



VITOR GABRIEL PEREIRA JUNTA

**A INFLUÊNCIA DA ESTRUTURA DE HABITAT SOBRE AS
COMUNIDADES DE INVERTEBRADOS SUBTERRÂNEOS
DA REGIÃO DE SANTANA, BAHIA.**

LAVRAS-MG

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Orientador

Prof. Dr. Rodrigo Lopes Ferreira

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Resumo: O presente trabalho visa avaliar a influência da heterogeneidade de habitat, através de atributos paisagísticos, físicos, tróficos e microclimáticos sobre composição e riqueza de invertebrados cavernícolas em distintas escalas amostrais, assim podendo contribuir com uma melhor conservação dos ambientes subterrâneos. O trabalho é composto de dois artigos redigidos conforme as normas do periódico *Biodiversity and Conservation*. O primeiro artigo teve como objetivo entender quais fatores do habitat possuem influência sobre comunidade de invertebrados em 24 cavernas da região de Santana-BA. A distância da entrada e as distâncias geográficas entre cavernas mostraram importantes para composição e riqueza das comunidades, juntamente com a heterogeneidade de habitat, representada por diferentes substratos usados como abrigos e recursos tróficos. O segundo capítulo traz um olhar mais específico para a quinta maior caverna do Brasil, a Gruta do Padre. Aqui, além de tentar entender quais fatores do habitat influenciam as comunidades de invertebrados, a caverna é definida como um novo Hotspot de Biodiversidade Subterrânea, com 25 espécies estritamente subterrâneas. Dentro da Gruta do Padre, os fatores do habitat que mais tiveram relação com a fauna foram a distância da entrada e os diferentes níveis de altitude da caverna. A presença de recursos tróficos e diferentes substratos inorgânicos também foi relevante para as comunidades.

Palavras-chave: Cavernas; Invertebrados; Habitat; Comunidade.

Abstract: This work aims to evaluate the influence of habitat heterogeneity, through landscape, physical, trophic, and microclimatic attributes on the composition and richness of cave invertebrates at different sample scales, thus being able to contribute to better conservation of subterranean environments. The work consists of two articles written according to the rules of the journal *Biodiversity and Conservation*. The first article aimed to understand which habitat factors influence the invertebrate community in 24 caves in the region of Santana-BA. Distance from the entrance and geographic distances between caves were important for community

composition and richness, along with habitat heterogeneity, represented by different substrates used as shelters and trophic resources. The second chapter takes a more specific look at the fifth largest cave in Brazil, Gruta do Padre cave. Here, in addition to trying to understand which habitat factors influence invertebrate communities, the cave is defined as a new Hotspot of Subterranean Biodiversity, with 25 strictly subterranean species. Within the Gruta do Padre cave, the habitat factors that were most closely related to the fauna were the distance from the entrance and the different altitude levels of the cave. The presence of trophic resources and different inorganic substrates was also relevant for the communities.

Keywords: Caves; Invertebrates; Habitat; Community.

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INTRODUÇÃO

Os padrões de distribuição das espécies têm sido a anos foco de trabalhos visando entender fatores estruturantes de comunidades (Dunson & Travis, 1991; Kolasa & Pickett, 1991; Cushman & McGarigal, 2004; Steinitz *et al.*, 2006; Talley, 2007; Pacheco *et al.*, 2020). Tanto fatores bióticos, quanto abióticos foram levados em consideração em trabalhos anteriores, buscando entender esses padrões de distribuição. Dentre os agentes bióticos, podem ser destacadas as interações intra e interespecíficas, como a predação e a competição (Dunson & Travis, 1991). A respeito dos fatores abióticos, pode-se salientar a temperatura, umidade relativa do ar, salinidade, quantidade de abrigos, tipo de substratos, entre outros (Dunson & Travis, 1991; Pacheco *et al.*, 2020).

A heterogeneidade de habitat também vem sendo um fator importante estudado por diversos autores na busca de respostas aos padrões de distribuição das espécies em uma determinada área (Amarasekare & Nisbet, 2001; Cornell, 2010; Yang *et al.*, 2015; Stein *et al.*, 2015; Vargas-Mena *et al.*, 2020; Souza-Silva *et al.*, 2021). Assim, diferentes usos, por parte das espécies, de diferentes microhabitats são decretórios para a coexistência de múltiplas populações (MacArthur & Levins, 1967; Tilman, 1982; Chesson, 2000a; Mehrabi *et al.*, 2014; Souza-Silva *et al.*, 2021). A ideia da influência da heterogeneidade de habitat sobre as características das comunidades pode ser aplicada a diferentes ambientes, inclusive aos ambientes subterrâneos, onde essa heterogeneidade pode afetar as características das comunidades cavernícolas. (Pacheco *et al.*, 2020; Souza-Silva *et al.*, 2021)

Os ambientes subterrâneos são caracterizados pela ausência permanente de luz, temperaturas estáveis durante todo ano (normalmente próxima à média anual do ambiente externo) e umidade relativa do ar tendendo à saturação e oligotrofia (Howarth, 1983). Dessa maneira, grande parte das fontes de energia para a rede trófica são alóctones (provenientes do ambiente externo),

podendo ser carregadas para o interior das cavernas através da ação da água, animais ou por raízes de plantas (Howarth, 1983; Polis *et al.*, 1997; Ferreira, 1998; Souza-Silva, 2003; Simon *et al.*, 2007; Culver & Pipan, 2009). Organismos autótrofos quimiossintetizantes também podem ser de grande importância nutricional para os animais que compõem a fauna cavernícola (Howarth, 1983, Sarbu *et al.*, 2018).

Em consequência a essas limitações impostas pelos ambientes cavernícolas, as espécies animais colonizadoras desses habitats são limitadas e possuem usualmente pré-adaptações que possibilitam sua existência nesses locais. Os organismos habitantes das cavernas usualmente são classificados por um sistema contendo três categorias, proposto por Schinner-Racovitza e modificado por Sket (2008). Os (a) troglóxenos são organismos que passam parte de seu ciclo de vida dentro das cavidades, mas ainda precisam do meio externo para completar todo seu ciclo de vida; os animais (b) troglófilos são os que conseguem manter populações viáveis tanto fora, quanto dentro das cavernas; e os (c) troglóbios são as espécies que possuem suas populações restritas ao ambiente cavernícola durante todo seu ciclo de vida.

Os animais classificados como troglóbios comumente podem apresentar adaptações em resposta às pressões seletivas presentes nos ambientes subterrâneos, chamadas troglomorfismos. Essas adaptações podem ser morfológicas, como redução de estruturas oculares, perda da pigmentação melânica e alongamento de apêndices; fisiológicas, como diminuição da taxa metabólica e estratégia de vida K; ou comportamentais (Romero & Green, 2005). Além disso, são raros e endêmicos (em sua maioria) devido a uma longa história evolutiva em ambientes tão estáveis como as cavernas, e acabam se tornando organismos sensíveis a mudanças no sistema, tais quais pequenas variações de temperatura e umidade (Mammola *et al.*, 2019; Culver & Pipan, 2009).

A fauna cavernícola brasileira passou a ser estudada a partir de 1980, especialmente nos estados de São Paulo, Goiás, Minas Gerais, Bahia, Paraná, Mato Grosso e Ceará (Dessen *et al.*, 1980; Pinto-da-Rocha, 1995). Entretanto, muitos estudos foram feitos dentro de Unidades de Conservação e por isso encontram-se fragmentados e escassos em determinadas regiões do Brasil (Ferreira, 2005; Ferreira *et al.* 2010; Gnaspi e Trajano, 1994; Pinto-da-Rocha, 1995; Souza-Silva, 2008; Trajano, 2000; Zepon & Bichuette, 2017).

Diante do exposto, entender os aspectos ecológicos da fauna subterrânea é de suma importância, já que, apenas os levantamentos taxonômicos podem não ser eficientes para a conservação da biodiversidade cavernícola (Trajano *et al.* 2010). Logo, compreender como os padrões de riqueza e composição são influenciados pelos fatores que atuam na manutenção e estruturação dessas comunidades no tempo e espaço é invariavelmente necessário para a conservação das espécies cavernícolas (Legendre *et al.* 2005; Jost *et al.*, 2010).

A distância das entradas, disponibilidades de recursos alimentares e heterogeneidade de habitats mostram-se como fatores que exercem forte influência sobre as características das comunidades de invertebrados em cavernas (Prous, 2005; Oliveira, 2014; Pellegrini *et al.*, 2016; Gomes, 2017; Zepon & Bichuette, 2017), já que esses animais tendem a buscar por microhabitats preferenciais dentro dos ambientes subterrâneos (Culver & Pipan, 2009; Mammola *et al.*, 2016; Souza-Silva & Ferreira, 2009; Souza-Silva *et al.* 2021).

Tais microhabitats podem compreender componentes orgânicos como detritos vegetais, guano e outros tipos de matéria orgânica, assim como componentes físicos, como frestas, espaços sob rochas, corpos d'água, entre outros (Ferreira *et al.*, 2007; Culver & Pipan, 2009; Souza-Silva *et al.*, 2011; Simões *et al.*, 2015; Gomes, 2017). Dessa forma, a heterogeneidade de habitat pode agir sobre a estrutura das comunidades de invertebrados cavernícolas por meio

da disponibilização de diferentes habitats para a fauna, agindo então sobre a distribuição e riqueza das espécies (Zagmajster *et al.*, 2018).

Logo, este estudo visa analisar a estrutura de habitat como subsídio à compreensão dos mecanismos responsáveis pela composição e riqueza de comunidades subterrâneas na região de Santana, estado da Bahia. Para tal, foram utilizados parâmetros paisagísticos, físicos, tróficos e microclimáticos em diferentes escalas amostrais.

A dissertação é composta por dois artigos redigidos nas normas do periódico *Biodiversity and Conservation*. O primeiro artigo visa elucidar quais características do habitat influenciam a riqueza e composição de comunidades de invertebrados cavernícolas em 24 cavernas da região de Santana-BA. O segundo artigo foca as análises em uma importante caverna da região, a Gruta do Padre. Na quinta maior caverna do mundo, além de entender quais fatores do habitat influenciam nas comunidades de invertebrados subterrâneos, o objetivo foi também defini-la como um novo Hotspot de Biodiversidade Subterrânea, graças às 25 espécies troglóbias encontradas.

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SEGUNDA PARTE-ARTIGOS

Artigo 1: Are there choices in the darkness? Habitat selection and conservation of cave invertebrates in a Brazilian semi-arid region

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Are there choices in the darkness? Habitat selection and conservation of cave invertebrates in a Brazilian semi-arid region

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Abstract

Habitat characteristics are key factors for fauna distribution inside caves, as distinct species usually present different microhabitat requirements. Hence, this work aimed to understand which habitat traits influence the richness and composition of invertebrate communities in 24 caves located in southwestern Bahia state, in a semi-arid area. Landscape, physical, trophic, and microclimatic traits were used as predictors and were analyzed in both meso and microscale inside the caves. In total, 338 species from 37 orders and at least 93 families were found, with 41 of them considered troglobitic. The results showed that the distance from the nearest entrance and the geographic distances between caves were important for communities' composition and richness, along with habitat heterogeneity, represented by different substrates used as shelters and trophic resources.

Introduction

Different environmental traits can lead to several responses from the associated biota, thus determining singular distribution patterns (Tews *et al.* 2004; Odum & Barret 2006). Habitat heterogeneity is one of the keys factors for the distribution patterns since a higher heterogeneity usually allows the coexistence of a higher number of species (Yang *et al.* 2015; Stein *et al.* 2015; Vargas-Mena *et al.* 2020; Pacheco *et al.* 2020; Souza-Silva *et al.* 2021).

In subterranean environments, habitat heterogeneity is also determinant for species distribution, even considering that the subterranean realm many times presents unique habitat characteristics (Pacheco *et al.* 2020; Souza-Silva *et al.* 2021). From theories proposed at the beginning of the XX century to advances in sampling techniques and analysis achieved at the end of the same period, important mechanisms for the definition of subterranean biodiversity started to be unraveled (Mammola *et al.* 2016; Rabelo *et al.* 2020).

Even though the subterranean environments are not limited to caves, they are amongst the most important subterranean habitats, and are the most studied subterranean habitats due to its dimensions (Juberthie *et al.* 1980; Mammola *et al.* 2016; Rabelo *et al.* 2020; Pacheco *et al.* 2020; Souza-Silva *et al.* 2021). These natural cavities are known for having peculiar characteristics, such the higher climatic stability when compared to the epigeal surrounding environments, with stable temperature all over the year and high humidity (Howarth 1980, 1983). These habitats are also characterized by the absence of light and a tendency towards the oligotrophy (Culver & Pipan 2009).

Among the mechanisms determining the subterranean biodiversity, the distance from the caves' entrances stands out (Ficetola *et al.* 2018; Mammola 2019; Souza-Silva *et al.* 2021; Furtado *et al.* 2022). Since most cave trophic resources come from the external habitats and are transported to caves through their entrances, a higher distance from the entrance limits the organic supply availability for the communities (Tobin *et al.* 2013; Moseley 2008; Ficetola *et al.* 2018; Mammola 2019). Moreover, regardless of the general stability, caves present a gradient of conditions from near-to-entrance (usually more unstable) to deep zones (Moseley 2009; Tobin *et al.* 2013; Lunghi *et al.* 2014; Prous *et al.* 2015; Mammola & Isaia 2018; Lunghi & Manenti 2020; Souza-Silva *et al.* 2021). This zonation creates distinct microhabitats for cave species, which vary regarding substrate types as well as climatic and

photic properties (Moseley 2008; Souza-Silva *et al.* 2011b; Du Preez *et al.* 2015; Lunghi *et al.* 2017; Mammola & Isaia 2017; Mammola 2019; Lunghi & Manenti 2020; Mammola *et al.* 2020; Souza-Silva *et al.* 2021).

The uniqueness of the environmental traits found in caves usually selects pre-adapted species, which are the only capable to colonize these habitats. It also creates singular evolutive pressures that may lead to restricted subterranean species, called troglobitic, which can maintain viable populations exclusively in subterranean environments (Racovitza 1907; Sket 2008).

The evolution in subterranean habitats can produce, in the restricted species, adaptations. These adaptations can be morphological (named troglomorphisms), such as reduction in ocular structures, loss of melanic pigmentation, and appendage elongation. It also can be physiological, like metabolic rate reduction and K life strategy, and even behavioral (Romero & Green 2005). These animals are, in their majority, rare and endemic, being very sensitive to slight variations in their habitats, as in temperature and moisture content (Culver & Pipan 2009; Mammola *et al.* 2019).

Troglobitic species descend from epigeal ancestors that migrate to subterranean environments as became isolated, for example by past climatic events, such as retractions of rain forests. Accordingly, caves can function as a refuge for fauna in moments of climate change, besides being a showcase of the fauna from the past (Culver & Sket 2000; Sobral-Souza *et al.* 2015).

The western Bahia state, located in the Brazilian northeast, is a semi-arid region that presents areas with high speleological potential. The region of the municipalities of Santana, Santa Maria da Vitória, and Canápolis, presents approximately 120 registered caves (CECAV 2022) and is home of important caves, such as the Gruta do Padre cave, with along two other caves (Gruta do Cipó Cave and Gruta da Bananeira Cave) represent the longest hydrological subterranean system from Brazil. Considering the high potential and the few studies accomplished in this area, the main objective of this work was to identify habitat variables determining the richness and composition of cave invertebrates. Using landscape, physical, trophic, and microclimatic traits of the caves, these hypotheses were tested: *i)* species richness will reduce with the increase of distance from the entrance; *ii)* geographically closer caves will present more similar communities, and *iii)* invertebrates will respond to different habitat

components and habitat heterogeneity on the cave floor. In addition, we discuss the importance of preserving this high-potential area for subterranean biodiversity.

Material and Methods

Study area

The sample campaigns occurred between August 23 and September 02 of 2021, and May 23 and June 02 of 2022 in the municipalities of Santana, Santa Maria da Vitória e Canápolis, Bahia State, Brazil. This transition zone between Seasonal Dry Forests and Caatinga is classified by Köppen (1936) as Aw, with dry winters and rainy summers, and has a high potential for endemic species (Dinerstein 2017). The high potential found in this area may be due to many paleoclimatic changes caused by expansions and retractions of the Amazonian and Atlantic rains forests (Sobral-Souza *et al.* 2015). The area is located within the limits of the Corrente River basin and its affluents which are an important tributary of the São Francisco River. For security reasons, the samples were conducted only in the dry season, since many caves in this region present subterranean rivers with wide capitation basins, which make the rainy season highly dangerous for those intending to visit such caves (Fig. 1; Table 5).

This area is inserted in the Bambuí Group, the largest carbonate formation in the country, with more than 145,000 Km², and more than 6,000 caves registered (Auler *et al.* 2019). It is important to highlight that this region represents a priority area for the Brazilian Speleological heritage conservation according to the map published by the Centro Nacional de Pesquisa e Conservação de Cavernas – CECAV.

Field procedures

Sampling design

The composition and richness of cave invertebrates, as well as the habitat structure traits, were determined along 122 transects (mesoscale sampling - 10 × 3 m each) distributed on the floor of 24 caves, from the entrances to their deeper regions. Quadrats (micro-scale sampling - 1 m²) were placed in triplicates inside the limits of each transect (Fig. 2), totalizing 366 quadrats (Souza-Silva *et al.* 2021). Invertebrate sampling was executed by visual search along the transects and quadrats (Souza-Silva *et al.* 2021). The sampling in the quadrats allowed the detection of small-size and low-mobility species,

which could then be carefully searched in the remaining transect if discovered. The sampling was first conducted in the quadrats and later in the respective transect, always by three collectors, and was only finished when all the invertebrates had been sampled and/or accounted for. Since the several sampling regions along the cave presented a significant structural distinction, the time spent searching for invertebrates in each transect was variable. Moreover, direct intuitive search approaches were used in different cave locations to increase the discovery of troglotic and stygotic species (Wynne et al. 2019). Invertebrates were preserved in labeled vials containing 70% ethanol. In laboratory, the specimens were sorted with a Stemi 508 (ZEISS) stereomicroscope, identified until the lowest possible taxonomic level, and separated into morphotypes (Oliver & Beattie, 1996). Potential troglotic species were identified by the presence of troglomorphic characteristics, such as pigmentation and eye reduction, and appendage elongation, among others (Culver & Pipan 2009). Furthermore, experts in several taxa were contacted to help to identify particular troglomorphic characteristics (specialists are acknowledged further on). The voucher specimens were deposited in the Collection of Subterranean Invertebrates of Lavras (ISLA), linked to the Center of Studies on Subterranean Biology (CEBS) of the Federal University of Lavras (UFLA).

Environmental traits in different scales

The measurement of the habitat structure traits in the transects was carried out according to the methodology used by Souza-Silva *et al.* (2021). In order to visually quantify the surface area occupied by various organic and inorganic substrates, each transect was divided into 10 parts (1 x 3 m) (Fig. XX). The area occupied by each substrate along the whole transect was then calculated by sum. The same researcher characterized all transects to reduce observer error. The humidity and temperature were measured using a digital thermo-hygrometer that was set up on the ground in the center of each transect. Photographs (4000 x 3000 pixels) of each quadrat taken at the researcher's chest height with a Canon Powershot SX60HS camera at the closest possible angle to a 90° angle were used to calculate the percentage of each substrate in the quadrats. Posteriorly, Photographs were analyzed with the aid of *ImageJ 1.53K* software. The distances were obtained by a laser tape measure or by the plot of each transect on the map. For the definition of the Micro Drainage Basins, the function “Channel Network

and Drainage Basins” from the SAGA Next Generation plugin was used with the aid of a Digital Elevation Model (DEM) in the QGIS 3.22.11 software. The DEM was also used to extract the altitude information for each sector. The sectors with 600m or higher in elevation were classified as Recharge Zones and those under this altitude were classified as Discharge Zones.

Data analysis

Pre-analysis routine

All the analyses were run in the R Studio 2022.07.02 Build 576 software. Prior to the analysis of invertebrate fauna composition and richness, the correlation between the variables was tested with the help of the *CHART.CORRELATION* function from the ‘*PerformanceAnalytics*’ package and the variables with correlation value > 0.70 were excluded from the models. The functions *VIF* and *VIF.CCA* from the ‘*Car*’ package was used to test the multicollinearity of variables, and the ones that present valor > 10 were discarded. A Shapiro-Wilk test was executed, to verify the normality of the data, using the *SHAPIRO.TEST* function from the package ‘*Stats*’. A Mantel test was performed to try the spatial autocorrelation between the samples, in mesoscale, for all the fauna types.

Mesoscale abiotic features

All the substrates in each sector were evaluated and classified into the following classes: guano - GU; feces - FZ; carcass - CRC; roots - RZ; litter - SER; vegetal debris - DTV ($< 10\text{mm}$); fine branch - GALF (11 - 30 mm); medium branch - GALM (31 - 50 mm); coarse branch - GALG (65 - 250 mm); trunk - TRO ($> 250\text{mm}$); termite mounts - TM; water streams - ST; water pond - WP; drip water - DP; phanerogams - FG; actinomycetes - ACT; another organic substrate - OTO; concrete floor - RC; rough rock - RR; large rock - XB (1000 - 4000mm); medium rock - MB (500 - 1000mm); small rock - SB (250 - 500mm); cobbles - CB (64 - 250mm); coarse gravel - CAG (16 - 64mm); fine gravel - CAF (2 - 16mm); sand - ARE (0.06 - 2mm); silt - SEF (≤ 0.05 mm); hardpan - HP; speleothems - ES; calcite rafts - JNS; concreted calcite raft - JNC; stalactite - ESTC; stalagmite - EST; micro travertine - MTR; travertine - TRA; rough flowstone - ESR; flowstone - ESC; worm acorn - BM; retraction cracks - GRR; gastropod shell - COG.

Based on those classes and using a Shannon-Weaver Index (Buttigieg & Ramette 2014), were determined the Substrate Diversity (all classes), the Shelter Diversity (WP, DP, XB, MB, SB, CB, CAG, CAF, ES, ESTC, EST, MTR, TRA, BM, GRR, COG) and the Trophic Resources Diversity (GU, FZ, CRC, RZ, SER, DTV, GALF, GALM, GALG, TRO, FG, ACT, OTO) for each sector. The classes were also used to generate, by sum, the Shelter Availability (WP, DP, XB, MB, SB, CB, CAG, CAF, ES, ESTC, EST, MTR, TRA, BM, GRR, COG) and the Trophic Resources Availability (GU, FZ, CRC, RZ, SER, DTV, GALF, GALM, GALG, TRO, FG, ACT, OTO) for each sector.

For their use in analysis with individual substrate classes, a few categories were grouped in order to reduce variables. DTV, GALF, GALM, GALG, and TRO were grouped into **DTV**, while ES, JNS, JNC, EST, MTR, and TRA have grouped into the class **ES**.

The abiotic attributes were then divided into *Landscape features*, such as *Micro Drainage Basins*, *Water Zones*, and *Caves*; *Physical features*, which comprise the *Distance of each transect from the nearest entrance*, *Substrate Diversity*, *Shelter Diversity*, and *Shelter Availability*; *Trophic Resources* grouped *Trophic Resources Diversity* and *Trophic Resources Availability*; the *Microclimatic Variables* considered were *Temperature* and *Moisture*.

Micro-scale abiotic features

For the micro-scale, the same substrate classes were evaluated, and the same groupings were made. The diversities and availabilities were calculated equally for the sectors. The *Landscape*, *Physical* and *Trophic* features were also formed similarly to the mesoscale. For the quadrats, the microclimatic variables were not measured.

Habitat traits determining the communities' richness and composition

In order to understand the potential correlation between total, troglobitic, and non-troglobitic invertebrate richness with *physical*, *trophic*, and *microclimatic* traits, Generalized Linear Models (GLM) and Generalized Linear Mixed Models (GLMM) were performed, with sectors and quadrats as sample units, using two models for each fauna group. The first model (Succinct Model) used *Microclimatic Variables*, *Distance from the nearest entrance*, *Diversities* (general substrate, shelter, and trophic resources), and *Availabilities* (shelter and trophic resources). The second model (Long Model) used

Microclimatic Variables, *Distance from the nearest entrance*, *Diversities* (general substrate, shelter, and trophic resources), and each substrate class individually. For the micro-scale, the *Microclimatic Variables* were not used.

For those models, the Poisson family was adopted because it better fitted the data. For the evaluation of model overdispersion, the function *CHECK_OVERDISPERSION* from the package ‘*Performance*’ was used. To obtain r^2 values of the GLMMs was used the function *r.squaredGLMM* from the ‘*MuMIn*’ package, while the function *r.squaredLR* from the ‘*piecewiseSEM*’ package was used to obtain r^2 values of the GLMs.

To evaluate the possible correlation between total, troglobitic, and non-troglobitic invertebrate composition with *landscape*, *physical*, *trophic*, and *microclimatic* variables a Distance-Based Redundancy Analysis (dbRDA) was performed (Clarke *et al.* 2014), with sectors and quadrants as sample units, using two models for each fauna group. The first model (Succinct Model) used *Landscape features*, *Microclimatic Variables*, *Cave*, *Distance from the nearest entrance*, *Diversities* (general substrate, shelter, and trophic resources), and *Availabilities* (shelter and trophic resources). The second model (Long Model) used *Landscape features*, *Microclimatic Variables*, *Cave*, *Distance from the nearest entrance*, *Diversities* (general substrate, shelter, and trophic resources), and each substrate class individually. For the micro-scale, the *Microclimatic Variables* were not used.

Results

Richness and composition of cave invertebrates

Considering all 24 caves (with samplings in the transects, quadrats, and other habitats) 2,754 specimens were found, totalizing 338 species from 37 orders and at least 93 families (Fig. 4). The most expressive group was Araneae, with 66 species distributed in 17 families, totalizing 1,062 individuals. The spiders were followed by Diptera and Coleoptera, with 49 and 37 species respectively.

Cave-restricted species

The sampled caves presented at least 41 cave-restricted species belonging to 8 higher taxa and 14 families (Fig. 5; Table 1). The eight higher taxa were Crustacea (11), Arachnida (11), Hexapoda (10

spp.), Myriapoda (5), Mollusca (1), Nemertea (1), Annelida (1) and Osteichthyes (1) (Table 1). The richness of the observed obligate cave fauna was Gastropoda (1), Nemertea (1), Oligochaeta (1), Isopoda (10), Amphipoda (1), Polydesmida (4), Sympleyla (1), Blattodea (1), Collembola (4), Coleoptera (2), Hemiptera (1), Orthoptera (1), Pseudoscorpiones (2), Araneae (4), Opiliones (3), Palpigradi (2), and Siluriformes (1).

It is very important to highlight that from all 41 troglobitic species, 25 are found in the Gruta do Padre Cave, which represents more than 60% of the species. Furthermore, only 14% of the obligate cave species from this area are currently described. Such described species are *Coarazuphium tessai* (Godoy & Vanin 1990); *Phaneromerium cavernicolum* (Golovatch & Wytwer 2004), *Spelaeogammarus santanensis* (Koenemann & Holsinger 2000), *Eusarcus cf. cavernicola* (Hara & Pinto-da-Rocha 2010), *Pectenoniscus santanensis* (Cardoso *et al.* 2020) and *Chaimowiczia tatus* (Cardoso *et al.* 2021). Although Hara & Pinto-da-Rocha (2010) considered that *E. cavernicola* may represent an assembly of species that cannot be identified by external and genital characteristics, herein we considered the population of the Gruta do Padre cave (and other related caves) as a troglobitic species, due to the strong troglomorphic traits they present.

Habitat traits determining the communities' richness and composition

On the mesoscale, the Mantel test revealed the existence of spatial autocorrelation for the general and non-troglobitic fauna ($p=0.0004$); therefore, the geographic distances between transects explain 20.43% and 20.17% respectively of the variation in the composition. For the troglobitic fauna, the geographic distances were not significant.

For the composition of communities, the *Distance from the nearest entrance* was significant for all models, both at the meso and micro-scale. The *Cave* itself was also an important variable, presenting significance except for the troglobitic fauna at the microscale. *Shelter Availability* was significant only for the general and troglobitic invertebrate fauna in the mesoscale. On the other hand, *Trophic Availability*, , was only related to the general and non-troglobitic fauna at the micro-scale. *Trophic Diversity* showed a significant correlation only with the non-troglobitic fauna on the micro-scale, for both long and succinct models. Concerning individualized substrates, guano (GU), vegetal debris

(DTV), and course gravel (CAG) were important in some of the models. GU only was significant at the microscale for general and non-troglobitic fauna, while DTV was only important for the general fauna on the microscale. CAG was significant for both non-troglobitic fauna at the mesoscale, and troglobitic fauna at the micro-scale (Table 2).

For community richness, the *Distance from the nearest entrance* did not show significance only for the troglobitic fauna in the long model of the mesoscale. The correlation between *Distance from the nearest entrance* and Richness was positive for the troglobitic fauna and negative for general and non-troglobitic. The *Temperature* always presented a negative correlation and was significant for the general and non-troglobitic fauna of the succinct model, and general fauna of the long model. The *Trophic Availability* was only important for the general fauna at the micro-scale, presenting a positive correlation (Table 3).

For the individual substrates, GU had a negative correlation with the troglobitic fauna on the mesoscale, but showed a positive correlation with general and non-troglobitic fauna on the micro-scale, similar to DTV. Carcass (CRC), Phanerogams (FG), Sand (ARE), and Worm Acorn (BM) were also found to be positively significant, whereas Hardpan (HP) and Speleothems (ES) were negatively correlated, affecting general and non-troglobitic fauna at the mesoscale. On the mesoscale, Actinomycetes (ACT) were positively significant for the troglobitic fauna, while small rock (SB) and flowstone (ESC) were important for the general fauna, with negative and positive correlations, respectively. On the microscale, concrete floor (RC) showed a positive correlation with the non-troglobitic fauna, while rough rock (RR) and retraction cracks (GRR) had negative significance for general and troglobitic fauna, respectively.

Discussion

Distance from the entrance and its relationship with communities' traits

For all habitat traits analyzed, the *distance from the nearest entrance* stood out as the most important. In terms of community richness, only the troglobitic fauna from the long model at the mesoscale did not respond to the *distance*. In all other models, the general and non-troglobitic fauna responded negatively

to an increase in the *distance from the nearest entrance*, while the troglobitic fauna showed a positive response. For species composition, the *Distance from the nearest entrance* was important for all types of fauna, models, and scales, demonstrating its strong relationship with the fauna distribution. It is important to note that most of the general fauna is composed of non-troglobitic species, which may bias the results towards this group.

The decrease observed in the richness of general and non-troglobitic fauna is the anticipated response for this trait, given that the distance from the cave entrance is a well-known limiting factor for species distribution. The restrictive influence of the distance from the entrance is linked to the reduction of trophic resources, as well as the habitat heterogeneity, which decreases from near-to-entrance to deep zones of the caves (Tobin *et al.* 2013; Moseley 2008; Ficetola *et al.* 2018; Mammola 2019; Souza-Silva *et al.* 2021).

In this way, greater distances limit the amount of accessible energy for the communities, as the majority of cave trophic resources are transported from the entrances (Tobin *et al.* 2013; Moseley 2008; Ficetola *et al.* 2018; Mammola 2019; Souza-Silva *et al.* 2021, Furtado *et al.* 2022). Additionally, since zones closer to the entrances present less stable climatic conditions, a gradient of temperature, moisture, and sunlight is created. This gradient can also be observed on the cave floor, leading to the simplification and homogenization of substrates in deeper zones (Prous *et al.* 2004; Prous *et al.* 2015; Souza-Silva *et al.* 2021; Furtado *et al.* 2022).

On the other hand, the restricted subterranean fauna richness shows a contrary response to that of the general and non-troglobitic fauna. The higher climatic stability found in the deeper zones of the caves favors the existence of troglobitic fauna due to their adaptations. These adaptations include reduced metabolic rates and cuticle thinning, which increase the risk of desiccation and may limit the distribution of these species to areas with minor temperature and moisture variations, often found in deeper areas (Tobin *et al.* 2013; Lunghi *et al.* 2014 and 2017; Kozel *et al.* 2019; Souza-Silva *et al.* 2021).

This preference of troglobitic organisms for stabler habitats can, on the other hand, be associated with a cave characteristic initially thought to limit the communities, the oligotrophy. However, as demonstrated by Hüppop (2005), the K-strategy life history adopted by these animals, combined with

their reduced metabolic rates, enables troglobitic fauna to survive for extended periods without food. Thus, restricted fauna is commonly more prevalent in areas with fewer trophic resources, allowing them to avoid non-troglobitic competitors (Sket 1999; Deharveng & Bedos 2000; Souza-Silva *et al.* 2021). It is important to emphasize that an increase in organic matter in these oligotrophic zones can be detrimental to troglobites, as it may attract more competitive and energetically needed non-troglobites (Sket 1999; Souza-Silva *et al.* 2021).

Similarity between caves thus geographic position

The Mantel test performed revealed that geographic distances between transects account for 20.43% of the variation in the composition of the general fauna and 20.17% on the non-troglobitic fauna communities. This indicates that sampling units that are closer to each other exhibit a greater similarity in fauna than those that are farther apart.

As demonstrated in previous studies, spatial autocorrelation can imply that cave communities can affect the composition of adjacent subterranean habitats (Christman *et al.* 2015; Jaffé *et al.* 2016; Jaffé *et al.* 2018). The findings of this study support this notion, but only for the general and non-troglobitic fauna, as the troglobitic composition did not exhibit any spatial autocorrelation.

This lack of influence of geographic distance on the restricted fauna may suggest that, unlike in other studies, this region does not have a highly interconnected subterranean environment, or at least the troglobitic species are not utilizing these connections (Ferreira 2005; Souza-Silva *et al.* 2011; Auler *et al.* 2014; Christman *et al.* 2005; Jaffé *et al.* 2016; Jaffé *et al.* 2018).

On the other hand, the spatial autocorrelation observed in the composition of general and non-troglobitic fauna suggests that, despite the subterranean connections between caves being unused, non-restricted species may still disperse in epigeal environments (Ribera *et al.* 2019). These surface movements by animals raise concerns for the overall conservation of the area, as degraded regions can obstruct the dispersal of fauna and negatively impact the subterranean diversity.

It is important to note that spatial autocorrelation, and thus the potential for epigeal dispersal movements of fauna, can account for approximately 20% of the variation in composition. However, a

higher explanatory value is attributed to the grouping of other analyzed variables, such as *physical*, *trophic*, and *climatic*.

Invertebrates' response to habitat heterogeneity

The *trophic* and *physical* attributes play a highly significant role in defining cave invertebrate communities in the semi-arid regions of Brazil. Since caves are naturally oligotrophic environments, they depend greatly on the surface, with the majority of the energy of the system originating from epigeal zones (Tobin *et al.* 2013; Moseley 2008; Ficetola *et al.* 2018; Mammola 2019).

The presence and diversity of trophic resources are not only important for the existence of cave invertebrate communities, but they also affect their composition and richness. This suggests that different types of organic matter may provide a higher number of specific niches, allowing for greater richness and creating differences in composition. These findings align with previous research on non-troglobitic fauna (Schneider *et al.* 2011; Souza-Silva *et al.* 2011; Ladle *et al.* 2012; Ferreira 2019; Pacheco *et al.* 2020a; Furtado *et al.* 2022).

Among the different types of organic matter observed in the sampled caves, bat guano and vegetal debris were found to be the most significant. Bat guano serves as one of the primary energy sources for invertebrate fauna, particularly in caves that are permanently dry (Ferreira & Martins 1999; Souza-Silva *et al.* 2011). However, the guano production in caves may vary seasonally due to external vegetation fluctuations and, as a result, food availability for bats (Faria 1996; Souza-Silva *et al.* 2011). The transport of guano into caves is crucial because the piles formed by bats can sustain entire invertebrate communities that vary depending on the age and composition of the deposit (Ferreira & Martins 1999; Ferreira 2019). Furthermore, guano deposits may be critical for establishing richer cave communities, as they attract colonizer species that initiate a complex process, resulting in more extensive trophic webs (Ferreira & Martins 1999; Ferreira 2019; Pacheco *et al.* 2020a).

Due to the prevalence of deciduous species in the limestone vegetation of this region, a significant amount of leaves and small branches accumulate in the litter during the dry season (Crowther 1987; Brina 1998; Souza-Silva *et al.* 2011). In turn, this accumulated vegetal debris frequently finds its way into caves via flood pulses during the rainy season. These floods typically carry substantial volumes of

water downstream, transporting organic matter and other substrates into caves (Minshall et al. 1983; Carrling 1987; Downes & Street 2005). Therefore, as shown by Souza-Silva et al. (2011), the dependence of cave communities on external vegetal material causes the trophic dynamics of subterranean habitats to be influenced by seasonal changes outside the cave environment.

The positive correlation observed between cave invertebrate communities and organic matter is generally applicable to non-restricted fauna. However, when considering troglobitic species, this correlation may not hold. Restricted subterranean animals typically have reduced metabolic rates and K-strategy life history, enabling them to endure periods of starvation (Hüppop 2005; Souza-Silva et al. 2021). Consequently, due to the greater energetic demand and superior competitive ability of non-troglobitic species, troglobites tend to seek for locations with limited organic matter, thus avoiding competitors (Sket 1999; Deharveng & Bedos 2000; Souza-Silva et al. 2021).

It is essential to emphasize that the distribution of trophic resources inside caves is not uniform, with the majority concentrated near the entrance, creating an energy gradient that decreases towards deeper areas (Tobin et al. 2013; Moseley 2008; Ficetola et al. 2018; Mammola 2019; Souza-Silva et al. 2021, Furtado et al. 2022). This gradient encompasses not only trophic resources but also physical and microclimatic conditions (Prous et al. 2004; Prous et al. 2015; Souza-Silva et al. 2021; Furtado et al. 2022).

The data obtained from this study demonstrate that the presence of different types of substrates can have a positive effect on the composition and richness of cave invertebrate communities. This supports the notion that greater substrate diversity and variation in climatic traits generate more microhabitats, enabling a greater number of species to occupy the habitats by creating new niches (Prous et al. 2004; Prous et al. 2015; Souza-Silva et al. 2021; Furtado et al. 2022). However, troglobitic species respond differently from non-troglobitic fauna, displaying distinct responses to different substrates. This contrasting response may be attributed to the high level of specialization of restricted organisms, which seek specific conditions within subterranean habitats (Pacheco et al. 2020a; Souza-Silva et al. 2021).

Conservation of subterranean environments in Santana region

The Santana region lies in a transition zone between the Caatinga and Seasonal Dry Forests. While some caves have entrances that are covered by native vegetation, it is evident that the original forests surrounding the caves have been replaced by pastures and other monocultures. Deforestation in the caves' surroundings can have a direct and indirect impact on the energetic dynamics of subterranean invertebrate communities. The loss of vegetation in the cave surroundings can directly reduce the amount of litter available to be transported to hypogean environments. This litter, derived from vegetation, is one of the most significant food sources for cave invertebrates in the region, and its depletion can disrupt the entire subterranean trophic web (Crowther 1987; Brina 1998; Souza-Silva et al. 2011). Indirectly, deforestation can limit the availability of bat food, which may decrease the input of guano to the caves. Bat guano is one of the most critical energy sources for invertebrates in permanently dry caves, and its absence can pose a threat to entire communities (Faria 1996; Ferreira & Martins 1999; Souza-Silva et al. 2011; Ferreira 2019).

Despite the high speleological potential in the Santana region, many locals are unfamiliar with most of the caves, even the larger ones like the Gruta do Padre Cave. Fear or lack of opportunities may contribute to this lack of awareness, which is not unique to the region but extends to the Brazilian population as a whole. It is estimated that there are almost 23 thousand caves registered in the country, but this represents only a small fraction of the real potential, which is around 300 thousand caves (CECAV 2022). Similarly, in the Santana region, unexplored limestone outcrops are easy to find. This study alone identified nine previously unregistered caves out of 25 sampled, highlighting significant gaps and unprospected areas.

It is crucial to emphasize that the general lack of knowledge among the population, both nationally and in Santana, is also attributable to the research community as a whole, not just in Brazil but worldwide. Regrettably, many researchers overlook the local population when conducting their work, neglecting to communicate scientific findings in a more accessible manner, which is equally true for speleology.

Clearly, legislation plays a crucial role in protecting the speleological heritage. However, despite regulations and management controls, some damage to cave ecosystems is still caused by locals, and

effective monitoring is a challenging task in a vast country like Brazil. Sometimes, due to lack of awareness or for basic survival needs, locals can inadvertently cause harm to subterranean environments and their surroundings. Therefore, it is imperative to provide accessible information about the legislation and the significance of cave habitats for the local communities in Santana's region. This could foster a sense of ownership and stewardship among locals, and they could become new and powerful allies in the conservation of the unique and fragile cave ecosystems of the Brazilian semi-arid region.

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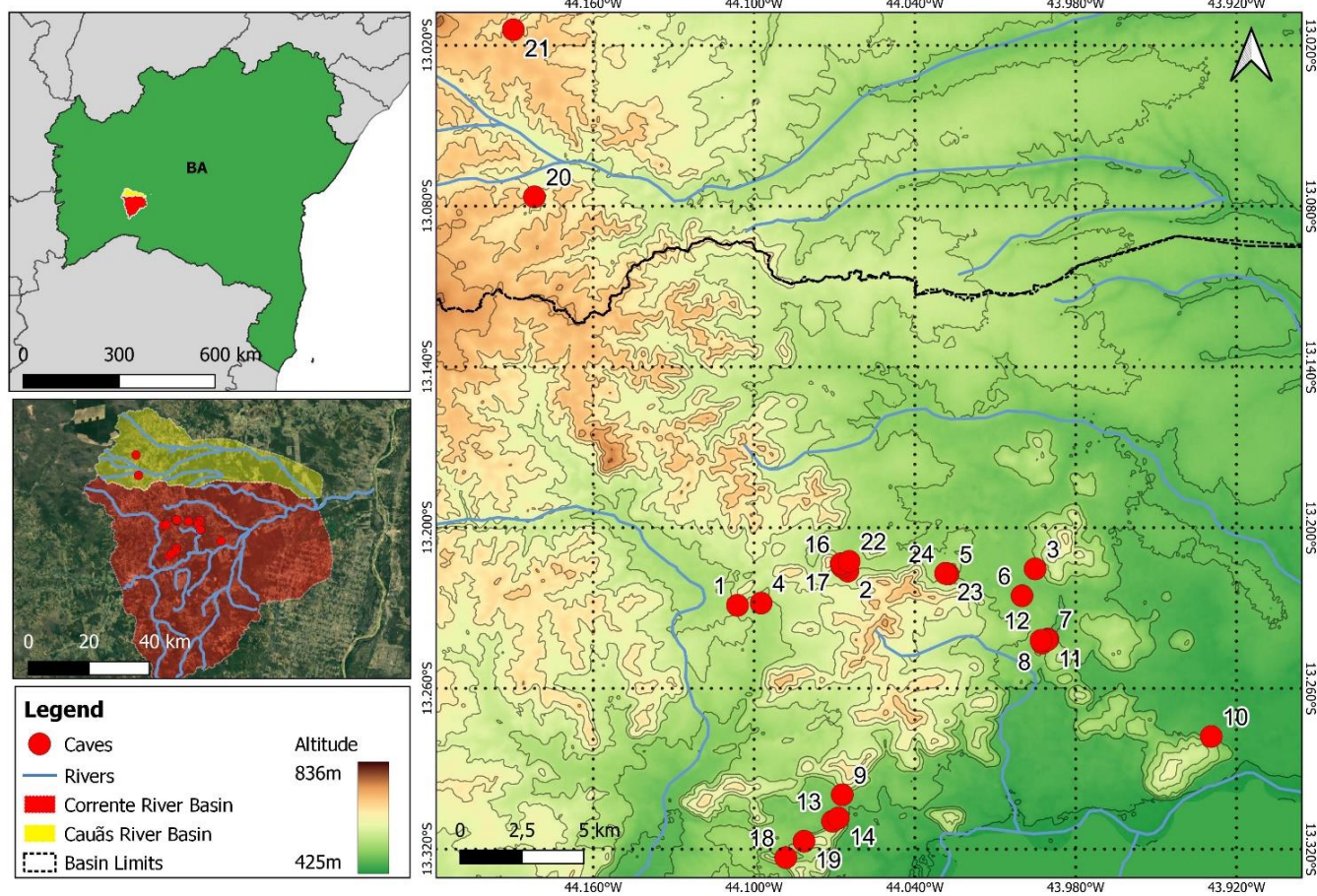


Figure 1. Location map of the caves sampled in Santana region. Caves identification are in table 5.

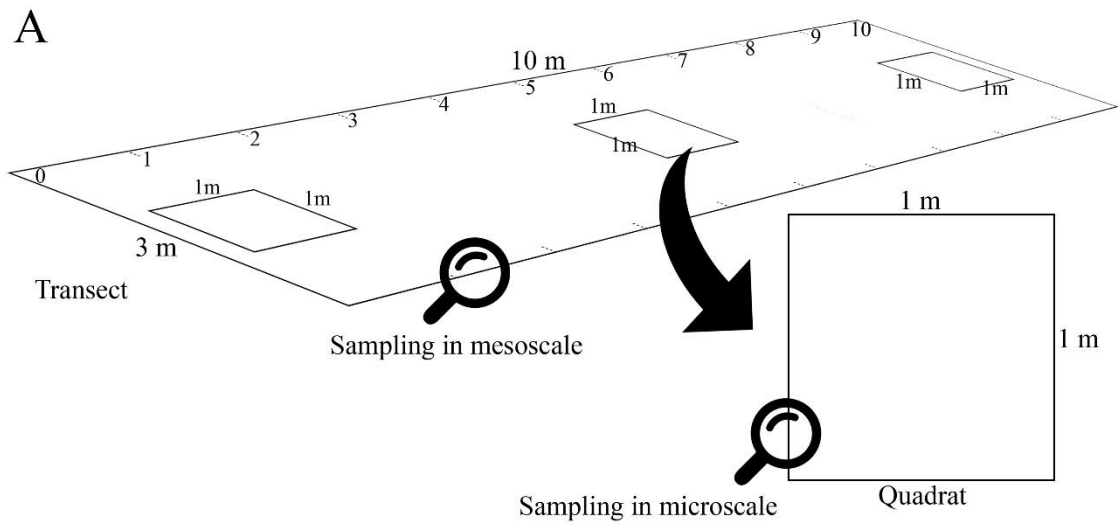


Figure 2. Sampling method. A—sampling method scheme showing both meso and microscale; B—sampling method being apply in one of the caves.

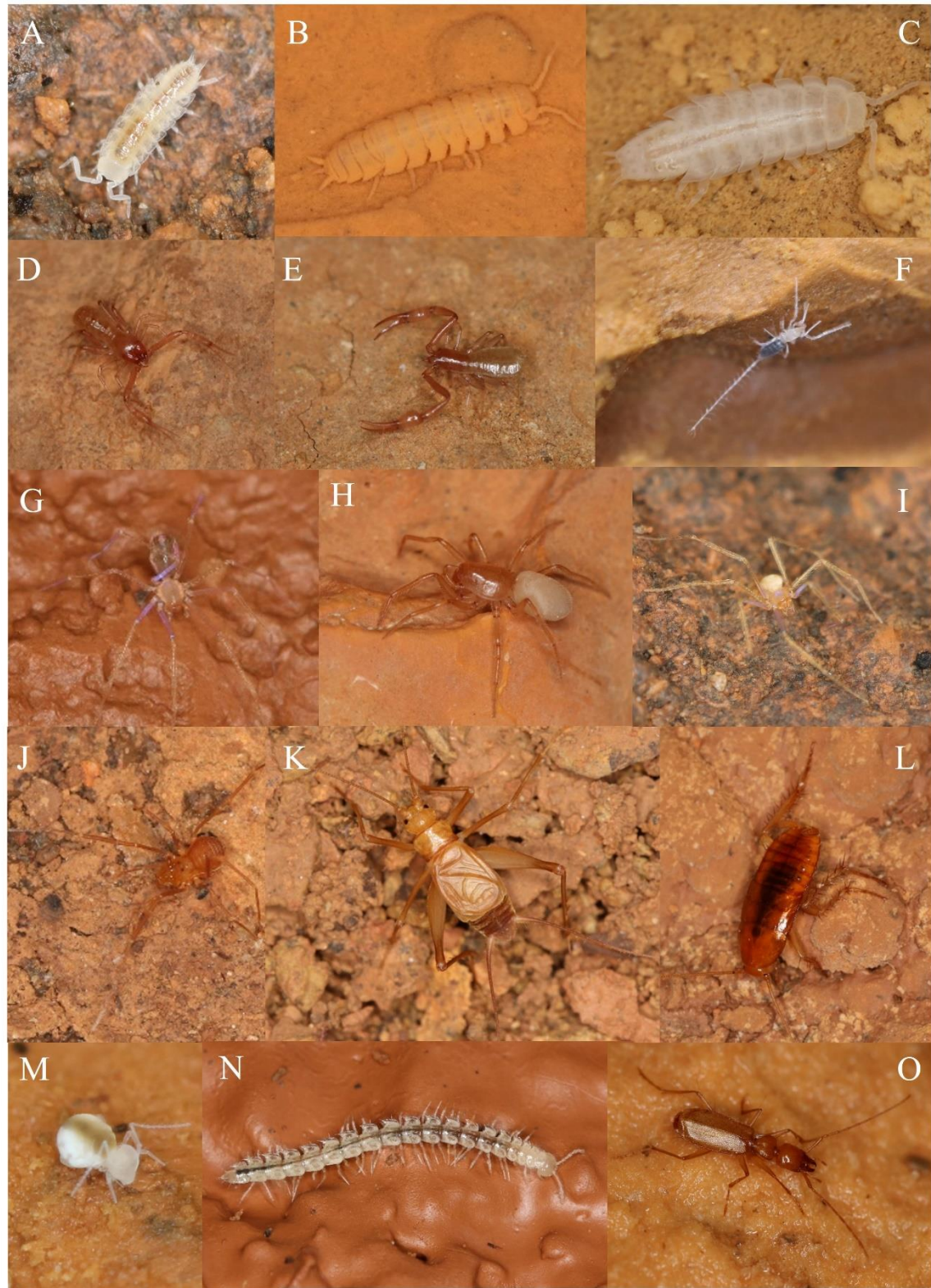


Figure 3. 15 of the 41 troglomorphic species found in Santana region. A– *Pectenoniscus santanensis*; B– *Chaimowiczia tatus*; C– *Chaimowiczia tatus*; D– *Pseudochthonius* sp1; E– Ideoroncidae sp1; F– *Eukoenia* sp2; G– Ochyroceratidae sp1; H– Caponidae sp1; I– Ochyroceratidae sp2; J– Escadabiidae sp1; K– *Endecous* sp1; L– Blattidae sp1; M– Arrhopalitidae sp1; N– *Phaneromerium cavernicolum*; O– *Coarazuphium tessai*.

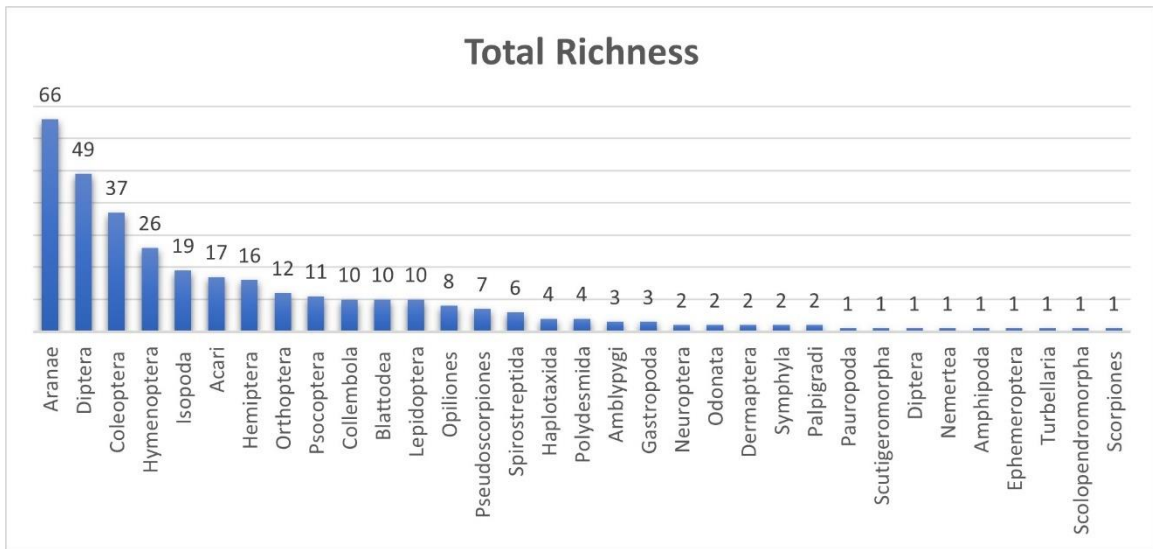


Figure 4. Main invertebrate groups richness of Santana region caves.

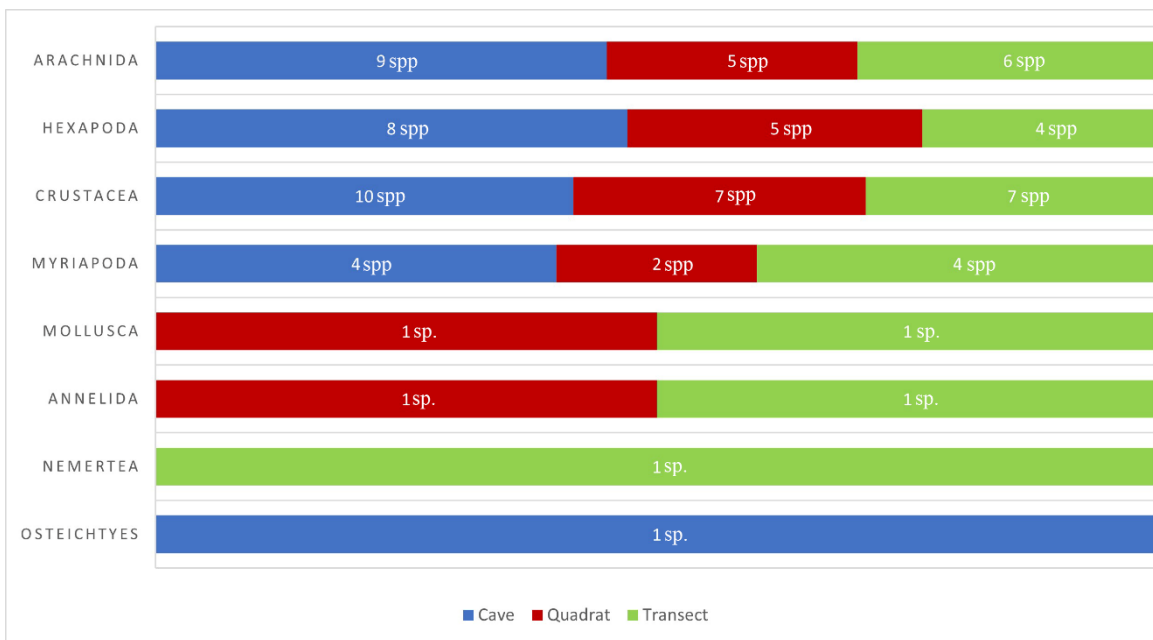


Figure 5. Richness of troglobitic species groups by sample scale in Santana region caves. There are 41 restricted species in total.

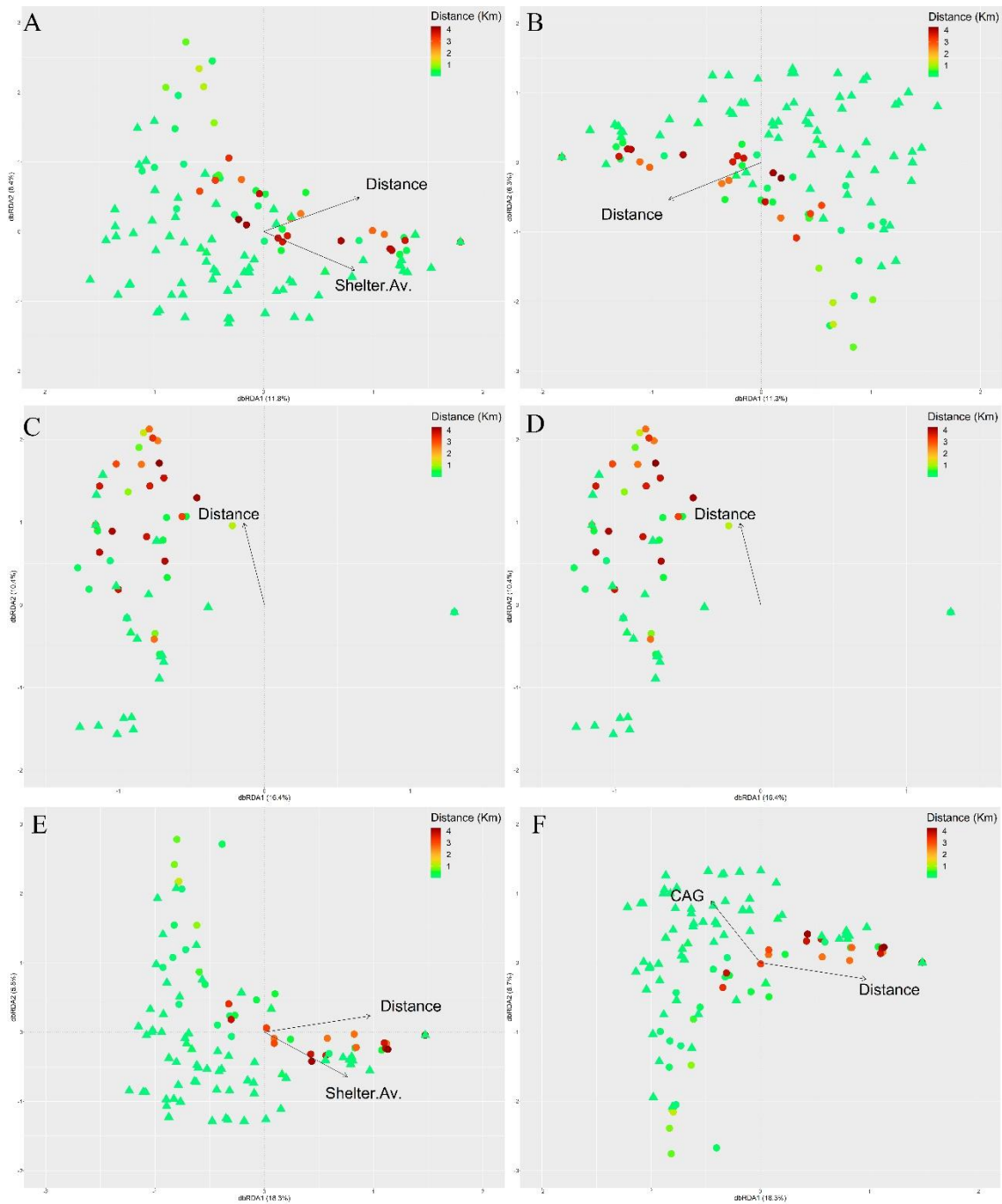


Figure 6. Distance-based Redundancy Analysis (dbRDA) on the mesoscale, succinct (A, C, E) and long (B, D, F) models. A and B—general fauna; C and D—troglobitic fauna; E and F—non-troglobitic fauna.

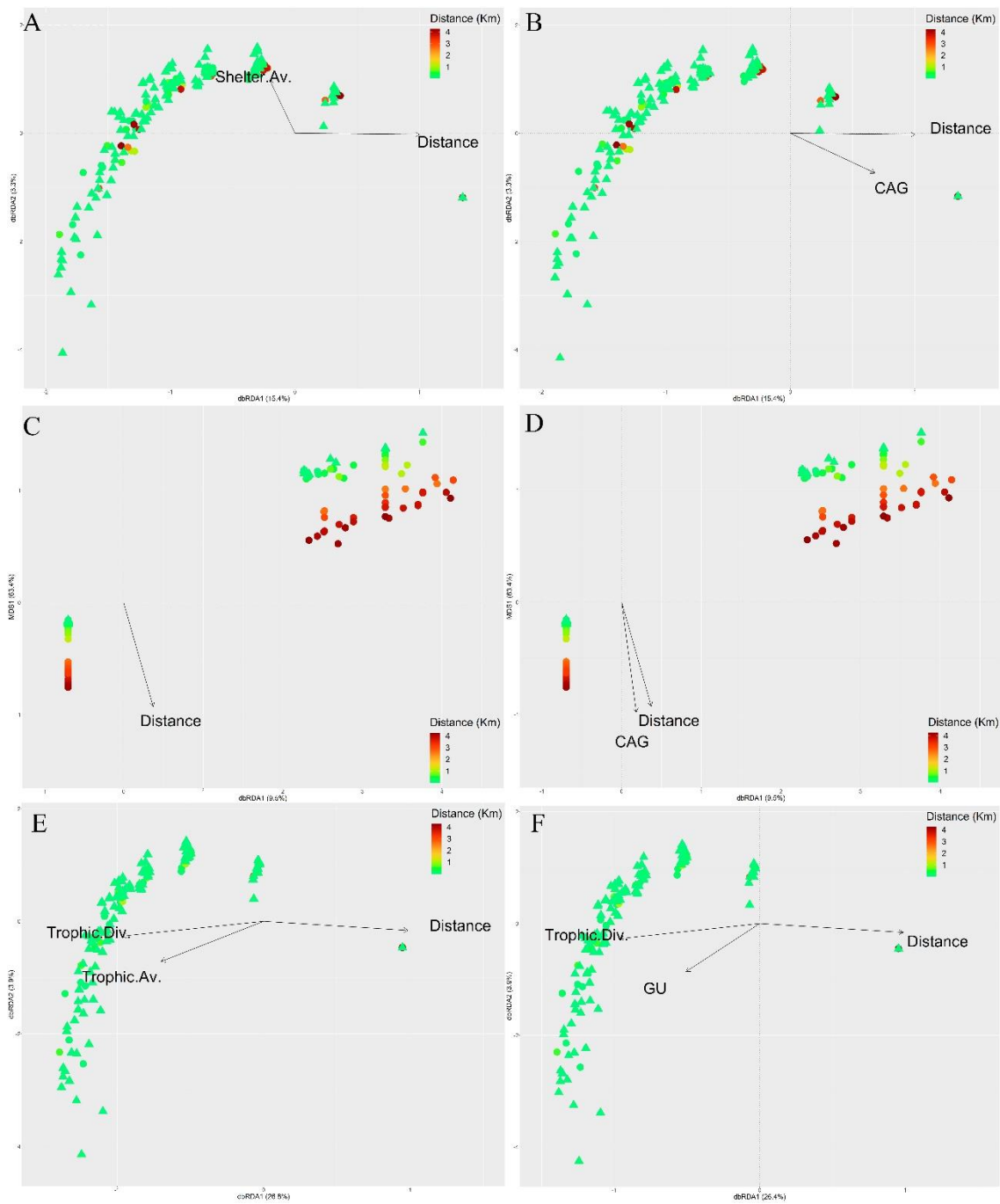


Figure 7. Distance-based Redundancy Analysis (dBRDA) on the microscale, succinct (A, C, E) and long (B, D, F) models. A and B—general fauna; C and D—troglobitic fauna; E and F—non-troglobitic fauna.

Taxons	Species and Morphotypes	Ca	Qu	Sec
Amphipoda	<i>Spelaeogammarus santanensis</i>	+		
Aranae	Ochyroceratidae sp1	+	+	+
	Ochyroceratidae sp2		+	+
	Ochyroceratidae sp3		+	+
	Prodidomidae sp2	+		
	Caponidae sp1	+		
Blattodea	Blattidae sp1	+		
Coleoptera	<i>Clivina</i> sp1	+	+	
	<i>Coarazuphium tessai</i>	+		
Entomobryomorpha	Paronellidae sp2	+	+	+
Gastropoda	Gastropoda sp3	+		+
Oligochaeta	Lumbricina sp3	+		
Hemiptera	Kinnaridae sp1	+	+	+
Isopoda	<i>Chaimowiczia tatus</i>	+		
	<i>Pectenoniscus santanensis</i>	+	+	+
	Platyartridae sp1		+	+
	Styloniscidae sp1	+	+	+
	Styloniscidae sp2		+	+
	<i>Pectenoniscus</i> sp3			+
	Philosciidae sp1	+		
	<i>Trichorhina</i> sp2			+
	Calabozoidea sp1		+	
	<i>Xangoniscus</i> sp1	+		+
Nemertea	Nemertea sp1	+		
Opiliones	Escadabiidae sp1	+		+
	Escadabiidae sp2	+		
	<i>Eusarcus cavernicola</i>	+	+	+
Orthoptera	<i>Endecous</i> sp1	+	+	+
Palpigradi	<i>Eukoeningenia</i> sp1	+	+	
	<i>Eukoeningenia</i> sp2	+	+	
Poduromorpha	Poduromorpha sp1			+
Pseudoscorpiones	<i>Pseudochthonius</i> sp1	+	+	+
	Garypoidea sp1	+	+	+
Polydesmida	<i>Phaneromerium</i> sp1	+	+	+
	<i>Phaneromerium</i> sp2			+
	<i>Phaneromerium</i> sp3	+	+	+
	<i>Phaneromerium</i> sp4	+		
Symphyla	Symphyla sp1	+		
Symphypleona	Arrhopalitidae sp1	+	+	+
	Arrhopalitidae sp2	+		
Siluriformes	<i>Pimelodella</i> sp1	+		

Table 1. Troglotic species

and the sample scale where they were found.

Table 2. P-values for Distance-based Redundancy Analysis (dbRDA).

Variables	Mesoscale						Microscale					
	Succinct Model			Long Model			Succinct Model			Long Model		
	General	T	n-T	General	T	n-T	General	T	n-T	General	T	n-T
Cave*	0.005	0.005	0.005	0.005	0.005	0.005	0.005		0.005	0.005		0.005
Distance*	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Shelter Av.	0.045	0.050										
Trophic Av.							0.005		0.005			
Trophic Div.									0.035			0.005
GU										0.005		0.005
DTV										0.005		
CAG						0.025						0.050
Explanation	41.14%	33.51%	45.74%	39.75%	33.51%	45.75%	24.31%	9.52%	36.86%	24.66%	10.29%	36.63%

Table 3. P-values and Estimate values for the GLM and GLMM on the mesoscale.

Variables	Mesoscale											
	Succinct Model						Long Model					
	General		T		n-T		General		T		n-T	
	P	Est.	P	Est.	P	Est.	P	Est.	P	Est.	P	Est.
Distance*	1.61E-05	-0.273	0.003	0.265	1.79E-09	-0.553	1.73E-07	-0.350			3.09E-14	-0.671
Temperature	0.010	-0.156			0.007	-0.187	0.040	-0.130				
Trophic Av.												
GU									0.026	-3.349		
DTV												
CRC							0.020	0.070			0.033	0.065
FG							0.001	0.085			1.00E-04	0.095
ACT									0.041	1.895		
RC												
RR												
SB							0.049	-0.096				
ARE							1.028	0.111			0.021	0.134
HP							0.035	-0.107			0.038	-0.112
ES							0.015	-0.133			0.007	-0.156
ESC							0.022	0.113				
BM							0.027	0.114			0.013	0.155
GRR												
R²/R²M	38.80%		15.74%		60.42%		56.05%		31.52%		79.38%	

R²C

65.73%

80.71%

60.86%

Table 4. P-values and Estimate values for the GLM and GLMM on the microscale

Variables	Microscale											
	Succinct Model						Long Model					
	General		T		n-T		General		T		n-T	
	P	Est.	P	Est.	P	Est.	P	Est.	P	Est.	P	Est.
Distance*	8.44E-08	- 0.390	2.79E-08	0.507	7.64E-13	- 1.055	6.45E-09	- 0.390	1.40E-06	0.475	1.85E-14	- 1.091
Temperature												
Trophic Av.	0.001	0.120										
GU							0.015	0.075			0.003	0.092
DTV							0.001	0.119			3.00E-04	0.142
CRC												
FG												
ACT												
RC											0.029	0.121
RR							0.041	- 0.116				
SB												
ARE												
HP												
ES												
ESC												
BM												
GRR									0.047	- 0.471		
R²/R²M	24.34%		10.55%		64.15%		28.82%		16.25%		48.00%	
R²C	39.40%				72.21%							

Table 5. The 24 sampled caves' coordinates. Sirgas 200; UTM 23S.

Map ID	Cave	Long. X	Lat. Y
1	Gruta Cãnion da Baixa Verde Cave	596844	8537362
2	Gruta do Padre Cave	601311	8538762
3	Gruta Labirinto do Toxodon Cave	608884	8538822
4	Gruta do Boqueirao Cave	597800	8537451
5	Gruta da Pedra Escrevida I Cave	612496	8532062
6	Gruta das Duas Cobras Cave	608351	8537716
7	Gruta do Tunel II Cave	609390	8535922
8	Gruta Sãn Geraldo Cave	609156	8535758
9	Olho D'água do Cumbra Cave	601050	8529525
10	Racha Bovina Cave	615975	8531872
11	Gruta do Tunel I Cave	609348	8535882
12	Gruta Couve-Flor Cave	609111	8535870
13	Gruta do Geraldo Cruz Cave	600656	8528434
14	Fenda Obliqua Cave	600891	8528568
15	Gruta do Cedro Cave	601082	8538937
16	Gruta do Cedrãn Cave	601040	8539058
17	Gruta do Cedrículo Cave	601044	8539067
18	Gruta do Pajeú Cave	598760	8526941
19	Gruta Cristal Cave	599497	8527612
20	Gruta do Salobro Cave	588687	8554258
21	Gruta da Grota Cave	587858	8561161
22	Gruta do Leãn Cave	601365	8539161
23	Gruta Cinquentona Cave	605268	8538657
24	Gruta da Pedra Escrevidinha Cave	605264	8538648

Artigo 2: Habitat selection of cave invertebrates in a new South American hotspot of subterranean biodiversity

Artigo redigido conforme as normas do periódico *Biodiversity and Conservation*.

Habitat selection of cave invertebrates in a new South American hotspot of subterranean biodiversity

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Abstract

The Gruta do Padre Cave, the fifty largest cave in Brazil, is the home of 25 cave-restricted species, thus becoming the fourth hotspot of subterranean biodiversity in South America. To understand the fauna associated with this cave were performed composition and richness analysis to access how these cave communities respond to habitat characteristics, such as climatic variables, different substrates, presence of shelters, and food resources. The results demonstrate that the *Distance from the nearest entrance* and the *Zone* within the cave where the transects are placed are the main factors for fauna distribution. Also, habitat heterogeneity demonstrates a correlation with the fauna richness and composition, with shelter and food availability as decisive traits. The information accomplished herein demonstrates the great importance of the conservation of Gruta do Padre Cave as a new hotspot of subterranean biodiversity and highlights the urge to protect the cave in all its complexity, since this is a very heterogeneous cave with unique habitats within it.

Introduction

Several studies have demonstrated that the cave linear development tends to be strongly related to the species richness in such environments (Simões *et al.* 2015; Souza-Silva *et al.* 2020; Souza-Silva *et al.* 2021; Rabelo *et al.* 2021). This relation is probably because bigger caves tend to present both greater areas and higher habitat heterogeneity. However, the large linear development can be limiting, especially in caves with few entrances. It is well known that caves rarely present primary production, excepting a few subterranean chemautotrophically based ecosystems (Sarbu & Kane 1996; Galassi *et al.* 2017) and entrance zones (Souza-Silva *et al.* 2011; Prous *et al.* 2015). Thus, the main source of energy in caves comes from external environments, corresponding to the organic matter transported by physical or biological agents (Souza-Silva *et al.* 2011; Ferreira 2019). Hence, in large caves with few entrances, the organic matter hardly reaches deep areas, limiting the available energy for the communities (Tobin *et al.* 2013; Moseley 2008; Ficetola *et al.* 2018; Mammola 2019).

As well as the large extension of the caves, the presence of rivers also contributes to the maintenance of richer communities (Simões *et al.* 2015). Rivers and streams act as trophic resource carriers, especially in the case of allogenic drainages, taking energy from external habitats and bringing it to deeper cave zones. Additionally, microclimate traits, such as temperature and moisture, are usually linked to the presence of great water bodies (Souza-Silva *et al.* 2011b; Lobo *et al.* 2015; Simões *et al.* 2015; Souza-Silva *et al.* 2020).

Furthermore, despite the general stability observed in caves, a gradient of climate conditions is usually observed from near-to-entrance zones (which are more variable) to deeper areas (Moseley 2009; Tobin *et al.* 2013; Lunghi *et al.* 2014; Prous *et al.* 2015; Mammola and Isaia 2018; Lunghi and Manenti 2020; Souza-Silva *et al.* 2021). This zonation generates distinct microhabitats for cave fauna, which varies not only in climatic and photic characteristics but also in the types of substrates they present (Moseley 2008; Souza-Silva *et al.* 2011b; Du Preez *et al.* 2015; Lunghi *et al.* 2017; Mammola and Isaia 2017; Mammola 2019; Lunghi and Manenti 2020; Mammola *et al.* 2020; Souza-Silva *et al.* 2021).

Cave-restricted species (troglobitic species) usually present adaptations to the cave environments, which can be morphological, physiological, reproductive, or even behavioral (Romero & Green, 2005).

Furthermore, those species are usually rare and endemic because of their long evolutive history in such stable environments, which makes them sensitive to changes in their ecosystems, such as slight variations of temperature and moisture (Culver & Pipan 2009; Mammola *et al.* 2019).

The troglobitic species are so unique that caves with many cave-restricted species are classified as Hotspots of Subterranean Biodiversity (HSB). According to Culver and Sket (2000), a cave or a cave-system must have 20 or more cave-restricted species to be considered a hotspot. However, Culver *et al.* (2021) raised the cutoff to 25 species, arguing that the global list was too lengthy. It is important to consider, nevertheless, that 25 species is another arbitrary cutoff (as the originally purposed number of 20 species), that probably results from an analysis preferably considering temperate regions. Thus, it is important to mention that caves located in tropical areas will rarely present up to 25 cave-restricted species. Hence, we herein opted by keeping the original concept of HSB (Culver & Sket, 2000).

Considering the original definition of Culver and Sket (2000), there are currently three HSB in Brazil: the Toca do Gonçalves cave (northeastern Brazil) with 22 troglobitic species; the Areias Cave System (southeastern Brazil) with 28 troglobitic species and the Água Clara Cave System (northeastern Brazil), with 30 troglobitic species (Souza-Silva & Ferreira 2016; Souza-Silva *et al.* 2021). Of those three HSB, only one (Areias Cave System) is protected within the limits of a State Conservation Unit (Parque Estadual Turístico do Alto Ribeira). The two remaining are in unprotected areas and are currently exposed to several anthropogenic threats (Souza-Silva & Ferreira 2016; Souza-Silva *et al.* 2021).

Unfortunately, Brazilian speleological legislation does not protect these important areas. From 1990 onwards, Brazilian caves were fully protected, but in 2008, a decree determined that Brazilian caves should be classified according to their relevance degree, allowing some caves to be destroyed for mineral resources exploitation. Only those caves classified as presenting maximum cultural, geological, and/or biological value should be preserved (Decree nº 6.640). However, a new decree (Decree nº 10.935) from 2022, started to allow the destruction of even those caves with maximum relevance.

The Gruta do Padre cave is one of the largest known caves in Brazil, with 16,400 meters of mapped galleries, representing the fifth longest cave in the country. Additionally, it is part of the most extensive subterranean hydrological system in Brazil (Rubbioli *et al.* 2019). Considering the uniqueness and high

biological relevance of this Cave, the main goal of this study was to identify the variables determining the spatial distribution of invertebrates along with presenting the fourth hotspot of subterranean biodiversity in the Neotropical region. Furthermore, as this cave presents areas with highly distinct conditions (upper dry galleries and lower stream conduits), variables describing the physical, trophic, and microclimatic attributes of the cave were used to test three hypotheses: *i*) upper and lower areas within the cave will present distinct communities regarding the species composition; *ii*) species richness will be reduced in areas far from the cave entrances; and *iii*) invertebrates will respond to different habitat components and habitat heterogeneity on the cave floor in the distinct cave compartments (upper and lower areas). In addition, we discuss the impacts over this cave and argue about the importance of preserving this unique new South American hotspot of subterranean biodiversity.

Material and Methods

Study area

The Gruta do Padre cave is located at the Santana municipality (Fig. 1), in southwestern Bahia state, and is inserted in the Bambuí Group, the largest carbonate region in Brazil, with 146,378 Km² of area and approximately 6,302 registered caves. The Santana municipality is placed in a transition zone between the Caatinga and Seasonal Dry Forests and has a high potential for endemic species (Dinerstein *et al.*, 2017). This high potential probably resulted from several paleoclimatic changes in the Brazilian semi-arid caused by the expansions and retreats of Atlantic and Amazonian humid forests (Sobral-Souza; Lima-Ribeiro; Solferini, 2015). The climate in the area is the Aw (Köppen, 1936), with dry winters and rainy summers. Due to the strong tropical rains that occur in the region during the summer, safe access to the cave is only possible in dry periods (March to October).

The Padre Cave comprises the bigger portion of the longest subterranean hydrologic system in the country, formed by a long subterranean stretch of the Santo Antônio River (Auler *et al.*, 2019). This river flows through four caves before reaching the Corrente River. Firstly, the Santo Antônio River becomes underground in a small nameless cave, which siphons after the entrance. In a second moment, the river reappears in the Cipó Cave, with approximately 2.76 Km of extension, sinks again, and

reappears in the Padre Cave. Into this cave, it flows through 6.2 Km of large conducts until sinks. Finally, the river reappears in the Bananeira Cave, the twenty-fifth longest cave in Brazil, with its 6.55 Km of waterlogged conducts, and then flows into Corrente River (Auler *et al.*, 2019).

The Padre Cave has two entrances, located around 1.7 Km far from each other. The upstream entrance is also locally known as Lapa do Cedro Cave or Lapa D'água Cave (Fig. 2–B) and presents rupestrian paintings and archeological engravings indicating its past use by native populations. The downstream entrance, known as Padre Cave or Santo Antônio Cave, was used as a peregrination point for religious in the 20th century. Although both entrances have a narrow area of dry forest protecting them (Fig. 2–A), pastures and monocultures are dominant in the surrounding landscape (Auler *et al.*, 2019).

The cave can be dived into three distinct levels. The lower level (Fig. 2–D), where the Santo Antônio river flows, is the longest, with very deep areas and a high ceiling, reaching 40 meters. The second level is located around 45 meters above the river level and comprises giant galleries (Fig. 2–C), some of which present almost 50 meters in width. These galleries are composed of different kinds of substrates, such as downed blocks, sandy areas, and speleothems. The third and last level is smaller and can be found 53 meters above the Santo Antônio river (Auler *et al.*, 2019).

Field procedures

Sampling design

The richness and composition of cave invertebrates, as well as the habitat structure traits, were determined along 53 transects (meso-scale sampling - 10 × 3 m each) distributed on the caves' floor (Fig. 3), from the entrances to the deeper regions of the cave, encompassing both the lower level (river conduit) and the upper level (upper dry galleries). Quadrats (micro-scale sampling - 1 m²) were inserted in triplicates within the limits of each transect (Fig. 2), totalizing 159 quadrats (Souza-Silva *et al.* 2021). Invertebrate sampling was done by visual search along the transects and quadrats (Souza-Silva *et al.* 2021). The sampling in the quadrats allowed the detection of low mobility and small-size species, which could then be thoroughly searched in the remaining transect if detected. The sampling was first

performed in the quadrats and later in the respective transect, always by three collectors, and was only completed when all the invertebrates had been sampled and/or accounted for. The search time varied among each sampling unit since the different sampling areas presented a considerable structural distinction along the cave. Additionally, to maximize the detection of troglobitic and stygobitic species, direct intuitive search techniques were also applied in other cave areas (Wynne *et al.* 2019). Invertebrates were preserved in properly labeled jars containing 70% ethanol. In laboratory, the specimens were sorted with a Stemi 508 (ZEISS) stereomicroscope, identified until the lowest possible taxonomic level, and separated into morphotypes (Oliver & Beattie, 1996). Potential troglobitic species were identified by the presence of troglomorphic traits, such as eyes and pigmentation reduction, appendage elongation, among others (Culver & Pipan, 2009). Furthermore, specialists in different taxa were also consulted to assist in the detection of specific troglomorphic traits (specialists are acknowledged further on). The voucher specimens were deposited in the Collection of Subterranean Invertebrates of Lavras (ISLA), linked to the Center of Studies on Subterranean Biology (CEBS) of the Federal University of Lavras (UFLA).

Environmental traits in different scales

The survey of the habitat structure parameters in the transects was performed according to the methodology used by Souza-Silva *et al.* (2021). Each transect was subdivided into 10 sections (1 × 3 m) (Fig. 3), in which the surface area occupied by distinct organic and inorganic substrates was visually quantified. Then, a sum was made to obtain the area occupied by each substrate throughout the entire transect. To minimize observer error, all transects were characterized by the same researcher. A digital thermo-hygrometer positioned at the ground at the center of each transect was used to measure the temperature and humidity. The proportion of each substrate in the quadrats was determined through photographs (4000 × 3000 pixels) taken of each quadrat as close as possible to a 90° angle with a Canon Powershot SX60HS camera, at the researcher's chest high. Photographs were posteriorly analyzed with the aid of *ImageJ 1.53K* software. Each sector's position in the cave was plotted on its map and then the distances to the nearest entrance were obtained. The coordinates of each sector in the cave were obtained by the plot in the map with the aid of *QGis 3.22* software.

Data analysis

Pre-analysis routine

All the analyses were performed in the R Studio 2022.07.02 Build 576 software. Before running the analysis for invertebrate fauna composition and richness, the correlation between the variables was tested with the aid of the *CHART.CORRELATION* function from the ‘*PerformanceAnalytics*’ package. Variables with correlation values higher than 0.70 were excluded from the models. Variables correlated with more than one other variable and with less specificity were favorite to exclusion. Using the functions *VIF* and *VIF.CCA* from the ‘*Car*’ package, the multicollinearity of variables was tested, and those presenting values higher than 10 were excluded. Firstly the variable with highest value was excluded, then the test was repeated, and the process continue until no higher than 10 value was found. A Shapiro-Wilk test was executed, to verify the normality of the data, using the *SHAPIRO.TEST* function from the package ‘*Stats*’. A Mantel test was performed to try the spatial autocorrelation between the samples, in both meso and microscales.

Mesoscale abiotic features

All the substrates in each sector were evaluated and classified into the following classes: vegetal debris - DTV (< 10mm); fine branch - GALF (11 - 30 mm); medium branch - GALM (31 - 50 mm); coarse branch - GALG (65 - 250 mm); trunk - TRO (> 250mm); water pond - WP; drip water - DP; coleoptiles - COL; smooth rock - RL; concrete floor - RC; large rock - XB (1000 - 4000mm); medium rock - MB (500 - 1000mm); small rock - SB (250 - 500mm); cobbles - CB (64 - 250mm); coarse gravel - CAG (16 - 64mm); fine gravel - CAF (2 - 16mm); sand - ARE (0.06 - 2mm); silt - SEF (≤ 0.05 mm); hardpan - HP; speleothems - ES; calcite rafts - JNS; concreted calcite raft - JNC; stalagmite - EST; micro travertine - MTR; travertine - TRA; rough flowstone - ESR; flowstone - ESC; worm acorn - BM; retraction cracks - GRR; owl acorn - BCO; pinnacle - PIN; another inorganic substrate - OTI.

Based on those classes and using a Shannon-Weaver Index (Buttigieg and Ramette 2014), the Substrate Diversity (all classes), the Shelter Diversity (WP, DP, XB, MB, SB, CB, CAG, CAF, ES, JNS, JNC, EST, MTR, TRA, ESR, ESC, BM, GRR, BCO) and the Trophic Resources Diversity (DTV,

GALF, GALM, GALG, TRO) were calculated for each sector. The classes were also used to generate, by simple summation, the Shelter Availability (WP, DP, XB, MB, SB, CB, CAG, CAF, ES, JNS, JNC, EST, MTR, TRA, ESR, ESC, BM, GRR, BCO) and the Trophic Resources Availability (DTV, GALF, GALM, GALG, TRO) for each sector.

To reduce variables in analysis with individual substrate classes, some variables were grouped. DTV, GALF, GALM, GALG, and TRO were grouped into DTV (vegetal debris). The classes ES, JNS, JNC, EST, MTR, and TRA were grouped into the class ES (speleothems).

The abiotic attributes were then separated into *Physical features*, which comprise the *Distance of each transect from the nearest entrance*, the *Cave Zone* (Shallow, River, or Deep), *Substrate Diversity*, *Shelter Diversity*, and *Shelter Availability*. The *Trophic Resources* grouped *Trophic Resources Diversity* and *Trophic Resources Availability*. The *Microclimatic Variables* considered were *Temperature* and *Moisture*.

Microscale abiotic features

For the quadrats, the same substrate classes were evaluated, and the same groupings were formed. The diversities and availabilities were calculated in the same manner as for the sectors. The *Physical* and *Trophic* features were also formed similarly to the mesoscale. For the microscale, the microclimatic variables were not measured.

Habitat traits determining the communities' richness and composition

To understand the possible correlation between total richness, troglobitic, and non-troglobitic invertebrate richness with *physical*, *trophic*, and *microclimatic* variables, Generalized Linear Models (GLM) and Generalized Linear Mixed Models (GLMM) were performed, with sectors and quadrats as sample units, using two models for each fauna type. In the first model (Succinct Model) were used *Microclimatic Variables*, Distance, Diversities (general substrate, shelter, and trophic resources), and Availabilities (shelter and trophic resources). The second model (Long Model) used *Microclimatic Variables*, Distance, Diversities (general substrate, shelter, and trophic resources), and each substrate class individually. For the microscale, the *Microclimatic Variables* were not used.

For those models, the Poisson family was adopted because it better fitted the data. For the evaluation of model overdispersion, the function *CHECK_OVERDISPERSION* from the package ‘*Performance*’ was used. To obtain r^2 values of the GLMMs the function *r.squaredGLMM* from the ‘*MuMIn*’ package was used, while the function *r.squaredLR* from the ‘*piecewiseSEM*’ package was used to obtain r^2 values of the GLMs.

To evaluate the possible correlation between total, troglobitic, and non-troglobitic invertebrate composition with *physical*, *trophic*, and *microclimatic* variables a Distance-Based Redundancy Analysis (dbRDA) was performed (Clarke *et al.* 2014), with sectors and quadrants as sample units, using two models for each fauna type. In the first model (Succinct Model) were used *Microclimatic Variables*, *Zones*, *Distance*, *Diversities* (general substrate, shelter, and trophic resources), and *Availabilities* (shelter and trophic resources). The second model (Long Model) used *Microclimatic Variables*, *Zones*, *Distance*, *Diversities* (general substrate, shelter, and trophic resources), and each substrate class individually. For the microscale, the *Microclimatic Variables* were not used.

Distinction among Zones of the Gruta do Padre Cave

The troglobitic fauna proportion was obtained by the division of the richness of cave-restricted fauna by the total richness for both micro and mesoscale. To access the influence of the distance from the nearest entrance in the troglobitic fauna proportion, Linear Models were used. To access the differences in the average habitat traits in each Areas of the Gruta do Padre Cave, a Kruskal-Wallis’s test was used. Finally, for those significantly different variables, a Nemenyi Post Hoc test was used to understand which area differs from the others.

Results

Richness and composition of cave invertebrates

Considering the whole Padre Cave (with samplings in the sectors, quadrants, and other habitats) 1,811 specimens were collected, totalizing 115 species. These were grouped into 31 orders and at least 61 families (Fig. 4). The richest group was Araneae, with 22 species distributed in 11 families, totalizing

277 individuals. The spiders were followed by Diptera and Coleoptera, with 15 and 14 species respectively.

Cave-restricted species

The Gruta do Padre Cave has at least 25 cave-restricted species belonging to 8 higher taxa and 14 families (Fig. 5 and 6). The eight higher taxa were Hexapoda (8 spp.), Arachnida (6), Crustacea (5), Myriapoda (2), Mollusca (1), Nemertea (1), Annelida (1) and Osteichthyes (1) (Table 1). The richness of the observed obligate cave fauna was Gastropoda (1), Nemertea (1), Oligochaeta (1), Isopoda (4), Amphipoda (1), Polydesmida (1), Sympleyla (1), Blattodea (1), Collembola (4 spp.), Coleoptera (2), Orthoptera (1), Pseudoscorpiones (1), Araneae (1), Opiliones (2), Palpigradi (2), and Siluriformes (1). However, only 24% of the obligate cave species from the Gruta do Padre Cave are described. Such described species are *Coarazuphium tessai* (Godoy & Vanin, 1990); *Phaneromerium cavernicolum* (Golovatch & Wytwer, 2004), *Spelaeogammarus santanensis* (Koenemann & Holsinger, 2000), *Eusarcus cf. cavernicola* (Hara & Pinto-da-Rocha, 2010), *Pectenoniscus santanensis* (Cardoso et al. 2020) and *Chaimowiczia tatus* (Cardoso et al. 2021). It is important to note that although the type series of *E. cavernicola* presents specimens from Gruta do Padre cave, Hara & Pinto-da-Rocha (2010) attested that “it is possible that what we know as *E. cavernicola* is actually an assembly of species that cannot be recognized by external and genitalic features”. Considering that the population observed in the Gruta do Padre Cave is strongly troglomorphic (much elongated and depigmented when compared to other populations, we are herein considering that it constitutes a troglobitic species.

Habitat traits determining the communities' richness and composition

For the communities' composition, the *Zone* of the cave where the samples were made was the most important factor, being significant for all models in both meso and microscale. The *Distance from the nearest entrance* was the second more important trait, not showing significance for the troglobitic fauna in the mesoscale. The *Shelter* and *Trophic Resources Availability* were important to the general and non-troglobitic fauna of the mesoscale and the non-troglobitic fauna in the microscale. The *Shelter Diversity* was significant for the general and troglobitic species on the mesoscale, while the *Trophic Resources*

Diversity was important to general and non-troglobitic fauna on the microscale. The *Temperature* presented correlation only to general and non-troglobitic species on the mesoscale. From the individualized substrates, *Vegetal Debris* (DTV) was important to general and non-troglobitic fauna on both scales, while *Course Gravel* (CAG), *Speleothems* (ES) and *Concrete Floor* (RC) were important to non-troglobitic fauna (Table 1) (Figs 7 and 8).

For the communities' richness, the *Distance from the nearest entrance* was the most important trait, being significant to non-troglobitic fauna on the mesoscale and general and non-troglobitic species on microscale, always with negative effects. The *Temperature* was important negatively on mesoscale for general and non-troglobitic fauna. The *Shelter* and *Trophic Resources Availability* shown positive correlation only with non-troglobitic fauna on micro and mesoscale respectively. The *Substrate Diversity* was important to troglobitic species on microscale, with negative effects. *Smooth Rock* (RL) and *Small Rock* (SB) presented correlation to non-troglobitic fauna on mesoscale, with positive and negative effects respectively. *Hardpan* (HP) was negatively important to general and non-troglobitic fauna on mesoscale, while *Speleothems* (ES) was positively important to general and non-troglobitic on microscale (Table 2 and 3).

For the mesoscale scale, the proportion of troglobitic fauna among all the communities shows a significant correlation with the *Distance from the nearest entrance* ($p=0.002$) (Est= 0.134), with an explanation of 17.46% for the linear model. In the microscale, there was no significant correlation revealed by the linear model.

Differences between Zones of the Gruta do Padre Cave

The cave *Zones* (Shallow, River, Deep) within Gruta do Padre Cave differed in Shelter Availability, Trophic Availability, Shelter Diversity, and Temperature. The Shelter Availability in Shallow zones was significantly different from River and Deep Zones in both scales. In the case of Trophic Resources Availability and Shelter Diversity, only Deep and Shallow zones were statistically contrasting, in both scales. To the *Microclimatic Variables*, the average Temperature varied among the Shallow and Deep zones (Table 5).

Regarding the average richness, the meso and microscale respond in the same manner, with the total and non-troglobitic richness of the Deep differing significantly from the Shallow and the River zones. The troglobitic fauna richness, on the other hand, is different between the three zones (Table 5).

Discussion

Distance from entrance and Cave Zones affecting the communities

Two habitat traits have emerged as crucial factors influencing the distribution of invertebrate species within the Gruta do Padre Cave. These are the *distance from the nearest entrance* and the *cave zones*. The distance from the entrance is a key determinant of community distribution, as demonstrated by various studies on cave communities. This is due to the limiting effect of long distances within the cave, which leads to a decrease in trophic resources and habitat diversity from the entrance to deeper regions of the cave (Tobin et al. 2013; Moseley 2008; Ficetola et al. 2018; Mammola 2019; Souza-Silva et al. 2021). Organic matter is one of the most significant inputs into caves, primarily transported from external habitats through the entrances. As a result, the availability of organic matter decreases in the deeper zones of the cave (Sarbu & Kane 1996; Moseley 2008; Souza-Silva et al. 2011; Tobin et al. 2013; Prous et al. 2015; Galassi et al. 2017; Ficetola et al. 2018; Mammola 2019).

The distance from the entrance also affects habitat heterogeneity, as caves exhibit a gradient of features from the entrance to the deeper regions. The proximity to the entrance is characterized by less stable climatic conditions, resulting in gradients of temperature, moisture, and sunlight. This gradient is also reflected in the cave floor, with the substrates becoming increasingly simpler and homogeneous as one moves towards deeper zones (Prous et al. 2004; Prous et al. 2015; Souza-Silva et al. 2021; Furtado et al. 2022). In the case of Gruta do Padre Cave, which is a vast cave (16.5 km) with only two entrances, these effects are particularly noticeable. As one moves from the entrances to deeper areas, there is a reduction in organic matter and habitat heterogeneity, which directly affects the cave fauna.

The richness of the general and non-troglobitic fauna decreases as one moves from near the entrance to the deeper zones of Gruta do Padre Cave, likely due to the decrease in food sources and habitat complexity (Prous et al. 2004; Prous et al. 2015; Souza-Silva et al. 2021; Furtado et al. 2022).

Conversely, the troglobitic fauna responds differently, with richness increasing towards the deeper regions of the cave. Troglobitic species are highly adapted to living in areas with very limited resource availability, making them attracted to the greater climatic stability observed in the deeper regions of the cave. Furthermore, many troglobitic species are vulnerable to fluctuations in microclimate conditions due to thin cuticles, which increase the risk of desiccation. Therefore, these species tend to occur in areas with minimal temperature and moisture fluctuations, which are typically found in deeper areas of caves (Tobin et al. 2013; Lunghi et al. 2014 and 2017; Kozel et al. 2019; Souza-Silva et al. 2021). In Gruta do Padre Cave, this preference for stable locations is particularly evident in the River zone. While the richness of non-troglobitic fauna significantly decreases towards deeper areas of the zone, the richness of troglobitic species remains relatively constant. This is likely due to the presence of large water bodies, such as the Santo Antônio River, which can maintain stable microclimatic conditions such as temperature and moisture, enabling the existence of troglobitic species throughout the entire river's length (Souza-Silva et al. 2011b; Lobo et al. 2015; Simões et al. 2015; Souza-Silva et al. 2020).

However, despite their climatic stability, deeper zones are often characterized by a lack of food resources, as noted by Souza-Silva et al. (2011) and Prous et al. (2015). This scarcity of trophic resources can limit the presence of many species. However, unlike the non-troglobitic fauna, cave-adapted species usually exhibit K-strategy life histories and reduced metabolic rates, enabling them to flourish in oligotrophic environments (Hüppop, 2005). Therefore, troglobitic species can evade stronger and more energy-demanding competitors, typically non-troglobitic species, by inhabiting areas with limited food sources (Sket, 1999; Deharveng & Bedos, 2000; Souza-Silva et al., 2021). This preference for resource-poor regions is evident in the Deep zone of Gruta do Padre Cave, where only troglobitic species are present.

Nevertheless, it is noteworthy that although the distance from the nearest entrance is a significant factor in determining the distribution of fauna inside Gruta do Padre Cave, the levels or cave zones where the communities reside also play a crucial role. Notably, the availability of food resources is relatively higher at the Santo Antônio River level, spanning both shallow and deep zones. This finding

supports the notion that rivers can transport trophic resources through the cave, providing energy to communities in diverse locations (Simões et al. 2015).

The variation in habitat complexity also varies between the levels of Gruta do Padre Cave. In the upper dry level, the habitat heterogeneity decreases towards deeper zones, while in the stream level, the complexity increases. This is because the near-to-entrance stream zone consists mainly of sediment banks composed of silt, sand, and gravel, while the deep stream zone features more complex habitats, such as travertines, waterfalls, and a variety of speleothems. These distinctive features between the upper dry and stream levels also contribute to the unique characteristics of Gruta do Padre Cave, with each zone hosting a distinct community with unique species and habitat characteristics.

Habitat heterogeneity affecting communities

In the Gruta do Padre Cave, the diversity of habitats, characterized by both trophic and physical traits, plays a vital role in determining the distribution of invertebrate communities. It is well-established that in cave environments, greater habitat diversity can facilitate the coexistence of a larger number of species, by creating new niches in unique microhabitats (Prous et al. 2004; Prous et al. 2015; Souza-Silva et al. 2021; Furtado et al. 2022). The Gruta do Padre Cave supports this notion by highlighting the importance of both shelter and substrate diversity for sustaining its communities. The environmental complexity, particularly of substrates, provides additional microhabitats, thereby reducing niche overlap and competition among species (Poulson & Culver 1969; Ferreira & Souza-Silva 2001; Tews et al. 2004; Stein et al. 2014; Pellegrini et al. 2016; Resende & Bichuette 2016; Pacheco et al. 2020a).

However, the response of troglobitic species to habitat heterogeneity differs from that of non-troglobitic species. While an increase in shelter availability and habitat complexity may lead to a higher number of individuals, it does not necessarily result in higher species richness due to the high specialization of these animals (Pacheco et al. 2020a). This suggests that cave-restricted species have specific preferences for the conditions inside Gruta do Padre Cave (Pacheco et al. 2020a; Souza-Silva et al. 2021).

The composition and richness of invertebrate communities in Gruta do Padre Cave strongly depend on the availability and diversity of trophic resources. The different types of organic matter available provide a greater variety of niches, allowing for higher species richness and causing changes in community composition in different areas of the cave. This pattern is consistent with previous studies of non-troglobitic animals (Schneider et al. 2011; Souza-Silva et al. 2011; Ladle et al. 2012; Ferreira 2019; Pacheco et al. 2020a; Furtado et al. 2022).

Among all the types of organic matter found in the Gruta do Padre Cave, vegetal debris (DTV) was the most prevalent and crucial for the increase in richness of non-troglobitic fauna. The vegetation around the cave consists mainly of deciduous species, which leads to an accumulation of leaves and small branches in the litter during the dry season (Crowther 1987; Brina 1998; Souza-Silva et al. 2011). During the rainy season, flood pulses transport large volumes of this stored vegetal debris and other substrates downstream into the cave (Minshall et al. 1983; Carrling 1987; Downes & Street 2005). Thus, external seasonal events affect the trophic dynamics of subterranean habitats, as cave populations rely on vegetal debris from the external environment (Souza-Silva et al. 2011).

Although the positive relationship between cave invertebrate populations and organic matter is relevant for non-restricted fauna, it varies for troglobitic species. Restricted subterranean fauna often has low metabolic rates and a K strategy life history, enabling them to tolerate periods of hunger (Hüppop 2005; Souza-Silva et al. 2021). Due to increased energetic needs and better competitive fit of non-troglobitic species, troglobites choose areas with minimal organic materials, avoiding competition with other species (Sket 1999; Deharveng & Bedos 2000; Souza-Silva et al. 2021). Therefore, the response to organic matter availability differs depending on the trophic specialization and ecological requirements of each species.

Conservation of a new Hotspot of Subterranean Biodiversity

The Gruta do Padre Cave stands out as a unique cave in the Brazilian semiarid region, owing to its extensive linear development, voluminous conduits and chambers, and the presence of the Santo Antonio River, as well as the remarkable and exclusive fauna that inhabit the cave.

Out of the fauna surveyed in this cave, 25 species are cave-restricted, thereby designating the Gruta do Padre Cave as a Subterranean Biodiversity Hotspot, as per Culver and Sket (2000). This finding places the cave on the South American roster of subterranean hotspots, along with three other caves: the Toca do Gonçalves Cave in the Campo Formoso municipality (Bahia state) (Souza-Silva & Ferreira, 2016); the Areia Cave System in the Iporanga municipality (São Paulo state) (Souza-Silva & Ferreira, 2016), and the Água Clara Cave System in the Carinhanha municipality (Bahia state) (Souza-Silva et al. 2021). The inclusion of the Gruta do Padre Cave in this elite list highlights the crucial importance of the semiarid region for subterranean biodiversity in Brazil since three of the four hotspots are situated in the Caatinga, the only semi-arid biome in Brazil.

The high biodiversity and endemism of troglobitic fauna in the Caatinga domain may be related to the climatic history of this dry region (Souza-Silva et al., 2021). During the Last Glacial Maximum, the area where these cave systems are located was covered by humid tropical forests (Collevatti et al., 2013). As these forests began to recede to their current locations, the ancestors of the current troglobitic species were likely "trapped" inside caves (Wang et al., 2004; Souza-Silva & Ferreira, 2016; Polhemus & Ferreira, 2018). However, the lack of hotspots in South America may also be related to the scarcity of well-studied areas or caves (Deharveng & Bedos, 2012; Souza-Silva et al., 2021), as Brazil still has vast expanses of karst areas that have not undergone speleological surveys.

Many of the 25 troglobitic species are endemic to the Padre Cave, which highlights the need to protect this cave since it may be the only habitat for such species. Nonetheless, the differences between distinct zones of the cave underscore the importance of preserving the unique attributes of each zone.

The different zones of Gruta do Padre Cave exhibit varying physical and microclimatic conditions, resulting in divergent faunal composition and richness. These contrasts may also create barriers within the cave, with species found at the River level unable to survive in the Shallow or Deep dry zones, and vice versa. Consequently, the cave's fauna may be not only endemic to Gruta do Padre Cave but also to specific regions of the cave. Even so, the River level may be the most critical area for conservation in Gruta do Padre Cave as it harbors the highest number of restricted species. The Santo Antônio River is

a significant feature of the cave, influencing climatic conditions such as temperature and moisture and transporting resources throughout the cave's extensive course.

It is crucial to emphasize that the Padre Cave constitutes just one component of a vast cave system that encompasses the largest subterranean hydrological system in Brazil (Auler et al. 2019). The conservation of both the Cipó and Bananeira Caves (which are also trespassed by the Santo Antônio River) is also vital for the preservation of the Santo Antônio River. This river, aside from being critical to the invertebrate communities of the Padre Cave, is a significant tributary of the Corrente River, which serves as the primary water source for the population of Santana municipality, Bahia State. Therefore, any negative impacts on this critical cave system could have far-reaching consequences, not only for the troglobitic and non-troglobitic invertebrates but also for more than 26,000 residents living in the semiarid northeast of Brazil, an area that already experiences drought conditions (IBGE 2020).

It is essential to note that despite the native vegetation covering both entrances of the Gruta do Padre Cave, the majority of the surrounding areas have been deforested. The natural Caatinga and Dry Forests in the region have historically been replaced by pastures, resulting in a direct impact on the energetic dynamics of the subterranean invertebrate communities in the Gruta do Padre Cave. The reduction of native vegetation in the surrounding areas of the Gruta do Padre Cave could lead to a decline in the total organic resources available to be transported to subterranean environments. Since the organic matter of vegetal origin is the most crucial trophic source inside this cave, any decrease in its availability could jeopardize the entire subterranean trophic web of the Gruta do Padre Cave.

Despite being a Hotspot of Subterranean Biodiversity, the Gruta do Padre Cave unfortunately lacks protection under Brazilian speleological legislation. Since 2008, a decree has stipulated that Brazilian caves must be classified according to their level of importance. Only those caves classified as having maximum cultural, geological, and/or biological value are supposed to be preserved (Decree nº 6.640). However, a new decree (Decree nº 10.935) implemented in 2022 permits even the destruction of caves with maximum relevance (Ferreira et al. 2022). Consequently, since the Gruta do Padre Cave is not situated within any protected area, the entire speleological heritage within the cave is currently at risk.

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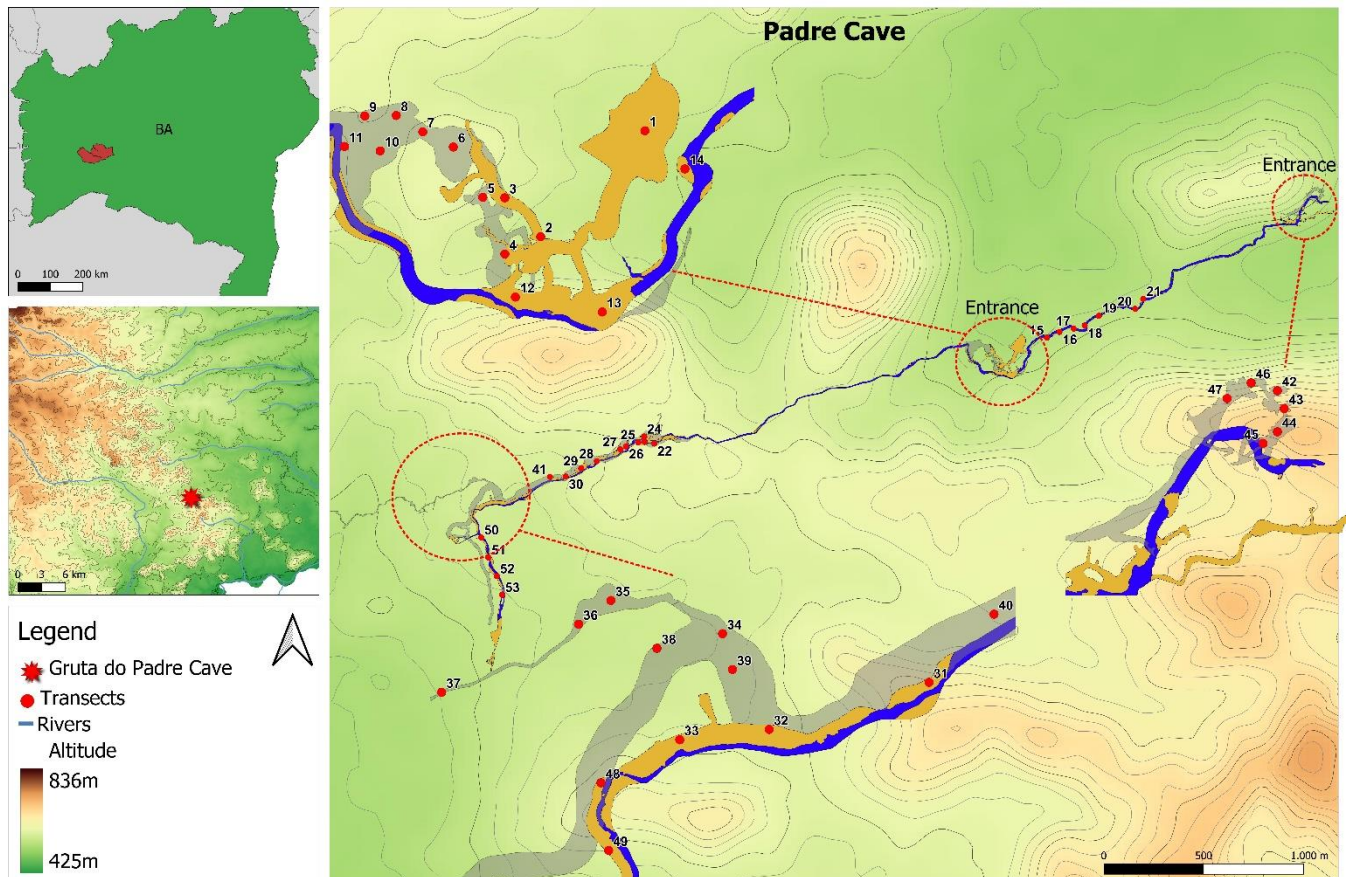


Figure 2. Location map of Gruta do Padre Cave with the sampling transects plotted.

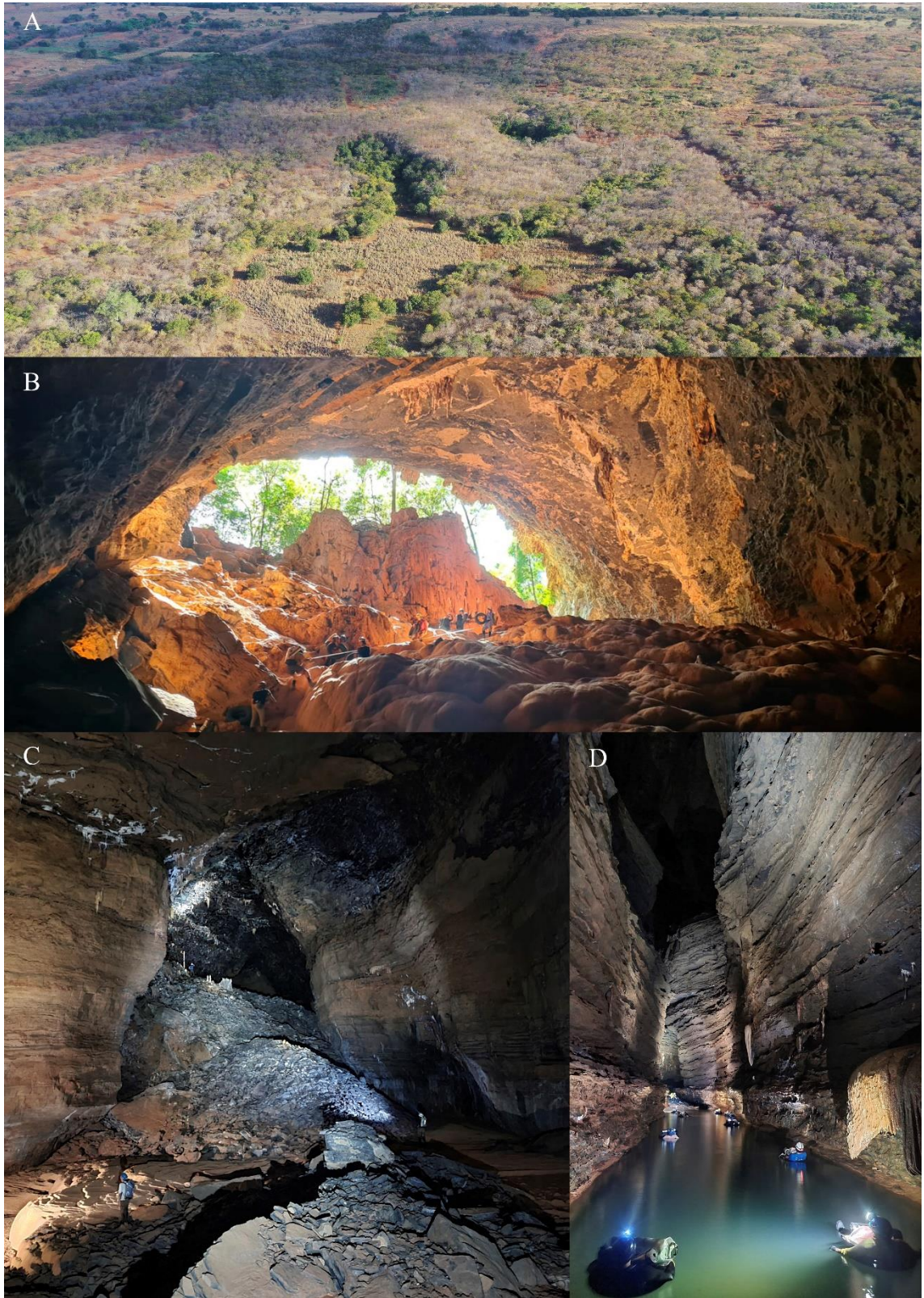


Figure 3. Study area, Gruta do Padre Cave. A–aerial view from the landscape and Gruta do Padre Cave’s entrance area; B–entrance; C–upper dry gallery; Santo Antônio River level.

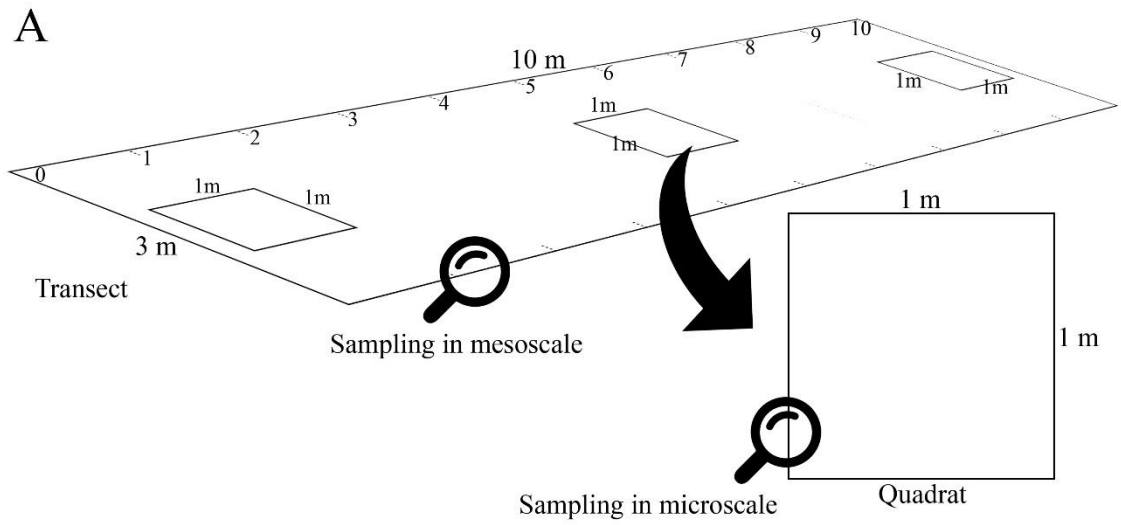


Figure 4. Sampling method. A—sampling method scheme showing both meso and microscale; B—sampling method being apply in Gruta do Padre Cave gallery.

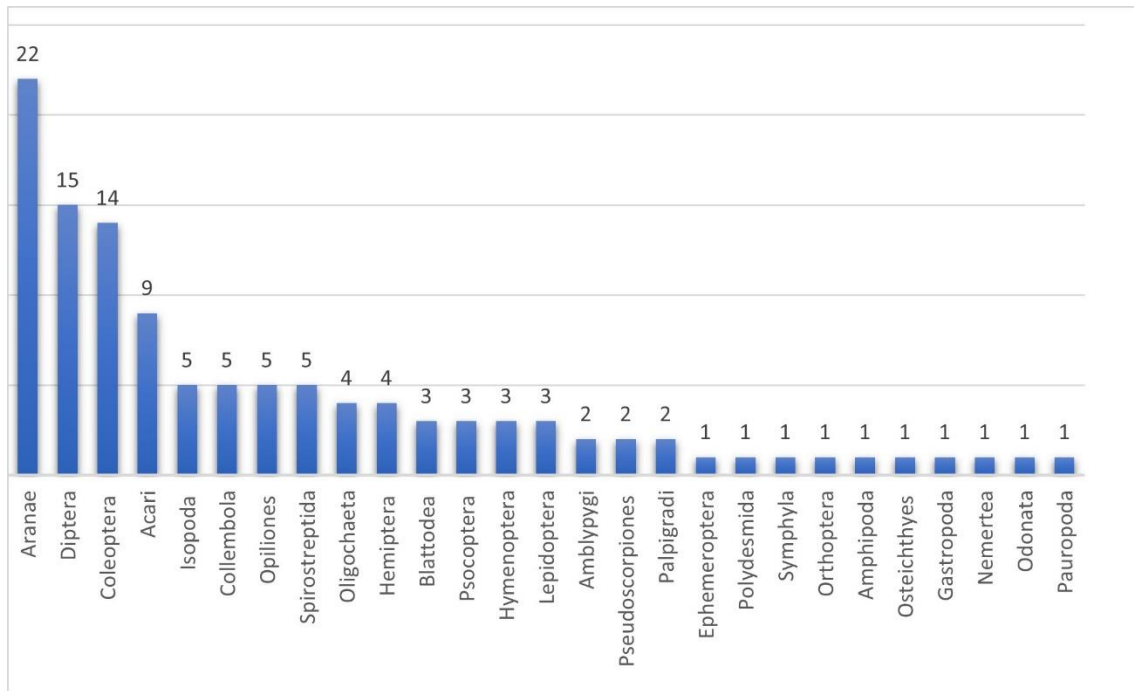


Figure 5. Main invertebrate groups richness of Gruta do Padre Cave.

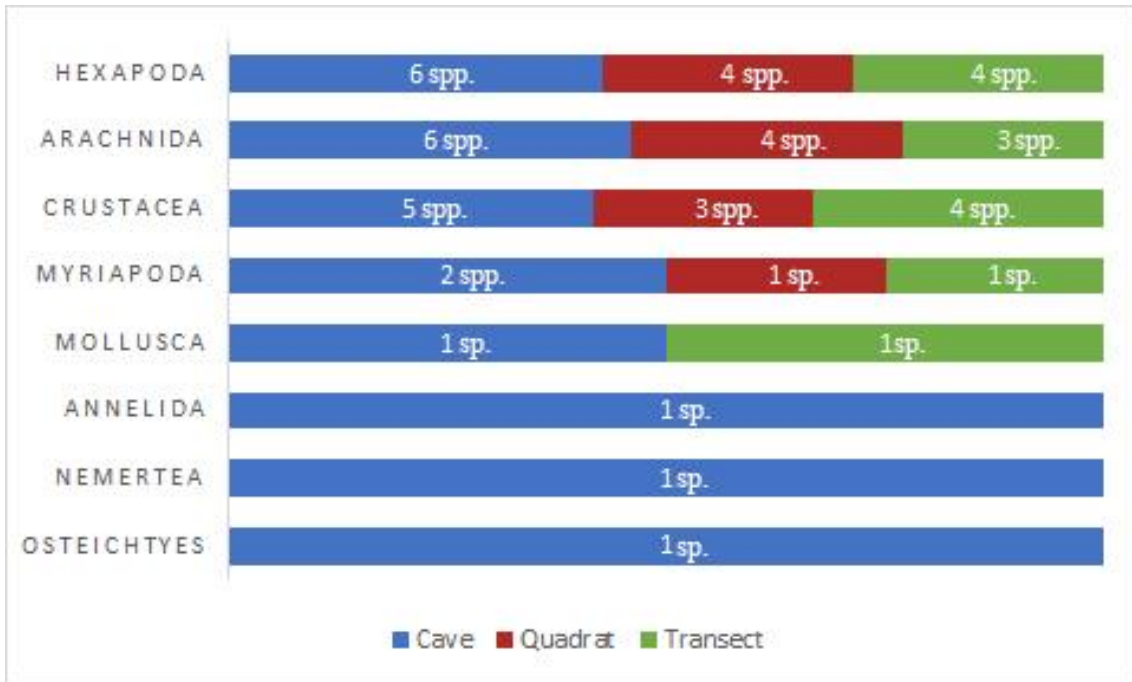


Figure 6. Richness of troglobitic species groups by sample scale in Gruta do Padre Cave. There are 25 restricted species in total.



Figure 7. 17 of the 25 troglobitic species found in the Gruta do Padre Cave. A–*Phaneromerium cavernicolum*; B– *Spiripockia* sp1.; C– *Chaimowiczia tatus*; D– *Xangoniscus* sp1.; E– *Pectenoniscus santanensis*; F– *Spelaeogammarus santanensis*; G– *Eukoenenia* sp1.; H– *Eukoenenia* sp2.; I– *Pimelodella* sp1.; J– *Eusarcus* cf. *cavernicola*; K– Ochyroceratidae sp1; L– *Pseudochthonius* sp1; M– Escadabiidae sp1; N– Lumbricina sp3; O– *Endecous* sp1; P *Coarazuphium tessai*; Q– Blattidae sp1.

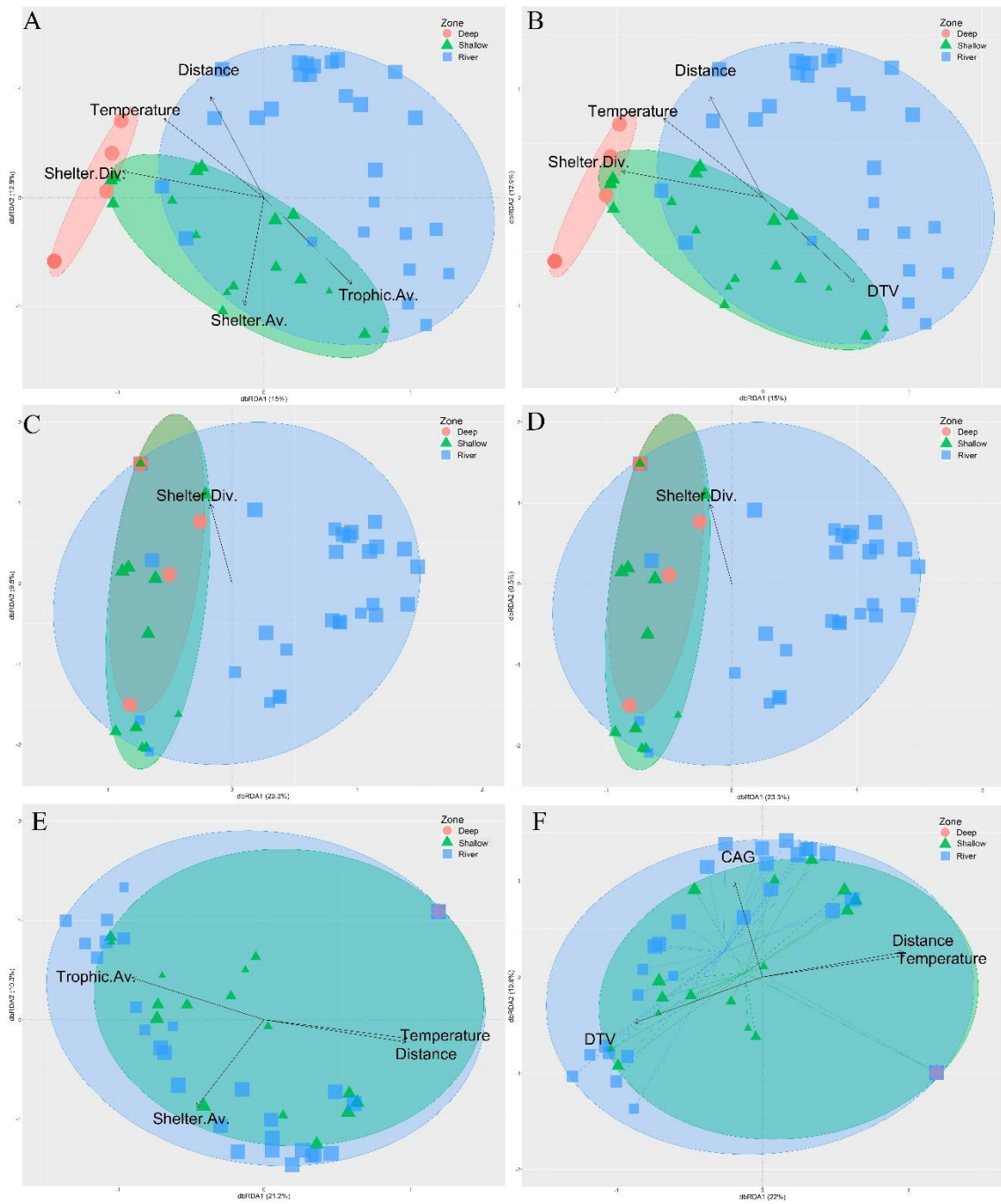


Figure 8. Distance-based Redundancy Analysis (dbRDA) on the mesoscale, succinct (A, C, E) and long (B, D, F) models. A and B—general fauna; C and D—troglobitic fauna; E and F—non-troglobitic fauna.

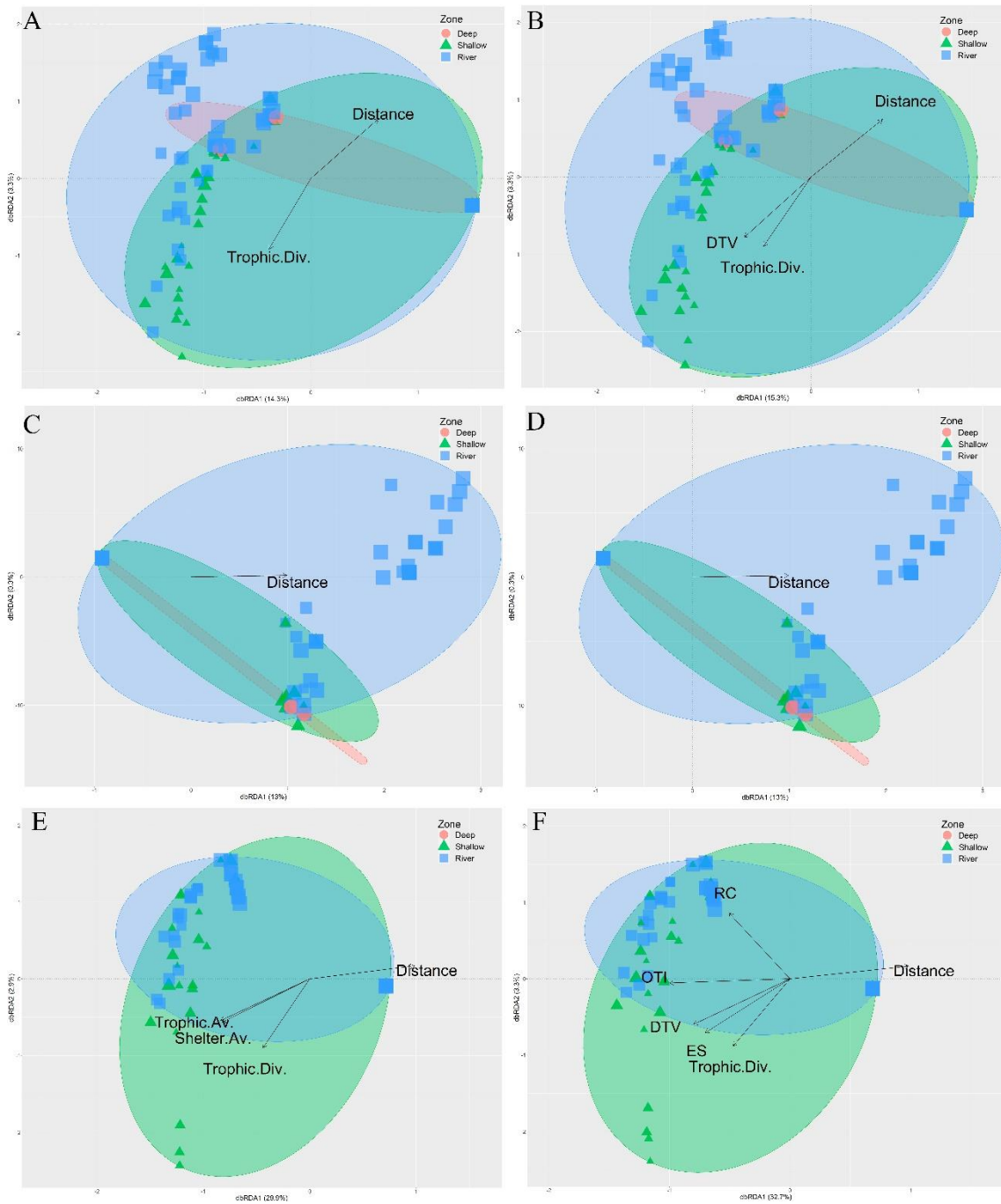


Figure 9. Distance-based Redundancy Analysis (dBRDA) on the microscale, succinct (A, C, E) and long (B, D, F) models. A and B—general fauna; C and D—troglobitic fauna; E and F—non-troglotic fauna.

Taxons	Species and Morphotypes	Ca	Qu	Sec
Amphipoda	<i>Spelaeogammarus santanensis</i>	+		
Aranae	Ochyroceratidae sp1			
Blattodea	Blattidae sp1	+		
Coleoptera	<i>Clivina</i> sp1	+	+	
	<i>Coarazuphium tessai</i>	+		
Entomobryomorpha	Paronellidae sp2	+	+	+
Gastropoda	Gastropoda sp3	+		+
Oligochaeta	Lumbricina sp3	+		
Isopoda	<i>Chaimowiczia tatus</i>	+		
	<i>Pectenoniscus santanensis</i>	+	+	+
	Platyartridae sp1		+	+
	Styloniscidae sp1	+	+	+
	<i>Xangoniscus</i> sp1	+		+
Nemertea	Nemertea sp1	+		
Opiliones	Escadabiidae sp1	+		+
	<i>Eusarcus cff. cavernicola</i>	+	+	+
Orthoptera	<i>Endecous</i> sp1	+	+	+
Palpigradi	<i>Eukoenenia</i> sp1	+	+	
	<i>Eukoenenia</i> sp2	+	+	
Poduromorpha	Poduromorpha sp1			+
Pseudoscorpiones	<i>Pseudochthonius</i> sp1	+	+	+
Polydesmida	<i>Phaneromerium</i> sp1	+	+	+
Symphyla	Symphyla sp1	+		
Symphyleona	Arrhopalitidae sp1	+	+	+
Siluriformes	<i>Pimelodella</i> sp1	+		

Table 1. Troglotic species

and the sample scale where they were found.

Variables	Mesoscale						Microscale					
	Succinct Model			Long Model			Succinct Model			Long Model		
	Gene ral	T	n-T	Gene ral	T	n-T	Gene ral	T	n-T	Gene ral	T	n-T
Zone*	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Distance*	0.005		0.005	0.005		0.005	0.005	0.010	0.005	0.005	0.010	0.005
Shelter Av.	0.005		0.020							0.035		
Trophic Av.	0.015		0.005							0.030		
Shelter Div.	0.040	0.005		0.005	0.005							
Trophic Div.							0.020		0.005	0.035		0.005
Temperat ure	0.010		0.040	0.030		0.025						
DTV				0.005		0.005				0.005		0.005
CAG						0.005						
ES												0.005
RC												0.049
Explanati on	42.26	33.15	41.28	40.09	33.15	42.28	19.50	13.53	34.40	21.37	13.53	38.84
	%	%	%	%	%	%	%	%	%	%	%	%

Table 2. P-values for Distance-based Redundancy Analysis (dbRDA).

Table 3. P-values and Estimate values for the GLM and GLMM on the mesoscale.

Variables	Mesoscale											
	Succinct Model						Long Model					
	General		T		n-T		General		T		n-T	
	P	Est.	P	Est.	P	Est.	P	Est.	P	Est.	P	Est.
Distance*				6.00E-04	-0.447					0.003	-0.450	
Temperature	0.026	-0.218					0.015	-0.216			0.031	-0.235
Shelter Av.												
Trophic Av.				0.048	0.189							
Substrate Div.												
RL										0.029	0.170	
SB										0.020	-0.381	
HP						0.014	-0.319			0.024	-0.618	
ES												
R²/R²M	40.07%				59.51%		67.08%				91.37%	
R²C	65.38%				72.96%		67.37%					

Table 4. P-values and Estimate values for the GLM and GLMM on the microscale

Variables	Succinct Model						Long Model					
	General		T		n-T		General		T		n-T	
	P	Est.	P	Est.	P	Est.	P	Est.	P	Est.	P	Est.

Distance *	4.09E-07	- 0.44 6	1.93E-11	- 1.12 0	1.23E-05	- 0.42 0	1.74E-09	- 1.10 0
Temperature								
Shelter Av.			0.021	0.23 6				
Trophic Av.								
Substrate Div.		0.02 2	- 0.45 2					
RL								
SB								
HP								
ES					0.047 6	0.10 6	0.023	0.14 2
R²/R²M	22.82%	15.81%	51.25%	33.00%			55.18%	
R²C	23.46%							

Table 5. Average values for variables in different zones of Gruta do Padre Cave.

	Mesoscale				Microscale			
	Shallow	River		Deep	Shallow	River		Deep
		Shallow	Deep			Shallow	Deep	
Total Richness	6.76	10.20	5.00	0.75	1.51	2.07	0.87	0.08
T Richness	1.41	2.70	2.83	0.75	0.23	0.50	0.68	0.08
n-T Richness	5.35	7.50	2.17	0	1.27	1.57	0.18	0
Temperature	24.3	24.6	25.5	26.0	-	-	-	-
Shelter Av.	70.8	14.0	40.8	30.6	38.43	14.76	23.11	27.97
Trophic Av.	2.98	4.23	0.73	0	2.57	2.40	0.05	0
Shelter Div.	1.02	0.322	0.695	0.56	0.28	0.14	0.16	0.33
Trophic. Div.	0.029	0.075	0	0	0.049	3.00E-04	0	0