Do different relevance attributes indicate the same conservation priorities? A case study in caves of southeastern Brazil

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Abstract: In the last decade, the scientific community brought to the debate gaps that slow down the advance of knowledge regarding global biodiversity. More recently, this discussion has reached subterranean environments, where these gaps are even more dramatic due to the relict and vulnerable nature of their species. In this context, we tested ecological metrics related to some of these gaps, checking if the biological relevance of the caves would change depending on ecological attributes related to each metric. The study was carried out in caves from southeastern Brazil, located in a region presenting a high richness of troglobitic species restricted to a narrow geographical extent. Thus, we verified: (a) the cave invertebrate communities' vulnerability with the Vulnerability Index and the Importance Value for Cave Conservation; (b) the distribution and endemism of the troglobitic species with the Endemism Index; (c) the phylogenetic diversity of the troglobitic species considering the average taxonomic distinction ($\Delta+$), their richness and evenness. We observed a considerable change in the ordering of the caves' biological relevance according to each tested attribute (index). We discussed how each of these metrics and their attributes indirectly relate to: (a) the preservation and maintenance of the phylogenetic diversity of subterranean communities; (b) the spatial restrictions of different groups, where the greater their restrictions, the greater their vulnerability; (c) the preservation of caves with high biological relevance considering these different attributes together. Thus, we recommend the use of different metrics so that different ecological attributes can be considered, supporting actions that aim to preserve caves in highly altered regions. Finally, we find that the most biologically important cave in the region is not protected (Gruta da Morena Cave). We warn that this cave needs to be contemplated by a conservation unit in the region urgently.

Keywords: Ecological metrics, cave ecology, nature conservation, subterranean biology, knowledge shortfalls
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INTRODUCTION

There is a long list of factors that negatively influence the knowledge regarding the diversity of the invertebrates on the planet, from purely political issues to major scientific dilemmas (Cardoso et al., 2011a, b). These factors are treated as large deficits that slow down the advance of knowledge about biodiversity (Hortal et al., 2015). For invertebrates, they were originally compiled by Cardoso et al. (2011b), which discussed how the negligence regarding this group can harm strategies for their conservation. More recently, other studies have expanded this issue to biodiversity in general (Hortal et al., 2015) and for subterranean

environments (Ficetola et al., 2019; Mammola et al., 2019). In general terms, these gaps involve the taxonomic impediment, the species' distribution, and their biological, ecological, and evolutionary aspects (Cardoso et al., 2011b; Hortal et al., 2015; Ficetola et al., 2019; Mammola et al., 2019).

Since subterranean environments are known to shelter highly specialized and relict fauna (Poulson & White, 1969), inevitably these deficits become even more dramatic. The obligate subterranean fauna usually presents disconnected and limited distributions to one or a few caves, as a result of biogeographical and historical factors that caused its isolation (Ribera et al., 2018). Additionally, these

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relictual patterns are even observed in non-obligate cave fauna (Bryson et al., 2014; Yoshizawa et al., 2019). Naturally, the isolation allied to the extinction of epigean populations causes the high phylogenetic singularity of the subterranean fauna at the local level (Poulson & White, 1969; Gibert & Deharveng, 2002). Thus, their phylogenetically closest relatives are usually observed only when considering a regional or global scale (Benítez-Álvarez et al., 2020).

However, the actual distribution of most subterranean species remains unknown, especially in tropical regions (Zagmajster et al., 2010, 2018), and is well-known only for certain groups whose phylogeny is well resolved (Zagmajster et al., 2018). In addition, karst landscapes are under huge human pressure across the globe (Mammola et al., 2019), sometimes leading to the destruction of subterranean habitats (Whitten, 2009). Even when considering local-scale impacts, the high connectivity through the rocky interstices and the karst hydrology favor the disturbances dissemination, thus enhancing the extension of the affected areas (Grünewald, 2001; Mulec, 2014; Mulec et al., 2017). Hence, studies aiming to describe subterranean species, as well as to determine their distributions and other ecological aspects that fill these gaps have become a real race against time (Mammola et al., 2019).

Given this scenario, studies started to propose priority caves for conservation by using different indexes of biological relevance developed specifically for these environments (Simões et al., 2014; Souza-Silva et al., 2015; Nitzu et al., 2018; Rabelo et al., 2018). Some of those indexes consider the degree of the anthropization of caves and aspects of their biological community (Donato et al., 2014; Simões et al., 2014; Souza-Silva et al., 2015), while others consider the distribution range of cave-restricted species, thus measuring the degree of endemism in caves (Nitzu et al., 2018). There are also highly sophisticated indexes developed for groundwater species, considering their distribution, niche, population, and evolution (Fattorini et al., 2020).

Thus, by comparing caves considering the phylogenetic singularity of specialized organisms, their distribution, and the whole subterranean community in a context of high anthropic pressure, would the ordering of priority of these caves remain the same? To answer this question, we assessed the subterranean invertebrate community in a group of caves in southeastern Brazil, according to: (a) the phylogenetic diversity among the obligate subterranean organisms with the **Average taxonomic distinction** (Warwick & Clarke, 1995), **species richness**, and **evenness**; (b) The distribution and the degree of endemism in each cave with the **Endemicity Index** (Nitzu et al., 2018); (c) The biological relevance and the vulnerability of the caves, with the **Vulnerability Index** (Simões et al., 2014) and the **Importance Value for Cave Conservation (IV-CC)** (Borges et al., 2012). Considering these different attributes, we assumed *a priori* that the caves could present different priorities according to each metric. Thus, this work discusses the ecological implications of using these different metrics in a conservation context.

MATERIAL AND METHODS

Study area

The study was conducted in 15 limestone caves located in the municipalities of Cordisburgo (13 caves) and Curvelo (2 caves), Minas Gerais, Brazil (Table 1, Fig. 1). Cordisburgo karst is part of the Bambuí Geological Group (from the Upper Proterozoic) widely distributed in the states of Minas Gerais, Goiás, and Bahia (Auler & Farrant, 1996). Four of the inventoried caves (Gruta de Maquiné, Gruta do Salitre, Gruta IV, and Gruta I caves) are located inside the *Monumento Natural Estadual Peter Lund*, a conservation unit established in 2005 by the government of the Minas Gerais State (Decree N° 44.120, 2005). The local vegetation is composed of semideciduous seasonal forest present on top of hills and surrounding the karst features, becoming denser and covering karst outcrops, especially in rainy periods (Travassos, 2010).

Table 1. Physical attributes of the 15 caves sampled at Cordisburgo karst area, Minas Gerais, Brazil: Cave localization in Latitude (Lat.) and Longitude (Long.) expressed in decimal degrees and datum WGS84. Entrance width (or summed width for caves with multiple entrances) (EW), Number of entries (NE); Sampled extension (SE). (*) indicates caves inside the limits of Monumento Natural Estadual Peter Lund.

Caves	Lat	Long	EW (m)	NE	SE (m)
Lapinha do Atamis	-19.142032°	-44.355766°	10	1	120
Gruta do Tobogã	-19.197336°	-44.332360°	15	2	300
Gruta IV*	-19.119370°	-44.351518°	1	1	60
Gruta Lagoa da Pedra	-19.081591°	-44.358186°	12	1	141
Gruta da Mata	-19.171889°	-44.313071°	2	2	224
Lapa da Onça	-19.186165°	-44.364858°	4	2	144
Gruta Santo Amaro I	-19.067175°	-44.342033°	30	1	273
Gruta Santo Amaro II	-19.067138°	-44.341913°	12	2	560
Gruta da Morena	-19.168794°	-44.338828°	70	7	2800
Gruta da Represinha	-19.131460°	-44.353629°	1	1	80
Gruta I*	-19.126847°	-44.355111°	3	1	20
Gruta II	-19.126847°	-44.368090°	8	1	30
Gruta do Salitre*	-19.121918°	-44.351499°	5	1	1098
Gruta do Meio	-19.169971°	-44.339082°	25	3	200
Gruta de Maquiné*	-19.122568°	-44.351116°	27	1	1312

Fig. 1. Cordisbugo karst area and caves sampled. Four caves are located inside the Monumento Natural Peter Lund, shown in orange (see inset A).

The ecoregion is the Cerrado (Olson et al., 2001), which is considered a global biodiversity hotspot (Myers et al., 2000). The climate is humid subtropical influenced by monsoon (CWA in Köppen classification), with hot summers and rainfall ranging from 1,300 to 1,600 mm annually (Alvares et al., 2013).

Determining abiotic and biotic parameters

The sampled extensions and the cave entrances widths (in its highest extensions) were determined with laser measuring tapes and from cave maps when available. Direct intuitive search and manual collection of invertebrates were carried out along the accessible extension of each cave, with special attention given to organic deposits and micro habitats (Wynne et al., 2018, 2019; Souza-Silva et al., 2020). In order to standardize the sampling effort, the same team of five biologists carried out the collections in the fifteen caves, and each collector spent on average 1 minute per square meter of cave floor searching for invertebrates. Terrestrial invertebrates were collected with tweezers and brushes, and aquatic invertebrates were collected with tweezers and entomological nets in puddles and under running water, and then stored in 70% alcohol (Rabelo et al., 2018; Souza-Silva et al., 2020). In the laboratory, these organisms were separated into morphotypes (Oliver & Beattie, 1996) and identified to the lowest possible taxonomic level with taxonomic keys and assistance from specialists. Data based on morphotypes still have the robustness to effectively replace data based on described species (Oliver & Beattie, 1996). All the collected specimens are deposited in the Subterranean Invertebrate

Collection of Lavras (ISLA) of the Center of Studies on Subterranean Biology (www.biologiasubterranea.com.br/en).

The determination of potentially troglotic species was carried out through the identification of troglomorphisms, which are indicative of isolation and evolution in subterranean environments (Christiansen, 1962; Barr, 1968; Poulson & White, 1969; Novak et al., 2012). All species presenting troglomorphisms were considered troglotes. Thus, by standardization, the term “troglotic species” is used throughout the text.

Testing the different ecological metrics

Applying the Vulnerability Index (Simões et al., 2014)

According to the original methodology proposed by Simões et al. (2014), three components were used to calculate the Vulnerability Index (hereinafter referred to as VI): the richness of troglotic species (TbR), the biological relevance (BR), and the human impacts on caves (HI). Following the VI original conception, the highest richness value of troglotic species found was divided by four, creating categories of low, average, high, and extreme richness, which received weights from 1 to 4, respectively. The biological relevance (BR) was obtained based on the total species richness (TR) and relative richness (RR). The relative richness was obtained from an adaptation of the original index method. Here, with the richness and sampled extension data, we used the SAR (Species-area relationship) function of the BAT package (Cardoso et al., 2015) in R 4.0.2 software (R Core Team, 2021), to verify which model had the best fit for the species/area relationship (see Supplementary Information,

[Supplementary Tables S1 and S2](#)). With the model, we extracted the residual values for each cave, which served as a metric to indicate the relative richness. The highest values of TR and RR were divided by four, creating categories of low, average, high, and extreme richness. The categories of RR received weights from 1 to 4 and those of TR received weights from 2 to 8. These weights were then summed and the highest result was divided by four, thus establishing the categories of the biological relevance (BR): low, average, high, and extreme, which also received weights from 1 to 4.

The impacts observed in each cave were considered as three distinct types of changes that can lead to depletion (a), enrichment (b), or modification (c) of microhabitats, organic, or fauna resources. These changes were classified and weighed according to their potential (tenuous-1, intense-2), their permanence (short-1, continuous-3), and the scope (localized-1, general-2). Subsequently, the values of these items were summed to obtain the final value of each impact. As in the biological component of the index, the largest sum of impacts measured in the caves was divided by four to define four categories, from low to extreme, which were weighted from 1 to 4. Finally, the summed values of the components of the richness of troglotic species (TbR), biological relevance (BR), and impacts (HI) determined four categories, obtained by dividing the largest sum by four, thus indicating the degree of vulnerability of the cavities: Extreme (E), High (H), Average (A) and Low (L) (Simões et al., 2014).

Applying the Importance Value for Cave Conservation (IV-CC) index (Borges et al., 2012)

The Importance Value for Cave Conservation (IV-CC) is a multi-criteria index based on a scoring approach originally proposed by Borges et al. (2012) to rank the Azorean caves according to their conservation priorities. This index includes nine different indicators that reflect biological and geological information, as well as the management features of the caves. The biological indicators originally used were the species richness of troglotic arthropods (Strogl); the species richness of Azorean endemic arthropods (including troglonites, trogloniles and troglonenes) (Send); and the number of rare species, which is equivalent to the number of troglonites that occur in only one island (Srare). In our study, the indicator "Send" is the number of troglonites that occur in a single cave. We used only information about the troglotic species here due to the difficulties of determining whether the trogloniles and troglonenes are endemic to the region. The majority of invertebrates sampled are not identified at the species level and many of them can belong to new taxa. Furthermore, we also adapted the indicator "Srare". It represents herein the number of troglonites occurring in up to three caves, which is following the concept of rare troglonites present in the Brazilian law that regulates the use and exploration of the speleological heritage (Ministério do Meio Ambiente, 2009).

The other indicators represent the geology, threats, and management features of the caves: show cave (Show), based on information about the cave size and

diversity of scenic structures; geology (Geo), based on the relevance of speleothems and other geological structures; difficulty of exploration (Dif. Expl.), based on a gradient of cave progression by visitors; integrity (Integrity), based on the percentage of the cave that remains in its pristine state; the anthropogenic threats index (Threats), based on the presence and possible consequences of human disturbance in the epigean environment; and accessibility (Access), based on how easily people can access the cave. This information was obtained from direct observation of the characteristics of each cave and its surroundings during the fieldwork and from technical reports. The list of the indicators describing geology, threat, and management features and their respective scores is available in Appendix 1 of Borges et al. (2012).

Since the value of some indicators may be related, we applied generalized linear models (GLM) with natural logarithm link functions before generating the formula for calculating the index, in order to avoid problems of collinearity between the variables. We performed an iterative partial regression analysis, in which each iteration extracts the variability of a predictor that is independent of that formerly chosen. The total number of troglotic species (Strogl.) was selected as the first indicator to be included without any transformation in the IV-CC calculations due to its major importance to cave conservation. Subsequently, we regressed the second indicator (B) against "Strogl.", obtaining its residuals (rB). In successive steps, each indicator (e.g., C) is regressed against the previously included values in a multiple regression analysis to obtain its residuals (rC). The other indicators were added in the formula according to the decreasing order of their r^2 values resulting from a GLM regression of each index with "Strogl.". Thus, in our study, we obtained the following formula to calculate the IV-CC index for each cave:

$$\text{IV-CC} = [(\text{Strogl.} / \text{Strogl. max}) + (\text{RSrare} / \text{RSrare max}) + (\text{RSend.} / \text{RSend. max}) + (\text{RGeo} / \text{RGeo max}) + (\text{RShow} / \text{RShow max}) + (\text{RIntegrity} / \text{RIntegrity max}) + (\text{R1/Access} / \text{R1/Access max}) + (\text{RThreats} / \text{RThreats max}) + (\text{R1/Dif.Expl.} / \text{R1/Dif.Expl. max})] / 9$$

In the IV-CC index, the residual variance (R) of each of the additional indicators for a given cave is divided by the maximum residual value (max) obtained within all caves. Furthermore, we used the inverses of the indicators Dif. Expl. and Access, as suggested by Borges et al. (2012). This index has a maximum value of 1. Finally, caves were classified as above or below the IV-CC overall mean, following the original publication of the index.

Applying the Endemicity Index (Nitzu et al., 2018)

The Endemicity Index (hereinafter referred to as EI), proposed by Nitzu et al. (2018), was used to list the caves according to the endemism values of troglotic species, based on their presence/absence data. The higher the EI value assigned to the cave, the greater the number of single-occurrence species in it. The ranges of occurrences of species in the caves

were initially defined based on a logarithmic scale (4 intervals in this study: in 1 cave; in 2 caves; in 3 or 4 caves; from 5 to 9 caves). Formulas and calculations are available in the [Supplementary Data](#). In the sequence, each species received a weight according to its occurrence class ($W = 1$; $W = 2$; $W = 4$; $W = 9$), which were used to calculate the EI of each cave. In the end, the caves were classified according to their EI values, in decreasing order (Nitzu et al., 2018). EI classes were obtained by dividing the highest value found by four to establish four distinct categories (Extreme = $EI > 9.83$; High = $EI \leq 9.83$ e > 6.55 ; Average = $EI \leq 6.55$ e > 3.28 ; Low = $EI \leq 3.28$).

Applying the Average Taxonomic Distinctness (Warwick & Clarke, 1995) and *Pielou's evenness* (Pielou, 1966)

The average taxonomic distinctness (hereinafter referred to as Δ^+) was calculated from the presence/absence of the troglotic species, considering a 95% confidence interval (the funnel) obtained in a simulation with 1000 permutations. Δ^+ is defined as the average length to phylogenetic nodes between two random species occurring in a sample, through a taxonomic hierarchy that receives different weights according to each of its levels (Clarke & Warwick, 1998, 1999). This metric is treated as a more robust alternative compared to other diversity indexes, as it does not depend on the sample size and the sampling effort applied between different samples (Clarke & Warwick, 1998; Clarke & Gorley, 2006). Average values are used in their construction and independent of the number of species or abundance values at the samples (Clarke & Warwick, 1998; Clarke & Gorley, 2006). The Δ^+ values converge from the values of taxonomic diversity (Δ) and taxonomic distinctness (Δ^*) when presence/absence data are used (Clarke & Warwick, 1998). In practical terms, the greater the Δ^+ , the greater the phylogenetic diversity of the community being assessed. For their calculation, the respective weights were used: Phylum (weight 100), class (weight 80), order (weight 60), family (weight 40), and morphotypes (weight 20). Finally, caves were classified as above or below the Δ^+ overall mean.

The Pielou's evenness (Pielou, 1966) was calculated in order to show the homogeneity of taxa variability in each cave. This index varies from 0 to 1, where values next to 1 represent communities whose species had more homogeneous relative abundances. This

metric was calculated using the raw abundances of troglotic species.

Final classification of the caves according to the parameters evaluated

The order of relevance of the caves was made by observing the overlapping of the indices that use some form of categorization in their methodology (Extreme, High, Average, and Low in EI and VI, the last with their components of richness of troglotic species and total richness; Above or Below the overall mean in Δ^+ and IVCC). Pielou's evenness was not used to categorize the caves because it devalues those caves with rare or very dominant species. In this way, caves ranked in the highest categories of at least two indexes were considered to be of extreme relevance (Cardoso et al., 2021). The other caves were ranked first according to their richness of troglotic species and second according to their total richness, aiming at the subsequent preservation of the highest number of species.

A correlation matrix was built to verify the possible correlations between all evaluated indexes, the richness of troglotic species, and the total richness. In this step, Spearman correlations were used after attempts to normalize the data did not have a satisfactory effect.

The Δ^+ were calculated with the Primer6 software (Clarke & Gorley, 2006). The IV-CC index, the Pielou's evenness, and the correlations matrix with the scatterplots were performed in R 4.0.2 software (R Core Team, 2021).

RESULTS

Biotic parameters

A total of 34,445 invertebrates distributed in 749 morphotypes were recorded, belonging to 48 orders and at least 167 families (Supplementary Information, [Supplementary Table S3](#)). The richest orders were Coleoptera (20.5% of the total species found), Diptera (15.8%), and Araneae (10.8%) (Fig. 2). Dermaptera, Geophilomorpha, Scolopendromorpha, Lithobiomorpha, Nematoda, Nemertea, Hirudinea, Ostracoda, Megaloptera, Plecoptera, Protura are not shown in the figure because they presented only one species each. The average richness was 80.73 (± 47.84) species per cave. With 214 species, the Gruta da Morena Cave showed the highest total richness and had the largest sampled extension (2,800 meters).

Fig. 2. Richness and composition of the main higher taxa of the 15 caves sampled at Cordisburgo karst area, Minas Gerais, Brazil.

Among the 15 sampled caves, 14 presented troglotitic species (Supplementary Information, [Supplementary Table S4](#)). Regarding the richness of these species, the Gruta de Maquiné Cave, with 16 species, and the Gruta da Morena Cave, with 15 species, presented the highest concentrations of troglotitic species.

In total, 40 troglotitic species were found (Supplementary Information, [Supplementary Table S4](#)), some of which are shown in Figure 3. These species are distributed in the orders Palpigradi (Eukoeneriidae), Isopoda (Styloniscidae and Platyarthridae), Araneae (Ochyroceratidae, Oonopidae, Prodidomidae), Mesostigmata, Opiliones (Escadabiidae and Cryptogeobiidae), Pseudoscorpiones (Chthoniidae), Coleoptera (Carabidae and Staphylinidae), Collembola (Cyphoderidae and Arrhopalitidae), Polydesmida (Oniscodesmidae, Pyrgodesmidae, and Trichopolydesmidae), Polyxenida (Hypogexenidae), Diplura (Japygidae) and Zygentoma. We also found troglotitic species of Nemertea, Hirudinea and

Pauropoda. The most frequent troglotitic species was Cyphoderidae sp1 (Collembola), presenting 2,731 individuals distributed in nine caves. The troglotitic spider *Lygromma ybyguara* from Gruta de Maquiné was also included in the list of species that occur in this cave. Although no individual of this species was found in this study, it is an element of the troglotauna already reported from the Cordisburgo region.

Priority caves according to the Vulnerability Index

Considering the biotic components of the Vulnerability index, the Gruta da Morena and Gruta de Maquiné caves presented an extreme richness of troglotitic species (TbR). Gruta do Salitre and Gruta da Represinha caves presented average richness and the remaining caves presented low richness of troglotitic species. Three caves presented extreme biological relevance (BR), five presented high biological relevance, and seven presented average biological relevance.

Fig. 3. Some of the obligate cave species from Cordisburgo karst area, Minas Gerais, Brazil: A) *Eukoeneria maquinensis* (Palpigradi); B) *Eukoeneria sagarana* (Palpigradi); C) *Spaeleoleptes spaeleus* (Escadabiidae); D) Ochyroceratidae (Araneae); E) *Spinopilar moria* (Cryptogeobiidae); F) Mesostigmata; G) Chthoniidae (Pseudoscorpiones); H) Oniscodesmidae (Diplopoda); I) Trichopolydesmidae (Polydesmida); J) Hypogexenidae (Polyxenida); K) Styloniscidae (Isopoda); L) *Trichorhina* sp. (Platyarthridae); M) *Pectenoniscus* sp. (Styloniscidae); N) *Oxarthrus* sp. (Pselaphinae).

The impacts observed in the caves and their surroundings are listed in Table 2 and some of them are shown in Figure 4. The most common use observed around the caves was the replacement of the original vegetation by pasture for cattle breeding and *Eucalyptus* crops. Other impacts are those resulting from visiting the caves, such as trampling, garbage, and artificial lighting. Gruta de Maquiné Cave was included in the

category of extreme degree of impact. Gruta Lagoa da Pedra and Gruta da Morena caves were classified in the category of average degree of impact and the other caves in the category of low degree of impact.

After all, Gruta de Maquiné and Gruta da Morena caves were considered extremely vulnerable. Among the other caves, two presented high vulnerability and eleven had average vulnerability (Table 3).

Table 2. Human impact assessment in the 15 caves at Cordisburgo karst area, Minas Gerais state, Brazil. (Alt) alterations; (a) depletion; (b) enrichment; (c) modifications; (Pot) potential; (I) intense; (T) tenuous; (Per) permanence; (S) short; (C) continuous; (Ext) extension; (G) general; (L) localized; (W) weights; (Σ) final impact weight ($= \Sigma W_{Pot} + \Sigma W_{Per} + \Sigma W_{Ext}$).

Code. Human Impacts	Alt	Pot	W	Per	W	Ext	W	Σ
1. Deforested surroundings	-	-	-	-	-	-	-	1
2. Agropastoral practice surroundings	-	-	-	-	-	-	-	1
3. Saltpeter extraction	c	T	1	S	1	L	1	3
4. Drainage exploitation in caves	a	I	2	C	3	L	1	6
5. Palaeontological excavations	c	T	1	S	1	L	1	3
6. Trampling by humans in caves	c	I	2	C	3	G	2	7
7. Electric illumination in caves	b+c	T+I	1+2	C	3+3	G	2+2	13
8. Garbage/trash in caves	c	T	1	S	1	L	1	3
9. Construction sites in caves	c	T	1	S	1	L	1	3
10. Dam at the cave entrance (damming water inside the cave)	c	T	1	C	3	L	1	5
11. Dam at the cave entrance (preventing water from entering the cave)	a+c	I	2+2	C	3+3	G	2+2	14
12. Killing of bats in caves	a+c	I	2+2	S	1	L	1	6
13. Road near caves	c	T	1	C	3	L	1	5

Fig. 4. Human impacts observed in the caves sampled in Cordisburgo karst area and their surroundings: A) Water reservoir near the entrance of the Gruta da Represinha Cave; B) Dam in entrance of Gruta Lagoa da Pedra Cave; C) Broken speleothems in Gruta da Mata Cave. Impacts in Gruta de Maquiné Cave: D) Masonry constructions at the entrance; E) Spotlights for artificial lighting; F) Power cables to support artificial lighting; G) Lamp bulb covered by calcite; H) Lampenflora with a Collembola in the detail.

Table 3. Classification of the 15 caves sampled in this study according to their degree of vulnerability, assessed by the index proposed by Simões et al. (2014). The impacts observed in each cave are indicated as numerical superscripts, according to Table 2. The following acronyms are employed: (Σ HI; TbR; TR; RR; BR) sum of all impacts, richness of troglobitic species, total richness, relative richness and biological relevance of each cave, respectively; (HIC; TbRC; TRC; RRC; BRC) category of each cave according to its degree of impact, the richness of troglobitic species, total richness, relative richness, and biological relevance, respectively (L = low; A = average; H = high; E = extreme); (HIW; TbRW; TRW; RRW; BRW) weights assigned to each cave according to its category of the degree of impact, richness of troglobitic species, total richness, relative richness, and biological relevance; (HI+TbR+BR) sum of the weights assigned to each cave according to its category of the degree of impact, richness of troglobitic species and biological relevance; (VI) vulnerability index.

Caves ^{impacts from table 2 codes}	Σ HI	HIC	HIW	TbR	TbRC	TbRW	TR	TRC	TRW	RR	RRC	RRW	BR	BRC	BRW	HI+TbR+BR	VI
Gruta de Maquiné ^{1,5,6,7,8,9,13}	35	E	4	16	E	4	75	A	4	-48.5154	L	1	5	A	2	10	E
Gruta da Morena ^{6,8}	10	A	2	15	E	4	214	E	8	12.96424	H	3	11	E	4	10	E
Gruta do Salitre ³	3	L	1	7	A	2	146	H	6	33.63336	E	4	10	E	4	7	H
Gruta da Represinha ¹	1	L	1	5	A	2	52	L	2	-7.33188	A	2	4	A	2	5	A
Gruta I	0	L	1	1	L	1	54	A	4	-2.20606	H	3	7	H	3	5	A
Gruta do Meio	0	L	1	1	L	1	108	H	6	42.41648	E	4	10	E	4	6	H
Lapinha do Atamis ^{1,2}	2	L	1	0	L	1	54	A	4	-7.41576	A	2	6	H	3	5	A
Gruta do Tobogã ^{1,2,13}	7	L	1	1	L	1	101	A	4	30.20678	E	4	8	H	3	5	A
Gruta Lagoa da Pedra ^{1,2,11}	16	A	2	1	L	1	48	L	2	-14.5098	A	2	4	A	2	5	A
Gruta da Mata ^{1,2,12}	8	L	1	3	L	1	79	A	4	12.16615	H	3	7	H	3	5	A
Gruta Santo Amaro II ^{1,2,8}	5	L	1	3	L	1	54	A	4	-30.3384	L	1	5	A	2	4	A
Gruta II ^{1,2,10}	7	L	1	2	L	1	100	A	4	43.27297	E	4	8	H	3	5	A
Gruta IV	0	L	1	1	L	1	32	L	2	-26.2899	L	1	3	A	2	4	A
Lapa da Onça ^{1,2}	2	L	1	3	L	1	52	L	2	-10.6661	A	2	4	A	2	4	A
Gruta Santo Amaro I ^{1,2}	2	L	1	1	L	1	42	L	2	-27.3866	L	1	3	A	2	4	A

Priority caves according to the IV-CC index

The scores of all indicators included in the IV-CC index for each cave sampled in this study are available in Table 4. This table also shows the ranking of caves according to the decreasing value of the IV-CC index.

Among those with a score higher than the overall mean (0.005), there are large caves (Gruta do Salitre, Gruta do Tobogã, and Gruta da Morena), small caves (Gruta da Mata and Lapa da Onça), and also Gruta de Maquiné, a large show cave included in a preserved area.

Table 4. Richness of troglobitic species (Stroglo), of endemic troglobionts (Send) and rare troglobionts (Srare) of the 15 caves studied, as well as scores of the indicators selected to describe their geology, threat, and management features (Show, Geo, Dif. Expl., Integrity, Threats, and Access). In the last column, the 15 caves sampled in this study are ranked according to the Importance Value for Conservation Index (IV-CC). The caves with an IV-CC value higher than the overall mean (0.005) are shown in bold.

Caves	Stroglo	Send	Srare	Show	Geo	Dif. Expl.	Integrity	Threats	Access	IV-CC
Gruta do Salitre	7	5	7	5	4	1	5	5	1	0.297
Gruta da Mata	3	1	1	3	1	5	5	3	3	0.262
Gruta do Tobogã	1	0	0	5	1	1	5	3	3	0.174
Lapa da Onça	3	2	2	2	1	5	5	3	3	0.129
Gruta da Morena	15	12	14	5	3	2	5	2	3	0.117
Gruta de Maquiné	16	9	14	5	4	5	1	2	5	0.078
Gruta I	1	0	0	1	1	5	5	5	1	-0.009
Gruta do Meio	1	0	1	4	1	2	5	3	3	-0.014
Gruta Santo Amaro II	3	0	1	4	1	5	5	3	3	-0.019
Gruta II	2	1	1	1	1	5	3	2	3	-0.041
Gruta IV	1	0	1	1	1	5	5	5	2	-0.07
Gruta da Represinha	5	1	4	1	1	1	5	3	3	-0.103
Gruta Santo Amaro I	1	0	1	3	1	5	5	3	3	-0.132
Lapinha do Atamis	0	0	0	2	1	5	5	3	3	-0.197
Gruta Lagoa da Pedra	1	0	0	2	1	5	3	2	3	-0.307

Priority caves according to the Endemicity Index

The Gruta da Morena and Gruta de Maquiné caves presented the highest number of single-occurrence troglobitic species (12 and 9, respectively). Hence, both were considered as presenting maximum relevance according to the EI. Except for Gruta do Salitre Cave,

with 5 single-occurrence species and presenting average relevance, the other caves presented wide-distributed troglobitic species, thus presenting low relevance according to the EI. The species occurrence data (W) and the caves classification according to the EI are shown in Table 5.

Table 5. Occurrence of troglobitic species according to the classes of the EI: W = 1, species restricted to a single cave; W = 2, species occurring in two caves; W = 4, species occurring in 3 or 4 caves; W = 9, species occurring between 5 and 9 caves. The last column shows the final classification of the caves according to the Endemicity Index: E = Extreme; H = High; A = Average; L = Low.

Caves	W=1	W=2	W=4	W=9	EI
Gruta da Morena	12	2	-	1	13.11 (E)
Gruta de Maquiné	9	3	3	1	11.36 (E)
Gruta do Salitre	5	1	1		5.75 (A)
Lapa da Onça	1	2	1	1	2.36 (L)
Gruta da Represinha	2	-	1	-	2.25 (L)
Gruta da Mata	1	-	1	1	1.36 (L)
Gruta II	1	-	-	1	1.11 (L)
Gruta Santo Amaro II	-	-	2	1	0.61 (L)
Gruta IV	-	1	-	-	0.5 (L)
Gruta do Meio	-	1	-	-	0.5 (L)
Gruta Santo Amaro I	-	-	1	-	0.25 (L)
Gruta I	-	-	-	1	0.11 (L)
Gruta Lagoa da Pedra	-	-	-	1	0.11 (L)
Gruta do Tobogã	-	-	-	1	0.11 (L)
Lapinha do Atamis	-	-	-	-	0 (L)

Classification of caves according to the Average Taxonomic Distinctness and Pielou's evenness

The taxonomic distinctness analysis showed that 8 of the 13 caves with troglobitic species have values of $\Delta+$, ranging from 72 in Gruta da Represinha Cave to 80.57 in Gruta da Morena Cave (Fig. 5). The other five caves had $\Delta+$ values equal to zero because only one troglobitic species was recorded in them. Except for those caves with $\Delta+$ values equal to zero, all other caves were within the 95% confidence interval (the funnel). Gruta do Salitre, Gruta da Represinha and Lapa da Onça caves presented values of $\Delta+$ below

the average value for the region (central horizontal line). With the largest number of troglobitic species, Gruta da Morena Cave presented the highest average taxonomic distinction ($\Delta+ = 80.57$) followed by Gruta II, Gruta da Mata, and Gruta Santo Amaro II caves ($\Delta+ = 80$). The $\Delta+$ values of the other caves are shown in Table 6.

With 3 troglobitic species, Lapa da Onça cave had de highest evenness value ($J = 0.95$). On the other hand, Gruta de Maquiné Cave, the richest in terms of troglobitic fauna with 16 species, had the lowest evenness value ($J = 0.11$) (see Table 6).

Fig. 5. Values of the $\Delta+$ (AvTD) using the presence/absence data of troglobitic species in caves of Cordisburgo karst area. The funnel represents the interval with 95% of confidence after 1000 permutations. The horizontal line represents the $\Delta+$ overall mean for the region.

Final classification of the caves

The correlations between metrics are shown in Figure 6, which shows, for example, a significant positive correlation between the troglobitic richness and all indexes analyzed, except with the VI. The final classification of the caves according to the metrics is shown in Table 6. When comparing the rankings obtained by the caves according to the different

metrics, it is possible to see that their ordering changed substantially.

The Gruta da Morena Cave was the most recorded cavity in the highest categories of the indexes tested (6 times), followed by the Gruta de Maquiné (5) and Gruta da Mata (2) caves. Because these cavities have been indicated in these categories at least twice, they have been classified as being of extreme relevance.

Table 6. Comparison of the caves classification according to the different metrics tested and the number of metrics that they lead. E = Extreme relevance; H = High relevance; A = Average relevance; L = Low relevance; Aa = Above average value; Ba = Below average value; TbS = Richness of trogloditic species; TR = Total Richness; AvTD = Average Taxonomic Distinctness; J = Pielou's evenness; VI = Vulnerability Index; IVCC = Importance Value for Cave Conservation; EI = Endemicity Index. *Ordination made according to the TbS and TR components of VI. The caves in the highest categories are in bold. Final rank (based on records in at least two highest categories of the metrics evaluated).

Cave	TbS*	Cave	TR*	Cave	AvTD	Cave	J
Gruta de Maquiné	16 (E)	Gruta da Morena	214 (E)	Gruta da Morena	80.57 (Aa)	Lapa da Onça	0.951891454
Gruta da Morena	15 (E)	Gruta do Salitre	146 (H)	Gruta da Mata	80 (Aa)	Gruta II	0.918295834
Gruta do Salitre	7 (A)	Gruta do Meio	108 (H)	Gruta Santo Amaro II	80 (Aa)	Gruta Santo Amaro II	0.897608283
Gruta da Represinha	5 (A)	Gruta do Tobogã	101 (A)	Gruta II	80 (Aa)	Gruta do Salitre	0.670139825
Gruta da Mata	3 (L)	Gruta II	100 (A)	Gruta de Maquiné	76.33 (Aa)	Gruta da Represinha	0.668920666
Gruta Santo Amaro II	3 (L)	Gruta da Mata	79 (A)	Gruta do Salitre	73.33 (Ba)	Gruta da Mata	0.451259687
Lapa da Onça	3 (L)	Gruta de Maquiné	75 (A)	Lapa da Onça	73.33 (Ba)	Gruta da Morena	0.198998754
Gruta II	2 (L)	Gruta I	54 (A)	Gruta da Represinha	72 (Ba)	Gruta de Maquiné	0.117065171
Gruta I	1 (L)	Lapinha do Atamis	54 (A)	Gruta I	0 (Ba)	Gruta I	0
Gruta do Meio	1 (L)	Gruta Santo Amaro II	54 (A)	Gruta do Meio	0 (Ba)	Gruta do Meio	0
Gruta do Tobogã	1 (L)	Gruta da Represinha	52 (L)	Lapinha do Atamis	0 (Ba)	Lapinha do Atamis	0
Gruta Lagoa da Pedra	1 (L)	Lapa da Onça	52 (L)	Gruta do Tobogã	0 (Ba)	Gruta do Tobogã	0
Gruta IV	1 (L)	Gruta Lagoa da Pedra	48 (L)	Gruta Lagoa da Pedra	0 (Ba)	Gruta Lagoa da Pedra	0
Gruta Santo Amaro I	1 (L)	Gruta Santo Amaro I	42 (L)	Gruta IV	0 (Ba)	Gruta IV	0
Lapinha do Atamis	0 (L)	Gruta IV	32 (L)	Gruta Santo Amaro I	0 (Ba)	Gruta Santo Amaro I	0
Cave	VI	Cave	IVCC	Cave	EI	Cave	Final rank
Gruta de Maquiné	10 (E)	Gruta do Salitre	0.297 (Aa)	Gruta da Morena	13.11 (E)	Gruta da Morena	6 (E)
Gruta da Morena	10 (E)	Gruta da Mata	0.262 (Aa)	Gruta de Maquiné	11.36 (E)	Gruta de Maquiné	5 (E)
Gruta do Salitre	7 (H)	Gruta do Tobogã	0.174 (Aa)	Gruta do Salitre	5.75 (A)	Gruta da Mata	2 (E)
Gruta do Meio	6 (H)	Lapa da Onça	0.129 (Aa)	Lapa da Onça	2.36 (L)	Gruta do Salitre	0 (7 TbS)
Gruta da Represinha	5 (H)	Gruta da Morena	0.117 (Aa)	Gruta da Represinha	2.25 (L)	Gruta da Represinha	0 (5 TbS)
Gruta I	5 (A)	Gruta de Maquiné	0.078 (Aa)	Gruta da Mata	1.36 (L)	Gruta Santo Amaro II	0 (3 TbS, 54 TR)
Lapinha do Atamis	5 (A)	Gruta I	-0.009 (Ba)	Gruta II	1.11 (L)	Lapa da Onça	0 (3 TbS, 52 TR)
Gruta do Tobogã	5 (A)	Gruta do Meio	-0.014 (Ba)	Gruta Santo Amaro II	0.61 (L)	Gruta II	0 (2 TbS)
Gruta Lagoa da Pedra	5 (A)	Gruta Santo Amaro II	-0.019 (Ba)	Gruta IV	0.5 (L)	Gruta do Meio	0 (1 TbS, 108 TR)
Gruta da Mata	5 (A)	Gruta II	-0.041 (Ba)	Gruta do Meio	0.5 (L)	Gruta do Tobogã	0 (1 TbS, 101 TR)
Gruta II	5 (A)	Gruta IV	-0.07 (Ba)	Gruta Santo Amaro I	0.25 (L)	Gruta I	0 (1 TbS, 54 TR)
Gruta Santo Amaro II	4 (A)	Gruta da Represinha	-0.103 (Ba)	Gruta I	0.11 (L)	Gruta Lagoa da Pedra	0 (1 TbS, 48 TR)
Gruta IV	4 (A)	Gruta Santo Amaro I	-0.132 (Ba)	Gruta Lagoa da Pedra	0.11 (L)	Gruta Santo Amaro I	0 (1 TbS, 42 TR)
Lapa da Onça	4 (A)	Lapinha do Atamis	-0.197 (Ba)	Gruta do Tobogã	0.11 (L)	Gruta IV	0 (1 TbS, 32 TR)
Gruta Santo Amaro I	4 (A)	Gruta Lagoa da Pedra	-0.307 (Ba)	Lapinha do Atamis	0 (L)	Lapinha do Atamis	0 (0 TbS, 54 TR)

Fig. 6. Scatterplot matrix with Spearman correlations calculated using the different metrics (AvTD = Average Taxonomic Distinctness; J = Pielou's evenness; VI = Vulnerability Index; IV.CC = Importance Value for Cave Conservation; EI = Endemicity Index), the richness of troglitic species and the total richness. The * indicates significant correlations with $p < 0.05$, ** correlations with $p < 0.01$ and *** correlations with $p < 0.001$. Red lines correspond to the linear fit. Grey regions correspond to the 5% confidence intervals.

DISCUSSION

The results corroborated our hypothesis indicating that the ordering of biological relevance of the caves is altered considerably according to the application of distinct metrics. The raw values of most of the metrics (except the IVCC and VI, which had a positive correlation with TR) did not show significant relationships with the total species richness. This independence reinforces its applications in subterranean communities, minimizing the risk of misinterpretations due to possible correlations. However, except VI, all metrics were highly dependent on the richness of troglitic species. This was already expected because this is a parameter with great weight in its constructions.

Furthermore, despite these correlations, it is crucial to note that caves with few troglitic species (except Gruta da Morena Cave, with 15 troglitic species and $\Delta^+ = 80.57$) still showed high values of Δ^+ , revealing a high taxonomic singularity among their species. In the same way, caves with fewer species of single occurrence but with many wide-distributed species (and consequently with lower values of EI) still showed high values of Δ^+ . This indicates that the troglitic species widely distributed in the region contribute a lot to the phylogenetic diversity of each cave.

After comparing all of the metrics analyzed, the cave with the greatest total richness (TR), with the largest number of troglitic species with limited distributions (EI), and with the highest taxonomic singularity

among these species (Δ^+), was the Gruta da Morena Cave. This cave is unfortunately located outside the limits of the conservation unit located in the region and is not covered by any protection measure. As noted, the biological relevance of the caves obtained by different metrics changes substantially, so one should consider such differences when applying a given metrics for conservation purposes. Although in certain places other parameters are evaluated for the definition of priority areas for conservation (in Brazil, for example, scenic beauty, historical and cultural relevance, among others, are frequently included), we recommend that the parameters tested here to be taken into account for the definition of priority caves for conservation. Otherwise, caves that have unique ecological attributes in the local context may not be included in preservation measures, as was the case of the Gruta da Morena Cave.

Regarding the indexes applied, in recent years, Δ^+ has been used in ecological and conservation studies to illustrate caves with high phylogenetic diversity in tropical regions (e.g., Gallão & Bichuette, 2015; Bichuette et al., 2019; Souza-Silva et al., 2020). As informed in the methodology, Δ^+ was originally developed as an alternative to traditional biodiversity measures, such as the Shannon index, being applied to coastal gradients under increasing environmental contamination (Warwick & Clarke, 1995). For conservation purposes, phylogenetic diversity is a central point as it is related to the functional and evolutionary diversity of the community (Winter et al., 2013). In this sense, it is argued that the preservation of species that diverge in their phylogeny at the highest hierarchical levels results in the maintenance of evolutionary and functional processes that tend not to overlap (Cadotte et al., 2011).

However, we recommend the use of Δ^+ with caution. If its application is carried out considering the entire cave community, its values can be highly inflated by the para-epigeal community, which is notoriously much more diverse (Prous et al., 2004, 2015). Furthermore, caves with few troglotic species can have high Δ^+ values (as we can see in the Gruta da Mata Cave, with three troglotic species and Gruta II, with two troglotic species). This fact occurs because the more species are found in a cave, the greater the chance of phylogenetic redundancy between them (and functional redundancy, especially due to their truncated trophic webs, e.g., Gibert & Deharveng, 2002). Hence, if only this metric is used, caves with few species can be considered a priority over others with more species and consequently with greater phylogenetic and even functional redundancy. This is the opposite of what conservation biology proposes, where the more species preserved, the better. In this way, it is highly advisable that other ecological attributes be used together with Δ^+ , as presented here.

Regarding Pielou's equitability index, the interpretation of the results presented should be made, again, with great caution. Although the results showed that the Lapa da Onça Cave presents the community whose species has the most uniform abundances, in a conservation context we believe that this result

should never be used alone because of the rarity of species. For example, the Gruta de Maquiné Cave has the lowest equitability value as a consequence of highly abundant species in detriment of very rare ones. For this reason, we do not recommend the use of the equitability index for conservation studies of subterranean environments. In those cases, the uniformity ranked by this index masks one of the most striking characteristics of these environments, which is the rarity and endemism of species.

Considering the relictual nature of cave species distribution, the EI was developed to favor species that occur in narrow geographical areas (Nitzu et al., 2018). Its logarithmic scale of occurrences favors species with single or double occurrences, which ends up satisfying the context of isolation and high endemism of the subterranean environments (Nitzu et al., 2018). In this work, although the inventoried caves are located very close to each other (within a 16 km radius), 30 unique occurrences were recorded in only seven caves. Twelve of these single-occurrence troglotic species were recorded only in the Gruta da Morena Cave. The application of EI, however, must take into account that other sampling events may increase the records of troglotic species in other caves, impacting the distribution intervals (W) used in EI. Bearing this in mind, areas where there is a wide knowledge of subterranean fauna compiling several sample events can result in very safe diagnoses (Nitzu et al., 2018). Even so, its application reinforces the unique occurrences of troglotic species, which was evidenced in Cordisburgo karst area thanks to the description of some species observed in other sampling events and whose occurrences were shown to be the same in this work. For example, *Eukoenenia sagarana* (Souza & Ferreira, 2012) and *Eukoenenia maquinensis* (Souza & Ferreira, 2010) occur only in Gruta da Morena and Gruta de Maquiné caves, respectively, being highly dependent on specific microhabitats and consequently vulnerable to disturbances in the environment.

Regarding Vulnerability Index, the impacts recorded in the region, as deforestation and dams in the caves, have great potential to destabilize the fragile cave environment. The original vegetation of the Cordisburgo region was partially replaced by agriculture and *Eucalyptus* crops (Travassos, 2010), activities that often overlap the locations of the caves (visible in Fig. 1). This removal of the pristine vegetation cover weakens the soil structure, which in the rainy season causes sediments to be carried through the drainages, silting the cave ducts and reducing the availability of habitat. The dams at the cave entrances completely alter the water regime of the caves, which is one of the main responsible for the resource input in aquatic habitats (Souza-Silva et al., 2012) and involuntary transport of fauna during flooding events (Pacioglu et al., 2019). Other important impacts observed are due to tourist activities and intense visitation, such as the installation of artificial lighting, access structures, and the presence of waste. These impacts, in turn, can result in the establishment of lampenflora (Piano et al., 2015) and severe changes

in the subterranean microbiota (Mulec, 2014; Mulec et al., 2017). Some caves of Cordisburgo attract a lot of visitors mainly due to their scenic and scientific relevance (Travassos, 2010). There are criticisms regarding the lack of control of this activity, especially because caves of great scientific importance are outside the conservation unit that exists in the region (Trajano et al., 2009).

Considering the biological components of VI, one of the main advantages of this index is to consider both the troglomorphic and troglone fauna in the counting of the total richness of the caves. Although not restricted to these environments (what makes them therefore neglected by other indexes), these organisms are crucial for the maintenance of the subterranean trophic dynamics and safeguard a portion of the biodiversity available on the surface (Gibert & Deharveng, 2002; Jaffé et al., 2016). VI also increases the relevance of caves with high species densities, which at first sight can be disregarded because they have small dimensions (Simões et al., 2014; Rabelo et al., 2018). This was the case of the Gruta do Meio Cave, which presented a great relative richness value despite having average dimensions, identified as the fourth most relevant cave according to VI.

On the other hand, the application of the IV-CC index resulted in the indication of different caves as priorities for conservation when compared to the other indexes. Despite using some biological attributes similar to those used in the VI and EI indexes, it also includes geological and management features to predict the conservation value of the caves. While Gruta da Morena or Gruta de Maquiné appears as a priority for conservation according to the other indexes, the IV-CC indicates the Gruta do Salitre as having a greater conservation value. This is related to the fact that all troglonites that occur in this cave are rare and most of them are endemic, having not been recorded in other caves. In addition, it is a large cave with a high diversity of geological structures, including rare speleothems. Yet, it presents a well-preserved surrounding area, since it is within the limits of Monumento Natural Estadual Peter Lund.

In general terms, the high subterranean biodiversity of the Cordisburgo karst seems to reflect a pattern of the high richness of troglonitic species that extends through the north-central regions of the state of Minas Gerais (Rabelo et al., 2018) and northeastern Brazil (Souza-Silva & Ferreira, 2016; Trajano et al., 2016). However, many of these regions have not yet been explored with the necessary scientific scrutiny. In our study, of the 40 troglonitic species listed, only five are formally described: *Eukoeneria sagarana* (Souza & Ferreira, 2012), *Eukoeneria maquinensis* (Souza & Ferreira, 2010), *Spinopilar moria* (Kury & Pérez-González, 2008), *Spaeleoleptes spaeleus* (Soares, 1966), and *Lygromma ybyguara* (Rheims & Brescovit, 2004), all of them included in some category of threat in the Red Book of Brazilian Threatened Fauna (ICMBio/MMA, 2018).

The high number of species not described and whose ecology remains obscure to science still feed the gaps addressed in the introduction. Furthermore, even

though many of them are experiencing a continuous decline in the quality of their habitats, they cannot be targets for conservation actions because they are not formally described. The description and exploration of the biological and ecological aspects of the species observed in this and other regions are fundamental for the gaps explored in this work to be filled, aiming for a better synthesis of global subterranean biodiversity.

CONCLUDING REMARKS

Far beyond mere classes of relevance outlined for conservation strategies, conservation indexes and other biodiversity metrics should be explored mainly by emphasizing which ecological parameter is being verified. As already known, the perfect index or metric, capable of satisfying several ecological parameters, does not exist if used alone. They must be applied together to thoroughly explore different ecological aspects of the communities (Gallardo et al., 2011). As discussed, the indexes must be applied considering their limitations so that a more accurate application and critical interpretation be possible. Another important point is that even if correlated, the ordering of relevance pointed out by distinct metrics can be completely different. Despite the several shortfalls in biodiversity knowledge (Cardoso et al., 2011b; Hortal et al., 2015; Mammola et al., 2019) and the several ecological indexes that have been extensively explored in the last decades (e.g., Gallardo et al., 2011; Rabelo et al., 2018; Cardoso et al., 2021), inexplicably, its use still seems to remain inefficient for decision making in some places. In this sense, although other attributes are considered for the establishment of conservation units in Brazil, we observe that the most unique cave in the Cordisburgo Karst region regarding its fauna is not inserted in any conservation unit. Thus, we recommend the Gruta da Morena Cave be contemplated by some conservation units urgently.

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and MSS performed data collection. DAA, MFVRS, and MSS performed the analyses. DAA, MFVRS, MSS, and RLF led the pertinent discussions to the elaboration of the manuscript. DAA and MFVRS designed the final version of the manuscript with the approval of all authors.

SUPPLEMENTARY MATERIAL

Two supplementary files are provided. [Supplementary Information](#) contains four tables with the values of the models testing the fit of species/area relation, the residuals of the chosen model, the richness of the taxa and the distribution of the troglobitic species. [Supplementary Data](#) contains the raw data and software codes used to calculate the metrics.

REFERENCES

- Alvares, C.A., Stape, J.L., Sentelhas, P.C., De Moraes Gonçalves, J.L., Sparovek, G., 2013. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, 22(6), 711-728.
<https://doi.org/10.1127/0941-2948/2013/0507>
- Auler, A.S., Farrant, A.R., 1996. A brief introduction to karst and caves in Brazil. *Proceedings of the University of Bristol Speleological Society*, 20(3), 187-200.
- Barr, T.C., 1968. Cave ecology and the evolution of troglobites. In: Dobzhansky, T., Hecht, M.K., Steere, W.C. (Eds.), *Evolutionary biology* (1st ed.). Springer, Boston, p. 35-102.
https://doi.org/10.1007/978-1-4684-8094-8_2
- Benítez-Álvarez, L., Leal-Zanchet, A.M., Ocegueda-Figueroa, A., Ferreira, R.L., Bento, D.M., Braccini, J., Sluys, R., Riutort, M., 2020. Phylogeny and biogeography of the Cavernicola (Platyhelminthes: Tricladida): Relicts of an epigeal group sheltering in caves? *Molecular Phylogenetics and Evolution*, 145, 106709.
<https://doi.org/10.1016/j.ympev.2019.106709>
- Bichuette, M.E., Simões, L.B., Zepon, T., von Schimonsky, D.M., Gallão, J.E., 2019. Richness and taxonomic distinctness of cave invertebrates from the northeastern state of Goiás, central Brazil: A vulnerable and singular area. *Subterranean Biology*, 29, 1-33.
<https://doi.org/10.3897/subtbiol.29.30418>
- Borges, P.A.V., Cardoso, P., Amorim, I.R., Pereira, F., Constância, J.P., Nunes, J.C., Barcelos, P., Costa, P., Gabriel, R., Dapkevicius, M.L., 2012. Volcanic caves: Priorities for conserving the Azorean endemic troglobitic species. *International Journal of Speleology*, 41(1), 101-112.
<https://doi.org/10.5038/1827-806X.41.1.11>
- Bryson, R.W., Prendini, L., Savary, W.E., Pearman, P.B., 2014. Caves as microrefugia: Pleistocene phylogeography of the troglomorphic North American scorpion *Pseudouroctonus reddelli*. *BMC Evolutionary Biology*, 14(9), 16.
<https://doi.org/10.1111/j.1095-8312.1996.tb01434.x>
- Cadotte, M.W., Carscadden, K., Mirotchnick, N., 2011. Beyond species: Functional diversity and the maintenance of ecological processes and services. *Journal of Applied Ecology*, 48(5), 1079-1087.
<https://doi.org/10.1111/j.1365-2664.2011.02048.x>
- Cardoso, P., Borges, P.A.V., Triantis, K.A., Ferrández, M.A., Martín, J.L., 2011a. Adapting the IUCN Red List criteria for invertebrates. *Biological Conservation*, 144(10), 2432-2440.
<https://doi.org/10.1016/j.biocon.2011.06.020>
- Cardoso, P., Erwin, T.L., Borges, P.A.V., New, T.R., 2011b. The seven impediments in invertebrate conservation and how to overcome them. *Biological Conservation*, 144(11), 2647-2655.
<https://doi.org/10.1016/j.biocon.2011.07.024>
- Cardoso, P., Rigal, F., Carvalho, J. C., 2015. BAT - Biodiversity Assessment Tools, an R package for the measurement and estimation of alpha and beta taxon, phylogenetic and functional diversity. *Methods in Ecology and Evolution*, 6(2), 232-236.
<https://doi.org/10.1111/2041-210X.12310>
- Cardoso, R.C., Ferreira, R.L., Souza-Silva, M., 2021. Priorities for cave fauna conservation in the Iúçu karst landscape, northeastern Brazil: a threatened spot of troglobitic species diversity. *Biodiversity and Conservation*, 30(5), 1433-1455.
<https://doi.org/10.1007/s10531-021-02151-5>
- Christiansen, K., 1962. Proposition pour la classification des animaux cavernicoles. *Spelunca*, 2, 75-78.
- Clarke, K.R., Gorley, R.N., 2006. *Primer v6: User manual/Tutorial* (1st ed.). Primer-E Ltd.
- Clarke, K.R., Warwick, R.M., 1998. A taxonomic distinctness index and its statistical properties. *Journal of Applied Ecology*, 35, 523-531.
<https://doi.org/10.1046/j.1365-2664.1998.3540523.x>
- Clarke, K.R., Warwick, R.M., 1999. The taxonomic distinctness measure of biodiversity: Weighting of step lengths between hierarchical levels. *Marine Ecology Progress Series*, 184, 21-29.
<https://doi.org/10.3354/meps184021>
- Donato, C.R., Ribeiro, A.S., Souto, L.S., 2014. A conservation status index, as an auxiliary tool for the management of cave environments. *International Journal of Speleology*, 43(3), 315-322.
<https://doi.org/10.5038/1827-806X.43.3.8>
- Fattorini, S., Fiasca, B., Di Lorenzo, T., Di Cicco, M., Galassi, D.M.P., 2020. A new protocol for assessing the conservation priority of groundwater-dependent ecosystems. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 1-22.
<https://doi.org/10.1002/aqc.3411>
- Ficetola, G.F., Canedoli, C., Stoch, F., 2019. The Racovitza impediment and the hidden biodiversity of unexplored environments. *Conservation Biology*, 33(1), 214-216.
<https://doi.org/10.1111/cobi.13179>
- Gallão, J.E., Bichuette, M.E., 2015. Taxonomic distinctness and conservation of a new high biodiversity subterranean area in Brazil. *Anais Da Academia Brasileira de Ciencias*, 87(1), 209-217.
<https://doi.org/10.1590/0001-3765201520140312>
- Gallardo, B., Gascón, S., Quintana, X., Comín, F.A., 2011. How to choose a biodiversity indicator - Redundancy and complementarity of biodiversity metrics in a freshwater ecosystem. *Ecological Indicators*, 11(5), 1177-1184.
<https://doi.org/10.1016/j.ecolind.2010.12.019>
- Decree N° 44.120/2005. Cria o Monumento Natural Estadual Peter Lund, no município de Cordisburgo, Minas Gerais, Brasil.
- Gibert, J., Deharveng, L., 2002. Subterranean ecosystems: A truncated functional biodiversity. *BioScience*, 52(6), 473-481.
[https://doi.org/10.1641/0006-3568\(2002\)052\[0473:seatfb\]2.0.co;2](https://doi.org/10.1641/0006-3568(2002)052[0473:seatfb]2.0.co;2)
- Grünwald, U., 2001. Water resources management in river catchments influenced by lignite mining. *Ecological Engineering*, 17(2-3), 143-152.
[https://doi.org/10.1016/S0925-8574\(00\)00154-3](https://doi.org/10.1016/S0925-8574(00)00154-3)
- Hortal, J., De Bello, F., Diniz-Filho, J.A.F., Lewinsohn, T.M., Lobo, J.M., Ladle, R.J., 2015. Seven shortfalls that beset large-scale knowledge of biodiversity.

- Annual Review of Ecology, Evolution, and Systematics, 46, 523-549.
<https://doi.org/10.1146/annurev-ecolsys-112414-054400>
- ICMBio/MMA, 2018. Livro Vermelho da Fauna Brasileira Ameaçada de Extinção: Volume 7 - Invertebrados. ICMBio, Brasília, 727 p.
- Jaffé, R., Prous, X., Zampaulo, R., Giannini, T.C., Imperatriz-Fonseca, V.L., Maurity, C., Oliveira, G., Brandi, I.V., Siqueira, J.O., 2016. Reconciling mining with the conservation of cave biodiversity: A quantitative baseline to help establish conservation priorities. *PLoS ONE*, 11(12), 1-17.
<https://doi.org/10.1371/journal.pone.0168348>
- Kury, A.B., Pérez-González, A., 2008. The first cave-dwelling spinopilar mello-leitão 1940 (Opiliones gonyleptidae tricommatinae), described from a Brazilian cave. *Tropical Zoology*, 21(2), 259-267.
- Mammola, S., Cardoso, P., Culver, D.C., Deharveng, L., Ferreira, R.L., Fiser, C., Galassi, D.M.P., Griebler, C., Halse, S., Humphreys, W.F., Isaia, M., Malard, F., Martinez, A., Moldovan, O.T., Niemiller, M.L., Pavlek, M., Reboleira, A.S.P.S., Souza-Silva, M., Teeling, E.C., Wynne, J.J., Zagmajster, M., 2019. Scientists' warning on the conservation of subterranean ecosystems. *BioScience*, 69(8), 641-650.
<https://doi.org/10.1093/biosci/biz064>
- Ministério do Meio Ambiente, 2009. Instrução Normativa N° 1, 2009: Estabelece metodologia para classificação do grau de relevância das cavidades naturais subterrâneas.
- Mulec, J., 2014. Human impact on underground cultural and natural heritage sites, biological parameters of monitoring and remediation actions for insensitive surfaces: Case of Slovenian show caves. *Journal for Nature Conservation*, 22(2), 132-141.
<https://doi.org/10.1016/j.jnc.2013.10.001>
- Mulec, J., Oarga-Mulec, A., Šturm, S., Tomazin, R., Matos, T., 2017. Spacio-temporal distribution and tourist impact on airborne bacteria in a cave (Škocjan Caves, Slovenia). *Diversity*, 9(3), 1-12.
<https://doi.org/10.3390/d9030028>
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. *Nature*, 403, 853-858.
<https://doi.org/10.1038/35002501>
- Nitzu, E., Vlaicu, M., Giurginca, A., Meleg, I.N., Popa, I., Nae, A., Baba, S., 2018. Assessing preservation priorities of caves and karst areas using the frequency of endemic cave-dwelling species. *International Journal of Speleology*, 47(1), 43-52.
<https://doi.org/10.5038/1827-806X.47.1.2147>
- Novak, T., Perc, M., Lipovšek, S., Janžekovič, F., 2012. Duality of terrestrial subterranean fauna. *International Journal of Speleology*, 41(2), 181-188.
<https://doi.org/10.5038/1827-806X.41.2.5>
- Oliver, I., Beattie, A.J., 1996. Invertebrate morphospecies as surrogates for species: A case study. *Conservation Biology*, 10(1), 99-109.
<https://doi.org/10.1046/j.1523-1739.1996.10010099.x>
- Olson, D.M., Dinerstein, E., Wikramanayake, E.D., Burgess, N.D., Powell, G.V.N., Underwood, E.C., D'amico, J.A., Itoua, I., Strand, H.E., Morrison, J.C., Loucks, C.J., Allnutt, T.F., Ricketts, T.H., Kura, Y., Lamoreux, J.F., Wettengel, W.W., Hedao, P., Kassem, K.R., 2001. Terrestrial ecoregions of the world: A new map of life on Earth. *BioScience*, 51(11), 933.
[https://doi.org/10.1641/0006-3568\(2001\)051\[0933:teotwa\]2.0.co;2](https://doi.org/10.1641/0006-3568(2001)051[0933:teotwa]2.0.co;2)
- Pacioglu, O., Ianovici, N., Filimon, M.N., Sinitean, A., Iacob, G., Barabas, H., Pahomi, A., Acs, A., Muntean, H., Părvulescu, L., 2019. The multifaceted effects induced by floods on the macroinvertebrate communities inhabiting a sinking cave stream. *International Journal of Speleology*, 48(2), 167-177.
<https://doi.org/10.5038/1827-806X.48.2.2239>
- Piano, E., Bona, F., Falasco, E., La Morgia, V., Badino, G., Isaia, M., 2015. Environmental drivers of phototrophic biofilms in an Alpine show cave (SW-Italian Alps). *Science of the Total Environment*, 536, 1007-1018.
<https://doi.org/10.1016/j.scitotenv.2015.05.089>
- Pielou, E.C., 1966. The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology*, 13, 131-144.
[https://doi.org/10.1016/0022-5193\(66\)90013-0](https://doi.org/10.1016/0022-5193(66)90013-0)
- Poulson, T.L., White, W.B., 1969. The cave environment. *Science*, 165, 971-981.
- Prous, X., Ferreira, R.L., Jacobi, C.M., 2015. The entrance as a complex ecotone in a Neotropical cave. *International Journal of Speleology*, 44(2), 177-189.
<https://doi.org/10.5038/1827-806X.44.2.7>
- Prous, X., Ferreira, R.L., Martins, R.P., 2004. Ecotone delimitation: Epigean-hypogean transition in cave ecosystems. *Austral Ecology*, 29(4), 374-382.
<https://doi.org/10.1111/j.1442-9993.2004.01373.x>
- R Core Team., 2021. R: A language and environment for statistical computing. (4.0.3). R Foundation for Statistical Computing. <https://www.r-project.org/>
- Rabelo, L.M., Souza-Silva, M., Ferreira, R.L., 2018. Priority caves for biodiversity conservation in a key karst area of Brazil: comparing the applicability of cave conservation indices. *Biodiversity and Conservation*, 27(9), 2097-2129.
<https://doi.org/10.1007/s10531-018-1554-6>
- Rheims, C.A., Brescovit, A.D., 2004. A new cave spider of the genus *Lygromma* Simon (Araneae, Prodidomidae) from Minas Gerais, Brazil. *Revista Ibérica de Aracnologia*, 9, 325.
- Ribera, I., Cieslak, A., Faille, A., Fresneda, J., 2018. Historical and ecological factors determining cave diversity. In: Moldovan, O.T., Kováč, L., Halse, S. (Eds.), *Cave ecology* (1st ed.). Springer, Cham, p. 229-254.
https://doi.org/10.1007/978-3-319-98852-8_10
- Simões, M.H., Souza-Silva, M., Ferreira, R.L., 2014. Cave invertebrates in northwestern Minas Gerais state, Brazil: endemism, threats and conservation priorities. *Acta Carsologica*, 43, 159-174.
<https://doi.org/10.3986/ac.v43i1.577>
- Soares, H.E.M., 1966. Novos opiliões da coleção "Otto Schubart" (Opiliones: Cosmetidae, Gonyleptidae, Phalangodidae). *Papéis Avulsos Do Departamento de Zoologia Do Estado de São Paulo*, 18(11), 103-115.
- Souza, M.F.V.R., Ferreira, R.L., 2010. *Eukoeneria* (Palpigradi: Eukoeneriidae) in Brazilian caves with the first trogloliotic palpigrade from South America. *Journal of Arachnology*, 38(3), 415-424.
<https://doi.org/10.1636/ha09-112.1>
- Souza, M.F.V.R., Ferreira, R.L., 2012. A new highly troglomorphic species of *Eukoeneria* (Palpigradi: Eukoeneriidae) from tropical Brazil. *Journal of Arachnology*, 40(2), 151-158.
<https://doi.org/10.1636/ha11-26.1>
- Souza-Silva, M., Bernardi, L.F.O., Martins, R.P., Ferreira, R.L., 2012. Transport and consumption of organic detritus in a Neotropical limestone cave. *Acta Carsologica*, 41(1), 139-150.
<https://doi.org/10.3986/ac.v41i1.54>

- Souza-Silva, M., Ferreira, R.L., 2016. The first two hotspots of subterranean biodiversity in South America. *Subterranean Biology*, 19(1), 1-21.
<https://doi.org/10.3897/subtbiol.19.8207>
- Souza-Silva, M., Iniesta, L.F.M., Ferreira, R.L., 2020. Cave lithology effect on subterranean biodiversity: A case study in quartzite and granitoid caves. *Acta Oecologica*, 108, 103645.
<https://doi.org/10.1016/j.actao.2020.103645>
- Souza-Silva, M., Martins, R.P., Ferreira, R.L., 2015. Cave Conservation Priority Index to adopt a rapid protection strategy: A case study in Brazilian Atlantic Rain Forest. *Environmental Management*, 55(2), 279-295.
<https://doi.org/10.1007/s00267-014-0414-8>
- Trajano, E., Gallão, J.E., Bichuette, M.E., 2016. Spots of high diversity of troglobites in Brazil: the challenge of measuring subterranean diversity. *Biodiversity and Conservation*, 25(10), 1805-1828.
<https://doi.org/10.1007/s10531-016-1151-5>
- Trajano, E., Secutti, S., Mattox, G.M.T., 2009. Epigean and subterranean ichthyofauna in Cordisburgo karst area, eastern Brazil. *Biota Neotropica*, 9(3), 277-281.
- Travassos, L.E.P., 2010. Considerações sobre o carste da região de Cordisburgo, Minas Gerais, Brasil. *Tradição Planalto*, Belo Horizonte, 102 p.
- Warwick, R.M., Clarke, K.R., 1995. New "biodiversity" measures reveal a decrease in taxonomic distinctness with increasing stress. *Marine Ecology Progress Series*, 129(1-3), 301-305.
<https://doi.org/10.3354/meps129301>
- Whitten, T., 2009. Applying ecology for cave management in China and neighbouring countries. *Journal of Applied Ecology*, 46(3), 520-523.
<https://doi.org/10.1111/j.1365-2664.2009.01630.x>
- Winter, M., Devictor, V., Schweiger, O., 2013. Phylogenetic diversity and nature conservation: Where are we? *Trends in Ecology and Evolution*, 28(4), 199-204.
<https://doi.org/10.1016/j.tree.2012.10.015>
- Wynne, J.J., Howarth, F.G., Sommer, S., Dickson, B.G., 2019. Fifty years of cave arthropod sampling: Techniques and best practices. *International Journal of Speleology*, 48(1), 33-48.
<https://doi.org/10.5038/1827-806X.48.1.2231>
- Wynne, J.J., Sommer, S., Howarth, F.G., Dickson, B.G., Voyles, K.D., 2018. Capturing arthropod diversity in complex cave systems. *Diversity and Distributions*, 24(10), 1478-1491.
<https://doi.org/10.1111/ddi.12772>
- Yoshizawa, K., Lienhard, C., Yao, I., Ferreira, R.L., 2019. Cave insects with sex-reversed genitalia had their most recent common ancestor in West Gondwana (Psocodea: Prionoglarididae: Speleketorinae). *Entomological Science*, 22, 334-338.
<https://doi.org/10.1111/ens.12374>
- Zagmajster, M., Culver, D.C., Christman, M.C., Sket, B., 2010. Evaluating the sampling bias in pattern of subterranean species richness: Combining approaches. *Biodiversity and Conservation*, 19(11), 3035-3048.
<https://doi.org/10.1007/s10531-010-9873-2>
- Zagmajster, M., Malard, F., Eme, D., Culver, D.C., 2018. Subterranean biodiversity patterns from global to regional scales. In: Moldovan, O.T., Kováč, Ľ., Halse, S. (Eds.), *Cave Ecology* (1st ed.). Springer, Cham, p. 195-228.
https://doi.org/10.1007/978-3-319-98852-8_9