7	800	23.1	1.569948	1.007947	0	0	25
S3	93.3	1175	20.2	1.075196	0.818742	0	0	89
S4	90.3	1612.5	22.6	1.464032	0.798204	0	0	28
S5	95.8	2112.5	21	1.717874	1.382366	0	0	56
S6	92.9	2737.5	22.6	0.809573	0.758937	0	0	14
S7	91.6	3050	22.7	0.965731	0.537861	0	0	75
S8	96	3612.5	23.6	0.656424	0.693147	0	0	6
S9	95.7	2487.5	21.7	1.140927	1.074092	0	0	25
S10	89.9	2925	22.8	1.023693	1.376096	0	0	30
S11	83.2	3487.5	23	0.838235	0.666278	0	2	26
S12	84.6	1157.5	22.8	1.034266	0.562565	0	0	46
S13	83	1687.5	23	1.248351	1.424147	0.686962	4.5	31.5
S14	92.8	1250	22.4	1.52428	1.411826	0	0	56
S15	84.9	562.5	22.8	1.399856	1.176697	0	0	47
S16	91.6	2500	22.8	0.618946	0.906985	0	2.2	12
S17	67	375	24.5	1.29189	1.278792	0.636514	0.6	46
S18	60.6	312.5	24.5	0.948503	0	0.846366	8.5	30
S19	56.6	750	24.5	1.323457	1.016285	0	0	76
S20	75	250	21.5	0.958118	0.237378	0	8.5	23.5

S21	86.8	562.5	21.3	0.784168	0.410116	0	16	84
S22	80.8	1062.5	23.6	0.346515	0	0	0	11

Supplementary material II. The values of temperature (ºC), humidity (%), distance from the entrance (m), substrate diversity, resource diversity, shelter diversity, availability of resources, and shelter of the quadrats of Três Cobras Caves. Div: diversity and Avail: availability.

Quadrats	Distance (m)	Substrate Div.	Shelter Div.	Resource Div.	Resource Avail.	Shelter Avail.
S1Q1	370	0.143599	0.143599	0	0	10000
S1Q2	375	0	0	0	370.2872	10000
S1Q3	380	0	0	0	375	10000
S2Q1	795	0.945565	0	0	380	3489.99
S2Q2	800	0.795024	0.53749	0	795.9456	3170.4
S2Q3	805	1.295253	0	0	801.3325	993.296
S3Q1	1170	0.679941	0.679941	0	806.2953	10000
S3Q2	1175	0.323491	0.323491	0	1171.36	10000
S3Q3	1180	0	0	0	1175.647	10000
S4Q1	1607.5	0.619915	0	0	1180	1680.95
S4Q2	1612.5	0.35141	0.071983	0	1608.12	9289.76
S4Q3	1617.5	1.305957	0.320967	0	1612.923	5697.93
S5Q1	2107.5	0.958129	0.74393	0	1619.127	3893.696
S5Q2	2112.5	0.32931	0	0	2109.202	8980.67
S5Q3	2117.5	0	0.487857	0	2112.829	3388.48
S6Q1	2732.5	0.730987	0	0	2117.988	90.346
S6Q2	2737.5	0.574964	0	0	2733.231	0
S6Q3	2742.5	1.183058	0.675031	0	2738.075	3550.27
S7Q1	3045	0.489634	0.139771	0	2744.358	8801.56
S7Q2	3050	1.001159	0.543326	0	3045.629	6255.48
S7Q3	3055	0.972144	0.661389	0	3051.544	8613.92
S8Q1	3607.5	0	0	0	3056.634	0
S8Q2	3612.5	0.60568	0.60568	0	3607.5	10000
S8Q3	3627.5	0.278114	0	0	3613.711	0
S9Q1	2482.5	0.678768	0	0	3627.778	5845.89
S9Q2	2487.5	0.715583	0	0	2483.179	253.59
S9Q3	2492.5	0.708999	0	0	2488.216	1072.49
S10Q1	2920	0.781737	0.202497	0	2493.209	1430.33
S10Q2	2925	1.098076	0.693135	0	2920.984	6512.94
S10Q3	2930	0.818818	0.67346	0	2926.791	208.52
S11Q1	3482.5	0.132405	0	0	2931.492	0
S11Q2	3487.5	0	0	0	3482.632	0
S11Q3	3492.5	0.045211	0	0	3487.5	0

S12Q1	1152.5	0	0	0	3492.545	10000
S12Q2	1157.5	0.672957	0	0	1152.5	6001.35
S12Q3	1162.5	0.137912	0.057175	0	1158.173	9840.94
S13Q1	1682.5	0.405187	0.405187	0	1162.695	10000
S13Q2	1687.5	0.381522	0.381522	0	1683.31	10000
S13Q3	1692.5	0.958877	0.363776	0	1688.263	7560.36
S14Q1	1245	1.32625	1.01813	0	1693.823	7453.47
S14Q2	1250	0.90612	0.505106	0	1247.344	4372.65
S14Q3	1255	0.232072	0.232072	0	1251.411	10000
S15Q1	557.5	0.862972	0.566001	0	1255.464	3651.26
S15Q2	562.5	1.653583	1.449162	0	558.929	7639.92
S15Q3	567.5	0	0	0	565.6027	10000
S16Q1	2495	0	0	0	567.5	10000
S16Q2	2500	0.228256	0	0	2495	9395.31
S16Q3	2505	0.156039	0.156039	0	2500.228	10000
S17Q1	370	0.323686	0.195519	0	2505.312	9702.16
S17Q2	375	0	0	0	370.5192	10000
S17Q3	380	0.363306	0.363306	0	375	10000
S18Q1	307.5	0.106012	0	0	380.7266	9779.24
S18Q2	312.5	0.165765	0	0.350716	307.606	9645.15
S18Q3	317.5	0.826416	0	0	313.0165	6018.02
S19Q1	745	0.659011	0.648078	0	318.3264	2145.72
S19Q2	750	0.372046	0.372046	0	746.3071	10000
S19Q3	755	0.000001	0.475699	0	750.7441	2370.15
S20Q1	245	0.685979	0.108358	0	755.4757	8084.23
S20Q2	250	0.323472	0	0	245.7943	9007.32
S20Q3	255	0	0	0	250.3235	10000
S21Q1	557.5	0.653366	0	0	255	6400.9
S21Q2	562.5	0.691129	0	0	558.1534	4682.48
S21Q3	567.5	0.222514	0.222514	0	563.1911	10000
S22Q1	1057.5	0.199098	0	0	567.945	501.98
S22Q2	1062.5	0	0	0	1057.699	0
S22Q3	1067.5	0	0	0	1062.5	0

500