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Aquatic biota of different karst habitats in epigean and subterranean systems of Central Brazil – visibility versus relevance of taxa

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Abstract

The karstic area of São Domingos, central Brazil, holds extensive drainage systems. In order to understand its biodiversity, various volumes of water were filtered with planktonic nets in stretches of subterranean and superficial rivers on five different occasions. We sampled four drips (152L), three calcite pools (368L), two subterranean rivers fed mainly by percolation water (6,395L), two subterranean rivers fed mainly by water coming from a sinkhole (4,175L) along different caves, one resurgence (158L), and four epigean rivers (101,690L). Physical and chemical variables were measured at some sites. Canonical Correlation Analysis was used to verify relationships between taxa and environment. The degree of similarity of the biota was assessed by cluster analysis (Sorensen, single linkage). There were records of exclusive taxa in epigean and subterranean samples, mainly in drips, which harbour the most unique fauna. The high richness of taxa presently recorded reveals the potential of the vadose zone biota in the tropical region, which was neglected in studies on Brazilian subterranean biodiversity. According to our results, the unsaturated zone tropical fauna may have different composition compared to that from temperate habitats. The studied communities were dominated by rotifers, while crustacean are predominant in the latter. The hypothesis can be clarified with the increase of long term studies and taxa identification at species level, besides the use of complementary sampling methods.

Keywords

Subterranean habitats, epigean rivers, aquatic biota, Rotifera, conservation, Neotropical region

Introduction

Most of the subterranean habitats develop in karst systems, which are discontinuous geomorphic systems formed in soluble rocks and characterized by the presence of aquifers and conduits, with subterranean drainage (Dreybrodt 1988). These systems originate most caves that can reach hundreds of kilometres. The caves provide indirect human access to the hypogean environment in such a way that a large portion of information on subterranean ecosystems is derived from studies performed in them. Generally, the structure of karst systems consists in a sum of different horizons that change with depth. The vadose or unsaturated zone is the layer between the soil and the phreatic or saturated zone (Mangin 1994). The vadose zone is heterogeneous, with dissolution voids and fissures in which water percolates downwards by a multiphase process before reaching the phreatic zone (Ford and Williams 2007). In the upper section of the superficial layers of karstifiable rocks, a particular zone is formed - the epikarst (Mangin 1973). This consists of a heterogeneous system of openings in which surface water percolates through soil, retained for variable periods, potentially for years at a time (Jones et al. 2004).

The waters that refuel the epikarst can infiltrate through different voids (i.e. fractures, conduits or shafts), which vary in physical and chemical properties (Musgrove and Banner 2004, Williams 2008) (Figure 1). As such, epikarst acts as a transitional zone between epigean and subterranean environments, where organic matter and other resources are transported and redirected. The faunistic flow can also occur in these conduits where the environmental filters offer ideal conditions for a few species. Thus, the composition of unsaturated communities depends on different physical and chemical variables at each depth level (Pipan et al. 2008, Moldovan et al. 2012, Pipan and Culver 2012).

In these habitats an exceptionally rich fauna occurs, best described in some European karst areas (Pipan et al. 2008, Brancelj and Camacho 2009, Galassi et al. 2009, Meleg et al. 2011, 2012, Moldovan et al. 2012, Cottarelli et al. 2012) and in West Virginia (USA; Pipan and Culver 2005). The organisms that live in the unsaturated zone of the karst are occasionally carried by trickling water to the interior of caves. Local seasonality influences this process to the extent that the intensity of rainfall alters the volume of infiltration and the connectivity between surface and underground environment. This process is extremely variable and especially important to the understanding of the karst ecosystem functions (Bonacci et al. 2009).

The vadose zone fauna in temperate zones is mainly composed of small crustaceans, oligochaetes, nematodes, acari, and molluscs, less than 1 mm to several centimetres in body size (Gilbert et al. 2009). In general, stygobiotic biodiversity in Europe is dominated by Crustacea (Pipan et al. 2008, Brancelj and Camacho 2009, Meleg et al. 2011, 2012). Among these, copepods represent the most common group, with Harpacticoida outnumbering Cyclopoida in diversity and abundance (Moldovan et al. 2007, Meleg et al. 2011, Moldovan et al. 2012, Cottarelli et al. 2012). Their spatial and temporal distribution appears to be rather heterogeneous and has been related to a number of environmental parameters and cave characteristics, as topography, cave ceiling thickness and/or voids interconnectivity (Stoch 2000, Brancelj 2002, Pipan and Brancelj 2004a, 2004b, Sket et al. 2004, Camacho et al. 2006, Pipan et al. 2008,

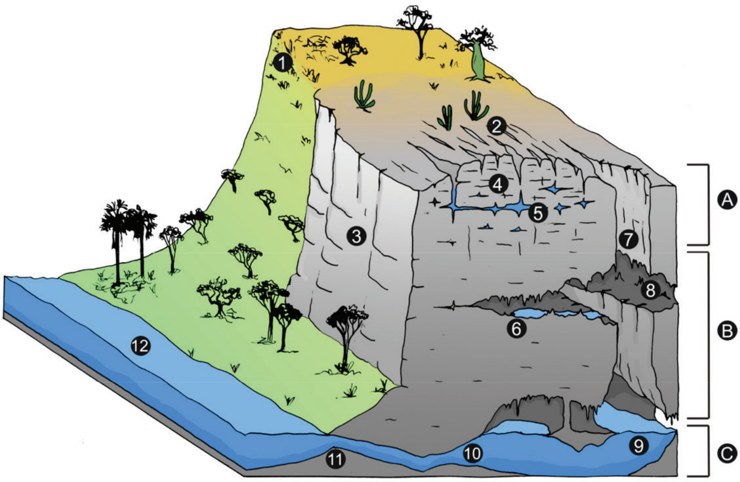

Figure 1. Horizons in karst: **1** soil **2** karst terrain **3** limestone outcrop; **4** epikarst **5** aquifer in epikarst **6** drips 7 doline **8** cave **9** and **10** subterranean river at the base level **11** resurgence **12** epigean river **A** epikarstic zone **B** vadose zone **C** phreatic or saturated zone. (Ilustration: Pedro Pereira Rizzato).

Meleg et al. 2011). Nevertheless, studies on stygofauna in tropical regions are scarce, and non-existent in relation to the unsaturated zone horizons.

As the terrestrial hypogean fauna is more visible and can be easily assessed, it has been studied in more detail in Brazil (Trajano and Gnaspini 1991, Pinto-da-Rocha 1995, Trajano and Bichuette 2006, 2010). The aquatic diversity, however, is clearly underestimated, as sampling methods of this biota are not always standardized and/or adequate (Trajano and Bichuette 2006). Sampling of these organisms require the use of nets with micrometric mesh in habitats like pools fed by percolating water, as well as from sifting of sediment for interstitial species and use of vacuum pumps.

The unique long-term study focusing on Brazilian subterranean aquatic fauna was conducted for fish populations (Bichuette and Trajano 2004). For example, the catfish *Ituglanis epikarsticus* and *I. ramiroi* are rare in cave habitats, being found respectively in a single set of pools in São Mateus Cave and in a side pool in São Bernardo Cave, both located in our study area. However, when it comes to aquatic microfauna studies, the only records of copepods are restricted to six species from two caves in Goiás state, central Brazil, aside from the sparse references in general publications, which adds three other species (Pinto-da-Rocha 1995).

Many studies have demonstrated that organic carbon may arrive in the form of animals dripping into caves through the epikarst (Simon et al. 2003, 2007, Pipan 2005), acting as an important resource for the subterranean habitats. The assessment of local subterranean fauna is important for understanding the functioning of karst aquifers for protection and conservation purposes.

As the study of the unsaturated zone biota is a new challenge for subterranean biology in Brazil, the main goal of this study was to elucidate part of the richness and distribution of the aquatic biota in different karstic horizons of São Domingos region and its surroundings, north-eastern Goiás state, central Brazil. Furthermore, we aimed to verify the correlation between this biota and some physical and chemical variables of the different habitats. The question of relevance *versus* visibility of this biota and its respective habitats was raised, considering that less visible species are yet important to the functioning of subterranean systems.

Material and methods

Study Area

The karst area of São Domingos and its surroundings represents one of the regional formations of the Bambuí geomorphologic unit, which constitutes the largest set of limestone areas favourable to caves development in Brazil. The region has an approximate area of 105, 200 km^2. In the region there are five large systems of caves, with up to 23 km of development (Figure 2).

Superficial rivers belonging to the Paranã Basin (Alto Tocantins) penetrate the limestone layers after draining an extensive arenitic region, forming large cave systems (Karmann and Setúbal 1984, Auler and Farrant 1996). As part of these, there are upper tributaries in the vadose zone fed mainly by infiltrating waters from the surface.

The area is part of a state-level Conservation Unit (Terra Ronca State Park - TeRSP), created 17 years ago but still with diverse land ownership problems. One aggravating factor is that some river sources that cross the cave systems are located outside the park. There are anthropic activities as livestock, extensive plantations and clandestine mining which on long-term are responsible for the adverse effects on local drainage systems, which include the subterranean domain.

The studied region belongs to the morphoclimatic Dominion of the Cerrados (Ab'Saber 1977), with a dry season between May and September, sometimes extending through October (Nimer 1979). Heavy showers characterize the rainfall regime during the rainiest periods (October through March), transporting a large amount of organic matter in the caves (Trajano and Bichuette 2010).

Sampling of aquatic invertebrates

The sampling area embodies subterranean systems and epigean rivers, sampled differently regarding frequency and volume since in some cases the availability of water was restricted. Abbreviations and characteristics of each sampled point are described in Table 1.

The region was sampled on five occasions: April and October 2011, and February, June and October 2012. The subterranean stretches included drips, subterranean rivers (base level) and water pools (Figure 3). The sampled caves and rivers are: Angélica, Bezerra, Buraco das Araras, São Bernardo, São Mateus and Revolucionários caves; the

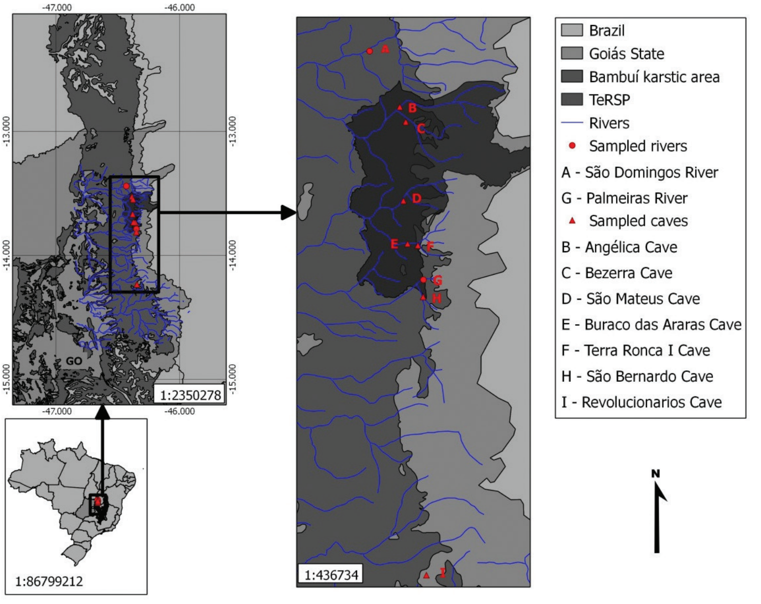

Figure 2. Study area map with details of subterranean cave systems and epigean rivers at São Domingos karst area and surroundings, Goiás state, central Brazil. In dark gray – limits of Terra Ronca State Park (TeRSP). **A** São Domingos river **B** Angélica Cave **C** Bezerra Cave **D** São Mateus Cave **E** Buraco das Araras Cave **F** Terra Ronca I Cave (sinkhole of Lapa river) **G** Palmeiras river **H** São Bernardo Cave **I** Revolucionários Cave (this cave is located outside the limits of Terra Ronca State Park).

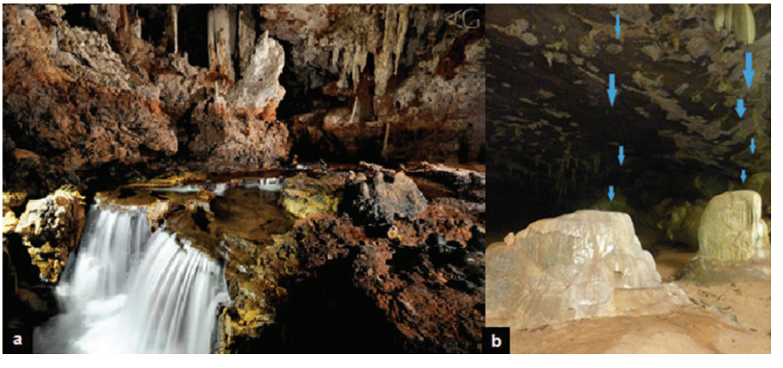

Figure 3. Subterranean river (**A** São Bernardo Cave) and drips (in blue) formed by infiltration water (**B** Angélica Cave – AngD01). Photography: a, Adriano Gambarini; b, Maria Elina Bichuette.

epigean Palmeiras, Angélica, da Lapa, São Domingos Rivers, and the Resurgence of Terra Ronca (Table 1). Revolucionários Cave and São Domingos River are outside the park area, but inserted in the same limestone lens. The Angélica Cave is the only locality sampled on every trip, as a long-term monitoring base.

Samples were taken with plankton nets (20µm mesh), which remained installed for periods varying from twenty minutes to three hours, according to the local con-

Table 1. Characteristics, volumes and months in which sampling was undertaken. Sampling sites in the karst area of São Domingos and surroundings (2011-2012). Caves: Angélica (Ang), Buraco das Araras (BAra), Bezerra (Bez), São Bernardo (SBer), São Mateus (SMat), Revolucionários (Rev); epigean rivers: Palmeiras (Palm), Angélica (Ang), São Domingos (SD); resurgence: Terra Ronca (RTR). D = drip; Ek = subterranean river fed mainly by percolation water; Ep = epigean; P =pool; S = subterranean. *Locality visited on the five occasions (segment of Angélica Cave, monitoring base); samplings were not performed during periods of inactive drips.

Points	Collection Occasions	Vol (L)	Characteristics
AngD01	Apr/2011; Feb/2012 *	83	Drips in the entrance zone of Angélica Cave. Water drips directly from the ceiling, with flow varying according to the season.
AngD02	Apr/2011*	3	Reduced dripping, with low flow rate, and ceasing during the dry season.
AngEk	Apr & Oct/2011; Feb, Jun & Oct/2012	3,596	River fed by percolation water, habitat of the small catfish *Ituglanis epikarsticus* Bichuette & Trajano, 2004. The water flows with moderate rate and little turbulence, and there is notable reduction in the river level during the dry periods.
AngEp	Jun/2012 & Oct/212	23,580	Epigean Angélica River, point close to the sinkhole.
BAraD	Oct/2011 & Oct/12	21	Water drips from ceiling speleothems.
BAraP	Oct/2011 & Oct/2012	45	Pool near the BurAraGot drip.
BAraEp	Oct/2012	6,000	Epigean Lapa River, entrance of Buraco das Araras Cave.
BezD	Jun/2012	45	Drips coming directly from the ceiling.
BezP	Jun/2012	113	Pool in the aphotic zone of Bezerra Cave. Water drips from the ceiling and remains puddled.
BezS	Feb & Jun/2012	2,603	Subterranean river in the aphotic zone of Bezerra Cave, base level.
SBerEk	Fev/2012	2.592	River in the aphotic zone of São Bernardo Cave.
SMatP	Feb/2012	210	Pool in São Mateus Cave. Water drips from the ceiling and forms a puddle.
RTR	Oct/2011	158	Lapa River at its epigean portion, resurgence of Terra Ronca Cave.
PalmEp	Apr/2011	110	Palmeiras River (epigean) near the sinkhole of São Bernardo Cave.
SDEp	Feb & Jun/2012	72,000	São Domingos River (epigean), near its sinkhole.
RevS	Jun/2012	1,545	Subterranean river in Revolucionários Cave.

ditions. Nets, installed under dripping water sites based on the device proposed by Brancelj (2004) were used to sample the fauna percolating through the unsaturated zone for similar periods. For the shallow stretches, the flow (used to calculate the filtered volume) was estimated from the velocity of the water surface. This velocity was determined by the time it took for a small plastic disc to travel a pre-determined distance. In the case of percolating water and pools, the volume was directly estimated using a graduated plastic container. The material was subsequently fixed (formaldehyde 4%) and packed. Afterwards, the samples were analysed in the laboratory of Limnology in the Biology Department of FFCLRP/USP (Ribeirão Preto campus), and the organisms were identified to the lowest possible taxonomic level.

Physical and chemical variables

The following physical and chemical variables of water were measured by using a Horiba multiprobe (model U50G): temperature (°C), pH, ORP redox potential (mv), conductivity (mS/cm), dissolved oxygen (mg/L), oxygen saturation (%), total of dissolved solids TDS (g/L), turbidity (NTU), depth (m) and salinity (%).

Data analysis

Relative taxa richness among sites was assessed. Furthermore, the richness of the taxa in epigean and hypogean environments was taken into account separately for comparison. Similarity in composition of aquatic invertebrates in the different habitats was analysed by cluster analysis (Sorensen, single linkage).

In order to study the species-environment relationship, Canonical Correspondence Analysis (CCA) was used, associating the measured environment variables with the matrix of taxa present at eleven sampled sites.

Finally, by means of simple linear regression (Pearson correlation), we verified the relationship between the sampling effort and the richness of taxa, as the volumes sampled in each segment varied. Analyses of similarity and CCA were performed using the software PAST (version 2.13) and R language (version 3.0.1), respectively.

Results

Thirty-six taxa were registered in all of the samples, belonging to different groups listed in decreasing order according to absolute/relative richness: Rotifera (23; 64%), Insect larvae (4; 11%), Copepoda-Cyclopoida, Calanoida and Harpacticoida (3; 8%), Cladocera (2; 5%), Dinoflagellata (1; 3%), Nematoda (1; 3%), Ostracoda (1; 3%) and Protozoa (1; 3%) (Table 2).

In decreasing order, the richest habitats (based on number of taxa present) were: AngEk (16); RTR (13); AngD02 (12); BAraP (10); BezS (9); PalmEp (8); AngD01, BAraD and AngEp (7); SBerEk, BezP, SMatP and RevS (5); SDEp and BAraEp (2); BezD (1). We summed up the richness of taxa from the samples of epigean and hypogean origin separately, totalling 21 and 30, respectively (Figure 4).

Some taxa were exclusive to one single sampling point: *Anuraeopsis fissa* and *Euchlanis* sp. (RTR); *Bosmina* sp. (PalmEp); *Anuraeopsis* sp. (AngEp); *Brachionus* sp. (SBerEk); Chaoboridae (AngEk); Simuliidae (BAraP), *Arcella costata* (AngD01), *Brachionus falcatus*, *Conochilus* sp., *Filinia* sp., *Kellicotia bostoniensis* and *Keratella cochlearis tecta* (AngD02). The group Calanoida was restricted to samples with epigean origin, and Harpacticoida was restricted to subterranean waters.

Cluster analysis for the 16 sites evidenced low similarity between them (*S*=0.32). The dendrogram also revealed that drips and epigean rivers are the most singular environments as they were more distant from other samples (Figure 5).

Table 2. Taxa list and number of individuals sampled in each point. Caves: Angélica (Ang), Buraco das Araras (BAra), Bezerra (Bez), São Bernardo (SBer), São Mateus (SMat), Revolucionários (Rev); epigean rivers: Palmeiras (Palm), Angélica (Ang), São Domingos (SD); resurgences: Terra Ronca (RTR). Undet= undetermined taxa. Sampling point abbreviations are described in Table 1.

Sampling points	Drips				Pools			Subterranean rivers				Epigean rivers				
	Ang 01	Ang 02	BAra	Bez	Bara	Bez	SMat	Ang Ek	SBer Ek	BezS	RevS	Palm Ep	Ang Ep	BAra Ep	SDEp	RTR
Taxa/ Sampled Volume (L)	83	3	21	45	45	113	210	3,802	2,592	2,603	1,545	110	23,580	6,000	72,000	158
Rotifera					3											
**Anuraeopsis fissa*																1
Anuraeopsis sp.													1			
Brachionus falcatus		1														
Brachionus sp.									2							
Chonochilidae								2								
Collotheca sp.	4	4			2			1		2	2	3			18	15
Conochilus sp.		1														
**Euchlanis* sp.																12
Filinia sp.		1														
Gastropus sp.	1															25
Hexarthra sp.			1											1		
Kellicotia bostoniensis		5														
Keratella cochelaris tecta		4														
Keratella cochelaris	4	1	1		2			2		4						
Lecanidae								3		2	2				18	
Lecane sp.	2															5
Lecane monostyla sp.								2								12
Ploimida			2					4					47			
Synchaeta sp.								1		2	1		46			
Testudinella sp.								1		2						

Trichocerca sp.		1								6			7			
Bdelloidea			3		3		2	7	2	4	3	3	7			55
Copepoda																
Cyclopoida adult		3	1		1			3				5				1
*Cyclopoida copepodito	1	1										2	46			
*Cyclopoida nauplius	1	2	1		1		2	9				3				9
Calanoida												4				2
Harpacticoida				2	4	2	1	1	2		1					
Cladocera									2			2				
Bosmina sp.												1				
Dinoflagellata																
Peridinium sp.						6		2								
Insecta	1	2	2			2	2	6	3	1						1
Chironomidae					1	4		4		4			7			
Chaoboridae								1								
Simuliidae					1											
Nematoda	5				8	2	1							4		2
Ostracoda																1
Protozoa																
Arcella costata	1															

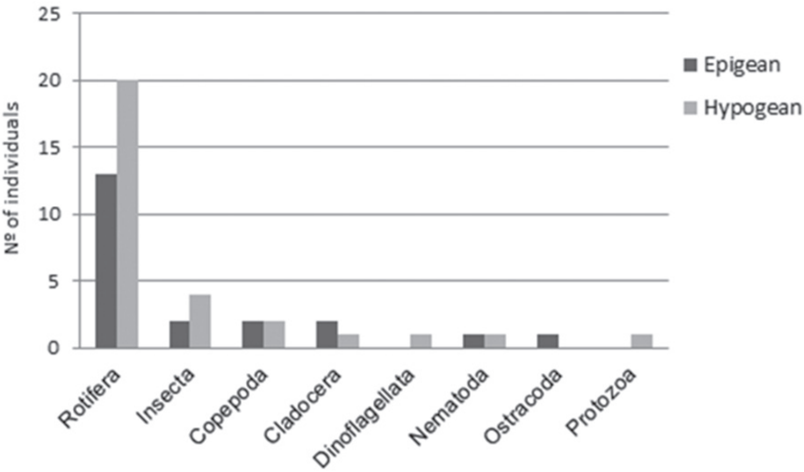

Figure 4. Number of taxa recorded in 16 sampling sites. Total taxa for epigean (21) and subterranean habitats (30).

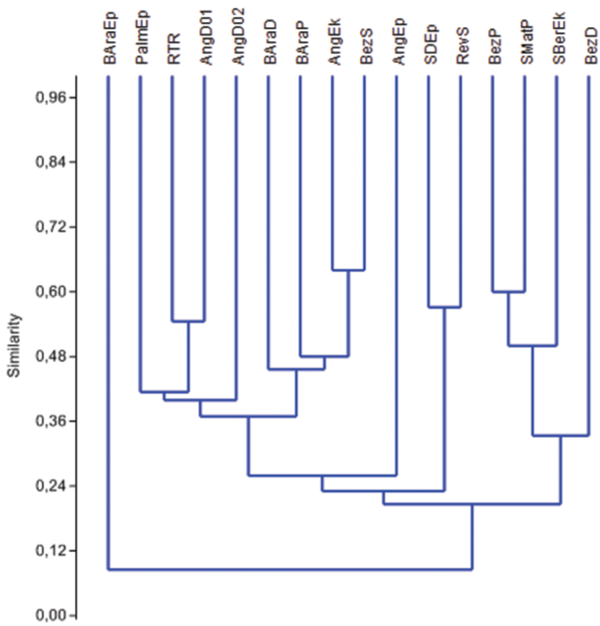

Figure 5. Similarity Cluster Analysis (Sorensen, single linkage) for 16 sampling sites from São Domingos karst area and surroundings, Goiás state, central Brazil. Abbreviations are described in Table 1.

In the resultant CCA (Figure 6), the taxa closest to the various environmental vectors are strongly associated with them. For this analysis, thirty-one taxa were recorded in 11 sampling points. The majority of hypogean taxa are close related to both high levels of conductivity and pH, and low ORP. The epigean taxa appear more related to

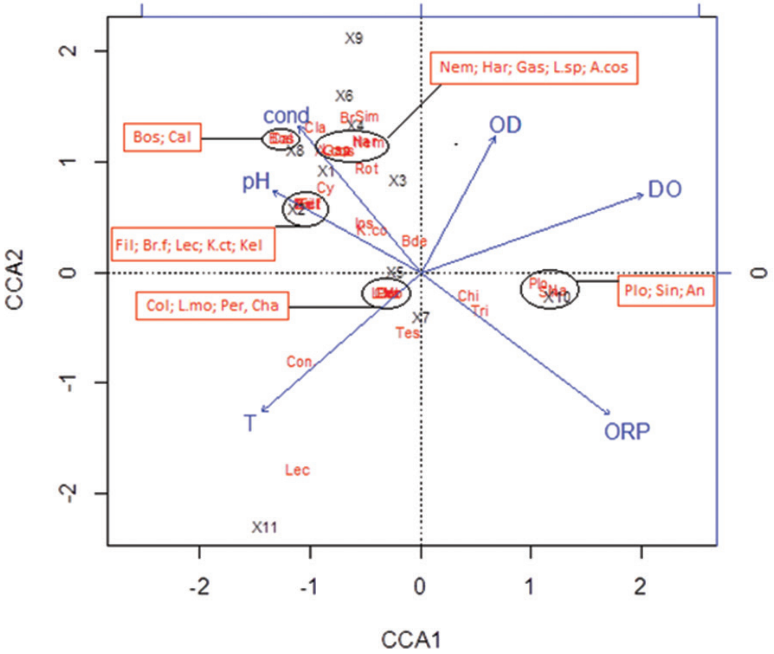

Figure 6. CCA for environmental variables and biotic data of 11 sampling sites from São Domingos karst area and surroundings, central Brazil (ORP= redox potential; T = temperature; OD= concentration of O_2; DO = oxygen saturation and cond=conductivity). Red abbreviations represent the taxa: Rot = Rotifera; An = *Anuraeopsis* sp.; Br.f = *Brachionus falcatus*; Br= *Brachionus* sp.; Col = *Collotheca* sp.; Con = *Conochilus* sp.; Fil = *Filinia* sp.; Gas = *Gastropus* sp.; Kel= *Kellicotia bostoniensis*; K.ct= *Keratella cochelaris tecta*; K.co = *Keratella cochelaris*; Le c= Lecanidae; L.sp = *Lecane* sp.; L.mo = *Lecane monostyla* sp.; Plo = Ploimida; Sin = *Synchaeta* sp.; Tes = *Testudinella* sp.; Tri = *Trichocerca* sp.; Bde = Bdelloidea; Cy= Cyclopoida; Cal = Calanoida; Har = Harpacticoida; Bos = Bosmina; Cla = Cladocera; Per = *Peridinium* sp.; Ins = Insecta; Chi = Chironomidae; Cha = Chaoboridae; Sim = Simuliidae; Nem = Nematoda; A.cos = *Arcella costata*. X words represent the sampling sites (described at Table 1): X1=AngD01; X2=AngD02; X3=BAraD; X4=BAraP; X5=AngEk; X6=SBerEk; X7=BezS; X8=PalmEp; X9=BAraEp; X10=AngEp and X11=SDEp.

high levels of OD and ORP, except for the SDEp site. In the same way, taxa near each other (Figure 6) have similar environmental preferences. The sampling point positions reflect the physical and chemical characteristics of each habitat.

Only six of the ten environmental variables that were measured held some importance in the distribution of the samples according to fauna: dissolved oxygen, oxygen saturation, redox potential, conductivity, pH and temperature (Table 3). Oxygen saturation and redox potential presented the highest correlation values with the two first ordination axes (0.7802 and 0.5588, respectively). The two first canonical axes represented 51% of the total variation (30% and 21%, respectively).

Pearson correlation did not show a significant relation between the volume of water filtered and the number of taxa recorded at each sampling point, since simple linear regression analysis presented a value of R= 0.10756.

Table 3. Physical-chemical variables of the 11 sampling points in São Domingos karst area and surroundings, central Brazil. Cond=conductivity, OD=concentration of O_2, OD=saturation of O_2, ORP= redox potential, T=temperature. Sampling points abbreviations are described in Table 1.

Sampling point	pH	Cond (mS/cm)	OD (mg/L)	DO (%)	ORP (mv)	T (°C)
AngD01	7.40	0.81	3.17	37.92	179.00	23.00
AngD02	9.80	0.47	4.25	50.50	24.30	22.60
BAraD	8.20	0.49	12.80	145.90	210.20	22.30
BAraP	8.10	0.54	15.60	170.50	220.00	22.00
AngEk	7.50	0.49	4.48	102.63	215.90	23.90
SBerEk	6.30	0.45	3.50	42.73	203.90	24.50
BezS	7.50	0.09	5.55	66.03	300.00	23.00
PalmEp	7.60	0.01	6.03	75.75	68.70	26.20
BAraEp	7.00	0.02	11.55	197.70	204.40	25.30
AngEp	6.70	0.01	6.96	163.09	331.60	22.90
SDEp	7.00	0.02	2.91	36.80	20.00	26.50

Discussion

Richness of the karstic waters of Goiás

In this preliminary approach, we studied the richness of taxa in habitats of karstic systems, which represents new data on tropical subterranean domain species. The fauna belonging to samples of percolating water were remarkable and responsible for the high number of recorded species in the studied habitats

Rotifers were the richest group in our study, representing more than half of the total richness recorded. The dominance of rotifers was associated with the increase in the trophic state of a system due to short life cycles and rapid reproduction, which favour this group in more dynamic, competitive and selective environments. Presently, it is known that rotifers are also dominant in several other aquatic environments, independent of the trophic state (Rocha et al. 1995). This group species have greater tolerance to different environmental conditions, and present short reproductive cycles and high renewal rate, providing an advantage for the formation of well-established populations (Neves et al. 2003, Segers 2008).

Diversity in subterranean water courses is frequently limited by competition, and especially limited by energetic resources and space (Culver 1994). In Brazilian karst, the subterranean aquatic microfauna certainly configures an important source in the diets of larger taxa, mainly for fish populations and aquatic invertebrate groups that inhabit the most inaccessible points of the unsaturated zone. Rotifers are considered a source highly rich in energy, as they make available a large quantity of renewable food through conversion of primary production in tissue that is easily assimilated by consumers (Esteves 1998). In dry periods, when the level of the phreatic zone tends to suffer brisk reductions, some fish become imprisoned in pools, without record of populations in base-level streams (e.g. populations of catfishes *Ituglanis epikarsticus* Bichuette & Trajano, 2004 of São Mateus Cave). Therefore, it is possible that rotifers act as an essential component of the food chain, offering rapid renewal of nutrients for the other trophic levels.

The low number of Copepoda occurrences in our samples must be due to environmental factors and/or interspecific relationships in the communities. In dry periods, the water volume flowing through the system can decrease, as well as the access to aquatic communities from unsaturated zone habitats. In fact, some of our sampling points could not be efficiently accessed in all periods, in part because of the reduced local rain rates. Besides, sampling frequency could have also affected our results to the extent that it did not offer access when abundant taxa of these communities occurred (i.e. different life cycles). We also have to consider that benthonic fauna was not well assessed.

The fauna distribution depends on the scale of the karst system and generally species change in number from the surface to the saturated zone (Gibert et al. 2009). The most relevant factors to the composition of aquatic biota consist of physical and chemical variables of water, the thickness of rocks and the rainfall regime, as they directly influence waters properties and volume of the karst systems (Pipan et al. 2006, Kogovšek 2010, Meleg et al. 2011, Pipan and Culver 2012). The most relevant physical and chemical factors explaining the composition of aquatic biota were considered the thickness of rocks and the rainfall regime, as they directly influence waters properties and volume of the karst systems (Pipan 2006, Kogovšek 2010, Meleg et al. 2011, Pipan and Culver 2012). The environmental variables used in our correlation analysis have low significance in explaining the different faunistic distributions. Certainly, there are other more important factors that have not been considered, such as the dissolved organic carbon and concentration of ions (e.g., sodium, nitrate and potassium). Until present, few studies have considered the levels of carbon, phosphorous and nitrogen in the waters (e.g., Pipan et al. 2006, Simon et al. 2007), even if the dissolved organic carbon is an important limiting factor (Simon and Benfield 2002). Even if we did not measure these values, we can infer the availability of organic carbon in percolation water for the high number of taxa found in drips.

We observed that drips samples have the most unique fauna of all the other subterranean habitats. In our study, it is important to highlight that the water volumes filtered in drips were much smaller than the volume of water filtered in other habitats. However, we encountered elevated richness and occurrence of taxa exclusive to these samples. Thus, we recorded the greatest richness of taxa from the least voluminous samples (drips of Angélica Cave and epigean resurgence), while some of the most voluminous ones had inferior richness (São Domingos, Lapa and Angélica epigean rivers). The total of taxa encountered in drips outnumbered all others samples, emphasizing the complexity of communities from the unsaturated zones.

For the environmental parameters considered, we have observed that there was a separation between epigean and hypogean sites in the diagram generated by CCA as well as in the dendrogram of faunistic similarity (except for SDEp and RevS sites). Both demonstrated that there were clear differences between the biota of epigean and groundwater environments while distinctions of hypogean habitats among themselves are less evident. When comparing each sample individually, we noticed faunistic distinction between them.

Epikarst and sinkholes are both responsible for supplying the aquatic hypogean realm, but they differ in their capacities to redirect and/or store water through the karst (Juberthie 2000). The small size of voids of the epikarstic and the vadose zone restricts the transport of particles and microorganisms, and also represents a much slower flow. However, the total area occupied by the unsaturated zone is much more ample and complex, permitting innumerable vertical and lateral connections. These factors interact in a heterogeneous way, generating various microhabitats and promoting the formation of distinct communities (Moldovan et al. 2012).

The waters that percolate the subterranean systems are filtered by soils on the karst surface and tend to suffer reduction of dissolved oxygen. Organic material, carbon, and diverse ions suffer reduction of dissolved oxygen, depending on the manner in which they are used and transformed through chemical processes by the interstitial and/or unsaturated zone biota. Water circulation in surface rivers promotes higher oxygenation rates than in the subterranean environment, which generally are formed by smaller and less dynamic or lentic systems (Malard and Hervant 1999, Pipan and Culver 2005).

Redox potential, the second most important variable in the analysis, has negative correlation to concentrations of oxygen, suggesting that reductive processes predominate in the absence of oxygen. Low levels of ORP can be associated with environmental pollution and/or high concentrations of ions, as occurs in waters with karstic origins (Drew and Hötzl 1999). The uncontrolled anthropic activities in the surroundings of the studied area can promote silting and pollution in drainages systems, including the subterranean domains. Most taxa recorded in subterranean habitats were rotifers, which appeared associated with alkaline pH and elevated conductivity. However, the other registered taxa were not strongly related to the measured parameters.

Fong and Culver (1994) found for some caves of the United States that the first order watercourses fed by percolation water have higher diversity than others of higher order. Generally, the water that drains directly from the ceiling can supply a better approximation of physical and chemical characteristics of the upper horizon. However, once in water pools, it frequently presents more different environmental conditions than in the epikarst.

Tropical versus temperate aquatic subterranean biota

Comparing the results obtained with studies on the unsaturated zone in temperate regions (Brancelj 2002, Pipan and Brancelj 2004a, Pipan et al. 2006; Moldovan et al. 2007, 2012, Meleg et al. 2011, Cottarelli et al. 2012), the tropical aquatic subterranean biota (mainly from the unsaturated zone) has a distinct composition. The richness demonstrated was very high compared to records from more extensive approaches, with a sampling effort along a couple of years (e.g., Pipan and Brancelj 2004a, Pipan et al. 2006). Furthermore, Copepoda was not the group with the highest diversity, as in samples of percolating water from many temperate regions, mainly in Europe. However, none of these studies have used nets with such thin mesh (smaller than 40μm), so the richness of Rotifera may have been underestimated. We also have to emphasize that the sampling method we used could have underestimated the number of benthonic taxa, such as Harpacticoida, commonly find in samples of groundwater (Pipan et al. 2008, Moldovan et al. 2012).

The presence of exclusive taxa at some sites emphasizes the singularity of the studied systems. There are faunistic differences between sites belonging to the same limestone lens and geographically neighbouring. Many species inhabiting the void network of the unsaturated zone are known to have a linear distribution of only a few hundred metres (Pipan et al. 2006, Moldovan et al. 2012), and a discontinuous distribution, caused by different environmental condition (Brancelj 2002, Meleg et al. 2011, Moldovan et al. 2012).

The structure of the unsaturated zone can vary significantly in different regions according to its lithology, geomorphic history, as well as environmental parameters and seasonal variables (Klimchouk 2004). The wide variability of rainfall regime in tropical zones is expected to impact on the tropical subterranean fauna and to offer different models of distribution and diversity than in temperate karst zones.

Final considerations

Nowadays, there is an incessant increase in agricultural, livestock and mining activities in many karst areas in Brazil. In the surroundings of the studied area, cattle and extensive plantations of soybean and cotton can trigger pollution and/or siltation of these streams. Besides, there are many land ownership problems and clandestine mining, which cause deforestation and endanger the integrity of the subterranean environments.

The richness and distribution of aquatic species vary dependent on water quality, and the unsaturated zone biota can be used as a bio-indicator for the impact of polluting actions (Dole-Olivier et al. 1994, Malard et al. 1996, Di Lorenzo et al. 2005, Galassi et al. 2009). The epikarstic zone acts as a reservoir as well as a disperser of toxic substances, considering its ample vertical and horizontal drainage system (Loop and White 2001, Williams 2008). Therefore, it is expected to find variations in the unsaturated zone communities also depending of the degree of pollution. The variations encountered in biological communities can be associated with local activities, being a useful tool in controlling aggressive human practices in the environment. Most stygobionts have a narrow distribution range, the risk of species extinction is thus expectedly high in face of the increase of multiple anthropogenic pressures (Danielopol et al. 2003, Gibert et al. 2009). In our samples, we could not identify stygobiont species because it requires more accurate taxonomic and ecological approaches. However, it is important to highlight that we found many taxa with distribution restricted to just one or few of the studied habitats.

To efficiently assess this particular fauna, it is necessary to constantly monitor the subterranean habitats by means of frequent sampling during different periods of the year.

As such, not only can species richness be recorded but it also can be verified whether there are differences in their distributions and abundances according to seasonality. The use of combined sampling methods (e.g. micrometric mesh nets and vacuum pumps) is also recommended to efficiently access both the benthonic and planktonic fauna.

In Brazil, studies on diversity of aquatic biota in subterranean environments are few and data is still sparse (Pinto-da-Rocha 1995, Bichuette and Santos 1998, Souza-Silva et al. 2012). No such works have been done on the karst unsaturated zone communities. The high richness of taxa presently recorded reveals the potential of unsaturated zone in the tropical region. This preliminary faunistic study represents the first approach on fauna inhabiting the unsaturated zone of the Brazilian karst and also a novelty considering the Neotropic karst systems.

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