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ECOSYSTEMS

The role of turnover in structuring subterranean aquatic communities in Southeastern Brazil

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Abstract: Similarly to other animal communities, the diversity of subterranean aquatic fauna is influenced by several factors and processes, such as habitat fragmentation, dispersion, environmental heterogeneity, and physical and chemical water characteristics. Here, we studied cave aquatic communities of the Alto Ribeira hydrographic basin, regarding troglobitic and non-troglobitic species, located in a single karst area to evaluate the influence of sub-basins in fauna differentiation. We investigated how abiotic variables (flow, electrical conductivity, temperature, pH, and substrate) influence the fauna composition and the contribution of beta diversity components (nestedness and turnover) in explaining communities' dissimilarities. Fauna composition differed between sub-basins, as most species did not co-occur in different caves. Caves with higher flow and substrate diversity were the richest. In addition, each cave community was influenced by a unique set of abiotic variables. Dissimilarity among caves was mainly explained by turnover, and our findings suggest the restricted species distribution could be due to ecological (e.g., limited dispersion capacity, tolerance to abiotic variables), hydrogeological (e.g., dispersion barriers, isolation of sub-basins), and historical (e.g., colonization, paleoclimatic events) factors and processes. Therefore, different elements are responsible for determining the composition of cave aquatic communities in different sub-basins, reflecting the variability within a single karst area.

Key words: abiotic variables, beta diversity, caves, conservation, dispersion, river basin.

INTRODUCTION

The subterranean environment is the set of interconnected spaces of the subsoil filled with water or air, ranging from millimetric cracks to large cavities, which when accessible to humans are called caves (Juberthie 2000). Caves are mainly formed in karstic rocks, *i.e.*, rocks that are soluble by chemical processes and have well-developed secondary porosity (Ford & Williams 2007), such as limestone and dolomite. They are part of an extensive environment with several spaces, and their main characteristics are the absence of light, relative environmental stability, such as a tendency to constant temperature and high relative humidity of the air, and dependence on allochthonous food resources (Barr 1967, Juberthie 2000, Poulson & Lavoie 2000). Due to the absence of primary producers, subterranean food webs have two trophic levels significantly represented: detritivores and predators (Gibert & Deharveng 2002). Subterranean populations tend to be less numerous, due to the lower food supply, than the epigean ones (Barr 1968).

The subterranean fauna is classified ecologically and evolutionarily into troglobites (inhabit exclusively the subterranean realm, where are their source population), troglophiles (can establish populations and have source populations in both subterranean and epigean realms), and trogloxenes (use the subterranean realm, but need to leave to complete their life cycle; have source populations in the epigean environment) (Barr & Holsinger 1985, Trajano & Carvalho 2017). Usually, troglobites have troglomorphisms, i.e., morphological, physiological, and behavioral modifications associated with isolation in the subterranean environment (Christiansen 1962, 2012). Some of the most common morphological troglomorphisms are reduction of eyes and melanic pigmentation and elongation of appendages (Barr & Holsinger 1985, Christiansen 1962, 2012). In Brazil, a Neotropical country, the subterranean communities are mainly composed of non-troglobite organisms, in contrast to North America and Europe (Trajano & Bichuette 2010).

In fluvial ecosystems, the main abiotic characteristics that influence the species distribution and abundance are the water flow, temperature, and substrate (Allan & Castillo 2007). The flow transports resources for the organisms and removes residuals from the environment, the water temperature influences the growth rates and life cycles, and the substrate provides surface and space where the animals can establish, forage, and shelter (Allan & Castillo 2007). Concerning the variation in the chemical characteristics of water, such as conductivity and alkalinity, the biological consequences are not significant when the conditions are near to the mean but are evident in extreme values (Allan & Castillo 2007). Areas with higher habitat heterogeneity (such as the spatial arrangement of patches, diversity of habitats, and environmental variability) provide higher surface area, more physical refuges, and higher or more varied supply of limiting resources, and, consequently, more ecological niches, promoting the diversity (Li & Reynolds 1995, Warfe et al. 2008, Palmer et al. 2010).

Several factors and processes control the diversity in the subterranean environment, such as time of dispersion and adaptation to habitats, spatial heterogeneity, food availability, niche overlap, predation, and competition (Poulson & White 1969, Gibert et al. 2009). In addition, hydrogeology, altitude, paleogeographic factors, and human activities can interact in complex ways to generate different patterns of species composition and diversity among regions (Gibert et al. 2009). Some authors (e.g., Culver & Sket 2000, Gibert & Deharveng 2002, Fernandes et al. 2013) have been discussing how fragmented habitats and dispersion restrictions in karstic areas result in many endemic species, sometimes restricted to a unique cave. The presence of shelters (Pellegrini et al. 2018), food resources (Souza-Silva et al. 2011), and habitat heterogeneity (Gibert et al. 2009) are relevant factors in determining species richness and structuring subterranean aquatic communities. Moreover, different factors can influence communities of different caves, such as the physical characteristics of stream channels (Pellegrini et al. 2018) and physical and chemical water characteristics (Simões et al. 2013, Pellegrini et al. 2018). In addition, caves and other subterranean habitats may have aquatic horizons, such as pools fed by dripping waters and running streams (Poulson & White 1969). These running streams frequently are surface base-level streams that enter caves by sinkholes. When crossing calcareous terrains, they have pH values from neutral to basic (Culver 1982, Bichuette & Trajano 2003).

In subterranean communities, alpha diversity (local) is generally lower than beta diversity (*i.e.*, variation in species composition of communities) (Malard et al. 2009, Stoch & Galassi 2010, Pipan et al. 2018). Beta diversity has two components: species turnover, which refers to the replacement of a species by another, and nestedness of assemblages, *i.e.*, when the community with fewer species is a subset of the richest community (Baselga 2010, 2012). The turnover implies the replacement of some species by others due to environmental or spatial ordering and historical restrictions (Qian et al. 2004, Baselga 2010). The nestedness reflects the result of a non-random process of species loss in consequence of any factor that promotes the orderly disaggregation of the assemblages (Gaston & Blackburn 2007, Baselga 2010). Thus, the differentiation of these two components is useful for understanding biogeographic, ecological, and conservation issues (Baselga 2010) and the diversity of cave communities (Pipan et al. 2018).

Studies of subterranean communities have been discussing the contribution of turnover in beta diversity at different scales: species distribution gradients in the different cave zones (entrance, twilight, and aphotic), with the spatial heterogeneity explained by microclimate, light availability, history of invasions, trophic entries, competition, among others (see Mammola 2019); temporal species turnover (Bichuette et al. 2017), mainly in the regions near to entrances (Mammola et al. 2017, Mammola 2019); and troglobitic species replacement in communities from different karst areas (Cardoso 2012, Fattorini et al. 2016, Pipan et al. 2018, Mammola 2019), due to the highly restricted migration of these species among areas (Gibert & Deharveng 2002, Pipan et al. 2018).

On the left bank of the Alto Ribeira hydrographic basin, South-eastern Brazil, the main tributaries cross limestone outcrops, forming several semi-isolated micro-basins (Bichuette & Trajano 2018). Some troglophilic aeglids populations occur between caves and the epigean routes of different sub-basins without the restrictions imposed by the limestone discontinuity (Fernandes et al. 2013). However, most streams form cave systems, and almost all of them have their own troglobitic and troglophilic populations, as in the case of the *Potamolithus* (Caenogastropoda, Tateidae) populations (Trajano et al. 2016, Bichuette & Trajano 2018).

In this study, we investigated the cave aquatic communities from a single karst area to evaluate the sub-basins' influence on the fauna composition. For this purpose, first, we compared the subterranean aquatic community composition in streams from the three Alto Ribeira sub-basins (Iporanga, Betari, and Pilões). We hypothesized that cave communities differ according to their sub-basins due to the relative isolation among these. Second, we investigated how different physical and chemical characteristics and habitat heterogeneity influence the fauna composition. We hypothesized that sites with higher flow and substrate diversity have higher richness and abundance values. Third, we verified the contribution of beta diversity components (turnover and nestedness) to the dissimilarity of these communities. We hypothesized that turnover explains most beta diversity between caves due to relative isolation among their sub-basins.

MATERIALS AND METHODS

Study area

The Parque Estadual do Alto Ribeira (PETAR) (24°27'36" S, 48°36'0" O) is located in the municipalities of Iporanga and Apiaí, state of São Paulo, Brazil (Figure 1a-c). Its predominant native vegetation is the Atlantic Forest, with the development of the Dense Ombrophilous Forest (Fundação Florestal 2010). The region is characterized by a warm climate, with generally low thermal ranges (Nimer 1977) and annual means between 20 °C and 22 °C (Karmann & Ferrari 2002), and a super humid climate, with no defined dry season. The total annual precipitation is around 1,500 mm and the

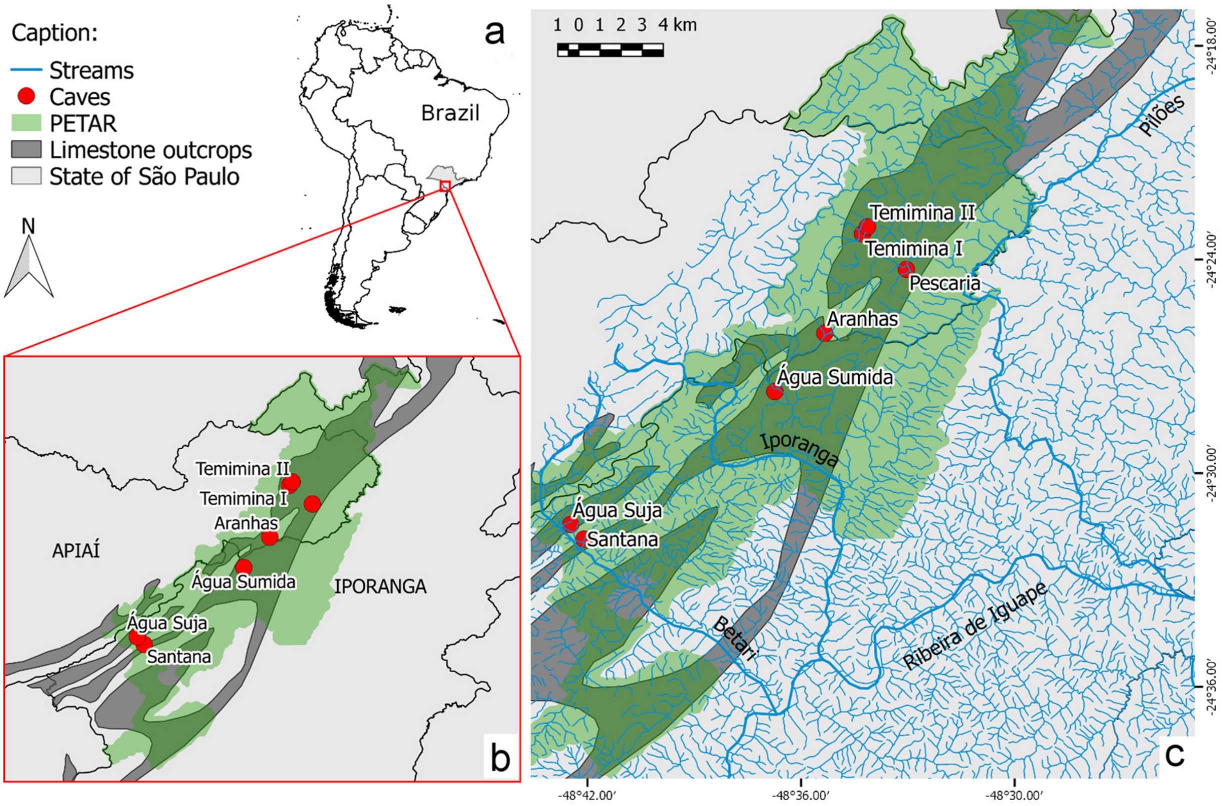


Figure 1. Study area. (a) South America, Brazil, and the state of São Paulo, (b) and (c) Parque Estadual Turístico do Alto Ribeira (PETAR), boundaries of Iporanga and Apiaí municipalities, streams of the Alto Ribeira basin, and studied caves.

precipitation in the driest and coldest month (July) is higher than 30 mm (Bichuette & Trajano 2018).

Geologically, the Alto Ribeira karst area is characterized by limestone rocks of the Açungui Group composed of Pre-Cambrian metasedimentary rocks (Karmann & Sánchez 1979). This karst area has four limestone outcrops interspersed with non-soluble rocks with northeast-southwest (NE-SW) directions, ranging from 1 km to 10 km in width and extending up to dozens kilometers in length (Trajano 1991) (Figure 1b-c). The PETAR has 474 caves (Fundação Florestal 2010), most of which represent accessible sections of groundwater circulation routes (general NE-SW orientation), with a convergent pattern (*i.e.*, water collected by many sinkholes is drained for few resurgences) (Karmann & Ferrari 2002). In general, trophic resources in terrestrial habitats of the PETAR caves are mainly organic matter dispersed throughout the caves, such as vegetal debris, bat guano, and otter feces (Fundação Florestal 2010). The same is observed for cave streams, which have little to moderate dispersed organic matter, but some caves have sites with a high accumulation of vegetal organic matter (Fundação Florestal 2010).

The Ribeira de Iguape River runs a total length of 470 km (CBH-RB 2019). Its hydrographic basin originates in the state of Paraná and flows into the Atlantic Ocean on the south coast of the state of São Paulo (CBH-RB 2019). The PETAR is located on the left bank of the middle to the upper course of the Ribeira river and is drained by the hydrographic basins of the Betari, Iporanga, and Pilões streams (Karmann & Ferrari 2002) (Figure 1b-c).

Despite the implementation of PETAR between 1987 and 1988, part of the caves, subterranean systems, and catchment basins, such as the headwaters of the Pilões, Iporanga, and Betari stream sub-basins, are outside the boundaries of the park and, therefore, are not protected (Karmann & Ferrari 2002, Bichuette & Trajano 2018). In addition, the PETAR region has contamination of terrestrial and aquatic environments resulting from mining (presence of heavy metals due to abandoned mines of lead, silver, and gold), agriculture (pesticides), and human settlements (domestic sewage) (Moraes et al. 2002).

Sampling

We carried out the samplings in the caves Água Suja (24°31’26,5” S, 48°42’28,5” W), Santana (24°54’33,8” S, 48°59’22,6” W) (Betari stream sub-basin), Aranhas (24°26’04.0 S, 48°35’20,0” W), Água Sumida (24°27’42,3” S, 48°36’44,1” W) (Iporanga stream sub-basin), Pescaria (24°24’16,6” S, 48°33’02,5” W), Temimina I (24°23’16,5” S, 48°34’16,5” W), and Temimina II (24°23’04,2” S, 48°34’07,6” W) (Pilões stream sub-basin) (Figure 1). We considered Temimina I and II caves as “Temimina cave” because only a daylight hole separates these two cavities, forming a system. All caves studied have streams that run along the entire length of their main conduits. The extension (horizontal projection) of each cave is in Table I.

In the six studied caves, we collected the fauna over five visiting occasions (July-2017, November-December-2017, April-2018, July-2018, and April-2019). It was not possible to carry out the field activity between November 2018 and March 2019 due to heavy rains in the region.

In each cave, ten sampling sites of 20 meters in length were delimited following the stream bed. The uniqueness and accessibility of the locations were also decisive for the delimitation of the sampling sites. Therefore, the distance between the sites could not be the same in all caves, as well as the distance between the sites and the cave entrances. However, all caves had a sampling site in the entrance zone.

Five sites in each cave were drawn to carry out sampling on every sampling occasion. The sampling effort in each site was 15 minutes

Table I. Extension (horizontal projection) of the caves; total volume of filtered water in streams for zooplankton collection; and mean values and their respective standard deviations for abiotic variables measured in caves of the Parque Estadual Turístico do Alto Ribeira (PETAR), state of São Paulo, Brazil.

Caves	Horizontal projection (m)	Volume (m^3)	Flow ($m^3 s^{-1}$)	Conductivity (mS cm^{-1})	Temperature (°C)	pH	Substrate (diversity)
CS	8,540	651.79	0.37 ± 0.20	0.19 ± 0.01	18.94 ± 0.24	7.45 ± 0.26	3.10 ± 1.22
SU	2,985	610.84	0.24 ± 0.12	0.12 ± 0.02	18.57 ± 0.20	7.61 ± 0.29	4.70 ± 1.19
AS	298	566.30	0.65 ± 0.46	0.14 ± 0.01	18.93 ± 0.81	7.75 ± 0.33	4.70 ± 1.10
AR	210	477.38	0.07 ± 0.04	0.03 ± 0.01	16.77 ± 0.99	6.72 ± 0.48	5.90 ± 1.76
PE	2,780	397.86	0.37 ± 0.20	0.14 ± 0.01	18.87 ± 0.57	7.51 ± 0.36	3.40 ± 1.36
TE	2,021 ‡	388.67	0.70 ± 0.29	0.05 ± 0.01	17.95 ± 0.79	7.26 ± 0.32	5.50 ± 1.63
Total/ mean	-	3092.84	**0.40 ± 0.33**	**0.11 ± 0.05**	**18.33 ± 1.02**	**7.38 ± 0.47**	**4.55 ± 1.72**

‡ The horizontal projections of the Temimina I (52 m) and Temimina II (1,969 m) caves were summed.
Abbreviations (caves): CS = Santana, SU = Água Suja, AS = Água Sumida, AR = Aranhas, PE = Pescaria, TE = Temimina.

using the qualitative direct search method, i.e., species were searched intensively in areas where they were considered most likely to be found (Weinstein & Slaney 1995), and using 10 *Surber* samplers (250 µm mesh), each with an area of 900 cm^2 (0.09 m^2), which were arranged randomly. Invertebrates were fixed in situ in 70% ethanol, except for some decapod crustaceans and mollusks that were anesthetized with benzocaine crystals and later fixed in 4% formalin and preserved in 70% ethanol. Fish were anesthetized with benzocaine crystals, fixed in 10% formalin, and preserved in 70% ethanol, following the animal welfare protocols set out in Law N° 11,794 of October 8, 2008 (Brasil 2008). When a species was found frequently and its identity was known, its individuals were only counted to avoid the euthanasia of specimens.

We sampled all ten sites in every cave with plankton nets (20 µm mesh) throughout the duration of the study. Two sites were drawn to be sampled in every sampling occasion. We collected approximately 200 ml of water at each sampling site and used 4% formalin to preserve the fauna. We estimated the filtered water volume for zooplankton collection by calculating $Vol = A \cdot V \cdot T$, where Vol corresponds to the volume (m^3), A the area of the plankton net opening (m^2), V the flow velocity ($m\ s^{-1}$), and T the filtering time (s).

At each sampling site, we measured the maximum depth of the stream using a plastic meter (PVC) and the mean width using a laser distance meter (Leica Disto D2). We calculated the flow (volume of water that passes in a section of the stream per unit of time) using the formula $Q = A \cdot V$, where Q is the flow ($m^3\ s^{-1}$), A is the area (width x depth of the section) (m^2), and V is the flow velocity ($m\ s^{-1}$). We calculated the flow velocity by counting the time that a floating object (falcon-type conical tube) used to travel two meters. We repeated such measurements three times and then calculated the average velocity. The values of electrical conductivity, temperature, and pH were measured using a digital water quality monitoring meter (Tri-Meter pH/Ec&Temp-983, RoHS).

The characterization of inorganic substrates according to particle diameter followed the proposal by Cummins 1962 and Minshall 1984 (apud Allan & Castillo 2007): clay (< 0.063 mm), sand (0.063 to 2 mm), gravel (2 to 16 mm), pebbles (16 to 64 mm), and rock blocks (> 64 mm). Thus, we visually determined the substrate types according to their components (clay, sand, gravel, pebbles, rock blocks, vegetal organic matter, and their possible combinations, e.g., sand and gravel; sand, rock blocks, and vegetal organic matter, etc). We considered how many types of substrates were available in each sampling site as a measure of substrate diversity.

We identified specimens to the least inclusive taxonomic level (Operational Taxonomic Units / OTUs) using specialized bibliography (Wilson & Yeatman 1959, McCafferty 1983, Costa et al. 2006, Krantz & Walter 2009, Mugnai et al. 2010, Segura et al. 2011, Rafael et al. 2012). We identified gastropods of the genus *Potamolithus* (Tateidae) according to Bichuette & Trajano (2018). The material was also analyzed by experts from different taxonomic groups to obtain more accurate identifications. Additionally, we defined ecological-evolutionary categories of the taxa comparing them with the epigean fauna collected during sampling occasions and literature data.

The specimens were deposited in the Zoological Collection of the Laboratório de Estudos Subterrâneos (LES) of the Universidade Federal de São Carlos (UFSCar) (Curator: ME Bichuette). Images were taken with a Leica DFC 295 video camera attached to a Leica M205C with a Planapo 1.0x objective. Figures were produced from stacks of images using LAS (Leica Application Suite v. 3.7).

Research ethics

All applicable institutional and national guidelines for the care and use of animals were followed. The collection permits were granted by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (nº 28992-11) and Instituto Florestal do Estado de São Paulo (process SMA n° 260108 – 002.120/2017). The permission to use vertebrate animals in research was granted by Comissão de Ética no Uso de Animais (CEUA) - UFSCar (nº 8246020517).

Data analysis

We verified the relationship between the sampling effort (filtered water volume for zooplankton collection) and the richness and abundance of taxa using Pearson's correlation, as done by Simões et al. (2013), as the volumes sampled in each sampling site varied. The analysis was performed using the R software version 3.6.2 (R Core Team 2019).

We determined the richness and abundance of species in total and for each cave. Rotifera were not considered in this study. We performed a Nonmetric Multidimensional Scaling (nMDS) (Kruskal 1964) to analyze the composition of subterranean communities of the different Alto Ribeiro sub-basins. This is a robust method of unrestricted ordering (Oksanen 2004) that preserves the ordering relationships between objects, *i.e.*, dissimilar objects are plotted distant in the order space, and similar objects are near each other (Legendre & Legendre 1998). Furthermore, it is not required the data to follow the Gaussian distribution (Oksanen 2004). We used the Bray-Curtis dissimilarity distance, which is recognized as a good measure of ecological distance for species abundance (Legendre & Anderson 1999), and the double standardization transformations of Wisconsin, as suggested by Oksanen (2004). We used vegan version 2.5-6 (Oksanen et al. 2019) and ggplot2 packages (Wickham 2016) of R software version 3.6.2 (R Core Team 2019).

To verify whether the fauna composition differs among sub-basins and caves, we performed a non-parametric multivariate analysis of variance (PERMANOVA) (Anderson 2001) using the Bray-Curtis dissimilarity distance and 9,999 permutations, followed by pairwise analyses using the Bray-Curtis distance, p-value corrections of Bonferroni, and 9,999 permutations. We used vegan version 2.5-6 (Oksanen et al. 2019) and pairwiseAdonis version 0.0.1 (Martinez-Arbizu 2020) packages of R software version 3.6.2 (R Core Team 2019).

We performed a Canonical Correspondence Analysis (CCA) (Legendre & Legendre 1998), a multivariate technique that explains niche separation along environmental gradients, to verify how physical, chemical, and habitat heterogeneity variables influence the fauna distribution. The variables used were flow (Q), electrical conductivity (EC), temperature (T), pH, and substrate. For substrate data, we considered the substrate diversity, i.e., how many types of substrates were available in each sampling site, as a measure of environmental heterogeneity. Data from abiotic variables were standardized for analysis. Thereafter, we performed a significance test using Monte Carlo permutations (Hope 1968). The analyses were performed using the vegan package version 2.5-6 (Oksanen et al. 2019) of R software version 3.6.2 (R Core Team 2019).

The beta diversity among multiple sites, *i.e.*, the dissimilarity of Sørensen (β_{SOR}) among caves, was decomposed into Simpson dissimilarity (β_{SIM}, which describes species turnover among communities) and into nestedness component resulting from dissimilarity (β_{SNE}, which is the difference between β_{SOR} and β_{SIM}) (see Baselga 2010).

Similarly, the beta diversity between each pair of caves was decomposed, according to Baselga (2010), into:

$$\beta_{sor} = \beta_{sim} + \beta_{sne} = (b+c) / (2a + b + c) = [b/(b + a)] + [c - b)/(2a + b + c)][a/(b + a)]$$

where β_{sor} is the Sørensen dissimilarity (total dissimilarity between communities); β_{sim} is the Simpson dissimilarity (= turnover); β_{sne} is the nestedness component resulting from dissimilarity; a is the number of shared species; b is the number of unique species from the poorest site; c is the number of unique species from the richest site. For this, we used the betapart package version 1.5.1 (Baselga et al. 2018) of R software version 3.6.2 (R Core Team 2019), as done by Pipan et al. (2018).

RESULTS

On average, we filtered 51.5 m^3 of water per sampling site for zooplankton collection (Table I). According to Pearson's correlation, there is no linear correlation between the filtered water volume and the taxa richness ($r^2 = 0.011$) and abundance ($r^2 = 0.002$) in each sampling site, *i.e.*, the filtered water volume did not influence the number of species and individuals.

We recorded 117 species, of which three were classified as troglomorphic (possibly troglobite), one as troglobite, 79 as troglophile, two as accidental, and 32 as uncertain classification (Figure 2 and Supplemenatry Material -Appendix S1). The troglobitic species, a snail of the genus *Potamolithus* (Caenogastropoda, Tateidae), was recorded only in Pescaria cave (Figure 2n). It has a reduction/absence of eyes and pigmentation (body and periostracum), presenting an intraspecific variability of these characters. In Santana cave, two troglomorphic species were recorded: a blind amphipod of the genus *Hyalella* (Amphipoda, Hyalellidae) (Figure 2c), the only troglomorphic arthropod found in the studied caves, and a flatworm of the genus *Girardia* (Tricladida, Dugesiidae) (Figure 2e). Another troglomorphic species of the genus *Girardia* was recorded in Aranhas cave (Figure 2i). These *Girardia* species present reduced eyes and body pigmentation.

The richest cave was Água Sumida (61 species), followed by Aranhas (48), Temimina (44), Água Suja (29), Pescaria (23), and Santana (14) (Appendix S1). In addition, of the 117 species, 60 occurred in a unique cave: five in Santana, six in Água Suja, 12 in Temimina, five in Pescaria, 17 in Água Sumida, and 15 in Aranhas (Appendix S1). For example, the four species of *Potamolithus* did not co-occur; of the three species of *Hyalella*, one occurred in Aranhas and Água Sumida caves (Iporanga sub-basin), while one occurred only in Santana cave, and another in Temimina cave; and of the five species of *Girardia*, two occurred exclusively in Santana cave and two exclusively in Aranhas cave (Figure 2 and Appendix S1).

We recorded a total of 3,444 individuals (1,620 collected and 1,824 sighted). *Potamolithus* was the most abundant taxon (1,617 individuals). The Pescaria cave had the highest number of individuals (1,040), followed by Temimina (777), Santana (501), Água Sumida (447), Água Suja (410), and Aranhas (269) (Appendix S1).

Subterranean communities of the different Alto Ribeira sub-basins

The nMDS (two dimensions) presented a reasonable ordering (stress = 0.1391). According to the ordination diagram, it is possible to visualize the grouping of caves located in the Betari sub-basin differing from the Iporanga and Pilões sub-basins. These, in turn, overlapped due to the Água Sumida cave (Figure 3).

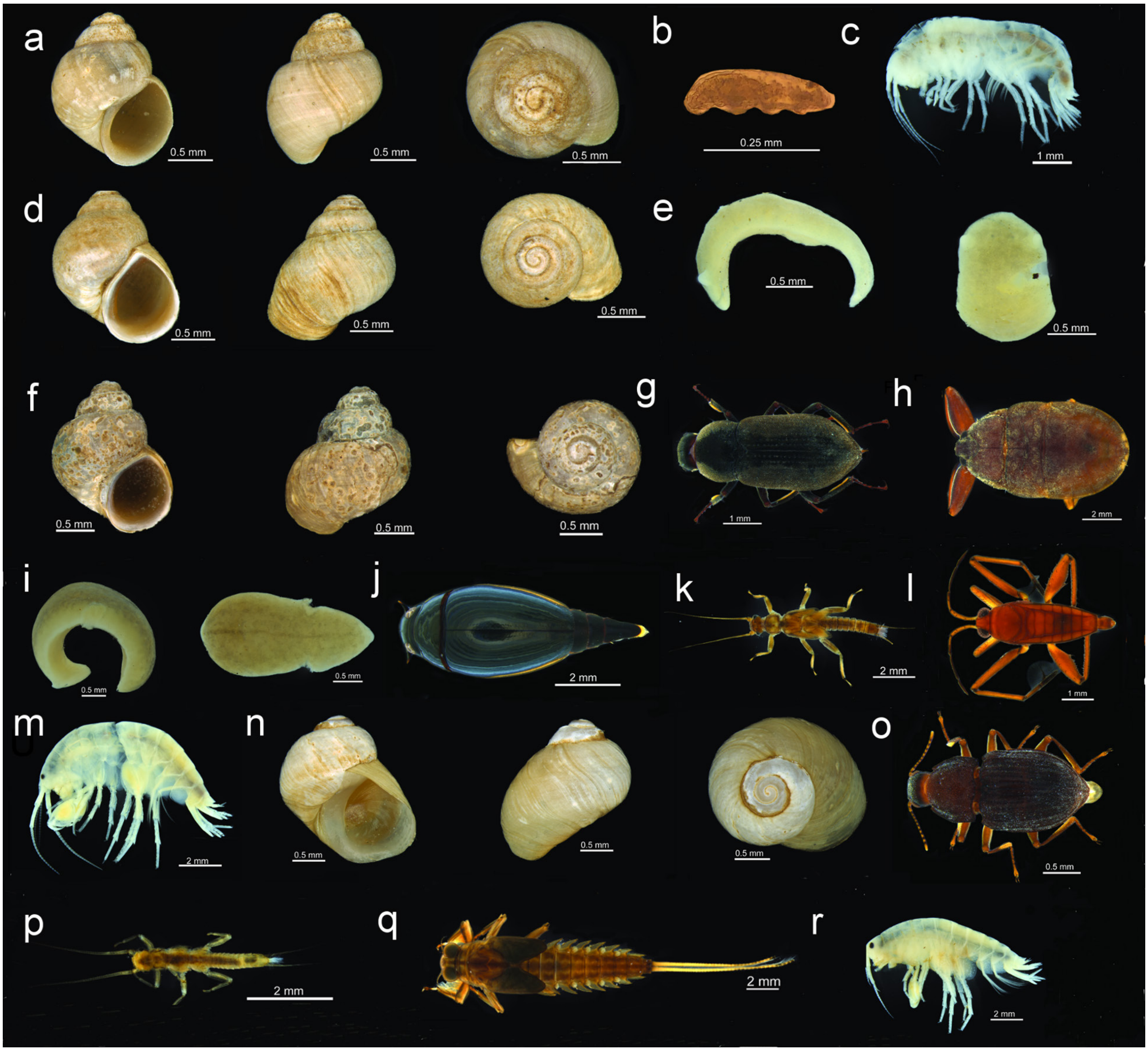


Figure 2. Examples of recorded species in the studied caves, Parque Estadual Turístico do Alto Ribeira (PETAR), state of São Paulo, Brazil. Species occurring exclusively in the Água Suja cave: (a) opening, dorsal, and apical view of *Potamolithus* sp. 4 (Gastropoda, Tateidae), **(b)** Tardigrada sp. Species occurring exclusively in the Santana Cave: (c) *Hyalella* sp. n. 8 (Amphipoda, Hyalellidae), (d) opening, dorsal, and apical view of *Potamolitus* sp. 5, (e) lateral and dorsal view of *Girardia* sp. n. 1 (Tricladida, Dugesiidae). Species occurring exclusively in the Água Sumida cave: (f) opening, dorsal, and apical view of *Potamolithus* sp. 6, (g) Dryopidae sp. 3 (Coleoptera), (h) Naucoridae sp., (Hemiptera). Species occurring exclusively in the Aranhas cave: (i) lateral and dorsal view of *Girardia* sp. 5, (j) *Gyretes* sp. (Coleoptera, Gyrinidae), (k) *Paragrypopterix* sp. 1 (Plecoptera, Gripopterygidae), (l) Veliidade sp. 1 (Hemiptera). (m) The species *Hyalella* sp. n. 1 occurred in both Água Sumida and Aranhas caves. Species occurring exclusively in Pescaria cave: (n) opening, dorsal, and apical view of *Potamolithus* sp. 9, (o) *Xenelmis* sp. 2 (Coleoptera, Elmidae). Species occurring exclusively in the Temimina cave: (p) *Tupiperla* sp., (q) *Grypopterix* sp. (Plecoptera, Gripopterygidae), (r) *Hyalella* sp. n. 2. Photos: LBR Fernandes (except b); Edition: JS Gallo and T Zepon.

The polygons of each sub-basin indicate that the composition differed between the sub-basins (Figure 3), as evidenced by PERMANOVA (r^2 = 0.28933, p = 0.0001); and confirmed by pairwise analysis (p_{adj} = 0.003 between each pair of sub-basins). The composition also differed between the caves (r^2 = 0.6524, p = 0.0001); which was confirmed by the pairwise analysis (p_{adj} = 0.015 between each pair of caves).

Fauna composition related to abiotic characteristics

We identified 25 types of substrates in all caves. The mean values and standard deviations of the abiotic variables measured for each cave are in Table I.

The eigenvalues of the first two axes of the CCA were 0.78 and 0.29, respectively, and they explain 70.95% of the data variation (axis 1 = 51.40% and axis 2 = 19.55%). The scores indicated that the substrate, conductivity, temperature, and pH variables are more explained by axis 1 (-0.99, 0.98, 0.77, and 0.71, respectively), while the flow is more explained by axis 2 (-0.76), and that the correlation between species and abiotic variables is strong (axis 1 = 0.94 and axis 2 = 0.73). According to the Monte Carlo significance test, there is a relationship between the distribution of species and the variables tested (p = 0.001). For each abiotic variable tested, this relationship was also significant, with conductivity explaining most of the data variation (r^2 = 0.8698, p = 0.0009), followed by temperature (r^2 = 0.5189, p = 0.0009), flow (r^2 = 0.3944, p = 0.0009), substrate diversity (r^2 = 0.3672, p = 0.0009), and pH (r^2 = 0.2240, p = 0.0029).

The ordering diagram of the abiotic variables in relation to the sampling sites of each cave indicates that the values of the abiotic variables are similar in the Pescaria and Água Suja caves (Figure 4a), and the Água Sumida and Temimina caves are associated with higher water flow (Figure 4a). The Temimina and Aranhas caves showed higher substrate diversity at their sampling sites (Figure 4a). Santana cave

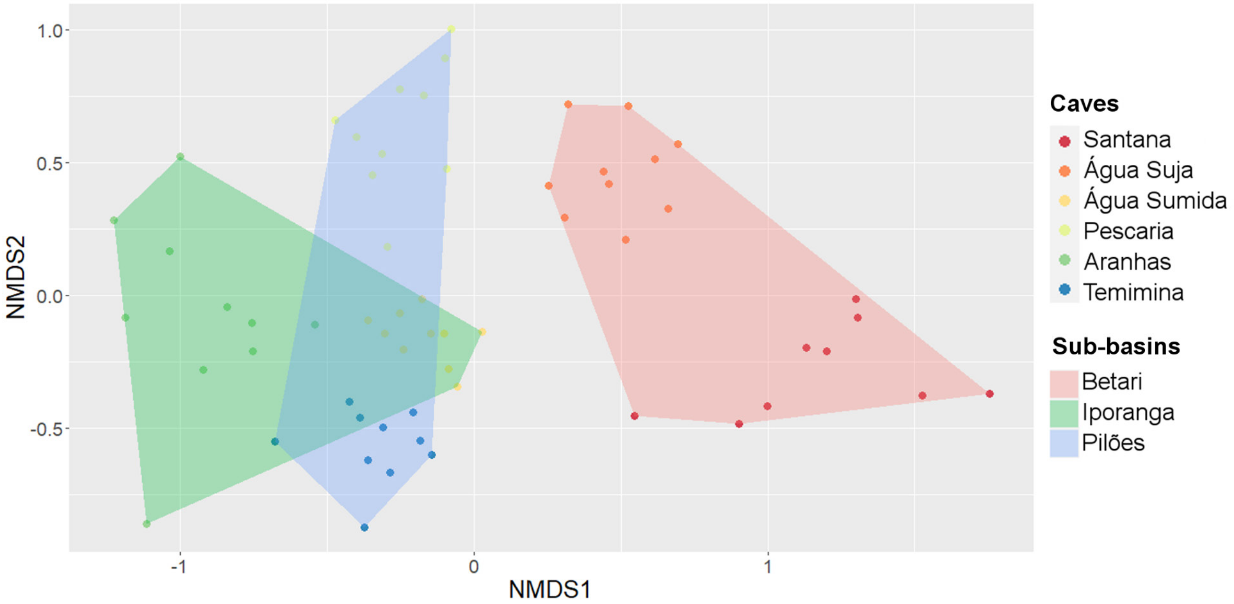


Figure 3. Nonmetric Multidimensional Scaling (nMDS) diagram of subterranean aquatic communities in the different sub-basins of the Alto Ribeira, Parque Estadual Turístico do Alto Ribeira (PETAR), state of São Paulo, Brazil.

presented higher values of conductivity and lower substrate diversity (Figure 4a).

The ordination diagram of abiotic variables in relation to species indicates that, since the points are concentrated near the diagram center, most species are associated with moderate values of flow, temperature, and pH (Figure 4b). In addition, species are positively associated with sites with higher substrate diversity and lower conductivity values (Figure 4b). Plecoptera, Odonata, and Trichoptera were mainly recorded in sites with higher flow and substrate diversity, while Gastropoda and Tricladida were in sites with lower substrate diversity and low to moderate water flow (Figure 4b). Siluriformes were recorded in sites with lower substrate diversity and low to moderate conductivity values (Figure 4b). Regarding the troglomorphic species, *Hyalella* sp. n. 8, *Girardia* sp. n. 1 and *Girardia* sp. 5 were found in lentic habitats, under rock blocks, and in the aphotic zones of the caves. The troglobitic *Potamolithus* sp. 9 was found in habitats with low to moderate water flow, under rock blocks, and throughout all the cave zones.

Partitioning of beta diversity (turnover and nestedness)

When considering the species composition, we found a high dissimilarity among the caves (β_{SOR} = 0.78), most of which was explained by turnover (β_{SIM} = 0.67) (85.9%) and a small part by nestedness (β_{SNE} = 0.11) (14.1%).

Considering each pair of caves, the turnover explained most of the beta diversity (Figure

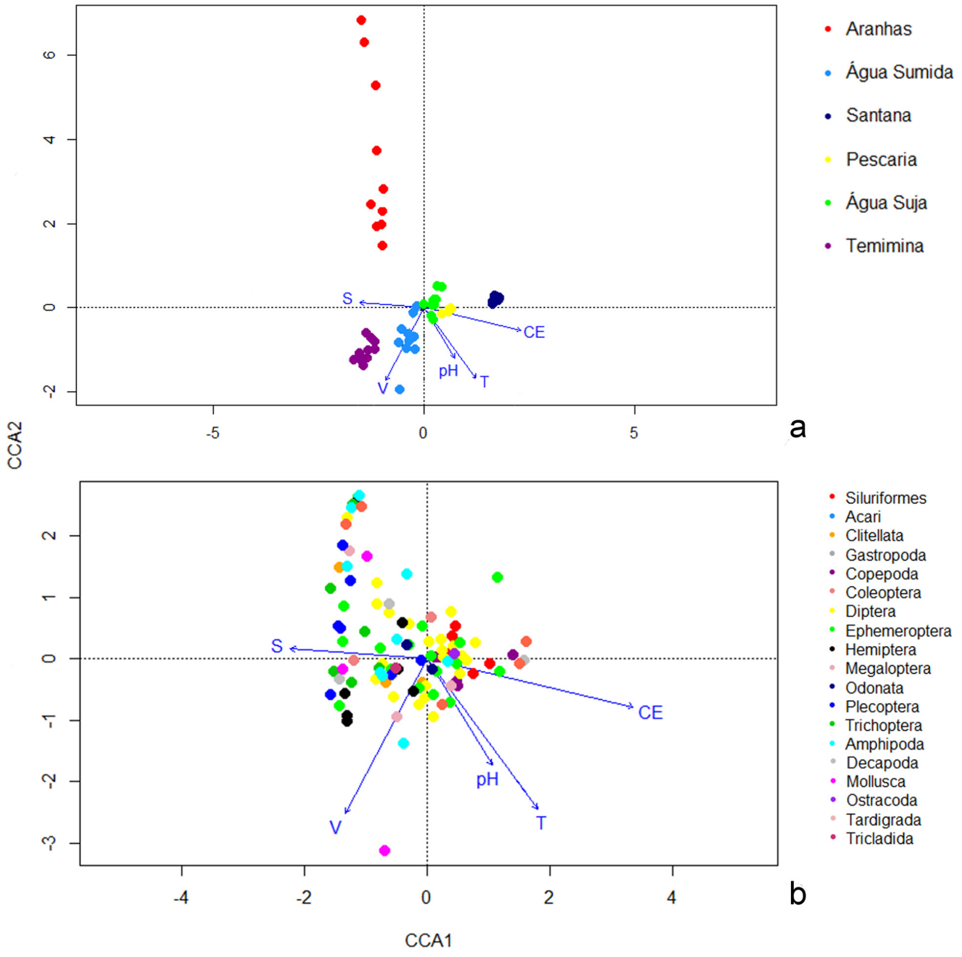
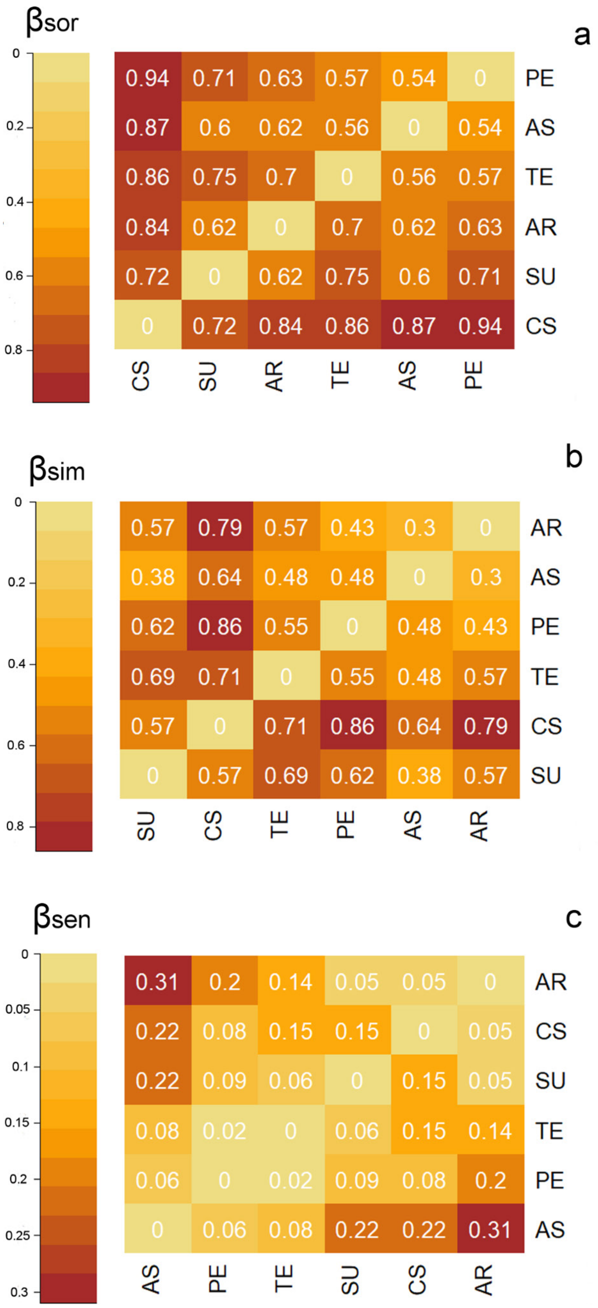


Figure 4. **Canonical Correspondence Analysis (CCA) diagrams for the abiotic variables in relation to (a) the sampling sites and (b) the species recorded in caves of the Parque Estadual Turístico do Alto Ribeira (PETAR), state of São Paulo, Brazil. Caption: abiotic variables: T = temperature, CE = conductivity, S = substrate, V = flow.**

5a-c). The total dissimilarity values (β_{sor}) and the contribution of turnover (β_{sim}) and nestedness (β_{sne}) to the beta diversity are in Figure 5a-c. In relation to the total dissimilarity (β_{sor}), Santana cave presented the most distinct composition, differing more from Pescaria, Água Sumida, Temimina, and Aranhas caves, and was more similar to Água Suja cave. The caves Água Sumida, Aranhas, and Temimina were the least dissimilar (Figure 5a). Considering the turnover (β_{sim}), Santana cave also presented the highest dissimilarity values in relation to Pescaria and Aranhas caves (Figure 5b). The dissimilarity due to nestedness (β_{sne}) contributed little to the difference in composition between the caves (Figure 5c).



CS	SU	AR	TE	AS	PE	
0.94	0.71	0.63	0.57	0.54	0	PE
0.87	0.6	0.62	0.56	0	0.54	AS
0.86	0.75	0.7	0	0.56	0.57	TE
0.84	0.62	0	0.7	0.62	0.63	AR
0.72	0	0.62	0.75	0.6	0.71	SU
0	0.72	0.84	0.86	0.87	0.94	CS

SU	CS	TE	PE	AS	AR	
0.57	0.79	0.57	0.43	0.3	0	AR
0.38	0.64	0.48	0.48	0	0.3	AS
0.62	0.86	0.55	0	0.48	0.43	PE
0.69	0.71	0	0.55	0.48	0.57	TE
0.57	0	0.71	0.86	0.64	0.79	CS
0	0.57	0.69	0.62	0.38	0.57	SU

AS	PE	TE	SU	CS	AR	
0.31	0.2	0.14	0.05	0.05	0	AR
0.22	0.08	0.15	0.15	0	0.05	CS
0.22	0.09	0.06	0	0.15	0.05	SU
0.08	0.02	0	0.06	0.15	0.14	TE
0.06	0	0.02	0.09	0.08	0.2	PE
0	0.06	0.08	0.22	0.22	0.31	AS

Figure 5. Partition of beta diversity (species composition) between each pair of caves in the Parque Estadual Turístico do Alto Ribeira (PETAR), state of São Paulo, Brazil. (a) Total dissimilarity (β_{sor}), (b) dissimilarity by turnover (β_{sim}), and (c) dissimilarity by nestedness (β_{sne}). Caves caption: CS = Santana, SU = Água Suja, AS = Água Sumida, AR = Aranhas, PE = Pescaria, TE = Temimina.

DISCUSSION

The subterranean aquatic fauna composition of the different sub-basins (Betari, Iporanga, and Pilões streams) is explained by the singularities found in the communities of each cave, as each one has exclusive taxa. Thereby, corroborating Trajano et al. (2016), the streams that flow through caves in the Alto Ribeira valley have their unique populations.

The Água Sumida (Iporanga) and Temimina (Pilões) caves were similar regarding environmental gradients, with higher flow and high species richness. The flow influences the transport of energy resources for the fauna and, therefore, an increase in water flow can lead to an increase in the richness (Allan & Castillo 2007). In addition, locations with continuous flow contribute to water oxygenation, influencing the occurrence of fauna according to the ecological characteristics of each species, such as functional food groups and oxygen demand (Collier 1994, Pellegrini et al. 2018).

The Temimina (Pilões) and Aranhas (Iporanga) caves showed a positive relationship between the number of species and substrate diversity. Locations with higher substrates diversity have higher environmental heterogeneity, offering different microhabitats, shelters, and food resources (such as accumulation of vegetal organic matter that are an important source of nutrients in the subterranean habitats), where different groups of insects can establish (Palmer et al. 2010, Pellegrini et al. 2018), such as Plecoptera, Trichoptera, and Odonata.

Gastropods of the genus *Potamolithus* and free-living flatworms such as planarians (Tricladida) were found under rock blocks and mainly in sites with low to moderate water flow. Bichuette & Trajano (2003) observed that the spatial distribution of *Potamolithus* gastropods is limited by some environmental conditions; these animals are not found in places with strong flow or lacking substrates for fixation (such as rocks). The environmental conditions in which we recorded the *Potamolithus* corroborate Bichuette & Trajano (2003). Furthermore, the presence of shelters, such as rock blocks, is also important in determining the richness of aquatic invertebrates, as they provide a higher surface area for colonization and refuge (Palmer et al. 2010). This was also observed in quartzitic caves in Minas Gerais, Brazil, where most of the substrate consisted of exposed matrix rock and, therefore, the presence of shelters was a key factor for the occurrence of aquatic insects (Pellegrini et al. 2018).

In the studied PETAR caves, except the Santana, most of the taxa were related to lower conductivity values, contradicting what was observed in limestone caves in the state of Goiás, Brazil (Simões et al. 2013). The Santana cave presented higher conductivity values and lower species richness. This cave has a higher extension when compared to the other studied caves and, consequently, the stream that runs through its interior remains in contact with the limestone for longer. As higher values of conductivity and pH may be due to the limestone dissolution process that provides calcium ions to the water (Gillieson 2009, Taylor & Ferreira 2012), the higher extension of the Santana cave explains the observed conductivity values. Therefore, the extension of the caves can influence the physical and chemical variables of their streams, which, in turn, influence the fauna composition.

Thus, we observed that different sets of physical and chemical variables influenced the communities in different caves of PETAR. In the study by Pellegrini et al. (2018), the community of each cave was also influenced by a unique set of physical and chemical factors. This emphasizes that the occurrence of fauna in specific microhabitats depends on the ecological characteristics of each species (Collier 1994) and that different abiotic variables contribute to the fauna differences between caves.

The troglobitic and troglomorphic species recorded in PETAR caves occurred in habitats with similar abiotic characteristics where troglophilic species of the same genera were found. However, the troglomorphic *Hyalella* and *Girardia*. occurred exclusively in aphotic zones and specific sites in the caves. This might be related to environmental stress intolerance of troglobites since they have evolved in a relatively stable environment (Culver 1982, Trajano 2000). Therefore, troglobites are considered fragile due to their susceptibility to climatic fluctuations (Trajano 2000). Furthermore, many troglobites have small geographic ranges and low population densities, resulting in small population sizes (Culver 1982, Trajano 2000), as observed for *Hyalella* sp. n. 8, *Girardia* sp. n. 1 and *Girardia* sp. 5

Considering the entire aquatic community (troglobites and non-troglobites), turnover is the main component of beta diversity in the studied

PETAR caves. Most of the beta diversity was also explained by the turnover component in a study carried out with obligate subterranean copepod communities present in the epikarst of karst areas in Slovenia (Pipan et al. 2018). However, unlike what we observed, the nestedness component had a significant contribution when considering inter-drip and inter-cave scales, as few rich drips largely determined the species diversity (Pipan et al. 2018). Our study differs from that observed by Fattorini et al. (2016) for subterranean copepod communities in water springs from different karst areas in Italy. These authors observed that turnover was the main component of beta diversity when considering only troglobitic species, and nestedness was the main component for non-troglobitic species. Fattorini et al. (2016) discussed that troglobitic species have restricted distributions due to historical factors (survivors of ancient lineages that became extinct in surface water bodies) or as a result of the fragmentation of a wider original distribution.

Patterns of spatial replacement (turnover) suggest the existence of any barrier or selective differentiation between fauna (Baselga 2010). This could be the case for the studied area, for which paleoclimatic models explained the diversity of troglobites (Trajano 1995, Trajano & Britski 1998, Fernandes et al. 2013, Bichuette & Trajano 2018). According to such models, the interglacial phases would allow the colonization of caves and the establishment of troglophilic populations, while the dry glacial phases resulted in the replacement of forests by open vegetation and in the interruption of epigean drainage, isolating these troglophilic populations in the subterranean environment (Trajano 1995, Trajano & Britski 1998).

The geology of the area where the caves are located also influences the diversity of their communities whether the colonization or dispersion in subterranean routes is facilitated (Barr 1967, Dole-Olivier et al. 2009). At PETAR, insoluble rocks such as phyllites are interspersed with limestone outcrops (Karmann & Sánchez 1979), which probably represent barriers and limit subterranean dispersion. These barriers could facilitate the process of isolation and speciation, as observed for troglobitic species of *Aegla* crabs and *Potamolithus* gastropods that occur in the region (Fernandes et al. 2013, Bichuette & Trajano 2018), and lead to a higher number of endemic species with limited distribution ranges (Barr 1967, Barr & Holsinger 1985, Fernandes et al. 2013). This could also explain the restricted distribution of other troglobitic species found in the studied caves (*e.g.*, *Hyalella* sp. n. 8 and *Girardia* sp. n. 1).

In addition to hydrogeology, the limited dispersal capacity is another factor that can contribute to the restricted distribution of species (Bichuette & Trajano 2018). For example, the small size and slow locomotion of troglobitic and troglophilic *Potamolithus* from the Alto Ribeira region favor the isolation in confined areas (Bichuette & Trajano 2018). The restricted distributions of these gastropods to micro-basins and/or caves (Bichuette & Trajano 2003, 2018) can also be explained by habitat preferences (see above discussion on environmental variables). Conversely, troglophilic species with higher dispersal capacity could access different limestone outcrops through epigean routes and/or could be distributed in caves located in different sub-basins, as observed for *Aegla schmitti* (Fernandes et al. 2013), which was found in different caves in our study. Moreover, according to Fattorini et al. (2016), non-troglobitic species show distribution patterns mainly influenced by ecological constraints at present.

The mosaic distribution of different character states in the different troglobitic and troglophilic species of *Potamolithus* observed by Bichuette & Trajano (2018), combined with the high degree of habitat fragmentation, provides evidence of an independent origin of these species from one or more unknown epigean ancestors that live on some of the main tributaries of the Alto Ribeira. According to the authors, these ancestral populations would have colonized upstream micro-basins and possibly isolated in epigean headwaters during the dry phases of paleoclimatic cycles, originating some troglophilic species. These species established populations in subterranean habitats and, later, isolated in this realm they speciated as troglobites (Bichuette & Trajano 2018).

Therefore, the troglophilic populations that we recorded could have colonized the upstream streams, and species with low dispersal capacity could be not able to access other streams from other sub-basins. Such factors allied to the environmental preferences and tolerances of each taxon would explain the high contribution of the turnover component in the beta diversity. Thus, our findings suggest the restricted distribution observed for troglobitic and troglophilic *Potamolithus* seems to reflect the distribution of cave communities in PETAR. In addition, in the case of species of uncertain classification, most of which the juveniles are aquatic and the adults are terrestrial winged, and taxa with greater dispersal capacity, our findings suggest that restrictions to certain caves could be due to specific abiotic conditions and resources, and habitat preference, i.e., to local and current ecological factors (Fattorini et al. 2016).

Our results corroborate the premise that conservation efforts should target multiple communities (in this case, caves), when the turnover component has a greater contribution to their beta diversity, instead of focusing only on the richest communities (Baselga 2010). In addition, the Alto Ribeira karst area is an area of high troglobite diversity (Trajano et al. 2016), where the caves have highly dissimilar communities. Therefore, impacts on a single cave could lead to a high biodiversity loss.

The protection of subterranean fauna depends on several actions besides the maintenance of caves in conservation units, such as fauna management and monitoring. Efforts must also be made to protect the entire influence area of the caves, such as drainage recharge regions (Trajano et al. 2012). Such actions are urgent, as the catchment basins and part of subterranean systems are located outside the boundaries of PETAR (Karmann & Ferrari 2002), and pollution in aquatic and terrestrial environments has already been identified in the region (Moraes et al. 2002). However, such actions have not yet been properly implemented, since the PETAR Speleological Management Plan, carried out between 2008 and 2009 (Fundação Florestal 2010), was only approved in 2018 with the publication of Resolution No. 57 of the Secretaria do Meio Ambiente do Estado de São Paulo, which establishes the rules and guidelines for internal and buffer zones of the park (São Paulo 2018).

CONCLUSION

Subterranean aquatic communities can have many endemic species and/ or species with restricted distribution, even in caves present in the same sub-basin. Furthermore, communities from different caves can be influenced by different physical and chemical variables, as the occurrence of fauna in specific microhabitats depends on the ecological characteristics of each species.

The dissimilarity between cave communities from different sub-basins within a single karst area, considering troglobitic and non-troglobitic species, was mainly due to the turnover.

Our findings suggest this is influenced by ecological (e.g., habitat preference, tolerance to environmental conditions, dispersal capacity), hydrogeological (e.g., isolation between sub-basins, dispersal barriers), and historical (e.g., colonization, paleoclimatic events) factors and processes. This high dissimilarity observed between PETAR caves shows that their communities are unique, and conservation efforts are needed for their effective protection.

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SUPPLEMENTARY MATERIAL

Appendix S1.

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TZ contributed to the study conception and design, data collection, analysis, and interpretation, manuscript preparation, and critical revision, adding intellectual content. MEB contributed to the study conception and design, data collection, analysis, and interpretation, manuscript preparation, and critical revision, adding intellectual content.