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IS AN OUTSTANDING ENVIRONMENT ALWAYS PRESERVED? WHEN THE MOST DIVERSE CAVE IN SUBTERRANEAN SPECIES BECOMES ONE OF THE MOST ENDANGERED IN A LANDSCAPE.

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ABSTRACT

In this paper, we present unprecedented data sampled since 2010 from the richest cave regarding troglobitic fauna from the Arcos-Pains-Doresópolis (APD) Brazilian speleological province, the Éden limestone cave. Therefore, we assessed the cave biodiversity in dry and wet periods to highlight troglobites and troglophiles terrestrial and aquatic (stream and epikarst) fauna, besides of qualifying human activities that can directly or indirectly affect the cave. We also included here data gather from Zampaulo thesis (2010) that used the same sampling methods. The Éden cave harbor at least 93 troglophilic invertebrates species belonging to 66 families, being Araneae (23 spp.), Diptera (13 spp.), Coleoptera (10 spp.), Acari (7 spp.), Hymenoptera (7 spp.) and Collembola (4 spp.) the richest taxa. Furthermore, 13 invertebrates species with troglomorphic traits were found: *Paratricommatus* sp. (Opiliones, Cryptogeobiidae), Chthoniidae sp. (Pseudoscorpiones, Chthoniidae), two Styloniscidae spp. (Isopoda), Blattellidae sp. (Blattodea), *Arrhopalites* sp. (Collembola, Arrhopalitidae), three Carabidae spp. (Coleoptera), Symphyla sp., *Pseudonannolene ambuatinga* (Spirostreptida, Pseudonannolenidae), Sphaerodesmidae sp. (Polydesmida) and Harpacticoida sp (Crustacea). The cave harbor at least 16,25% of local biodiversity of obligate cave fauna from the APD province. The invertebrate fauna showed distinct richness and abundance values according to upper and lower cave levels. The lower level features a higher number of species when compared to the upper level, probably due to the stream presence and high humidity. The cave is inserted in a drainage basin with intense agricultural, pastures, mining and urban activities. Therefore, the main concern is that conservation will not only depend on actions focused on the cave, but should also focus on the intense social and economic conflicts in the small drainage basin.

KEY WORDS: Invertebrates, diversity; cave fauna; endemic species; troglobites; conservation

1. INTRODUCTION

Biodiversity loss and species extinction far exceed conservation resources around the globe and the perspective is getting increasingly worse (Wilson 1992, Amano and Sutherland 2013). It has been imperative to identify priorities to conservation in order to protect endangered caves with a significant number of unique and threatened species (Souza-Silva et al. 2015). The delimitation of biodiversity *Hotspots*, which includes areas with outstanding concentration of endemic species subject to exceptional habitat loss, has shown to be an important strategy (Myers et al. 2000, Culver and Sket 2000, Culver and Pipan 2009). Karst systems are notably vulnerable and fragile to human impacts compared to most other natural ecosystems. The efficient surface drainage down to an array of subterranean conduits transmits the surface pollution in an incredible speed. Human activities undertaken upstream of karst terrains are liable to lead to major environmental degradation (Ford 2004). The rising concern with global biodiversity contributed to produce relevant information concerning species diversity patterns, also including different groups of cave organisms (Culver and Sket 2000).

Extensive studies on subterranean biodiversity are unevenly distributed with most part of the data from northern temperate karst areas. Culver and Sket (2000) working with patterns of subterranean biodiversity introduced the concept of *Hotspots of subterranean biodiversity*. They identified 20 subterranean systems (18 caves and two wells) that matched their criteria (20 or more obligate subterranean species per site). These systems are mainly distributed in temperate regions (five in Slovenia, five in France, three in North America, one in Australia, one in South East Asia and one in an Atlantic island) (Culver and Sket 2000). Culver and Pipan (2009) added another 16 hotspots of subterranean biodiversity to those 20 previously defined by Culver and Sket (2000) and Souza-Silva and Ferreira (2016) described the first two hotspots of subterranean biodiversity in South America. However, since tropical subterranean habitats are not as extensively studied as temperate ones, many other hotspots may exist in South America. Furthermore, studies regarding subterranean biodiversity in Brazil are still quite fragmented, and most part of the sampling efforts were historically concentrated in some regions of the country (Souza-Silva et al. 2015). Some of these areas deserve special attention due to their biodiversity and number of obligate cave species: Alto do Ribeira State Park, Intervales State Park, Peruaçu National Park, Serra da Bodoquena National Park, Arcos-Pains-Doresópolis speleological province and scattered karst areas located at the northeastern part of the country (Trajano and Bichuette 2010, Souza-Silva et al. 2015, Souza-Silva and Ferreira 2016).

Subterranean habitats are notably detached with distinct species occurring in caves separated only by a few kilometers. In many cases, the number of troglobites listed in a single cave is not incredibly high (less than 20 species) but when the entire region is considered, this value can increase significantly (Culver and Sket 2000). In fact, great part of the subterranean biodiversity described takes into account the sum of different species from adjacent sites. On the other hand, it is important not to neglect individual sites (e.g. single cave systems) as some of them show outstanding biodiversity and singularities (Souza-Silva and Ferreira 2016). The protection of subterranean habitats and karst landscapes may start with the protection of a single cave, which can constitute an important step for preserving larger karst areas (Culver and Sket 2000, Ford 2004, Souza-Silva et al. 2015).

The identification of key karstic areas as well as very important caves regarding subterranean biodiversity is essential to assure the protection of a variety of endemic and threatened species (Souza-Silva et al. 2015). Therewith, the purpose of this paper is to analyze the subterranean biodiversity in aquatic and terrestrial compartments inside one single limestone cave in Brazil and characterize human activities and impacts inside and in the surroundings to highlight their importance to the conservation of the local subterranean biodiversity facing different threats.

2. MATERIAL AND METHODS

2.1. Study site

The fieldwork was performed in the Éden cave (20.384577; 45.666798; 712 m asl) during two sampling events (dry and rainy periods). The cave is inserted in limestone outcrops of the Bambui geological group and is located near the town of Pains, Minas Gerais state, Brazil (Fig. 1). The area is an important Brazilian speleological province (Arcos-Pains-Doresópolis - APD) with more than 2500 known caves. This number represents approximately 32% of all known caves in the Minas Gerais state (Cecav 2017). The region is characterized by warm and humid summers and dry winters with temperatures ranging from 23.3ºC in January (hottest) to 16.3ºC in July (coldest) with an annual average temperature of 20.7ºC and local annual average precipitation of 1344 mm³ (Menegasse et al. 2002). Most part of the native vegetation in the region has been removed and replaced by monocultures (especially *Eucalyptus*) and pastures. The original phytophysiognomy was a deciduous seasonal forest, currently confined to areas of limestone outcrops (Alves de Melo et al. 2013).

The Éden cave is one of the largest known caves in this region with approximately 1.931 meters long of linear development (Fig. 1). The cave comprises a wide dissolution gallery, conditioned by North/South lineaments with linear morphology. It consists of a lower level trespassed by a stream and an upper level without stream (Cadamuro 2007). During the rainy period a big amount of water can move from the epikarst compartments to the upper level through speleothems and fractures (Fig. 2). The cave has only one natural entrance, a small skylight that gives access to the lower level and an artificial opening resulted from past mining activities. This artificial entrance is now closed by an iron gate.

The cave can be classified as a "pecilotrophic" system due to its considerable size and the noticeable shifts in the amount of organic resources available in different cave compartments. However, great part of the cavity is considered "oligotrophic" (upper level) showing a striking absence of coarse particulate organic matter (CPOM) on both dry and rainy periods. At the upper level, during the rainy period, the water dripping from the ceiling and speleothems can probably transport dissolved organic carbon (DOC) to this cave compartment. The cave's lower level, receives coarse and fine particulate organic matter carried by the stream such as leaves, wood debris and carcasses. The skylight located at a small separate chamber in this level also contributes to the carbon input with the falling of leaves and branches, although this contribution is restricted to the area near this entrance.

2.2. Field procedures

2.2.1. Invertebrates sampling events in terrestrial habitats

We surveyed the cave's invertebrate fauna in the rainy (January) and dry (July) periods of 2012. An intense search was carried out at the cave floor and walls with special attention to potential biotopes. The invertebrates were captured with the aid of fine brushes and tweezers and kept in 70% ethanol vials (Sharrat et al. 2000, Ferreira 2004, Souza-Silva et al. 2011). Previously known species had their abundance estimated by visual counts in field, avoiding unnecessary sampling and their position plotted in the cave´s floor plan (Ferreira, 2004, Bento et al. 2016). It is important to highlight that pitfall traps were not installed in the Éden cave, this method is considered to have low efficiency when applied to most tropical caves and can represent a threat to fragile troglobiotic populations (Weinstein and Slaney 1995).

2.2.2. Invertebrates sampling events in epikarstic

We installed “epikarstic” traps underneath ceiling and speleothem drips in order to sample the invertebrates that inhabit the cave “epikarst” (Pipan and Culver 2013). The traps consisted of 500ml plastic vials and one bucket in which we opened horizontal slits and attached a fine mash in order to drain the water and retain the organisms that came from the epikarst during the rainy period at the upper level only (Fig. 2). The traps remained in the cave for a period of three months. The water retained inside the traps, was filtered with a zooplankton net, stained with rose bengal and stored in 70% ethanol plastic vials. We also surveyed all water filled pools and travertines for aquatic fauna.

2.2.3. Invertebrates sampling events in the subterranean stream

We sampled the benthic fauna during the dry period of 2012 in triplicates using surber nets (bottom area = 300mm x 300mm; 250µm mesh size). The cave´s lower conduit becomes flooded during strong rains, very common in the rainy period. We selected three sampling transects equally distributed along the cave stream (upstream-midstream-downstream). Three sub-samples were taken of each transect. Samples were preserved in plastic vials containing 70% ethanol and taken to the laboratory for sorting and identification.

2.2.4. Vertebrates sampling events and other records

We sampled the cave´s fish fauna during the dry period only. We utilized kicking nets (4x2m 5mm mash size) and seines (80cm diameter; 1mm mash size) to capture the fish in an upstream direction. All specimens were fixed in 10% formalin and latter preserved in 70% ethanol. The specimens were identified and lodged at the Laboratory of fish Ecology, Ecology Department of Lavras Federal University, Minas Gerais State, Brazil. We did not use any specific method for amphibians, reptiles and mammals, but we photographed such organisms when they were visualized inside the cave.

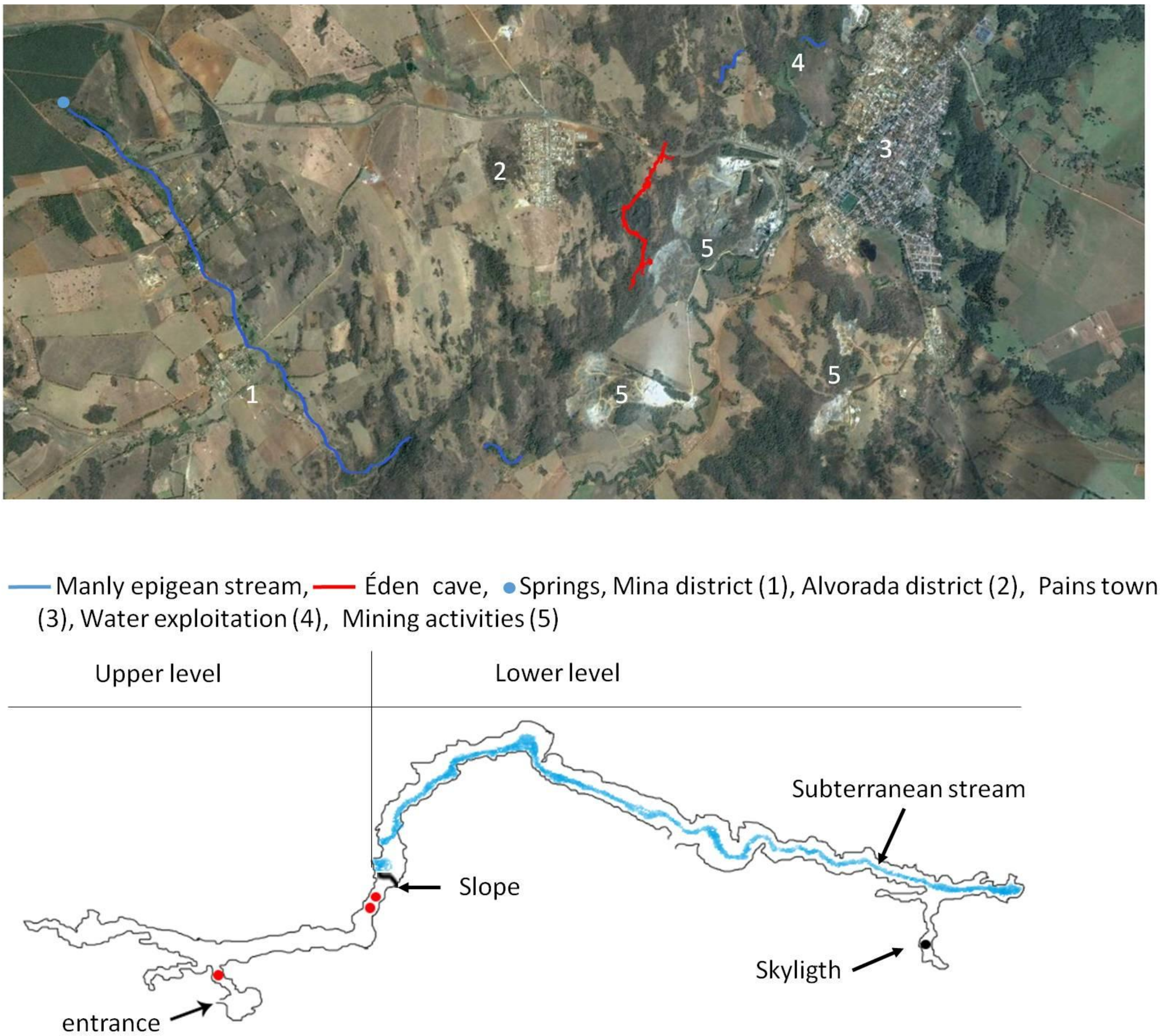

Figure 1. Aerial view from Éden cave surroundings, Pains, MG, Brazil, (year 2012) showing some human activities and Éden cave map with entrances, epikarst sampling points (red dots) skylight (black dot) and lower and upper levels delimitation

2.3. Laboratory procedures

All the invertebrate sampled in terrestrial and aquatic compartments were sorted in the laboratory under a stereomicroscope and identified to the lowest possible taxonomic level and separated into "morphotypes" with the aid of taxonomic keys and were lodged in the collection of invertebrates of the Subterranean Biology Study Center, Biology Department, General Zoology Section, Federal University of Lavras (ISLA), Center of studies on Subterranean Biology, Minas Gerais State, Brazil.

2.4. Data Analyses

We characterized the different cave compartments (lower level with a stream - and upper level without stream) and seasons (rainy - R and dry periods - D) using the richness (S), diversity (Shannon-Wiener), dominance, e evenness. In addition, the total species richness found in the cave was determined (by means of the sum of the species observed in the dry in the rainy season). The Bray-Curtis similarity index (Magurran 2004) was calculated in order to compare the communities found in each season and in each cave compartment. Finally, Beta diversity (turnover or β) was calculated using data of presence and absence, through the index of Harrison (1992), modified by Whittaker

(1960), in order to compare samples of different sizes. *β*Harrison = {[(S/a) - 1]/(N - 1)}9100). Where S = total species richness values, a = average richness values and N = number of samples. This measure ranges from 0 (no turnover) to 100 (each sample has a unique set of species) (Koleff et al. 2003). It was assumed here that ecologically stable cave systems present lower species exchanges over time and consequently lower values of *β* diversity.

The average richness of the high taxa sampled in terrestrial microhabitats, was compared between upper and lower levels of the cave and between both seasons, by average of T test for independent samples (Zar 1984). Finally, the composition and richness of the invertebrate fauna collected in the cave (excepting the epikarst fauna) were compared to those collected by Zampaulo in august 2009 (Zampaulo 2010), who used the same methodology for collecting fauna during the rainy season (Appendix I).

3. RESULTS

3.1. Invertebrates composition, abundance and richness

We sampled 107 invertebrates species belonging to at least 30 high taxa (and at least 66 families) in terrestrial and aquatic compartments of the Éden cave during dry and rainy periods belonging to at least 66 families (Appendix I, Fig. 2). The richer groups were Araneae (23 spp.), Diptera (13 spp.) and Coleoptera (10 spp.). Other groups found are Acari (7 spp.), Hymenoptera (7 spp), Collembola (4 spp.), Gastropoda (4 spp.), Ensifera (3 spp.), Opiliones (3 spp.), Auchenorrhyncha (2 spp), Polydesmida (3 spp.), Turbellaria (3 spp.), Blattodea (2 spp.), Lepidoptera (2 spp.), Oligochaeta (2 spp.), Psocoptera (3 spp.), Isopoda (2 spp.), Spirostreptida (2 spp.), Copepoda, Trichoptera, Megaloptera, Odonata, Ephemeroptera, Sternorrhyncha, Palpigradi, Pseudoscorpiones, Schizomida, Spirobolida, Symphyla and Lithobiomorpha, the last groups with one species each.

Regarding abundance, we counted 8,815 specimens distributed in Collembola (3,347 specimens), Acari (1,362), Ensifera (1,074), Blattodea (555), Coleoptera (445), Spirostreptida (431), Opiliones (285), Lepidoptera (241), Symphyla (164), Oligochaeta (155), Turbellaria (126), Diptera (100), Gastropoda (98), Araneae (90), Psocoptera (86), Hymenoptera (84), Isopoda (80), Megaloptera (20), Auchenorrhyncha (14), Pseudoscorpiones (14), Ephemeroptera (9), Spirobolida (9), Palpigradi (7), Polydesmida (5), Trichoptera (5), Copepoda (4), Lithobiomorpha (2), Odonata (1), Schizomida (1) and Sternorrhyncha (1) (Fig. 2).

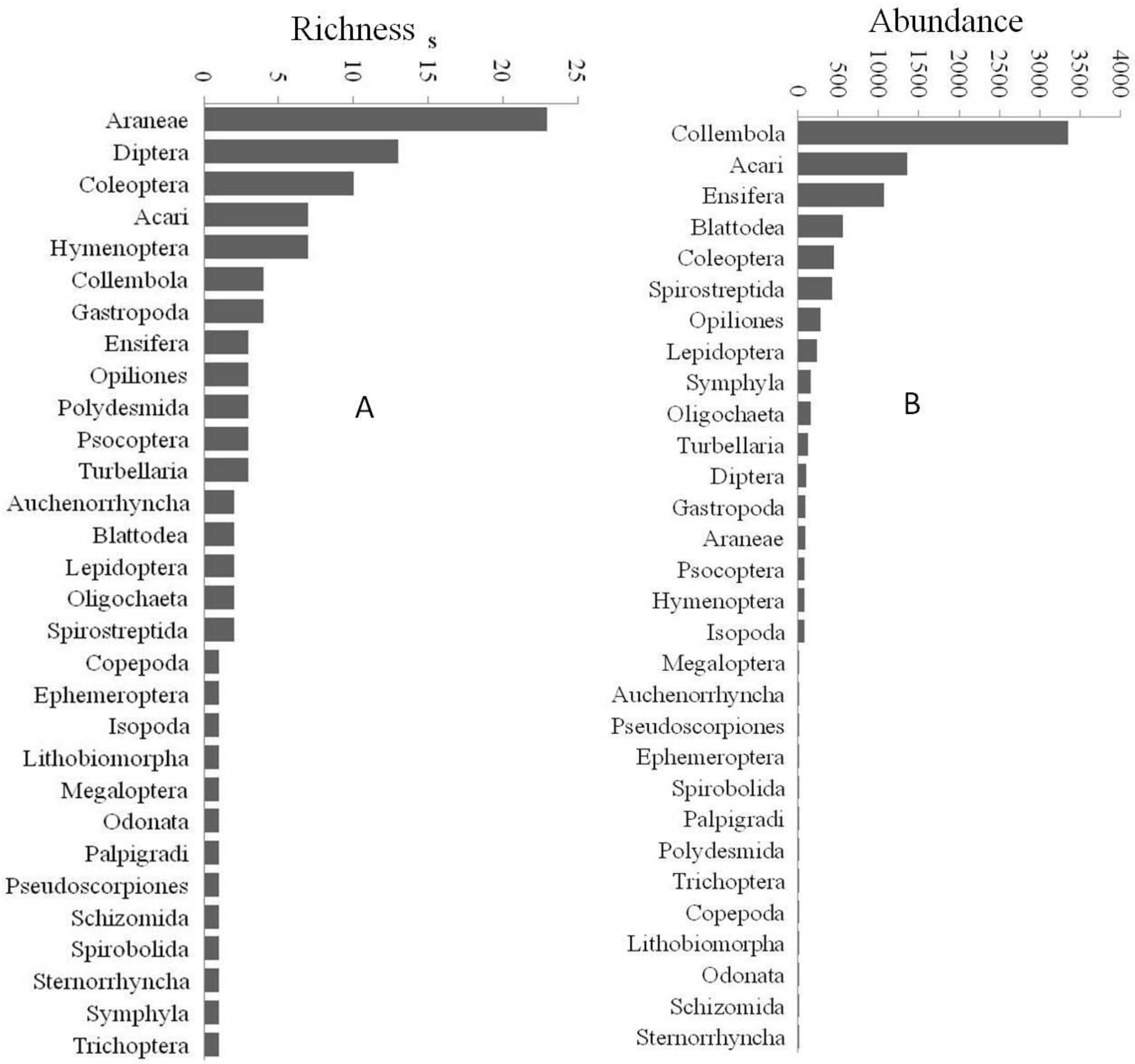

Figure 2. Richness and abundance of invertebrates high taxa sampled in terrestrial and aquatic compartments of the Éden cave during dry and Rainy periods, Pains, MG, Brazil.

3.2. Invertebrates species with troglomorphic traits

Among all sampled invertebrates species, we found 13 species with troglomorphic traits (Fig. 3): one species of Opiliones (*Paratricommatus* sp. - Cryptogeobiidae), one of the Pseudoscorpiones (Chthoniidae sp.), two Isopods species (Styloniscidae spp.), one Blattellidae species (Blattodea), one Collembola species (Arrhopalitidae *Arrhopalites* sp.), three Coleoptera species (Carabidae spp.), one species of the Symphyla, one species of the Spirostreptida (*Pseudonannolene ambuatinga*, Iniesta and Ferreira 2013 - Pseudonannolenidae), one Sphaeriodesmidae (Polydesmida) and one Copepoda (Harpacticoida) collected from epikarstic compartments. Furthermore, the cave shelters a variety of phylogenetically distinct obligate subterranean species that, in some cases, can present very large populations: *Arrhopalites* sp. (1,306 specimens); Blattellidae sp. (373 specimens); *Pseudonannolene ambuatinga* (159 specimens); *Paratricommatus* sp (110 specimens).

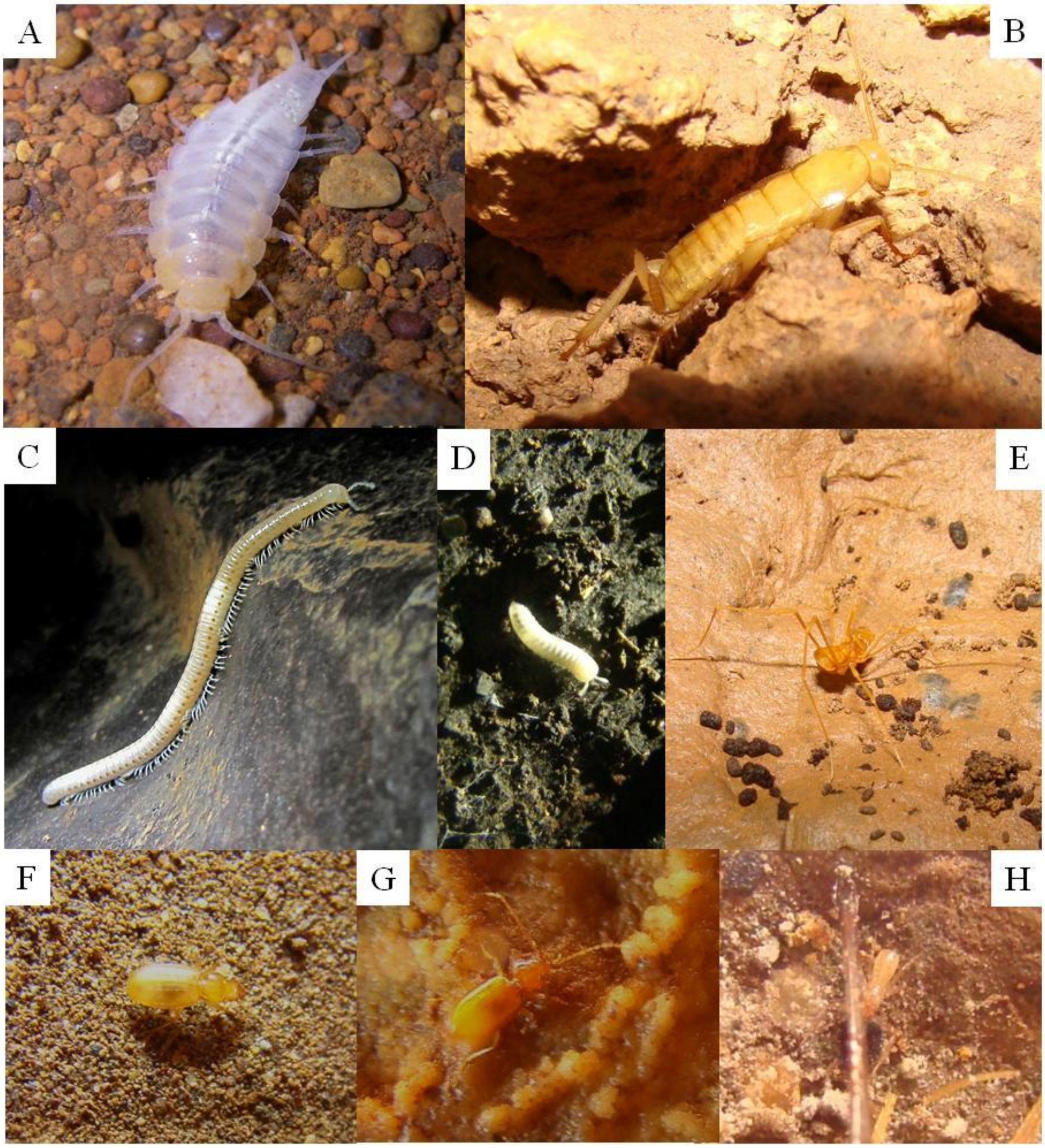

Figure 3. Some obligate cave species: (A) Isopoda (Styloniscidae); (B) Blattodea; (C) Spirostreptida (*Pseudonannolene ambuatinga*); (D) Sphaeriodesmidae; (E) Opiliones (*Paratricommatus* sp.); (F) Coleoptera (Carabidae); (G) Coleoptera (Carabidae – second species); and (H) Chtoniidae (Pseudoscorpiones), sampled in Éden cave.

3.3. Vertebrate's composition and richness

We also recorded eight fish species inside the cave: *Astyanax lacustris* (Characidae), *Astyanax fasciatus* (Characidae), *Hemigrammus marginatus* (Characidae), *Imparfinis minutus* (Heptapteridae), *Pimelodella lateristriga* (Pimelodidae), *Pseudopimelodus charus* (Pseudopimelodidae), *Hoplias malabaricus* (Erythrinidae) and *Hypostomus lima* (Loricariidae). Furthermore, two species of Anura were recorded: *Scinax fuscovarius* (Hylidae) and *Leptodactylus* sp (Leptodactylidae). A single species of bat, *Carollia perspicillata* (Phyllostomidae) was recorded during all visits to the cave.

3.4. Epikarst invertebrate fauna

The epikarst traps revealed the presence of copepods (Harpacticoida). Only four specimens of Harparticoida were sampled in drip water that pass-through speleothems during rainy periods. However, the Styloniscidae isopods also seems to use the epikarst compartments, since they are abundant during rainy periods (they migrate to the macrocaves spaces, probably searching for food), but are rare during dry periods, thus indicating they migrate to the epikast during this season when the travertine pools inside the cave are dry (Appendix I).

3.5. Invertebrate in the subterranean stream

The subterranean stream features a very low aquatic invertebrate diversity. We collected a total of 10 species of aquatic invertebrates in the cave stream: four species of Gastropoda, two species of Diptera (Chironomidae and Stratiomyidae), one species of Trichoptera (Hydropsychidae), one species of Oligochaeta, one species of Odonata (Ghomphidae) and one species of Coleoptera (Scirtidae).

3.6. Invertebrates sampled by Zampaulo 2010: composition and richness

Zampaulo (2010) observed, in a single collection performed in august 2009 (dry period), 78 invertebrate species belonging to at least 42 families from Coleoptera (14 spp.), Araneae (10 spp.), Collembola (10 spp.), Dipter (5 spp.), Orthoptera (4 spp), Hemiptera (3 spp.), Mollusca (3 spp.), Opiliones (3 spp.), Trichoptera (3 spp.), Blattodea (2 spp.), Hymenoptera (2 spp), Megaloptera (2 spp.), Acari (4 spp) and Anellida, Isopoda, Ephemeroptera, Lepidoptera, Lithobiomorpha, Odonata, Palpigradi, Polydesmida, Pseudoscorpiones, Psocoptera, Spirostreptida, Symphila, Turbellaria one espécie cada (Appendix I). Furthermore, Zampaulo (2010) found 8 troglomorphic species: Styloniscidae sp., Trichomatinae sp. (in fact, *Paratricommatus*, sp.), *Arrhophalites* sp., Blattodea sp. Carabidae spp. (two species), Pselaphinae sp., and Spirostreptida sp. However, the Pselaphinae, in fact, do not represent a troglobitic species.

3.7. Temporal and Spatial distribution of the invertebrate communities

During the rainy period, we found 66 invertebrate species, 50 species occurring at the lower level and 30 at the upper level. Twenty-five species occurred in both lower and upper level in this period. During the dry period, 77 species were found, 64 occurring at the lower level and 25 at the upper level. Nineteen species occurred in both lower and upper level in this period. The invertebrate fauna showed distinct richness and abundance values per cave “compartments” with the lower level featuring a higher number of species when compared to the upper level (Fig. 4).

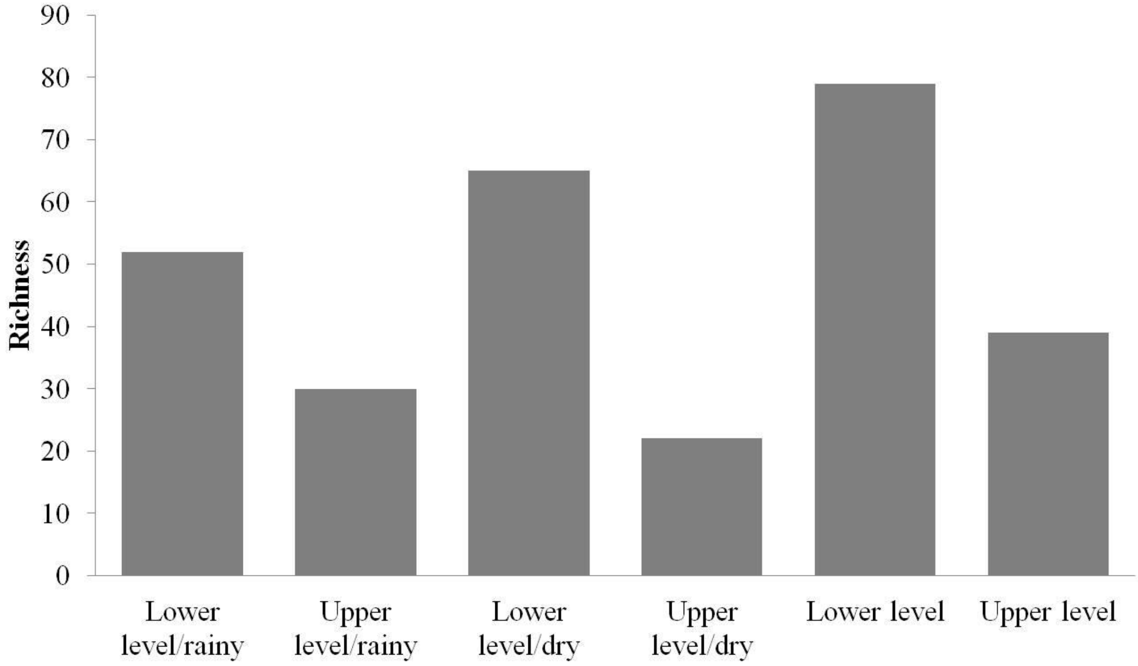

Figure 4. Absolute species richness during rainy period at lower level and upper level and dry period at lower level and upper level and lower and upper levels in Éden Cave, Pains, MG, Brazil.

The taxa Oligochaeta, Turbellaria, Gastropoda, Acari, Ephemeroptera, Polydesmida and Symphyla, were only collected at the lower level of the cave. On the other hand, Palpigradi, Hemiptera, Isopoda, Copepoda (epikarst) and Schizomida were collected only at the upper level of the cave. Coleoptera and Hymenoptera were not collected at the upper level in the dry period (Fig. 5).

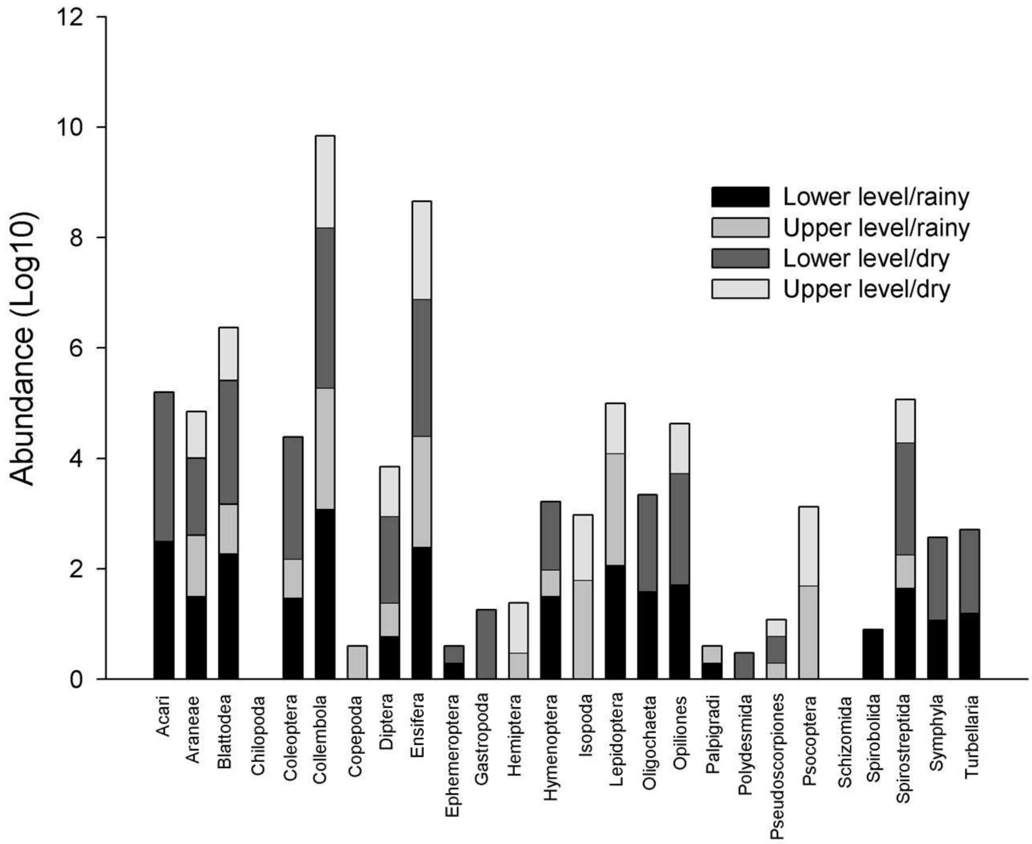

Figure 5. Absolute high taxa abundance during rainy and dry periods at lower and upper levels in Éden Cave, Pains, MG, Brazil.

The values of species richness, abundance, dominance and diversity varied seasonally and per cave levels (table 1). The lower level always presented the highest richness and absolute and average abundances of invertebrate fauna in both the dry and rainy periods (Figs 5 and 6). However, significant differences in high taxa richness and abundance were observed between lower and upper levels only during dry period (tables 2 and 3).

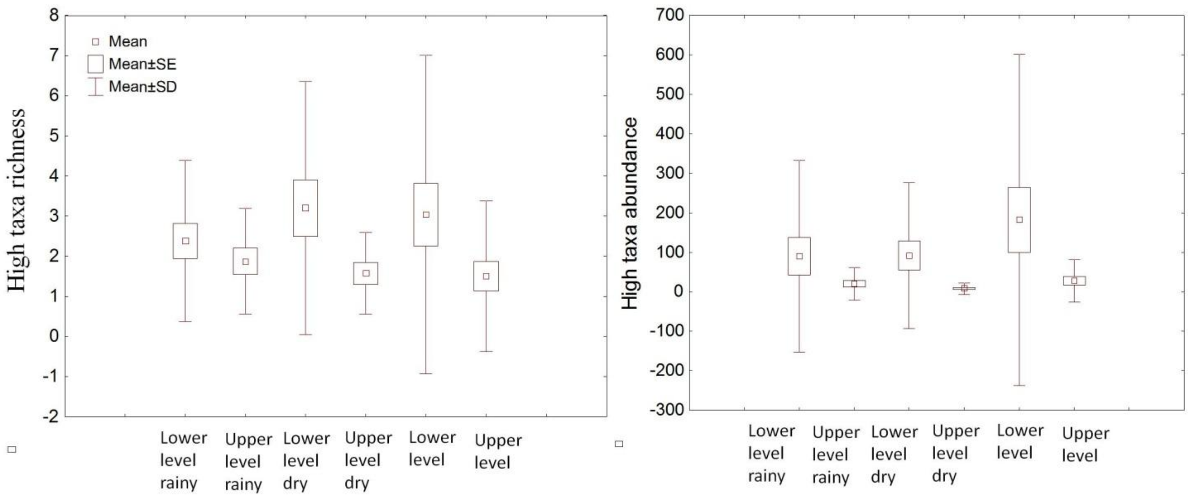

Figure 6. Average high taxa richness and abundance during rainy and dry periods at lower and upper levels in Éden Cave, Pains, MG, Brazil.

Table 1. Values of species richness, abundance, dominance and diversity during rainy and dry periods in lower and upper levels at Éden cave, Pains, MG, Brazil.

	Lower level/rainy	Upper level/rainy	Rainy period	Lower level/dry	Upper level/dry	Dry period
Richness	50	30	66	64	22	74
Abundance	2352	521	2873	2384	208	2592
Dominace	6.312	4.636	8.163	8.101	3.934	9.287
Diversity	0.8014	0.9492	0.9159	1.136	1.044	1.178

Table 2. T-test for high taxa richness between lower and upper levels, during dry and rainy periods in Éden cave, Pains, MG, Brazil. Variables were treated as independent samples. Significant diferences* p ≤ 0,05. Average (A), freedom of degree (Df), Standard deviation (Std.Dev).

Variables1 and 2	A1	A2	tvalue	Df- Value	P value	Cases1	Cases2	Std.Dev. 1	Std.Dev.2
Lower level rainy vs Upper level rainy	2,38	1,88	0,87	35,00	0,39	21,00	16,00	2,01	1,31
Lower level rainy vs Upper level dry	2,38	1,57	1,39	33,00	0,17	21,00	14,00	2,01	1,02
Lower level rainy vs Upper level	2,38	1,50	1,55	45,00	0,13	21,00	26,00	2,01	1,88
Lower level dry vs Upper level rainy	3,20	1,88	1,57	34,00	0,13	20,00	16,00	3,16	1,31
Lower level dry vs Upper level dry	3,20	1,57	1,86	32,00	0,07	20,00	14,00	3,16	1,02
Lower level dry vs Upper level*	**3,20**	**1,50**	**2,28**	**44,00**	**0,03**	**20,00**	**26,00**	**3,16**	**1,88**
Lower level vs Upper level rainy	3,04	1,88	1,13	40,00	0,26	26,00	16,00	3,96	1,31
Lower level vs Upper level dry	3,04	1,57	1,35	38,00	0,18	26,00	14,00	3,96	1,02
Lower level vs Upper level	3,04	1,50	1,79	50,00	0,08	26,00	26,00	3,96	1,88

Table 3. T-test for high taxa abundance between lower and upper levels, during dry and rainy periods in Éden cave, Pains, MG, Brazil. Variables were treated as independent samples. Significant diferences* p ≤ 0,05. Average (A), freedom of degree (Df), Standard deviation (Std.Dev).

Variables1 and 2	A1	A2	tvalue	Df-Value	P value	Cases1	Cases2	Std.Dev. 1	Std.Dev.2
Lower level rainy vs Upper level rainy	90,46	20,04	1,46	50,00	0,15	26,00	26,00	242,78	41,05
Lower level rainy vs Upper level dry	90,46	8,00	1,73	50,00	0,09	26,00	26,00	242,78	14,60
Lower level rainy vs Upper level	90,46	28,04	1,28	50,00	0,21	26,00	26,00	242,78	53,74
Lower level dry vs Upper level rainy	91,69	20,04	1,93	50,00	0,06	26,00	26,00	184,88	41,05
Lower level dry vs Upper level dry *	**91,69**	**8,00**	**2,30**	**50,00**	**0,03**	**26,00**	**26,00**	**184,88**	**14,60**
Lower level dry vs. Upper level	91,69	28,04	1,69	50,00	0,10	26,00	26,00	184,88	53,74
Lower level vs Upper level rainy	182,15	20,04	1,96	50,00	0,06	26,00	26,00	419,28	41,05
Lower level vs Upper level dry *	**182,15**	**8,00**	**2,12**	**50,00**	**0,04**	**26,00**	**26,00**	**419,28**	**14,60**
Lower level vs Upper level	182,15	28,04	1,86	50,00	0,07	26,00	26,00	419,28	53,74

3.8. Temporal and spatial similarity of the invertebrates cave fauna

The species composition also varied according to the cave level and season. We built a dendrogram based on the Bray-curtis similarity index to illustrate this variation (Fig. 7). There is a visible difference in faunal similarity between cave levels, as a result of a distinct distribution of species along the cave habitats. The dendrogram separated two major group categories based on the composition of the invertebrate fauna. The lower level further contributes to the overall cave richness and abundance being subject to a greater number of species substitutions.

The overall turnover of the cave communities between the lower and upper levels corresponded to 66%. In the rainy season (January 2012), the turnover of the cave between lower and upper conduits corresponded to 65%. In the dry season (June 2012) it accounted for 72%. The overall turnover of the cave communities between the dry and rainy periods corresponded to 40%.

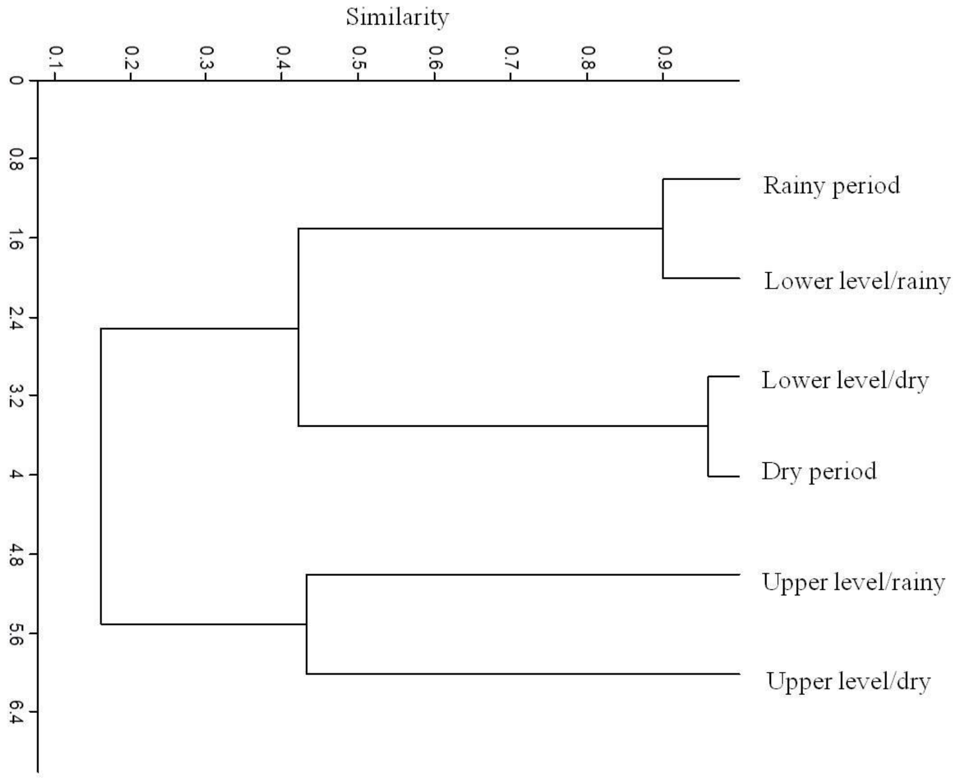

Figure 7. Faunal similarity dendrogram between lower and upper levels and rainy and dry periods in Éden Cave, Pains, MG, Brazil.

3.9. Human activities inside and surrounding the cave

Although we did not measure the environmental impacts in the Éden cave we could visually identify some damage caused by human activities. An artificial entrance and a gate was created by mining companies (Fig. 8A, B, C). At the upper level´s floor, there are collapsed speleothems along with big rock blocks due to the past use of explosives in the quarry. The subterranean stream conditions are worrying; we noticed a great amount of fine sediment deposited on the bottom of the stream, indicating intense silting through its course (Fig. 8D and E).

The surroundings of the cave are highly modified by human activities such as agriculture, pastures, urban expansion and past mining activities (Fig. 1). The cave is located within a mining area adjacent to the city of Pains (Fig. 1). A stream reaches the cave’s lower level through an upwelling and flows for approximately 700 meters until it reaches a sinkhole. Before entering the cave, the stream runs through an impacted watershed subject to different types of human activities. The watershed’s natural vegetation has been long removed. Pastures, agriculture and domestic sewage are among some of the most damaging activities affecting this water course.

Figure 8. Human alterations and consequent impacts inside and surroundings of the Éden cave. Artificial entrance closed by a gate (A), limestone exploitation by mining activities (B and C). Subterranean stream silting (D and C) because of human activities in the upstream drainage basin (deforestation, agropastoral and urban activities).

4. DISCUSSION

Despite the Arcos, Pains, Doresópolis (APD) province represent the highest concentration of caves in South America (with many troglobitic species recorded) and have received intense impacts historically, there are few publications regarding cave biology of the area. The first report on cave fauna for the APD province was made by Álvares and Ferreira (2002), which described the first troglobitic species for the region (the carabid beetle *Coarazuphium pains*). Latter, Ferreira (2004), in a study conducted in 63 caves, reported 18 obligate cave species. Zampaulo (2010) complemented the studies started by Ferreira (2004) and recorded 1,574 invertebrate species, from which 79 species presented troglomorphic traits. Other studies regarding cave ecology were performed in the area, focusing on the population ecology of a harvestmen species inhabiting caves (Machado et al. 2001); ecotone delimitation between epigean and hypogean ecosystems (Prous et al. 2004); transitory aquatic taxocenosis in caves (Souza-Silva et al. 2012); subterranean stream benthic communities (Taylor and Ferreira 2012) and detritus processing in caves (Souza-Silva et al. 2012).

Furthermore, from 2002 on, new taxa were described for the region, as the troglobitic millipede *Pseudonannolene ambuatinga* (Iniesta and Ferreira 2013) and the palpigrade *Eukoenenia cavatica* (Souza and Ferreira 2016). Troglophilic species were also described, as the millipedes *Pseudonannolene saguassu* (Iniesta and Ferreira 2013), *Pseudonannolene robsoni* and *Pseudonannolene rosineii* (Iniesta and Ferreira 2014), the bug *Zelurus gerevatinga* (Ferreira et al. 2016), the barklice *Psyllipsocus spinifer* and *Psyllipsocus falcifer* (Lienhard and Ferreira 2014), the whipspider *Charinus jibaossu* (Vasconcelos et al. 2014), the spider *Symphytognatha carstica* (Brescovit et al. 2004), and the mites *Cyta troglodyta* (Hernandes et al. 2011) and *Ornithodoros cavernicolous* (Dantas-Torres et al. 2012).

4.1. The singularity of the Éden invertebrate cave fauna

The total number of troglophilic species (93) found in the Éden cave is considerably high when compared to the average richness of the 296 surveyed caves in the region (35 ± 19.1 species/ cave) (Zampaulo 2010). Moreover, the Éden cave also presented a singular and distinct invertebrate community when compared to other caves of the area, featuring many endemic species and the highest number of troglobites known for the province. The cave harbor at least 16,25 % of local biodiversity of obligate cave fauna in the APD province, and this high richness of troglobitic fauna is probably related to the cave's dimension, with consequent habitats heterogeneity and the presence of groundwater, as for other caves rich in obligate cave fauna in Brazil (Souza-Silva and Ferreira 2016).

Many studies in tropics have shown that the number of troglophilic and troglobitic species can increase with the increase of the cave's extension (Ferreira 2004, Souza-Silva et al. 2011). Such relationship occurs due to the fact that larger caves can present higher habitat and resource availability, which are decisive factors for the subterranean fauna maintenance (Culver et al. 2006, Simões et al. 2015), allowing higher number of species to establish themselves (Souza-Silva et al. 2011). Besides that, according to Simões et al. (2015) the presence of subterranean streams are factors that can determine the community structure, increasing the species number and the similarity between the caves. Cave streams, besides increasing the humidity, can transport debris from epigean habitats to caves, providing food resources for the fauna (Poulson and Lavoie 2001, Souza-Silva et al. 2011b).

Besides the size, the Éden cave stands out from the entire APD karst area concerning the invertebrate richness and to the number and phylogenetically distinct obligate cave species present. From the 13 cave-dwelling species listed in this study, 12 represent undescribed species and only one is formally described in the literature (*Pseudonannolene ambuatinga* – Iniesta and Ferreira 2013). Any information concerning the biology and ecology of these species is yet inexistent. This fact reflects the incipient knowledge on the tropical subterranean fauna and the need for more studies and experts. Troglobites are considered very fragile due to its low density and its populations are commonly known for only a handful of specimens (Trajano 2001, Culver and Pipan 2009). Accordingly, the large number of individuals found for some of the obligate subterranean species in the Éden cave represents a rare event in nature (Culver and Pipan 2009, Glavaš et al. 2016). In this context, the Éden cave may be preserved to be used as a natural laboratory, fostering a variety of future biological studies.

4.2. Temporal and spatial distribution of the invertebrates cave fauna

It is well established that habitat heterogeneity and food availability are important selective factors in cave ecosystems (Culver et al. 2006, Ferreira and Martins 2009, Schneider et al. 2011). The quality and distribution of resources within the cave habitat are important factors that influence the composition, abundance and spatial organization of the cave fauna (Culver and Pipan 2009, Ferreira and Martins 2009, Schneider et al. 2011). Confirming this trend, Humphreys (1991) found that moisture and organic matter combined have a positive effect on the number of organisms in cave patches in Australia. Finally, seasonal events related to stochastic flow pulses are responsible to change the amount of available organic resources though time in the subterranean environment, which may also have severe implications on the composition and distribution of the cave fauna (Souza-Silva et al. 2011b, Bento et al. 2016).

The resource distribution within the Éden cave is patchy, as great part of the upper level features very low organic resources. The lower level shows the opposite scenario, with a considerable amount of organic matter carried by the stream. It is not surprising that the cave invertebrate community would respond to this energetic and moisture availability distinctness. So, the distribution of cave invertebrates in the cave can be influenced by this feature, resulting in disproportionately greater richness and abundance values for the lower level. It is interesting to highlight that this discrepancy between cave levels becomes even more pronounced during the dry period. During these seasons, the species richness decreases in the upper level and increases in the lower. We believe that shifts in the amount of available organic matter and moisture within cave levels are causing the observed community variation. During the dry periods, the amount of dripping and percolating water was severely reduced in the upper level (R. L. Ferreira, personal communication). The reduction on water volume may have led to a decrease in the available dissolved organic matter (DOM) in this cave compartment during part of the year, which in turn, possibly resulted in a reduction in the number of organisms. The increase in invertebrate richness in the lower level may result from a higher detritus retention rate during the dry season, thus providing larger amounts of organic resources for the community to explore, as occurs in other tropical caves (Souza-Silva et al. 2011b). However, further studies based on experimental approaches are needed to fully elucidate these questions.

Although the presence of a subterranean stream in the Éden cave has severe implications determining the structure of the cave community through its positive effects (moisture and organic matter input) on the terrestrial fauna, it is interesting to notice that its fish fauna and aquatic invertebrate community have extremely low diversity. This fact may be attributed to several reasons, and the causes of the observed low diversity patterns remain unclear and demand further research, but we can suggest stream silting as an important impact for aquatic fauna. Taylor and Ferreira (2012) sampling benthic invertebrates in a 100m long subterranean stream also located in the APD province found 22 species of the Gastropoda, Bivalvia, Hemiptera, Coleoptera, Ephemeroptera, Trichoptera, Diptera, Annelida, Turbellaria, Acari, Ostracoda, Copepoda, Cyclopoidea and Nematoda, reflecting in a high richness of the aquatic community.

However, we noticed a high quantity of silt in the stream channel causing a sedimentation gradient in a downstream direction in Eden cave. The homogeneous muddy substrate found in the stream bed of the Éden cave might have negative effects on the aquatic community through the loss of habitat heterogeneity and complexity (Allan and Castillo 2007), thus potentially causing a reduction on the diversity of benthic invertebrates.

4.3. Regional conflicts threatening the Éden cave integrity

The APD karst area represents the highest cave concentration in South America, being characterized by the presence of small cavities (average linear projection of 102.7 m) (Zampaulo 2010, Cecav 2017). Most part of this area has been subject to a severe fragmentation of its original vegetation due to decades of intense logging and agricultural exploitation. The remaining forest formations are restricted to the margins of São Miguel River and to the limestone rocky outcrops due to their particular morphological conditions, which make these areas unsuitable for agricultural and pastoral use. At the other hand, they receive considerable threat posed by the advance of mining activities (Teixeira and Dias 2003, Henriques-Junior 2006). During the 60´s, several mining companies and calcination industries were settled in this region and became the main economic activities. The carbonate rocks have been overexploited since then, thereby causing irreversible damage to the karst landscapes as well as to natural cavities (Ferreira and Martins 2001, Cherem and Magalhães-Junior 2007). At this date, this region represents one of the major conflicts between biological conservation and mining activities in Brazil. Protection actions of natural areas of biological interest usually cause socio-environmental conflicts due to several economic and social interests over a target area. In general, conflicts occur when different social and economic values lead to different interpretations such as use restrictions and responsibilities. Such tensions can lead to situations from small conflicts to even physical violence and death, in the most extreme cases (Hodge 2014).

The land use on the Éden cave surroundings poses serious threats to the subterranean biodiversity, as the Éden cave is situated in an extremely vulnerable area considered as a priority to conservation (Zampaulo 2010). The serious impacts posed on this fragile subterranean system have become a matter of public health care. The water pollution along with the removal of the native vegetation in the cave`s watershed, are liable to cause major water quality problems (Allan and Castillo 2007). This scenario can become very dangerous since studies utilizing fluorescent dye (Rhodamine Wt) revealed that the water utilized by the population of Pains city is collected from a spring (S.A.A.E) that receives considerable amount of water from the karst system in which the Éden cave is located (Freitas 2009).

In the year 2009, an attempt to protect the cave and its surroundings was made through the proposition of a conservation unit: “Gardens of Éden Natural Monument” (Decreto Municipal 40/2009). At this time, there was no information on the cave fauna yet, but its known singular dimensions and speleothems were enough to support the proposition. Unfortunately, this attempt failed and the conservation unit was never created. Thereby we strongly advise taking emergency actions in this situation, the Éden cave and its watershed must be urgently protected. Government authorities must reconsider creating a conservation unit to ensure the cave´s protection (as its surroundings).

Further biological and environmental information are needed to accurately delimitate the landscape area for the conservation unit to be created and to maximize the protection of natural resources. Therefore, the delimited area proposed in the 2009 official document must be reviewed. Studies on the aquatic habitat biotic integrity should be performed on the major streams that reach the Éden cave and provide water supply to the population of Pains town. The ongoing activities responsible of causing impacts on river ecosystems must be identified and stopped, and habitat restoration measures must be taken.

The protection of caves and their aquifers should be based on a land use policy design that comprises a central protection area near springs or pristine recharge areas and surrounding core areas of the immediate protection area that require severe protection and restriction and depends on knowledge aquifer recharge, hydrogeological anisotropy and depurative capacity (Ford 2004, Souza-Silva et al. 2015).

5. CONCLUSIONS

Since the Éden cave represent a cave with high biodiversity of obligate fauna that is currently highly endangered by human activities and economic and social conflicts, it´s very important and emergencial the establishment of a conservation unit that protects an epigean surrounding area of the whole hydrological micro basin which incorporates the Éden cave. In addition, it is imperative to revitalize the basin that feeds the underground drainage; control of urban growth around the cave; awareness of rural and urban dwellers and mining companies involved in direct impacts on a cave;

monitoring of terrestrial and aquatic invertebrate communities and epikarst with emphasis on troglobitic species.

The above-proposed actions should represent the first step towards a broader environmental protection program focused on strategic karst areas in the Arcos-Pains-Doresópolis speleological province.

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Appendix I. Species composition, richness and abundance counted in three sample events at the Éden cave in lower and upper levels. Richness/Abundance (R/A), data from Zampaulo 2010 (Z2010).

Order	Taxons	Rainy Period				Dry Period				Dry Period	
		Lower		Upper		Lower		Upper		Z 2010	
		R	A	R	A	R	A	R	A	R	A
Turbellaria	Geoplanidae (*Geoplana*)	1	1			1	2				
	Bipalidae	1	15			1	29			1	79
Oligochaeta	NI	2	39			2	56			1	60
Acari	Anystidae									1	3
	Ascidae									1	500
	Eviphididae					1	3				
	Neotenogyniidae (*Neotenogynum*)	1	3			1	1				
	Podocinidae (*Podocinum*)	1	1								
	Rhagidiidae	2	2			1	4			1	24
	Veigaiidae (*Veigaia*)	1	301			1	508			1	12
Araneae	Clubionidae					1	1				
	Corinidae (*Attacobius*)									1	1
	Ctenidae (*Ctenus/Isoctenus, Enoploctenus*)	1	10	2	7	1	2	1	4	1	3
	Linyphiidae (*Erigone*)									1	1
	Nemesiidae	1	2								
	NI	2	4	1	1	5	6				
	Oonopidae	1	2			2	2				
	Pholcidae (*Mesabolivar*)	1	10			1	11			1	2
	Symphytognathidae (*Anapistula*)					1	1				
	Theraphosidae					1	1				
	Theridiidae (*Theridion*, *Coleosoma*, *Nesticodes rufipes*)	1	2	2	4	1	1	1	1	5	7
	Theridiosomatidae (*Plato*)	1	1							1	1
	Uloboridae			1	1			1	1		
Opiliones	Gonyleptidae (*Liops, Mitogoniella taquara, Eusarcus astatus*)	3	52	1	1	3	103	1	8	3	121
Palpigradi	Eukoeneniidae (*Eukoenenia florenciae*)	1	2	1	2	1	1			1	2
Pseudoscorpiones	Chthoniidae			1	2	1	3	1	2	1	7
Schizomida	NI			1	1						
Copepoda	Harpacticoida			1	4						
Isopoda	Styloniscidae			2	63			2	15	1	2
Auchenorrhyncha	Cixiidae									1	2
	Cydnidae			1	3			1	8	1	1
Blattodea	NI	1	184	2	8	1	176	1	9	2	178
Coleoptera	Carabidae (*Coarazuphium*)	4	5	3	5	4	129			6	89
	Dryopidae									1	1
	Elmidae (*Heterelmis*)									1	9
	Scirtidae					1	1				
	Hydrophilidae									1	10
	NI	1	2			2	2				
	Staphylinidae/Pselaphinae	1	23			2	31			2	125
	Tenebrionidae									3	14
Collembola	Entomobryidae	2	18	1	67	2	457	2	36	1	597
	Arrhopalitidae (*Arrhopalites*)	1	1195	1	90	2	337	1	10	1	500
	Tomoceridae									2	40
Diptera	Brachycera			1	1						
	Chaoboridae	1	1			1	1				
	Chironomidae					1	1			1	6
	Chloropidae			1	1						
	Dolichopodidae			1	2			1	1		
	Mycetophilidae					2	2	1	1		
	Stratiomyidae										
	NI					1	1			1	18
	Phoridae (*Conicera*)	2	2			1	31	1	6	2	14
	Psychodidae (*Lutzomyia*)	1	3							1	7
	Sciaridae					2	2				
Ensifera	Phalangopsidae (*Eidmanacris, Endecous*)	2	252	2	101	3	298	1	59	5	364
Ephemeroptera	Leptophlebiidae	1	2			1	2			1	5
Hymenoptera	Eupelmidae							1	1		
	Formicidae (*Azteca*, *Solenopsis*, *Odontomachus*)	1	31	1	2	3	15			2	34
	NI			1	1						
Lepidoptera	Noctuidae (*Hypena*)	1	117	1	104	1	1	1	8	1	10
	Tineidae			1	1						
Megaloptera	Corydalidae									2	20
Odonata	Libellulidae									1	1
	Gomphidae					1	1				
Psocoptera	Psyllipsocidae	1	1	1	49			1	23		
	Ptiloneuridae							1	4		
	Lepidopsoscidae									1	9
Sternorrhyncha	Coccoidea									1	1
Trichoptera	Psychomiidae									3	5
	Hydropsychidae					1	1				
Gastropoda	NI	1	1								
	Valloniidae					1	18			1	15
	Mycetopodidae					1	1			1	1
	Subulinidae					1	3			1	63
Lithobiomorpha	Anopsobiidae	1	1							1	1
Polydesmida	Sphaeriodesmidae	1	1								
	Paradoxosomatidae					1	2				
	Chelodesmidae					1	1			1	1
Spirobolida	NI	1	8			1	1				
Spirostreptida	Pseudonannolenidae (*Pseudonannolene ambuatinga, P. saguassu*)	2	45	1	4	2	107	1	6	1	269
Symphyla	Scutigerellidae	1	12			1	31			1	121