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## The role of microhabitats in structuring cave invertebrate communities in Guatemala

Gabrielle S.M. Pacheco <sup>1\*</sup>, Marconi Souza Silva <sup>1</sup>, Enio Cano <sup>2</sup>,  
and Rodrigo L. Ferreira <sup>1</sup>

<sup>1</sup>Universidade Federal de Lavras, Departamento de Ecologia e Conservação, Setor de Biodiversidade Subterrânea, Centro de Estudos em Biologia Subterrânea, Caixa Postal 3037, CEP 37200-900, Lavras, Minas Gerais, Brasil

<sup>2</sup>Escuela de Biología, Facultad de Ciencias Químicas y Farmacia, Universidad de San Carlos de Guatemala, Ciudad Universitaria, Zona 12, 01012, Guatemala City, Guatemala

**Abstract:** Several studies have tried to elucidate the main environmental features driving invertebrate community structure in cave environments. They found that many factors influence the community structure, but rarely focused on how substrate types and heterogeneity might shape these communities. Therefore, the objective of this study was to assess which substrate features and whether or not substrate heterogeneity determines the invertebrate community structure (species richness and composition) in a set of limestone caves in Guatemala. We hypothesized that the troglobitic fauna responds differently to habitat structure regarding species richness and composition than non-troglobitic fauna because they are more specialized to live in subterranean habitats. Using 30 m<sup>2</sup> transects, the invertebrate fauna was collected and the substrate features were measured. The results showed that community responded to the presence of guano, cobbles, boulders, and substrate heterogeneity. The positive relationship between non-troglobitic species composition with the presence of guano reinforces the importance of food resources for structuring invertebrate cave communities in Guatemalan caves. Furthermore, the troglobitic species responded to different substrate features when compared to non-troglobitic species. For them, instead of the presence of organic matter, a higher variety of abiotic microhabitats seem to be the main driver for species diversity within a cave. The high specialization level of troglobitic organisms might be the reason why they respond differently to environmental conditions. The findings of this study highlight the importance of biological surveys for understanding cave biodiversity and give insights on how this biodiversity might be distributed within a cave. Conservation measures should keep in mind the target organisms and if such measures aim to protect a broad variety of organisms, then one should aim to preserve as many microhabitats and trophic resources as possible.

**Keywords:** arthropods, habitat heterogeneity, substrate composition, troglobites, Central America

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### INTRODUCTION

Patterns of species distribution and factors structuring communities have always been central subjects for ecological studies (Dunson & Travis, 1991; Kolasa & Pickett, 1991; Cushman & McGarigal, 2004; Steinitz et al., 2006; Talley, 2007). For caves, understanding these patterns and factors is fundamental for conservation purposes, as the organisms that inhabit these environments are known to be relatively sensitive to many stressors, both natural and anthropic (Howarth, 1983; Mammola, 2019; Mammola et al., 2019).

Several studies have tried to elucidate the main environmental factors driving the invertebrate community structure (species richness and composition) in cave environments. The main factors highlighted as important are seasonality (Tobin et al., 2013; Mammola et al., 2015; Bento et al., 2016; Lunghi et al., 2017; Kozel et al., 2019), lithology (Souza-Silva et al., 2011b), landscape structure (Christman et al., 2016; Pellegrini et al., 2016; Mammola & Leroy, 2017), distance from cave entrance (Ferreira & Martins, 1998; Prous et al., 2004; Tobin et al., 2013; Kozel et al., 2019), linear development of the cave (Simões et al., 2015; Pellegrini & Ferreira, 2016), presence

\*gabrielle.pacheco@hotmail.com

of water (Simões et al., 2015), resource availability (Ferreira & Martins, 1998; Gers, 1998; Jaffé et al., 2016; Ferreira, 2019), microclimate (Mammola et al., 2015; Lunghi et al., 2019) and speleogenesis (Sendra et al., 2014; Jiménez-Valverde et al., 2017). However, rare examples focused on how the substrate types and substrate heterogeneity might be shaping these communities within a cave (Prous et al., 2015; Pellegrini et al., 2016; Zepon & Bichuette, 2017).

It is currently known that invertebrates have a preferential selection of microhabitats inside the caves (Culver & Pipan, 2009; Moseley, 2009; Souza-Silva & Ferreira, 2009; Mammola et al., 2016). Such preferences for specific types of microhabitats are due to a variety of reasons, including behavioral, physiological, and morphological adaptations (Howarth, 1983; Zepon & Bichuette, 2017). These microhabitats encompass different abiotic (lakes, water bodies, puddles, types of substrates, and rocks of different textures and sizes) and biotic (roots, guano, vegetal debris, and carcasses) features (Ferreira et al., 2007; Culver & Pipan, 2009; Souza-Silva et al., 2011a; Simões et al., 2015). Therefore, the habitat heterogeneity increases the microhabitat availability for the invertebrate fauna (Zagmajster et al., 2018).

Organisms that are specialized to live in caves (i.e., troglobites) are, in many cases, rare and endemic. They are vulnerable to stochastic events, environmental disturbances, and anthropic impacts (Gibert & Deharveng, 2002; Culver & Pipan, 2009; Mammola et al., 2019). Furthermore, underground life provides environmental ecosystem services, such as the decomposition of organic matter, pollination, and insect control, features that have been attracting the attention of conservation researchers (Culver & Pipan, 2009; Mammola et al., 2019).

Guatemala has a variety of caves in quite distinct regions, although in a small territory. There is a reasonable amount of scientific work on the taxonomy of cave invertebrates in the country (see Reddell, 1981; Strinati, 1994; Reddell & Veni, 1996), but there is a noticeable gap when it comes to their ecology. This study, therefore, aimed to evaluate which substrate features influence the invertebrate community structure and how the substrate heterogeneity influences the invertebrate community structure in a set of Guatemalan caves. We hypothesized that places with higher substrate heterogeneity have higher species richness than places with lower substrate heterogeneity and that the troglobitic species respond differently to the habitat structure than non-troglobitic species because they are more specialized to live in subterranean habitats.

## MATERIALS AND METHODS

### Study area

Guatemala is a tropical country that features several environmental conditions within a relatively small geographic area (Strinati, 1994). With three different climate types (Equatorial Monsoon, Equatorial savannah with dry winter and Warm temperate climate with dry winter) (Kottek et al., 2006; Peel et

al., 2007), the country features an average of 3,000 to 4,000 mm of rain every year, with rains concentrated between May and October (Kottek et al., 2006; Peel et al., 2007). There is a set of limestone karstic landscapes that cover about one-third of its area. The karstic landscapes from Guatemala are divided into four main geologic domains, mostly in the northern portion of the country: Huehuetenango, Alta Verapaz, Izabal, and Petén (Strinati, 1994). This study was conducted in 10 limestone caves from two of these karstic regions: Alta Verapaz and Petén, on a region known as Northern Lowlands (Fig. 1; Table 1).

The caves sampled had different characteristics. They varied as to the presence/absence of water bodies, dripping activity, and the number of entrances. The caves also had different uses. Furthermore, most of the caves had remains of Mayan rituals, such as clay pots, candles, bones, and traces of bonfires.

Cueva Aktun Kan, Cúpula de los Murciélagos, Ventanas de Seguridad, Cueva Blanca, and Gruta de Lanquín have different levels of touristic activity. Cueva Aktun Kan and Gruta de Lanquín are located in places with high international tourism influx and are, therefore, highly explored touristically with almost no control whatsoever. The other touristic caves, in the other hand, are located in an area predominantly visited by local tourists and have more controlled adventure tourism. Cueva del Venado, Cueva el Rostro, Cueva de Arcilla, Cueva Coral, and Cueva Chipix do not have touristic activity.

### Field procedures

All data were collected in 30 m<sup>2</sup> (10 m x 3 m) transects throughout the caves. All transects were placed at least 25 m from each other. The number of transects per cave ranged from one to eight (Table 1), with a total of 25 transects. In this study, the number of transects per cave was not standardized for logistical reasons, therefore it was best suited to use the transects as sampling units instead of the caves. Since there are no studies that state how far apart cave sampling units should be in order to assume independency, we have assumed a 25 m distance between transects as a measure of independence among transects, especially considering the highly heterogeneous substrates sampled within each cave.

The invertebrate fauna was collected manually in the transects through direct intuitive searches (Wynne et al., 2019) and the sampling method was exhaustive (i.e., the researchers only stopped once there were no more invertebrates to be collected or accounted for). The invertebrates were collected with the aid of brushes and tweezers and placed in vial containing a solution of 70% ethanol for further examination and identification in the laboratory. When the abundance of invertebrates of the same species in a transect was high, a few specimens were collected and the remaining individuals were accounted for in the field notebook.

Afterwards, the substrate was characterized according to a field protocol adapted from Peck et al. (2006) and Hughes and Peck (2008). This protocol consists of dividing each transect into 10 sections of

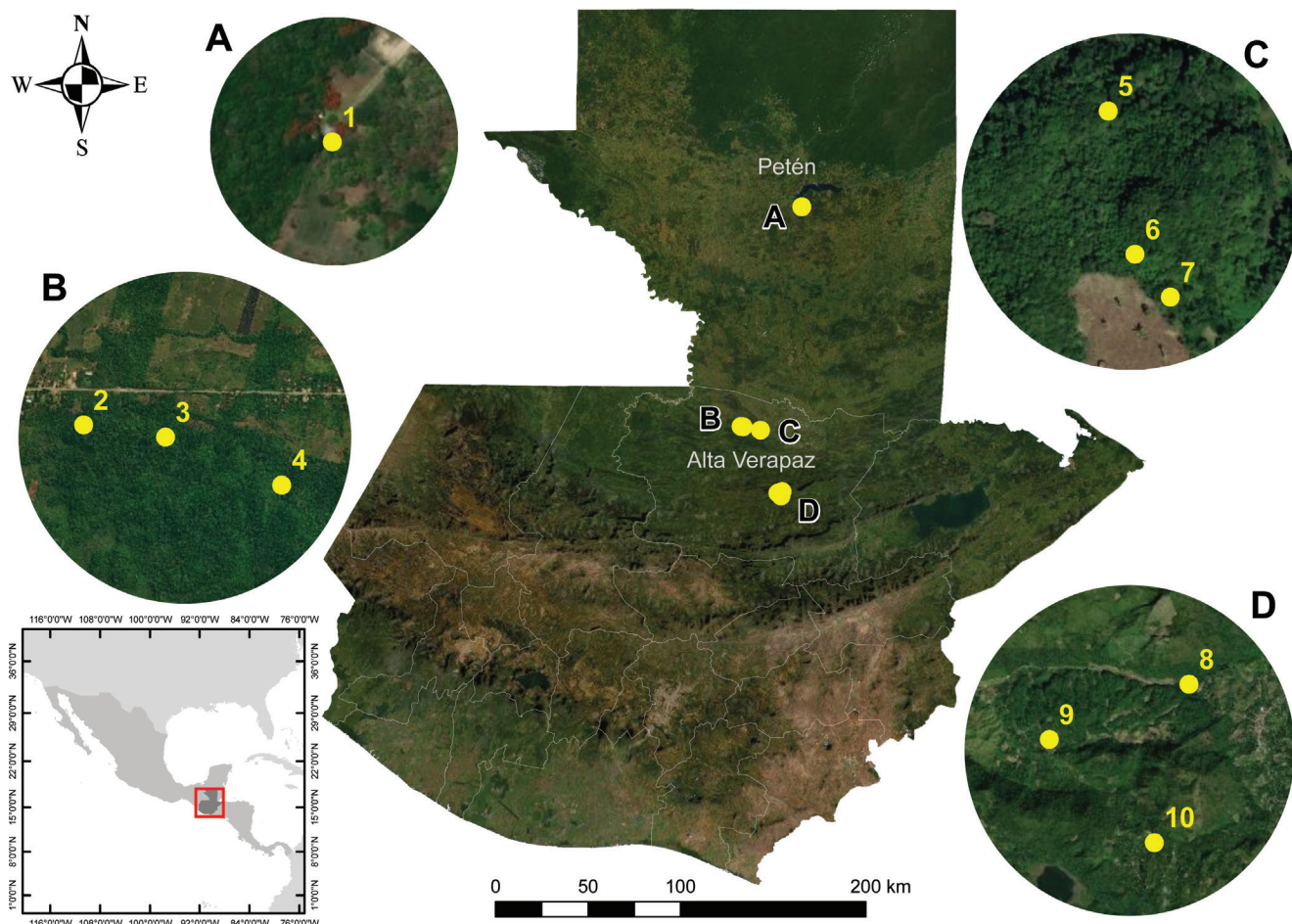


Fig. 1. Location of the ten caves sampled in Guatemala. A = Petén: 1) Cueva Aktun Kan. B, C and D = Alta Verapaz: 2) Cúpula de los Murciélagos; 3) Cueva del Venado; 4) Ventanas de La Seguridad; 5) Cueva Blanca; 6) Cueva El Rostro; 7) Cueva de Arcilla; 8) Gruta de Lanquín; 9) Cueva Coral, and 10) Cueva Chipix.

Table 1. Cave names, coordinates UTM - Datum: WGS-84 (X, Y), altitude (Alt), and number of transects sampled in each cave (N).

Cave name	Department	Municipality	X	Y	Zone	Alt (m)	N
Cueva Aktun Kan	Petén	Flores	191539	1871065	16Q	174	3
Cúpula de los Murciélagos	Alta Verapaz	Raxruhá	801025	1758019	15P	215	3
Cueva del Venado	Alta Verapaz	Raxruhá	801412	1757969	15P	218	3
Ventanas de Seguridad	Alta Verapaz	Raxruhá	801964	1757753	15P	220	2
Cueva Blanca	Alta Verapaz	Raxruhá	811098	1756305	15P	180	2
Cueva El Rostro	Alta Verapaz	Raxruhá	811125	1756173	15P	208	1
Cueva de Arcilla	Alta Verapaz	Raxruhá	811159	1756134	15P	187	1
Gruta de Lanquín	Alta Verapaz	Lanquín	179249	1724774	16P	299	8
Cueva Coral	Alta Verapaz	Lanquín	820737	1723971	15P	482	1
Cueva Chipix	Alta Verapaz	Lanquín	178710	1722477	16P	642	1

one meter each and estimating the percentage of each substrate type. In this case: guano, roots, leaves, twigs, trunks, waterbody, water puddle, actinomycetes, fine sediment (0.06 – 64 mm), cobbles (64 – 1000 mm), boulders (1000 – 4000 mm), matrix rock (>4000 mm) and human interventions (bridges, pathways, and steps made of concrete).

Because we have chosen a visual estimation method for evaluating the substrate percentages, three steps were taken in order to minimize observer error: (i) all transects were characterized by the same person; (ii) the percentage values obtained in the 10 sections in a transect summed and divided by 10 (arithmetic mean), resulting in a single percentage value for each substrate type in the transect and (iii) these percentage ranges allocated into five categories:

<1% = **0**; 1 – 5% = **1**; 5 – 25% = **2**; 25 – 50% = **3**; 50 – 75% = **4**; 75 – 100% = **5**.

### Species identification and troglomorphisms

Invertebrates were identified until the lowest possible taxonomic level with the aid of taxonomic keys and then grouped into morphospecies (Oliver & Beattie 1996). This approach was chosen due to a large number of new species found in caves, combined with the lack of literature and specialists on some of the taxa. Still, specimens of the taxa Acari, Diplura, Isopoda, Opiliones, Palpigradi, and Pseudoscorpiones were identified by specialists.

Specimens that featured morphologic traits that indicate adaptations to life in the cave environment were considered to be potentially troglobitic. These

characteristics classically include the elongation of locomotor and sensory appendages, increased number of trichobothria, absence or reduction of ocular structures, body pigmentation, and wings (Barr, 1968; Hoch & Ferreira, 2012; Novak et al., 2012).

### Data analysis

To test the influence of the percentage of each substrate type (guano, roots, leaves, twigs, trunks, waterbody, water puddle, actinomycetes, fine sediment, cobbles, boulders, matrix rock, human interventions) and substrate heterogeneity on species composition and richness, Distance-Based Linear Models (DistLM) were performed in Primer-e and Permanova + Software (Anderson et al., 2008). The model selection was made using the  $R^2$  selection criterion and forward selection procedure. These models had the transects as sample units and only significant predictor variables were included in the final best models. To observe the effects on species composition, we built the resemblance matrix using the Bray Curtis similarity index with the invertebrate data transformed into the squared root, in order to mitigate the effect of large species abundances and to observe the effects on species richness, we built models using an Euclidean Distance matrix.

To test our main hypotheses, the above-cited analyses were performed for non-troglobitic species and then repeated using only troglobitic species. Significance was regarded at  $p \leq 0.05$ .

## RESULTS

A total of 10,354 specimens were registered in the 25 sampled transects. They were distributed into 38 orders, at least 78 families and 177 species, 24 of which were considered troglobites (Fig. 2 and [Supplementary Material](#)). The troglobitic fauna includes one species of Araneae (Corinnidae), one Coleoptera (Tenebrionidae), two Diplura (Campodeidae), five Collembola (four Entomobryidae and one Symphypleona), one Geophilomorpha, two Isopoda (Philosciidae and Styloniscidae), two Zygentoma (Nicoletiidae), two Opiliones (Samoidae and Stygnopsidae), four Polydesmida, and four Pseudoscorpiones (one Bochicidae and three Chthoniidae). The highest non-troglobitic species richness was found in the transects of Gruta de Lanquín (29 spp.), Cueva Chipix (29 spp.), and Cueva Aktun Kan (28 spp.), while the highest troglobitic species richness was found in the transects of Gruta de Lanquín (6 spp.), Cueva Blanca (4 spp.), and Cueva Aktun Kan (4 spp.).

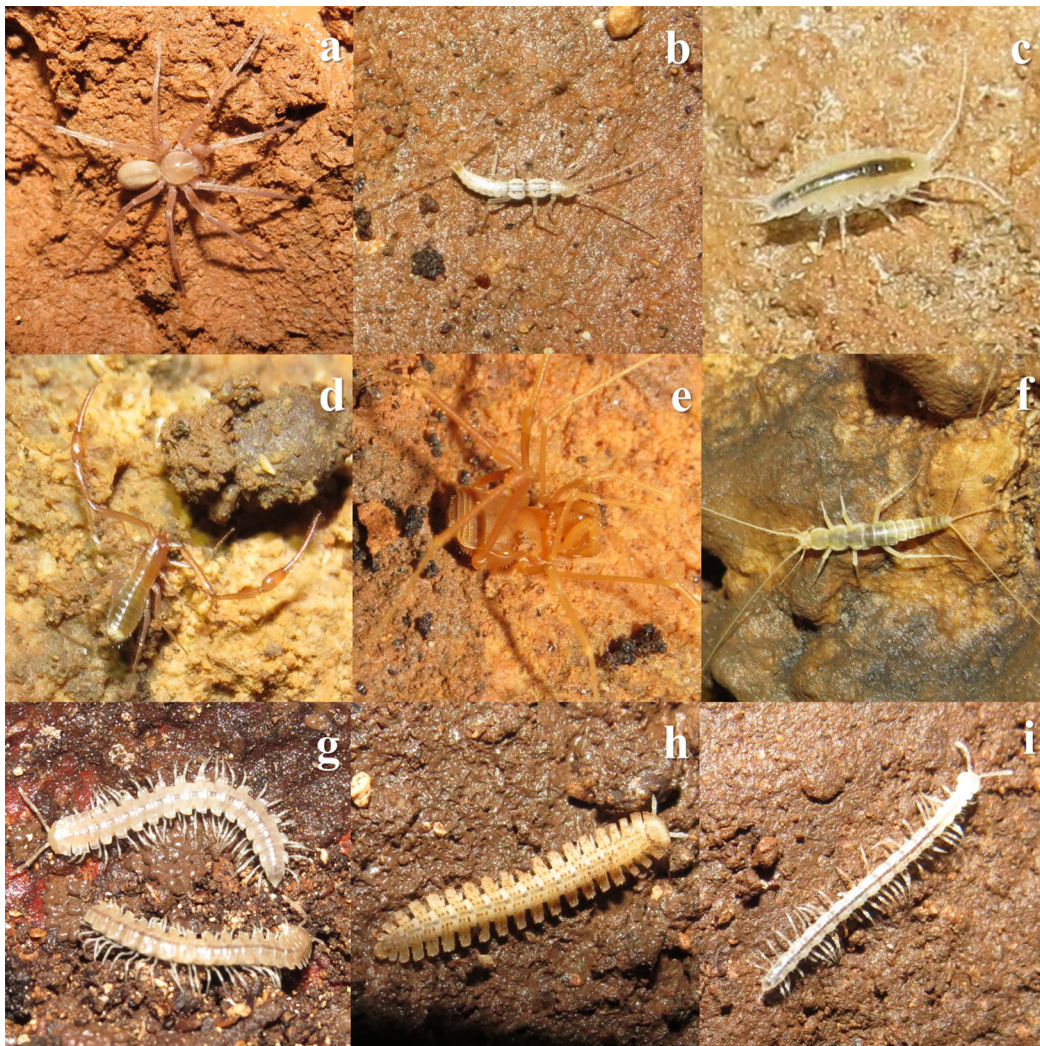


Fig. 2. Some of the troglobitic species sampled in Guatemala caves. a) Araneae (Corinnidae); b) Diplura (Campodeidae, *Juxtacampa* sp. n.); c) Isopoda (Styliniscidae); d) Pseudoscorpiones (Ideoroncoidae, *Typhloroncus guatemalensis*); e) Opiliones (Stygnopsidae); f) Thysanura (Nicoletiidae, *Anelpistina* sp. n.); g) Polydesmida (Chelodesmidae); h) Polydesmida (Pyrgodesmidae); i) Polydesmida (Paradoxosomatidae).

The most abundant substrate type was fine sediment, followed by matrix rock, guano, cobbles, and boulders, present in 24, 20, 16, 14, and 11 of the 25 transects, respectively ([Supplementary Material](#)). Each transect had an average of 4.19 (sd =  $\pm 1.48$ ) different types of substrates. The transects with higher substrate heterogeneity were located in Cueva Chipix (transect 10,  $H' = 2.008$ ), Cueva Coral (Transect 9,  $H' = 1.864$ ), and Gruta de Lanquín (transects 1 and 4,  $H' = 1.748$ ) ([Supplementary Material](#)).

The non-troglobitic species composition variation was best explained by the presence of guano, cobbles,

and substrate heterogeneity, which explained together 23.1% of the variation on the community on the sequential tests and 27.9% of the non-troglobitic species richness variation was best explained by the substrate heterogeneity (Table 2). As for the troglobitic species, the variation in species composition was best explained in the model by the presence of boulders, substrate heterogeneity, and cobbles, totalizing 24.3% of explanation power on the model, while 20% of the troglobitic species richness variation was best explained by the presence of boulders (Table 2).

Table 2. Summary of the DistLM models describing troglobitic and non-troglobitic species composition variations (**Subs heter** = Substrate heterogeneity; **Prop** = Proportion of explanation of the model; **R<sup>2</sup>adj** = R squared adjusted).

			Guano	Cobble	Boulder	Subs heter
Species composition	Non-Troglobitic	Prop	0.111	0.065	-	0.056
		R <sup>2</sup> adj	0.073	0.102	-	0.123
		Pseudo-F	2.896	1.738	-	1.547
		p-value	<0.01*	<0.01*	-	<0.05*
	Troglobitic	Prop	-	0.064	0.099	0.080
		R <sup>2</sup> adj	-	0.083	0.058	0.129
		Pseudo-F	-	0.616	2.417	2.116
		p-value	-	<0.05*	<0.01*	<0.05*
Species richness	Non-Troglobitic	Prop	-	-	-	0.279
		R <sup>2</sup> adj	-	-	-	0.247
		Pseudo-F	-	-	-	8.904
		p-value	-	-	-	<0.01*
	Troglobitic	Prop	-	-	0.2	-
		R <sup>2</sup> adj	-	-	0.163	-
		Pseudo-F	-	-	5.511	-
		p-value	-	-	<0.05*	-

## DISCUSSION

The species richness and composition found in this study are unprecedented for Guatemala. Previous studies focused mostly on descriptions of new species and thus failed to showcase the broad diversity of invertebrate species in Guatemalan caves (Barr, 1973; Gertsch, 1973; Schultz, 1977; Platnick & Pass, 1982; Rodriguez & Hobbs Jr, 1990; Espinasa & Zhuang, 2009; Viana & Ferreira, 2019) with rare exceptions (Mitchell & Reddell, 1973; Reddell, 1981; Reddell & Veni, 1996).

The linear models showed that the troglobitic species composition is more influenced by the substrate heterogeneity than the non-troglobitic, but only non-troglobitic species richness is influenced by substrate heterogeneity. Thus, for troglobitic species, the substrate heterogeneity might not be providing suitable habitats to a larger number of species, but instead for a larger number of individuals. This is probably a result of the high specialization of troglobitic organisms. While for the non-troglobitic fauna, the variety of substrates provides suitable habitats for an important increase in both species richness and composition.

In our study, non-troglobitic species behaved as already showed in the literature, as it is known that heterogenous habitats can shelter a larger number of species by providing more available habitats,

allowing a decrease in ecological niche overlap and reducing competitive exclusion (Poulson & Culver, 1969; Ferreira & Souza-Silva, 2001; Tews et al., 2004; Stein et al., 2014; Pellegrini et al., 2016; Resende & Bichuette, 2016). Environments formed by the agglomeration of gravel, for example, comprise void spaces that can provide terrestrial invertebrate species with microhabitats, food resources, protection, and refuge (Mammola et al., 2016).

Since most caves can be characterized as oligotrophic environments when compared to the surface environments (Fiser, 2019; Fong, 2019; Trontelj, 2019), the local presence of organic resources often means that these places have a higher diversity of invertebrate fauna. Several authors have already shown the importance of guano deposits and vegetal debris inside the cave environment (Decu, 1986; Gnaspini, 1989; Ferreira & Martins, 1998; Ferreira & Martins, 1999; Ferreira et al., 2000; Ferreira et al., 2007; Santana et al., 2010; Schneider et al., 2011). Beyond providing habitat availability, they also act as the main food sources for the lower levels of the food web, allowing the occurrence of greater species richness (Pellegrini & Ferreira, 2012; Pellegrini & Ferreira, 2013; Jaffé et al., 2016; Ferreira, 2019). The direct and significant relationship between non-troglobitic species composition with the presence of guano reinforces the importance of food resources for the structuring of Guatemalan cave invertebrate communities.

The presence of guano tends to be of extreme importance in permanently dry caves (Ferreira et al., 2007; Ferreira, 2019). In this study, even though most of the caves had water bodies, the guano was still a strong structurer for the cave invertebrate communities. Here, the presence of guano seems to be more important for the invertebrates than the presence of other types of organic matter (actinomycetes and vegetal debris). The abundance of guano in a cave can eventually attract a huge variety of invertebrates, most of them non-troglobites, which can lead to the emergence and establishment of large invertebrate populations and consequently a high level of biological interactions onto it. The presence of these communities on an ephemeral resource such as guano might repel the troglobitic species, which are often more sensitive, from these habitats (Ferreira et al., 2007), although there are exceptions (Ferreira, 2019).

Accordingly, the troglobitic species seem to be influenced differently from the non-troglobitic within the caves, responding to different substrate features when compared to non-troglobitic species. For them, the presence of organic matter, regardless of its source, does not seem to be the main driver for species diversity within a cave. Instead, troglobitic species composition seems to be more related to a higher variety of abiotic microhabitats (cobbles, boulders, and substrate heterogeneity) and troglobitic species richness, to the presence of boulders. The high specialization level of troglobitic organisms might be the reason why they respond differently to environmental conditions, in this case, substrate characteristics and heterogeneity. The lack of a statistically significant relationship between troglobitic species richness and substrate heterogeneity is a sign of such specialization level since instead of filling all available ecological niches and habitats, they seek for specific conditions inside the cave environment. In other words, substrate heterogeneity can influence which troglobitic species are inhabiting a cave but not how many. Troglobitic organisms might have suffered different ecological pressures over time when compared to troglaphiles and troglaxenes, hence occupying an entirely different niche. Being adapted to the dark and oligotrophic cave environment, they can inhabit places that are not tolerable for most cave dwellers (Kozel et al., 2019).

The relatively low explanatory values obtained in the linear models were expected due to the methodology chosen for this study. There is likely a range of other parameters influencing these communities. Therefore, future studies should incorporate as many variables as possible to further investigate and elucidate the main factors influencing cave invertebrate fauna.

The findings of this study highlight the importance of biological surveys for understanding cave biodiversity and give insights on how this biodiversity might be distributed within a cave. Conservation biologists should keep in mind to target as many microhabitats as possible to maximize the effectiveness of protection measures. Furthermore, it provides a scientific biological background for the creation of new parameters for the management and conservation of caves in Guatemala.

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