

Microhabitat heterogeneity and organic resources drive invertebrate diversity in a remote Brazilian limestone cave

Rayanne Lays Sant'Ana Merlo

Centro de Estudos em Biologia Subterrânea, Departamento de Ecologia e Conservação,
Instituto de Ciências Naturais, Universidade Federal de Lavras
Programa de Pós-Graduação em Ecologia Aplicada
<https://orcid.org/0000-0002-5466-1563>

Marconi Souza Silva

Centro de Estudos em Biologia Subterrânea, Departamento de Ecologia e Conservação,
Instituto de Ciências Naturais, Universidade Federal de Lavras
Programa de Pós-Graduação em Ecologia Aplicada
E-mail: marconisilva@dbi.ufla.br
<https://orcid.org/0000-0002-3184-5319>

Leandro Mata da Rocha Melo

Centro de Estudos em Biologia Subterrânea, Departamento de Ecologia e Conservação,
Instituto de Ciências Naturais, Universidade Federal de Lavras
Programa de Pós-Graduação em Ecologia Aplicada
<https://orcid.org/0000-0002-6351-8654>

Rodrigo Lopes Ferreira

Centro de Estudos em Biologia Subterrânea, Departamento de Ecologia e Conservação,
Instituto de Ciências Naturais, Universidade Federal de Lavras
Programa de Pós-Graduação em Ecologia Aplicada
e-mail: drops@ufla.br
<https://orcid.org/0000-0003-3288-4405>

RESUMO: Os ecossistemas subterrâneos são caracterizados pela estabilidade ambiental e pela limitação de recursos tróficos, sustentando uma fauna com adaptações especializadas à vida na escuridão. Apesar de sua relevância ecológica, muitas cavernas brasileiras permanecem pouco estudadas, particularmente no bioma Pantanal, restringindo nosso conhecimento sobre a biodiversidade subterrânea. Este estudo investigou os efeitos de variáveis ambientais sobre a riqueza, composição e diversidade taxonômica de invertebrados na remota caverna calcária Ricardo Franco. Foram registradas assembleias de invertebrados, as condições microclimáticas (temperatura e umidade) e a diversidade e disponibilidade de substratos tróficos e de abrigo em duas escalas espaciais: mesoescala (setores de 30 m²) e microescala (quadrantes de 1 m²), ao longo da cavidade. Identificamos 30 espécies, incluindo seis troglóbios: *Potiticoara brasiliensis*, *Megagidiella azul*, *Novamundoniscus* sp., *Pirassunungoleptes* sp., *Chernetidae* sp. e *Chelodesmidae* sp. Na mesoescala, a riqueza de espécies diminuiu com o aumento da distância da entrada, mas aumentou com maior heterogeneidade do habitat e disponibilidade de recursos orgânicos; a composição de espécies também foi influenciada pela distância. Na microescala, nenhuma variável explicou significativamente a riqueza ou a composição, embora a diversidade de recursos tróficos tenha mostrado um papel potencial na estruturação das comunidades. A diversidade taxonômica foi principalmente influenciada pela temperatura, umidade e disponibilidade de abrigos, que juntas explicaram mais de 90% da variação. Esses resultados evidenciam como os gradientes ambientais e a complexidade do habitat atuam na organização das assembleias subterrâneas, enquanto a presença de fauna troglóbica na Caverna Ricardo Franco acrescenta um peso adicional ao seu valor ecológico como uma área prioritária para ações de conservação.

Palavras-chave: fauna cavernícola, troglóbios, Pantanal, distinção taxonômica, conservação.

ABSTRACT: Subterranean ecosystems are characterized by environmental stability and limited trophic resources, supporting fauna with specialized adaptations to life in darkness. Despite their ecological relevance, many Brazilian caves remain poorly studied, particularly in the Pantanal biome, restricting our understanding of subterranean biodiversity. This study investigated the effects of environmental variables on invertebrate richness, composition, and taxonomic diversity in the remote Ricardo Franco limestone cave. Invertebrate assemblages, microclimatic conditions (temperature and humidity), and the diversity and availability of trophic and shelter substrates were recorded at two spatial scales: mesoscale (30 m² sectors) and microscale (1 m² quadrats), along gradients of distance from the entrance. We identified 30 species, including six troglobites: *Potiiocoara brasiliensis*, *Megagidiella azul*, *Novamundoniscus* sp., *Pirassunungoleptes* sp., Chernetidae sp., and Chelodesmidae sp. At the mesoscale, species richness declined with increasing distance from the entrance but increased with greater habitat heterogeneity and organic resource availability; species composition was also shaped by distance. At the microscale, no variable significantly explained richness or composition, though trophic resource diversity showed a potential role in structuring communities. Taxonomic diversity was mainly driven by temperature, humidity, and shelter availability, which together explained over 90% of variation. These results underscore the influence of environmental gradients and habitat complexity on the structuring of subterranean assemblages, while the presence of troglobitic fauna within Caverna Ricardo Franco further elevates its ecological significance, establishing it as a conservation priority.

Keywords: cave fauna, troglobites, Pantanal, taxonomic distinction, conservation.

1. INTRODUCTION

Subterranean environments are unique ecosystems characterized by stable abiotic conditions, including the absence of light, high relative humidity, constant temperature, and limited trophic resources (Culver & Pipan, 2019). These conditions impose ecological constraints that favor the evolution of highly specialized organisms, such as troglobitic species, which often display morphological traits including loss of pigmentation, eye reduction, and elongation of sensory appendages (Sket, 2008; Deharveng et al., 2024). Despite their relevance for understanding evolutionary, biogeographical, and ecological processes, subterranean faunas remain among the least studied worldwide and are frequently overlooked in conservation planning (Mammola et al., 2019; Cardoso et al., 2022).

In Brazil, research in subterranean ecology has advanced substantially in recent decades, with most studies concentrated in the karst regions of Minas Gerais and Bahia, which host the highest numbers of recorded caves and known species (Souza-Silva et al., 2020; Ferreira et al., 2023; Reis-Venâncio et al., 2024; Rocha Melo et al., 2025). These efforts have revealed complex patterns of species distribution, diversity, and endemism, alongside growing threats from mining, agriculture, and unregulated tourism (Cardoso et al., 2022). However, vast portions of the country (particularly the Central-West, South, and North regions) remain under sampled, limiting our understanding of large-scale ecological patterns. This paucity of data hinders biodiversity assessments and impedes the development of effective conservation and management strategies (Cordeiro et al., 2014).

Among Brazil's neglected subterranean regions, the Pantanal stands out as one of the largest wetland systems on Earth and a biome renowned for its exceptional epigeal biodiversity (Alho & Sabino, 2011). In contrast, its subterranean fauna remains largely unknown (Cordeiro et al., 2014). Ricardo Franco Cave, also known as Hell Cave, is located in Forte Coimbra, municipality of Corumbá (Mato Grosso do Sul), within this biome. The cave holds considerable historical significance: it was explored in the 18th century by Colonel-Engineer Ricardo Franco de Almeida Serra during boundary surveys between Portuguese and Spanish territories (Coelho, 2005) and later visited by the naturalist Alexandre Rodrigues Ferreira, who documented it during his expedition to the western regions of the colony (Martins & Abreu, 2022).

Geographically, Ricardo Franco Cave lies within a small, isolated limestone outcrop near the borders of Brazil, Paraguay, and Bolivia. Despite its historical prominence, the cave remains largely unexplored from an ecological perspective. The only biological record comes from a broader survey of subterranean biodiversity in the Serra da Bodoquena region (Cordeiro, 2014), which reported the presence of troglobitic and threatened species. However, no study has yet addressed the role of environmental factors (such as humidity, temperature, substrate diversity and availability, or distance from the entrance) in shaping its cave fauna. This knowledge gap is especially concerning given the evidence of anthropogenic disturbances, including old structural modifications and occasional tourist visitation.

Recent studies have demonstrated that the organization of subterranean invertebrate communities is strongly influenced by environmental gradients, which act as ecological filters shaping species distributions (Prous et al., 2015; Rocha Melo et al., 2025). Key factors include microhabitat availability, substrate heterogeneity, organic matter input, and microclimatic stability (Souza-Silva et al. 2021; Vaz et al. 2025). Species responses to these variables may vary depending on the spatial scale of analysis, highlighting the importance of multi-scale approaches to adequately

capture patterns of occupancy and diversity (Pacheco et al., 2020; Junta et al., 2025). In general, heterogeneous environments sustain higher species richness by providing a broader range of niches and promoting coexistence (Tews et al., 2004; Pacheco et al., 2020).

In the case of Ricardo Franco Cave, the lack of targeted ecological investigations limits our understanding of how such variables influence the local fauna. Assessing the role of physical, microclimatic, and trophic factors in shaping species richness, composition, and taxonomic diversity is essential for robust environmental evaluations and for developing effective management strategies, particularly in caves subject to anthropogenic disturbance (Souza-Silva et al., 2015; Mammola et al., 2020).

Given this context, the present study aimed to evaluate the influence of environmental variables on the richness, composition, and taxonomic diversity of terrestrial invertebrates in Ricardo Franco Cave. Specifically, we examined the effects of distance from the entrance, relative humidity, temperature, and the diversity and availability of trophic and shelter substrates. We hypothesized that areas closer to the entrance would harbor greater species richness and distinct community composition, driven by higher environmental heterogeneity and increased organic matter input.

2. MATERIAL AND METHODS

Study Area

This study was conducted in Ricardo Franco Cave (417081.38 E; 7800764.80 S), located in the Forte Coimbra region, municipality of Corumbá, Mato Grosso do Sul, Brazil. The cave lies within the Pantanal, a region designated as a National Heritage site in 1988. Covering approximately 160,000 km² (of which about 140,000 km² are within Brazilian territory), the Pantanal is one of the largest wetland systems on Earth (Junk et al. 2006). The regional climate is classified as Tropical Savanna (Aw) under the Köppen system, and the dominant vegetation corresponds to the Cerrado biome. The Pantanal represents about 1.8% of Brazil's total area (IBGE 2019), extending across the states of Mato Grosso and Mato Grosso do Sul, with roughly 65% of its extent in the latter (Coelho, 2005; Cordeiro et al., 2014; Martins & Abreu, 2022).

Ricardo Franco Cave lies on the opposite bank of the Paraguay River, about 200 km from Serra da Bodoquena National Park. It is situated within the boundaries of Forte Coimbra, a fortification established in 1775 to defend the frontier, first contested between Portugal and Spain, and later between Brazil and Paraguay (Figure 1). The fort, now registered by IPHAN, currently houses military facilities and residences associated with the Third Border Company, preserving an important part of regional history (Coelho, 2005). The fact that this cave is located within an area legally protected as historical heritage by IPHAN also facilitates access control and helps maintain its current state of conservation.

Geologically, Ricardo Franco Cave developed in dolomitic and calcitic limestone. Evidence of historical human use is widespread, including marks of cannon fire, inscriptions (e.g., a record of a visit by Marshal Rondon), and several modifications to the cave interior and its surroundings. Notable alterations include remnants of a deactivated lighting system, an unused external generator, and a masonry staircase built prior to 1876, connecting the entrance to the main hall, where a permanent lake is located (Cordeiro et al., 2014).

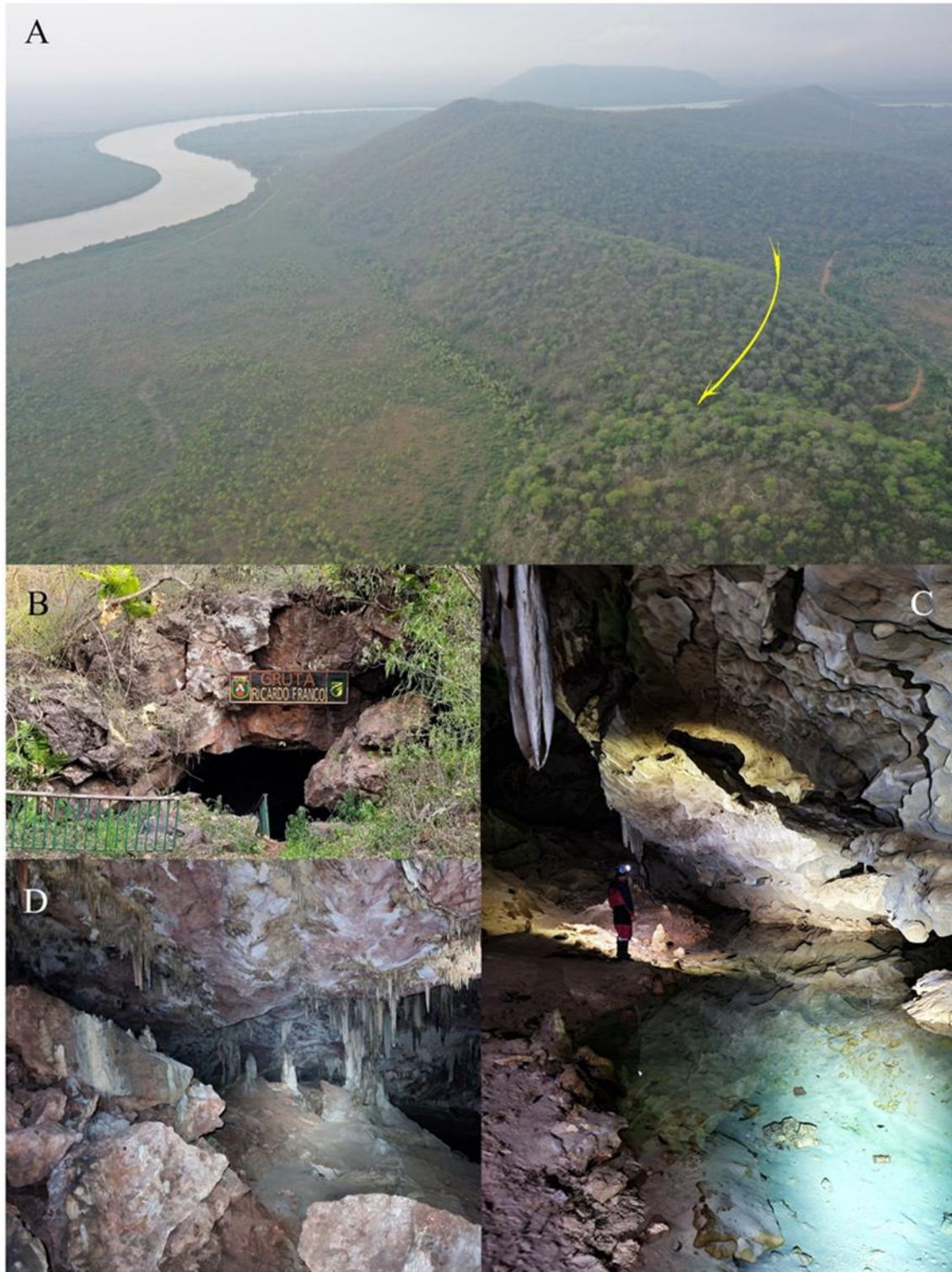


Figure 1. The Forte Coimbra region is located in the municipality of Corumbá, Mato Grosso do Sul State, Brazil. A) Aerial view of the landscape of Forte Coimbra and the Paraguay River (the yellow arrow indicates the entrance of the cave); B) Entrance of Ricardo Franco Cave; C) Interior of the Cave, highlighting the lake inside the cave; D) Speleothems present inside the cave. Photos: Rodrigo Lopes Ferreira.

Sampling design

Sampling was carried out at two spatial scales: (i) the mesoscale, represented by 12 sectors measuring 3×10 m each, and (ii) the microscale, consisting of 36 quadrats of 1 m^2 (Figure 2, Figure 3). Within each sector, three quadrats were established, one at each extremity and one in the

central area. Sampling extended from the cave entrance to the innermost accessible zones, thereby encompassing the main environmental gradient.

Temperature, relative humidity, and distance from the nearest entrance were measured using a thermohygrometer and a laser tape measure. For each sector, the thermohygrometer was placed at floor level in the central area for approximately 15 minutes to obtain microclimatic values (Souza-Silva et al., 2021). Biotic and abiotic data were collected during a single field campaign conducted in September 2022.

Sampling abiotic data

At the sector level (mesoscale), each 3×10 m sector was subdivided into ten perpendicular subsections measuring 3×1 m. Within each subsection, visual inspection was used to estimate the proportion of the area occupied by different substrate classes. For each class, percentage values from all subsections were summed and divided by 100 to obtain a single value per sector (Pacheco et al., 2020; Souza-Silva et al., 2021).

At the quadrat level (microscale), three representative 1 m^2 quadrats, located at the beginning, middle, and end of each sector were photographed with the camera positioned perpendicular to the ground (90°) prior to invertebrate collection. The images were processed in *ImageJ* software (Rasband, 1997) to quantify the proportion of each substrate class. As at the mesoscale, the percentage values for each class were summed and divided by 100, resulting in a single value per quadrat (Pacheco et al., 2020; Souza-Silva et al., 2021).

Invertebrate sampling

Invertebrates were collected through exhaustive active visual searches and manual capture using tweezers and brushes. Specimens were preserved in vials containing 70% ethanol, following established protocols (Pacheco et al., 2020; Souza-Silva et al., 2021). To increase the likelihood of detecting troglobitic species, complementary surveys were conducted outside the predefined sectors and quadrats, encompassing a variety of microhabitats throughout the cave. However, only specimens collected within the designated sampling units were included in the statistical analyses, whereas records obtained outside these units were used exclusively to compile the overall species inventory of Ricardo Franco Cave.

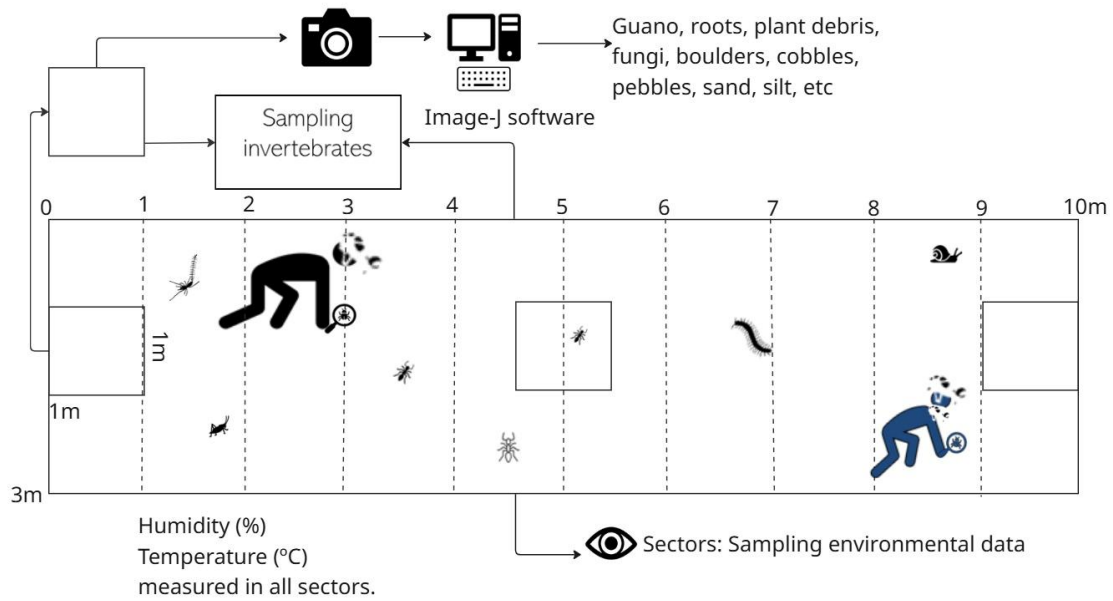


Figure 2. Diagram of invertebrate sampling and abiotic variables in quadrats and sectors inside Ricardo Franco Cave.

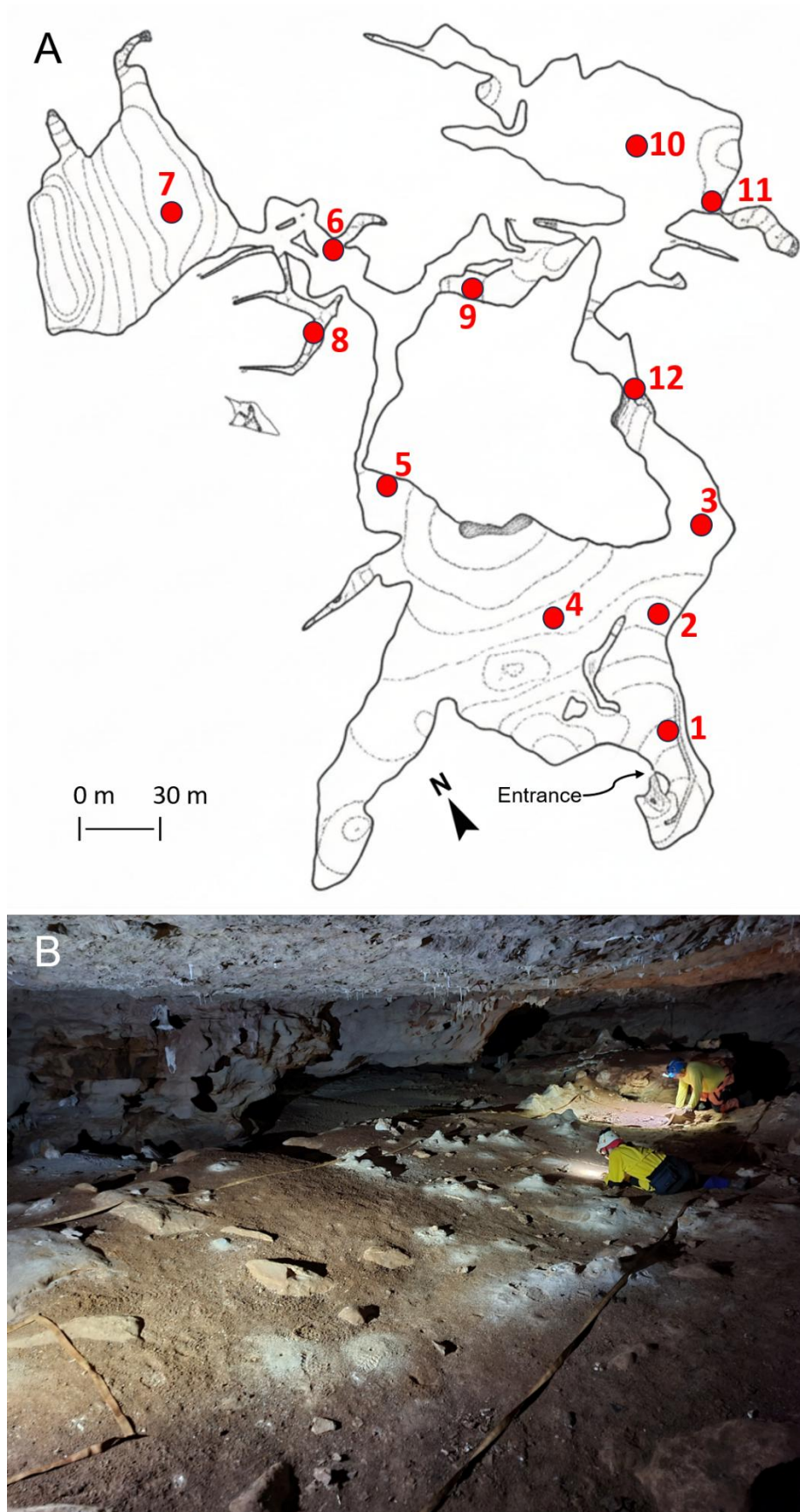


Figure 3. Distribution of sampling points in Ricardo Franco Cave. (A) Cave map showing the twelve sampling sectors (red points). Contour lines represent variations in floor topography, and the arrow indicates the location of the entrance. (B) Sampling activity in one of the surveyed sectors.

Sorting and identifying invertebrates

Invertebrates were sorted and identified to the lowest possible taxonomic level using available literature (Oliver & Battie, 1996) and with the assistance of specialists in Isopoda, Acari, Orthoptera, and Pseudoscorpiones. Potential troglobitic species were recognized based on troglomorphic traits indicative of evolutionary adaptation to subterranean environments (Culver & Pipan, 2019; Aden, 2005).

All identified morphotypes were deposited in the Lavras Subterranean Invertebrates Collection (ISLA), housed at the Center for Studies in Subterranean Biology, Federal University of Lavras (CEBS–UFLA), Minas Gerais, Brazil (biologiasubterranea.com.br).

Data analyses

Substrates recorded in the sectors and quadrats were classified into three categories according to their ecological functions: *trophic*, *shelter*, and *general*. Trophic substrates included roots (RO), guano (GU), fine twigs (TB), and phanerogams (PH), as these provide direct or indirect food resources for cave fauna. Shelter substrates (those offering potential refuge for invertebrates) included rough rock (RR), boulders (BO), blocks (BL), coarse gravel (CG), fine gravel (FG), and fine twigs (TB). General substrates encompassed both organic and inorganic elements, such as water bodies (WB), sand (SA), silt/clay/mud (SCM), hardpan (HP), undifferentiated inorganic substrate (IS), and speleothems (SP), which, although not directly functioning as food or shelter, contribute to habitat heterogeneity.

Resource diversity (trophic) and shelter diversity were calculated using the Shannon–Weaver index (H'), based on the proportional cover of trophic and shelter substrate types, respectively (Buttigieg & Ramette, 2014). Resource and shelter availability were determined from the summed proportional cover of the substrate classes in each group (Souza-Silva et al., 2021; Reis-Venâncio et al., 2022).

To visualize substrate distribution across transects and quadrats, a shade plot was generated after square-root transformation of the data. Substrate types were grouped through cluster analysis using Whittaker's Similarity Index to identify spatial associations and reorder substrate classes by similarity in floor distribution (Whittaker, 1952; Clarke et al., 2014).

The relationships between the diversity and availability of trophic and shelter resources and their distance from the cave entrance were examined using simple linear models (lm function, *stats* package). Residual normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was checked via diagnostic plots (*graphics* package). The aim was to characterize the spatial distribution of physical and trophic components along the entrance–deep zone gradient. All analyses, including those described in subsequent sections, were performed in R (R Core Team, 2019).

To assess factors influencing cave-dwelling species richness at different spatial scales, we applied generalized linear models (GLMs) and generalized linear mixed models (GLMMs). At the mesoscale (sector level), GLMs with a Poisson distribution were fitted using species richness as the response variable. Explanatory variables included shelter diversity, trophic resource diversity, and distance from the cave entrance. Additional models incorporated combinations of biotic and abiotic predictors, such as temperature, relative humidity, shelter availability, and food resource availability. Model selection followed a stepwise procedure based on the Akaike Information Criterion (AIC). Goodness-of-fit was evaluated by the ratio of deviance residuals to residual degrees of freedom, with values close to 1 indicating adequate fit and absence of overdispersion.

Residual patterns were further checked via simulations in the DHARMA package (Hartig, 2016) to confirm the suitability of the Poisson distribution.

At the microscale (quadrat level), GLMMs were used with species richness as the response variable. Explanatory variables included resource availability, shelter availability, distance from the entrance, and the diversity of both resources and shelters. Sector was included as a random effect. Poisson GLMMs provided a better fit than negative binomial models, based on AIC values, residual diagnostics from DHARMA, and visual inspection of residual normality and homoscedasticity.

To evaluate the influence of substrate diversity and availability, temperature, humidity, and distance from the entrance on species composition, we applied distance-based linear models (DistLM) using a Bray–Curtis similarity matrix. Model selection followed the corrected Akaike Information Criterion (AICc) as recommended by Anderson et al. (2008). Distance-based redundancy analysis (dbRDA) was then used to jointly assess model fit and quantify the proportion of explained variation (Anderson et al. 2008). Variation in species composition among sampling units was further visualized with non-metric multidimensional scaling (NMDS) combined with bootstrap resampling (Clarke et al. 2014).

Taxonomic Distinction ($\Delta+$) was calculated in PRIMER 7 using 24 of the 30 species identified to at least the family level (Anderson et al. 2008; Souza-Silva et al. 2020). The analysis considered five hierarchical taxonomic levels with progressively decreasing weights: Phylum (100), Class (80), Order (60), Family (40), and “Species” (morphotype – 20). These data generated a species-by-sampling unit matrix from which $\Delta+$ values were computed.

To identify environmental variables significantly influencing $\Delta+$ at each spatial scale, we performed distance-based linear models (DistLM) using an Euclidean distance matrix and the same environmental predictors applied in the community composition analyses. Models were built using a forward selection procedure, starting from a null model and sequentially adding variables with the highest explanatory power (Anderson et al. 2008).

3. RESULTS

Describing Habitat Structure inside the sample units

At the microscale, Spearman correlation analysis revealed significant negative relationships between distance from the entrance and both resource diversity ($R_s = -0.313$, $p < 0.001$) and resource availability ($R_s = -0.286$, $p = 0.0017$), indicating that resource diversity and availability decrease progressively toward the deeper cave zones.

At the mesoscale, Spearman correlations also showed significant negative relationships between distance from the entrance and shelter diversity ($R_s = -0.332$, $p = 0.039$), trophic resource diversity ($R_s = -0.397$, $p = 0.012$), and resource availability ($R_s = -0.390$, $p = 0.014$). These results indicate that habitats closer to the entrance are generally more diverse and provide greater resource availability.

Substrate composition also varied with distance from the entrance across both spatial scales. At the microscale, speleothems, guano, and inorganic substrates were more common in the cave’s interior, whereas roots, coarse gravel, and speleothems predominated near the entrance (Figure 4A). At the mesoscale, inorganic substrates and fine gravel dominated interior sectors, while roots and hardpan were more frequent in entrance-proximal areas (Figure 4B).

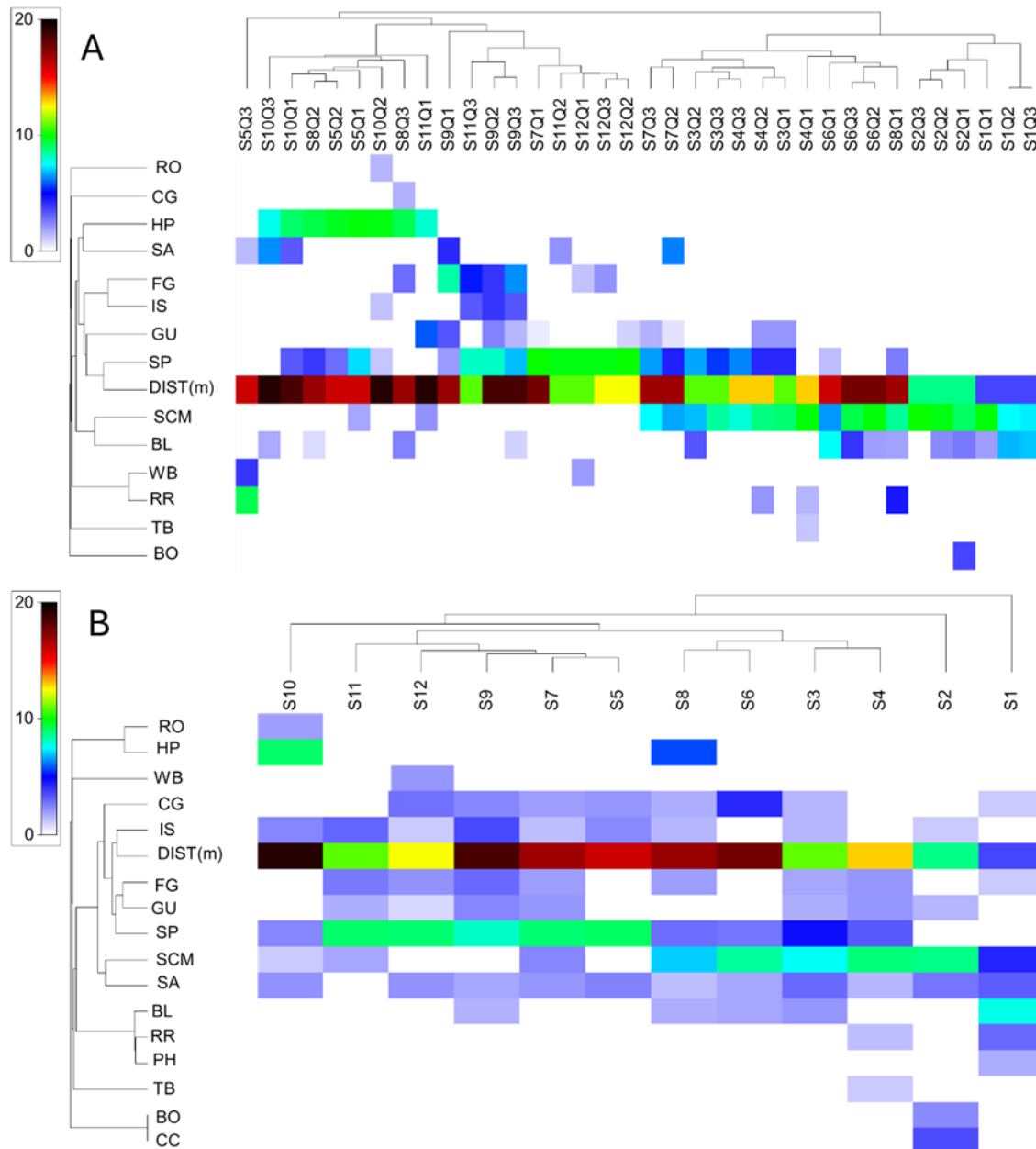


Figure 4. Shadeplot representing the variations in percentage and distribution of each substrate class, as well as the relationship of each class with the distance from the entrance (DIST) in quadrats (A) and sectors (B). The grouping of substrate classes was based on the Whittaker index, and the intensity of the color indicates the amount of each substrate class in a given sector and quadrant. RO: roots; CG: coarse gravel; HP: harpan; SA: sand; FG: fine gravel; IS: inorganic substrate; GU: guano; SP: speleothem; DIST: distance from the entrance; SCM: silt/clay/mud; BL: blocks; WB: water bodies; RR: rough rock; TB: thin branch; BO: boulder; PH: phanerogams; CC: contraction crevice.

Species Composition and richness of cave invertebrates

Considering the sampling effort, a total of 30 species were recorded, with 25 occurring at the mesoscale (sectors) and 17 at the microscale (quadrats). Overall, 15 orders and 24 families of invertebrates were identified. The order Araneae exhibited the highest richness, with six species, followed by Diptera with four species, and Psocodea and Opiliones with three species each.

Five additional species were recorded outside the sampled quadrats and sectors: Tabanidae sp. 1 (Diptera), Chernetidae sp. 2 (Pseudoscorpiones), Gonyleptidae sp. 13 (Opiliones), Chelodesmidae sp. 1 (Polydesmida), and *Tytius* sp. 1 (Scorpiones). Six species displayed

troglomorphic traits: *Potiicoara brasiliensis* (Spelaeogriphacea: Spelaeogriphidae), *Megagidiella azul* (Amphipoda: Artesiidae), *Novamundoniscus* sp. (Isopoda: Dubioniscidae), *Pirassunungoleptes* sp. (Opiliones: Zalmoxidae), Chernetidae sp. 1 (Pseudoscorpiones), and Chelodesmidae sp. 1 (Polydesmida) (Figure 5).



Figure 5. Troglomorphic species found in the Ricardo Franco Cave, located in Forte Coimbra. A – *Potiicoara brasiliensis* (Spelaeogriphacea: Spelaeogriphidae); B – *Megagidiella azul* (Amphipoda: Artesiidae); C – *Novamundoniscus* sp. (Isopoda: Dubioniscidae); D – *Pirassunungoleptes* sp. (Opiliones: Zalmoxidae); E – Chernetidae (Pseudoscorpiones); F – Chelodesmidae (Polydesmida). Photos: Rodrigo Lopes Ferreira.

Environmental influence on invertebrate Community Structure

At the mesoscale, distance from the cave entrance was the only significant predictor of reduced species richness ($R^2 = 0.50$, $p = 0.003$; Figure 6). Neither shelter diversity nor organic

resource diversity showed a significant effect on richness (Table 1). At the microscale, none of the evaluated variables significantly influenced species richness (Table 1).

Table 1. Results obtained from the generalized linear model (GLM) applied at the meso-scale. Statistically significant effects ($p < 0.05$) are indicated with an asterisk ().*

Mesoscale	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	2.573158	0.600590	4.284	1.83e-05 ***
Distance from the entrance	-0.003360	0.001135	-2.960	0.00307 **
Trophic diversity	-0.158400	0.604635	-0.262	0.79334
Shelter diversity	0.074249	0.332297	0.223	0.82319

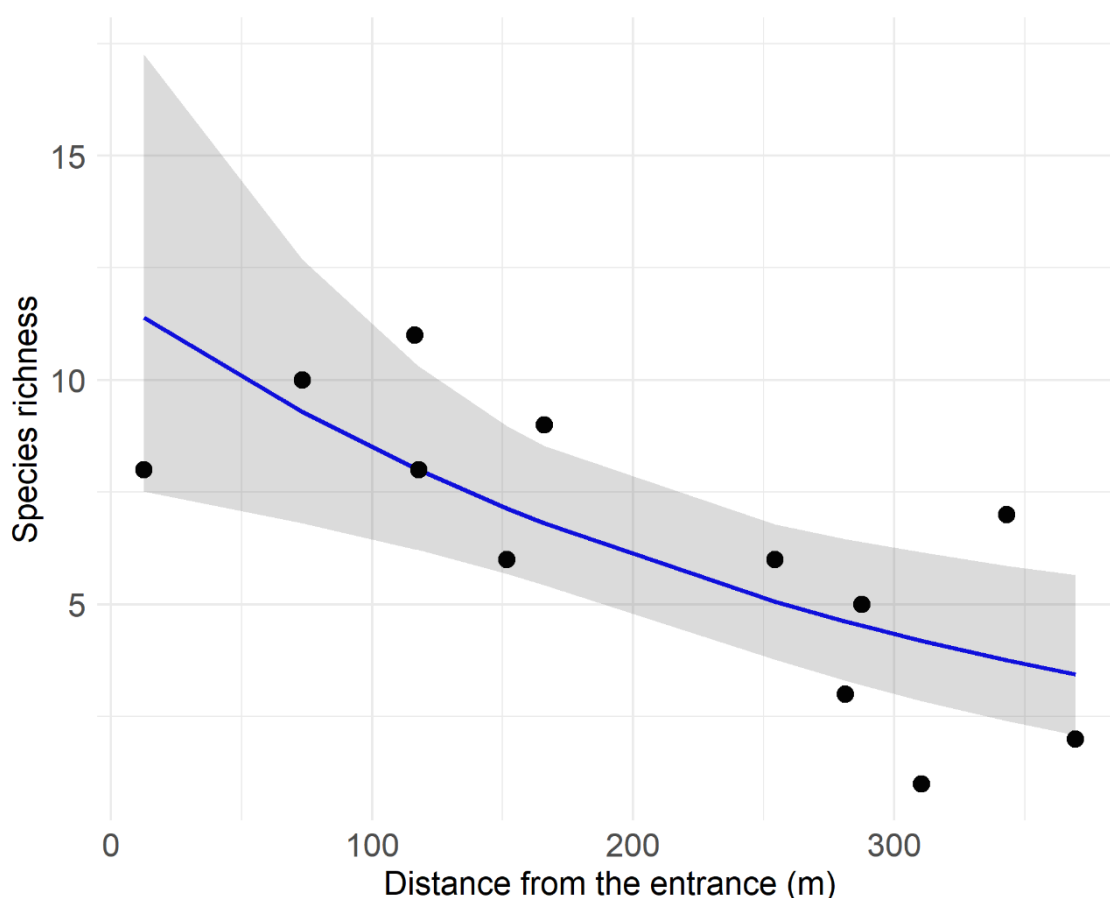


Figure 6. The GLM applied to the mesoscale revealed a negative relationship between species richness and distance from the entrance.

DistLM analysis revealed that, at the microscale (quadrats), none of the environmental variables significantly explained variation in community composition. In contrast, at the mesoscale (sectors), distance from the cave entrance was the only variable that significantly explained species composition, accounting for 21.14% of total variation.

The dbRDA ordination at the mesoscale confirmed that distance from the entrance was the strongest driver of community composition. The first two dbRDA axes together explained 40.9% of the total variation in faunal composition (Figure 7).

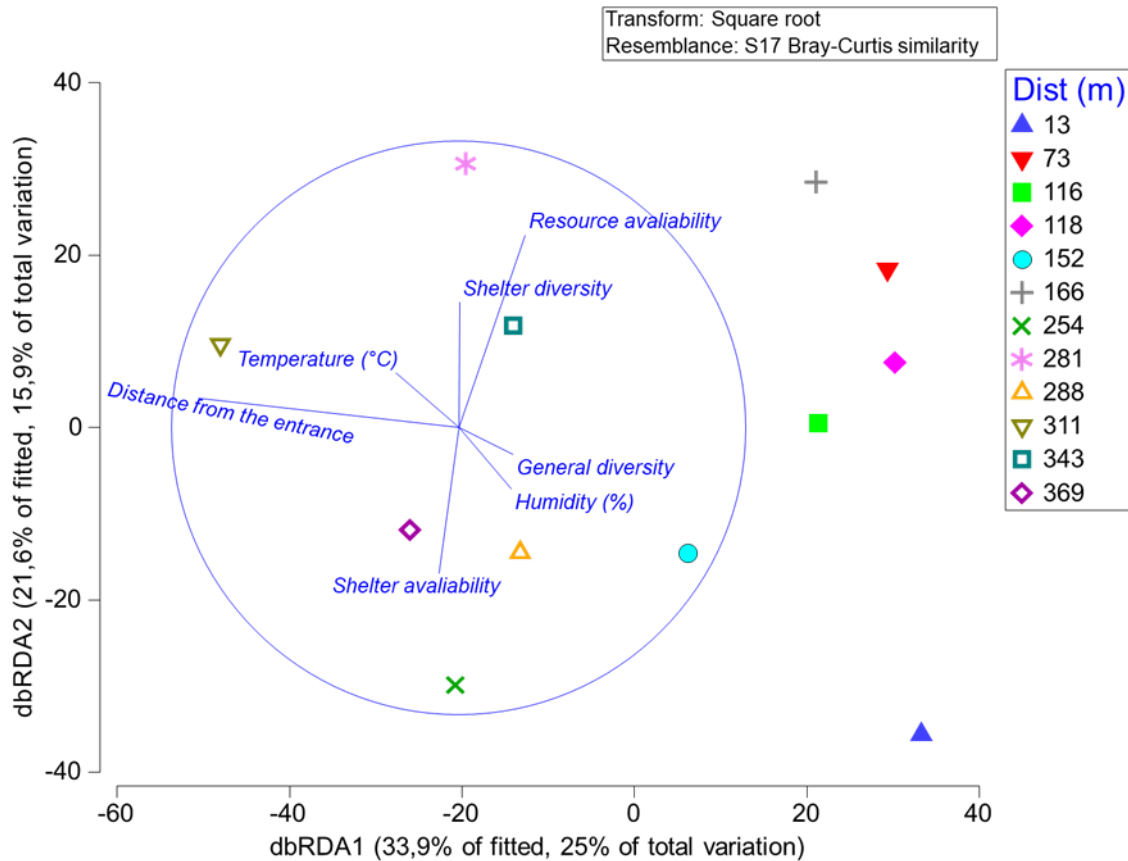


Figure 7. Distance-based Redundancy Analysis (dbRDA), using Bray-Curtis similarity (square root transformation), represents the relationship between environmental variables and subterranean fauna composition. The vectors indicate the direction and magnitude of the influence of physical, microclimatic, and trophic variables: distance from the entrance (m), temperature (°C), relative humidity (%), availability of shelters (disp.shelter), availability of trophic resources (disp.trophic resource), overall substrate diversity (DIVG), and shelter type diversity (DIV.AB). The symbols represent the different sampled sectors, coded according to the distance from the entrance.

Taxonomic distinction on the invertebrate community

At the mesoscale (sectors), sequential tests from the DistLM analysis identified temperature (Pseudo-F = 15.75, $p = 0.02$, $R^2 = 61.17\%$), relative humidity (Pseudo-F = 5.73, $p = 0.039$, $R^2 = 15.10\%$), and shelter availability (Pseudo-F = 16.13, $p = 0.003$, $R^2 = 15.86\%$) as the most influential predictors of taxonomic distinction. The final model, incorporating these three variables, explained 92.13% of the total variation in taxonomic distance between samples. These results indicate that microclimatic factors (temperature and humidity) and habitat structural complexity (shelter availability) play a key role in shaping the taxonomic diversity of invertebrate communities in Ricardo Franco Cave.

At the microscale (quadrats), none of the evaluated environmental variables (including distance from the entrance, resource and shelter availability, and substrate diversity) showed a significant relationship with variation in taxonomic distinction.

4. DISCUSSION

The results from Ricardo Franco Cave indicate that distance from the entrance is a key factor in structuring invertebrate communities, particularly at the mesoscale. Species richness declined significantly with increasing distance from the entrance, reflecting an ecological gradient widely documented in subterranean systems (Culver & Pipan, 2009; Lunghi et al., 2017; Souza-

Silva et al., 2021). This pattern is consistent with the reduction in environmental heterogeneity and the decline in allochthonous resource availability deeper inside the cave. In addition to richness, species composition also varied significantly along this gradient, indicating turnover likely driven by environmental filtering (Prous et al., 2015). Inner zones, characterized by more stable and oligotrophic conditions, tend to favor more specialized taxa, whereas entrance areas harbor generalist or troglomorphic species, which are typically more abundant and diverse (Prous et al., 2015; Souza-Silva et al., 2021).

Substrate distribution within the cave reinforces this interpretation. Organic and heterogeneous substrates (e.g., roots and gravel) were more frequent near the entrance, while fine inorganic materials, guano, and speleothems predominated in deeper zones. This structural shift along the entrance–interior gradient generates greater microhabitat diversity and trophic input in the transition zone between epigeal and hypogean environments (Reis-Venâncio et al., 2022).

At the microscale, no clear patterns emerged in richness or composition, suggesting that key variables relevant to this scale may not have been included in the analyses. Processes such as species interactions (predation, competition, mutualisms), succession dynamics, and biological invasions can strongly influence community structure but remain difficult to quantify and are rarely addressed in cave ecological studies (Da Rocha Melo et al., 2025). These findings highlight the need to expand the set of variables considered at fine spatial scales and to incorporate ecological processes that remain understudied in subterranean systems.

The adoption of multiple spatial scales is increasingly recognized as important in ecological research, as different species may respond differently depending on the observational scale and sampling frame (Wiens, 1989; Pacheco et al., 2020; Souza-Silva et al., 2021; Furtado-Oliveira et al., 2022; Vaz et al., 2025). In this study, the strong influence of distance from the entrance at the mesoscale, contrasted with the absence of effects at the microscale, suggests that the entrance–interior gradient and structural heterogeneity of Ricardo Franco Cave become apparent only when broader environmental variation is captured. This distinction underscores that both ecological inference and management recommendations are scale-dependent, emphasizing the importance of multi-scale designs in subterranean biodiversity research (Lovell et al., 2002).

The observed variation in taxonomic distinction (Δ^+) suggests that communities inhabiting sectors with greater structural complexity and microclimatic stability tend to harbor more distantly related lineages, resulting in greater taxonomic distance. The finding that temperature, humidity, and shelter availability together explain more than 90% of this variation indicates that these factors act as ecological filters shaping species composition and taxonomic diversity a pattern consistent with reports from other subterranean systems (Lunghi et al., 2017; Mammola & Isaia, 2018; Souza-Silva et al., 2021).

Climatic stability is widely recognized as a major determinant of biodiversity patterns in both surface and subterranean ecosystems (Tews et al., 2004; Souza-Silva et al., 2021). Among these factors, humidity plays a particularly critical role for invertebrates, whose small body size and thin cuticle make them highly susceptible to desiccation (Strachan et al., 2015; O'Donnell, 2022). In caves, high humidity levels can be maintained through multiple mechanisms, including limited atmospheric exchange with the surface, low thermal variation, condensation of water vapor, percolation and infiltration from soil and rock, and the presence of internal or entrance-proximal water bodies (Buecher et al., 1999). Together, these conditions generate the environmental stability that supports the persistence of specialized cave fauna (Howarth, 1983; Culver & Pipan, 2009; Souza-Silva et al., 2011).

From a conservation perspective, the results of this study reinforce the urgent need to recognize Ricardo Franco Cave as a priority area for protection and management. The occurrence of troglobitic species, combined with the cave's historical and cultural significance, highlights its status as a natural heritage of exceptional ecological and cultural value, whose preservation is essential. The absence of formal protection, coupled with increasing anthropogenic pressures in the Pantanal including unregulated tourism and potential indirect impacts from landscape alterations intensifies the risk of irreversible biodiversity loss (Mammola et al., 2020; Furtado-Oliveira et al., 2022). In this context, the incorporation of Ricardo Franco Cave into management and conservation programs would represent a crucial step toward safeguarding its ecological and cultural attributes.

Although several studies have addressed strategies to mitigate tourism impacts on cave fauna, evidence shows that without proper planning, tourism may compromise visitor safety, degrade cave integrity, and negatively affect local fauna (Lobo, 2006; Faille et al., 2015; Furtado-Oliveira et al., 2022). The persistence of troglobitic organisms in Ricardo Franco Cave underscores the importance of maintaining key ecological processes, including microclimatic stability and the preservation of minimal resource inputs (Souza-Silva et al., 2021; Vaz et al., 2025). Therefore, modified caves should be subject to continuous monitoring and evaluation, based on biological and ecological criteria such as the presence of endemic species, population trends, and fauna responses to tourism impacts, as recommended in recent conservation frameworks for subterranean ecosystems (Mammola et al., 2019; Cardoso et al., 2022).

Finally, this study contributes to filling critical knowledge gaps on ecological patterns in subterranean environments of the Pantanal, a region still poorly explored from the perspective of cave biology. The multiscale approach employed revealed distinct mechanisms structuring subterranean biodiversity, emphasizing the roles of distance from the entrance, substrate heterogeneity, and microclimatic stability. Future research should broaden the scope of variables considered, incorporating ecological interactions, invasion dynamics, and succession processes, in order to establish a more comprehensive foundation for conserving subterranean biodiversity in this biome.

5. FINAL REMARKS

The multiscale analysis demonstrated that distance from the entrance is the primary factor structuring the invertebrate fauna of Ricardo Franco Cave, influencing both species richness and community composition at the mesoscale. Microclimatic stability and shelter availability also emerged as key determinants of taxonomic distinctness. Together, these findings underscore the importance of incorporating spatial scale and environmental heterogeneity into cave management strategies. The occurrence of troglobitic species, combined with the cave's historical and cultural significance, reinforces its prioritization in conservation programs aimed at preserving ecological integrity and mitigating anthropogenic impacts.

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