UNIVERSIDADE FEDERAL DE LAVRAS

LEANDRO MATA DA ROCHA MELO

EXPLORING THE ECOLOGY OF INVERTEBRATE COMMUNITIES IN SUBTERRANEAN HABITATS OF A SUBTROPICAL KARST

LAVRAS–MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ecologia Aplicada, área de concentração em Ecologia e Conservação de Recursos Naturais em Paisagens Fragmentadas e Agroecossistemas, para a obtenção do título de Doutor.

Prof. Dr. Marconi Souza Silva
Orientador

Prof. Dr. Rodrigo Lopes Ferreira
Coorientador

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LEANDRO MATA DA ROCHA MELO

EXPLORANDO A ECOLOGIA DAS COMUNIDADES DE INVERTEBRADOS EM HABITATS SUBTERRÂNEOS DE UM CARSTE SUBTROPICAL.

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Dr. Luis Claudio Paterno Silveira, UFLA
Dr. Nelson Henrique de Almeida Curi, Unilavras
Dr. Leonardo Sousa Carvalho, UFPI
Dr. Kleber Makoto Mise, Minoru Assessoria Ltda

Dr. Marconi Souza Silva
Orientador
Dr. Rodrigo Lopes Ferreira
Coorientador

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" A natureza pode suprir todas as necessidades do homem, menos a sua ganância."

Mahatma Gandhi

RESUMO GERAL

Ambientes subterrâneos representam locais em que a sua biodiversidade ainda é pouco explorada, com vastas extensões ainda não mapeadas e espécies desconhecidas. Esses ecossistemas cavernícolas abrigam uma riqueza de espécies que se especializou às condições únicas desses ambientes, como a tendência a estabilidade ambiental, ausência de luz e escassez de recursos. No entanto, esses ambientes estão sob crescente ameaça devido às atividades humanas, como desmatamento, atividade agropecuária, mineração, urbanização, que comprometem a integridade dos habitats subterrâneos. No estado do Paraná, que se destaca como um dos líderes em desmatamento no Brasil, os ambientes cavernícolas enfrentam desafios ainda mais acentuados. O desmatamento resulta na diminuição do aporte de recursos, como matéria orgânica e nutrientes, aos sistemas subterrâneos, exacerbando sua característica natural de oligotrofia. Esse empobrecimento de recursos tem um impacto direto na biodiversidade de cavernas, levando à perda de espécies. Além disso, as atividades antrópicas também podem causar danos físicos diretos às cavernas, seja por meio de construção de estradas, mineração ou poluição. Assim, este estudo teve como objetivo investigar as variáveis que influenciam a composição e a riqueza de invertebrados em cavernas do Paraná, além realizar uma revisão sistemática para compreender quais variáveis estão sendo testadas em estudos sobre o tema e identificar lacunas do conhecimento. Realizamos uma atualização da fauna troglóbia na região metropolitana do estado do Paraná e identificamos cavernas prioritárias para conservação na região de estudo. Descobrimos que tanto a temperatura quanto a diversidade de abrigo exercem influência sobre a composição da fauna em mesoescala, enquanto a distância da entrada das cavernas e a distância entre caverna são determinantes significativas da fauna em microescala. Identificamos um total de 28 espécies troglóbias, além de 9 cavernas como de extrema prioridade de conservação. A revisão revelou que diversas variáveis têm sido testadas na área de ecologia de comunidades de invertebrados cavernícolas, porem lacunas do conhecimento ainda permanecem, como o estudo de relações ecológicas em ambientes cavernícolas, sucessão ecológica, interações bióticas, ecologia funcional e metacomunidades. Ao identificar as variáveis ambientais que estruturam as comunidades cavernícolas, podemos direcionar esforços para mitigar os impactos negativos e promover práticas de conservação mais eficazes. Além disso, ao identificar cavernas prioritárias para conservação, podemos concentrar recursos e esforços onde são mais necessários, maximizando o impacto das medidas de proteção.

Palavras-chave: Ecologia; Caverna; Paraná.

ABSTRACT

Subterranean environments represent locations where biodiversity is still underexplored, with vast expanses yet to be mapped and unknown species. These cave ecosystems harbor a wealth of species that have specialized to the unique conditions of these environments, such as the tendency for environmental stability and resource scarcity. However, these environments are under increasing threat due to human activities such as deforestation, agriculture, mining, and urbanization, which compromise the integrity of subterranean habitats. In the state of Paraná, which stands out as one of the leaders in deforestation in Brazil, cave environments face even more acute challenges. Deforestation results in a decrease in the supply of resources, such as organic matter and nutrients, to subterranean systems, exacerbating their natural oligotrophic characteristics. This resource depletion has a direct impact on cave biodiversity, leading to species loss. Additionally, anthropogenic activities can also cause direct physical damage to caves, whether through road construction, mining, or pollution. Thus, this study aimed to investigate the variables that influence the composition and richness of invertebrates in caves in Paraná, as well as to conduct a systematic review to understand which variables are being tested in studies on the topic and to identify knowledge gaps. We updated the troglobitic fauna in the northern region of Paraná and identified priority caves for conservation in the study area. We found that both temperature and shelter diversity influence fauna composition at a mesoscale, while the distance from the cave entrance and the distance between caves are significant determinants of fauna at a microscale. We identified a total of 28 troglobitic species and 9 caves as of extreme conservation priority. The review revealed that various variables have been tested in the field of cave community ecology, but knowledge gaps still remain, such as the study of ecological relationships in cave environments, ecological succession, biotic interactions, functional ecology, and metacommunities. By identifying the environmental variables that structure cave communities, we can direct efforts to mitigate negative impacts and promote more effective conservation practices. Additionally, by identifying priority caves for conservation, we can concentrate resources and efforts where they are most needed, maximizing the impact of protection measures.

Keywords: Ecology; Cave; Paraná.

INDICADORES DE IMPACTO

O estudo de ambientes subterrâneos torna-se cada vez mais urgente diante das crescentes ameaças antrópicas que comprometem esses ecossistemas pouco explorados. Esta tese aborda cavidades subterrâneas da Mata Atlântica, um dos biomas mais impactados do mundo, com foco na conservação da fauna cavernícola e dos ambientes subterrâneos da região metropolitana de Curitiba. Para isso, foram elaborados cinco manuscritos que analisam os principais fatores que estruturam a comunidade de invertebrados na região e destacam a necessidade de preservação das cavernas estudadas. Além da produção científica, o trabalho investiu em materiais de divulgação científica e educação ambiental, incluindo cartilhas e vídeos voltados ao público regional. Esses materiais visam sensibilizar a sociedade sobre a importância da conservação dos ambientes subterrâneos e aproximar a população da fauna local, incentivando um maior engajamento na proteção desses ecossistemas. Dessa forma, esta pesquisa se alinha a três das oito temáticas da Política Nacional de Extensão: Comunicação (1), Educação (4) e Meio Ambiente (5). Além disso, o estudo está diretamente relacionado a três dos 17 Objetivos de Desenvolvimento Sustentável (ODS) da ONU: ODS 4 – Educação de Qualidade: A produção e disseminação de conhecimento por meio de artigos científicos, materiais didáticos e ações de divulgação promovem a educação ambiental e científica, ampliando o acesso à informação sobre a fauna subterrânea e sua conservação. ODS 13 – Ação Contra a Mudança Global do Clima: Os ambientes subterrâneos são influenciados pelas condições climáticas da superfície, e a degradação do meio ambiente pode intensificar os impactos das mudanças climáticas sobre as espécies adaptadas a esse ambiente. A pesquisa contribui para a compreensão dessas interações e reforça a importância da conservação dessas áreas frente às mudanças ambientais globais. ODS 15 – Vida Terrestre: A proteção da biodiversidade subterrânea é essencial para a manutenção dos ecossistemas e da funcionalidade ecológica das cavernas. O estudo evidencia a importância da fauna cavernícola e alerta para a necessidade de medidas de conservação voltadas para esses ambientes, que frequentemente são negligenciados em políticas de proteção ambiental. Ao integrar ciência, educação e conservação, esta tese reforça a urgência de proteger os ecossistemas subterrâneos e contribui para uma maior conscientização sobre a biodiversidade e os serviços ecossistêmicos fornecidos por esses ambientes.

IMPACT INDICATORS

The study of subterranean environments is becoming increasingly urgent due to the growing anthropogenic threats that compromise these little-explored ecosystems. This thesis addresses subterranean cavities in the Atlantic Forest, one of the most impacted biomes in the world, focusing on the conservation of cave fauna and subterranean environments in the metropolitan region of Curitiba. To achieve this, five manuscripts were developed to analyze the main factors structuring the invertebrate community in the region and highlight the need for the preservation of the studied caves. In addition to scientific production, the research invested in science communication and environmental education materials, including booklets and videos aimed at the regional public. These materials seek to raise awareness about the importance of conserving subterranean environments and to engage local communities in protecting these ecosystems. Thus, this research aligns with three of the eight themes of the National Extension Policy: Communication (1), Education (4), and Environment (5). Furthermore, the study is directly related to three of the 17 United Nations Sustainable Development Goals (SDGs): SDG 4 – Quality Education: The production and dissemination of knowledge through scientific articles, educational materials, and outreach initiatives promote environmental and scientific education, expanding access to information about subterranean fauna and its conservation. SDG 13 – Climate Action: Subterranean environments are influenced by surface climate conditions, and environmental degradation can intensify the impacts of climate change on species adapted to these habitats. This research contributes to the understanding of these interactions and reinforces the importance of conserving these areas in the face of global environmental changes. SDG 15 – Life on Land: Protecting subterranean biodiversity is essential for maintaining ecosystems and the ecological functionality of caves. The study highlights the importance of cave fauna and calls for conservation measures targeted at these environments, which are often overlooked in environmental protection policies. By integrating science, education, and conservation, this thesis emphasizes the urgency of protecting subterranean ecosystems and contributes to greater awareness of biodiversity and the ecosystem services provided by these environments

SUMÁRIO

PRIMEIRA PARTE

1 APRESENTAÇÃO

Esta tese é composta por cinco manuscritos, que serão submetidos a periódicos científicos visando a sua publicação. Teve como objetivo central, avaliar os padrões de composição e riqueza de invertebrados em cavernas na Mata Atlântica, na região metropolitana de Curitiba, Paraná, em diferentes escalas, investigando como as características dos habitats influenciam a estrutura da comunidade de invertebrados. Além disso, empregamos o Índice de Prioridade de Conservação de Cavernas (CCPi) para identificar áreas e cavernas que demandam prioridade de medidas de conservação. Realizamos também uma revisão sistemática sobre fatores ambientais que estruturam a comunidade de invertebrados em cavernas e produzimos materiais com intuito de contribuir em atividades de educação ambiental na região.

O primeiro manuscrito que será submetido ao periódico internacional “Community Ecolgy” investigou a influência das variáveis de substratos físicos e tróficos, microclima, distância da entrada e distância de áreas de mineração sobre a estruturação espacial das comunidades de invertebrados em 23 cavernas calcárias no norte do estado do Paraná, Brasil. As cavernas foram analisadas em diferentes escalas, utilizando quadrantes (1x1m) como microescala, setores (3x10m) como mesoescala, e as faixas calcárias Capiru, Votuverava e Itaiacoca como macroescala, para investigar as respostas das comunidades em relação às variáveis mencionadas. Os resultados obtidos neste estudo destacam a influência significativa de variáveis como a diversidade de abrigo e distância da entrada, na composição das comunidades de invertebrados em cavernas calcárias do Paraná. Essas descobertas contribuem para nosso entendimento sobre a ecologia desses ambientes subterrâneos, com implicações práticas para a conservação.

O segundo manuscrito, que será submetido à revista “Zoologia” compara a riqueza de organismos troglóbios conhecidos em 23 cavernas no estado do Paraná antes e após a realização deste estudo. Nossos resultados indicam que a fauna troglóbia do Paraná era praticamente desconhecida até o início deste trabalho. Mesmo cavernas sujeitas a uma única coleta durante nossa pesquisa revelaram uma diversidade de espécies troglóbias superior ao conhecimento prévio. A aplicação de métodos de coleta em micro e mesoescalas, incluindo uma análise minuciosa de substratos, demonstrou ser efetiva para a coleta eficiente desses organismos. Esses achados têm implicações significativas para a biodiversidade e a conservação de espécies endêmicas e ameaçadas. A elucidação de uma riqueza significativa de espécies troglóbias anteriormente não documentadas destaca a necessidade de intensificar os esforços de pesquisa e conservação nas cavernas do Paraná. Essa diversidade recém-descoberta enriquece o conhecimento científico e destaca a importância de estratégias de conservação mais abrangentes e direcionadas para esses ambientes subterrâneos.

No terceiro manuscrito, que será submetido ao periódico "Anthropocene", empregamos o Índice de Prioridade de Conservação de Cavernas (CCPi) para classificar as 23 cavernas estudadas na região metropolitana do estado do Paraná, visando avaliar sua relevância para a conservação. Para alcançar tal classificação, conduzimos um levantamento dos impactos humanos em cada cavidade, que foi realizado concomitantemente às coletas de invertebrados em cada uma das cavernas avaliadas. Além disso, utilizamos dados sobre a riqueza biológica de cada caverna. Os resultados obtidos revelaram que 9 cavernas são de extrema prioridade de conservação, indicando uma necessidade imediata de medidas protetivas. Outras 11 cavernas foram classificadas como de alta prioridade, demandando atenção especial para a preservação. Duas cavernas foram designadas com prioridade média, e apenas uma caverna apresentou baixa prioridade de conservação. Os resultados deste estudo orientam ações prioritárias de conservação, através do uso de uma metodologia robusta que pode ser aplicada em futuros esforços de preservação em ambientes subterrâneos. Essa estratégia visa assegurar a proteção efetiva desses ecossistemas no estado do Paraná, promovendo a sustentabilidade e a manutenção dos ecossistemas subterrâneos.

O quarto manuscrito, submetido ao periódico "Community Ecology" se trata de uma revisão sistemática sobre os fatores ambientais que estruturam a comunidade de invertebrados em cavernas, para isso, realizamos buscas sistemáticas sobre o tema no Web of Science e Scopus, resultando na seleção de 136 artigos. A partir desses estudos, foram extraídas informações para a elaboração da revisão. Nosso objetivo foi elucidar os avanços significativos alcançados na área e, além disso, propor novas perspectivas para o futuro das pesquisas relacionadas à ecologia de invertebrados cavernícolas. Identificamos que Brasil e Itália são os países com maior colaboração científica na área, considerando o número de artigos publicados. Observamos, também, que o avanço do conhecimento nesse campo tem ocorrido ao longo dos últimos 32 anos. No entanto, constatamos que as variáveis preditoras analisadas para a estruturação de comunidades de invertebrados cavernícolas têm se repetido nos estudos, enquanto outros aspectos da ecologia de ambientes subterrâneos permanecem negligenciados. Com base nisso, destacamos os avanços realizados e propomos novos desafios para aprofundar a compreensão da ecologia de comunidades de invertebrados cavernícolas.

O último manuscrito foi desenvolvido para criar materiais de educação ambiental, visando apoiar atividades na região metropolitana de Curitiba, uma área fortemente impactada por atividades antrópicas. Foram elaboradas duas cartilhas, sendo a primeira direcionada a alunos do ensino fundamental I e II, e a segunda voltada para um público mais amplo, abrangendo todas as idades. Ambas têm um foco regional, destacando a fauna cavernícola local. Além disso, foram produzidos três vídeos que apresentam o desenvolvimento do trabalho, destacam os principais resultados obtidos, a experiência profissional adquirida pelos alunos envolvidos no projeto, e discussão de questões como a regulamentação de estudos em ambientes cavernícolas no Brasil. Espera-se que esses materiais possam ser utilizados por diversas instituições na região metropolitana de Curitiba, contribuindo para a conscientização da população sobre a importância da preservação dos ambientes cavernícolas e da fauna associada.

SEGUNDA PARTE

ARTIGOS

Article 1:
Multiscale influences of environmental factors shaping cave invertebrates in a subtropical Atlantic Rainforest karstic area

This article is to be submitted to the journal Community Ecology

Leandro Mata da Rocha Melo[1,2], Rodrigo Lopes Ferreira[1,2], Marconi Souza Silva[1,2],
[1]Centro de Estudos em Biologia Subterrânea, Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, Campus Universitário, P.O. Box 3037, Lavras CEP 37200-000, Minas Gerais, Brasil
[2]Programa de Pós-Graduação em Ecologia Aplicada, Universidade Federal de Lavras, Lavras CEP 37200-000, Minas Gerais, Brasil

Leandro Mata da Rocha Melo - https://orcid.org/0000-0002-6351-8654
Marconi Souza Silva - https://orcid.org/0000-0002-3184-5319
Rodrigo Lopes Ferreira - https://orcid.org/0000-0003-3288-4405
corresponding author: marconisilva@ufla.br

Abstract

Caves host a diverse array of invertebrate species, yet the factors shaping their community structure, particularly in tropical regions, remain underexplored. This knowledge gap exists due to the difficulty of accessing these habitats and the limited number of studies on cave fauna across different regions. Understanding the ecological patterns of cave invertebrates is essential for implementing effective conservation measures. This study investigated the influence of physical, trophic, and microclimatic factors on the composition and richness of terrestrial invertebrate communities in caves within the Atlantic Rainforest of Paraná State, southern Brazil. Data were collected from 23 caves located across three limestone belts: 12 caves in the Itaiacoca belt, 6 in the Capiru belt, and 5 in the Votuverava belt. The research assessed habitat characteristics and invertebrate fauna through active sampling at different spatial scales: caves, sectors (3x10 m), and quadrats (1x1 m). A total of 357 invertebrate species were recorded, representing at least 55 distinct orders. At the microscale (within quadrats), invertebrate composition was influenced by the distance from the cave entrance and the distance between caves. At the mesoscale (within sectors), the diversity of shelters and temperature significantly influenced species composition. Furthermore, the different limestone belts showed differences in species composition, suggesting that local characteristics of each belt play a crucial role in shaping these communities. Species richness at the microscale was positively associated with trophic diversity and resource availability. However, the tested variables did not significantly influence species richness at the mesoscale. These findings enhance our understanding of the factors affecting subterranean invertebrate communities and provide insights for the conservation of caves in the region.

Keywords: Caves; Diversity; Ecology; Gap.

Introduction

Understanding how environmental factors, such as physical characteristics, trophic components, and microclimatic conditions shape the community structure of terrestrial invertebrates in cave ecosystems is essential for unraveling the ecological dynamics and biodiversity within these unique habitats (Tobin et al., 2013; Souza-Silva et al., 2021; Cardoso et al., 2022). In cave environments, where light is absent, nutrient inputs are limited, and microclimatic conditions remain highly stable, invertebrate communities have evolved adaptations that significantly differ from those of their surface-dwelling counterparts (Schiner, 1854; Racovitza, 1907; Howarth, 1983; Sket, 2008).

The physical characteristics of caves, such as substrate type, moisture levels, and structural complexity, influence habitat suitability. Meanwhile, trophic factors, including energy sources and food web dynamics, affect species interactions and community structure (Venarsky & Huntsman, 2018; Mammola & Isaia, 2018). Microclimatic conditions, particularly temperature and humidity, are also critical in shaping these communities, as many cave-dwelling invertebrates depend on specific environmental stability to survive (Souza-Silva et al., 2021). Understanding these relationships offers valuable insights into how cave invertebrates persist and evolve under such specialized conditions. This knowledge is important for the conservation and management of these fragile ecosystems (Mammola, 2019).

Cave-dwelling animals exhibit distinct preferences for specific microhabitats within these environments. These preferences are influenced by the behavioral, physiological, and morphological characteristics associated with their ecological and evolutionary classifications, which include trogloxenes (species that use caves but do not live in them exclusively), troglophiles (species that can thrive both inside and outside caves), and troglobites (species exclusively adapted to cave environments) (Schiner, 1854; Racovitza, 1907; Howarth, 1983; Sket, 2008).

Studying the diversity of invertebrate communities in caves is important for understanding the ecological patterns determining cave biodiversity and microhabitat preferences. This research helps us understand the factors that influence the community of subterranean invertebrates. Furthermore, it provides knowledge that can be used to develop strategies for preserving these unique ecosystems (Trevelin et al. 2019; Souza-Silva et al. 2021; Reis-Venâncio et al. 2022 and 2024).

Research in cave habitats has explored factors such as substrate heterogeneity, trophic resources, and microclimatic conditions (temperature and humidity). These studies consider various elements, including the cave's linear extent, distance from the entrance, speleogenesis, and the presence of water bodies such as puddles, lakes, and streams. Additionally, they account for favorable microclimates, substrate structures (e.g., speleothems, rock fragments of varying sizes and textures), and the availability of organic resources like guano, roots, and carcasses (Tobin et al., 2013; Mammola et al., 2015; Bento et al., 2016; Lunghi et al., 2017; Kozel et al., 2019; Cardoso et al., 2022).

It is evident that any disturbance to the cave environment can directly impact its microhabitats and associated fauna. Due to the isolation and environmental stability of caves, their integrity is highly susceptible to natural or anthropogenic disturbances, which in turn can significantly affect the resident fauna (Ferreira & Horta, 2001; Souza-Silva et al., 2015). These environmental changes can alter the resources available to species and influence their interactions. The responses to such changes vary among different taxa and can be either positive or negative. Since each invertebrate species relies on specific resources, any modification in the availability or nature of these resources due to microhabitat changes is likely to affect these species (Hamilton-Smith, 2004; Romero, 2009; Faille et al., 2015; Pacheco et al., 2020).

From this perspective, this study was designed to evaluate the influence of trophic, physical, and microclimatic variables on the structure of invertebrate communities in caves within the Atlantic Rainforest, located in three distinct limestone belts (Capiru, Itaiacoca, and Votuverava). The analysis considered both local and regional scales. To achieve these objectives, the following hypotheses were proposed: (*i*) Diversity patterns in caves are influenced by the specific characteristics of the limestone belts (Capiru, Itaiacoca, and Votuverava). The distinct interconnectivity potential of each belt may limit the dispersal of invertebrate fauna between discontinuous features. This limitation is particularly significant for terrestrial species restricted to subterranean habitats, as adjacent non-carbonate formations act as barriers, thereby confining species distributions; (*ii*) Local habitat predictors (such as the highly restrictive conditions of subterranean environments) are the primary determinants of species presence or absence in caves, outweighing regional-scale predictors. Consequently, diversity patterns are driven by niche-based processes operating at a microscale, such as microhabitat occupation, resource availability, and specific microclimatic conditions.

Methodology

Study area

This study was conducted in caves located in the limestone belts Capiru, Votuverava, and Itaiacoca, located in the metropolitan region of Curitiba in the state of Paraná, Brazil, encompassing the municipalities of Colombo, Sengés, Castro, Campo Largo, Rio Branco do

Sul, Almirante Tamandaré, Itaperuçu, Cerro Azul, Adrianópolis, and Doutor Ulysses (Figure 1). The limestone belts Itaiacoca, Votuverava, and Capiru consist of metasedimentary rocks of the Açungui Carbonate Group. This region is characterized by a humid subtropical climate, mild winters and well-distributed rainfall throughout the year. It is part of the Atlantic Rainforest biome, containing Araucaria Forest (Mixed Ombrophilous Forest) and the Atlantic Rainforest proper (Dense Ombrophilous Forest) (Passos 1986).

Several proposals for delimiting these belts have been presented over the years (Almeida, 1956; Bigarella & Salamuni, 1956; Fiori et al., 1990; Reis Neto, 1994). In this study, we used the division used by Sessegolo et al. (2006), where the Capiru Belt encompasses all the metasediments of the Açungui Group to the south of the Lancinha Fault and is located at the base of this carbonate group. The Votuverava Belt, formed by carbonatic rocks (Scholl et al., 1980), is located north of the Lancinha Fault, between the Capiru and Itaiacoca Belts. Finally, the Itaiacoca Belt, located between the Serra de Itaiacoca in Paraná and the Taquari Mirim River Valley in São Paulo (Reis Neto, 1994), is composed of a complex of metavolcanic and metasedimentary rocks, for which there are several stratigraphic proposals (Almeida, 1956; Trein et al., 1985; Reis Neto, 1994).

Twenty-three caves were sampled, twelve of these in the Itaiacoca limestone belt, five caves located in the Votuverava limestone belt, and another six caves located in the Capiru limestone belt (Figure 1, Table 1).

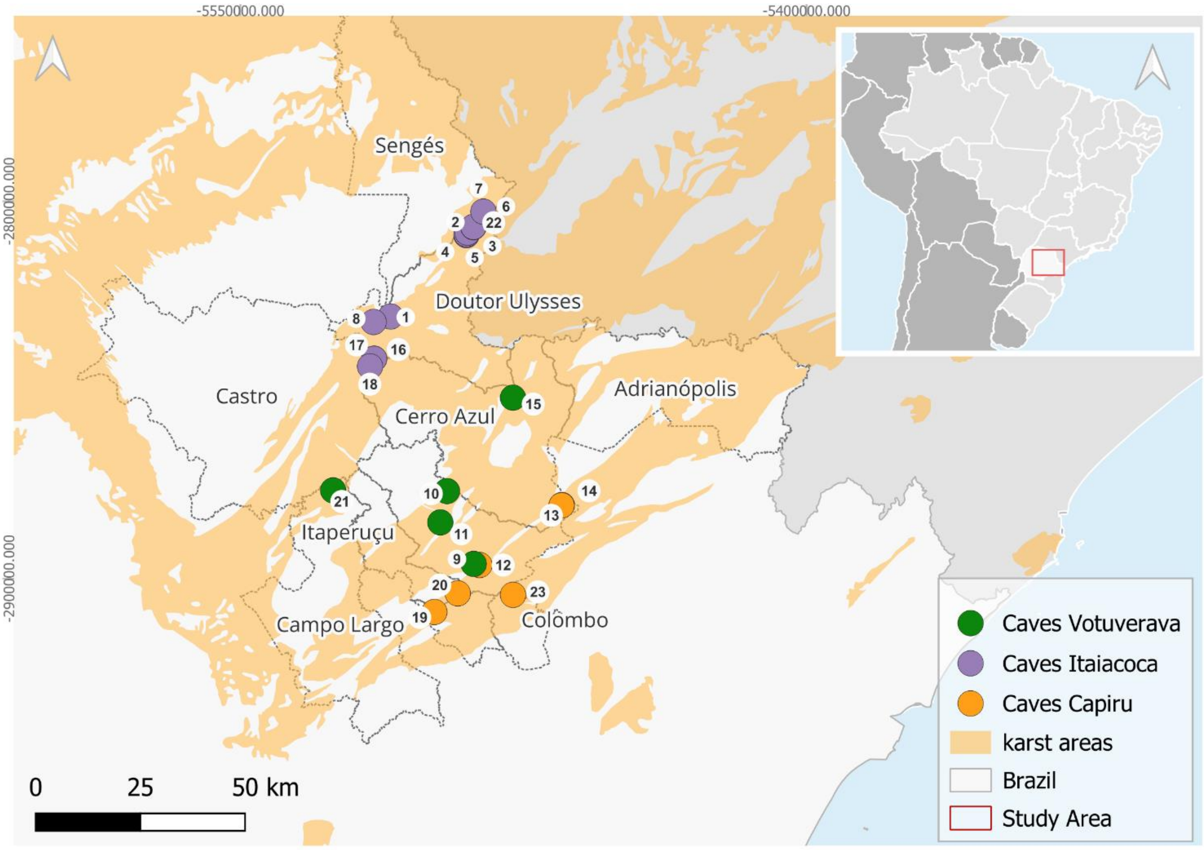

Figure 1. The municipalities are located in the metropolitan region of Curitiba, Paraná state, Brazil, and the studied caves. The circles represent the caves located in the limestone belts Votuverava (purple), Itaiacoca (green), and Capiru (Orange). The numbers represent the caves studied. 1-Varzeão cave; 2-Casa de Pedra cave; 3-Ressurgência do Feital cave; 4-Dá a volta cave; 5-Arco de Pedra cave; 6-Pinhalzinho cave; 7-Pocinho cave; 8-Malfazido cave; 9-Toquinhas cave; 10-Piedade cave; 11-Bromados cave; 12-Lancinha cave; 13-Fadas cave; 14-Jesuítas cave; 15-Bom Sucesso cave; 16-Chiquinho cave; 17-Chiquinho II cave; 18-Pinheiro Seco cave; 19-Ermida cave; 20-Itaperussu cave; 21-Pinheirinho cave; 22-Pocinho II cave; 23-Bacaetava cave.

Table 1. Caves sampled in the Curitiba metropolitan region, Paraná, Brazil. Abbreviations of the cave names (Abbrev), limestone belts (belt), latitude (lat), and longitude (Long) in UTM, the number of sectors (Se), and quadrats (Quad). Itaiacoca (ITA), Votuverava (VOT), Capiru (CAP), belts. Time (in minutes) spent collecting inside sectors (STS) and quadrats (STQ) average/standard deviation.

Cave name	Belt	Lat	Long	Set	STS	Qua	STQ
Varzeão	ITA	7275042	651909	8	29.38/9.38	24	11.33/4.54
Malfazido	ITA	7273761	647782	4	26.5/8.2	12	7.17/6.15
Ressurgência. do Feital	ITA	7294326	670403	4	32.45/9.44	12	8/10
Dá a volta	ITA	7294572	670427	2	32/9	6	11,5/5
Arco de Pedra	ITA	7294581	670419	1	47/0	3	17/3.22
Casa de Pedra	ITA	7294947	670668	1	34/0	3	12/4,36
Pocinho	ITA	7296230	672123	4	32/5.77	12	13.25/7.36
Pocinho II	ITA	7296306	672130	1	39/0	3	14/7.55
Pinhalzinho	ITA	7299940	674573	5	23.6/8.68	15	10/5.82
Chiquinho	ITA	7264965	647839	2	38/10	6	18/9.62
Chiquinho II	ITA	7264954	647825	3	27.03/4.93	9	9.22/4.22
Pinheiro Seco	ITA	7263142	646809	6	31/11.15	18	10.56/7.46
Bom Sucesso	VOT	7255243	681136	3	33.33/8	9	15/4.15
Bromados	VOT	7225641	663298	4	36/19.76	12	14.33/8.77
Piedade	VOT	7233100	664985	3	31/6.99	9	14/5.96
Pinheirinho	VOT	7233572	637641	5	24.2/7.19	15	10.33/11.42
Toquinhas	VOT	7215636	671190	3	17.33/3.29	9	10/4.37
Bacaetava	CAP	7208130	680519	6	22.17/6.55	18	7.5/5.5
Ermida	CAP	7204278	661607	2	54.5/10.5	6	22.5/11.35
Itaperussu	CAP	7208651	667277	4	26.15/8	12	7.25/4.66
Jesuítas	CAP	7229413	692528	4	36.15/14	12	13/6
Fadas	CAP	7229326	692470	1	27/0	3	9/3
Lancinha	CAP	7215358	672566	4	19/4.55	12	7.3/6

Habitat structure measurement

The characterization of the habitat structure on the cave floor was carried out through visual inspection and quantification of the surface area occupied by distinct organic and inorganic components within sectors and quadrats (Figure 2). The substrates were categorized as trophic, shelter, and general (Table S1). Each sector was characterized according to the protocol applied by Pacheco et al. (2020) and Souza-Silva et al. (2021), in cave environments. For this purpose, the sector was divided along its longest extent into ten perpendicular sections of one meter by three meters. The percentages of area occupied by distinct substrate elements, both organic and inorganic, were measured in these ten sections. Subsequently, to obtain a single value for each substrate, the percentages of areas occupied by each type of substrate were summed and divided by 100 (Souza-Silva et al. 2021).

On the other hand, in the quadrats, the substrate components were evaluated using photographs taken in loco. Digital photographs (4000 x 3000 pixels) were taken in the field in a vertical position (90º) using a Canon Powershot SX50 HS camera. The photographs of each quadrant were analyzed using IMAGE J software (Rasband 1997), in which the present substrates were measured according to their occupation area (Pacheco et al. 2020). Temperature and humidity measurements were taken in each sector with the help of a digital thermometer hygrometer placed on the floor of these sectors for at least fifteen minutes (Souza-Silva et al. 2021).

Determination of geographical distances between the caves and the distances to the mines

The geographic distance between caves was determined by recording the geographical coordinates of the cave entrances using a Garmin 64s GPS. Google Earth Pro software (Google LLC, 2024) was used to plot the cavity points and measure both the distance between caves and their proximity to mining areas. These distance measurements were then used to assess the potential influence of proximity to mining activities on the composition and richness of cave invertebrate fauna.

Sampling of Invertebrates

The sampling of invertebrates was carried out in sectors (3x10m) and quadrats (1x1m) separately through direct intuitive Search (DIS) (Wynne et al. 2019, Pacheco et al. 2020), and active collection with the aid of tweezers and brushes moistened with 70% alcohol (Figure 2). A total of 80 sectors and 240 quadrats were sampled, with the number of sectors per cave varying depending on the extent of the cavity and its physical characteristics (Table 1). The collections were conducted in sectors and quadrats by biologists with cave fauna experience, as Souza-Silva et al. (2011) recommended. The time in minutes spent in each sample unit was recorded using a digital stopwatch (Table 1). A sampling event was conducted per cave in separate campaigns in November 2022, April, and July 2023.

Figure 2. Sampling invertebrates in sectors and quadrats inside the caves, using tweezers, brushes, and containers with 70% alcohol. Photos of Jesuítas Cave, in Cerro Azul, Capiru limestone belt.

Sorting and identification of invertebrates

All invertebrate specimens were collected and kept in vials containing 70% alcohol for later identification and separation into morphotypes (Oliver & Beattie, 1996; Furtado-Oliveira et al., 2022). Specialists confirmed the identification of Acari, Isopoda, Hemiptera, Pseudoscorpiones, and Orthoptera. The samples are deposited in the collection of cave invertebrates of Lavras (ISLA) of the Center for Studies in Subterranean Biology (CEBS), at the Federal University of Lavras (UFLA) (biologiasubterranea.com.br).

Determination of troglomorphisms in invertebrates

The identification of potentially troglobitic species was based on the presence of troglomorphic traits, which serve as indicators of isolation and evolutionary adaptation to cave environments. Commonly observed troglomorphisms included the reduction or complete loss of ocular structures and pigmentation, as well as the elongation of sensory and locomotor appendages (Culver & Pipan, 2019). Certain taxa displayed distinct troglomorphic adaptations, such as elongated appendages (e.g., Polydesmida, Pseudoscorpiones), elongated appendages and tergites coupled with depigmentation and reduced cuticle sclerotization (e.g., Chilopoda), wing reduction (e.g., Hemiptera), and the enlargement of claws (unguis) or basal teeth on unguiculi (e.g., some Collembola). These morphological traits have been widely used as diagnostic markers in cave fauna studies (Bento et al., 2016). To enhance the accuracy of identification, specialists in various taxa were consulted to evaluate specific troglomorphic characteristics, and their contributions are duly acknowledged. For this study, we classified species as troglobitic only when they exhibited clearly observable and well-documented troglomorphic features (Sket, 2008).

Data analysis

The categories of substrates presented in Supplementary Material I were used to obtain the diversity of general substrate, shelter diversity, and trophic resource diversity through the Shannon-Weaver index (Buttigieg & Ramette 2014). Shelter availability for invertebrates was calculated by the sum of coarse gravel, rocky blocks, and organic debris in each transect. For trophic resource availability, we considered guano, feces, roots, organic debris, cryptogams, phanerogams, algae, actinomycetes, basidiomycetes, animal carcasses, and pteridophytes.

Spearman's correlation analysis was used to evaluate the influence of distance from the entrance on the diversity and availability of substrates, shelter diversity and availability, and trophic diversity and availability on the cave floor, using quadrats and sectors as sample units (Gallucci, 2019).

ANOSIM based on Euclidean distance was used to identify differences in the composition of substrate elements, substrate diversity, shelter diversity, and trophic diversity between limestones belts and caves, using sectors and quadrats as sample units (Buttigieg and Ramette 2014; Clarke et al. 2001). A shade plot Graphic model was used to show the data of the matrix of substrate elements according to the spatial distribution inside transects and quadrats along the caves (after being transformed into a square root). The distributions of substrates on the cave floor were also reordered in a clustering analysis using the Whittaker Association Index (Whittaker 1952; Clarke et al., 2014).

Analysis of variance, or ANOVA, was used to compare differences in the average between different groups of variables (species richness, distance from the caves to the nearest mining, temperature, humidity, diversity and availability of substrates, diversity and availability of shelters, and trophic resource diversity and availability (Gallucci 2019).

The similarity of troglobitic and non-troglobitic fauna was obtained using the Bray-Curtis index and contrasted on a multidimensional metric scale (MDS) with resampling by the Bootstrap method. ANOSIM based on the Bray-Curtis index was used to identify differences in the composition of invertebrate fauna between carbonate bands, using cave quadrats and sectors as sample units (Anderson et al., 2015).

The influence of substrate characteristics on the composition, (Bray-Curtis similarity), was evaluated through a distance-based linear model (DistLM), Forward step-wise method, and the Akaike model selection criterion corrected for small samples (AICc) (Anderson et al. 2015). Distance-based redundancy analysis (dbRDA) was subsequently used to evaluate the percentage of data adjustment to the model and the proportion of variation explanation (Clarke & Gorley, 2006). These analyses were made independently for sectors and quadrats and non-troglobitic fauna and troglobitic fauna (Pacheco et al., 2020). The included variables were: substrate diversity, shelter diversity, trophic diversity, temperature, humidity, geographical distance between the caves, distance from the entrance, and distance from mining as predictor variables.

To obtain invertebrates' abundance and richness values, counts of individuals and morphotypes of each sample unit were performed (alpha diversity). The individual-based rarefaction curve was used to obtain the expected species in each carbonate band separately for sectors and quadrats (Chao & Jost 2012).

Generalized Linear Models (GLMs) were used to examine the influence of predictor variables such as temperature, humidity, distance from the entrance, trophic resource diversity, shelter diversity, general substrate diversity, trophic resource availability, shelter availability, and distance from mining on the species richness at mesoscale (sectors). A second model was created by adding all types of substrates mentioned in Supplementary Material I as predictor variables. We evaluated multicollinearity between all variables using Spearman's correlation (Peterson and Carl, 2020). Variables with correlation values greater than 70% ($r^{\hat{}} \geq 0.7$) were excluded from the analyses to mitigate potential collinearity problems (Schober et al., 2018). As the Spearman coefficient shows only paired correlations (Zuur, et al., 2009), we also used variance inflation factors (VIF) in the models. Predictors with values greater than three were removed and then re-analyzed. We use the negative binomial error distribution for the models, since the Poisson distribution showed overdispersion (Zuur et al., 2009).

To analyze the effects of all substrate variables, resources, and microclimatic characteristics on morphospecies richness at the microscale (quadrats), we performed mixed generalized linear models (GLMM), assuming the sector as a random effect. The predictors and response variables included in the model were consistent with mesoscale models. We assumed the negative binomial distribution for the model, based on AIC criteria, residual normality, and parameter significance. We assessed the model fit by visually inspecting the spatial distribution of residuals and using the 'simulateResiduals' function from the DHARMa package (Hartig, 2022). The model with the lowest Akaike Information Criterion (AIC) value was selected.

We used a series of statistical and ordination analyses to evaluate the phylogenetic distance (distinctness) between cave communities and the influence of environmental variables such as humidity, temperature, distance from the entrance, trophic diversity, shelter diversity, overall diversity, resource availability, and shelter availability. To measure phylogenetic distance, we converted the hierarchical taxonomic identity into a clustering representation that quantified the distance between different taxonomic levels. These distances were then normalized by the number of levels, following the method described by Clarke & Warwick (1999). We then used the comdist function from the picante package in R to calculate the MPD values, which provided the distances between each pair of communities. Presence/absence data of species were transformed into binary matrices and standardized using the 'decostand' function of the 'vegan' package. We applied Principal Coordinates Analysis (PCoA) to examine the similarity between communities based on the Jaccard distance matrix calculated by the 'vegdist' function of the 'vegan' package. Environmental variables were associated with PCoA results through a generalized linear regression (glm), followed by segmented regression to identify points of change in the relationship between phylogenetic distance and environmental variables at micro and mesoscales. Model significance was evaluated by AICc comparison, and results were interpreted based on trends identified by segmented models.

Results

Habitat structure characterization in cave floors

The substrates on the cave floors are detailed supplementary materials I and II, including their abbreviations and classifications as resource, shelter, or general. The analysis of similarity (ANOSIM), based on Euclidean distance, did not reveal significant differences in the composition of substrates between the limestone belts for sectors and quadrats. The proportions of substrate components on the floor and their relationship with the distance from the entrance are represented in figure S1.

Among the analyzed sectors, it was found that substrate elements such as hardpan, guano, speleothems, and boulders are associated with the distance from the entrance, occurring in more distant locations from the cave entrance. On the other hand, elements such as carcasses, snail shells, algae, woods, and basidiomycetes show a lower association with the distance from the entrance, occurring in locations closer to the entrance. In the quadrats, the substrate elements most significantly associated with the distance from the entrance were guano, hardpan, gravel, and rough rock. These substrates occurred in locations distant from the entrances. On the other hand, woods, basidiomycetes, animal carcasses, and sloped floors were found in quadrats near the entrances.

The overall diversity of the substrates decreased with the distance from the entrance in the Votuverava and Itaiacoca caves, while it remained constant in the Capiru cave. As the distance from the entrance increases, both trophic diversity and shelter diversity reduce in all ranges. This pattern is evident when considering the availability of resources and shelters, showing a gradual decrease in distance from the entrance (Figure S2). Thus, we observe that the regions near the cave entrances exhibit a more significant habitat heterogeneity, contrasting with the deeper areas, which tend to have more homogeneous habitats.

Invertebrates species richness

As mentioned, 23 caves were sampled, covering 80 sectors and 240 quadrats. However, invertebrate fauna was recorded in only 200 quadrats, leaving 40 quadrats without any invertebrate collections. The survey identified 357 invertebrate species distributed across at least 55 distinct orders, with some representatives depicted in Figure S3. Among the limestone formations, the Capiru belt exhibited the highest average species richness, with 53.8 species (SD = 20.6), followed by the Itaiacoca formation with an average of 52.6 species (SD = 19.8), and the Votuverava formation with an average of 48.2 species (SD = 21.9). These averages encompass invertebrates collected from sectors, quadrats, and general cave collections. Across all scales, the orders Araneae, Diptera, and Acari stood out as the most species-rich groups (Figure 3).

Regarding troglobic fauna, 28 species have been identified: Four Palpigradi: (Eukoeneniidae) – *Eukoenenia* sp1, *Eukoenenia* sp2, *Eukoenenia* sp3 e *Eukoenenia* sp4; Two Pseudoscorpiones: (Chtoniidae) – *Pseudochthonius* sp1 e *Pseudochthonius* sp2; Four Opiliones: (Gonyleptidae) – Gonyleptidae sp1 e Gonyleptidae sp2; Cryptogeobiidae sp1 e Cryptogeobiidae sp2; Three Araneae: (Hahniidae) – Hahniidae sp1; (Prodidomidae) – Prodidomidae sp1 e Prodidomidae sp2; Seven Collembola: (Hypogastruridae) – *Acherontides aff. eleonorae*; (Arrhopalitidae) – *Arrhopalites* sp1 e *Arrhopalites* sp2; (Entomobryomorpha) – Entomobryomorpha sp1 e Entomobryomorpha sp2; (Symphypleona) – Symphypleona sp1 e Symphypleona sp2; Three Isopoda: (Philosciidae) – Philosciidae sp1; (Plathyarthridae) – Plathyarthridae sp1 e *Trichorhina* sp1; Four Diplopoda: (Pyrgodesmidae) – *Peridontodesmella* sp1, *Peridontodesmella* sp2 e Pyrgodesmidae sp3; (Oniscodesmidae) – *Crypturodesmus* sp1; and one Stylommatophora: (Systrophiidae) - Happia sp1.

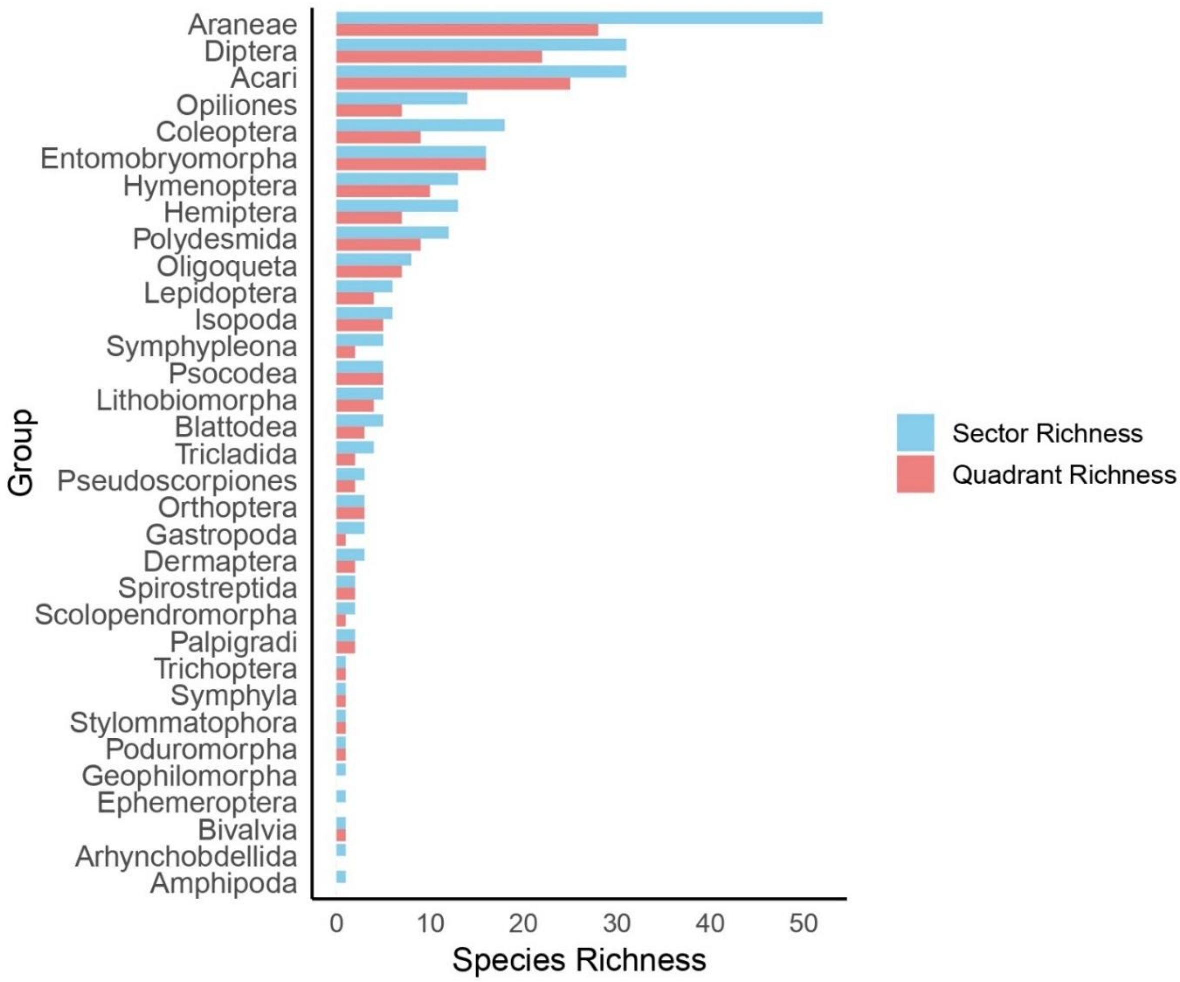

Figure 3. The richness of invertebrates in different taxa was observed in 23 caves in the state of Paraná. The blue lines represent the richness in the sectors, and the red lines represent the richness in the quadrats.

The rarefaction curves based on individual abundance, analyzed separately for sectors and quadrats across the limestone formations, indicate that species richness in the sectors is likely higher than what was sampled. This suggests a significant potential for additional species diversity in these caves, particularly in the Capiru and Votuverava formations, which require more comprehensive sampling. In contrast, at the microscale, the observed species richness in the quadrats closely approximates the expected richness across all three limestone formations studied (Figure 4).

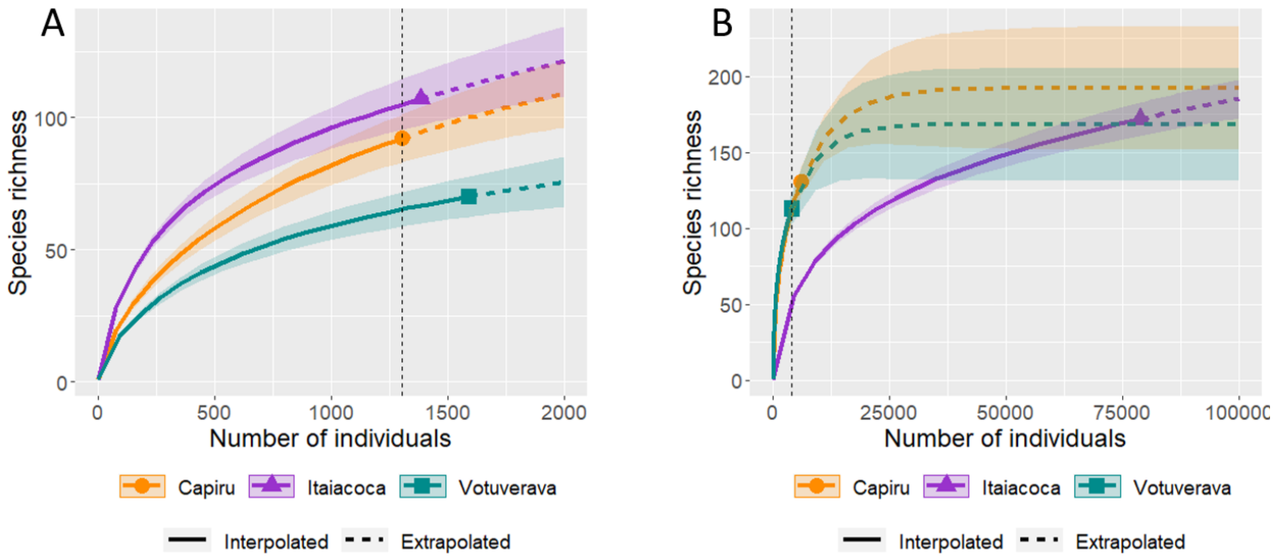

Figure 4. Rarefaction curves based on individuals. A) Rarefaction curve performed on a microscale (quadrants) B) Rarefaction curve performed on a mesoscale (sectors). The black dashed line represents the community with the lowest abundance, the continuous colored lines represent the richness and abundance of collected species, and the dashed lines represent the extrapolation of the data.

The model used to investigate the response of species richness at the microscale revealed a significant positive relationship between trophic resource diversity ($p = 0.03$, R^2=0.277) and trophic resource availability ($p = 0.02$, R^2=0.277). (Figure 5). At the mesoscale, none of the variables tested significantly influenced fauna richness.

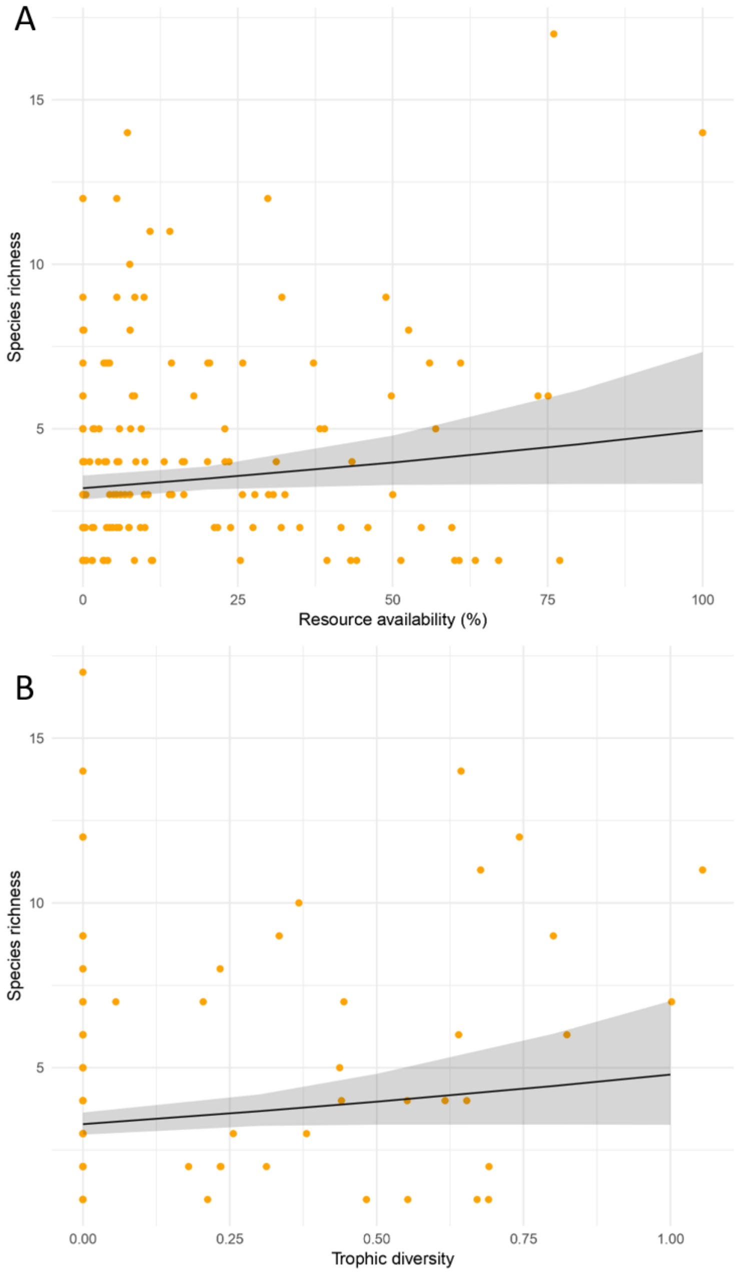

Figure 5. The relationship between the species richness present and the availability of trophic resources (A), and diversity of trophic resources (B), on a micro scale.

Cave fauna composition

The ANOSIM analysis revealed significant differences in the species composition of cave fauna within the quadrats. The Itaiacoca and Votuverava formations exhibited the greatest similarity, followed by Itaiacoca and Capiru, while Votuverava and Capiru showed differences in fauna composition (Rglobal = 0.1, p = 0.01). Similar patterns were observed at the sector level, with the highest similarity between Itaiacoca and Votuverava, followed by Itaiacoca and Capiru. However, significant differences in fauna composition were again noted between Votuverava and Capiru, reflected by a global R-value of 0.14 and a p-value of 0.01.

The ANOSIM similarity analysis for the caves revealed significant differences in fauna composition (global R = 0.23, p = 0.011). However, no significant differences were detected in fauna composition when the caves were grouped by limestone belts. Similarly, for troglobites, no significant differences in fauna composition were observed among the caves or limestone belts. Figure 6 illustrates the dispersion of similarity around the mean for quadrats, sectors, and caves, considering both the total fauna and troglobites.

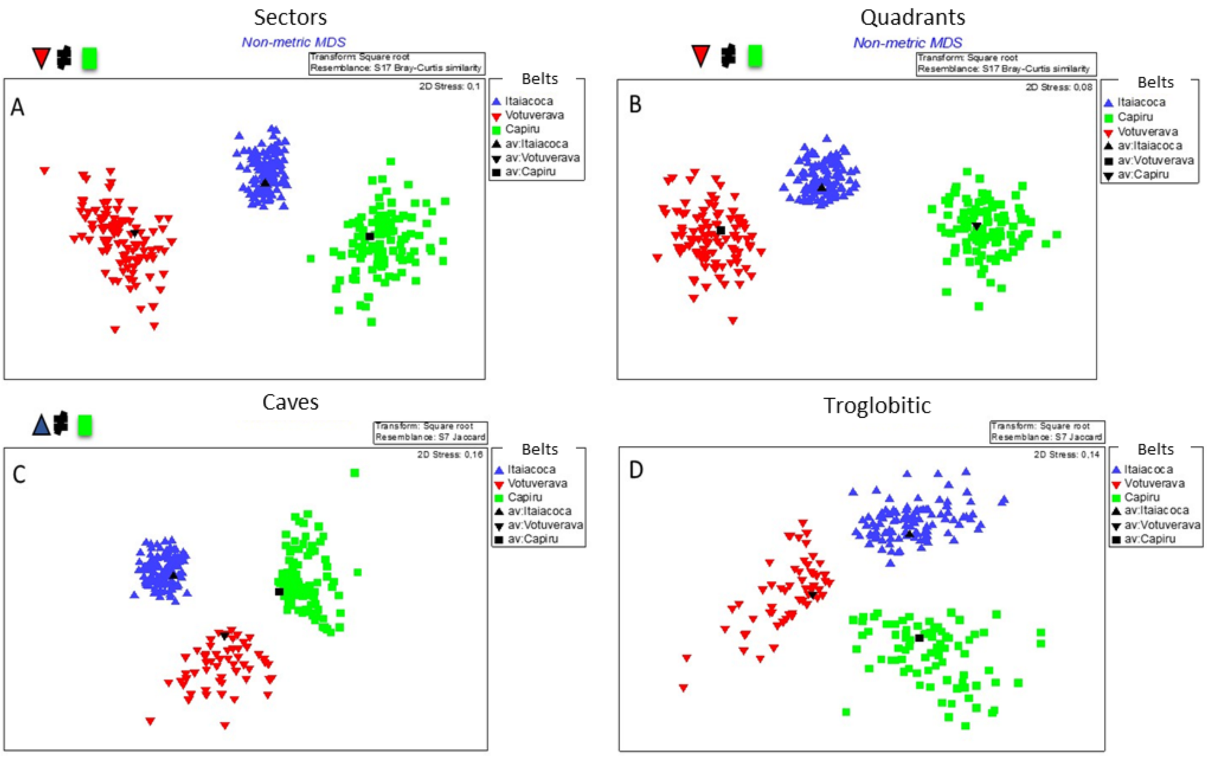

Figure 6. Multidimensional scaling (MDS) showing the dispersion around the mean (av) in fauna similarity in sectors (A), quadrats (B), caves (C) and troglobites (D). The dispersion of the points indicates the variation in the fauna composition within each limestone belt.

The DistLM analysis revealed that in the sectors, only shelter diversity and temperature acted as predictor variables of variations in composition (AICc=661.9; R^2=0.06; P=0.04). The variables that significantly determined the composition for the quadrats were the distance from the entrance and the distance between the caves (AICc=1685.6; R^2=0.025; P=0.001). The Distance-Based Redundancy Analysis (dbRDA) in the sectors indicated 46% model fit and 9% response to the tested variables. The dbRDA had a model fit of 54.9% for the quadrats, and 3.5% of the tested variables determined the fauna variation.

The influence of environmental variables on the phylogenetic distinctness in invertebrate communities

The predictive variables significantly influencing the phylogenetic distinctness of invertebrate communities at the microscale were shelter diversity and trophic resource diversity. Increases in these variables were associated with greater phylogenetic distinctness. Conversely, trophic resource availability demonstrated a positive but nonlinear effect: beyond a certain threshold, further increases in resource availability ceased to enhance phylogenetic distinctness and instead negatively impacted it. Additionally, distance from the entrance had a significant negative effect; greater distances from the entrance corresponded to reduced phylogenetic distinctness among species (Figure 7).

At the mesoscale, trophic diversity and resource availability exhibited patterns similar to those observed at the microscale, suggesting that resource diversity plays a stronger role in driving phylogenetic distinctness than mere resource availability in the sampled caves. Humidity also showed a positive effect on phylogenetic distinctness up to an inflection point, beyond which additional increases in humidity negatively impacted it (Figure 7). Other tested variables did not have a significant influence on phylogenetic distinctness. Furthermore, the segmented model consistently provided a better fit to the data. Detailed results are presented in Table 3.

Table 3: Results of segmented regression model analyses evaluating the relationship between phylogenetic distance (distinctness) and different predictive variables at the micro (quadrat) and mesoscales (sectors). Entrance distance (DE), Trophic resource diversity (TRD), Temperature (TEP), General substrate Diversity (GSD), Humidity (HUM), Diversity Shelter (SHD), Shelter Availability (SHA), Trophic Resource Availability (TRA)

Scale	Predictor variables	Adjust R^2	Pseudo-F	p-value
Meso	**DE**	**0.0002**	**1.418**	**0.108***
	TRD	**0.0123**	**79.936**	**0.0221***
	TEMP	0.0024	15.3696	0.954
	GSD	0.0002	1.33308	0.94
	HUN	**0.003**	**18.8689**	**0.0002***
	SHD	0.0008	5.27062	0.948
	SHA	0.0009	5.64207	0.207
	TRA	**0.004**	**24.9079**	**3.23E-14***
Micro	**DE**	**0.0001**	**23.4017**	**2.68E-06***
	TRD	**0.0001**	**12.6473**	**0.044***
	GSD	0.0001	10.0541	0.73
	SHD	**0.001**	**87.4273**	**2.00E-16***
	SHA	0.0004	10.9461	0.637
	TRA	**0.0004**	**10.5011**	**0.0003***

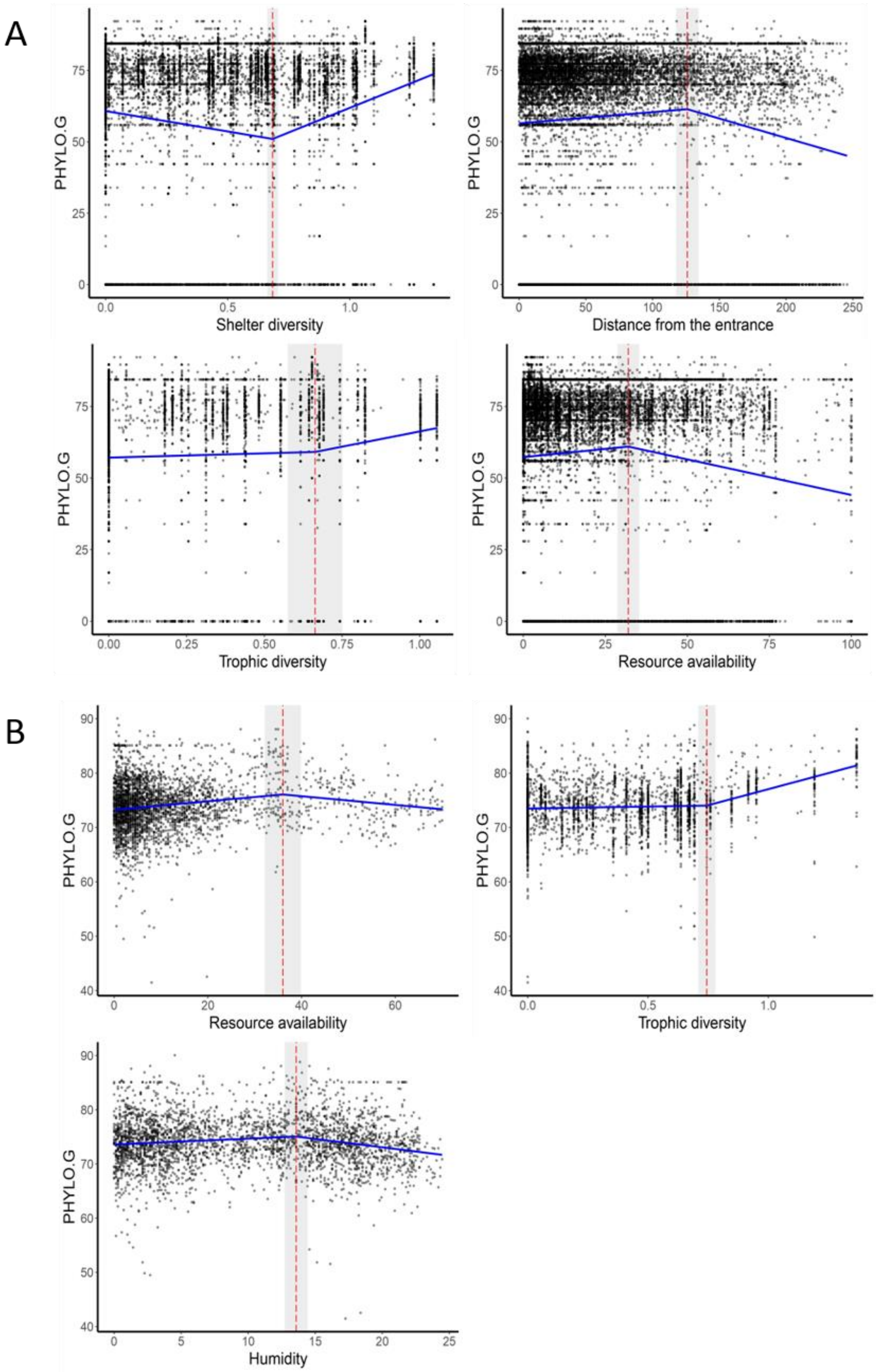

Figure 7. The thresholds in the relationship between phylogenetic distance and significant predictor variables. A) Microscale, B) Mesoscale. The red dashed vertical lines indicate significant change points ($p < 0.05$), while the shaded gray area represents the standard error around the estimate.

Discussion

The results demonstrated that the sampled caves exhibited a pronounced gradient of shelter and resource diversity and availability, extending from the entrances to the deeper regions. However, no significant differences in substrate composition were detected across the limestone belts.

The faunal composition was influenced by different factors depending on the spatial scale analyzed. At the mesoscale, shelter diversity and temperature emerged as key determinants, while at the microscale, composition was shaped by the distance from the entrance and the distance between caves. Furthermore, analysis of the caves revealed significant variations in fauna similarity between the Capiru and Itaiacoca limestone belts. Even between the geographically closer belts, Capiru and Votuverava, similarity varied significantly at both meso- and microscales.

These findings suggest that the distance between caves may act as a barrier to species dispersal, fostering the formation of distinct non-troglobitic invertebrate communities across different limestone belts. These variations are likely driven by local processes and conditions, such as the specific environmental characteristics of subterranean habitats and niche-based ecological processes operating on smaller scales, including the availability and diversity of resources and shelters (Mammola et al., 2020; Souza-Silva et al., 2020).

None of the tested variables significantly explained variation in species richness at the mesoscale. However, at the microscale, trophic diversity and shelter availability were found to significantly influence species richness.

At the microscale, shelter diversity and trophic resource diversity were key factors affecting the phylogenetic distinctness of invertebrate communities. Trophic resource availability initially had a positive effect; however, beyond a certain threshold, an excess of resources led to a decline in phylogenetic distinctness. Additionally, distance from the cave entrance negatively impacted distinctness, with greater distances corresponding to reduced phylogenetic distinctiveness.

At the mesoscale, trophic diversity and resource availability followed similar trends observed at the microscale, emphasizing that resource diversity had a more pronounced effect on phylogenetic distinctness than resource abundance. Humidity also played a role, positively contributing to distinctiveness up to an optimal level, after which excessive humidity resulted in a decline in phylogenetic distinctness.

Spatial variations in faunal similarity

When examining factors at both micro and mesoscale levels, significant differences in faunal composition were observed only between the Capiru and Votuverava belts. Surprisingly, these two belts showed greater dissimilarity with each other compared to the Itaiacoca belt, despite Itaiacoca being geographically farther away. While the literature often suggests that greater distances between caves correlate with distinct faunal compositions (Souza-Silva et al., 2020; Sovie et al., 2022), our analysis revealed that the two geographically closer belts, Capiru and Votuverava, exhibited the highest dissimilarity. This unexpected result indicates that the proximity of these two belts does not facilitate species exchange as might be anticipated. These findings suggest that specific local factors unique to the Capiru and Votuverava belts play a predominant role in shaping faunal composition at both micro and mesoscale levels, irrespective of geographic distances. This underscores the importance of incorporating local environmental variables and scale-specific analyses when assessing biological diversity in subterranean ecosystems.

Additionally, it is important to note that our understanding of the dispersal mechanisms of various subterranean species remains limited, particularly at the species level. This knowledge gap suggests significant variability in dispersal capabilities, with some species potentially exhibiting restricted movement. Conversely, other species may demonstrate greater dispersal abilities than expected (Lidicker and Stenseth, 1992; Jordan et al., 2020). This variability further emphasizes the need for detailed studies on species-specific behaviors and ecological interactions to better understand faunal dynamics in these environments.

This finding underscores that even among closely situated caves, non-troglobite invertebrate communities can display considerable variations in species composition, supporting previous research (Simões et al., 2015; Zagmajster et al., 2018; Mammola et al., 2020). Differences in community composition between nearby caves are often attributed to substrate characteristics and microclimatic conditions. These factors create highly heterogeneous microhabitats within caves, shaping the diversity and distribution of species. This heterogeneity contributes to reduced faunal similarity between caves (Pellegrini et al., 2016; Pacheco et al., 2020; Mammola et al., 2020; Souza-Silva et al., 2020).

In addition to substrate diversity, temperature plays a significant role in influencing faunal composition at the mesoscale. Subterranean species specialized for deeper cave regions often exhibit lower tolerance to temperature fluctuations. These species benefit from the thermal stability offered by deeper regions, as opposed to those near the entrance, which experience greater variability (Colado et al., 2022). In the context of climate change, subterranean species are particularly vulnerable to temperature variations, which could have substantial impacts on community composition (Deutsch et al., 2008; Arribas et al., 2012; Colado et al., 2022).

At the microscale, the distance from the entrance and the distance between caves emerged as significant factors influencing faunal composition. The gradient of conditions and resources extending from the entrance to deeper cave zones is well-documented in the literature. This gradient fosters distinct microhabitats, occupied by various species and enabling diverse ecological interactions throughout the cave. Areas further from the entrance tend to be more restrictive for many species due to the more homogeneous and harsher environmental conditions prevailing in these deeper zones (Prous et al., 2015; Mammola & Isaia, 2018; Lunghi & Manenti, 2020).

It is important to note that caves within the Capiru belt are located near mining areas, which often result in a range of negative impacts on subterranean environments (Donato et al., 2014). Proximity to mining activities raises concerns about significant alterations to surface ecosystems, which, in turn, may affect adjacent caves. Environmental instabilities on the surface can lead to changes in the richness and composition of biological communities within nearby caves (Cardoso et al., 2022). This highlights the critical role of these environments as refuges for various species (Tscharntke et al., 2002; De Fraga et al., 2023).

Influence of Environmental variables on species richness

Species richness in caves is influenced by several factors, including the availability of organic resources, substrate heterogeneity, and microclimatic conditions (Ferreira et al., 2007; Tobin et al., 2013; Pellegrini et al., 2016; Pacheco et al., 2020; Souza-Silva et al., 2021). However, scale analysis can uncover distinct patterns regarding how these variables affect species richness. At the microscale, higher trophic diversity and resource availability were observed to favor the presence of a greater number of species. The accumulation of organic matter in caves creates favorable conditions for colonization and establishment, leading to increased species richness (Schneider et al., 2011; Souza-Silva et al., 2012).

Resource diversity is particularly significant in sustaining a broader range of species, facilitating the development of complex food webs (Moore et al., 2004; Schneider et al., 2011; Venarsky & Huntsman, 2018). Additionally, diverse resources provide various spaces that increase the availability of shelters and niches. These spaces act as refuges from environmental fluctuations and predation, as well as breeding sites, while also offering microclimatic conditions favorable to certain invertebrate species (Moore et al., 2004; Ferreira et al., 2009; Cardoso et al., 2022).

Influence of variables on phylogenetic distinctiveness

Segmented regression analyses were instrumental in identifying the extent to which various environmental variables significantly influenced the phylogenetic distinctiveness of cave species. The segmented model provided a superior fit compared to the linear model, underscoring its ability to capture complex relationships. Based on these findings, we recommend combining segmented and linear regression approaches as complementary tools to elucidate how environmental variables affect species diversity. This combination allows for a more nuanced understanding of environmental influences on the phylogenetic structure of cave invertebrate communities.

On a microscale, entrance distance showed a breakpoint at 126 meters, marking a significant shift in the relationship between phylogenetic distinctiveness and distance from the entrance. This pattern likely reflects the increasing challenges fauna face when transitioning from the entrance to deeper cave regions, including reduced light, diminished availability of organic resources, and decreased habitat heterogeneity (Prous et al., 2015; Souza-Silva et al., 2021). These harsh conditions favor the colonization of highly specialized species (Sket, 2008), thereby significantly shaping species distinctiveness.

Shelter diversity exhibited a strong positive relationship with phylogenetic distinctiveness, suggesting that caves with a greater variety of shelters tend to harbor phylogenetically diverse groups. A variety of shelter types enhances the structural complexity of these environments, creating diverse microhabitats that provide refuges, breeding sites, and microclimatic variability. This structural heterogeneity facilitates the coexistence of species with varying ecological requirements, thereby increasing phylogenetic diversity (Tews et al., 2004; Ferreira et al., 2009; Cardoso et al., 2022; Reis-Venâncio et al., 2022).

Trophic diversity and resource availability also influenced phylogenetic distinctiveness at both meso and micro-scales. Organic resources are closely linked to the surrounding landscape's integrity, which supplies the organic inputs essential for cave ecosystems (Schneider et al., 2011; Souza-Silva et al., 2012; Cardoso et al., 2022). However, the caves in this study are under constant threat from mining expansion and deforestation, jeopardizing the quantity and quality of resources entering these environments.

Segmented analysis revealed that the relationship between organic resource availability and taxonomic differentiation of fauna, initially, an increase in resources promotes greater phylogenetic distinction, but beyond a certain threshold, this positive effect diminishes. This phenomenon may result from a “saturation” effect, where abundant resources allow generalist and opportunistic species to dominate, outcompeting specialized species (Moore et al., 2004;

Schneider et al., 2011). Trophic diversity, more than resource abundance, showed a robust relationship with taxonomic distinction, indicating that resource variety plays a critical role in sustaining diverse invertebrate groups and enabling the coexistence of species with varied ecological niches.

Although caves are often considered oligotrophic environments with limited resource availability, recent studies have demonstrated that invertebrate richness is positively associated with both the quantity and diversity of organic resources. A diverse resource base reduces interspecific competition, creating ecological niches that support higher local species richness (Schneider et al., 2011; Tews et al., 2004). These findings suggest that in subterranean ecosystems, resource diversity may be more critical than absolute resource abundance for establishing and maintaining biodiversity (Reis-Venâncio et al., 2024).

These results underscore the importance of preserving cave-surrounding areas and maintaining the flow of organic matter into subterranean habitats. Protecting these inputs is vital for sustaining the diversity of invertebrate species within these fragile ecosystems.

Conclusion

It is important to acknowledge that the composition, richness, and phylogenetic distinctness of cave-dwelling fauna respond differently to environmental factors depending on the scale of analysis. This variability underscores the critical need to incorporate scale considerations into ecological research, particularly in studies of subterranean ecosystems.

Understanding how environmental factors such as temperature, humidity, and trophic and physical features influence cave fauna can provide valuable insights into their diversity and distribution. Moreover, examining these influences across multiple scales—from microhabitats within a single cave to broader regional contexts—can uncover significant patterns and relationships that might otherwise be overlooked.

These findings have important implications for developing effective conservation strategies for cave-dwelling species, which are often vulnerable to environmental changes and human activities. Additionally, future research should aim to explore additional scales and a broader range of environmental variables that may impact cave fauna diversity. Such investigations are particularly critical, as many aspects of subterranean invertebrate ecology remain underexplored.

By addressing these gaps, researchers can advance our understanding of subterranean biodiversity and devise more targeted and effective conservation measures to address the unique challenges faced by cave ecosystems.

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AUTHOR CONTRIBUTIONS

Rodrigo Lopes Ferreira and Marconi Souza Silva were responsible for the conceptualization, methodology, translation, and English corrections. Leandro Mata da Rocha Melo, Rodrigo Lopes Ferreira, and Marconi Souza Silva participated in field activities. Leandro Mata da Rocha Melo prepared the original draft. Leandro Mata da Rocha Melo and Marconi Souza Silva were responsible for the statistical analysis, artwork, reviewing, and editing. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data supporting this study's findings are available on request from the corresponding author. The Editor-in-Chief has waived the required archiving due to privacy or ethical restrictions.

CONFLICTS OF INTEREST

The corresponding author confirms on behalf of all authors that no involvement might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

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Supplementary material I. List of substrates found in the sampled caves, the abbreviations used, and their classification for analysis. Hardpan = Compacted clay. W=Width

Substrates type	Grain size	Abbreviation	Categories
Waterbody	Does not apply	WC	General
Sand	(2-0,06 mm)	SND	General
Silt/clay/mud	Does not apply	SCM	General
Speleothem	Does not apply	SP	General
Hardpan	Does not apply	HP	General
Inorganic substrate	Does not apply	OTI	General
Dropping of Speleothem	Does not apply	FR	General
Termite mounds	Does not apply	TM	General l
Shrinkage crack	Does not apply	CG	Shelter
Smooth Rock floor	Does not apply	SR	Shelter
rough rock floor	Does not apply	RR	Shelter
Big boulder	4000-250mm	BD	Shelter
Small Block	250-64mm	CBD	Shelter
Gravel	64-2mm	GR	Shelter
Snail Shell	Does not apply	CON	Shelter
Guano	Does not apply	GA	Trophic resource
Feces	Does not apply	FC	Trophic resource
Roots	Does not apply	RT	Trophic resource
Cryptogams	Does not apply	CR	Trophic resource
Phanerogams	Does not apply	PHA	Trophic resource
Algae	Does not apply	AL	Trophic resource
Actinomycetes	Does not apply	ACT	Trophic resource
Basidiomycetes	Does not apply	BAS	Trophic resource
Animal carcass	Does not apply	CRC	Trophic resource
Pteridophytes	Does not apply	PT	Trophic resource
Leaf litter	>10mm	LFL	Trophic resource /shelter
Branches	250-11mm (w)	BR	Trophic resource /shelter
Stem	>250mm (w)	TRU	Trophic resource /shelter

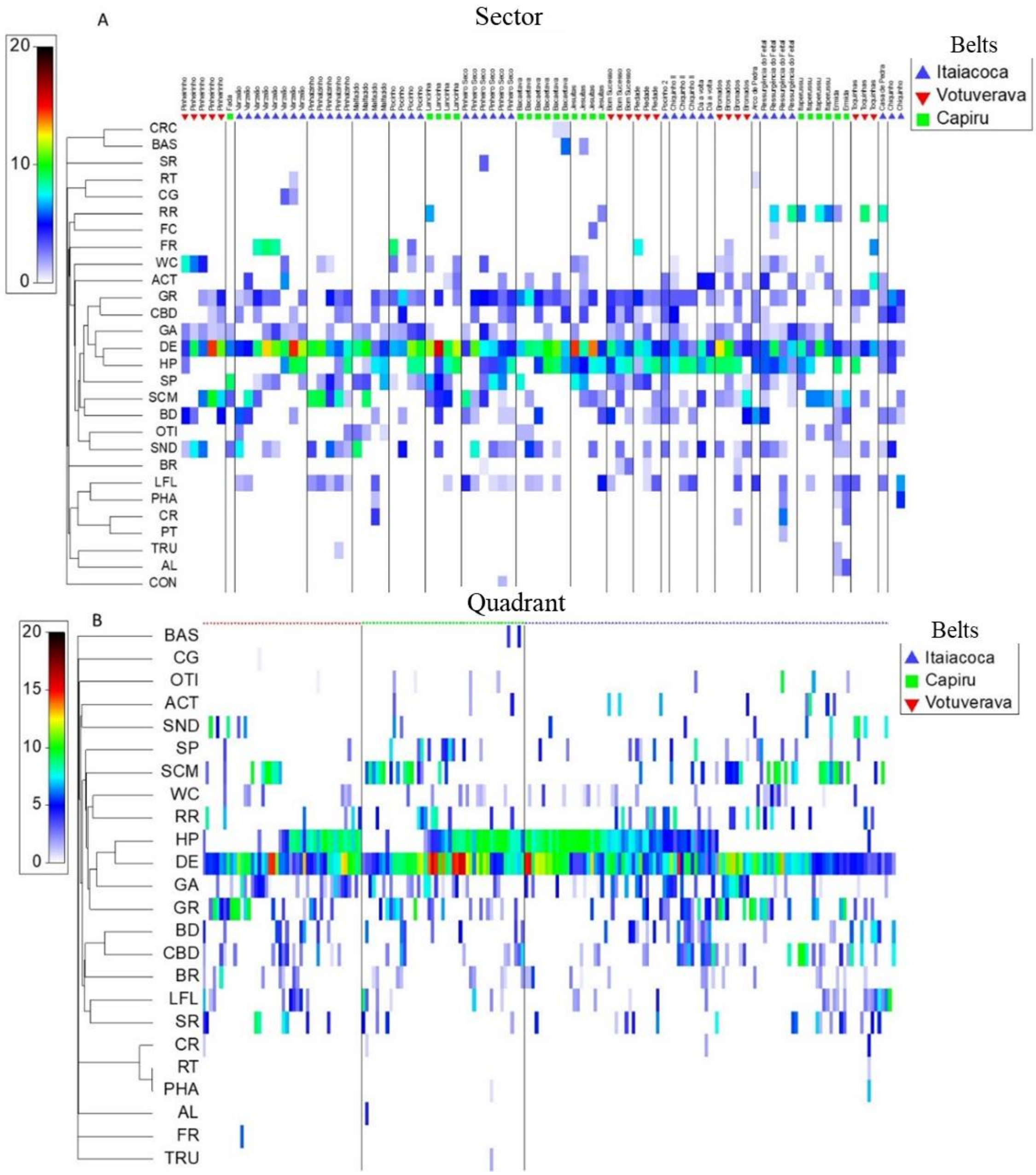
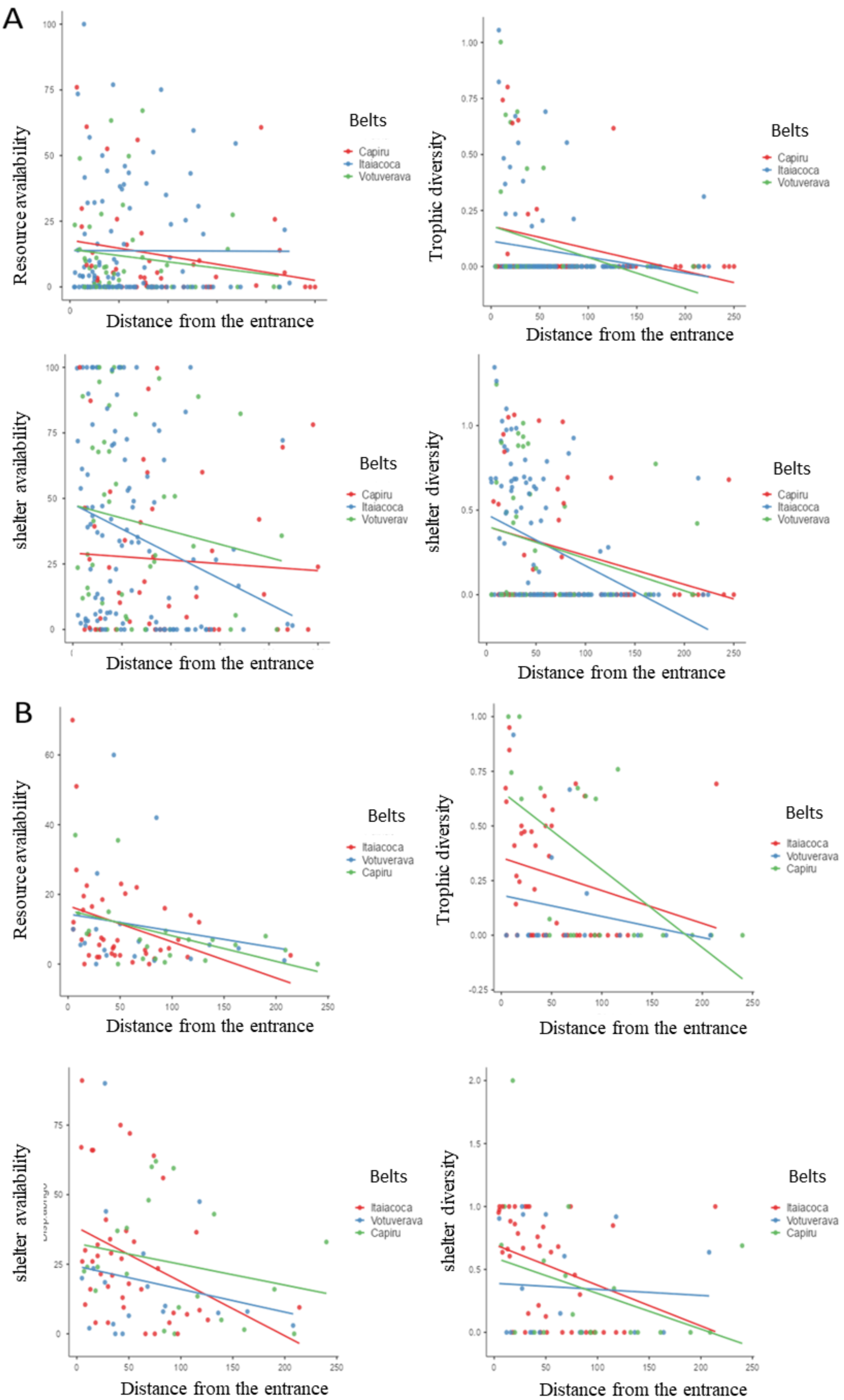


Figure S1. Variations in the distribution and percentage of area occupied (values from 0 to 20) by components of the substrate on the floor of the caves and their associations with the distance from the entrance (DE) in the sectors (A) and quadrats (B). The dendrogram on the left groups the sample units according to the Whittaker association index. Substrates type: WC-Waterbody; SND-Sand; SCM-Silt/Clay/Mud; SP-Speleothem; HP-Hardpan; OTI- Inorganic substrate; FR-Dropping of Speleothem; TM-Termite mounds; CG-Shrinkage crack; SR- Smooth Rock floor; RR- Rough rock floor; BD-Big Boulder; CBD- Small Block; GR-Gravel; CON- Snail Shell; GA-Guano; FC-Feces; RT-Roots; CR-Cryptogams; PHA- Phanerogams; AL-Algae; ACT-Actinomycetes; BAS-Basidiomycetes; CRC- Animal carcass; PT- Pteridophytes; LFL- Leaf litter; BR- Branches; TRU-Stem.



A
Resource availability
Distance from the entrance
Belts
Capiru
Itaiacoca
Votuverava
Trophic diversity
Distance from the entrance
Belts
Capiru
Itaiacoca
Votuverava
shelter availability
Distance from the entrance
Belts
Capiru
Itaiacoca
Votuverav
shelter diversity
Distance from the entrance
Belts
Capiru
Itaiacoca
Votuverava
B
Resource availability
Distance from the entrance
Belts
Itaiacoca
Votuverava
Capiru
Trophic diversity
Distance from the entrance
Belts
Itaiacoca
Votuverava
Capiru
shelter availability
Distance from the entrance
Belts
Itaiacoca
Votuverava
Capiru
shelter diversity
Distance from the entrance
Belts
Itaiacoca
Votuverava
Capiru

Figure S2. Relationship between diversity and availability of substrates, shelters, and trophic resource with the distance from the entrance in quadrats (A) and sectors (B). Diversity (Div), Availability (Disp), Distance (dist).

Figure S3. Invertebrates collected in 23 caves from three carbonate bands in the metropolitan region of Curitiba, Paraná. A) *Pseudonannolene* sp. B) Hirudinida sp. C) Trichoptera sp. D) Rhagidiidae sp1. E) Staphylinidae sp. F) *Serracutisoma* sp1. G) *Endecous* sp. H) Macronyssidae sp. I) *Cryptops* sp. J) *Loxosceles* sp. K) Gonyleptidae sp2. L) Trombidiformes sp. M) Gonyleptidae sp3. N) *Thestylus aurantiurus* O) *Spelaeochernes* sp. P) *Zelurus travassosi*. Q) *Aegla* sp.

Article 2
A spot of troglobitic species diversity in a highly altered karstic area inside the Brazilian Atlantic Rainforest

Article to be submitted to the journal Zoologia.

Leandro Mata da Rocha Melo[1,2], Rodrigo Lopes Ferreira[1,2], Marconi Souza Silva[1,2]

1 - Centro de Estudos em Biologia Subterrânea, Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, Campus Universitário, P.O. Box 3037, Lavras CEP 37200-000, Minas Gerais, Brasil
2 - Programa de Pós-Graduação em Ecologia Aplicada, Universidade Federal de Lavras, Lavras CEP 37200-000, Minas Gerais, Brasil

Abstract

Cave-restricted fauna is highly specialized, endemic, and vulnerable to extinction pressures. However, the biodiversity of many karst areas remains underexplored due to limited studies, accessibility issues, and inefficient data collection, leading to gaps in knowledge and protection efforts. By reviewing existing literature and implementing fine-scale sampling methods in caves within the Capiru, Itaiacoca, and Votuverava limestone belts, we surveyed the troglobitic species composition richness, and taxonomic distinctness under pressures from the metropolitan region of Curitiba, in Paraná state, Brazil. This research hypothesizes that the three limestone belts will exhibit distinct invertebrate communities due to geographic distance, varying environmental conditions, and human activities. Additionally, the proximity to urban conurbations, municipality population density, altitude, and mining activities may indicate the landscape's conservation status and impact pressure. We sampled 23 caves and recorded 28 invertebrate species with troglomorphic traits, compared to seven historically reported. No significant differences in species composition, richness, or taxonomic distinctness were found between the Votuverava, Capiru, and Itaiacoca carbonate belts. Common disturbances included trampling (78.2%), trash deposition (65.2%), graffiti (65.2%), pasture cultivation (60.8%), and speleothem vandalism (60.8%). Urban conurbations, mining activities, and *Pinus* and *Eucalyptus* plantations can impact cave microhabitats, leading to siltation and trampling by increased visitation, as observed in Gruta Itaperussu and Ermida due to their proximity to cities, mining, and deforestation. An alarming finding was the higher troglobitic species richness in caves with more human impact (R^2=0.48, AICc=105.82, p=0.003). Our findings increase the known number of troglobitic species and reveal the factors threatening them, enhancing our understanding of caves' biodiversity and faunal identity in the Itaiacoca, Votuverava, and Capiru limestone belts. Revealing troglobitic diversity in those three limestone belts highlights the importance of conserving these subterranean ecosystems and can influence more effective protection and management policies.

Keywords: Troglobites**;** Cave Ecology; Invertebrate Conservation; Cave Fauna.

Introduction

The knowledge of ecological, biogeographical, and environmental threats is essential for biodiversity conservation. It is crucial to understand how many and which species exist, where they are located, how they interact with each other and with the environment, and the many habitat alterations (Venter et al., 2014; Pollock et al., 2017; Mckerrow et al., 2018; Belote et al., 2021). This knowledge is the foundation for developing effective conservation strategies. Understanding biodiversity is a key pillar for developing conservation policies and strategies. This includes identifying priority conservation areas, implementing protective measures, reducing threats to species, and promoting sustainable practices (Elgar, 2010). Awareness and information about the importance of biodiversity play fundamental roles in its conservation. As people gain a better understanding of the significance of biodiversity and the impacts of their actions on it, they become more likely to support conservation measures (Newing et al., 2011).

The formulation of global conservation priorities has significantly influenced the allocation of resources across expansive geographic areas. Nonetheless, various researchers have noted that the efficacy of global conservation prioritization in facilitating concrete conservation implementation has been limited (Mace et al., 2000; Brummitt et al., 2003; Whittaker et al., 2005; Brooks et al., 2010). Separate processes are necessary to identify conservation targets and priorities at much finer scales because even within a region, they are uniformly critical (Whittaker et al., 2005). Culver and Sket (2000) emphasized the importance of subterranean places with 20 or more troglobitic species to establish hotspots of subterranean biodiversity and call attention to conservation actions. Subsequently, due to the increasing identification of areas of high diversity worldwide, this threshold was adjusted to the presence of at least 25 species (Culver et al., 2021). Although tropical regions currently have a relatively low number of such identified sites, it is expected that with an increase in collections and the identification of new species, new hotspots will emerge in these areas (Culver et al., 2021, Souza-Silva et al., 2021, Ferreira & Souza-Silva 2023). Quantifying the biodiversity of subterranean invertebrates has presented enduring and substantial challenges over time (Culver et al., 2004; Ferreira, 2005; Wynne et al., 2019; Mammola et al., 2021). The complexity of subterranean ecosystems, coupled with the inherent difficulties in accessing and studying underground habitats, has contributed to the persistent challenges in accurately quantifying the biodiversity of subterranean invertebrates (Mammola et al., 2021).

One of the main gaps has been the need for more observation of specific microhabitats within caves. This is essential due to collection techniques that do not consider the small body size of many invertebrates, making visual detection difficult. Moreover, these organisms exhibit a distribution associated with microhabitats with conditions such as humidity, the presence of specific resources (such as plant debris, guano, and microbial biofilms), and location distant from cave entrances (Pacheco et al., 2020; Nicolosi et al., 2021; Souza-Silva et al., 2021). These issues make it essential to adopt a targeted approach to data collection, prioritizing specific microhabitats to reveal the diversity of troglobitic species (Pacheco et al., 2020; Reis-Venâncio et al., 2024).

Recent research has shown that the spatial distribution of terrestrial invertebrate communities in caves is closely linked to the availability of food resources and microhabitat requirements (Fereira and Martins, 2001; Prous et al., 2015; Souza-Silva et al., 2021; Pacheco et al., 2022). This implies that collection methods focused on specific microhabitats tend to reveal a substantially higher number of troglobitic species, even with a reduced number of sampling events (Cardoso et al., 2021; Souza-Silva et al., 2021; Bento et al., 2021). This approach can enhance data collection efficiency and proves crucial for understanding biodiversity in environments where conducting prolonged inventories is difficult or impractical.

In the state of Paraná, Brazil, where extensive carbonate belts harbor numerous caves, essential studies on invertebrate fauna have been conducted for decades, revealing the presence of endemic and threatened species (Pinto-da-Rocha, 1995; Sessegolo et al., 2006; Bená and Vanin, 2014). However, knowledge about troglobitic species is still incipient, especially considering the high species richness in these regions. Therefore, this study aims to provide an update on the diversity of troglobitic species in the caves of the Capiru, Itaiacoca, and Votuverava limestone belts (Açungui group), located in the metropolitan region of Curitiba, Paraná state, Brazil, prioritizing fine-scale sampling methods directed towards specific microhabitats. This research hypothesizes that the three limestone belts will exhibit distinct invertebrate communities due to geographic distance and varying environmental conditions. Additionally, the proximity to urban conurbations, population density, altitude, and mining activities may indicate the landscape's conservation status and impact pressure.

Considering the metropolitan region of Curitiba, as an environment altered by multiple anthropogenic activities, including uncontrolled urbanization, environmental pollution, and resource exploitation (Macedo, 2004), subterranean biodiversity, particularly troglobitic species, is expected to be significantly affected. Revealing troglobitic diversity in Paraná limestone belts can highlight the importance of conserving the subterranean ecosystems and influencing more effective protection and management policies. Investigating cave biodiversity in these areas can also provide information to guide more effective conservation and management measures to protect these ecosystems and their species specialized in subterranean life.

Methods

Area of Study

The study was conducted in 23 caves in three continuous limestone belts: Itaiacoca, Votuverava, and Capiru, placed in the Metropolitan Region of Curitiba (MRC), Atlantic Rainforest biome, Paraná state, Brazil (Figure 1). The MRC, as proposed by Nojima et al. (2009), is comprised of the municipalities of Almirante Tamandaré, Araucária, Campina Grande do Sul, Campo Largo, Campo Magro, Colombo, Curitiba, Fazenda Rio Grande, Pinhais, Piraquara, Quatro Barras, São José dos Pinhais, Balsa Nova, Bocaiúva do Sul, Contenda, Itaperuçu, Mandirituba, Rio Branco do Sul, Tunas do Paraná, Adrianópolis, Agudos do Sul, Cerro Azul, Doutor Ulysses, Lapa, Quitandinha e Tijucas do Sul.

The accessed municipalities in this study were Colombo, Sengés, Castro, Campo Largo, Rio Branco do Sul, Almirante Tamandaré, Itaperuçu, Cerro Azul, Adrianópolis and Doutor Ulysses (Figure 1, Table 1). In the Capiru belt, samples were made in Bacaetava cave, Ermida cave, Itaperussu cave, Jesuíta cave, Fadas cave, and Lancinha cave. In the Votuverava belt, caves sampled were Bom Sucesso, Bromados, Piedade, Pinheirinho, and Toquinhas. The caves Ressurgência do Feital, Pocinho, Pocinho 2, Dá a Volta, Malfazido, Pinhalzinho, Chiquinho, Chiquinho II, Arco de Pedra, Casa de Pedra, and Pinheiro Seco, were sampled in Itaiacoca belt (Table 1).

The Itaiacoca, Votuverava, and Capiru limestone belts consist of metasedimentary rocks from the Açungui Carbonatic Group, formed in the Late Proterozoic - 1.000 to 570 million years ago (Fiori, 1994). This region is characterized by a humid subtropical climate, with mild winters and well-distributed rainfall throughout the year and located within the Atlantic Rainforest biome, comprising forests with Araucaria (Mixed Ombrophilous Forest) and the Atlantic Rainforest itself (Dense Ombrophilous Forest) (Morrelato and Haddad 2000, IAT 2024). The region has a karst aquifer with 5,740 km^2 in length, covering the municipalities of Campo Magro, Almirante Tamandaré, Itaperuçu, Rio Branco do Sul, Colombo, Bocaiuva do Sul, Cerro Azul, Tunas do Paraná, Doutor Ulysses and Adrianópolis (Andrade et al. 2015). All the studied caves are in the Ribeira watershed (IAT, 2024).

The presence of these caves in a highly degraded area (IAT, 2024) in the metropolitan region of Curitiba raises concerns regarding potential anthropogenic impacts stemming from urbanization, mining activities, agriculture, tourism, deforestation, and other human interventions (Gondim et al., 2002; Macedo, 2004; Fritzsons et al., 2009; Mohebalian et al., 2022).

Sampling and identifying obligate cave invertebrates

The sampling of invertebrates was conducted in sectors (3x10 m) and quadrants (1x1m) using active collection methods aided by tweezers and brushes (Souza-Silva et al., 2021). Additionally, invertebrates were searched and collected in various locations within the cavities. Microhabitats were thoroughly inspected in this case to obtain a higher species richness (Souza-Silva et al., 2021). All collected material was stored in containers filled with 70% alcohol for subsequent sorting and separation of morphotypes, carried out at the Center for Studies in Subterranean Biology (CEBS) at the Federal University of Lavras (UFLA).

Potential troglobionts species were determined by identifying troglomorphisms traits, which indicate isolation and evolution in the cave environment (Culver and Pipan, 2019). We send some specimens to taxonomists to help confirm the potential troglobites (see acknowledgments section).

The term troglobionts refer to species that inhabit caves and various shallow subterranean and above-ground environments. Most subterranean species typically exhibit troglomorphy adaptations, including reduced eyes and pigment, increased size, elongated appendages, and additional sensory structures (Culver and Pipan, 2019). However, certain troglobionts may not display significant troglomorphy due to habitat volume, exposure to twilight, genetic variability, and other reasons. This variability suggests that some species may be classified as "eutroglophiles" (Deharveng et al., 2024). As a result, certain cases are considered potential troglobionts for terminological consistency, pending further detailed studies to clarify their categorization.

Voucher specimens were deposited in the Underground Invertebrate Collection of Lavras (ISLA), affiliated with the Center for Studies in Subterranean Biology (CEBS) at the Federal University of Lavras, Minas Gerais, Brazil (https://www.biologiasubterranea.com.br).

Literature Review

A bibliographic search was conducted in July 2023 to compile lists of troglobitic species identified in caves in Paraná state. A systematic search was conducted across various databases, including Scielo, Web of Science, and Google Scholar. The following search terms were utilized: "cave fauna and Paraná," "cave and Paraná," and "troglobites and Paraná." Information from the literature review on troglobitic cave fauna in the state provided a basis for comparing the species richness of troglobitic organisms collected in the present study.

In addition to comparing and ranking species richness at the national level, we conduct a comprehensive search in the scientific literature using databases such as Scielo, Web of Science, and Google Scholar, using specific search terms related to troglobitic species, caves, and geographical review on a national level. We used this database to rank the richness of caves in the metropolitan region of Curitiba (local relevance) and other areas with high troglobitic species richness in Brazil (National relevance).

Degree of impacts inside the caves and Surroundings areas

The degree of impact was qualified according to the Cave Conservation Priority Index (CCPi) proposed by Souza-Silva and colleagues (2015). Uses and impact determined human modifications. Tourist and religious activities were considered uses, while real impacts were trampling, illumination, and construction resulting from these activities (Souza-Silva et al., 2015).

Based on the method, the impact was considered based on potential modifications that can cause impoverishment, enrichment, or modifications of cave microhabitats and organic resources. Impoverishment reduces organic resources and biological diversity due to human activities in the cave. Enrichment is promoted by human activities that increase the number of organic resources. Finally, modifications are impacts that alter the physical structure of the cave without causing trophic enrichment or impoverishment. Each impact was considered based on modifications inside and outside the caves, being divided, according to their modification potential, into spatial extent within the cave and the duration of the impact in the cavity, receiving weights 1, 2, or 3. Intense potential refers to modifications that cause significant disturbance to the fauna and physical structure of the cave (weight 2). Tenuous potential refers to modifications causing minor disturbances to the fauna and physical structure (weight 1).

Short spatial extent includes modifications of potentially low spatial extent, affecting the physical structure and fauna locally (weight 1), when compared to modifications of broad space (weight 2), and finally, permanence refers to the interval of time that the impact will remain in the cave environment, thus divided into impacts of occasional permanence (weight 1) and impacts of constant permanence (weight 3) (Souza-Silva et al., 2015).

The 23 sampled caves were classified based on the sum of their impacts. The highest value found (55) served as the basis for categorizing the degree of impacts of the cavities: Extremely high (≥28), high (20-27), medium (14-20), and low (≤13). Each category received the following weights: Extremely high (4), high (3), medium (2), and low (1). Deforestation and agricultural activities always received a weight of 1 due to the difficulty of understanding the true impact of these activities in the underground environment (Souza-Silva et al., 2015).

Environmental features of the sampled caves

Most of the caves in this study were mapped (Sessegolo et al., 2006) using a standardized mapping methodology with a British Cave Research Association (BCRA) – 4C survey grade. The altitude above sea level and geographic position of the caves were obtained with a Global Positioning System (GPS) in decimal degrees (Table 2). We insert the location coordinates of the caves in the free software Qgis 3.34.6 (https://qgis.org/pt_BR/site/) to measure the shortest linear distance from the caves to a limestone extraction mine (DMA) and from an urban center (DUC).

Population size and mineral economy of the municipalities

Estimates of the population size of the studied municipalities were extracted from the IBGE database in March 2024 (https://www.ibge.gov.br/estatisticas/sociais/populacao/9103-estimativas-de_populacao.html). The database related to the mineral economy of each municipality (names of the mining companies or legal entities, types of minerals exploited, quantity of mining concession process), was extracted from the database of the Instituto Água e Terra do Paraná – IAT, the year 2019 (https://www.iat.pr.gov.br/Pagina/Economia-Mineral).

Data Analysis

Morphotype counts were conducted for each sample unit (alpha diversity) to obtain richness values of troglobitic invertebrates.

We performed the average taxonomic distinctness (Δ+) analysis using Phyla (weight 100), Class (weight 80), Order (weight 60), Family (weight 40), and morphotypes (weight 20) as variables for a matrix of morphotype distribution among sample units (Anderson et al., 2008). The average taxonomic distinctness (Δ+) analysis was conducted with the Primer 7 statistical software PRIMER-E version 7 (https://www.primer-e.com/).

The faunal compositional similarity was assessed using the Bray-Curtis index and contrasted on a multidimensional metric scale (MDS) with resampling via the Bootstrap method. Similarity Analysis (ANOSIM) was performed among the different limestone belts using caves as sampling units. Euclidean distance matrix was employed to identify differences in species richness, taxonomic distinctness of troglobitic invertebrates, and degree of human impacts among limestone belts using ANOSIM (Capiru, Itaiacoca, and Votuverava), and caves as sampling units (Anderson et al., 2015).

Distance-based linear models (distLM) were performed to evaluate the effects of proximity to mining activities, distance from the urban center, altitude, population density, and the degree of impact on troglobitic species richness and taxonomic distinctness. Models were built using the forward selection procedure for variable selection, starting with a null model and adding variables based on their explanatory power (Anderson et al., 2008). The corrected Akaike information criterion (AICc) was the model selection criterion, recommended for cases with many variables and few samples (Anderson et al., 2008). AICc is a metric that measures the quality and simplicity of a statistical model, providing a metric for model comparison and selection, where lower AICc values represent higher quality and simplicity according to this criterion.

Additionally, we present a visual representation of the troglobitic fauna data matrix in a shaded plot according to spatial distribution in the caves (after transformation to square root). The distribution of troglobites in the caves was reordered in a cluster analysis using the Whittaker Association Index (Whittaker 1952; Clarke et al., 2014). All analyses mentioned above were conducted in the statistical software PRIMER-E version 7 (https://www.primer-e.com/).

To investigate the correlations between the richness of troglobites and the variables human impact, mining distance, distance from urban centers, altitude, and population density, we conducted a Spearman correlation analysis. The Spearman correlation coefficient (ρ) was chosen because it is a non-parametric measure of association, suitable for data that do not meet the assumptions of normality or linearity (Zar, 1999). This method allows us to assess whether there is a monotonic relationship between the variables without assuming a strict linear relationship. Multiple linear regression analysis was used to test whether troglobite species richness is related to taxonomic distinctness.

Results

Literature Data

Historical studies on caves in Paraná document the presence of seven troglobitic species. Among them, two species stand out as mentioned in a comprehensive survey conducted by Pinto-Da-Rocha (1995): the collembolan *Acherontides* sp. and the diplopod *Katantodesmus* sp. Both species were associated with bat guano. It is important to note that *Katantodesmus* mentioned in Pinto-Da-Rocha (1995) comprises millipedes of the genus *Crypturodesmus*

(Trajano et al., 2000) and *Acherontides* sp., is *Acherontides* aff. *Eleonorae* (Palacios-Vargas and Gnaspini-Netto, 1992). Subsequently, two new records were added in Gruta da Lancinha, with the identification of *Ideoroncus cavicola* (Mahnert, 2001) and *Arrhopalites paranaensis* (Zeppelini, 2006) as new troglobitic species (Sessegolo et al., 2006).

In subsequent investigations, Gruta do Varzeão was revealed as the habitat of one more species restricted to caves, with the identification of the opilionid Tricommatinae sp. (Sessegolo et al., 2006). Posteriorly, a new species of carabid beetle, Coarazuphium ricardoi Benà and Vanin, 2014, collected in Gruta do Varzeão, was described. Finally, the amphipod *Hyalella formosa* Araujo et al., 2014, was described in a sandstone cave located in the city of Ponta Grossa. With these records, seven troglobitic species had been documented in the state of Paraná by the year 2014. Due to the non-occurrence of *Hyalella formosa* in the Açungui group, it was not considered in comparing troglobitic fauna.

Current Troglobitic Richness in Caves of Paraná

Regarding troglobic fauna, 28 species have been identified: Four Palpigradi: (Eukoeneniidae) – Eukoenenia sp1, Eukoenenia sp2, Eukoenenia sp3 e Eukoenenia sp4; Two Pseudoscorpiones: (Chtoniidae) – Pseudochthonius sp1 e Pseudochthonius sp2; Four Opiliones: (Gonyleptidae) – Gonyleptidae sp1 e Gonyleptidae sp2; Cryptogeobiidae sp1 e Cryptogeobiidae sp2; Three Araneae: (Hahniidae) – Hahniidae sp1; (Prodidomidae) – Prodidomidae sp1 e Prodidomidae sp2; Seven Collembola: (Hypogastruridae) – Acherontides aff. eleonorae; (Arrhopalitidae) – Arrhopalites sp1 e Arrhopalites sp2; (Entomobryomorpha) – Entomobryomorpha sp1 e Entomobryomorpha sp2; (Symphypleona) – Symphypleona sp1 e Symphypleona sp2; Three Isopoda: (Philosciidae) – Philosciidae sp1; (Plathyarthridae) – Plathyarthridae sp1 e Trichorhina sp1; Four Diplopoda: (Pyrgodesmidae) – Peridontodesmella sp1, Peridontodesmella sp2 e Pyrgodesmidae sp3; (Oniscodesmidae) – Crypturodesmus sp1; and one Stylommatophora: (Systrophiidae) - Happia sp1. (Figure 2).

Only six of the 23 sampled caves showed records of troglobitic species, as indicated in the synthesis by Pinto-da-Rocha (1995) and additional information from the literature (Sessegolo et al., 2001; Sessegolo et al., 2006; Benà and Vanin, 2014). These historical data showed that Gruta do Varzeão stood out for harboring the highest troglobitic richness, with four identified species.

The number of troglobites collected in this study was greater or equal in all caves to that found in previous studies. The caves of Pinheiro Seco, Pinhalzinho, Bom Sucesso, and Varzeão stood out for their high diversity of obligatory cave invertebrates (Figure 3A), with seven species in the Pinheiro Seco and Bom Sucesso, eight in Pinhalzinho and nine species in the Varzeão Cave. In contrast, previous records indicated four troglobitic species for Gruta Varzeão, two troglobitic species for Gruta Bom Sucesso, only one troglobitic species for Gruta Pinheiro Seco, and no troglobite occurrence for Gruta Pinhalzinho.

After this study, the fourteen caves for which no records of troglobitic organisms were made now present at least one obligatory cave-dwelling species (Table 1). The collembolan *Acherontides aff. eleonorae* demonstrates a wide distribution among the caves, being identified in 14 out of the 23 caves associated with the guano and tree trunks and in a large abundance (Figure 2P).

This study did not record troglobitic organisms in only three caves (13%): Gruta dá a Volta, Ponte de Pedra, and Casa de Pedra. The remaining caves (87%) presented at least one obligate cave species (Table 1).

Faunal distribution and similarity

The similarity analysis (ANOSIM) did not detect significant differences in the composition of substrates on the cave floors, faunal composition, and the richness of troglobitic species among the Votuverava, Capiru, and Itaiacoca carbonate belts. Figure 5 shows the spatial distribution of the relative troglobitic species richness, providing information on the cave's local relevance regarding the number of species. The Mann-Whitney test showed no significant difference in troglobitic species richness and taxonomic distinctness in caves with water compared to those without water.

The Itaiacoca range stood out with the highest richness of troglobitic species, totaling 20. Following this, the Capiru range presented 14 species, while the Votuverava range recorded the lowest diversity, with 11 species. Four species were found across all three ranges: *Trichorhina* sp., *Crypturodesmus* sp., *Happia* sp., and *Acherontides aff. elonorae*. Additionally, five other species were recorded in two of the ranges: *Pseudochtonius* sp.1, Entomobryomorpha sp. 1, Symphypleona sp.1, Symphypleona sp.2 and *Arrhopalites* sp.1. The remaining 23 species were exclusive to a single limestone range, of these, 16 species were found in only one cave (Fig. 6).

Regional relevance of the troglobite species richness

Of the 32 troglobitic species from Paraná, only five are currently described: *Coarazuphium ricardoi* (Bená and Vanin, 2014), *Acherontides eleonorae* (Palacios-Vargas and

Gnaspini-Netto, 1992), *Ideoroncus cavicola* (Mahnert, 2001), *Arrhopalites paranaenses* (Zeppelini, 2006) *and Hyalella formosa* (Araújo et al., 2014).

Figure 3A shows the local relevance of each cave and region in terms of the total number of troglobitic species. Figure 7 shows the national relevance of the metropolitan region of Curitiba in terms of the total number of troglobite species. The metropolitan region is twelve places on the relevance scale of total species richness. However, its relative importance (species per caves sampled) ranks eleventh (table 3).

Economic development, degree of impacts, and social conflicts

The activity of the extraction of limestone rocks was registered by at least 56 companies exploiting these resources. The number of companies with a vocation for mining activities per municipality is: Rio Branco do Sul (42 companies), Almirante Tamandaré (24), Castro (18), Itaperuçu (8), Cerro Azul (7), Colombo (5), Jaguariaíva (5), Sengés (4), Campo Largo (3), Adrianópolis (2), Bocaiúva do Sul (1), Doutor Ulysses (1). The number of mining concessions by municipalities until 2019 were: Rio Branco do Sul (35), Castro (14), Almirante Tamandaré (12), Cerro Azul (7), Itaperuçu (7), Jaguariaíva (5), Colombo (4), Sengés (4), Campo Largo (3), Adrianópolis (2), Bocaiúva do Sul (1), Doutor Ulysses (1). The products explored by municipalities are: Adrianópolis (Limestone), Almirante Tamandaré (Dolomitic Limestone and Dolomite), Bacaiúva do Sul (Dolomite), Campo Largo (Limestone), Castro (Limestone, Dolomitic Limestone, Dolomite and Marble), Cerro Azul (Limestone and Marble), Colombo (Limestone and dolomitic), Doutor Ulysses (Marmoré), Itaperuçu (Limestone and dolomitic), Jaguariaíva (Limestone and dolomitic limestone), Rio Branco do Sul (Limestone, Calcitic Limestone, Dolomitic Limestone, dolomitic and Marble), Sengés (limestone and dolomite).

Due to limestone outcrops, limestone is one of the primary sources of income for municipalities in the study area. In addition to the impacts on native vegetation, which indirectly affects the underground environment, limestone extraction can lead to cave siltation. Cavities such as Gruta Itaperussu and Ermida were likely silted due to their proximity to mining and deforestation areas. The average distance from caves to urban centers and mining activities was 16.5 kilometers (sd= 6.3) and 8.9 kilometers (sd= 5.9), respectively (Table 2).

The municipalities where the sampled caves are located have the following population estimates: Colombo 249,277; Sengés 19,441; Castro 72,125; Campo Largo 135,678; Rio Branco do Sul 32,635; Almirante Tamandaré 121,420; Itaperuçu 29,493; Cerro Azul 17,884; Adrianópolis 5,797; and Doutor Ulysses 5,525. Estimates of the population size of the studied municipalities were extracted from the IBGE database (https://www.ibge.gov.br/estatisticas/sociais/populacao/9103-estimativas-de-populacao.html).

In the 23 caves investigated, 14 distinct impacts were recorded (Table 2, Figure 8). The most frequently observed were trampling (78.2%), trash deposition (65.2%), graffiti (65.2%), pasture cultivation (60.8%), and vandalism in speleothems (60.8%) (Table 2).

Four of the 23 sampled caves are located within conservation units: Gruta da Lancinha, Gruta Jesuítas, Gruta Fadas, and Gruta Bacaetava. Only Gruta da Lancinha lacks infrastructure for tourist control and environmental education activities.

Troglobites diversity and distribution and their relationships with abiotic factors

The Spearman correlation revealed that the only variable related to troglobite richness per cave was the level of human impact ($p=0.005$, $R^2=0.31$), demonstrating a positive relationship, where more impacted caves have a more significant number of troglobites. None of the other tested variables (distance from urban centers, distance from mining, altitude, and population density) showed a relationship with fauna richness.

The multiple linear regression analysis revealed that troglobite species richness is positively related to taxonomic distinctness ($p=0.0005$, $R^2=0.64$, AICc=213.95), while the other tested variables (distance from urban centers, distance from mining, altitude, and population density) did not show significant relationships.

The taxonomic distinctness analysis revealed that a higher number of species tends to be associated with greater average taxonomic diversity (Delta+) and higher uniformity in diversity distribution (Lambda+) (Figure 4B).

Discussion

The results reveal an increase in species number from historical studies to this more recent investigation. The historical survey, which identified seven troglobitic species by 2014, highlights the importance of studies such as those by Pinto-Da-Rocha (1995), which recorded species like *Acherontides* aff. *eleonorae* and *Katantodesmus* sp. (currently *Crypturodesmus*). Subsequent advances included describing new species in the Lancinha Cave and the Varzeão Cave, and more recently, the record of *Hyalella formosa* in a sandstone cave (Araujo et al., 2014). The sampling in our study recorded 28 troglomorphic/troglobitic morphotypes, reflecting an increase in known diversity. Species such as *Eukoenenia* sp., *Pseudochthonius* sp., and various species of Collembola and Diplopoda were found, revealing a greater richness of invertebrates specialized to subterranean environments. At least one troglobitic species in 14 caves were recorded, indicating an expansion of knowledge about cave fauna.

Systematic studies are essential for comprehensively characterization of fauna within subterranean ecosystems, as demonstrated by studies conducted globally (Deharveng and Bedos, 2012; White and Culver, 2012). Beyond ensuring spatial precision in sampling locations and utilizing effective collection methods, the frequency of sampling efforts directly impacts the observed species richness (Souza-Silva and Ferreira, 2016). Given the complexity of subterranean habitats, assuming a complete species inventory is challenging, as further exploration often reveals additional diversity. Species accumulation curves in these systems typically do not reach an asymptote, signifying that undiscovered species likely remain (Souza-Silva and Ferreira, 2016).

Sampling within subterranean environments presents challenges due to limited accessibility to fissures, and interstitial habitats (Culver and Pipan, 2009; Trontelj et al., 2012; Ortuno et al., 2013). Therefore, repeated sampling efforts are necessary to document subterranean biodiversity accurately. However, rapid assessment methods may also be effective for comparative purposes, provided that standardized sampling protocols are consistently applied (Souza-Silva et al., 2015; Simões et al., 2015, Souza-Silva and Ferreira, 2016, Moutaouakil et al. 2024).

The Role of Subterranean Connectivity and Troglobite Species Distribution

Regarding the composition of troglobitic species, we hypothesize that it would vary among the different limestone belts due to the distance between the caves and the specific environmental conditions of each belt. However, our hypothesis was rejected because no significant differences were found in the invertebrate composition nor the substrate composition on the cave floors among the three analyzed belts.

The heterogeneity in the environmental characteristics of caves, such as microhabitats and trophic conditions, tends to promote diverse and heterogeneous communities (Howarth, 1993; Souza-Silva et al., 2011; Lunghi et al., 2014; Simões et al., 2015). The structural complexity of habitats and resource availability can increase the number of niches, allowing for the coexistence of more species (Tews et al., 2004; Schneider et al., 2011; Stein et al., 2014; Busse et al., 2018). Thus, the similarity in environmental conditions among the three limestone belts may explain the similarity in the composition of troglobitic species among them.

Other factors determining differences in the composition of cave species include the distance between caves, which limits species dispersion, and lithological variations, which promote the diversification of habitats and cave characteristics, even among nearby caves, resulting in distinct faunal compositions (Souza-Silva et al., 2020). The presence of bodies of water and the availability of trophic resources also contribute to this differentiation (Bento et al., 2016; Pacheco et al., 2020; Souza-Silva et al., 2021). However, the sampled caves exhibit the same lithology and are within nearby carbonate ranges.

The lack of significant differences in the composition of troglobite species among the three limestone belts suggests an existing subterranean connectivity between them. According to Mammola et al. (2020), connectivity is crucial in determining faunal composition, influencing biodiversity patterns and ecological interactions. This connectivity may facilitate species dispersal, reducing faunal differentiation across studied areas. Additionally, as Sovie et al. (2021) reported, biological communities can serve as indicators of landscape connectivity. Ecosystems with higher species composition similarity reflect greater connectivity, whereas those with high dissimilarity imply more isolation between sites. Based on this principle, our findings provide valuable insights into the current and historical connectivity of the studied limestone belts, aligning with observations by Sovie et al. (2021).

Regional Significance of Troglobite Species Diversity

While it is true that other regions in Brazil have reported a greater number of troglobite species largely due to more extensive sampling efforts and the exploration of a higher number of caves (Trevellin et al., 2019; Bento et al., 2021; Ferreira & Souza-Silva, 2023), we hypothesize that ongoing and thorough exploration of caves within the state of Paraná will lead to an increased inventory of troglobite species. The Atlantic Rainforest, where these caves are located, is recognized as one of the most diverse and ecologically significant biomes in the world. It is also classified as a global biodiversity hotspot, characterized by exceptionally high levels of endemism and species richness (Tabarelli et al., 2010; Joly et al., 2014). The increased focus and intensification of research efforts in this region will significantly enhance our understanding of the subterranean biodiversity. By conducting more comprehensive studies, researchers will be able to uncover the complex interactions and relationships among various subterranean organisms, as well as their roles in the overall ecosystem. This deeper investigation will lead to a clearer and more accurate depiction of the diversity that exists below the surface, ultimately contributing to a better understanding of the biological richness in these caves.

Research indicates that various epigean factors, including climate conditions and primary productivity, have a direct impact on cave fauna within neotropical regions. These factors play a crucial role in determining the richness of subterranean species (Bregović & Zagmajster, 2016; Christman et al., 2016; Culver et al., 2006; Mammola et al., 2019). In both tropical and temperate ecosystems, the availability of resources in the surface environment significantly influences the dynamics of subterranean ecosystems (Culver et al., 2006; Culver & Pipan, 2019). Specifically, areas characterized by lower primary productivity tend to provide insufficient resources for troglobite species, leading to intensified competition among these organisms and ultimately resulting in a negative impact on cave fauna (Culver & Pipan, 2019). Thus, it is plausible that the troglobite fauna in caves of the Atlantic rainforest, which boasts high primary productivity, remains underrepresented in existing studies that focus on this region.

Our own research has revealed that prior studies utilized a variety of collection methodologies, which often led to the collection of fewer organisms compared to the method employed in our study. Our approach is more systematic and detailed, focusing on specific microhabitats by dividing them into distinct sectors and quadrants. This method enhances efficiency in organism collection and facilitates a deeper understanding of the diversity of species present. As such, we strongly recommend utilizing this detailed methodological framework for future investigations into the troglobite fauna of Paraná, as it will likely yield richer data on species diversity and ecological interactions.

Economic Development and Impact Levels

Human activities have intensified globally, increasing threats to biodiversity and ecosystems (Dirzo & Raven, 2003). Tropical karst areas are particularly vulnerable to various impacts, especially mining (Auler & Piló, 2015; Souza-Silva et al., 2017). Our analysis shows that mining is a primary economic activity in the metropolitan region of Curitiba, with at least 56 companies extracting these resources. Mining can lead to the destruction of underground cavities and significantly impact surrounding vegetation, which in turn can alter environmental conditions inside caves, reducing the influx and quality of external resources entering these caves, directly affecting subterranean ecosystems (Culver & Pipan, 2019; Prous et al., 2015).

The positively associated of troglobite species richness and the level of human impact, highlights the vulnerability of species restricted to cave environments in the region, as they are concentrated in areas heavily impacted by activities such as mining, deforestation, and agriculture factors known to reduce species richness (Beynen & Townsend, 2005; Schiesari et al., 2013). Additionally, of the 32 troglobite species recorded in Paraná, only five have been formally described, underscoring the need for more effective conservation measures and ongoing monitoring to protect this fauna in the face of increasing anthropogenic pressures.

Another concerning factor is that only four caves are located within conservation units: Gruta da Lancinha, Gruta Jesuítas, Gruta Fadas, and Gruta Bacaetava. Although Gruta da Lancinha is within a fully protected area, there is no enforcement to prevent impacts. Thus, establishing a conservation unit, as decreed for Gruta da Lancinha (Decree No. 6538, May 3, 2006), does not guarantee protection without adequate measures to implement and enforce protective actions. Gruta Jesuítas and Gruta Fadas are within Parque Estadual de Campinhos, a well-structured protected area, and Gruta Bacaetava is within Parque Municipal Bacaetava, which also has adequate infrastructure and carries out important environmental education work (Menin et al., 2022). These initiatives help promote the understanding of natural values and engage visitors in conservation and protection activities (Antic et al., 2022).

Given that only four caves fall within conservation units, only 12 of the 32 troglobite species in Paraná caves (about 37.5%) are in protected areas. However, as Gruta da Lancinha is an ineffective conservation unit, only 9 of these species (28.1%) are effectively in areas considered protected. In Brazil, only 11.6% of known caves are located within conservation units (Sugai et al., 2015), and many of these units were established based on geological and archaeological features, often neglecting troglobite species (Souza-Silva et al., 2015). Among regions with a high concentration of troglobites, only the Parque Nacional da Furna Feia in the Apodi region of Rio Grande do Norte was created specifically to protect troglobite fauna (De Araújo et al., 2022).

In light of these challenges, it is important to recognize that future preservation measures will inevitably be influenced by economic and political pressures from the region’s urban and industrial growth. In this context, systematic studies to identify and preserve protected areas are essential to ensure conservation efforts keep pace with economic activities. The importance of these studies for comprehensive faunal characterization in caves is widely recognized globally (White & Culver, 2011; Deharveng & Bedos, 2018).

Based on our results, establishing a conservation unit in the Itaiacoca belt located farther from Curitiba and with fewer mining companies exploiting natural resources would effectively preserve much of the local biodiversity, minimizing impacts from urban development. Additionally, we emphasize that as new technologies and sampling methods become available, leveraging them is essential to expand our knowledge of subterranean biodiversity, overcoming inherent challenges and limitations in researching underground environments.

Final considerations

In the context of such a diverse and threatened biome like the Atlantic rainforest, this study highlights the importance of cave biodiversity in Paraná, emphasizing the richness and diversity of troglobitic species and the historical underestimation of this fauna. The results underscore the urgent need for further research and conservation actions, especially considering environmental pressures from human activities, such as mining. Adequate protection of these caves by creating new conservation units and using efficient sampling methodologies is essential to preserve troglobitic fauna and mitigate anthropogenic impacts on subterranean ecosystems.

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AUTHOR CONTRIBUTIONS

Rodrigo Lopes Ferreira and Marconi Souza Silva were responsible for the conceptualization, methodology, translation, and English corrections. Leandro Mata da Rocha Melo, Rodrigo Lopes Ferreira, and Marconi Souza Silva participated in field activities. Leandro Mata da Rocha Melo prepared the original draft. Leandro Mata da Rocha Melo and Marconi Souza Silva were responsible for the statistical analysis, artwork, reviewing, and editing. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data supporting this study's findings are available on request from the corresponding author. The Editor-in-Chief has waived the required archiving due to privacy or ethical restrictions.

CONFLICTS OF INTEREST

The corresponding author confirms on behalf of all authors that no involvement might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

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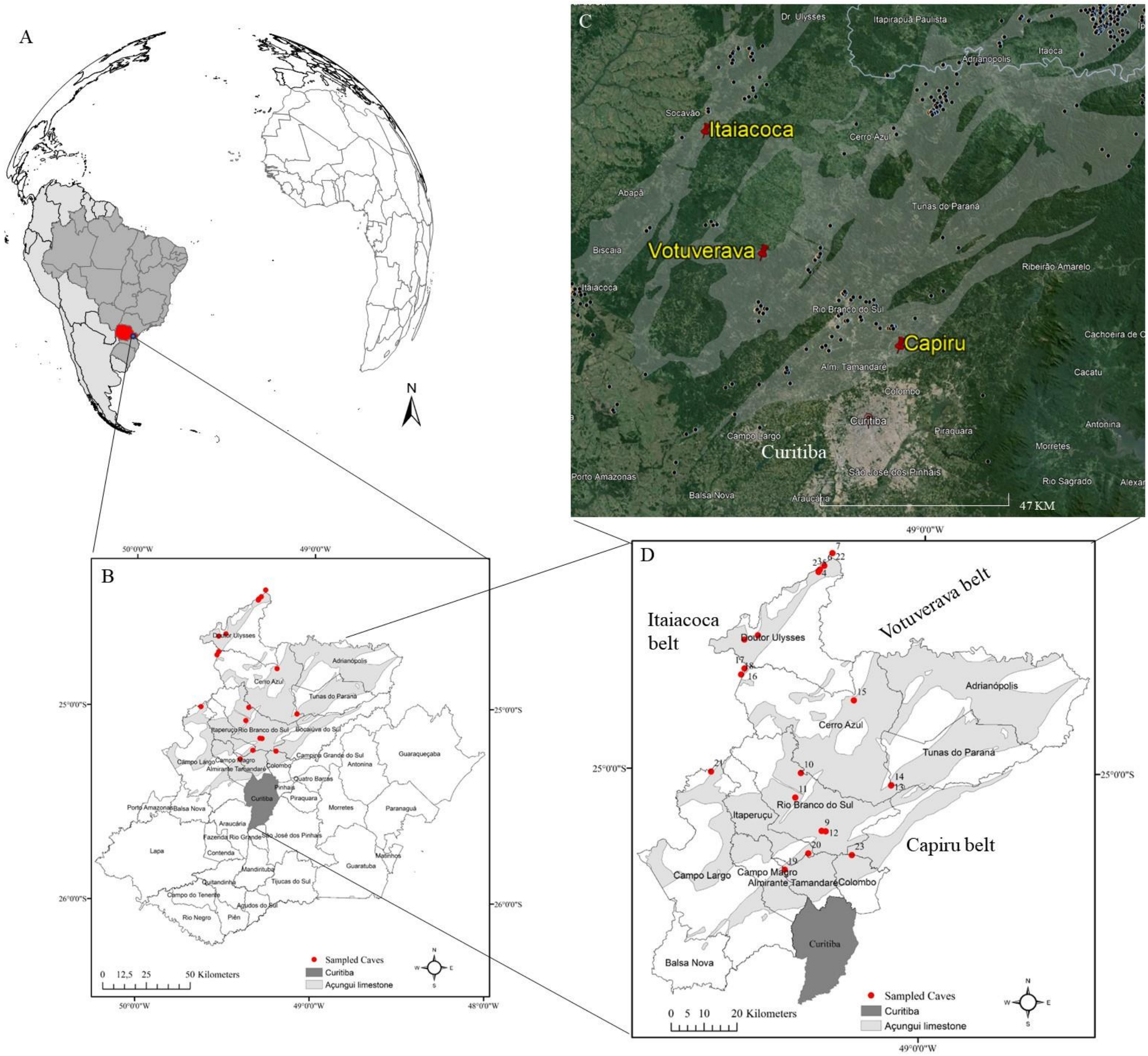

Figure 1. Map of Brazilian territory (A) and the location of municipalities (B and D) that make up the Metropolitan region of Curitiba (capital of the state of Paraná state with 1,773,718 people), https://cidades.ibge.gov.br/brasil/pr/curitiba/panorama. The Itaiacoca, Votuverava, and Capiru limestone belts (D). The red dots are the sampled caves. For more details see Table 1. 1) Varzeão; 2) Arco de Pedra; 3) Casa de Pedra; 4) Ressurgência do Feital; 5) Dá a Volta; 6) Pocinho; 7) Pocinho II; 8) Malfazido; 9) Toquinhas; 10) Piedade; 11) Bromados; 12) Lancinha; 13) Fadas; 14) Jesuítas; 15) Bom Sucesso; 16) Chiquinho; 17) Chiquinho II; 18) Pinheiro Seco; 19) Ermida; 20) Itaperussu; 21) Pinheirinho; 22) Pinhalzinho; 23) Bacaetava.

Figure 2. Some of the troglobitic species collected in Northern Paraná between the years 2022 and 2023 A) *Peridontodesmella* sp1; B) *Crypturodesmus* sp1; C) Pyrgodesmidae sp1; D) Gonyleptidae sp1; E) Gonyleptidae sp2; F) Cryptogeobiidae sp1; G) Cryptogeobiidae sp2; H) Pseudochthonius sp1; I) Prodidomidae sp1; J) *Eukoenenia* sp1; K) *Eukoenenia* sp2; L) *Eukoenenia* sp3; M) Platyarthridae sp1; N) *Trichorhina* sp1; O) Philosciidae sp1; P) *Acherontides* aff. *eleonorae*; Q) *Arrhopalites* sp1; R) *Happia* sp1.

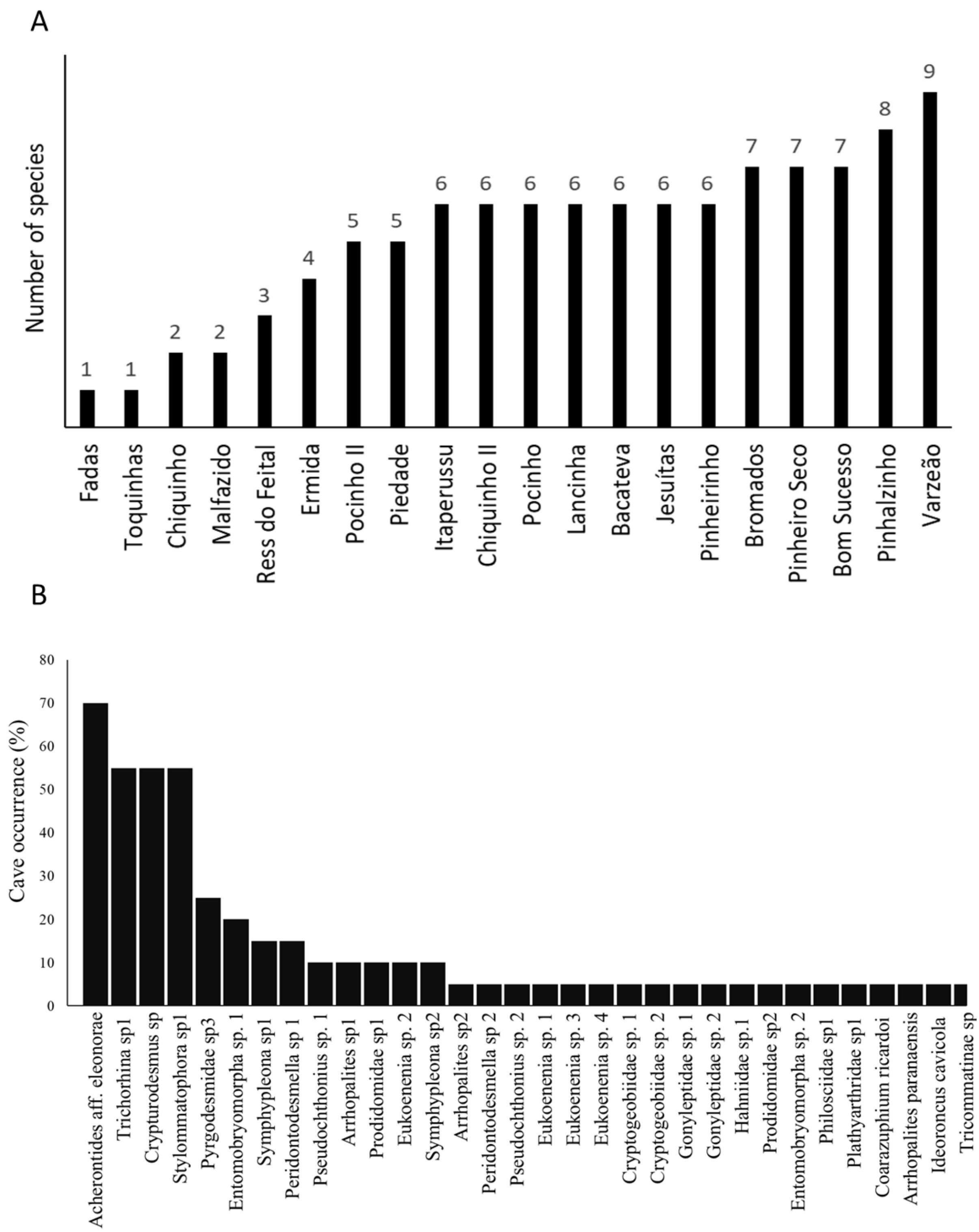

Figure 3. The number of troglobitic species (A) and species occurrence among 20 caves placed at the Curitiba Metropolitan region, Paraná state, Brazil.

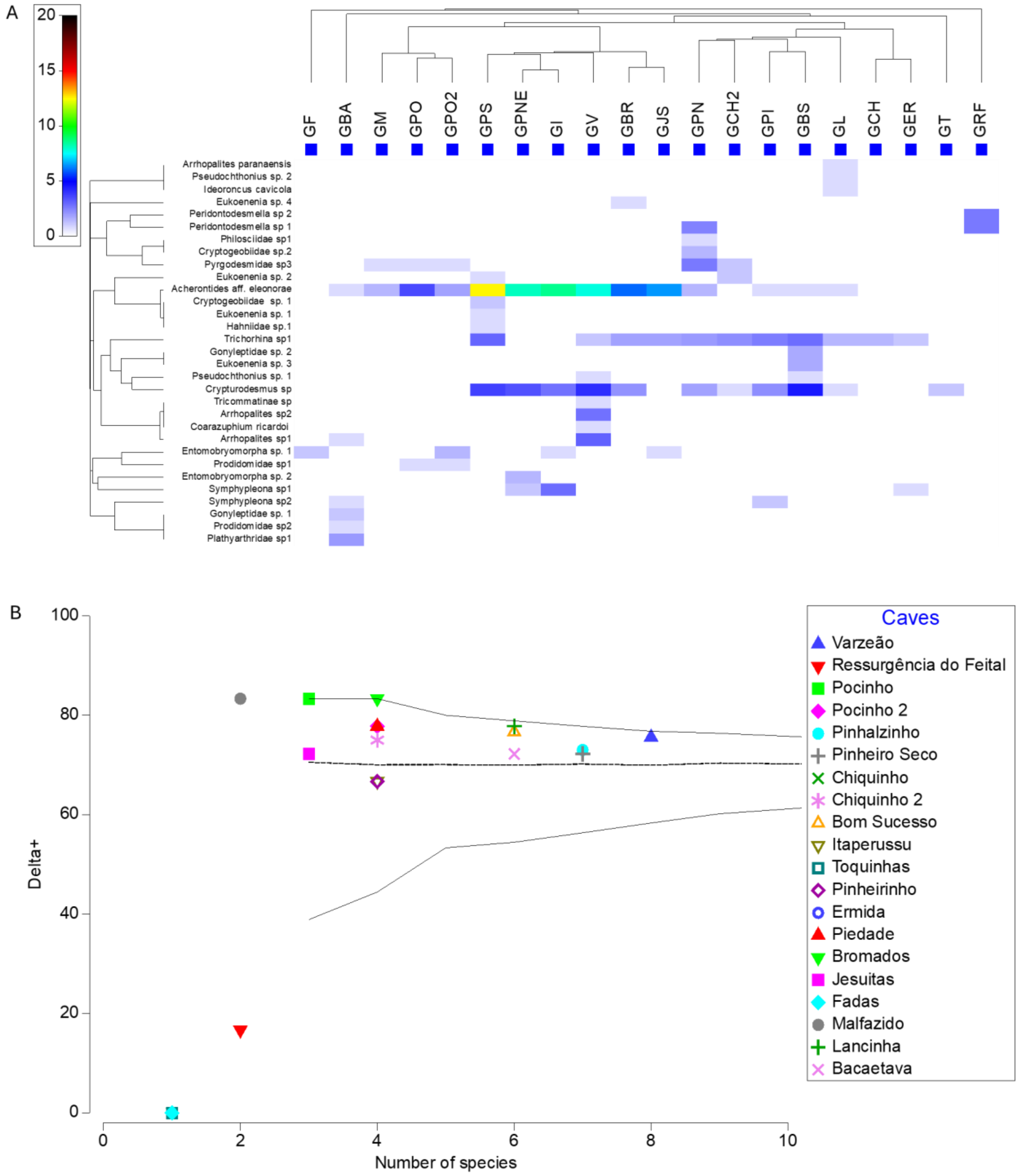

Figure 4. Troglobitic species distribution (A) among caves (up cluster analysis) and index of association among species occurrence inside caves at Paraná State, Brazil (left cluster analysis). Varzeão cave (GVZ), Ressurgência do Feital (GRF), Pocinho (GPO), Pocinho II (GPOII), Pinhalzinho (GPNA), Pinheiro Seco (GPS), Chiquinho (GCH), Chiquinho II (GCHII), Bom Sucesso (GBS), Itaperussu (GIG), Toquinhas (GT), Pinheirinho (GPNE), Ermida (GER), Piedade (GPI), Bromados (GBR), Jesuitas (GJS), Fadas (GF), Malfazido (GM), Lancinha (GL), Bacaetava (GBA). B) Relationship between the number of species and taxonomic distinctness for the sampled caves. Symbols represent different caves.

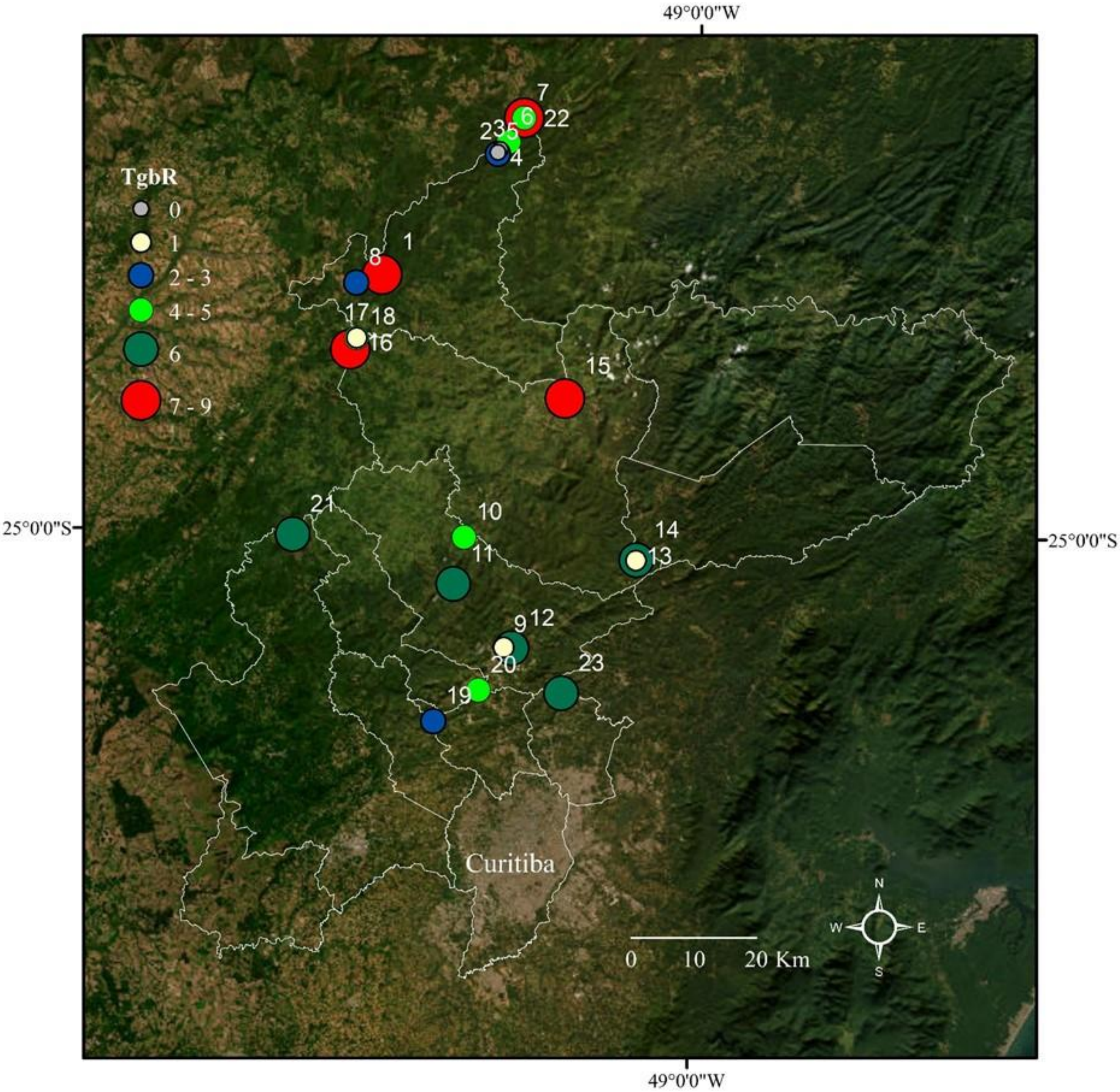

Figure 5. Spatial distribution of some caves and their troglobitic species richness in Curitiba metropolitan region, Paraná State, Brazil. 1) Varzeão; 2) Arco de Pedra; 3) Casa de Pedra; 4) Ressurgência do Feital; 5) Dá a Volta; 6) Pocinho; 7) Pocinho II; 8) Malfazido; 9) Toquinhas; 10) Piedade; 11) Bromados; 12) Lancinha; 13) Fadas; 14) Jesuítas; 15) Bom Sucesso; 16) Chiquinho; 17) Chiquinho II; 18) Pinheiro Seco; 19) Ermida; 20) Itaperussu; 21) Pinheirinho; 22) Pinhalzinho; 23) Bacaetava.

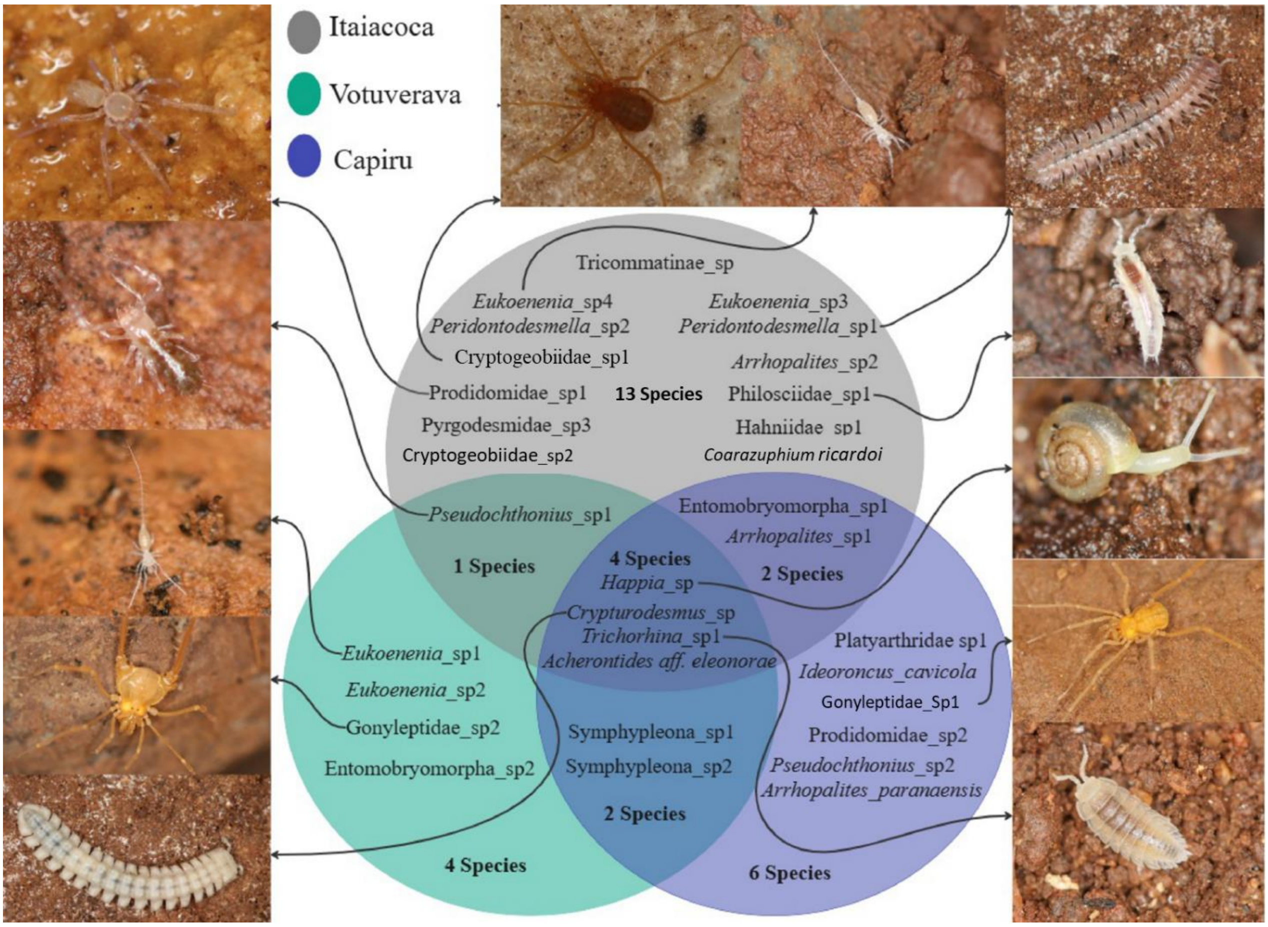

Figure 6. Venn diagram illustrating the distribution of troglobiotic species across the three limestone ranges: Itaiacoca, Votuverava, and Capiru.

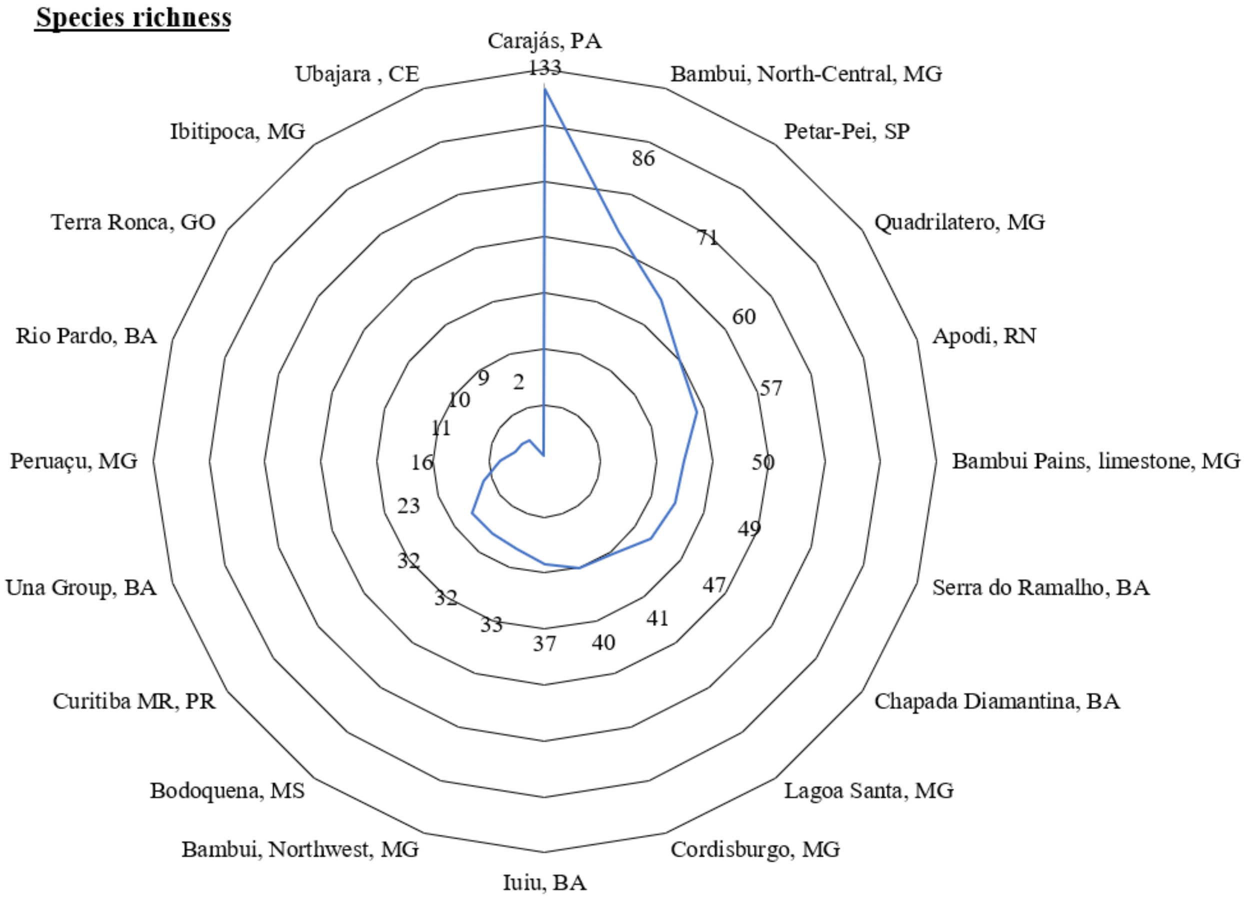

Figure 7. Brazilian areas of great biospeleological relevance. The numbers represent the total species richness. Some regions, such as Chapada Diamantina, have more than one lithology. Quadrilátero ferrífero and Carajás are iron ore and Ibitipoca is quartzite. Açungui represents the Metropolitan region of Curitiba (MR). States name: Pará (PA), Ceará (CE), Minas Gerais (MG), Goias (GO), Bahia (BA), Paraná (PR), São Paulo (SP), Mato grosso do Sul (MS), Rio Grande do Norte (RN).

Figure 8. Human impacts (HI) were observed on the surrounding epigean areas of the caves in the Curitiba metropolitan region, Paraná state, Brazil. Large-scale monoculture of *Eucalyptus* sp. (A), substitution of *Araucária angustifolia* forest (right side in picture B) by *Eucalyptus* sp., forest (left side in picture B), mining activities (C), urbanization (D), Construction and tourism inside a cave (E), Trampling on the cave floor (F), rock fragmentation inside a cave (G) and plastic bottle in the groundwater (H).

Table 1. Sampled caves, municipalities names, and troglobitic species occurrence registered by Pinto-da-Rocha 1995, Sessegolo et al. 2001, 2006 and Bená and Vanin (2014). Species occurrence after our samples in 2022 and 2023.

Caves	County	Occurrence of troglobites in Paraná until 2023	Occurrence of troglobites in Paraná after this study
Bom Sucesso	Cerro Azul	*Acherontides aff. eleonorae*, *Crypturodesmus* sp.*	*Crypturodesmus* sp., *Eukoenenia* sp. 1, *Eukoenenia* sp. 2, Gonyleptidae sp. 1, *Trichorhina* sp. *Pseudochthonius* sp 1.
Pinheiro seco	Castro	*Acherontides aff. eleonorae*.	*Acherontides aff. eleonorae*, *Crypturodesmus* sp., Cryptogeobiidae sp. 1., *Happia* sp., *Eukoenenia* sp. 2, *Trichorhina* sp, Hahniidae sp.
Varzeão	Dr. Ulysses	*Coarazuphium ricardoi*, *Acherontides aff. eleonorae*, *Crypturodesmus* sp., Tricommatinae sp.	*Acherontides aff. eleonorae*, *Crypturodesmus* sp. *Happia* sp. *Trichorhina* sp, *Pseudochtonius* sp1. *Arrhopalites* sp1, *Arrhopalites* sp2.
Ressurgência do Feital	Dr. Ulysses	None	*Peridontodesmella* sp 1., *Peridontodesmella* sp 2.
Pocinho	Dr. Ulysses	None	*Acherontides aff. eleonorae*, Prodidomidae sp 1., *Peridontodesmella* sp 1., *Happia* sp., Pyrgodesmidae sp. 3.
Pocinho II	Dr. Ulysses	None	*Acherontides aff. eleonorae*, Prodidomidae sp 1. Pyrgodesmidae sp. 3. Entomobryomorpha sp. 1
Pinhalzinho	Sénges	None	*Acherontides aff. eleonorae*, *Crypturodesmus* sp., *Peridontodesmella* sp 1., *Happia* sp. *Trichorhina* sp. Pyrgodesmidae sp. 3, Philosciidae sp, Cryptogeobiidae sp. 2.
Chiquinho	Castro	None	*Happia* sp. *Trichorhina* sp.
Chiquinho II	Castro	None	*Crypturodesmus* sp., *Happia* sp., *Eukoenenia* sp 3, *Trichorhina* sp. Pyrgodesmidae sp. 3.
Jesuítas	Cerro Azul	*Acherontides aff. eleonorae*	Entomobryomorpha sp. 1, *Acherontides aff. eleonorae*, Symphypleona sp 2, *Trichorhina* sp.
Fadas	Cerro Azul	None	Entomobryomorpha sp. 1.
Pinheirinho	Campo Largo	None	Entomobryomorpha sp. 2, *Acherontides aff. eleonorae*, Symphypleona sp1. *Happia* sp, *Crypturodesmus* sp.
Itaperussu	Itaperuçu	None	*Acherontides aff. eleonorae*, Symphypleona sp1, *Happia* sp, *Crypturodesmus* sp. Entomobryomorpha sp1.
Piedade	Rio Branco do Sul.	None	*Acherontides aff. eleonorae*, *Trichorhina* sp. *Happia* sp, *Crypturodesmus* sp.
Bromados	Rio Branco do Sul.	None	*Acherontides aff. eleonorae*, *Trichorhina* sp., *Happia* sp, *Crypturodesmus* sp, *Eukoenenia* sp4.
Malfazido	Dr. Ulysses	None	*Acherontides aff. eleonorae*, Pyrgodesmidae sp3.
Lancinha	Rio Branco do Sul.	*Ideoroncus cavicola*, *Arrhopalites paranaensis*	*Acherontides aff. eleonorae*, *Crypturodesmus* sp, *Trichorhina* sp, *Pseudochthonius* sp 2.
Bacaetava	Colombo	*Acherontides aff. eleonorae*	*Acherontides aff. eleonorae*, Symphypleona sp2, *Arrhopalites* sp1, Prodidomidae sp 2. Gonyleptidae sp 2., Plathyarthridae sp1.

Ermida	Almirante Tamandare	None	Symphypleona sp 1., *Trichorhina* sp, Stylommatophora sp.
Toquinhas	Rio Branco do Sul	None	*Crypturodesmus sp*.
Casa de Pedra	Dr. Ulysses	None	None
Dá a Volta	Dr. Ulysses	None	None

Table 2. Limestone belt (Belt), cave names, UTM coordinates (zone 22J), latitude (LAT), longitude (Long), Altitude asl (Alt), distance from urban center (DUC), distance from mining activities (DMA), Population density (PD), Human impacts weight (HI), troglobitic species richness (S), and taxonomic distinctness (TD) of the 20 limestone caves placed at the metropolitan region of Curitiba, Paraná, Brazil. Ressurgência do Feital (Ress. do Feit.), Bom Sucesso (B. Sucesso), Pinheiro Seco (P. Seco). *Presence of an allochthonous stream, **Presence of a travertine dam.

Belt	**Cave names**	**Lat**	**Long**	**Alt (m)**	**DUC (km)**	**DMA (Km)**	**PD**	**HI**	**S**	**TD**
Capiru	*Bacaetava[5,14,18,19,22,24,25]	7208130	680519	951	6	0.51	249.277	45	6	72.22
	*Ermida[6,14,15,18,23]	7203682	660082	950	7	0.11	121.42	16	3	83.33
	Fadas[22,23]	7229326	692470	915	10	2.5	17.884	10	1	0
	*Itaperussu[6,14,15,18,22,23,24]	7208651	667277	1006	1.6	0.77	29.493	35	5	66.67
	*Jesuitas[5,14,15,22,23,25]	7229413	692528	900	7	2.48	17.884	28	6	72.22
	*Lancinha[6,14,15,22,23]	7215358	672566	890	4	1.35	32.635	21	4	77.78
Itaiacoca	Arco de Pedra[6]	7294581	670419	890	16	4.9	5.525	1	0	0
	Casa de Pedra [15,23,26]	7294947	670668	900	17	4.5	5.525	18	0	0
	Chiquinho[6,7,22]	7264965	647839	790	20	11.31	72.125	5	2	0
	Chiquinho II[6,7,22]	7264954	647825	790	21	11.31	72.125	5	5	75
	Da a Volta [6,7,14,15,22,26]	7294581	670419	890	16	4.9	5.525	1	0	0
	*Malfazido[7,14,23]	7273761	647782	970	15	4.5	5.525	10	2	83.33
	**Pinhalzinho[6,14,15,22,23,25]	7300073	674618	950	16	2.32	19.441	18	7	68.89
	P. Seco[2,5,6,7,14,15,22,23,25,26]	7263142	646809	766	25	11.96	72.125	55	7	76.67
	*Pocinho[6,7,14,22,23,25]	7296230	672123	927	20	8.46	5.525	16	5	83.33
	Pocinho II[23,27]	7300073	674618	925	21	8.5	5.525	8	4	77.78
	*Ress.do Feit[6]	7294326	670403	850	23	7.8	5.525	1	2	16.67
	*Varzeão[14,15,22,23,25]	7275042	651909	820	12	8.21	5.525	18	9	75.60
Votuverava	B. Sucesso[6,7,14,15,22,26]	7255243	681136	350	5	6.76	17.884	16	7	76.67
	Bromados[14,15,22]	7225641	663298	818	15	3.2	32.635	13	6	83.33
	Piedade[15]	7233100	664985	520	20	7.25	32.635	3	4	77.78
	*Pinheirinho[5,6,15,22]	7233572	637641	517	40	25.12	135.678	17	6	66.67
	*Toquinhas[7,14,15,22,23]	7215482	671322	929	1.5	1.92	32.635	21	1	0
	Average			826.7	16.5	6.3		18	4.6	62
Standard	deviation			173.2	8.9	5.9		14	2.2	30.1

List of impacts on the caves and surrounding areas (superscript numbers): 2 changes due to detonation inside the cave, 5 constructions inside the cave, 6 deforestation, 7 agropastoral activities, 8 domestic sewage, 14 garbage, 15 graffiti, 18 mining activities nearby, 19 highway near the caves, 22 trampling, 23 vandalism in speleothems, 24 traffic vibrations road or explosions near the cave, 25tourism, 26 religious activities.

Table 3. National relevance in troglobite species richness in some areas of Brazil. Metropolitan region (MR). Lithology (Lito), Richness (troglobite richness), S/N Relative species richness, number of sampled caves (**n),** UTM coordinates, altitude (Alt). Places with caves considered hotspots of subterranean biodiversity (#), according to Culver and Sket 2000. Ferruginous rocks (Fe), Carbonates (Ca), and quartzite (Qua).

Speleological area name	Lito	Richness (S)	caves (n)	S/N	UTM E	UTM S	Zone	Alt (asl)	References
Carajás, PA	Fe	133	473	0,28	521442	9327413	22M	350	Trevellin, et.al 2019
Bambuí, North-Central, MG	Ca	86	48	1,79	589016	8280046	23 L	800	Rabelo et al. 2018
Quadrilátero Ferrífero, MG	Fe	60	17	3,53	607918	7765858	23K	1000	Souza-Silva et al. 2015
Apodi, RN	Ca	57	40	1,43	691611	9414023	23L	120	Bento et al 2021
Bambuí Pains, MG	Ca	50	300	0,17	430933	7747045	23 K	800	Ferreira et al 2022
#Serra do Ramalho, BA	Ca	41	7	7	633258	8418161	23L	500	Ferreira and Souza-Silva, 2023
Lagoa Santa, MG	Ca	41	80	0,51	614855	7827462	23K	800	Ferreira et al 2020
Cordisburgo, MG	Ca	40	15	2,67	571372	7885494	23K	800	Souza et al. 2021
Iuiú, BA	Ca	37	18	2,06	650604	8399855	23 L	800	Cardoso et al 2021
Bambuí, Northwest, MG	Ca	33	55	0,60	304122	8125007	23 K	650	Simões et al. 2015
Bodoquena, MS	Ca	32	84	0,38	429364	7899377	21K	450	Cordeiro et al., 2014
#Petar-Pei, SP	Ca	71	15	3.26	734786	7282715	22J	200	Souza-Silva and Ferreira 2016
*Curitiba MR, PR	Ca	32	28	1,39	682078	7230950	22J	900	This study 2023; Pinto-Da-Rocha 1995
#Una Group, BA	Ca	22	2	11,00	292401	8837813	24L	700	Souza-Silva and Ferreira 2016
Chapada Diamantina, BA	Ca	18	18	1,00	240080	8610663	24L	600	Souza-Silva et al. 2015
Peruaçu, MG	Ca	16	10	1,60	614944	8368379	23 L	730	Souza-Silva et al. 2015
Rio Pardo, BA	Ca	11	6	1,83	420374	8296354	24L	270	Souza-Silva et al. 2015
Terra Ronca, GO	Ca	10	12	0,83	355312	8500028	23L	700	Bichuette et al. 2019
Ibitipoca, MG	Qua	9	23	0,39	615534	7599638	23 K	1500	Souza-Silva et al. 2020
Ubajara, CE	Ca	2	3	0,67	286854	9578741	24M	600	Souza-Silva et al. 2015

Notes: Pará state (PA), Minas Gerais state (MG), Rio Grande do Norte state (RN), Bahia state (BA), Mato Grosso do Sul state, MS, São Paulo state (SP), Paraná state (PR), Goiás state (GO), Ceará (CE).

Article 3:

Identifying and Prioritizing the Cave Fauna Conservation in a Densely Populated and Exploited Subtropical Karst Area

Article to be submitted to the journal Anthropocene

Leandro Mata da Rocha Melo[1,2], Rodrigo Lopes Ferreira[1,2], Marconi Souza Silva[1,2],

1 - Centro de Estudos em Biologia Subterrânea, Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, Campus Universitário, P.O. Box 3037, Lavras CEP 37200-000, Minas Gerais, Brasil.
2 - Programa de Pós-Graduação em Ecologia Aplicada, Universidade Federal de Lavras, Lavras CEP 37200-000, Minas Gerais, Brasil. Corresponding author: marconisilva@ufla.br

Abstract – Environmental stability, limited food resources, and specialized fauna make cave ecosystems vulnerable to impacts and biodiversity loss. Understanding the impacts affecting caves, along with a detailed analysis of faunal composition and species richness, plays a role in formulating effective conservation measures. To identify and prioritize conservation needs, we categorized cave habitats in the Atlantic Rainforest within metropolitan regions highly threatened by human activities. We assessed conservation priorities, considering both human impacts and the biological richness of invertebrates. We tested the hypothesis that fauna suffers a high degree of impact related to population density near the caves and human activities in their surroundings and interiors. The caves showed a total of 357 species, including 28 troglobitic species, across 23 sampled caves. The main human alterations observed were deforestation, destruction of speleothems, trampling, littering, and graffiti. Results reveal that 39.1% of the caves were classified as an extreme priority for conservation actions, 47.8% as high priority, 8.7% as medium priority, and 4.3% as low conservation priority. The high degree of landscape alteration and the distribution of caves across three different limestone belts can impose challenges in establishing continuous conservation units; thus, the primary strategy may be to preserve significant areas surrounding the caves and invest heavily in environmental education practices. This crucial information should raise awareness and encourage effective measures to conserve these critical environments, safeguarding their diverse ecosystems and delicate ecological balance.

Keywords: Subterranean habitats, cave fauna, ecological index, Impacts, diversity**.**

Introduction

Caves are environments of significant geological, archaeological, paleontological, and ecological value, attracting the attention of scientists, spelunkers, and tourists worldwide (Cigna et al., 2013; Piano et al., 2022). However, preserving these ecosystems faces significant challenges due to threats ranging from human impacts to climate change (Mammola et al. 2019, Osborne 2019, Land and Peters 2023, Mammola et al. 2024, Knüsel et al. 2024). Cave environments generally feature high environmental stability and a permanent absence of light in regions far from the surface (Howarth 1983), leading to the absence of photosynthesizing organisms; thus, these ecosystems lack primary production by light and are therefore considered oligotrophic (Culver & Pipan 2009). They largely depend on the import of resources from the external environment and the movement of animals to import organic food resources (Culver & Pipan 2009). Consequently, changes in the external environment can cause significant impacts on the cave ecosystems' budgets and the organisms that inhabit them (Ferreira & Horta 2001; Souza-Silva et al. 2015; Cardoso et al. 2022).

Cave environments harbor a diversity of organisms that exhibit distinct ecological and evolutionary relationships, usually categorized as trogloxens once they use caves as shelters; troglophiles are facultative and can have an entire life cycle inside but have populations outside of caves. The most specialized are troglobites, restricted to living in subterranean environments, exhibiting morphological and physiological specializations that allow them to live exclusively in these locations (Sket 2008).

According to the diversity of ecological and evolutionary categories inside a cave, the degree of environmental services in both hypogean and epigean ecosystems can be high (Culver & Pipan 2014). Trogloxens such as bats and birds contribute to pollination, seed dispersal, and pest consumption outside, using caves as essential places for shelter and depositing guano for cave fauna (Kunz et al. 2011; Medellin et al. 2017; Leal and Bernard 2021; Canedoli et al. 2022, Tuneu-Corral et al. 2024). Besides that, caves may serve as a refuge for epigean fauna when external conditions undergo significant environmental variations (Cardoso et al. 2022; Fraga et al. 2023). Moreover, caves also perform other vital functions, such as maintaining the hydrological balance of the region and potentially acting as recharge sites for underground drainage systems (Griebler and Avramov 2015, Elliott, 2000; Boulton et al. 2003; Canedoli et al. 2022).

The uniqueness of subterranean environments creates extreme conditions that favor the colonization by microorganisms (Barton and Jurado 2007; Turrini et al. 2020). These organisms produce unique secondary metabolites to adapt. These substances may have broad biotechnological value, exhibiting antimicrobial, antifungal, antiviral, and anticancer properties (Turrini et al. 2020; Zada et al. 2021). This diversity of potential benefits highlights the value of these compounds for applications in various fields, such as agriculture, medicine, and food industries (Sadoway et al. 2013; Abdelghani et al. 2021). Thus, the interconnection between subterranean ecosystems and the biotechnological applications of microorganisms emphasizes the global importance of cave preservation in promoting biodiversity and contributing to sustainable development (Martin-Pozas et al. 2022).

Karst systems worldwide face increasing challenges due to rising human activities in surrounding areas and the growth of tourism in subterranean environments (Alexander 2021; Chiarini et al. 2022, Ferreira et al. 2022, Boulton et al. 2003). This reality demands urgent assessment and monitoring of the impacts on these ecosystems to avoid greater damage to the speleological heritage. To this end, various impact assessment indices have already been developed and applied in karst regions and caves (Calo and Parise 2006; Van-Beynen and Townsend 2005; Biswas 2010; Van-Beynen et al. 2012; Donato et al. 2014; Simões et al. 2014; Souza-Silva et al. 2015), providing valuable results that contribute to the conservation and restoration of threatened areas. However, most conservation efforts in subterranean biology lack a rigorous quantitative approach, which leads to a scarcity of evidence supporting the effectiveness of interventions in this field (Mammola et al. 2022).

Thus, this work aims to identify and prioritize the conservation of caves in a densely populated and exploited region in the Brazilian Atlantic Rainforest through the analysis of the impacts and biological relevance of the caves. To this end, we formulated the following hypothesis: a large number of caves will present extreme and high conservation priority due to their location in the metropolitan region of Curitiba, a densely populated area, with a high frequency of impacts associated with human alterations around the caves, including disturbances such as deforestation, waste deposition, and other landscape changes. Furthermore, these caves are situated in a global biodiversity hotspot (the Atlantic Forest), which is home to a high diversity of epigean species, and we expect this diversity to also be reflected in the hypogean environments, resulting in a rich troglobitic and non-troglobitic fauna within the cavities.

Methodology

Study Area

The Brazilian Atlantic Rainforest is known for hosting high endemism and diversity of organisms; however, it is also one of the most degraded and endangered biomes worldwide

(Lima et al. 2020; Diniz et al. 2022). The study encompassed an analysis of 23 limestone caves located in the Metropolitan region of Curitiba, state of Paraná (Figure 1), distributed among the municipalities of Colombo, Sengés, Castro, Campo Largo, Rio Branco do Sul, Almirante Tamandaré, Itaperuçu, Cerro Azul, Adrianópolis, and Doutor Ulysses.

The metropolitan region of Curitiba has an estimated population of 3,697,928 people in 2024, with all municipalities showing population growth compared to the 2022 census data, according to the Brazilian Institute of Geography and Statistics (IBGE, 2024). The main economic activities in the region include agriculture, the automotive industry, commerce, and the extraction of lime and limestone (Kmiecik et al. 2017).

These caves are situated in metasedimentary rocks belonging to the Açungui Carbonate Group, distributed across three limestone belts. The limestone belts contain formations named Itaiacoca, Votuverava, and Capiru, being adopted according to the proposition by Sessegolo et al. (2006). The carbonate rocks of the Açungui Group are organized into three geographical belts designated as Northwest Belt, Central Belt, and Southeast Belt (Passos 1986). In the context of the research, it is highlighted that the Central Belt houses the Votuverava Formation, covering an area of 201 km²; in the Southeast Belt, the Capiru Formation is found, extending over 291 km²; and finally, in the Northwest Belt, we have the Itaiacoca formation, with an area of 123 km² (Passos 1986). The study area exhibits extensively modified landscapes due to the plantation of Eucalyptus sp., Pinus sp., and limestone mining activities. and is located within the Atlantic Forest Biome.

The "Cave Conservation Priority Index (CCPi)"

To evaluate caves and priority areas (limestones belts) for conservation, we adapted the "Cave Conservation Priority Index (CCPi)" proposed by Souza-Silva and collaborators (2015). The CCPi assesses caves' conservation priority based on their degree of vulnerability. The vulnerability for each cave is obtained through the overlap of biological relevance (BR), which represents taxonomic uniqueness, biodiversity, and potential for interactions, and the degree of human impact (HI), which represents the threats from anthropogenic impacts (Souza-Silva et al. 2015).

Cave physical attributes and human impacts

Most of the caves in this study were mapped (Sessegolo et al., 2006) using a standardized mapping methodology with a British Cave Research Association (BCRA) – 4C survey grade. The altitude above sea level and geographic position of the caves were obtained with a Global Positioning System (GPS) in decimal degrees.

Total linear development (the size of the cave), sampled linear development (sum of the length of sampled conducts), and dimensions of cave entrances (highest vertical dimension and widest horizontal dimension) were determined in loco with the aid of a laser tape measure or taken from topographic maps of the cavities. In loco observations during field trips were made to assess, human uses and modifications within and around the caves. Furthermore, satellite images were analyzed to observe and confirm some large-scale modifications around caves (Deforestation, agriculture, dams, conurbation, roads, dolines, etc.).

Sampling invertebrates

In all sampled caves, it was conducted the active sampling of invertebrates, using tweezers and brushes in sectors (3x10m) and quadrants (1x1), in addition to general collections among the areas (Souza-Silva et al. 2021). Sampled invertebrates were stored in vials containing 70% alcohol for subsequent sorting and separation of morphotypes (Souza-Silva et al. 2021), carried out at the Center of studies on subterranean Biology (CEBS), Federal University of Lavras.

The identification of potential "troglobionts" species was conducted by identifying troglomorphisms, which indicate isolation and evolution in the cave environment (Christiansen 2012). The troglomorphisms frequently observed included the reduction or absence of ocular structures and pigmentation and the elongation of sensory and locomotor appendages (Culver and Pipan 2019). We have sent some specimens to taxonomists for assistance in confirming the potential troglobites (please see the acknowledgments section).

The terms "stygobionts" and "troglobionts" are used to describe species that live in caves, as well as in shallow subterranean and above-ground environments. Many of these subterranean species have specific adaptations known as troglomorphy. These adaptations can include reduced eyes and pigment, increased size, elongated appendages, and additional sensory structures (Culver and Pipan, 2019). However, some troglobionts may not show significant troglomorphy due to factors such as the size of their habitat, exposure to light, genetic variability, and other reasons. This variability suggests that some species may be classified as "troglophiles" (Deharveng et al. 2024). Consequently, certain cases are considered potential troglobionts for the sake of terminological consistency, pending further detailed studies to clarify their categorization.

All sampled specimens of invertebrates were deposited in the subterranean Invertebrates Collection of Lavras (ISLA), linked to the Center of studies on Biology subterranean (CEBS) at the Federal University of Lavras, Minas Gerais, Brazil (https://www.biologiasubterranea.com.br/en).

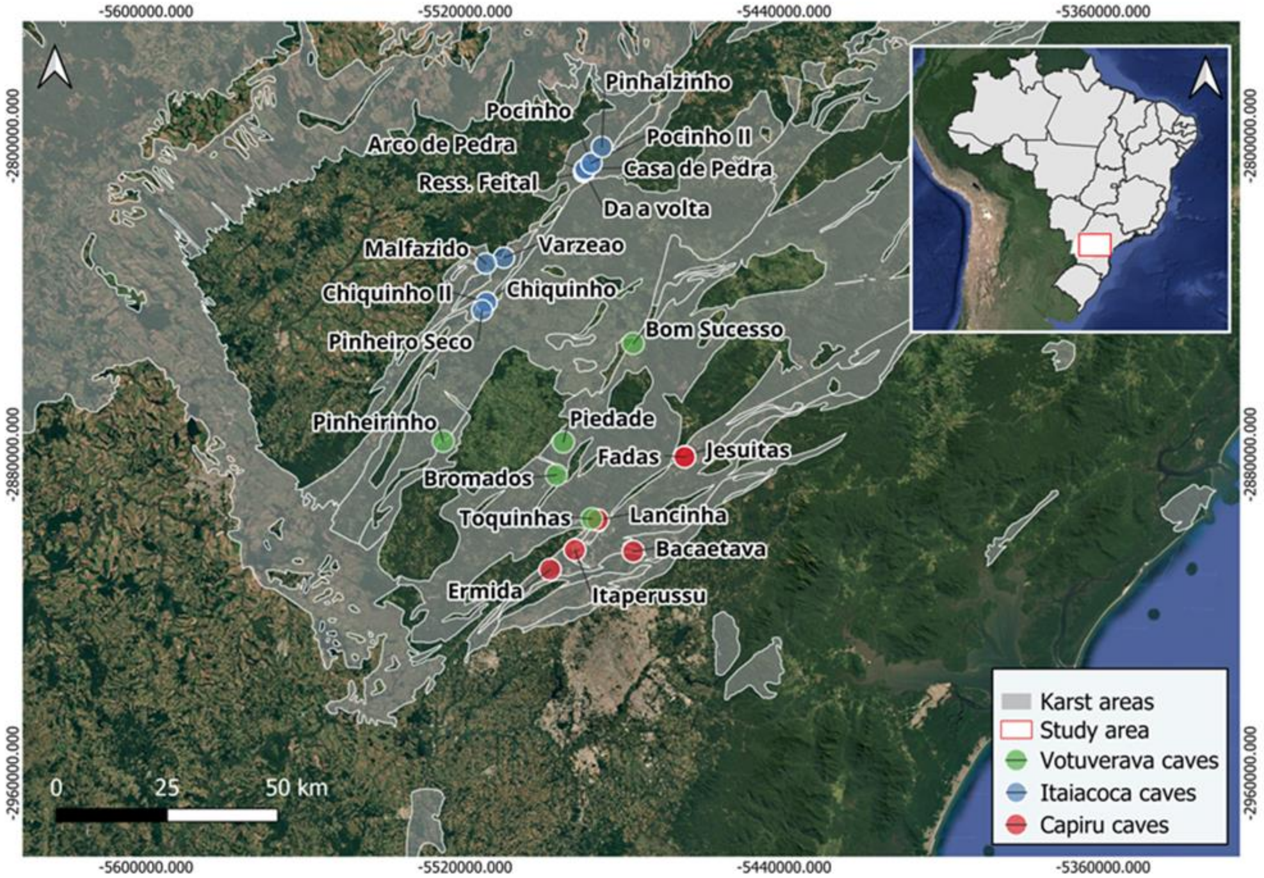

Figure 1. Location of the study area at different scales in Brazilian Atlantic Rainforest. The blue circles represent the cavities located in the Itaiacoca limestone belt; the green circles represent the caves in the Capiru belt, and the red circles represent the caves in the Votuverava belt.

Biological Relevance of the Caves

The analysis of biological relevance was determined through the overlap of three variables: richness of troglobitic species, non-troglobitic species richness, and relative non-troglobitic species richness. Due to the fragility of troglobitic species, these were evaluated separately, creating four categories based on the cave with the highest number of troglobites (9 spp./4): Extremely high (≥7), high (5-6 spp.), average (3-4 spp), and low (≤2). The following weights were assigned to each category: Extremely high (4), high (3), average (2), and low (1). The highest richness value of the non-troglobitic species richness found in each cave was also divided by four: 97spp./4; thus, the caves were divided into categories: Extremely high (≥74 spp.), high (50-73 spp.), Average (26-49 spp.), and low (≤25 spp.). Weights were given to each category to establish the degree of relevance of each cave. Each category received a weight: Extremely high (8), high (6), average (4), and low (2). The species richness categories of the non-troglobitic invertebrates receive double the weight due to their higher richness inside the caves, which maintain greater ecological interaction potential, supporting more resilient and balanced ecosystems (Souza-Silva et al. 2011).

Relative species richness was the number of species in the cave's extension sampled about the entrance superficial area (Souza-Silva et al. 2011). Relative species richness: Rtfr = [(Ttfr/ca)/Rcea], where Rtfr = relative species richness, ca = cave area, and cea = superficial area of the cave entrance (Souza-Silva et al. 2011). Therefore, this variable seeks to reduce the excessive contribution of para-epigean communities by considering the extent of the cave entrance in the analysis (Prous et al 2015). Thus, the highest relative richness found was used to create four categories (1.73/4): Extremely high (≥1.73 spp.), high (1.3-1.7 spp.), average (0.6-1.2 spp.), and low (≤0.5 spp.). Each category received the following weights: Extremely high (4), high (3), average (2), and low (1). Therefore, biological relevance was considered through the sum of the weights of troglobitic richness, total non-troglobitic species richness, and relative species richness. The biological relevance of each cavity was determined through the cave with the highest sum, divided into four categories (13/4): Extremely high (≥10), high (9-7), average (6-4), and low (≤3). Each category received a weight: Extremely high (4), high (3), average (2), and low (1).

Degree of Human Impact

The degree of impact was considered based on potential modifications that may lead to the depletion, enrichment, or alterations of the microhabitats and organic resources of the caves. Depletion includes the reduction of organic resources or biological diversity due to human activities in the cave. Enrichment is promoted by human activities that increase the amount of organic resources, and finally, alterations refer to impacts that modify the physical structure of the cave without causing trophic enrichment or depletion. Each impact was considered based on modifications both inside and outside of the caves, divided by their potential for modification, the spatial extent within the cave, and the duration of the impact within the cavity, receiving weights of 1, 2, or 3. Intense potential refers to modifications causing significant disturbance to the fauna and physical structure of the cave (weight 2). Low potential refers to modifications causing minor disturbances to the fauna and physical structure (weight 1). Short spatial extent encompasses modifications of potentially low spatial amplitude, affecting the physical structure and fauna locally (weight 1), compared to broad spatial modifications (weight 2), and finally, duration refers to the length of time that the impact will remain in the cavern environment, categorized as occasional duration impacts (weight 1) and constant duration impacts (weight 3). The caves were classified based on the sum of their impacts. The highest value found (55) served as the basis for categorizing the degree of impact weights of the cavities: Extremely high (≥28), high (20-27), average (14-20), and low (≤13). Each category received the following weights: Extremely high (4), high (3), average (2), and low (1). As Souza-Silva et al. (2015) proposed, deforestation activities always received weight 1 due to the difficulty in understanding the true impact of these activities on the subterranean environment.

Cave Priority for Conservation

The conservation priority for caves was determined based on the sum of the weights of biological relevance and human impacts. Each of these categories received the following weights: Extremely high (4), high (3), average (2), and low (1). The sum of the weights was calculated and the cave with the highest weight (8) was used to establish the categories of the CCPi: Extremely high (≥6), high (4-5), average (3), and low (≤2).

Results

Caves biodiversity

Overall, 357 species were found, which belong to 55 orders and at least 136 families. Of these species, 28 were considered troglobitic, which were distributed in 11 orders and at least 16 families. In which it is possible to observe some of their troglomorphic traits. Regarding the degree of endemism, the troglobitic species that presented the widest distribution was found in 14 caves, while the second most wide-ranging species occurred in 12 caves. Two species occurred in 11 caves, one specie in five caves, one in four caves, one in three caves, five species occurred in two caves, and finally, sixteen species found in only one cave. The highest total richness (Tr) recorded was 97 species observed in the Varzeão Cave, while the lowest total richness recorded was 23 species for Pocinho II cave. The highest richness of troglobiotic species (TrR) was 9 species recorded for Varzeão cave (Doutor Ulysses municipality). There was no record of troglobitic species in 3 caves (13%) of the sampled caves. However, the average number of troglobites per cave was 4.

Human Impacts (HI) and biological relevance (BR) of the caves

In the 23 caves investigated, 14 distinct impacts were recorded. Among them, the most frequently observed were trampling (78.2%), trash (65.2%), graffiti (65.2%), pasture cultivation (60.8%), and vandalism on speleothems (60.8%) (Figures 2 and 3). Human impact was extremely high for four caves (17.4%), high for 2 (8.7%), average for 7 (30.4%), and low for 10 (43.5%) (Figure 4).

Four caves (17.4%) showed an extremely high richness of troglobitic species (Tg/bR), while 10 (43.5%) showed high richness, four (17.4%) average richness, and 5 (21.7%) low richness (Figure 4). For the total species richness (TtfR), two caves (8.7%) were categorized as extremely high, 11 (47.8%) as high, 8 (34.8%) as average, and 2 (8.7%) as low. Regarding the relative species richness, one cave (4.3%) was considered highly relevant, while 22 (95.7%) were classified as low relevance. Biological relevance proved extremely high for eight caves, representing 34.8%. For the other 11 caves (47.8%), biological relevance was classified as high, while in four caves (17.4%), the biological relevance was average (Figure 4). It is important to highlight that no cave was categorized as having low relevance, indicating a tendency toward high relevance in this specific metric for the region.

Identifying and prioritizing the conservation of caves

Table 1 presents each cave's classification in the Cave Conservation Priority Index (CCPi) categories and the final conservation category according to the CCPi index. Nine caves (39.1%) were classified as extremely relevant, 11 (47.8%) as high, two (8.7%) as average, and one (4.3%) as low (Figure 4).

Figure 2: Human alterations inside the sampled caves. Construction of a religious altar in the Pinheiro Seco cave (A). Graffiti in the Itaperussu cave (B and D), Broken speleothems in the Pocinho cave (C), Broken speleothems in the Bom Sucesso cave (E), Presence of waste in the Itaperussu cave (F).

Figure 3: Human impacts outside the sampled caves. Construction near the caves (A), deforestation and monocultures near the caves (B), Deforestation and road around the Pocinho cave (C), Deforestation near the Pinheirinho cave (D), Mining activities near Itaperussu and Ermida caves (E and F).

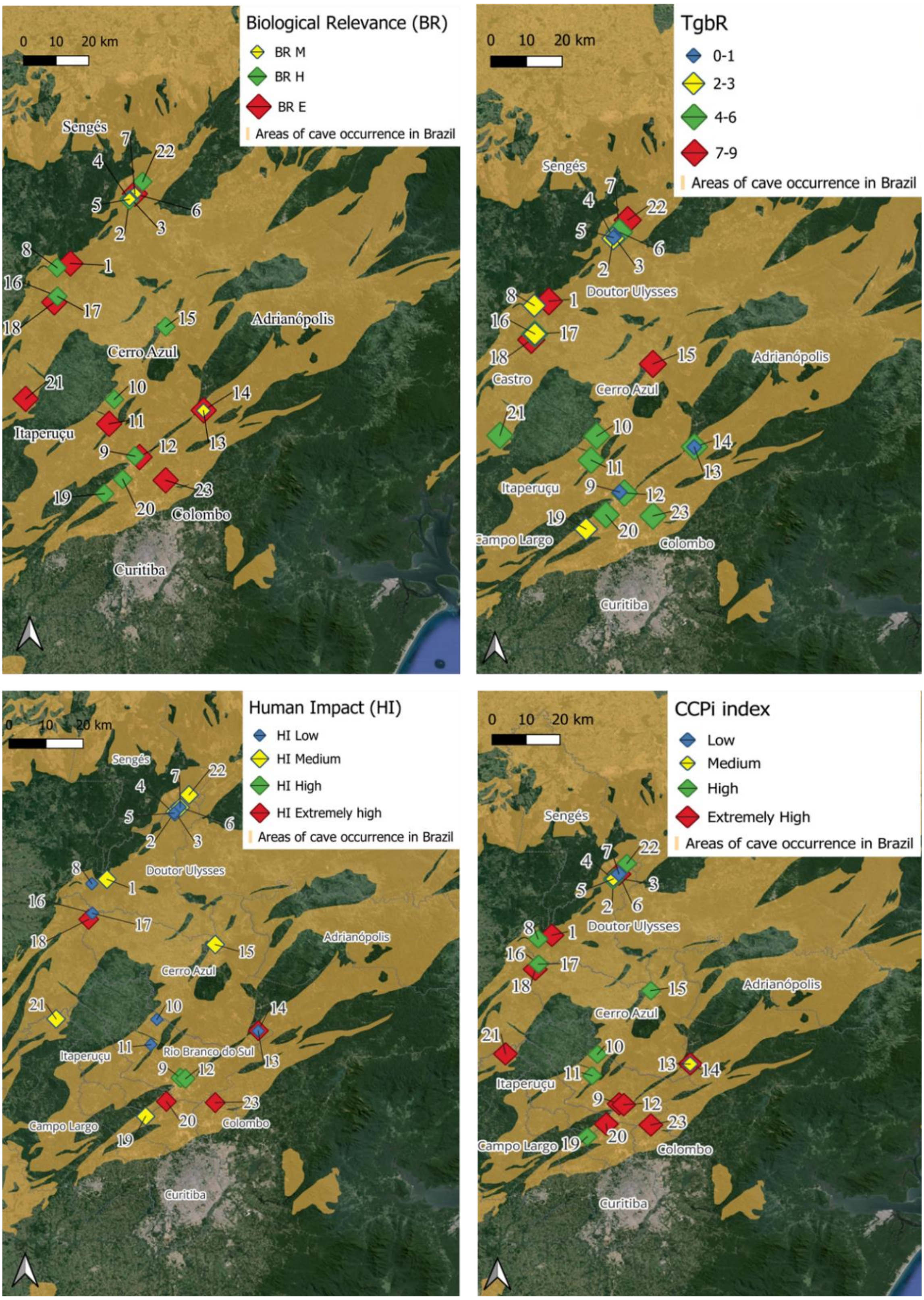

Figure 4: Distribution of the Biological relevance, Richness of troglobitic species, the degree of human impacts, and the final results of the cave conservation priority index (CCPi). Cave manes: 1) Varzeão; 2) Arco de Pedra; 3) Casa de Pedra; 4) Ressurgência do Feital; 5) Dá a Volta; 6) Pocinho; 7) Pocinho 2; 8) Malfazido; 9) Toquinhas; 10) Piedade; 11) Bromados; 12) Lancinha; 13) Fadas; 14) Jesuítas; 15) Bom Sucesso; 16) Chiquinho; 17) Chiquinho 2; 18) Pinheiro Seco; 19) Ermida; 20) Itaperussu; 21) Pinheirinho; 22) Pinhalzinho; 23) Bacaetava.

Table 1. Categorization of caves according to the cave conservation priority index (CCPi). Rio Branco (RB), Almirante (A.). ∑HI: Sum of human impacts per cave. Degree of human impacts (HI), number of troglobitic species (TgbR), Number of non-troglobitic species (TtfR), relative number of non-troglobitic species (RTtfR). Priorities for conservation actions(E) extremely high; (H) high; (A) average; (L) low.

Cave names impact	Municipality	Lat	Long	∑HI	HI	TgbR	Ttf R	RtfR	CCPi
Varzeão[14,15,22,23,25]	Dr, Ulysses	7275042	651909	18	A	9	97	0.0009	E
Arco de Pedra[6]		7294581	670419	1	L	0	54	0.002	H
Casa de Pedra[15,23,26]		7294947	670668	18	A	0	44	0.007	H
Ressurgência do Feital[6]		7294326	670403	1	L	2	52	0.002	H
Da a Volta[6,7,14,15,22,26]		7294572	670427	1	L	0	28	0.008	A
Pocinho[6,7,14,22,23,25]		7296230	672123	16	A	5	61	0.04	E
Pocinho dois[23,27]		7296306	672130	8	L	4	23	0.0039	L
Malfazido[7,14,23]		7273761	647782	10	L	2	70	0.0049	H
Toquinhas[7,14,15,22,23]	RB do Sul	7215636	671190	21	H	1	36	1.73	E
Piedade[15]		7233100	664985	3	L	4	35	0.004	H
Bromados[14,15,22]		7225641	663298	13	L	6	85	0.013	H
Lancinha[6,14,15,22,23]		7215358	672566	21	H	6	51	0.0023	E
Fadas[22,23]	Cerro Azul	7229326	692470	10	L	1	18	0.01	A
Jesuitas[5,14,15,22,23,25]		7229413	692528	28	E	6	71	0.0018	E
Bom Sucesso[6,7,14,15,22,26]		7255243	681136	16	A	7	33	0.08	H
Chiquinho[6,7,22]	Castro	7264965	647839	5	L	2	61	0.03	H
Chiquinho dois[6,7,22]		7264954	647825	5	L	5	42	0.022	H
Pinheiro Seco[2,5,6,7,14,15,22,23,25,26]		7263142	646809	55	E	7	59	0.0066	E
Ermida[6,14,15,18,23]	A. Tamandaré	7204278	661607	16	A	3	68	0.05	H
Itaperussu[6,14,15,18,22,23,24]	Itaperuçu	7208651	667277	35	E	5	45	0.02	E
Pinheirinho[5,6,15,22]	Campo Largo	7233572	637641	17	A	6	52	0.1	E
Pinhalzinho[6,14,15,22,23,25]	Sengés	7299940	674573	18	A	8	39	0.0008	H
Bacaetava[5,14,18,19,22,24,25]	Colombo	7208130	680519	45	E	6	70	0.0002	E

List of impacts on the caves and surrounding area (numbers overwritten): 2 changes due to blasting, 5 constructions, 6 deforestation, 7 agricultural and livestock activities, 8 domestic sewage, 14 litter, 15 graffiti, 18 mining activities nearby, 19 highways close to the caves, 22 trampling, 23 vandalism on speleothems, 24 vibrations from road traffic or explosions near the cave, 25 tourism, 26 religious activities.

Of the 23 studied caves, 20 (87%) showed extreme or high conservation priority. Among the 9 caves considered of extreme priority, only two are located within conservation units: the Jesuit Cave, situated in the Campinhos State Park, and the Bacaetava Cave, located in the Bacaetava Municipal Park in the municipality of Colombo, where conservation measures are implemented in both units. The other six caves classified as having extreme conservation priority are Pocinho, Toquinhas, Pinheiro Seco, Itaperussu, Pinheirinho, and Lancinha. With a total species richness of at least 36 organisms, including troglobitic organisms, and an impact classification ranging from average to extreme, these caves have a higher priority for protection and conservation. The suggested conservation measures for each cave of extreme relevance are listed in Table 2.

Table 2. Suggested priority actions to protect caves classified in the CCPi as extremely high (E). PM = management plan; IRN = inclusion in a natural reserve area; RA = restoration of surrounding areas; AEA = environmental education activities; RPM = Renew the management plan; APR = Actions of physical restoration.

Cave name	Municipality	Lat	Long	Actions to protect caves
Varzeão	Dr. Ulysses	7275042	651909	PM, IRN
Pocinho	Dr. Ulysses	7296230	672123	PM, RA
Toquinhas	RB do Sul	7215636	671190	PM, RA
Lancinha	RB do Sul	7215358	672566	PM, AEA
Jesuítas	Cerro Azul	7229413	692528	PM, AEA
Pinheiro Seco	Castro	7263142	646809	PM, RA, APR, AEA
Itaperussu	Itaperuçu	7208651	667277	PM, RA
Pinheirinho	Campo Largo	7233572	637641	PM, RA
Bacaetava	Colombo	7208130	680519	RPM

At least 11 caves were classified as high priority to receive conservation actions (Arco de Pedra, Casa de Pedra, Ermida, Ressurgência do Feital, Malfazido, Piedade, Bromados, Bom Sucesso, Chiquinho, Chiquinho 2, and Pinhalzinho. Two caves were classified with average priority (Dá a Volta and Fadas), while only the cave Pocinho 2 was classified as low priority. The eleven caves classified as highly relevant exhibit various characteristics that have elevated them to this category. Among these variables, the total species richness was crucial in five caves (Ermida, Arco de Pedra, Ressurgência do Feital, Malfazido, and Chiquinho). These caves received these classifications based on their extremely high and high levels of total species richness.

The Piedade, Bromados, Bom Sucesso, Chiquinho 2, and Pinhalzinho caves stand out due to the presence of troglobites species, categorizing them as extremely high and high relevance when considering the richness of these specialized organisms. Finally, the Casa de

Pedra cave falls into this category of high relevance due to the combination of moderate human impacts and average total non-troglobite species richness. The average relevance category encompassed only two caves. The only cave classified as low relevance, Pocinhos 2, is characterized by low total species richness and a lack of human impacts, justifying its inclusion in this category. Nevertheless, the discovery of four troglobitic species in this cave emphasizes the importance of conservation measures, even in lesser-relevant areas, aiming to protect this subterranean ecosystem. Among the limestone belts, the Capiru stands out for having the highest percentage of caves classified as extreme priority for conservation, with four out of the six sampled caves (66.7%) in this category. The Itaiacoca belt follows with three caves, but considering the 11 sampled caves, only 27.7% reached the extreme priority category. Finally, the Votuverava belt recorded two caves of extreme priority, representing 40% of the five sampled caves. Caves classified as average and low priority include two in the Itaiacoca belt and one in the Capiru, the latter in a conservation unit. In the Votuverava Range, no cave received an average or low-priority classification.

Discussion

The results of this study provide information about the conservation status of cave fauna in the metropolitan region of Curitiba, a densely populated and exploited region in the Atlantic Rainforest in Brazil. The findings highlight human-induced alterations such as deforestation, agriculture, monoculture plantations, damage in speleothems, human trampling, mining activities, and graffiti, indicating substantial pressure on the cave environments. Corroborating the study hypothesis, 39.1% of the caves are over extreme priority for receiving conservation actions, with an additional 47.8% with high priority, showing the urgent need for measures to protect these ecosystems. This classification underscores the significant threat most caves face, emphasizing the necessity for robust conservation strategies (Cardoso et al. 2021).

Besides that, the caves in the metropolitan region of Curitiba harbor numerous troglobitic species that have yet to be described, most of them terrestrial. These unique organisms, adapted to life in caves, are highly specialized, endemic, and often fragile (Slaney and Weinstein 1997). However, surface activities and direct human alterations within the caves threaten their survival. Habitat destruction, pollution, and changes to the subterranean environment could have devastating effects, potentially driving some of these undiscovered species to extinction before they are even described or studied (Beynen and Townsend, 2005; Mammola et al. 2024).

Furthermore, the study reveals the challenges posed by high landscape fragmentation and the disjunct distribution of caves across three different limestone belts, making it challenging to establish continuous conservation units. Therefore, it is crucial to focus on preserving the areas surrounding the caves and implementing environmental education practices as a critical strategy. This knowledge is essential in raising awareness of the need for effective actions to conserve these environments, safeguarding their biodiversity and ecological equilibrium (Souza-Silva et al. 2015, Mammola et al. 2019, Iannella et al. 2021). It can also influence public policies and conservation initiatives and educate the local community and visitors about the importance of these caves (Romero 2009, Wynne et al. 2021, Moutaouakil et al. 2024).

Despite their ecological significance, few caves are formally included within conservation units, further exacerbating the risks to their preservation (Sánchez-Fernández et al. 2021, Colado et al. 2023). The lack of legal protection leaves caves vulnerable to various human impacts, such as deforestation, siltation, visitation, graffiti, and trampling. Even those caves located within protected areas are not out of impact (Gunn et al. 2000, Romero 2009; Pellegrini et al. 2012; Souza-Silva et al. 2015; Pacheco et al. 2020; Osborne 2019; Furtado-Oliveira et al. 2022).

When choosing locations for creating protected areas, it's essential to carefully balance scientific factors such as biodiversity and human impact with short- and long-term socio-economic considerations, including political and societal needs (Souza-Silva and Ferreira 2016, Mammola et al. 2024).

Effects of human impacts on cave fauna

Deforestation followed by agricultural practices and/or monoculture plantations can substantially affect subterranean ecosystems. These activities can impact the surface water flow, affect subterranean aquatic habitats, disrupt native species, and change food webs (Ford and Williams 2007, Huntoon 1992, Liu et al. 2018). Additionally, the use of chemicals such as pesticides, herbicides, and fertilizers can significantly alter water quality, resulting in shifts in subterranean communities, changes in species diversity, and modifications in sediment composition (Havelkova et al. 2019; Gunn et al. 2000).

The disturbance of soils from machinery activities can also alter the landscape, affecting the recharge zones that supply groundwater to cave ecosystems. Additionally, guano deposits in caves can reveal historical shifts in agricultural practices by stratigraphy studies of the pollen grains preserved. Such studies provide evidence of changes in landscape vegetation over time

(Maher 2006, Marais et al. 2015). Inside the Atlantic rainforest, the widespread nature of agriculture makes its cumulative impact on subterranean aquatic ecosystems more extensive and generalized than localized disturbances (Moraes et al. 2002).

Cattle fecal matter introduced into sinkholes and underground streams is a well-known consequence of livestock activities. Cattle operations, particularly those without adequate waste management practices, result in elevated levels of nitrification and fecal bacterial contamination in karst waters (Boyer and Pasquarell 1996, 1999). This pollution has placed numerous hypogean species at risk of extinction (Koppelman and Figg 1995, Knüsel et al. 2024). The effects of permeability characteristics in karst regions can significantly delay changes in groundwater quality. Agricultural and industrial pollutants may take 3 to 50 years to seep through the soil and reach aquifers (Rozkowski 1998).

Excessive human traffic inside and increased human activity can accelerate the destruction of speleothems. In addition, trash left by visitors and graffiti alters the natural features, harming the aesthetic value of the caves (Romero 2009, Souza et al. 2021). Another concern is the disturbance to cave-dwelling fauna, which are highly adapted to stable environments and sensitive to slight changes. Uncontrolled visitation can alter the caves' temperature, humidity, and air quality, directly impacting species that depend on this delicate balance for survival (Romero 2009, Nicolossi et al. 2021). Therefore, creating sustainable tourism management policies with strict control over visitation and environmental education for tourists is essential. These actions must be accompanied by ongoing efforts to monitor and maintain the caves, ensuring that even those located within conservation units are not subjected to irreversible damage.

The vulnerability of the cave fauna in the metropolitan region of Curitiba

The Atlantic Forest is known for its exceptional biodiversity and high levels of species endemism globally (Myers et al. 2000). However, estimates indicate that only 11% to 16% of the original vegetation cover remains, highlighting a significant loss and fragmentation of this ecosystem (Ribeiro et al. 2009; Diniz et al. 2022). In our study, we found that deforested areas have been a persistent issue, impacting approximately 60.8% of the areas surrounding the caves we investigated.

The analysis of priority caves for conservation identified nine caves that are of extreme priority. This approach is essential for guiding effective preservation programs. Implementing agile methods, such as CCPi, aligns with research emphasizing the importance of time as a crucial factor in conservation (Souza Silva et al. 2015; Rabelo et al. 2018; Souza et al. 2021;

Wynne et al. 2021). This is particularly important in light of the urgent need to prevent extinction events in regions with high endemism and biodiversity, which face impacts similar to those historically experienced by the Atlantic Forest (Souza-Silva et al 2015, Rezende et al. 2018).

Souza-Silva et al. (2015) applied the CCPi methodology in a study of 100 caves across the Atlantic Rainforest biome, with only two caves located in Paraná. This extensive study identified only 14 caves as of extreme conservation priority, representing 4.6% of the caves investigated. In contrast, our study focused on 23 caves in Paraná, with 9 of them classified as of extreme conservation priority, indicating that 39.1% of the studied caves are at high risk. This underscores the area’s vulnerability and emphasizes the urgent need for conservation measures, especially considering these caves are located within the densely populated Curitiba metropolitan area.

One important thing is that the composition and richness of cave-dwelling fauna appear to be affected by the characteristics of the surrounding landscapes (Cardoso et al. 2021). However, the degradation of these external areas has been associated with a decrease in species richness and diversity within the caves themselves, suggesting that caves serve as essential refuges for various species under threat (Bush 2020; Fraga et al. 2023). This phenomenon underscores the critical role that cave ecosystems play in maintaining biodiversity, especially as they may offer stable habitats for species whose natural habitats are increasingly compromised by human activities.

Given caves crucial role as biodiversity refuges, conservation strategies must encompass direct cave protection and broader restoration efforts in their surrounding landscapes. This dual approach is important for minimizing ecological disruptions that could otherwise reach the caves, potentially affecting the pristine characteristics of the ecosystems. Protecting caves alone may not be sufficient if the surrounding landscapes continue to degrade, as the integrity of cave habitats is closely tied to the health of adjacent environments (Cardoso et al. 2021). To effectively protect caves and their associated habitats, it is essential to implement a comprehensive land use policy that incorporates a structured approach to regional planning. This strategy should involve of identifying and classifying of geographical units that reflect a diverse mosaic of land uses intermixed with vital conservation areas. Central to this framework should be establishing a primary protection zone that encircles critical natural resources, such as springs and pristine recharge areas (Ford 2005; Souza-Silva et al. 2015).

Furthermore, this central protection area would serve as a crucial hub for conservation, extending outward to encompass adjacent core regions that necessitate stringent protective measures and specific land use restrictions. These core areas must be managed with high scrutiny to safeguard the delicate ecosystems within karst landscapes. By coordinating these conservation efforts with regional planning initiatives, we can ensure that the integrity of both the karst caves and their aquifers is preserved, allowing for sustainability and ecological resilience in the long term (Ford 2005).

Given these findings, we advocate for a range of targeted conservation actions. For example, establishing legally protected areas around caves would help limit detrimental human impacts and provide a buffer zone that maintains the ecological balance. Specifically, we recommend prioritizing protection for sites like Gruta do Varzeão, which supports a particularly high diversity of both troglobitic and non-troglobitic species. This cave is an important habitat for unique species that rely on stable environmental conditions, making its conservation a priority. Such initiatives should also involve habitat restoration efforts in degraded areas to reinforce the ecological networks supporting cave biodiversity. Additionally, implementing stricter land-use regulations, controlling pollution sources, and raising public awareness about the ecological significance of cave ecosystems are all measures that can contribute to preserving these habitats.

Priority caves for conservation in the state of Paraná

According to the Brazilian Speleological Information Registry (CANIE), Paraná hosts 360 registered caves, including Gruta da Lancinha, which has been a tourist site for approximately 150 years, with daily visitor numbers reaching up to 50 people (Sessegolo et al. 2006; Rehme 1993). This cave is the most extensively studied subterranean fauna, previously documented with 76 invertebrate species, including two troglobitic species (Pinto-da-Rocha, 1993). Our study, however, identified at least six troglobitic species and a high level of human impact, factors that underscore its classification as a conservation priority. Supporting these findings, recent research (Bock et al., 2023) documented 43 degradation points, such as trash accumulation, graffiti, and habitat destruction, indicating improved management strategies to ensure effective preservation.

Gruta do Varzeão, Paraná's second-largest cave, also warrants urgent conservation action. It has the highest total species richness (97) and nine troglobitic species, making it the most biodiverse cave regarding invertebrates. Meanwhile, Gruta Jesuítas, previously thought to host a single troglobitic species, now shows at least six, revealing a need to update management plans in line with its newly recognized biological value.

Educational activities are already in place in Gruta do Bacaetava; however, the cave's high conservation priority suggests that additional monitoring and an updated fauna inventory are essential. Gruta Pinheiro Seco, notable for its high human impact score (55) and seven troglobitic species, similarly requires a comprehensive management plan to address surrounding area recovery and regulate tourism, given its culturais significance.

For 13 other caves classified as high, medium, or low priority, preventive conservation measures, including creating protected areas, restoration of surrounding habitats, and environmental education, are essential for the long-term preservation of these karst environments.

Finals remarks

The high population density and urbanization in the Curitiba metropolitan area have significantly impacted local natural ecosystems, exacerbating human influences on cave systems. Urban development, agricultural expansion, and resource extraction activities have resulted in habitat fragmentation, pollution, and disruption of critical ecological processes that sustain these cave environments. Consequently, the region's distinctive geological and biological characteristics face heightened risks, necessitating prioritized conservation strategies. The proximity to urban centers underscores the urgency of implementing protective measures and complicates the execution of effective conservation policies. Therefore, immediate and targeted interventions are essential to safeguard these vulnerable ecosystems against escalating anthropogenic pressures.

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Author Contributions

Rodrigo Lopes Ferreira and Marconi Souza Silva were responsible for the conceptualization, methodology, translation, and English corrections. Leandro Mata da Rocha Melo, Rodrigo Lopes Ferreira, and Marconi Souza Silva participated in field activities. Leandro Mata da Rocha Melo prepared the original draft. Leandro Mata da Rocha Melo and Marconi Souza Silva were responsible for the statistical analysis, artwork, reviewing, and editing. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data supporting this study's findings are available on request from the corresponding author. The Editor-in-Chief has waived the required archiving due to privacy or ethical restrictions.

CONFLICTS OF INTEREST

The corresponding author confirms on behalf of all authors that no involvement might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

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Article 4:

A review of the factors influencing invertebrate community structure in subterranean habitats

This article has been submitted to the journal Community Ecology.

Leandro Mata da Rocha Melo, Rodrigo Lopes Ferreira, Marconi Souza Silva

Centro de Estudos em Biologia Subterrânea, Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, Lavras, Minas Gerais, Brasil, CEP 37200-900

Leandro Mata da Rocha Melo ORCID:0000-0002-6351-8654 (Leandro.melo2@estudante.ufla.br)
Rodrigo Lopes Ferreira ORCID: 0000-0003-3288-4405 (drops@ufla.br)
Marconi Souza-Silva1 ORCID: 0000-0002-3184-5319 (marconisilva@ufla.br)
corresponding author: leandro.melo2@estuddante.ufla.br

Declarations
The authors declare no competing interests.

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Authorship statement: LMRM, RFL, and MSS, designed and directed the study. LMRM compiles data related to ecological studies related to invertebrate community structure in subterranean habitats. LMRM analyzed the data. LMRM wrote the first version of the survey with input from all authors. MSS and RLF made suggestions in artwork, writing, English, and editing. All authors have read and agreed to the published version of the manuscript.

Abstract

Subterranean ecosystems remain among the least explored on the planet, although studies have unveiled their biodiversity and potential for research across various ecological domains. Historically, the European continent pioneered research and knowledge production in this field. However, interest in the topic has been growing on other continents, particularly in recent decades. To understand historical trends in studies related to the structure of invertebrate communities in subterranean environments and to identify the main environmental factors shaping these communities, we conducted a systematic literature review encompassing terrestrial and aquatic cave habitats. Our analysis included 136 articles published between 1986 and 2025, identifying at least 57 environmental variables influencing the structure of subterranean assemblages. The period from 2014 to 2021 was marked by a notable increase in the quantity and quality of studies. Among the key factors, temperature and distance from cave entrances emerged as primary determinants of species composition and richness in subterranean aquatic fauna, with rising temperatures being a concern due to their potential negative impacts on these species. For terrestrial fauna, distance from the entrance proved significant: the farther from the entrance, the lower the species richness and diversity. Conversely, variables such as trophic potential, substrate heterogeneity, and cave size were identified as positive factors, promoting higher species richness in terrestrial cave habitats. Despite these advancements, significant knowledge gaps persist, particularly in biotic interactions, ecological succession, functional ecology, landscape ecology, and metacommunities. Addressing these gaps is essential to deepen our understanding of subterranean ecosystems and reduce existing knowledge disparities, promoting more effective conservation strategies for these unique environments.

Keywords: Ecology, Karst, assemblages, Cave fauna, Ecological Gaps.

Introduction

Subterranean environments are renowned for their unique ecosystems, housing a diverse array of species, many of which are endemic. However, these environments remain relatively understudied, particularly in tropical regions (Deharveng and Bedos 2012; Culver et al. 2021; Ferreira and Souza-Silva 2023).

Most caves around the world are found in carbonate rocks, such as limestone and dolomite, which are more susceptible to water's dissolution process. However, caves in other lithologies, such as quartzite, sandstone, iron ore, granite, gneiss, mica schist, phyllite, lava tubes, and even soil, are also recorded, although in smaller quantity and extent compared to carbonate rocks (Gillieson 1998; Ford 2007).

The environmental conditions of caves, such as the absence of light, constant humidity, and stable temperatures, promote the evolution of unique adaptations, making these environments natural laboratories (Poulson and White 1969). This uniqueness provides an opportunity to investigate ecological and evolutionary processes and mechanisms of biological specialization (Mammola et al. 2020; Souza-Silva et al. 2021).

Understanding the environmental factors and interactions that influence subterranean communities is important for unraveling the distribution patterns of cave-dwelling species, offering valuable insights into their structural dynamics (Kneitel and Chase 2004; Vellend 2010; Souza-Silva et al. 2021). This integrated knowledge aids in for the conservation of cave fauna across diverse global regions (Mammola et al. 2019).

However, despite the significance of these studies, substantial knowledge gaps persist in the ecology of subterranean habitats (Culver et al. 2004; Culver et al. 2008; Lunghi and Manenti 2020). The inherent complexity of subterranean research, coupled with challenges like inaccessibility and the difficulty of directly observing these communities, represents barriers that prevent comprehensive scientific inquiry (Ficetola et al. 2019; Mammola et al. 2019).

These gaps pose not only physical challenges but also constraints on our broader understanding of subterranean biodiversity and cave ecosystems. Therefore, in analyzing the factors shaping these communities, we aim not only to comprehend subterranean environments but also to address current knowledge gaps, fostering a more comprehensive and refined understanding.

Among the known factors structuring invertebrate communities in caves, the heterogeneity of microhabitats has emerged prominently (Pacheco et al. 2020; Souza-Silva et al. 2021). Varied cavities, crevices, like epigean environments, cavities, fissures, and various subterranean spaces offer diverse environmental conditions, shaping niches that influence the distribution and specialization of invertebrate communities (Culver et al. 2009). Furthermore, it is essential to recognize that interactions between organisms play a key role in determining species distribution patterns in their environments (Chase et al. 2002)

The unique conditions of caves, such as the absence of light and resource scarcity, are reflected in the morphological and physiological adaptations of cave-dwelling species. These extreme environments have driven a series of evolutionary adaptations, including the reduction of pigmentation and vision, as well as the development of specialized sensory organs (Sket 2008). These adaptations not only represent evolutionary responses to the harsh conditions of these habitats but also play roles in ecological interactions within subterranean communities (Sket 2008; Culver et al. 2015).

However, gaining a more comprehensive understanding of these communities requires looking beyond the physical components of subterranean habitats. The surrounding epigean landscapes play a significant role in shaping cave environments. Factors such as local geology, adjacent vegetation, and the proximity of water sources emerge as extrinsic elements that directly influence the environmental conditions of caves, thereby impacting their biodiversity (Galassi et al. 2009; Prous et al. 2015; Christman 2016; Jaffé et al. 2018).

The connectivity between subterranean and surface environments is of utmost importance. This intricate interaction affects the geographic distribution of cave-dwelling species and directly contributes to the structuring of these communities over time (Zaksek et al. 2009). The influence of cave surroundings extends to nutrient, water, and energy fluxes, which shape these subterranean ecosystems (Prous et al. 2015). Understanding the environmental factors that shape invertebrate communities in caves poses a significant challenge, particularly due to limited resources in many regions for conducting studies on broader spatial and temporal scales (Kadmon et al. 2004). Nevertheless, innovative and collaborative approaches in subterranean research, which integrate traditional methods like direct community observation with advanced techniques such as genetic analysis and environmental modeling, could be pivotal in addressing knowledge gaps and comprehending the fundamental factors influencing these communities (Culver and Pipan, 2009).

Thus, the objective of this review, through the integration of literature data, is to identify articles that analyzed how environmental characteristics influence the structure of invertebrate communities in caves and to assess whether the variables selected and tested by the authors effectively determine this structure. Additionally, we aim to explore the chronological evolution of research on this topic, investigate the most studied invertebrate taxa, and understand the factors structuring communities in hotspots of subterranean biodiversity. Our hypothesis posits that cave invertebrate communities are shaped by a complex interaction of various environmental factors, such as distance from the entrance, temperature, humidity, trophic diversity, trophic availability, and cave size. However, we anticipate that the most prominent habitat variables (such as temperature, humidity, luminosity, distance from the entrance, and resource availability) are generally the most rigorously tested statistically.

Methodology

Ecological Studies on invertebrate communities in Subterranean Environments

This systematic literature review aimed to identify publications discussing and/or testing environmental variables that influence the structure of invertebrate communities in caves, encompassing terrestrial, freshwater, marine, and anchialine subterranean environments. The search, selection, and inclusion of articles were illustrated in a flowchart (Figure 1), constructed using the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) framework (Liberati et al. 2009; Page et al. 2021), accessible at http://www.prisma-statement.org/.

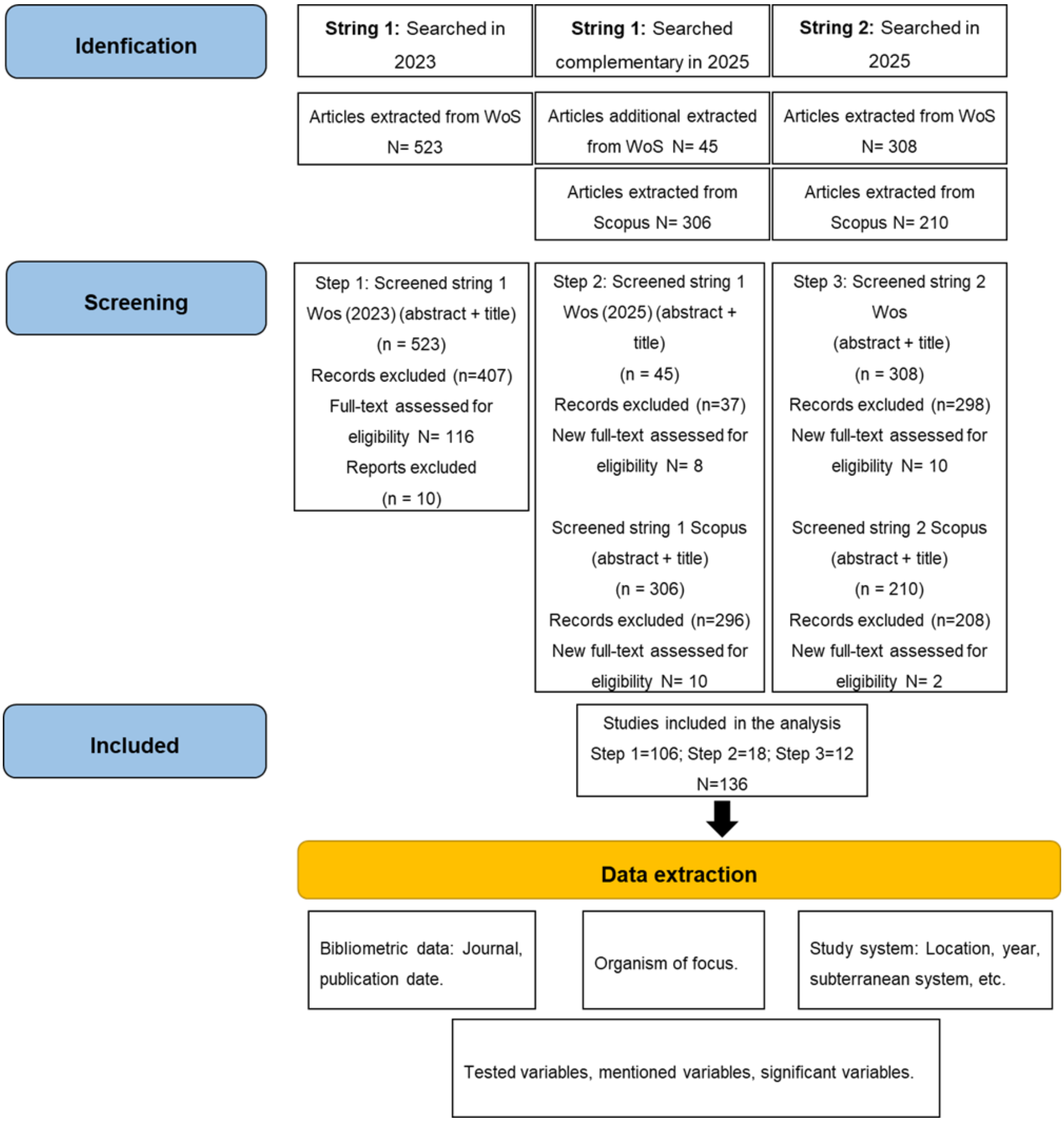

Fig 1 Sampled literature and extracted metadata. PRISMA diagram of the systematic review of articles extracted from WoS and Scopus, representing the flow of information through different phases of the systematic literature review and summary of the metadata collected for the databases.

Systematic Literature Search

In September 2023, we conducted the first standardized search in the Web of Science (WoS) database. We used various search terms to refine the process and exclude references that were not relevant to the objectives of this review. We chose to consider only articles in English, as the majority of scientific publications are in this language. This way, we avoided using broad terms like "Cave" and "Subterranean habitat" in isolation (Mammola et al. 2022). A search for the term "cave" in WoS resulted in 50,171 articles, while "subterranean habitat" yielded 1,622 articles, most of which were related to fields such as archaeology, paleontology, and geology, which were outside the scope of this study.

To increase specificity, we used the following search string (string 1): ("invertebrates" AND "cave" AND "ecology") OR ("fauna" AND "cave" AND "ecology") OR ("guano" AND "ecology" AND "invertebrates" AND "cave") OR ("management" AND "karst" AND "invertebrates" AND "cave") OR ("species richness" AND "cave") OR ("species composition" AND "cave") OR ("structuring" AND "invertebrates" AND "cave") OR ("hotspot" AND "cave").

This initial search resulted in 523 articles selected for review. Titles and abstracts were screened, and we adopted the following inclusion criteria: Articles that addressed ecological issues related to structuring invertebrate fauna in caves, testing or mentioning environmental variables that influence these communities. We excluded articles that: I) did not address subterranean ecosystems, II) were taxonomic studies, III) involved vertebrates, IV) did not address ecological issues, or V) were literature reviews.

After this screening, 407 articles were excluded because they did not meet the inclusion criteria. The full texts of the remaining 116 articles were analyzed, and 10 more were excluded because they did not evaluate or mention environmental factors that structure cave invertebrate fauna (Figure 1).

A new search was conducted in January 2025 using string 1 in the WoS and Scopus databases. In WoS, the new search identified 45 additional articles compared to the September 2023 search. After screening titles and abstracts, eight articles were included in the review. In Scopus, the same string 1 resulted in 306 articles. After excluding duplicates from WoS and articles that did not meet the inclusion criteria, 10 new articles were added to the review (Figure 1).

To increase the scope and minimize the loss of relevant articles, we created a second search string (string 2) using truncation: (ecolog* AND cave* AND invertebrat*) OR ("ecolog*" AND "invertebrat* communit*" AND cave*) OR ("ecolog* invertebrat*" AND cave*).

String 2 was applied in January 2025 to the WoS and Scopus databases. In WoS, the search resulted in 308 articles. After initial screening, 298 were excluded due to overlap with previous searches or failure to meet the inclusion criteria, resulting in the inclusion of 10 new articles. In Scopus, the same string yielded 210 articles, of which, after screening and exclusion of duplicates and articles that did not meet the inclusion criteria, 2 were included (Figure 1). At the end of the search and screening process, 136 articles were selected for the review.

Data extraction

All references included in the final database underwent extraction of specific information (Figure 1). To streamline data integration, the studies were systematically categorized based on several criteria: whether they addressed aquatic or terrestrial fauna, year of publication, year of the study, geographic location, study environment (freshwater, marine/brackish, or terrestrial), examined taxa, number of caves studied per article, number of collections conducted per study, tested variables, significant variables, variables mentioned but not tested, presence of subterranean hotspots, and relevant conclusions

Data analysis

Given the extensive variety of variables in the reviewed articles, we categorized them based on their interrelationships and synonymous terms to streamline the analysis. All variables extracted from our dataset were compiled to construct the chord diagram (Gu et al. 2014), as outlined in Table 1, along with their respective group categorizations to facilitate analysis.

The z-test for the difference in proportions (Argyrous 1997) was used to check for discrepancies in the proportion of studies where temperature held statistical significance between terrestrial and aquatic fauna.

All analyses were performed using R software, version 4.3.3 (R Development Core Team, 2024), with the ‘mass’ (Venables & Ripley 2002), ‘ggplot2’ (Wickham 2016), and ‘circlize’ (Gu et al. 2014) packages for data analysis and visualization.

Results

Literature timeline

The oldest publications identified in our search regarding how habitat structure and environmental factors influence the formation of cave invertebrate communities were from 1986 and 1991, with the works of Martín and Oromí and Humphreys, respectively. Martín and Oromí's study investigated the influence of low trophic availability on the structure of cave invertebrate communities. Humphreys' work demonstrated a significant relationship between the amount of water available in cave soils and the presence of troglobitic millipedes in an Australian cave.

Regarding the publication of articles in the field, there has been an increase in the number of studies over the years (Figure 2). The year 2021 stood out, recording the highest number of publications, with a total of 19 articles (Figure 2). Among the 136 selected articles, 33.8% (46 articles) studied the fauna of subterranean aquatic environments (supplementary material, articles 1 to 37, 48, 107, 112, 113, 115, 117, 123, 130, 136). Of these, 21 focused on marine caves (supplementary material, articles 1 to 12, 15, 16, 20, 27, 28, 35, 107, 113, 117), 22 on freshwater environments (supplementary material, articles 13, 14, 17, 18, 21, 22, 23, 25, 29, 30, 31, 32, 33, 34, 36, 37, 48, 112, 115, 123, 130, 136), and three on brackish environments (supplementary material, articles 19, 24, 26).

The remaining 90 articles (66.2%) (supplementary material, articles 38 to 47 and 49 to 106, 108 to 111, 114, 116 to 122, 124 to 129, 131 to 135) addressed a wider range of environments, exploring both the ecology and the factors that shape the terrestrial and aquatic invertebrate communities when present. The Postojna-Planina cave system, located in Slovenia, is the most intensively studied in the field of subterranean invertebrate ecology. Four articles focused on studies in caves within this system (Supplementary Material, articles 23, 33, 76, and 104). Additionally, nearby caves, such as Zguba Jama, have also attracted researchers' attention, being the subject of study in two articles (Supplementary Material, articles 21 and 108).

The number of caves studied per article ranged from a single cave to studies analyzing data from 844 caves (Jaffé et al. 2018). The majority of the studies focused exclusively on the invertebrate communities of a single cave, representing 41.2% of all the works included in this review. In eleven of these studies, only one sample was taken per cave (supplementary material, articles 5, 20, 55, 58, 60, 79, 88, 74, 107, 122, 134). On the other hand, the most extensively studied cave was the Monello cave, located in Italy (supplementary material, article 71), with 27 collections made over 14 months. Other studies involved 2 to 12 collections per cave (supplementary material, articles 6, 8, 10, 26, 12, 75, 14, 16, 18, 21, 25, 32, 54, 56, 71, 76, 77, 79, 80, 83, 85, 92, 93, 95, 96, 106, 114, 116, 125, 129, 132). Finally, 8 studies did not specify the number of collections (supplementary material, articles 11, 13, 19, 59, 89, 90, 118, 133).

Research that investigated data from up to 10 caves represented 27.9% of the total studies, while those that focused on up to 20 caves represented 8% of the dataset. Furthermore, 16.2% of the studies analyzed more than 20 caves. In contrast, 6.6% of the articles did not provide specific information on the number of caves used in the study.

Of the 136 studies included in this review, the majority, 119 (87.5%), were conducted exclusively in limestone caves. The distribution of the works is uneven, with only 5 (3.7%) focusing on iron caves, 4 (2.9%) on artificial cavities, 3 (2.2%) on lava tubes, 2 (1.5%) on quartzite caves, 2 (1.5%) on ice caves, and only 1 (0.7%) on granite caves.

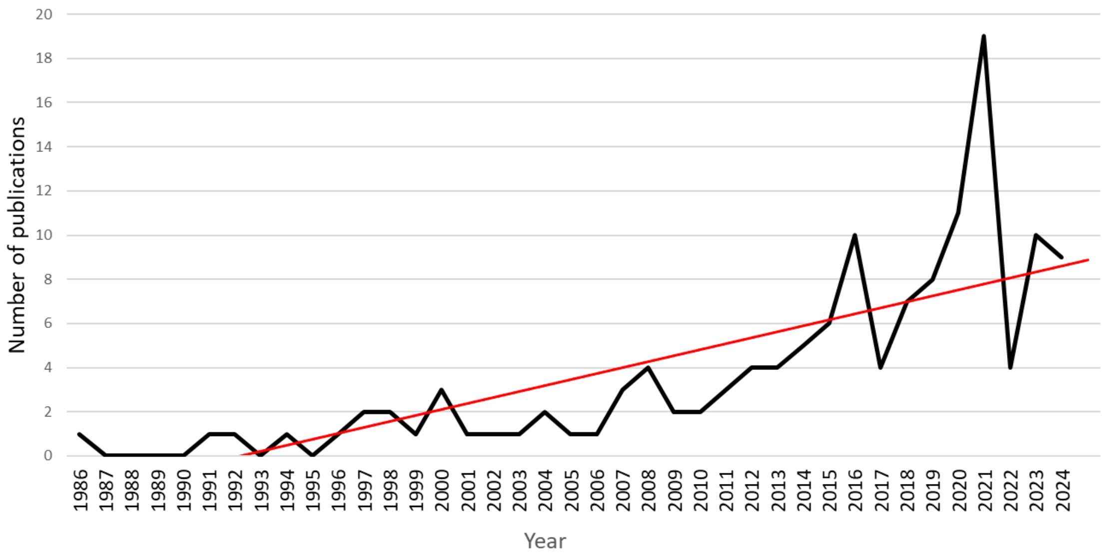

Fig 2 Number of publications over time. The black lines indicate the number of articles published annually, while the red line represents the linear trend.

Invertebrate taxa addressed and studied variables

Most studies (65.44%) addressed the entire invertebrate community present in caves. However, crustacean assemblages were evaluated in 8.82% of the studies included in this review. Sponges, beetles, and spiders, are also highlighted in ecological studies. Specifically, sponges were addressed in five articles related to marine subterranean environments, while the other mentioned taxa were the most studied in terrestrial subterranean environments (Table 2).

The studies tested 57 variables that potentially influence subterranean invertebrate communities/assemblages. Air and water temperature were the most tested variables, being evaluated in 62 articles and having a significant relationship with fauna distribution in 22 studies (Supplementary material, articles 9, 14, 21, 22, 23, 30, 45, 71, 74, 76, 88, 108, 109, 110, 121, 123, 124, 129, 130, 131, 133, 136). The distance from the cave entrance and substrate heterogeneity stood out as determinant environmental variables for community structure in terrestrial and aquatic environments. In 24 out of 31 cases where the distance from the entrance was tested, it was significant in determining the composition and species richness of invertebrates in caves (Supplementary material, articles 5, 11, 15, 16, 20, 21, 27, 28, 56, 62, 63, 64, 67, 78, 79, 83, 88, 108, 109, 113, 127, 128, 132, 134). Thus, the greater the distance from the entrance, the lower the richness and the less distinct the species composition, although there were exceptions, such as sponges, which showed a positive relationship with entrance distance in marine caves. Substrate heterogeneity was tested in 39 studies, proving significant in 20 of them (Supplementary material, articles 2, 3, 10, 12, 13, 14, 15, 41, 52, 68, 73, 81, 85, 88, 89, 106, 118, 122, 127, 133). Greater substrate heterogeneity was associated with a greater number of habitats, positively affecting species richness and composition.

The studies conducted in aquatic environments evaluated physical and chemical factors, highlighting pH, nitrogen concentration, sulfur, sulfuric acid, and CO_2 concentration. The main variables that showed associations with fauna distribution were pH, significant in four studies (Supplementary material, articles 17, 22, 23, 112), low oxygen concentration in groundwater was a significant factor for higher species richness in two studies (Supplementary material, articles 7 and 14). Nitrogen, sulfur, and sulfuric acid concentrations also influenced the fauna, with nitrogen having a positive effect on fauna richness and composition, while sulfur and sulfuric acid resulted in impoverished fauna (Supplementary material, articles 3, 8, 14, 27). Water temperature emerged as an important variable in determining the composition and richness of the fauna. In 18 studies where temperature was measured (Supplementary material, articles 6, 7, 9, 10, 13, 14, 16, 21, 22, 23, 24, 26, 28, 30, 34, 112, 115, 130), seven revealed a significant relationship with the fauna (Supplementary material, articles 9, 14, 21, 22, 23, 30, 130). Higher temperatures were negatively associated with the composition and richness of aquatic fauna, indicating that increased temperature in subterranean aquatic environments leads to significant loss of fauna.

Temperature exhibited a stronger effect on the structuring of aquatic fauna. The z-test for proportions revealed a significant difference ($p=0.04$) in the frequency of testing this variable and its significance between aquatic and terrestrial fauna.

In the 90 articles focused on terrestrial environments, variables such as distance from the entrance, trophic characteristics of the caves, substrate heterogeneity, seasonality, cave size, and the presence of organic resources like guano stood out as the main explanatory factors in structuring invertebrate communities in terrestrial caves.

The distance from the entrance negatively affects cave fauna richness and composition: the farther from the entrance, the lower the species richness and diversity. The availability of organic resources, such as guano, has a positive effect on invertebrate richness and serves as a crucial food source. Research highlights the importance of guano as a moist and sheltered microhabitat essential for the survival of various invertebrates (Souza-Silva et al. 2011; Ferreira 2019).

Additionally, larger caves generally exhibit higher species richness. Seasonality also influences fauna, with rainy periods and high humidity associated with greater species richness in caves. Although temperature was the most widely tested variable, assessed in 44 articles (Supplementary material, articles 44, 45, 46, 47, 48, 57, 59, 61, 62, 63, 64, 65, 68, 69, 71, 72, 74, 75, 76, 78, 79, 83, 85, 86, 88, 89, 91, 101, 102, 103, 108, 109, 110, 114, 120, 122, 124, 127, 128, 129, 131, 132, 133, 135), a positive relationship between temperature and fauna was identified in only ten studies. Thermal stability in caves is important for the maintenance of species specialized in living exclusively in this environment. Furthermore, the reduced temperature variation near cave entrances, compared to external environments, has been shown to favor the survival of transitional species that utilize both hypogean and epigean habitats (Supplementary material, articles 62, 68, 72, 76, 88, 110, 124, 129, 131, 133).

For the creation of the chord diagram and improved overall visualization of the data, 17 variable categories were established to group the 57 variables analyzed in the articles (Table 1). Notably, climatic factors emerged as the most densely studied group, followed by substrate heterogeneity and the physicochemical characteristics of groundwater. Analyzing the significant relationships, it was observed that distance from the entrance, trophic characteristics, substrate heterogeneity, cave lithological characteristics, and climatic factors were the elements with the highest number of statistically significant results. Data from studies in aquatic, terrestrial, and general environments can be seen in Figure 3.

Regarding the frequency of testing for each variable compared to the number of times it was found significant, it was observed that the variables most frequently chosen by authors are also those generating the highest number of significant results.

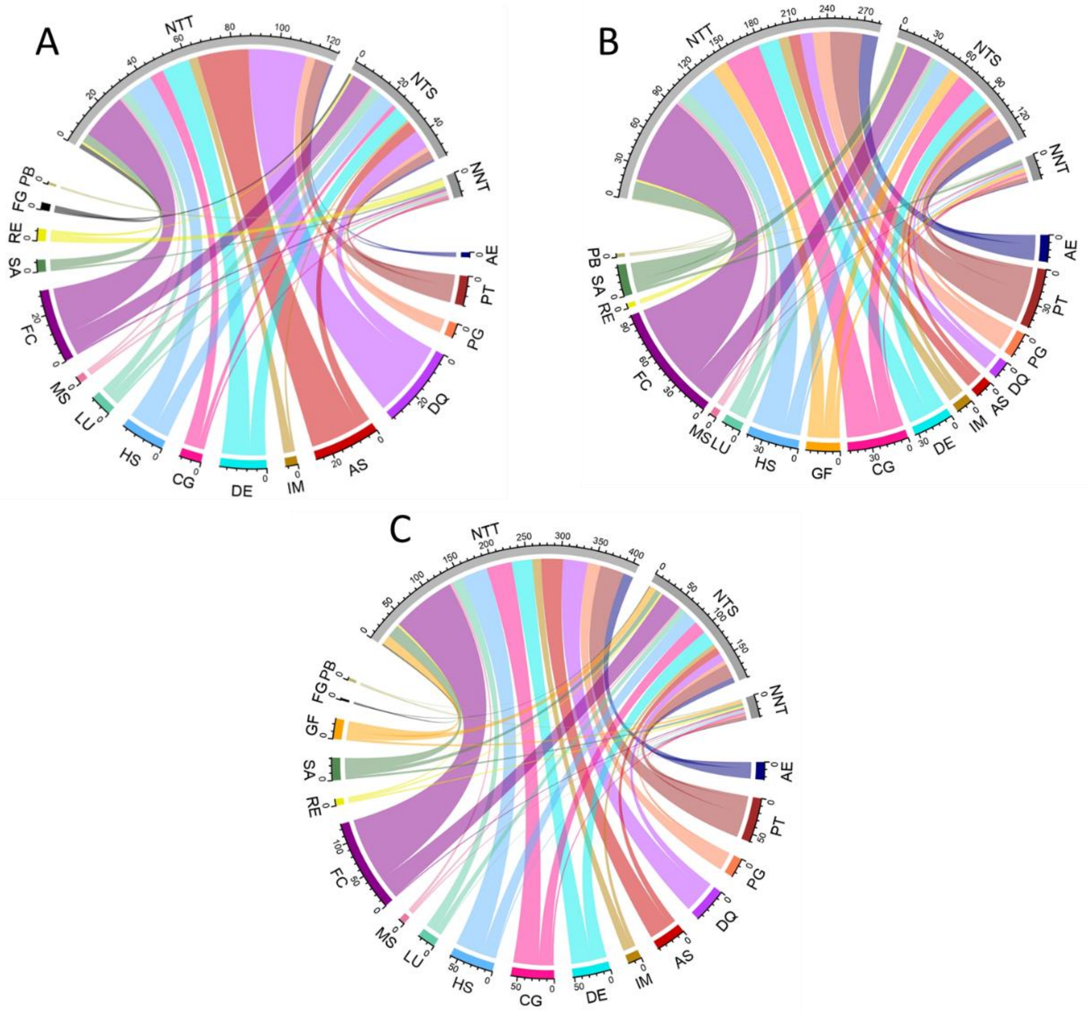

Fig 3 Chord diagram illustrating the interrelationships among the analyzed variables. NTT: Number of times each variable was statistically tested. NTS: Number of times each variable showed statistically significant results. NNT: Number of occasions where variables were mentioned as explanatory factors in community structuring, without corresponding statistical tests. A) Data from studies conducted in aquatic environments. B) Data from non-exclusively aquatic environments. C) Ensemble of all selected studies. Variables: AE = Epigean environment; PT = Trophic potential; PG = Geographical position; DQ = Chemical data; AS = Groundwater; IM = Impacts; DE = Distance from entrance; CG = Geological features; GF = Guano/Feces; HS = Substrate heterogeneity; LU = Luminosity; MS = Subterranean migration; FC = Climatic factors; RE = Ecological relationships; SA = Seasonality; FG = Gene flow; PB = Presence of chemoautotrophic bacteria

Studies on the hotspots of subterranean biodiversity

Eleven studies identified new subterranean biodiversity hotspots, adopting the definition proposed by Culver and Sket (2000). According to this approach, caves that harbor 20 or more troglobitic organisms are classified as subterranean biodiversity hotspots.

The most frequently mentioned factor to explain the diversity of troglobitic organisms, cited in four of the eleven reviewed articles, was the existence of subterranean connections that enable the migration of these organisms across vast underground areas (supplementary material, articles 92, 94, 96, 104). Furthermore, the presence of guano was pointed out as a relevant factor in three articles, with guano presence associated with higher diversity and richness of organisms (supplementary material, articles 93, 95, 97). However, it is important to note that no statistical analysis was performed to confirm these hypotheses.

The diversity of troglobitic organisms in caves is often attributed to the high heterogeneity of microhabitats. Four studies (supplementary material, articles 88, 92, 97, 118) investigated this hypothesis and obtained significant results that supported it.

The study conducted in the Águas Claras System (Souza-Silva et al. 2021), located in northeastern Brazil, stood out as the only one to analyze environmental factors, including variables such as temperature and humidity. The results reinforced the importance of these variables in the distribution of troglobitic fauna, indicating that variations in the cave's microclimate may have a direct effect on this specialized fauna, leading to the loss of species adapted to live in stable environmental conditions.

Other hotspots present specific characteristics, such as the Movile Cave in Romania, where the presence of chemoautotrophic bacteria plays a crucial role in sustaining the broad diversity of organisms restricted to the cave (Kumaresan et al. 2014). Movile Cave stands out as an isolated cave with no connections to the epigean environment, making this hotspot a unique case.

Most studied taxa

Despite the low number of studies focused on specific taxonomic groups, some stand out for presenting research on their ecology, specifically crustaceans, sponges, beetles, and spiders.

Crustaceans represent the most extensively studied group of cave invertebrates, with twelve studies included in this review (Supplementary material, articles 2, 3, 9, 10, 20, 28, 29, 30, 31, 33, 71, 76). The taxonomic level addressed varied between studies, with six articles investigating crustacean assemblages (Supplementary material, articles 2, 9, 10, 30, 31, 33), while two studies focused on the ecology of copepods (Supplementary material, articles 20, 76). Amphipoda, Isopoda, and studies on species of Niphargus spp. and Typhlatya spp. were the focus of one study each (Supplementary material, articles 3, 71, 29, 28).

Eleven environmental variables were found to be significantly related to the structuring of crustacean assemblages in subterranean environments. Substrate heterogeneity and temperature were the most frequently mentioned variables associated with the distribution of these assemblages, being cited in four studies each. Greater substrate heterogeneity is responsible for increased organism diversity, while temperature increases were related to a reduction in species richness (Supplementary material, articles 2, 3, 9, 10, 29, 30, 71). The presence of hydrological connections, identified in three studies, also resulted in increased faunal diversity (Supplementary material, articles 30, 31, 33). In two studies, the distance from the entrance was found to be an important parameter, with a higher distance from the entrance correlating with lower crustacean species richness in the caves (Supplementary material, articles 20, 28). Cave lithology was highlighted as a significant factor for crustacean species richness due to the high permeability of limestone, where cavities in this lithology offer a diverse and suitable habitat for various species of this group. Additionally, neutral water pH contributes to greater diversity and abundance of organisms. In contrast, factors such as high salinity, and elevated calcium and magnesium concentrations in the water, were associated with a reduction in species richness.

Sponges were investigated in five studies (Supplementary material, articles 5, 11, 15, 27, 113). The results of these studies indicated that the distance from the entrance positively influenced sponge density. Furthermore, the presence of greater microhabitat diversity (substrate heterogeneity) was considered significant in determining sponge composition and richness in cave environments (Supplementary material, article 15).

Among terrestrial invertebrates, beetles and spiders are the most studied groups. Beetles were examined in one study in Spain, another in Canada, and one in the Balkans (Supplementary material, articles 45, 70, 91). The Spanish study revealed that strictly cavernicolous beetles have low tolerance to high temperatures. Substrate heterogeneity played a role in the composition of beetle assemblages in the Balkans study, while in Canada, the presence of porcupine feces (Erethizon dorsatum) was suggested as a determining factor in beetle diversity. However, statistical tests to confirm this hypothesis were not conducted.

Regarding spiders, five studies were conducted (Supplementary material, articles 63, 65, 66, 124, 134). The studies observed significant variations influenced by seasonality. During rainy periods and higher humidity, spider abundance in caves increased. The distance from the entrance led to a decrease in species richness, while substrate heterogeneity and cave size also had positive effects on greater spider richness. Migration through subterranean connections was identified as a significant factor for greater spider fauna richness. Additionally, prey availability, such as crickets, beetles, and other spiders, was pointed out as a positive factor contributing to the richness and structuring of spider assemblages.

Discussion

The number of published studies has shown an increasing trend over the years, reflecting progress in this field of knowledge. To understand the complexities of cave invertebrate communities, researchers have extensively investigated various environmental variables, with particular emphasis on temperature, humidity, distance from cave entrances, and substrate heterogeneity. The distance from cave entrances has consistently proven to be a significant determinant of invertebrate community structure.

Furthermore, the frequency with which specific variables are tested affirms the relevance of the variables identified by authors in the dynamics of these communities. However, certain aspects, such as ecological interactions between species, remain overlooked in subterranean biology. Additionally, there is a gap in studies addressing the ecological factors that shape the composition and diversity of invertebrate fauna in subterranean biodiversity hotspots around the world.

Current scenario of cave community ecology studies

Subterranean environments represent one of the last frontiers of our world, with vast areas still unexplored (Ficetola et al. 2019). In the United States, more than 50,000 caves have been documented. In the Dinaric Alps, a small karst region in Europe, it is estimated that around 25,000 caves exist, with a large portion of them still unexplored (Niemiller & Zigler, 2013). In Brazil, although 23,000 caves are currently known, estimates suggest that the real number could exceed 300,000 subterranean cavities (CANIE 2022). These figures highlight the significant disparity between current knowledge and exploration potential, emphasizing the need to expand research and mapping efforts.

Despite the increasing trend in the number of studies published over the years, a decline in paper publications after 2021 has been noted. This decrease may be probably an effect of the Covid-19 pandemic, which had substantial impacts on research globally. Many projects were interrupted, and numerous laboratories were forced to close, directly affecting the continuity of studies and resulting in a reduction in published articles across various fields of knowledge during this period (Raynaud et al. 2021).

Review articles covering various topics related to cave environments commonly reveal a higher number of studies conducted in Europe compared to other continents (Mammola et al. 2022; Piano et al. 2022). This trend is likely influenced by historical and socioeconomic factors. Research in subterranean biology originated in Europe, which boasts over a century of research in this field. Additionally, socioeconomic factors, such as investments in subterranean research, are more robustly established in European countries (Meyer et al. 2015; Zizka et al. 2020).

Despite commencing their research activities in subterranean environments more recently compared to Europe (with only 3 publications until the year 2004 - supplementary material, articles, 26, 79, 134), North and South Americas have been increasingly prominent in recent years.

Brazil, in particular, stands out in the production of ecological studies related to cave invertebrate communities. This can be attributed to the establishment of new research groups focused on subterranean fauna in the country, along with increased research funding derived from cave compensation policies. The Brazilian legislation for cave protection, established by Decree No. 6,640 of 2008, classifies caves into four relevance categories (maximum, high, medium, and low). Although cave destruction is permitted for the high, medium, and low relevance categories, caves of maximum relevance are protected from any destruction. This legislative change has led to an increasing demand for scientific studies and cave monitoring, both to support the approval of projects and to deepen the knowledge about these ecosystems. As part of the legal requirements, projects that impact caves must carry out environmental or financial compensation, often through investments in scientific research. This need for compensation has resulted in the advancement and intensification of cave studies in Brazil since the implementation of the decree (Mirra 2019).

The relatively lower number of studies in Asia, Central America, and Africa may stem from various factors, including limited resources, insufficient awareness of the importance of these environments, or simply the absence of dedicated researchers in these specific regions (Hoffmann 2022).

The evident trend toward a greater quantity of studies focusing on carbonate caves could be attributed to their broader geographic distribution and historical factors (Souza-Silva et al. 2020). Carbonates cover approximately 20% of the Earth's surface, occurring on all continents, unlike iron-rich and siliciclastic rocks (Goldscheider et al. 2020). Carbonate formations also host a larger number of caves (and of greater extent) along with various karstic features that attract researchers due to their scenic beauty, biodiversity, and fragility (Poulson and White 1969). Moreover, limestone caves tend to harbor a diverse array of troglobites, further stimulating researchers' interest in exploring these ecosystems (Auler et al. 2005; Souza-Silva et al. 2021; Zagmajster et al. 2021; Camacho et al. 2021).

Studies that focus on only one cavity with a limited number of collections may lead to under-sampling of cave fauna due to the restricted number of collection events. Additionally, even in caves previously studied, new investigations may unveil results not captured during earlier sampling efforts (Ferreira and Souza-Silva 2023). Hence, conducting repeated collections in the same cave is advisable to gain a more comprehensive understanding of its community dynamics.

Exploring multiple caves offers an opportunity to discern patterns, variations, and trends transcending the unique characteristics of each underground environment (Pacheco et al. 2020;

Mammola et al. 2022). This broader approach contributes to a more holistic comprehension of the ecological factors influencing invertebrate communities across various spatiotemporal scales.

Consequently, studies that encompass diverse scales can furnish a thorough understanding of biological diversity, species interactions, and the underlying processes operating in different geographical regions (Field et al. 2009; Chase et al. 2000; Jansson and Dynesius 2002). Such an approach facilitates the examination of nuances, ranging from microhabitats within individual caves at a local scale to latitudinal patterns at a global scale (Kerr and Packer 1997; Willig et al. 2003). This enables the acquisition of insights that might be overlooked when employing only a singular scale of analysis.

Environmental factors structuring cave invertebrate communities

Given the global reach of this review, a significant contribution from several distinct environmental variables was anticipated to explain the composition and richness of invertebrates in cave ecosystems. Previous large-scale studies have investigated the influence of various factors linked to the geographical positioning of caves, including altitude, which affects climatic parameters such as temperature and humidity across different regions (Mammola et al. 2020; Souza-Silva et al. 2020). Consequently, explanatory variables for fauna distribution may vary based on the study's location and scale. However, we noticed that the variables most frequently examined in studies were those that most effectively elucidated fauna distribution patterns within caves. This suggests that researchers have been deliberate in selecting variables they perceive as crucial for investigation, demonstrating an effort to incorporate established variables from existing literature.

Nevertheless, it is imperative to acknowledge the importance of other variables that are often overlooked in understanding the structure and dynamics of subterranean invertebrate communities. Therefore, we advocate for the exploration of biotic interactions such as predation, mutualism, competition, and parasitism, along with considerations of ecological succession, functional ecology, landscape ecology, and metacommunity ecology. By incorporating these factors, studies can avoid the repetition of the same variables and broaden the scope of potential explanations for fauna distribution patterns.

Environmental factors affecting aquatic subterranean community structure

Environmental variations from the entrance to deeper areas impose restrictions on colonization, allowing only species adapted to oligotrophic conditions and the permanent absence of light to establish themselves (Mammola and Isaia 2018; Lunghi and Manenti 2020).

However, such gradients may favor specific groups of organisms over time. For instance, sponges exhibit higher abundance in regions farther from the entrance (Corriero et al. 2001; Slattery et al. 2013; Gerovasileiou and Voultsiadou 2016). This trait is attributed to the reduced competition for resources in distant regions, giving these organisms an adaptive edge compared to others (Corriero et al. 2001; Slattery et al. 2013).

On the other hand, temperature exerts a more pronounced influence on aquatic fauna than on terrestrial fauna, as evidenced by the significantly different proportions of studies yielding significant results ($p=0.04$). The lower thermal tolerance of aquatic fauna underscores their vulnerability in the face of ongoing climate change. Rising temperatures pose a critical threat to the survival and distribution of these species due to their susceptibility to thermal fluctuations (Chevaldonné et al. 2003; Kozel and Pipan 2020).

Subterranean habitats are characterized by environmental stability, which suggests that most cave organisms have a low capacity for adapting to thermal variations (Raschmanová et al. 2018). In this context, climate changes, such as temperature increases, could force species to retreat to deeper regions of caves, where food resources are even scarcer. In contrast, exotic species may benefit from these changes and colonize these subterranean environments (Wynne et al. 2014; Mammola et al. 2019). Detailed studies are needed to understand the impacts of climate change on subterranean fauna.

Environmental factors affecting terrestrial subterranean community structure

The organic deposits tend to enhance species richness (Schneider et al. 2011; Souza-Silva et al. 2012), serving as shelters and breeding grounds for various invertebrate species (Moore et al. 2004; Schneider et al. 2011; Souza-Silva et al. 2011). Consequently, multiple studies support the notion that diverse organic resources, such as litter, wood, and animal carcasses, foster intricate interactions, thereby contributing to the development of extensive food webs (Hills et al. 2008; Salgado et al. 2014). The complexity of these interactions underscores the importance of considering the nature of organic resources in comprehending trophic networks.

The role of substrate heterogeneity has gained prominence in recent studies aiming to unravel the link between species diversity and habitat variation (Pacheco et al. 2020; Souza-Silva et al. 2021). The presence of diverse substrates offers unique niches for different species to exploit (Canedoli et al. 2022; Cardoso et al. 2022), thereby enriching the richness and diversity of fauna in subterranean environments. It is worth noting that substrate heterogeneity is closely tied to external environments, as characteristics of surface habitats directly influence the quality and quantity of substrates found in caves (Souza-Silva et al. 2012; Souza et al. 2021).

Caves are not isolated ecosystems; rather, they are influenced by external environmental conditions. Consequently, activities such as deforestation, agriculture, and livestock farming have a direct influence on these subterranean ecosystems and their inhabitants (Pellegrini et al. 2016; Teodoro et al. 2021; Mcnie and Death 2017). This interplay between cave ecosystems and changes in their surroundings underscores the critical importance of preserving both caves and their surface ecosystems.

Subterranean fauna exhibits heightened abundance and reproductive activity during rain, due to the higher availability of organic resources. (Bento et al. 2016). This correlation between seasonality and fauna response underscores the susceptibility of cave ecosystems to temporal fluctuations, underscoring the necessity of considering these cycles in the management and conservation of subterranean environments. Understanding these seasonal dynamics not only aids in preserving biological diversity but also informs the development of effective and sustainable management strategies. This correlation between seasonality and fauna response highlights the susceptibility of cave ecosystems to temporal fluctuations, emphasizing the need to consider these cycles in the management and conservation of subterranean environments.

Most studied taxa

Crustaceans have been extensively studied due to their presence in both aquatic and terrestrial subterranean environments, highlighting their significance. This diverse group has been the subject of investigations, with particular focus on copepods, isopods, and amphipods, which are the most abundant and taxonomically diverse organisms in aquatic subterranean environments, while other taxa of aquatic invertebrates have few representatives in subterranean habitats (Gibert and Deharveng 2002).

Among terrestrial invertebrates, Araneae and Coleoptera have received significant attention. As for coleopterans, the attention given to this group in scientific studies can be partly attributed to beetles representing the most diverse group of terrestrial invertebrates in caves. However, other abundant groups, such as springtails, remain relatively understudied (Gibert and Deharveng 2002).

Studies focused on spiders can be attributed to the prominence of this group as study models for their ecological role as top predators in subterranean ecosystems. Additionally, spiders exhibit unique adaptations to the subterranean environment and demonstrate sensitivity to anthropogenic disturbances, characteristics identified in the study by Mammola and Isaia (2017) as stimulating factors for further research on spiders.

The significance of substrate heterogeneity in structuring spider and beetle communities suggests that specific microhabitat characteristics have a substantial influence on not only beetles and spiders in cave environments but may also influence various other groups, as observed in other studies (Pacheco et al. 2020; Souza-Silva et al. 2021).

Final remarks

This review enhances our understanding of current knowledge regarding cave invertebrate community ecology. By elucidating the factors influencing these communities, our analysis guides future research, thereby improving the efficacy of such endeavors. We advocate for the continuation of studies, recommending the exploration of new variables. This approach will help address existing knowledge gaps and establish comprehensive conservation strategies, crucial for safeguarding subterranean ecosystems and their diverse biodiversity, particularly amidst growing threats.

It is noteworthy that, even among taxa receiving more research attention, the number of articles remains relatively limited. These knowledge gaps stem from the absence of robust systematic sampling techniques for most taxonomic groups (Wynne et al. 2019). Addressing these gaps is essential to enhance our approach to and prioritization of ecological and conservation research in underground environments (Mammola et al. 2020).

Disclosure

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Table 1: Group of variables used for the creation of the chord diagram and variables included by created category.

Group of variables used in the chord diagram	Variables included in each category
Epigean Environment (AE)	Soil characteristics + Epigean fauna diversity + Presence of karst areas + Surrounding vegetation
Trophic potential (PT)	Trophic potential + Prey abundance + Type of litter + Presence of glow worms + Presence of bats
Geographical Position (PG)	Altitude + Latitude + Longitude + Distance between caves + Distance from coast
Chemical Data (DQ)	CO2 concentration + pH + Concentration of N, S, SO4 in water
Groundwater (AS)	Presence of water body + Electrical conductivity of water + Hydrological connection + Water flow + Water depth + Water salinity
Impacts (IM)	Deforestation + Impacts + Mining + Land use + Agriculture in the surroundings + Degree of habitat fragmentation
Distance from Entrance (DE)	Distance from entrance
Geological Features (CG)	Speleogenesis + Cave morphology + Number of entrances + Cave size + Lithology + Roof thickness + Geological formation age + Surface isolation degree
Guano/Feces (GF)	Guano + Feces
Substrate Heterogeneity (HS)	Substrate heterogeneity + Shelter availability
Luminosity (LU)	Luminosity
Subterranean Migration (MS)	Subterranean migration
Climatic Factors (FC)	Temperature + Humidity + Precipitation
Ecological Relationships (RE)	Predation + Competition
Seasonality (SA)	Seasonality
Presence of Chemoautotrophic Bacteria (PB)	Chemoautotrophic bacteria
Gene Flow (FG)	Gene flow

Table 2: Number of studies; when more than one taxon was studied per article, it was counted as a assemblage. FA: aquatic fauna; FT: terrestrial fauna. The table represents the countries where studies on different taxa and assemblages occurred and the number of studies related to aquatic and terrestrial fauna, as well as the total number of studies.

	Country FA	Country FT	FA	FT	Articles	% of total
Assemblages	Italy, Spain, Mexico, USA, Bermuda, Slovenia, Romania, England, New Zealand.	Brazil, USA, France, Belgium, Australia, Italy, Botswana, Canada, Guatemala, Spain, Turkey, Romania, China, Indonesia.	25	64	89	65,44
Crustacea	Spain, Croatia, Italy, France, Mexico, Canary Islands, Slovenia.	Italy, Slovenia.	10	2	12	8,82
Porifera	Ireland, Italy, Greece, Bahamas, Crimea.	-	5	0	5	3,67
Protozoa	Spain	-	1	0	1	0,73
Mollusca	Cyprus, Georgia	-	2	0	2	1,47
Bryozoa	France	-	1	0	1	0,73
Nematoda	Cuba	South Africa	1	1	2	1,47
Tricladida	Italy		1	0	1	0,73
Ants	-	Brazil	0	2	2	1,47
Troglobites		USA, Brazil, Slovenia	0	4	4	2,94
Coleoptera	-	Spain, Canada, Balkans, Poland	0	4	4	2,94
Orthoptera	-	India, USA	0	2	2	1,47
Diptera	-	Colombia, Brazil	0	2	2	1,47
Collembola	-	Romania, Slovakia	0	2	2	1,47
Araneae	-	Italy, Australia, Brazil	0	5	5	3,67
Blattodea	-	Australia	0	1	1	0,73
Mite	-	China	0	1	1	0,73
Total			46	90	136	100,00

Artigo 5

Uma jornada pela vida nas cavernas paranaenses: desenvolvimento de cartilhas e recursos digitais para atividades de educação ambiental.

Leandro Mata da Rocha Melo[1,2], Rodrigo Lopes Ferreira[1,2], Marconi Souza Silva[1,2],

[1]Centro de Estudos em Biologia Subterrânea, Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, Campus Universitário, P.O. Box 3037, Lavras CEP 37200-000, Minas Gerais, Brasil

[2]Programa de Pós-Graduação em Ecologia Aplicada, Universidade Federal de Lavras, Lavras CEP 37200-000, Minas Gerais, Brasil

Resumo

Devido às crescentes ameaças aos ambientes naturais causadas por atividades humanas, como a acentuada degradação da Mata Atlântica, as cavernas inseridas nesse bioma também estão sob constante pressão. Nesse cenário, o levantamento e divulgação de informações torna-se uma ferramenta essencial para o fortalecimento da sociedade nas tomadas de decisão realacionadas a presenvação dos ambientes naturais. Assim, o objetivo deste trabalho é apresentar materiais informativos produzidos através de resultados de pesquisas relacionadas a ecologia e conservação de cavernas e sua fauna associada, no estado do Paraná, Brasil. Para isso, elaboramos duas cartilhas educativas: "A Vida nas Cavernas", destinada ao público infantil e alunos do Ensino Fundamental I e II, e "Uma Jornada pela Vida nas Cavernas Paranaenses", voltada para jovens adultos, alunos do Ensino Médio e Superior. Além disso, disponibilizamos informações detalhadas sobre resultados das pesquisa, por meio de vídeos e publicações no site: https://cebsparana2023.mystrikingly.com. A primeira cartilha, "A Vida nas Cavernas", apresenta o ambiente cavernícola de forma lúdica, com personagens inspirados na fauna das cavernas do Paraná. Esses personagens explicam pontos importantes sobre o ecossistema subterrâneo e os impactos ambientais que afetam essas cavernas, além de como essas alterações podem prejudicar o funcionamento do ecossistema. Já a segunda cartilha, "Uma Jornada pela Vida nas Cavernas Paranaenses", utiliza imagens para ilustrar as principais características dos ambientes cavernícolas e da fauna encontrada nas cavernas do Paraná. Ela também traz descrições breves sobre os grupos coletados, tanto troglóbios quanto não troglóbios, além de alertas sobre os impactos ambientais enfrentados por essas cavernas. Os vídeos complementam o projeto, mostrando o envolvimento da equipe no desenvolvimento das atividades, com o objetivo de atrair o interesse do público e ampliar a divulgação do trabalho para alcançar o maior número possível de pessoas. Essas estratégias contribuirão para a uma maior divulgação de conhecimento, contribuindo para conservação das cavernas.

Palavras-chave: Cavernas; Educação; Conservação

Introdução

Poucos são os trabalhos acerca de ecologia e conservação de cavernas no Brasil e menos ainda se sabe a respeito de como este assunto deve ser tratado de forma didático-pedagógica nas escolas públicas, privadas, universidades e meios de produção, visando efetivamente, conscientizar a sociedade quanto às possibilidades e necessidades de uso e conservação destes ambientes (Souza-Silva et al. 2019). A construção de valores sociais, conhecimentos, habilidades, atitudes e competências voltadas para a conservação do meio ambiente, essencial à qualidade de vida e sua sustentabilidade é altamente dependente da divulgação e educação ambiental. Estes são componentes essenciais e permanentes da educação nacional, devendo estar presente, de forma articulada, em todos os níveis e modalidades do processo educativo, em caráter formal e não-formal. Como parte do processo educativo mais amplo, todos têm direito à educação ambiental (LEI No 9.795, DE 27 DE ABRIL DE 1999).

No intuito de promover a sustentabilidade das atividades humanas, práticas e conceitos da educação ambiental têm sido utilizados, de diferentes formas, com o objetivo de mudar atitudes dos cidadãos em relação à conservação e uso de recursos naturais (Caro et al 1994; Van Weelie & Wals 2002). No entanto, é importante destacar que existem diferentes entendimentos e abordagens de educação ambiental. Estas diferentes linhas trazem interpretações distintas sobre os conceitos e práticas, sendo que o entendimento de educação ambiental adotado, pode refletir em projetos com diferentes metodologias e, consequentemente, diferentes resultados (Rheinheimer & Guerra 2012). Estas diferentes ênfases educativas se relacionam aos modos de direcionamento da educação ambiental que pode ter uma visão mais popular, crítica, transformadora, política, comunitária, formal ou não formal (LEI No 9.795, DE 27 DE ABRIL DE 1999, Carvalho 2004, Loureiro 2004).

Desta forma, a eficiência dos programas educacionais deve ser avaliada para selecionar as abordagens mais adequadas (Souza-Silva et al 2014). Exposições zoológicas, campanhas de mídia ou filmes de animação com forte conteúdo conservacionista são usados para estimular atitudes positivas e aumentar o comprometimento do público para os esforços de conservação. Apesar de existirem muitas campanhas de divulgação e preocupações do público sobre a crise da biodiversidade, estas acções tendem a ser momentâneas (Novacek, 2008). Além disso, o conhecimento público relacionado às questões de uso e conservação da biodiversidade, ainda é pequeno (Pilgrim et al 2008; Robélia & Murphy, 2012). Assim, esforços de educação específicas sobre biodiversidade são necessários para promover atitudes positivas e esforços práticos de conservação (Lindemann-Matthies, 2006, Souza-Silva et al 2014).

Com uma maior difusão das atividades turísticas e de exploração mineral, há uma necessidade da conscientização para o incentivo de práticas sustentáveis no uso e conservação dos ambientes cársticos (paisagens onde ocorrem as cavernas) e sua biodiversidade, no intuito de se manter bens e serviços que promovam uma melhor qualidade de vida à população (Lobo et al. 2008, Donato et al. 2018, Souza-Silva et al. 2019).

Assim as atividades científicas são uma importante ferramenta para a conservação, pois, para assegurar a sustentabilidade do avanço científico e minimizar os impactos causados pelas atividades antrópicas, é importante manter um constante intercâmbio com a sociedade e dedicar atenção permanente ao desenvolvimento de novos pesquisadores. A divulgação eficaz da pesquisa, enriquece o conhecimento público e estimula o envolvimento da comunidade na ciência. Além disso, esse diálogo contínuo com a sociedade permite que os cientistas obtenham retorno valioso, identifiquem prioridades de pesquisa relevantes e promovam a aplicação prática dos resultados. Portanto, a conexão entre a atividade científica, divulgação de pesquisa, sociedade e formação de novos pesquisadores é um componente essencial para o progresso contínuo da ciência (Valerio & Pinheiro 2008, Martins 2015).

Diante do exposto, o presente trabalho tem como objetivo fomentar o desenvolvimento de ações educativas sobre o tema ecologia de ambientes subterrâneos, na forma de um site, vídeos e cartilhas educativas. Espera-se que tais ações contribuam para informar e formar cidadãos com senso crítico frente a tomada de decisões relacionadas a questões sociais, ambientais e econômicas que envolvam as cavernas e ambientes relacionados.

Metodologia

Desenvolvimento das cartilhas

As cartilhas foram desenvolvidas a partir da compilação dos resultados de projeto de pesquisa sobre a fauna cavernícola e os impactos diretos e indiretos aos ambientes subterrâneos da região metropolita de Curitiba, estado do Paraná, Brasil.

A cartilha "A Vida nas Cavernas" foi adaptada a partir da obra "A Aventura da Vida nas Cavernas" (Ferreira et al., 2008), que se baseou em um estudo realizado em 103 cavernas da Mata Atlântica brasileira. A adaptação teve como objetivo apresentar um foco regional, para representar a fauna das cavernas do Paraná de maneira lúdica, utilizando ilustrações nas quais os animais são retratados como personagens. Essas ilustrações narram as dificuldades que os invertebrados enfrentam devido aos impactos ambientais atuais na região. Além de conscientizar o público, a história foi criada para apresentar soluções práticas para mitigar esses problemas, destacando que é possível conciliar atividades humanas com a preservação do patrimônio espeleológico, promovendo a conservação de forma sustentável.

Além da estória, incluímos um jogo dos sete erros ao final da cartilha, destacando os impactos ambientais nas cavernas. Esse recurso foi projetado de maneira interativa para reforçar o aprendizado, permitindo que os leitores identifiquem e compreendam visualmente os problemas enfrentados pelos ecossistemas cavernícolas, promovendo a fixação do conhecimento de forma lúdica e educativa.

Na cartilha "Uma Jornada Pela Vida nas Cavernas Paranaenses", as imagens foram geradas com o auxílio de ferramentas de inteligência artificial (Magic Canva; Microsoft designer), proporcionando representações visuais dos ambientes cavernícolas. Além dessas ilustrações, também foram utilizadas fotografias para uma melhor representação do habitat. A narrativa busca apresentar as diferentes condições ambientais desde a entrada até as regiões mais profundas de uma caverna, assim como os grupos de organismos que habitam cada uma dessas regiões. Foram incluídas fotos de organismos troglóbios (estritamente cavernícolas) e não troglóbios coletados em cavernas do Paraná, acompanhadas de breves descrições que visam familiarizar a população com a fauna presente nesses ecossistemas subterrâneos.

Desenvolvimento dos vídeos

Em um primeiro momento identificamos os temas chave a serem abordados em cada vídeo, com o intuito de promover o trabalho realizado e ampliar o contato entre a pesquisa e a comunidade. Os vídeos foram planejados para cobrir aspectos desde o desenvolvimento do projeto até a conscientização sobre a preservação de cavernas no Brasil. Após a definição do conteúdo, o material foi produzido com técnicas de gravação e edição no intuito de garantir maior clareza das informações e a acessibilidade ao público-alvo. Elementos visuais, como fotos e vídeos das atividades de campo, foram incluídos para enriquecer a experiência do espectador.

Os vídeos foram disponibilizados por meio do site oficial do projeto e em plataformas digitais, visando aumentar a visibilidade e o alcance das ações realizadas.

Desenvolvimento do site

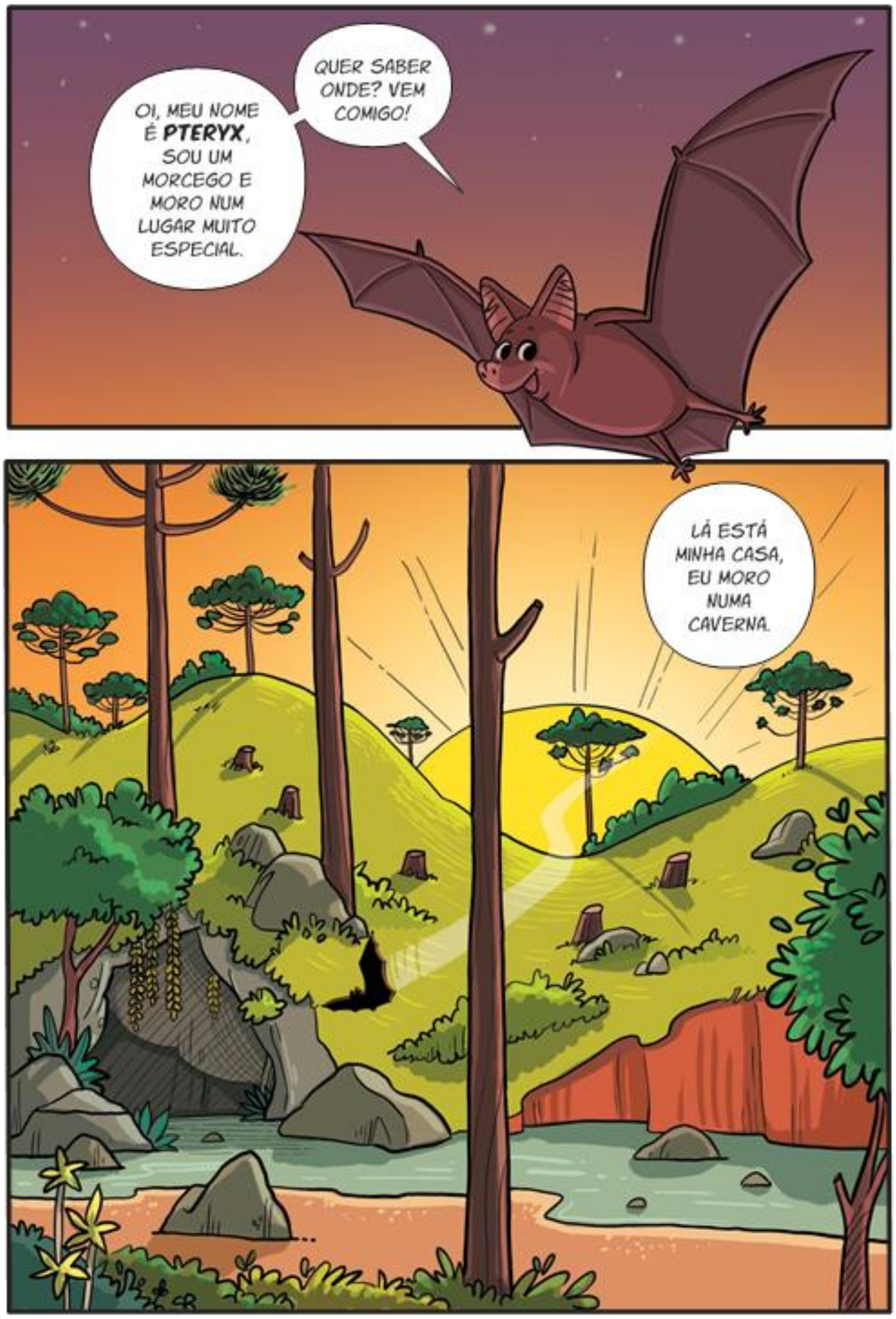
Para concentrar todo o material de divulgação produzido, foi criado um site utilizando o software Strikingly Website Builder (2024). No site, além das cartilhas e vídeos, foram disponibilizadas fotos capturadas durante as atividades de campo, proporcionando maior imersão aos leitores e visitantes.

O site foi organizado em diferentes seções para facilitar o acesso ao conteúdo, incluindo vídeos, cartilhas e demais materiais. Uma seção foi criada para armazenar todo o material que será publicado ao longo das atividades do projeto. Enquanto isso, resumos dos capítulos da tese estão disponíveis para consulta.

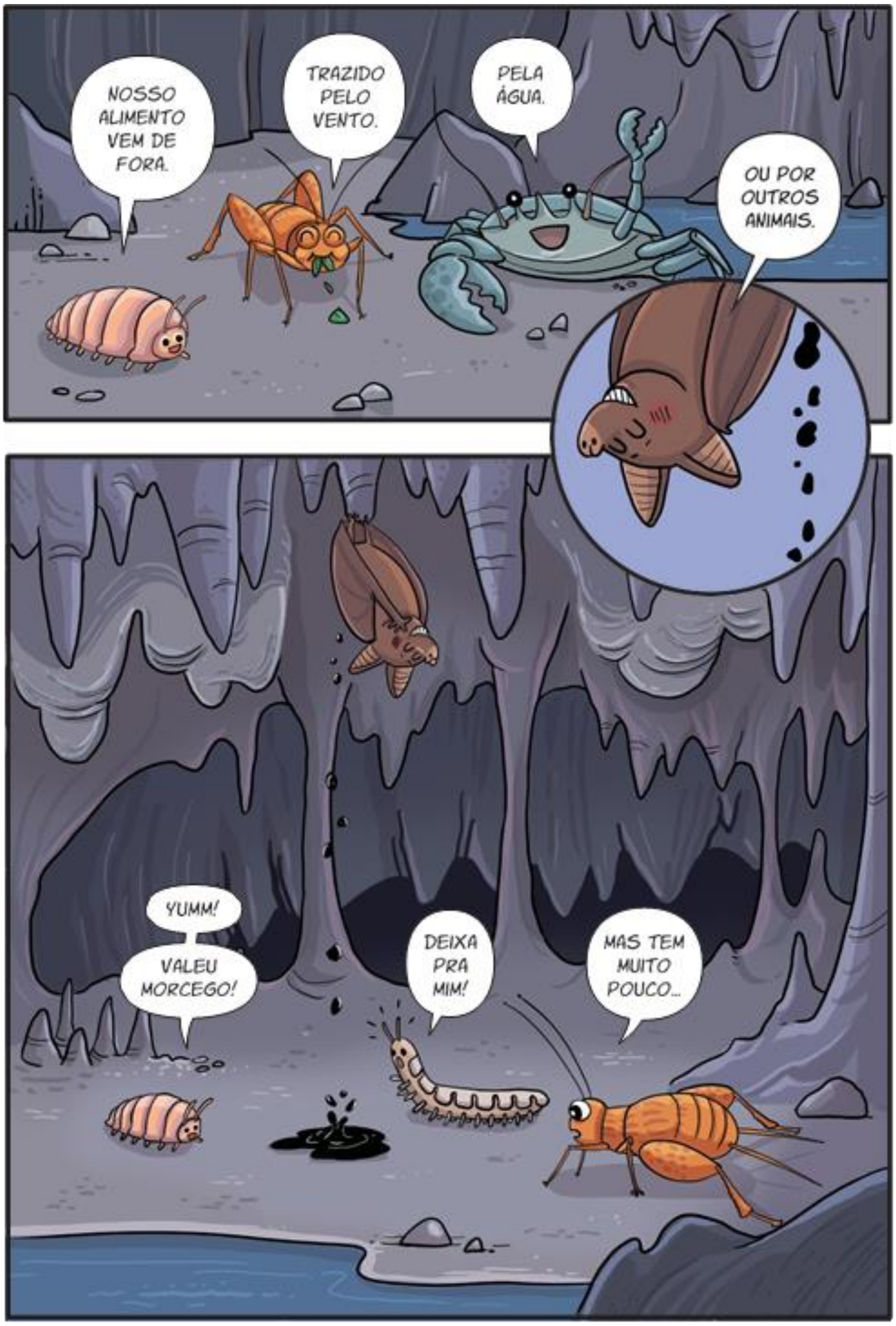
O site pode ser acessado através do seguinte endereço: https://cebsparana2023.mystrikingly.com/.

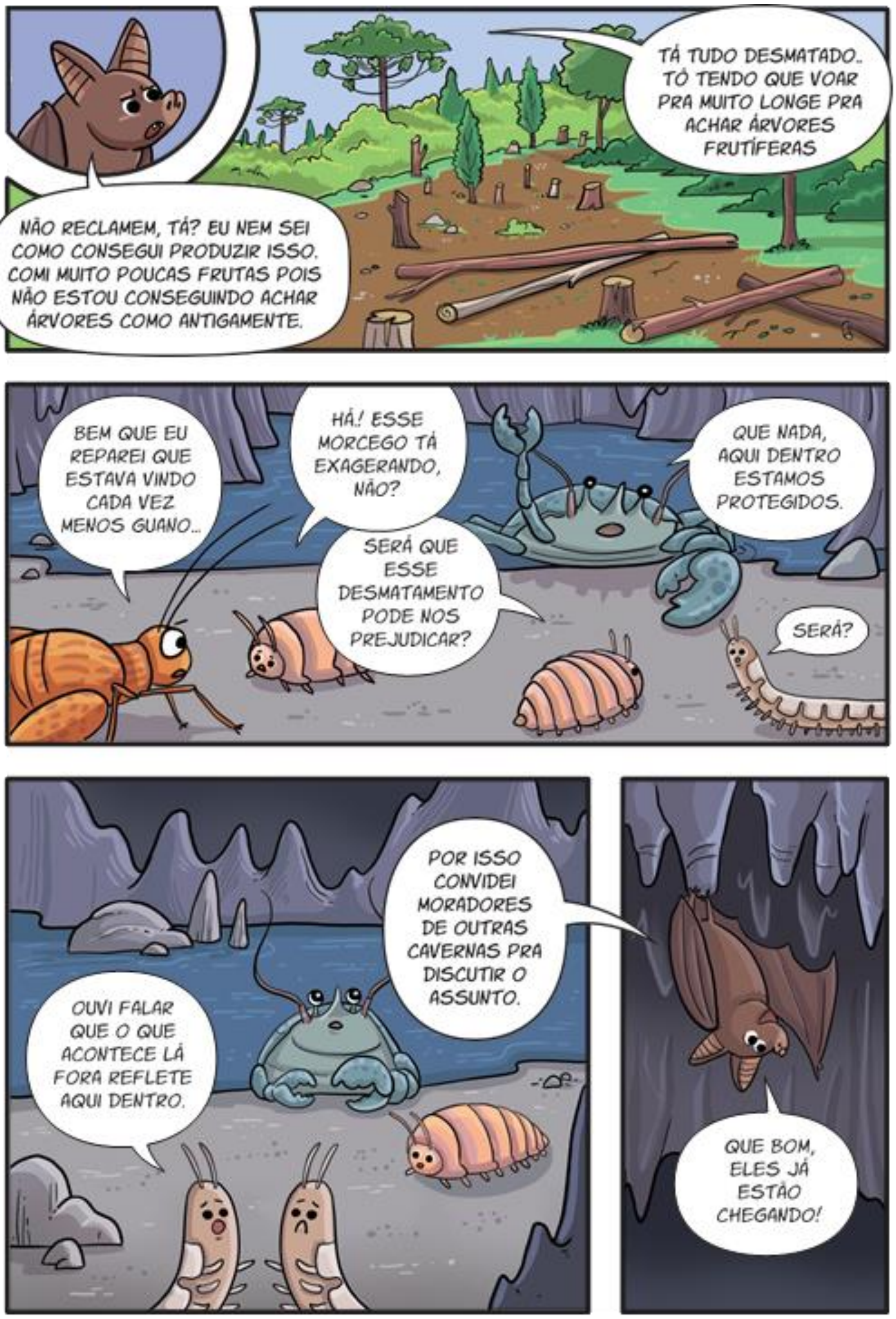
Resultados

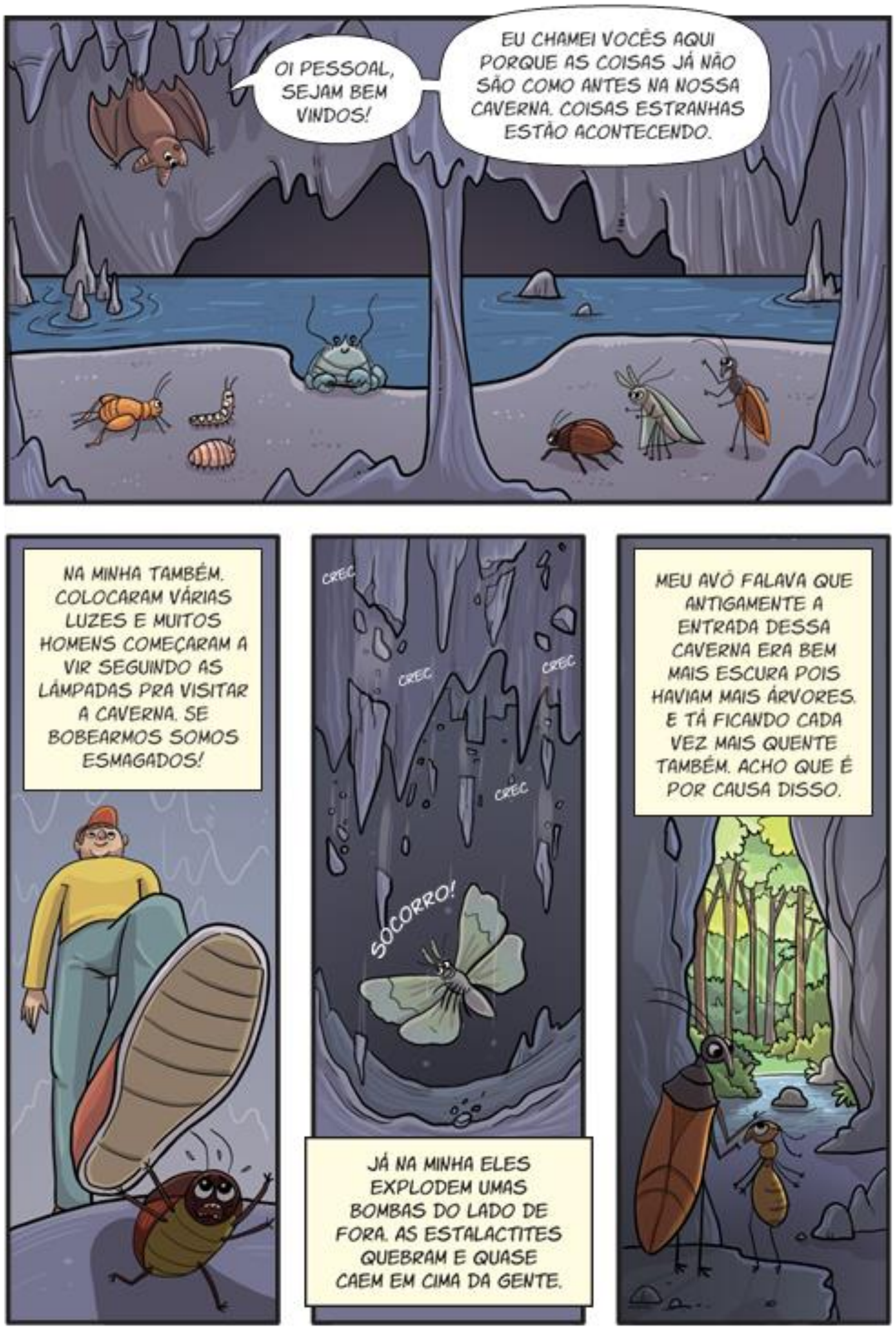
Cartilha 1: A Vida nas Cavernas


OI, MEU NOME É **PTERYX**, SOU UM MORCEGO E MORO NUM LUGAR MUITO ESPECIAL.
QUER SABER ONDE? VEM COMIGO!
LÁ ESTÁ MINHA CASA, EU MORO NUMA CAVERNA.


AS CAVERNAS SÃO GRANDES ESPAÇOS VAZIOS QUE SE FORMAM EM CERTOS TIPOS DE ROCHAS.
VOU TER QUE MOSTRAR AS COISAS RAPIDINHO, POIS TÔ ATRASADO PRA UMA REUNIÃO.
EM LOCAIS LONGE DA ENTRADA NÃO EXISTE LUZ E A TEMPERATURA É CONSTANTE.
POR CAUSA DISSO TAMBÉM NÃO EXISTEM PLANTAS POR AQUI.
MAS A ESCURIDÃO NÃO É PROBLEMA PRA MIM E MEUS AMIGOS, NÃO É MESMO, PESSOAL?
É ISSO AÍ!


NOSSO ALIMENTO VEM DE FORA.
TRAZIDO PELO VENTO.
PELA ÁGUA.
OU POR OUTROS ANIMAIS.
YUMM!
VALEU MORCEGO!
DEIXA PRA MIM!
MAS TEM MUITO POUCO...


TÁ TUDO DESMATADO.. TÔ TENDO QUE VOAR PRA MUITO LONGE PRA ACHAR ÁRVORES FRUTÍFERAS
NÃO RECLAMEM, TÁ? EU NEM SEI COMO CONSEGUI PRODUZIR ISSO. COMI MUITO POUCAS FRUTAS POIS NÃO ESTOU CONSEGUINDO ACHAR ÁRVORES COMO ANTIGAMENTE.
BEM QUE EU REPAREI QUE ESTAVA VINDO CADA VEZ MENOS GUANO...
HÁ! ESSE MORCEGO TÁ EXAGERANDO, NÃO?
QUE NADA, AQUI DENTRO ESTAMOS PROTEGIDOS.
SERÁ QUE ESSE DESMATAMENTO PODE NOS PREJUDICAR?
SERÁ?
OUVI FALAR QUE O QUE ACONTECE LÁ FORA REFLETE AQUI DENTRO.
POR ISSO CONVIDEI MORADORES DE OUTRAS CAVERNAS PRA DISCUTIR O ASSUNTO.
QUE BOM, ELES JÁ ESTÃO CHEGANDO!


OI PESSOAL, SEJAM BEM VINDOS!
EU CHAMEI VOCÊS AQUI PORQUE AS COISAS JÁ NÃO SÃO COMO ANTES NA NOSSA CAVERNA. COISAS ESTRANHAS ESTÃO ACONTECENDO.
NA MINHA TAMBÉM. COLOCARAM VÁRIAS LUZES E MUITOS HOMENS COMEÇARAM A VIR SEGUINDO AS LÂMPADAS PRA VISITAR A CAVERNA. SE BOBEARMOS SOMOS ESMAGADOS!
CREC
CREC
CREC
CREC
SOCORRO!
JÁ NA MINHA ELES EXPLODEM UMAS BOMBAS DO LADO DE FORA. AS ESTALACTITES QUEBRAM E QUASE CAEM EM CIMA DA GENTE.
MEU AVÔ FALAVA QUE ANTIGAMENTE A ENTRADA DESSA CAVERNA ERA BEM MAIS ESCURA POIS HAVIAM MAIS ÁRVORES. E TÁ FICANDO CADA VEZ MAIS QUENTE TAMBÉM. ACHO QUE É POR CAUSA DISSO.

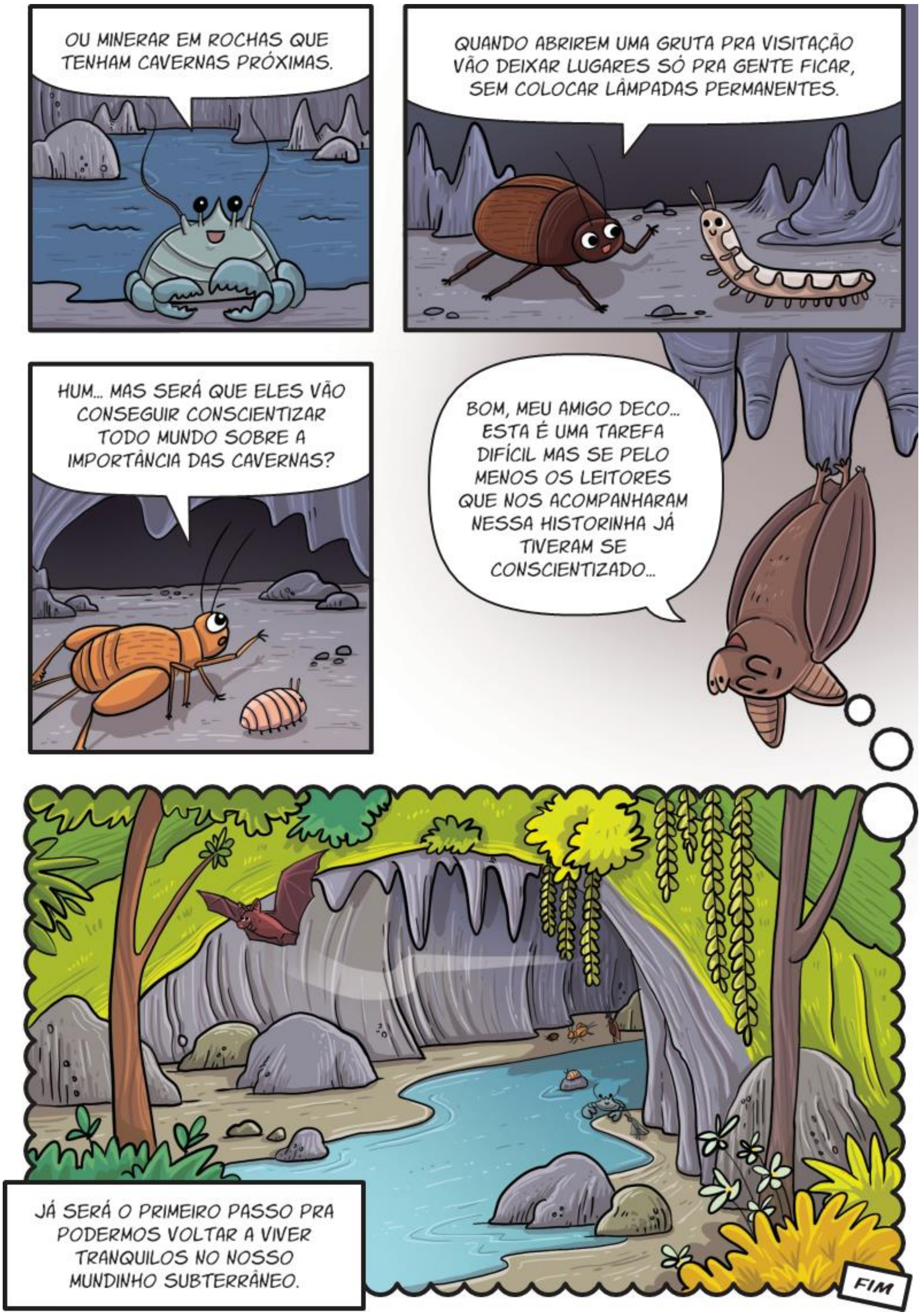

CABRUM!
MAS O QUE É ISSO?
É ÁGUA DA CHUVA!
MAS ELA NUNCA TINHA CHEGADO AQUI NESTE PONTO ANTES!
SÓ PODE SER POR CAUSA DO DESMATAMENTO. SEM AS ÁRVORES LÁ FORA A ÁGUA DA CHUVA ENTRA COM MUITO MAIS FORÇA AQUI DENTRO.
TCHÁÁÁ
BLARGH! ESSA ÁGUA TÁ TERRA PURA! QUASE NÃO CONSIGO RESPIRAR!
A ÁGUA QUE ANTES SÓ TRAZIA ALIMENTO AGORA SÓ TRAZ SUJEIRA!
BUÁÁÁ, O QUE ESTÁ ACONTECENDO? NOSSA VIDA ERA TÃO TRANQUILA NAS CAVERNAS! NOS SENTÍAMOS TÃO PROTEGIDOS QUE NEM LIGÁVAMOS PRA FALTA DE LUZ.


EU SEI O QUE ESTÁ ACONTECENDO. A CULPA É DOS HOMENS!
DOS HOMENS?!
ELES CORTAM AS ÁRVORES NA FRENTE DAS CAVERNAS PARA FAZER PASTO PARA O GADO...
EXPLODEM PAREDÕES DE CALCÁRIO ONDE AS CAVERNAS SE FORMAM PRA FAZER CIMENTO...
BOOOM!
SUPERLOTAM AS CAVERNAS DE TURISTAS COLOCANDO LUZES E NÃO DEIXANDO ESPAÇO PARA OS SERES QUE VIVEM ALI...
JOGAM ESGOTO EM RIOS QUE FLUEM PARA A CAVERNA.

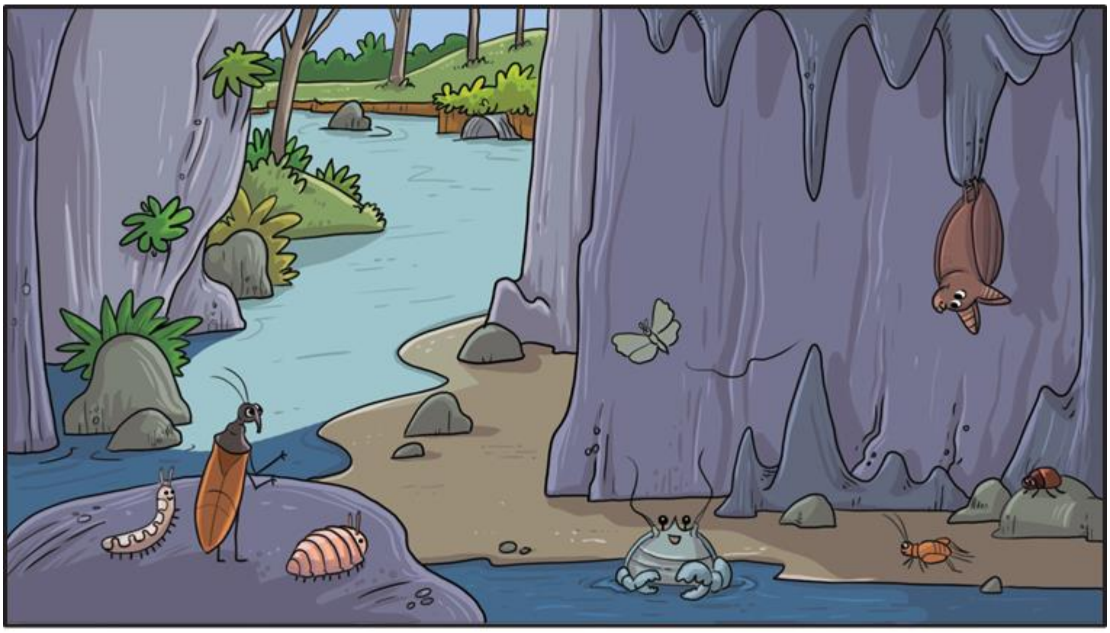

OH NÃO, ESTAMOS PERDIDOS! OS HOMENS VÃO ACABAR COM AS NOSSAS CAVERNAS!
NÃO É POSSÍVEL, TEM QUE HAVER UMA SOLUÇÃO.
OPS, PARECEQUE VEM VINDO ALGUÉM.
É UM HOMEM, FUJAM!
AI MEU DEUS, TOMARA QUE ELE NÃO VEJA A GENTE...
OLÁ AMIGUINHOS, COMO VOCÊS ESTÃO? ESTOU VINDO LÁ DE FORA E A SITUAÇÃO ESTÁ FEIA! COMO É QUE VOCÊS AINDA ESTÃO CONSEGUINDO VIVER AQUI?

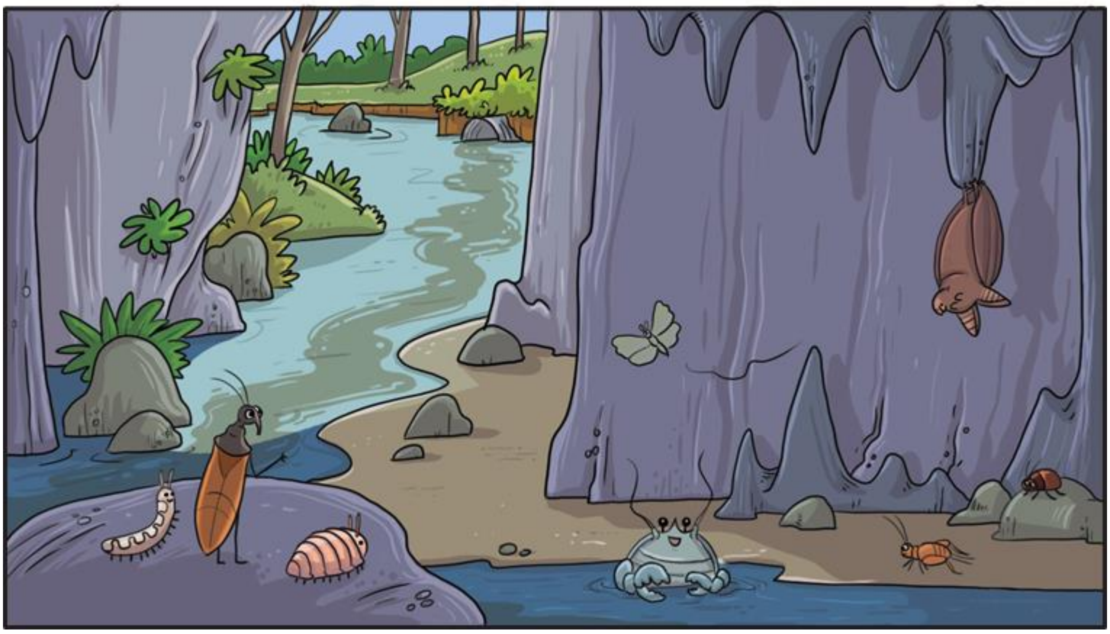

PERAÍ, QUEM É VOCÊ?
EU SOU UM BIOESPELEÓLOGO. DEDICO MINHA VIDA A ESTUDAR AS CAVERNAS E OS ANIMAIS QUE EXISTEM AQUI DENTRO. COM MINHAS PESQUISAS TENTO ENCONTRAR SOLUÇÕES PARA OS PROBLEMAS QUE PODEM AFETAR A VIDA DE VOCÊS. E PELO O QUE ESTOU VENDO AS COISAS NÃO ESTÃO NADA BOAS POR AQUI, NÃO É MESMO?
VOCÊS HUMANOS ESTÃO MALTRATANDO NOSSAS CAVERNAS.
SE CONTINUAR ASSIM VAMOS TER QUE NOS MUDAR PRA BEM LONGE.
ESTAMOS SOFRENDO MUITAS AGRESSÕES.
INFELIZMENTE.
NÃO SE PREOCUPEM, EU VOU CONVERSAR COM AS PESSOAS. ESSA SITUAÇÃO VAI SE RESOLVER.
FAZENDEIRO, EVITE DESMATAR AS ENTRADAS DAS CAVERNAS. A MATA SERVE DE PROTEÇÃO PARA AS GRUTAS E É VITAL PARA A MANUTENÇÃO DA VIDA.
PUXA, EU NÃO SABIA. NÃO VOU FAZER MAIS ISSO.


MINERADOR, ANTES DE COMEÇAR O EMPREENDIMENTO FAÇA UM BOM ESTUDO LOCAL PRA VER SE NÃO EXISTEM CAVERNAS PRÓXIMAS E SE ACHAR, MUDE O LOCAL DA MINA. AS EXPLOSÕES DANIFICARAM O AMBIENTE CAVERNÍCOLA.
OK, VOU TOMAR MAIS CUIDADO.
EMPRESÁRIO, QUANDO ABRIR UMA CAVERNA À VISITAÇÃO FAÇA UM ESTUDO PARA SABER O MELHOR LOCAL PARA AS PESSOAS PASSAREM SEM PREJUDICAR OS BICHOS.
OK, VOU FAZER ISSO.
ALPES
CAVERNAS
POXA, ESSES BIOESPELEÓLOGOS SÃO MUITO IMPORTANTES...
ENTÃO NEM TUDO ESTÁ PERDIDO.
À MEDIDA QUE O TRABALHO DOS BIOESPELEÓLOGOS FOR DIVULGADO, OS OUTROS HUMANOS APRENDERÃO A TOMAR MAIS CUIDADO COM AS CAVERNAS.
E NÃO VÃO MAIS DESMATAR NAS ENTRADAS DAS GRUTAS.


OU MINERAR EM ROCHAS QUE TENHAM CAVERNAS PRÓXIMAS.
QUANDO ABRIREM UMA GRUTA PRA VISITAÇÃO VÃO DEIXAR LUGARES SÓ PRA GENTE FICAR, SEM COLOCAR LÂMPADAS PERMANENTES.
HUM... MAS SERÁ QUE ELES VÃO CONSEGUIR CONSCIENTIZAR TODO MUNDO SOBRE A IMPORTÂNCIA DAS CAVERNAS?
BOM, MEU AMIGO DECO... ESTA É UMA TAREFA DIFÍCIL MAS SE PELO MENOS OS LEITORES QUE NOS ACOMPANHARAM NESSA HISTORINHA JÁ TIVERAM SE CONSCIENTIZADO...
JÁ SERÁ O PRIMEIRO PASSO PRA PODERMOS VOLTAR A VIVER TRANQUILOS NO NOSSO MUNDINHO SUBTERRÂNEO.
FIM

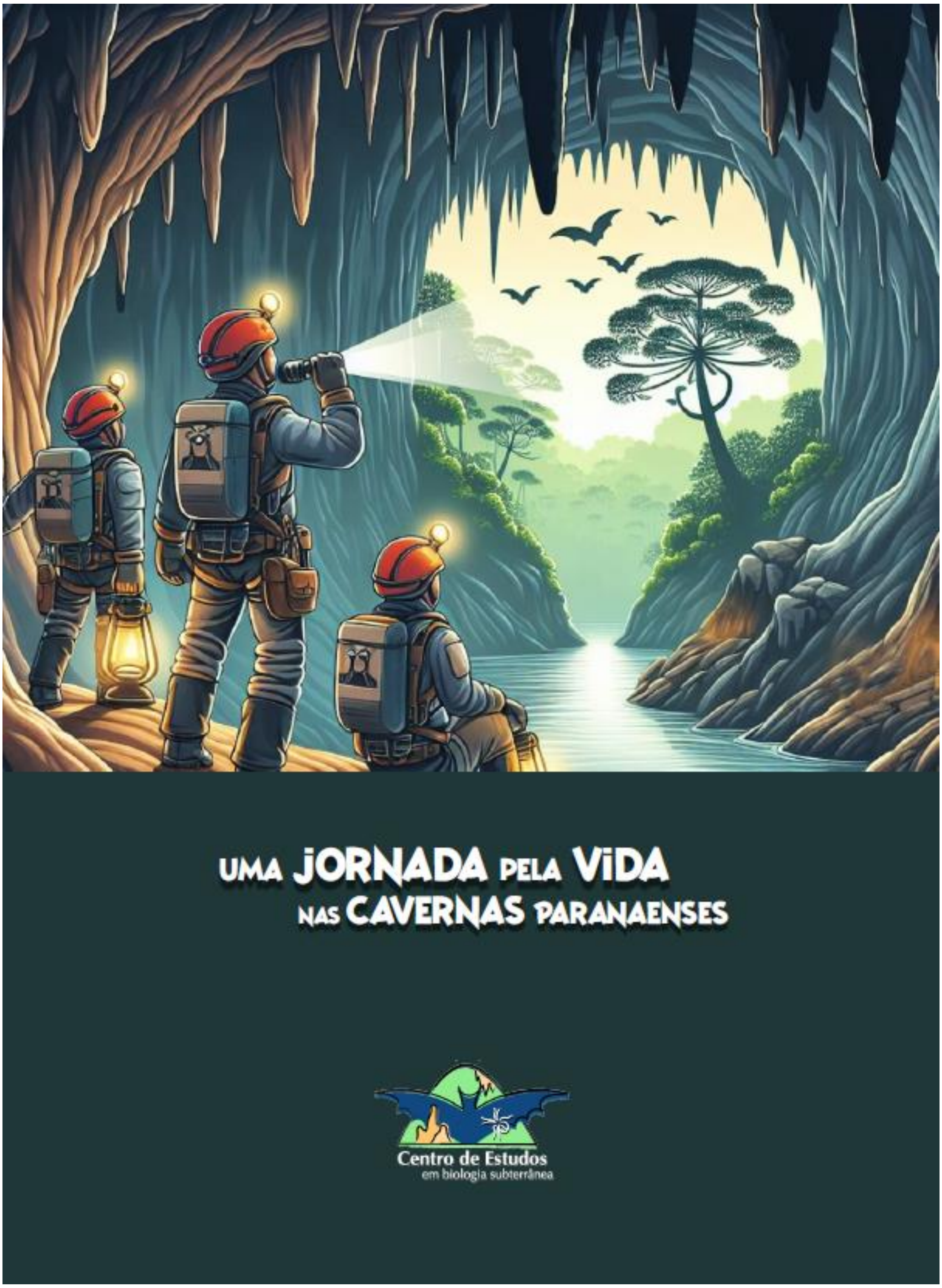
NA ﬁGURA B EXISTEM 7 ERROS GRAVES EM RELAÇÃO A ﬁGURA A, QUE PODEM PREJUDICAR OS NOSSOS AMIGUINHOS DAS CAVERNAS. VAMOS ENCONTRAR ESTES ERROS E AJUDAR A SALVAR AS CAVERNAS.





1. PATA DO PERCEVEJO, 2. ESTALACTITE À ESQUERDO EM CIMA, 3. OLHO DO MORCEGO, 4. ESTALACTITE À DIREITA EM CIMA, 5. ESGOTO NO RIO, 6. PEDRA PERTO DO GRILO, 7. PATAS DO SIRI

Cartilha 2: Uma Jornada Pela Vida nas Cavernas Paranaenses




BEM VINDO!
Convidamos a uma viagem ao interior das cavernas do Paraná, um mundo subterrâneo onde a escuridão abriga seres únicos e ainda pouco conhecidos.

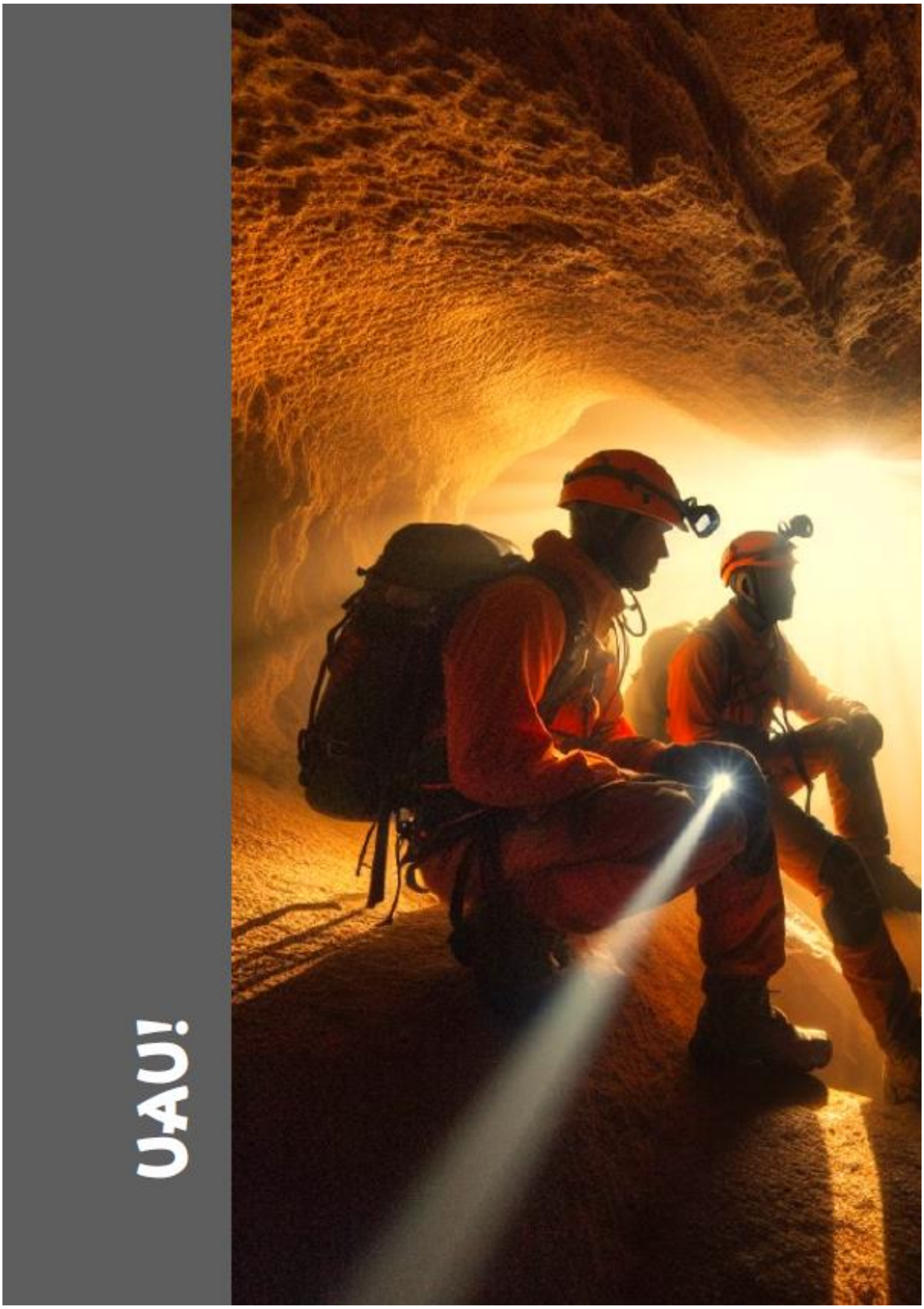
VAMOS EXPLORAR?

O entorno das cavernas é protegido por vegetação da Mata Atlântica úmida, com arvores altas e imponentes incluindo o Pinheiro do Paraná (*Araucaria angustifolia*). Embaixo da copa das árvores ocorrem muitas espécies de Musgos e samambaias, incluso o Samambaiaçu (*Dicksonia sellowiana*).


Acari:
Opilioacaridae
Restos de animais,
ossos e fósseis
Orthoptera:
Endecous sp
UAU!

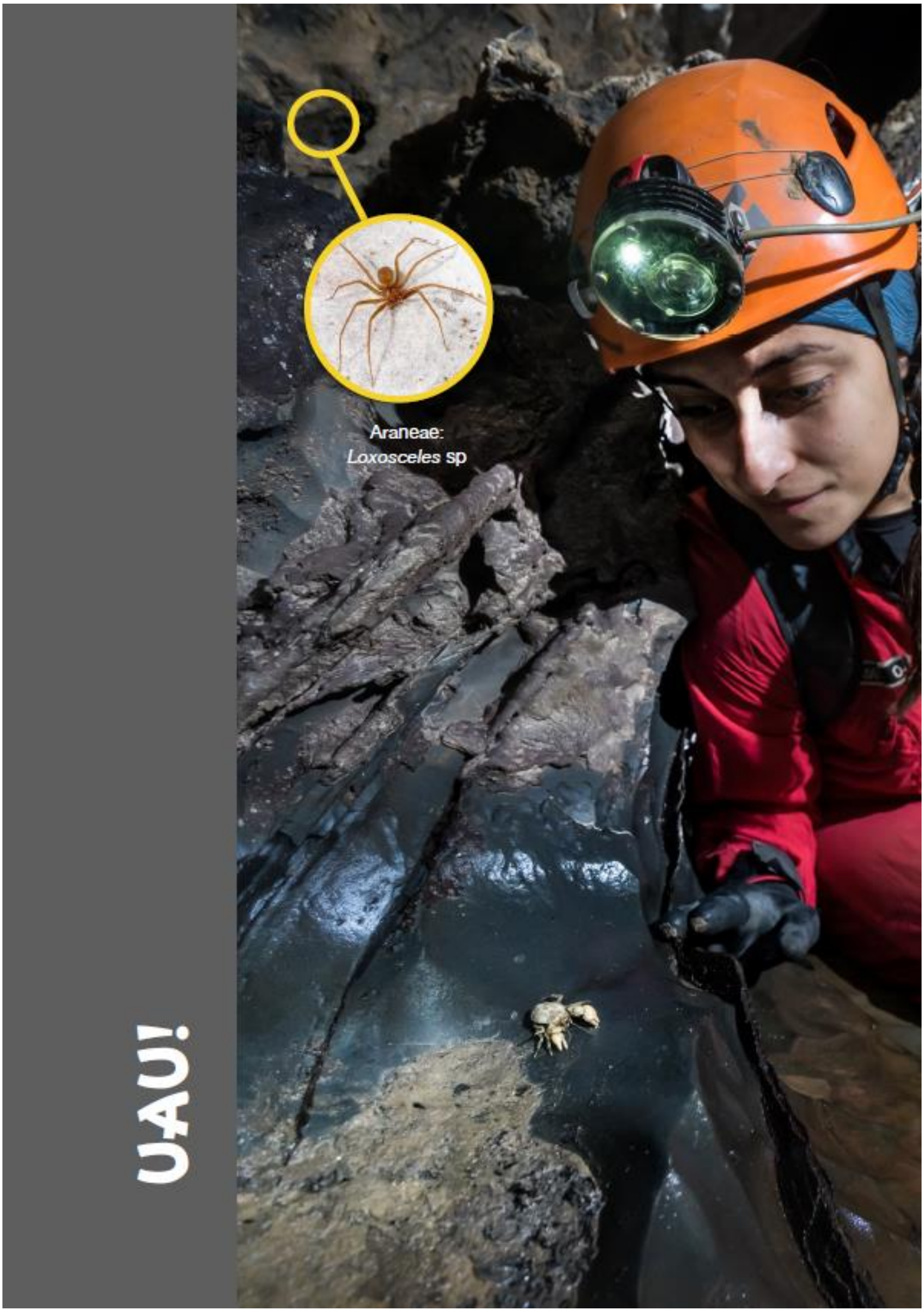
O que logo descobrimos?

Logo na entrada, ainda com um pouco de luz e plantas, já encontramos inúmeras espécies de pequenos animais chamados de invertebrados, que gostam da umidade das cavernas.
Muitos são trogloxenos podendo entrar e sair livremente das cavernas.
Um bom exemplo de trogloxeno são os morcegos. Eles usam as cavernas como abrigo diurno e saem a noite para se alimentar de Insetos, frutos, sementes nas matas do entorno das cavernas.
As fezes de morcegos depositadas nas cavernas (Guano) servem de alimento para invertebrados.


UAU!

Espeleólogos e biólogos?

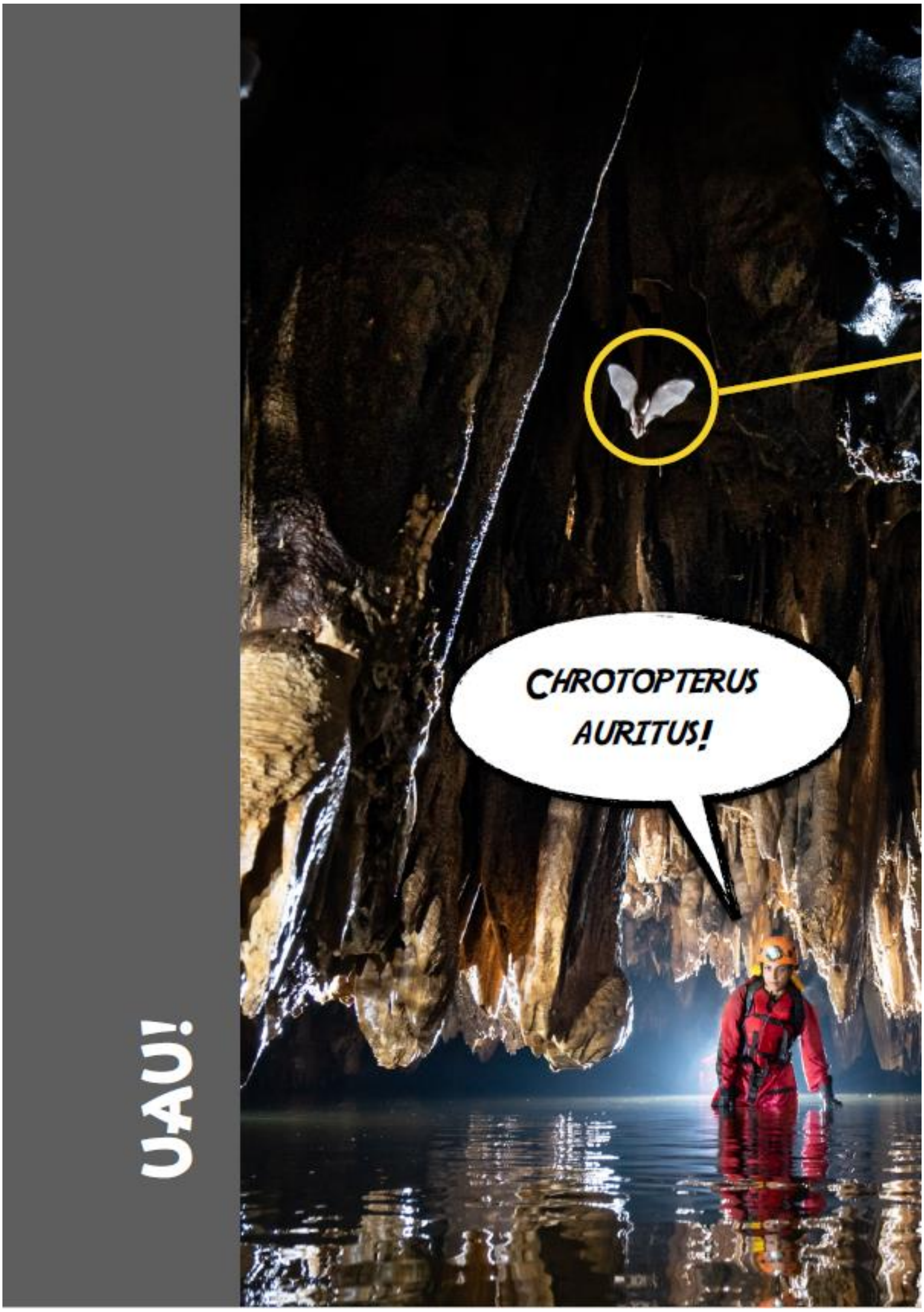
Isso mesmo, biólogos que estudam animais que vivem dentro das cavernas também são espeleólogos. Eles exploram os ambientes subterrâneos descobrindo e descrevendo novas espécies ainda desconhecidas da ciência. Essa especialidade se chama "bioespeleologia". Existem diferentes laboratórios de biologia subterrânea em universidades ao longo do Brasil. Se você gosta de biologia, de cavernas e de aventura, procure uma universidade ou centro de pesquisa perto de você para conhecer essa área fascinante!

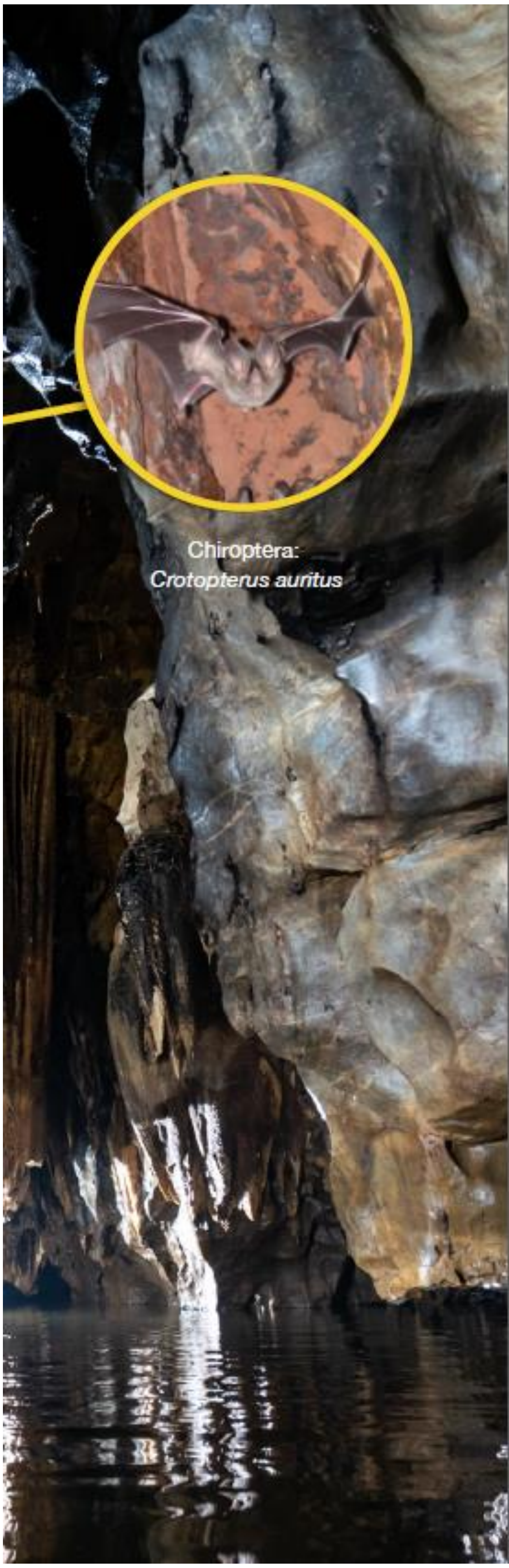

Araneae:
Loxosceles sp
UAU!

Decapoda:
Aegla sp

E o que é isso?

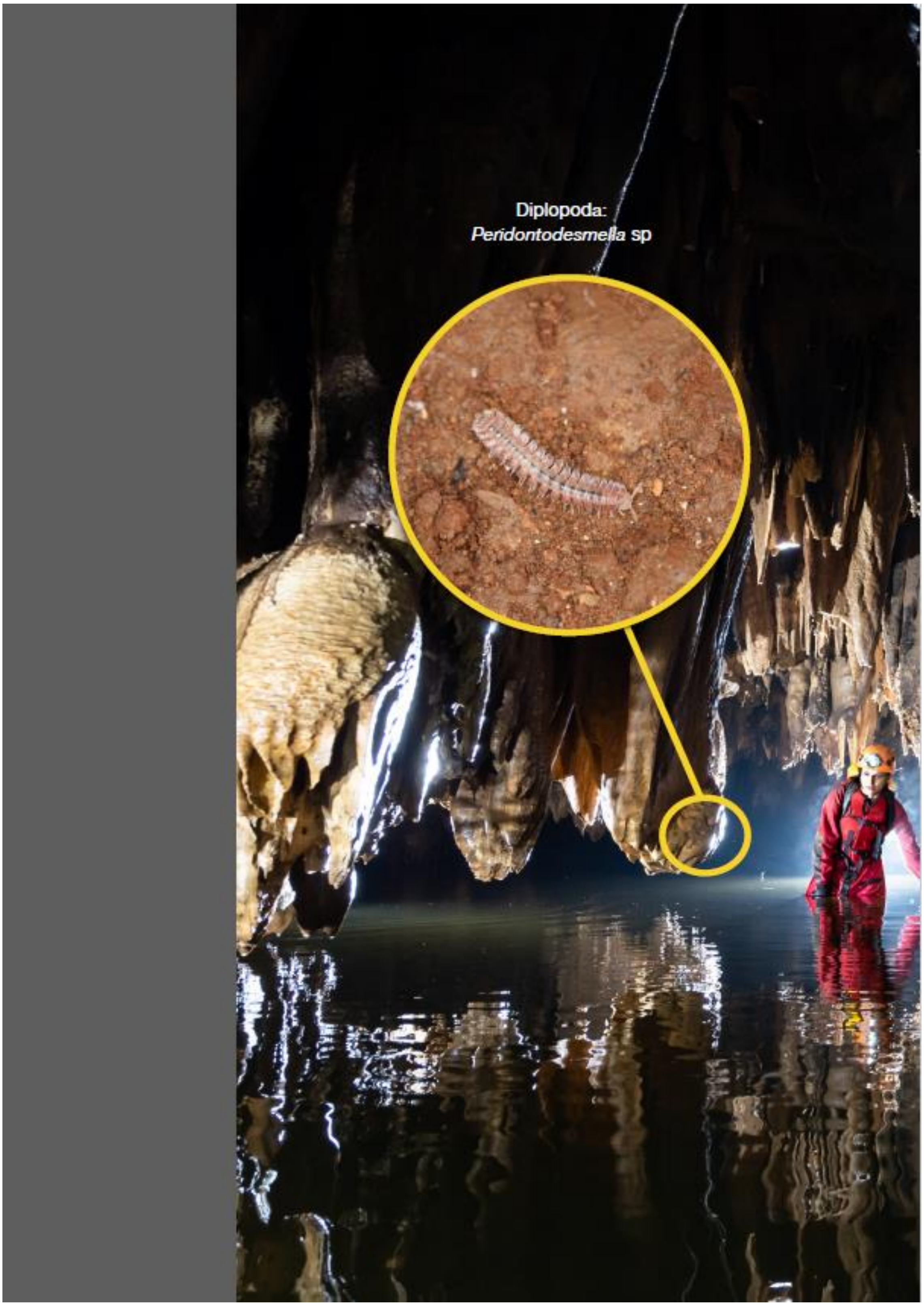
Um pouco mais adentro em uma região escura e mais úmida, encontramos uma fauna bem diferente e não há mais plantas aqui, pela escassez da luz e de nutrientes. Os invertebrados troglófilos são comuns. Troglófilos usam cavernas como local de vida, mas possuem indivíduos da mesma espécie vivendo fora de cavernas em locais semelhantes (solo de florestas, embaixo de pedras, buracos em troncos). Nas cavernas usam como alimentos as fezes dos morcegos e restos vegetais mortos que foram levados para dentro das cavernas pelo agua de rios e enxurradas.


UAU!
CHROTOPTERUS AURITUS!



animais restritos e especializados

Apesar da escassez de nutrientes nas partes mais distantes da entrada, a temperatura e umidade são constantes por quase todo o ano, fazendo com que essas condições sejam ideais para alguns grupos de invertebrados.
Portanto, ao adentrar nas cavernas, animais cada vez mais especializados a viver nesse ambiente (troglóbios), podem ser vistos e descobertos.


Diplopoda:
Peridontodesmella sp

Distante da superfície

Nos locais ainda mais profundos encontramos uma fauna mais especializada a viver nas cavernas, os troglóbios; Troglóbios são geralmente cegos e despigmentados. Entretanto, para compensar, possuem estruturas sensoriais (táteis e olfativas) altamente desenvolvidas. Na escuridão total utilizam outros sentidos para locomoção e capturar alimentos. Troglóbios são restritos às cavernas e incapazes de estabelecer populações fora. Essa especificidade de habitat lhes confere altíssima vulnerabilidade aos impactos ambientais que são frequentes.

Diversidade da fauna troglófila do Paraná

Foram identificadas 365 espécies de invertebrados em cavernas do Paraná, a maioria dos quais são troglófilos, adaptados para viver tanto no ambiente cavernícola quanto fora dele. Esses organismos desempenham um papel crucial no ecossistema das cavernas devido à sua capacidade de se adaptarem a diferentes condições ambientais, contribuindo para a biodiversidade desses sistemas.

Hirudinea: Arhynchobdellida
Utilizam cavernas como abrigo, sendo encontradas tanto em ambientes aquáticos como terrestres em cavernas do Paraná. Apesar de serem conhecidas como sanguessugas, as Arhynchobdellida são predadores de outros invertebrados (caramujos, minhocas, lesmas). Predam ovos, jovens e adultos.

Arachnida: Acari
A subclasse Acari é uma das mais abundantes em cavernas do Paraná, sendo representada por diversas espécies, evidenciando a importância desse grupo na ecologia subterrânea.

Conheça toda fauna identificada nas cavernas do Paraná no site do projeto

Scorpiones: Bothriuridae
Os escorpiões da família Bothriuridae, são pequenos e frequentemente de cor escura, apesar de estarem presentes em cavernas, não são encontrados com frequência. Esta família de escorpiões não abriga espécies de importância médica.

Araneae: *Loxosceles* sp
Aranha de coloração marrom, geralmente encontrada em cavernas secas, são consideradas de grande importância em saúde pública por possuírem um veneno necrosante, entretanto não são consideradas aranhas agressivas.

Pseudoscorpiones: Chernetidae
Invertebrado abundante em cavernas do Paraná, encontrados principalmente no solo e por baixo de pedras. São predadores de pequenos invertebrados.

Orthoptera: *Endecous* sp
Grilos que vivem em fendas e buracos no solo, sob serrapilheira e outros ambientes, sendo facilmente encontrados em cavernas. Possuem uma grande importância para o ecossistema das cavernas, uma vez que se alimentam de guano e detritos vegetais.

Hemiptera: Cixiidae
Pequenas cigarrinhas, com algumas espécies cavernícolas (troglóbias), que se alimentam principalmente de raízes da vegetação externa que penetram as cavidades em busca de umidade.

diversidade de fauna troglóbia do Paraná

O Paraná abriga uma elevada riqueza de espécies troglóbias que era anteriormente subestimada, destacando a importância de estudos em cavernas. Esses irão permitir a descoberta e proteção de mais espécies, destacando a necessidade contínua de estudar e proteger esses ambientes.
Alguns desses incríveis organismos vivem exclusivamente em uma única caverna e em pequenas populações, portanto qualquer impacto no ambiente pode apresentar uma grande ameaça a esses animais.

Palpigradi: *Eukoenenia* sp
Os Palpigradi são aracnídeos de pequeno porte e predadores, caracterizados por seus grandes flagelos. Devido ao seu tamanho diminuto, são difíceis de serem visualizados, o que requer atenção meticulosa por parte dos biólogos e especialistas para encontrá-los.

Diplopoda: *Crypturodesmus* sp
Troglóbio abundante em cavernas do norte do Paraná, se trata de um diplopoda despigmentado, se move lentamente no solo de cavernas, sendo encontrado principalmente em depósitos de guano.

Collembola: *Acherontides* sp
Trata-se do invertebrado troglóbio mais abundante nas cavernas do norte do Paraná, pode ser encontrado em diversas cavernas da região e em grandes populações, sempre associados a depósitos de guano.

Opiliones: Gonyleptidae

Nova espécie de opilião troglóbio, não oficialmente descrita, encontrado na gruta Bacaetava. Nas cavernas os opiliões podem ser predadores, saprófagos e/ou onívoros.

Pseudoscorpiones: Chtoniidae

Espécie troglóbia, apresentando corpo despigmentado, encontrado em baixa densidade e até o momento em apenas uma caverna no norte do Paraná. É um Aracnídeo semelhante aos escorpiões, entretanto, não possui o ferrão no final do abdome e não oferece risco a saúde humana.

Isopoda: Philosciidae

São tatuzinhos (crustáceos isópodos terrestres). Este troglóbio da foto ocorre nas cavernas do Norte do Paraná se alimentando de detritos vegetais e guano.

1) *Eukoenenia* sp 2) Arrhopalitidae sp 3) Philosciidae sp 4) Stylommatophora sp 5) *Eukoenenia* sp 6) *Trichorhina* sp.

Conheça toda fauna identificada nas cavernas do Paraná no site do projeto

Um patrimônio ameaçado

O norte do Paraná abriga as maiores cavernas do estado, dotadas de uma beleza cênica singular. No entanto, esse ecossistema está enfrentando uma série de ameaças provocadas pelas atividades humanas. A mineração, o desmatamento e o turismo intenso, desacompanhado de medidas adequadas, estão deixando um impacto significativo. Este impacto se manifesta na presença de lixo nas cavidades, no pisoteamento de animais e em outras formas de degradação.

Para garantir a preservação dessas cavernas, é urgente a implementação de medidas eficazes. É fundamental que todos nós, ao conhecermos mais sobre esse ambiente e os animais que lá vivem, possamos nós unir nesse desafio de preservação.

Desmatamento

O desmatamento exerce uma influência direta na quantidade de recursos vitais que são levados para dentro das cavernas. A destruição da vegetação nos arredores das cavernas resulta em uma redução significativa na transferência de restos vegetais para o interior das cavidades. Isso tem como consequência o empobrecimento do ambiente subterrâneo em termos de alimentos disponíveis para a fauna local, o que pode acarretar em seu declínio e, eventualmente, em sua extinção.

Mineração

A atividade de mineração pode levar a destruição completa de cavidades, resultando na perda de numerosas espécies. Muitas vezes, essas espécies desaparecem antes mesmo de serem descobertas e catalogadas por pesquisadores, privando-nos da oportunidade de conhecer sua existência.

Turismo desregulamentado

O turismo desregulamentado pode levar a uma série de impactos para as cavernas, como a destruição de espeleotemas, pisoteamento de animais, presença de lixo, pichações entre outros que são comumente vistos nas cavernas do norte do Paraná, que podem impactar diretamente a fauna e o ecossistema cavernícola.

Urbanização

Muitas das cavernas localizadas no norte do Paraná estão em proximidade com centros urbanos em expansão. O avanço da urbanização representa uma séria ameaça a esses ambientes naturais. Estabelecer áreas de preservação ao redor dessas cavernas para protegê-las do crescente desenvolvimento urbano é essencial. Essas medidas são importantes para garantir a conservação desses ecossistemas e para evitar sua degradação devido à expansão das cidades.

Termo de Compromisso de Compensação Espeleológica n.01/2021 · Coordenação Executiva · Gestão Operacional · Parceiro Executor

Série de Vídeos: Resultados do Projeto, Formação Profissional e Desafios na Proteção de Cavernas

Três vídeos foram desenvolvidos com o objetivo de ampliar a divulgação dos trabalhos realizados. O primeiro vídeo apresenta uma visão abrangente do desenvolvimento do projeto, destacando a rotina envolvida nas atividades de campo, além dos principais resultados obtidos, como a identificação das cavernas com maior biodiversidade no estado e aquelas mais afetadas por impactos ambientais. Voltado para o público em geral, o vídeo busca informar e conscientizar sobre a importância da preservação desses ecossistemas subterrâneos, mostrando de forma acessível as descobertas e os desafios enfrentados durante a pesquisa.

O segundo vídeo foca na evolução profissional dos alunos envolvidos no projeto, com o objetivo de inspirar e atrair novos potenciais estudantes que possam contribuir para a preservação do patrimônio espeleológico brasileiro. Por fim, o terceiro vídeo aborda os desafios relacionados à regulamentação e proteção das cavernas no Brasil, apontando possíveis caminhos e soluções para aprimorar esse cenário.

Os vídeos e cartilhas desenvolvidos estão disponíveis no site do projeto, acessível em: https://cebsparana2023.mystrikingly.com/videos. Além disso, as cartilhas também podem ser encontradas no site do Centro Nacional de Pesquisa e Conservação de Cavernas (CECAV), no link: https://www.gov.br/icmbio/pt-br/assuntos/noticias/ultimas-noticias/conheca-publicacoes-do-instituto-chico-mendes-e-parceiros-voltadas-para-o-publico-infantil.

Discussão

No Brasil, o impacto sobre o patrimônio espeleológico tem sido motivo de grande preocupação, especialmente devido ao crescimento do turismo desregulamentado e às pressões constantes de atividades como expansão urbana, mineração, desmatamento e agropecuária (Lino, 2001; Alexander, 2021; Chiarini et al., 2022). Como consequência, diversos estudos têm sido realizados para esclarecer os impactos ambientais causados pelo espeleoturismo e pelo avanço das atividades antrópicas (Ferreira et al., 2010; Souza-Silva et al., 2015; Silva et al., 2018).

Esses desafios são exacerbados na Mata Atlântica, onde recorrentes impactos resultaram na preservação de apenas 12,4% de sua cobertura vegetal original, conforme dados do Atlas da Mata Atlântica 2019-2020 (SOS Mata Atlântica, 2020). Nesse cenário, Ferreira et al. (2008) desenvolveram a cartilha “Aventura da Vida nas Cavernas” como parte de um trabalho focado na preservação de cavernas nesse bioma, destacando a importância da conservação dessas áreas.

Nesse contexto, acreditamos que um foco regional mais específico possa gerar maior engajamento do público. Por isso, desenvolvemos cartilhas voltadas para a região metropolitana de Curitiba. As cavernas e a biodiversidade associada estão sob pressão significativa devido às atividades antrópicas, ressaltando a necessidade de conscientização e preservação. Diante disso, a educação ambiental assume um papel importante para minimizar esses impactos, promovendo a conscientização sobre a importância dos ambientes cavernícolas, seu funcionamento e como nossas atividades podem afetá-los. A partir desse entendimento, é possível adotar medidas eficazes para garantir a preservação desses ecossistemas (Ferreira et al., 2008). Com esse objetivo, decidimos incluir a fauna das cavernas do Paraná em nossas cartilhas, buscando promover uma maior conexão e entendimento em relação à fauna local. Conforme sugerido por Nascimento et al. (2020), o uso de materiais com um contexto regional facilita a identificação do público com o conteúdo, promovendo uma maior assimilação e engajamento da população local.

A cartilha "A Vida nas Cavernas" foi desenvolvida como material didático complementar para o ensino fundamental I e II, alinhada aos conteúdos de Ciências. A cartilha aborda os principais problemas ambientais que afetam as cavernas da região metropolitana de Curitiba, destacando a singularidade da fauna subterrânea. Com uma abordagem lúdica, o material também apresenta medidas práticas que podem ser adotadas para mitigar esses impactos, incentivando a preservação desses ecossistemas.

A cartilha "Uma Jornada Pela Vida nas Cavernas Paranaenses" explora as características ambientais das cavernas e como essas condições se alteram ao adentrar uma cavidade, além de destacar a relação da fauna com esse gradiente ambiental. Utilizando imagens reais da fauna troglóbia e não troglóbia das cavernas da região metropolitana de Curitiba, o material foi desenvolvido principalmente para apoiar atividades de educação ambiental no Parque Estadual de Campinhos e no Parque Municipal Gruta do Bacaetava. Além disso, a cartilha pode complementar iniciativas de educação ambiental em toda a região.

O site e os vídeos foram desenvolvidos para um público amplo, mas com um foco especial em alunos de graduação, pesquisadores e interessados em espeleologia e bioespeleologia. Eles destacam as atividades de pesquisa por meio de vídeos e fotos, promovendo o projeto de forma acessível. Além disso, o site serve como um canal de divulgação para os trabalhos científicos que serão publicados como parte da pesquisa realizada.

O uso de vídeos na educação ambiental é defendido por autores, que apontam essa ferramenta como eficaz para apresentar conteúdos de ciências relacionados a questões ambientais, além de incentivar o questionamento crítico sobre os problemas ambientais presentes no cotidiano dos espectadores, tornando o aprendizado mais dinâmico e contextualizado (Kollas 2015; Guedes 2015). Abordar especificamente as questões das cavernas na região metropolitana de Curitiba por meio dessa mídia pode aproximar o público dos desafios locais, promovendo uma maior conscientização e engajamento com a realidade ambiental da região. Além disso, abordamos também questões de relevância nacional, demonstrando a amplitude das questões ambientais associadas aos ambientes cavernícolas.

Para que os produtos deste trabalho alcancem seus objetivos de conscientização e preservação, são fundamentais a parceria com as escolas da região metropolitana de Curitiba e as unidades de conservação. As instituições de ensino desempenham um papel central ao integrar a educação ambiental em suas práticas pedagógicas, enquanto as unidades de conservação, como o Parque Estadual de Campinhos e o Parque Municipal Gruta do Bacaetava, oferecem um cenário prático para vivenciar os conteúdos abordados. A sinergia entre educação formal e experiências em campo, aliada ao uso dos materiais didáticos produzidos, potencializa o impacto positivo das iniciativas, promovendo um maior entendimento da importância da preservação das cavernas e da fauna associada entre estudantes e a comunidade local.

Considerações Finais

Os materiais produzidos e apresentados, constituem um importante recurso pedagógico, com potencial para ser utilizado em ações de educação ambiental direcionadas a diferentes públicos e faixas etárias. Com ênfase na região metropolitana de Curitiba, esses materiais oferecem uma abordagem acessível para promover a conscientização sobre a preservação dos ecossistemas cavernícolas e sua biodiversidade. A utilização dessas ferramentas auxiliara na preservação das cavernas presentes na região.

Recomendamos a utilização deste material como uma ferramenta para fomentar a discussão sobre as pressões enfrentadas pelas cavernas e sua fauna associada, despertando na população a consciência sobre a importância de preservar esses ambientes, ao promover o entendimento de seu valor ecológico e sua relevância para todos, esperamos assim incentivar um maior compromisso com a proteção dessas áreas.

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TERCEIRA PARTE

3 Considerações Finais

Com base nos temas abordados nesta tese, concluímos que o estudo da ecologia de invertebrados cavernícolas tem revelado importantes descobertas sobre a biodiversidade e a conservação desses habitats subterrâneos. A pesquisa destacou o papel de variáveis ambientais como temperatura, distância da entrada, diversidade de abrigos e substratos na estruturação das comunidades de invertebrados, evidenciando a importância da análise em diferentes escalas espaciais para compreensão da composição e riqueza dessas espécies.

O segundo capítulo, que revisa a fauna troglóbia no estado do Paraná, ressalta a importância de um maior reconhecimento e valorização da biodiversidade subterrânea na região. O estudo demonstrou que, até o início da pesquisa, a fauna troglóbia do Paraná era amplamente desconhecida, e as coletas realizadas revelaram uma riqueza significativa de espécies previamente não documentadas. Essa descoberta demonstra a eficácia dos métodos de amostragem em diferentes escalas, que permitiram identificar uma diversidade de organismos troglóbios que enriquece o conhecimento científico, e aponta para a urgência em intensificar os esforços de conservação.

O estudo também trouxe à tona a importância da conservação de cavernas prioritárias no Paraná, demostrando que a região metropolitana de Curitiba apresenta um percentual alta de cavernas de extrema e alta prioridade de conservação, apontando a necessidade de ações imediatas para proteger esses ecossistemas frente à crescente ameaça de atividades humanas, como o crescimento urbano, a mineração e o desmatamento, que comprometem a fauna cavernícola e os processos ecológicos essenciais para sua sobrevivência.

Adicionalmente, a revisão sistemática demonstrou que as pesquisas na área têm sido amplamente repetitivas quanto às variáveis analisadas, deixando lacunas significativas em campos da ecologia como sucessão ecológica, interações bióticas e metacomunidades. Isso sugere a necessidade de uma diversificação nos enfoques das investigações para melhorar a compreensão das dinâmicas ecológicas em ambientes subterrâneos.

Para o futuro, é essencial direcionar esforços para explorar novas variáveis e metodologias que possam enriquecer a pesquisa em ecologia subterrânea. Isso contribuirá para um entendimento mais profundo das comunidades de invertebrados cavernícolas, além de auxiliar a desenvolver estratégias de conservação, assegurando a proteção da biodiversidade cavernícola em face das crescentes ameaças.

Por fim, ressaltamos a importância de desenvolver materiais de educação ambiental que abordem questões específicas da região de estudo. Esse enfoque regional facilita uma maior conexão entre a população e os problemas ambientais locais, promovendo o senso de responsabilidade e conscientização sobre a preservação. Através de estudos que podem ser divulgados por meio de cartilhas, vídeos e outros recursos didáticos, é possível engajar a comunidade de forma eficaz, incentivando práticas sustentáveis e a valorização do patrimônio natural da região.