

UNIVERSIDAD DE COSTA RICA
SISTEMA DE ESTUDIOS DE POSGRADO

**DIVERSITY, ECOLOGY AND CONSERVATION OF CAVE-DWELLING BATS IN
COSTA RICA AND BULGARIA**

Tesis sometida a la consideración de la Comisión del Programa de Posgrado de Doctorado
en Ciencias para optar al grado y título de Doctorado en Ciencias

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DEDICATION

This dissertation is dedicated to my ancestors who were not able to pursue academic careers as they were too busy surviving in Bulgaria. Their resilience and sacrifices made it possible for me to become a scientist and work for a better future. Doing scientific work is a privilege and I feel incredibly grateful for this opportunity.

“I'm a scientist and I know what constitutes proof. But the reason I call myself by my childhood name is to remind myself that a scientist must also be absolutely like a child. If he sees a thing, he must say that he sees it, whether it was what he thought he was going to see or not. See first, think later, then test. But always see first. Otherwise you will only see what you were expecting. Most scientists forget that.”

— Douglas Adams,

The Ultimate Hitchhiker's Guide to the Galaxy

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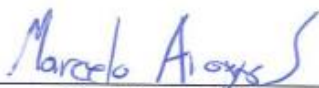
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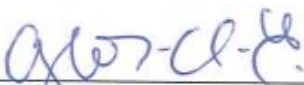
And finally, my research owes its existence to the sacrifice and noble cause of Alexandra Elbakyan to keep knowledge accessible to all.

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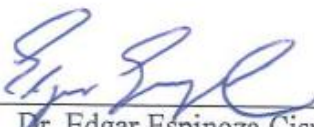
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PUBLICATION LIST

All parts of this thesis are written to be published in scientific journals. Chapters 1, 2 and 3 have been published in academic journals with an impact factor. Chapters 4 and 5 are not published yet but will be submitted to scientific journals soon. In addition, data from the dissertation was published in collaboration with other scientists.

Scientific publications of the dissertation

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RESUMEN

Esta tesis aborda las brechas de conocimiento en la ecología de los murciélagos cavernícolas en dos países distintos, específicamente Costa Rica y Bulgaria, con un enfoque sólido en la conservación. Inicialmente centrada en la región de Brunca en Costa Rica, el estudio se expandió para abarcar todo el país. Debido a que la pandemia ocasionada por Covid-19 restringió el trabajo de campo y los viajes a Costa Rica, se implementó la metodología original en Bulgaria, ampliando aún más el alcance geográfico de la investigación. No todos los aspectos de la investigación siguen una trayectoria paralela. Mientras que la fauna de cuevas y los murciélagos en Bulgaria han sido objeto de numerosos estudios, los refugios subterráneos en Costa Rica requerían una investigación más exhaustiva. En consecuencia, esta tesis dedica tres capítulos a este último tema. Se emplea una metodología uniforme en ambas ubicaciones de investigación, y el capítulo final integra datos de ambos países para presentar una visión integral. Utilizando conocimientos de ambas naciones, la tesis propone medidas de conservación adecuadas para ambos contextos.

El primer capítulo ofrece una revisión exhaustiva de la literatura, combinando los datos existentes sobre la fauna cavernícola en Costa Rica con investigaciones de campo originales. El segundo capítulo se centra en refugios cruciales de murciélagos en Bulgaria, proponiendo medidas de conservación urgentes. El tercer capítulo se dedica a explorar la diversidad de murciélagos cavernícolas en la región de Brunca en Costa Rica, identificando refugios vulnerables y evaluando la abundancia de murciélagos. El cuarto capítulo examina la influencia de varios factores ambientales y antropogénicos en los murciélagos cavernícolas. El último capítulo profundiza en el impacto de los nutrientes del guano de murciélagos en los ecosistemas superficiales, en particular el transporte de nitrógeno y fósforo en arroyos subterráneos.

Esta tesis adopta un enfoque pragmático y orientado a la conservación, generando no solo investigaciones científicas, sino también documentos estratégicos, capacitación y materiales de divulgación. Representa un esfuerzo sostenible a largo plazo destinado a mejorar el conocimiento y preservar los ecosistemas subterráneos y las colonias de murciélagos en cuevas en dos continentes, logrando plenamente sus objetivos y generando material adicional para futuros estudios.

OVERVIEW

This dissertation addresses knowledge gaps in cave-dwelling bat ecology in two distinct countries, namely Costa Rica and Bulgaria, with a strong conservation focus. Originally centered on Costa Rica's Brunca region, the study expanded to encompass the entire country. As the pandemic restricted fieldwork and travel to Costa Rica, the original methodology was performed in Bulgaria, further expanding the geographical scope of the research. Not all aspects of the research follow a parallel trajectory. While Bulgarian cave fauna and cave-dwelling bats have been subject to numerous studies, the Costa Rican underground roosts needed more thorough investigation. This dissertation accordingly dedicates three chapters to the latter. A consistent methodology is applied in both locations, with the final chapter integrating data from both countries to present a comprehensive view. Utilizing insights from both nations, the dissertation proposes conservation measures suitable for both contexts.

The first chapter offers a comprehensive literature review, combining the existing data on Costa Rican cave-dwelling fauna with original field research. Chapter two centers on crucial bat roosts in Bulgaria, emphasizing urgent conservation measures. Chapter three is dedicated to exploring the diversity of cave-dwelling bats in Costa Rica's Brunca region, identifying vulnerable roosts and assessing bat abundance. Chapter four examines the influence of various environmental and anthropogenic factors on cave-dwelling bats, while the last chapter delves into the impact of bat guano nutrients on surface ecosystems, particularly nitrogen and phosphorus transportation in underground streams.

This dissertation takes a pragmatic and conservation-oriented approach, yielding scientific research as well as strategic documents, capacity building, and outreach materials. It represents a sustainable, long-term effort aimed at enhancing knowledge and preserving underground ecosystems and cave bat colonies on two continents, fully achieving its objectives and generating additional material for future studies.

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LIST OF USED ABBREVIATIONS

ANOVA	Analysis of Variance
BCVI	Bat Caves Vulnerability Index
BG	Bulgaria
CR	Costa Rica
CIMAR	Centro de Investigación en Ciencias del Mar y Limnología
CS	Conservation Status
DIN	Dissolved inorganic nitrogen
GLM	General Linear Model
N	Nitrogen
NO ₂	Nitrites
NO ₃	Nitrates
NH ₄	Ammonium
P	Phosphorus
PO ₄	Phosphates
PCA	Principal Component Analysis
SINAC	Sistema Nacional de Áreas de Conservación

INTRODUCTION

Caves provide some of the most widely used roosting resources for ecologically important organisms such as bats (Altringham, 2011). Caves are essential resources for bats because they serve as a refuge from predators and inclement weather (Lausen & Barclay, 2002; Racey & Furey, 2016), and provide a critical venue for social interactions such as copulation and grooming (McCracken and Gustin, 1991, Ortega & Maldonado, 2006). Also, caves provide an important venue for rearing young (Altringham, 2011, Racey & Furey, 2016).

Cave-roosting bats in the temperate zones are known to exhibit preferences for caves that have very specific temperature conditions, particularly during hibernation (Daan & Wichers, 1968). Likewise in the tropics, studies show that temperature is the most important parameter affecting the spatial distribution of cave-dwelling bats (Avila-Flores & Medellin, 2004, Rodriguez-Duran, 1998). In addition to microclimatic conditions, the cave morphology could affect bat assemblages and their spatial distribution within the roosts (Peñuela-Salgado & Pérez-Torres, 2016, Brunet & Medellin, 2001). For example, the surface area of a cave could positively correlate with species richness (Brunet & Medellin, 2001), and the presence of cracks in the cave ceiling might also be affecting bat distribution (Peñuela-Salgado & Pérez-Torres, 2016). Even though many caves in Costa Rica have been surveyed (Goicoechea, 2015, Peacock & Hempel, 1993), there is still little information about their fauna or microclimatic conditions. Knowledge of cave structure is of potentially great importance in predicting species distributions among caves (Tuttle & Stevenson, 1977).

Despite the critical role of caves for many species of bats, these ecosystems suffer greatly from human activities, which endanger the long-term persistence of these colonies (Racey & Furey 2016). Threats to caves and cave-dwelling bats worldwide include pollution of water sources, frequent visitation, over tourism visitation, quarrying and mining, erosion, guano mining, nest collecting, and vandalism (Hutson et. al. 2001, Hobbs, 2004, Baker and Genty, 1998, Medellin et.al. 2017). Bats that roost in caves are particularly vulnerable to human activities due to their tendency to aggregate in large colonies in a single roost, as a single disturbance event could lead to the eradication of an entire colony (Racey & Furey 2016).

Karstic caves are formed by the activity of water, which is running underground through a network of underground and surface streams (Gunn 2004). Streams at the surface and in caves have the feature to transport nutrients such as Nitrogen (N) and Phosphorus (P) before their further utilization (Newbold et al. 1983). The nutrients enter streams from upstream runoff or atmospheric inputs and are incorporated into organic form by the decomposition of organic matter and excretion (Allan & Ibañez Castillo, 2009). In some systems animals are an important source of nutrients, for example birds (Ligeza and Smal 2003) and herbivorous mammals (Wolf et al. 2013) may contribute nutrients, or facilitate nutrient flux, within their habitat. The contribution of nutrients by seabirds in some places is so important that their sudden removal has been shown to change the structure of plant communities (Fukami et al. 2006). Nitrogen input by whales and seals, for example, can positively influence the primary production of marine ecosystems (Roman and McCarthy 2010; Smith et al. 2013). Another group of mammals that may significantly contribute nutrients to their ecosystems is bats.

Some of the most widely known ecological roles bats play in ecosystems include controlling insect pests in agroecosystems, pollination of plant crops, and seed dispersal of pioneer species fostering the recovery of degraded tropical ecosystems (Kunz et al. 2011). However, bats may also contribute nutrients to terrestrial and aquatic habitats that surround their roost sites (Culver & Pipan, 2009, Voigt et al. 2015).

Some caves are known to harbor colonies of several thousand and up to millions of bats, and guano accumulation from very large colonies may sum up to 50,000 kg/year (Sewall et al. 2003; Cardiff et al. 2009; Hristov et al. 2010). This may result in an extremely high and constant supply of nutrients to surrounding ecosystems. In caverns with underground streams, the water constantly removes guano deposits from the cave floor into adjacent aquatic systems (Anderson & Polis, 1999). This contribution can be extremely significant, particularly if bat colonies are very large (Culver & Pipan, 2009).

This dissertation aims to address important gaps in the knowledge about the ecology of cave-dwelling bats in Costa Rica and Bulgaria. Namely – the diversity and threats of cave-dwelling bats in their roosts. Another important question is study of the nutrient input of bat guano into surface ecosystems as a potential ecosystem service.

OBJECTIVES

Main Objective

Determine what environmental and human factors affect the diversity and distribution of cave-dwelling bats in Costa Rica and Bulgaria, and how bats affect habitats surrounding caves through nutrient deposition from their guano.

Specific objectives

- Assess bat species diversity and abundance in the caves in Costa Rica and Bulgaria.
- Identify the potential sources of human disturbance that bats face within their cave roosts.
- Determine the effect of cave morphology on bat occupancy.
- Analyze the contribution of bat guano to the nitrogen N and phosphorous P concentrations in rivers and determine the effect of these nutrients on the plant community close to the streams.

Hypotheses

Hypothesis 1

The size and complexity of a cave roost affect bat species richness and abundance. I propose that larger and more complex caves will have larger and more diverse bat colonies.

Hypothesis 2

The presence of anthropogenic activities in and around the cave roost affects species richness and abundance. I predict a significant negative relationship between the level of human disturbance, as measured by anthropogenic activities conducted in and around the cave, and bat species richness and abundance.

Hypothesis 3

Bat guano will significantly contribute to the nutrient content of water surrounding caves. Specifically, I predict that the nitrogen and phosphorus concentration will be higher in underground and aboveground rivers after the water has had contact with guano from the bat colonies.

Hypothesis 4

River organisms close to the cave entrances effectively assimilate the nutrients from the bat guano. Specifically, I predict that the $\delta^{15}\text{N}$ isotopic values of plants and vegetation growing close to cave entrances will be more similar to the $\delta^{15}\text{N}$ isotopic values of bat guano than to the signature of individuals of the same species but growing far away from the guano source.

STUDY AREA

The dissertation focused on caves and artificial underground habitats in Costa Rica and Bulgaria. In Costa Rica, 127 subterranean sites were examined, with 50 visited in person and the rest sourced from literature. Chapters three and four's fieldwork centered in the Puntarenas province due to its extensive karst area housing over 150 caves (Ulloa & Goicoechea 2013). In Bulgaria, research covered 107 bat-conservation sites, with 89 visited and the remainder assessed through literature data. The final chapter investigated caves and artificial sites with underground streams emerging as springs in both countries. Six cave systems were studied in Bulgaria, while Costa Rica's research encompassed eight sites—five natural caves and three artificial tunnels.

RESULTS

Each chapter of the dissertation addresses one or more of the objectives. The chapters are presented in the format in which they are published in scientific journals. The overview of the results in each chapter are listed below:

Chapter 1

This chapter fulfills the first specific objective of the dissertation, primarily evaluating bat species diversity within Costa Rican caves. The chapter extends this inquiry beyond bats to encompass all animal species, observed in caves. This broader scope places the study of cave-dwelling bats within the wider context of cave ecology. Serving as an introduction, this chapter fills the existing gaps in the knowledge and forms the foundational exploration of cave ecology within the country.

Chapter 2

The second chapter addresses two specific objectives of the dissertation: study of bat species diversity and abundance, and the potential sources of human disturbance in the caves for Bulgaria. This chapter evaluated conservation priorities and identified the most vulnerable underground bat roosts in Bulgaria using the Bat Cave Vulnerability Index and proposed measures to adequately protect sites.

Chapter 3

The third chapter of the dissertation focused on the diversity and abundance of cave-dwelling bats in the Brunca region of Costa Rica, which contains the largest karst area of the country. This chapter addresses two specific objectives of the dissertation: study of bat species diversity and abundance, and the potential sources of human disturbance in the caves for Costa Rica. This chapter determined the most important and vulnerable bat roosts in the region and showed the need for urgent conservation actions.

Chapter 4

This chapter aims to evaluate which anthropogenic and environmental factors, both underground and on the surface, contribute to the changes in bat diversity and abundance. The chapter answers the first and second hypotheses of the dissertation, that the size of the roost or the presence of anthropogenic activities affect bat species richness and abundance. In addition to diversity and abundance, this chapter explores the functional diversity of bats as an important diversity measurement.

Chapter 5

The last chapter of the dissertation studied the contribution of bat guano to the nitrogen and phosphorous concentrations in underground rivers and determine the effect of these nutrients on the plant community close to the cave entrances. This chapter answers two of the hypotheses of the dissertation – that bat guano significantly contributes to the nutrient content of water surrounding caves and that vegetation close to the cave entrances effectively assimilate the nutrients from the bat guano.

Supplementary material

The supplementary material represents the conservation contribution of this dissertation – guidelines for the future monitoring of cave-dwelling bats in Costa Rica. Monitoring day roosts is a highly accurate method for estimating colony size, and can be used to establish the protection of rare or declining species. This document uses the established methods for surveillance of cave-dwelling bats, described in the literature, and adapts them to the specific conditions of Costa Rica.

Chapter 1. Cave-dwelling fauna of Costa Rica – current state of knowledge and future research perspectives

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Abstract: This study focused on the cave fauna of Costa Rica, which has remained relatively understudied despite the presence of more than 435 recorded natural caves and artificial subterranean sites. We collected and reviewed all available literature data on cave fauna in Costa Rica and created the first comprehensive review of the existing information. In addition, we report new records from field surveys conducted between 2015 and 2018. This study reported approximately 123 animal species, whereas the remaining records (n = 82) represented taxa that could not be identified at the species level. Data were collected from 127 locations throughout the country, with new cave fauna records from 41 sites. Notably, we reported the first occurrence of the true bug *Amnestus subferrugineus* (Westwood 1837) within Costa Rican caves, which represents an addition to the country's faunal inventory. As this study highlights the knowledge gaps in the subterranean fauna, it will serve as an important stepping stone for future research and conservation efforts related to caves in Costa Rica.

Introduction

Caves are important habitats and roosts for a large number of animal taxa, such as bats and other highly specialized organisms, adapted to specific conditions, which usually consist of the absence of light, high humidity, and almost constant temperature (Romero 2009). Because of their isolation from the surface and other subterranean systems across evolutionary time, caves can provide refuge for numerous endemic species in a confined area, making them intriguing subjects for research (Culver and Pipan, 2009). Tropical regions are fascinating for biospeleological research because of their high biodiversity and the presence of large underground systems (Deharveng and Bedos, 2012). Despite the recent rapid progress in the study of cave-dwelling organisms in tropical regions such as Brazil (Campos-Filho et al. 2023), numerous areas and taxonomic groups remain underexplored (Niemiller et al. 2018 and Wynne et al. 2021). With its diverse and relatively understudied cave-dwelling fauna, Central America has enormous potential for speleological discoveries (Day and Koenig 2002, Taylor et al. 2011, Pacheco et al. 2020). Expanding research efforts in these areas is essential to better understand the unique biological communities in caves and their ecological roles.

Costa Rica is a small country located in the Neotropical region. It is a natural bridge between North and South America, and has been estimated to hold at least 5% of the world's biodiversity (Avalos 2019). Despite the existing knowledge, this country has great potential for taxonomic investigation and discovery. The country has a limestone surface area of approximately 430 km² with numerous karst landscapes and more than 435 described caves (Ulloa et al. 2011, Grupo Espeleológico Anthros 2023). Although the carbonated platforms in Costa Rica cover less than 1% of the country's area, several karstic systems exceed a kilometer in length (Ulloa 2009a). In addition to limestone caves, Costa Rica has several volcanic caves with a total length of 2.2 km (Ulloa and Alvarado, personal communication). The dimensions of the largest cave systems in Costa Rica are modest compared with those in other parts of Central and South America. However, Costa Rican caves are undoubtedly captivating research objects that have been attracting numerous expeditions since the first speleological explorations in 1943 (Goicoechea 2015).

The earliest records of cave fauna in Costa Rica were from 1965 to 1969, with studies on some cave-dwelling bat species (Armstrong 1969). Long-term research on Seba's short-tailed bats (*Carollia perspicillata*) was conducted in the late 1970s and the early 1980s in Santa Rosa National Park (Heithaus and Fleming 1978; Fleming and Heithaus 1986). Costa Rican caves grabbed the attention of the US National Speleological Society (NSS) and different European speleological groups, and several expeditions were conducted in the country, providing a tremendous scientific contribution (Hempel 1989, Peacock and Hempel 1993). Several dedicated biospeleological studies have been conducted in the country, with one describing a new species of stygobiont (Hobbs 1991). The existing biospeleological data are summarized in the book "Introduction to Speleology" (Alpizar et al. 2006). Cave-dwelling bats have been the topic of recent studies (Cubero and Artavia 2016, Deleva and Chaverri 2018, Mitchell et al. 2018). Unfortunately, all previous efforts have covered only a portion of caves in the country. Many caves and taxonomic groups remain unknown.

This study aimed to provide an overview of the current state of knowledge regarding the cave-dwelling fauna of Costa Rica. Given that previous efforts have provided valuable, albeit scattered, information, we sought to systematize the existing data on the cave fauna of Costa Rica and add original preliminary research from our field expeditions. We hope that this study will provide insights for new studies and conservation efforts in Costa Rican cave-dwelling animals.

Methods

Literature review

We searched for literature sources that mentioned cave-dwelling fauna in Costa Rica, including but not limited to peer-reviewed articles, expedition reports, conference papers, short notes, and dissertations. We conducted searches using Google Scholar, Web of Science, and ResearchGate. We searched separately using each of the keywords "cave," "underground," "subterranean," "cave fauna," "speleology," "troglobiont", "troglobite," "stygobiont," "stygobite" in combination with the keyword "Costa Rica," using the Boolean operator "and." We examined the references in the articles obtained during the search for additional relevant sources. We searched for studies published in Spanish by translating the

keywords and performing the search with the same word combinations. Furthermore, we checked the expedition reports of the Anthros Speleological group (Grupo Espeleológico Anthros 2023) and the archives of the University of Costa Rica's library (UCR 2023). The last search was performed in October 2022.

Field research

In addition to the literature review, we also included preliminary data from observations of cave-dwelling animals during speleological expeditions between December 2015 and August 2018. The research sites included natural caves, artificial tunnels, and abandoned mines. We used direct observations inside the roosts where the specimens were documented with photographs. Field guides were used to identify animals at the species level (Henderson 2011). A small number of invertebrates was collected and preserved in 96% ethanol. The collected material was distributed for further identification among specialists in the different taxonomic groups. Bats were divided into two categories, following the assessment of Sagot and Chaverri (2015): 1) cave-dependent – only known to roost in caves or cave-like structures, and 2) not cave-dependent – roosting in caves as well as in other types of roosts. We created a dataset for each record of cave-dwelling fauna, which included the following attributes: location, site type, protected area name (if applicable), conservation status of the species, and citation (if applicable).

Spatial data

We used GIS software (ArcGIS Desktop 10.8.1) to create the maps. The locations of the sites were obtained from the database of the Anthros Speleological Group (Grupo Espeleológico Anthros 2023). We included information on the origin of each subterranean site: karst, volcanic, marine, artificial or unknown. We used publicly available geospatial data from the National Geographical Institute to determine whether the sites were located in protected areas (SNIT 2023). Because of the sensitive species inhabiting the subterranean sites, we did not disclose the exact coordinates of each cave, as unregulated visitation may further affect these sensitive resources. Therefore, we plotted site locations at a low resolution following the best practices for generalizing sensitive species occurrence data (Chapman 2022).

Conservation status

We determined the management status of all species identified by cross-checking each species with the IUCN Red List (IUCN 2023), appendices of the Convention on the Conservation of Migratory Species of Wild Animals (CMS Convention 2023), the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2023), and the Costa Rican Law for the protection of wildlife (Ley de Conservación de Vida Silvestre, MINAE 2017).

Results

We identified a total of 62 sources reporting organisms in Costa Rican caves. Data from the published sources contained 773 records, with 123 unique organisms identified at the species level. The articles and reports were written in Spanish (33), English (25), French (4), and Italian (1). Of these, one-third (23) were peer-reviewed, and the earliest study was published in 1965. The majority of the studies (43) were published after 2003. Our study included 186 observations (both vertebrates and invertebrates) from 42 sites, four of which had no prior biospeleological records. The combined literature data and field research provided information for 127 locations, accounting for 30% of the 435 described subterranean sites in Costa Rica.

Conservation status of Costa Rican caves and cave-dwelling fauna

Regarding the level of protection, most sites (91) were located outside of protected areas. The categories of the protected areas were national park (25), national wildlife refuge (4), forestry reserve (2), and protected zone (2) (Figure 1). The subterranean sites across the country's specific administrative regions, also known as "Conservation Areas", were distributed as follows: Osa (63), Central Region (16), Tempisque (16), Guanacaste (7), Huetar Norte (6), La Amistad Pacifico (6), Pacifico Central (4), Arenal Tempisque (3), La Amistad Caribe (2). Most sites were concentrated in the two largest karst areas in the country: Barra Honda, which is located in the Tempisque Conservation Area (Figure 1A), and the Zona Sur Karst Area in the Osa Conservation Area (Figure 1B). Fifteen of the 16 sites in

Barra Honda were located within the borders of the Barra Honda National Park; only five of the 63 sites in Zona Sur had some level of state protection. Data on six of the sites were unavailable.

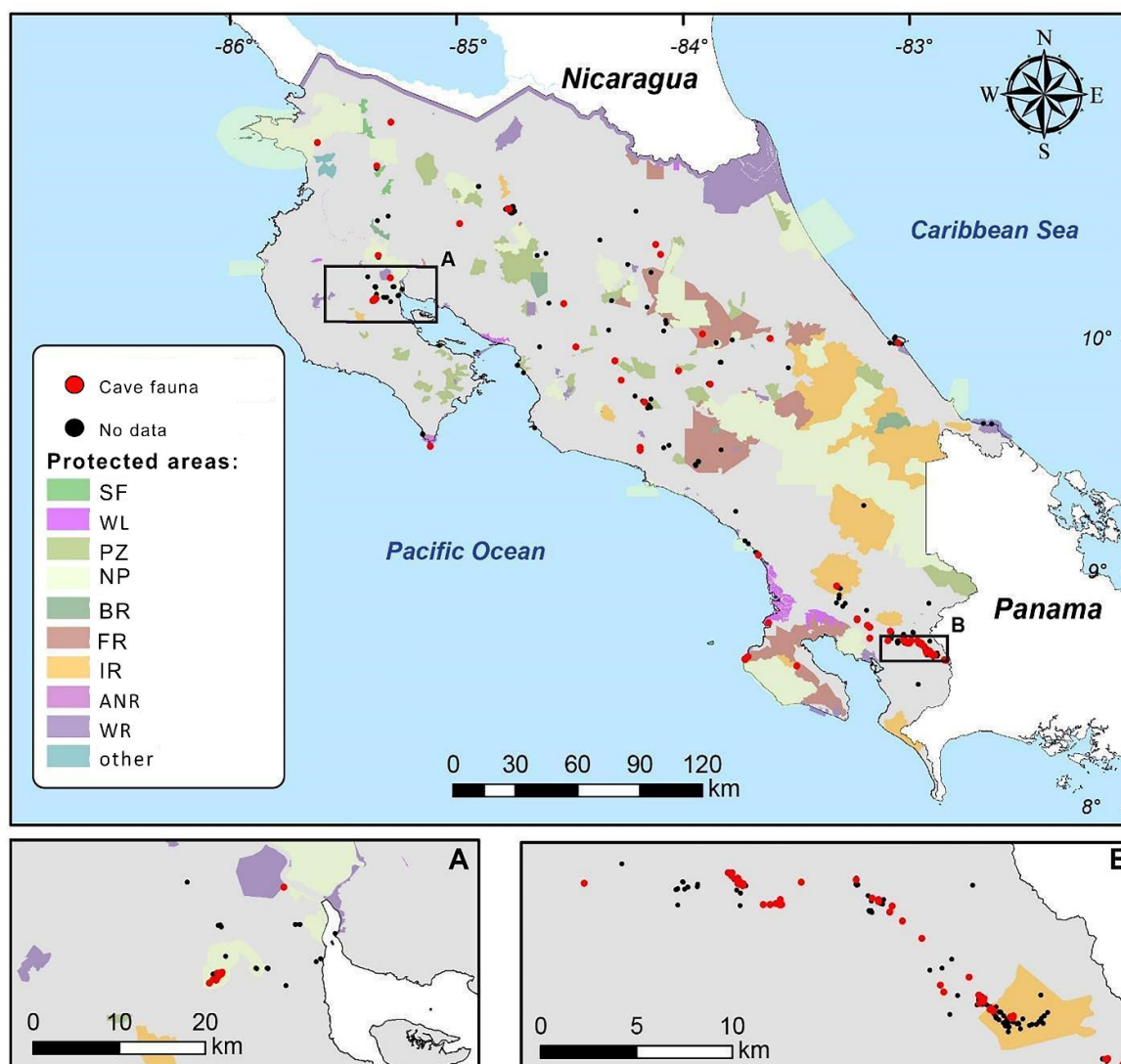


Figure 1 Costa Rica's subterranean sites: sites with records of cave fauna (red) vs. sites without records of cave fauna (black). The state-protected areas are presented in different colors: state farm (SF), wetland (WL), protected zone (PZ), national park (NP), biological reserve (BR), forest reserve (FR), indigenous reserve (IR), absolute natural reserve (ANR), and wildlife refuge (WR). The maps inserted at the bottom represent two karstic areas in Costa Rica with the most significant clusters of caves: **A** Barra Honda karst area and **B** Zona Sur karst area.

The distribution of global IUCN Red List conservation status among the species of cave-dwelling fauna was: “Least Concern” (75), “Not Evaluated” (43), “Data Deficient” (3), “Vulnerable” (2). Five species were included in the appendices of the CITES Convention. The Costa Rican Law for Wildlife Protection included nine species listed as “Reduced or Threatened population” and one species listed as “Endangered.” No species were included in the appendices of the CMS Convention. The conservation status of all species is presented in Tables 1, 2, and 3 and Supplementary Material 1.

Faunistic diversity in Costa Rican caves

Approximately 123 animal species were recorded in Costa Rican caves, along with 82 other records that could not be identified at the species level. Bats (Chiroptera) were the most studied vertebrate group with 36 species, followed by 26 species of other mammals (e.g., opossums, rodents, or cats), 11 reptiles (Reptilia), and eight amphibians (Amphibia). Additionally, seven species of cave-dwelling fish (Actinopterygii) were identified. Only three species of birds (Aves) were observed in caves. Studies on invertebrate diversity were predominantly represented by insects (Insecta) with 81 reported taxa, followed by 43 arachnids (Arachnida), 11 springtails and bristletails (Entognatha), eight crustaceans (Malacostraca), and several other unique taxa, including snails, millipedes, clitellate worms, mollusks, centipedes, copepods, various worms, bristle worms, garden centipedes, and flatworms, each represented by one or a few species (Figure 2).



Figure 2 Number of animal taxa reported from subterranean sites in Costa Rica. **A** Vertebrates: 1. Chiroptera, 2. Mammalia (excluding bats) 3. Reptilia 4. Amphibia 5. Actinopterygii 6. Aves. **B** Invertebrates: 1. Insecta 2. Arachnida 3. Entognatha 4. Malacostraca 5. Unknown 6. Diplopoda 7. Clitellata 8. Gastropoda 9. Bivalvia 10. Chilopoda 11. Thecostraca 12. Oligochaeta 13. Polychaeta 14. Symphyla 15. Turbellaria.

Invertebrates

Mollusca

Snails and slugs (Gastropoda) were reported from 11 sites, and shellfish (Bivalvia) from one marine cave. Snails from the subfamily Subulininae have been observed in Costa Rican caves. The only species of snail identified at the species level was the miniature Awlsnail (*Subulina octona* (Bruguière, 1789)), which was observed in an abandoned gold mine in the Osa Peninsula.

Crustacea

Subterranean crustaceans included *Macrobrachium carcinus* (Linnaeus, 1758) (Figure 3A), *Potamocarcinus magnus* (Rathbun, 1896), and *Ptychophallus montanus* (Rathbun, 1898). The troglophilic freshwater crab *Pseudothelphusa puntarenas* (Hobbs, 1991) from the Emus cave is currently the only known cave-dwelling species described from Costa Rica (Figure 1B). Although it does not have any external troglomorphic modifications, the crab was observed only underground. The other crustaceans observed belonged to the classes

Copepoda and Malacostraca (Bathynellacea and Isopoda) (Figure 3C). A single observation of goose barnacles (*Pollicipes elegans* (Lesson, 1831)) (Figure 3D) was reported from a marine cave.



Figure 3 Crustaceans from Costa Rican caves. **A** bigclaw river shrimp (*Macrobrachium carcinus*) **B** freshwater crab from Emus cave (*Pseudothelphusa puntarenas*) **C** woodlice (*Armadillidae*) **D** goose barnacles (*Pollicipes elegans*).

Arachnida

A minimum of 16 mites and ticks (Acari) were identified (Table 1). The reported species of mites belong to the superfamily Hydrachnellae and the families Arrenuridae, Limnesiidae, Omartacaridae, and Torrenticolidae of the order Trombidiformes. Other identified mites belonged to the families Ascidae, Dinychidae, Macronyssidae, Spinturnicidae, Uropodidae (order Mesostigmata), and Torrenticolidae (order Trombidiformes). Several studies mentioned ticks and mites only at the order or family levels.

Spiders (Araneae) were often observed in caves, but we could not find relevant peer-reviewed publications on their diversity. We report observations of spiders in the families Attidae, Ctenidae, Dysderidae, Lycosidae, Theraphosidae, Theridiidae, Theridiosomatidae, and Trechaleidae (Figure 4). Harvestmen (Opiliones) consisted of two identified species, *Pachylicus hispidus* Goodnight & Goodnight, 1983 from the family Phalangodidae and *Panopiliops inops* Goodnight & Goodnight, 1983 from the family Zalmoxidae. Both species were reported from the Damas cave. The remaining records (10) only mention the order without providing further details.

False scorpions (Pseudoscorpiones) were reported from five Costa Rican caves, but none of the reviewed studies provided species-level information. True scorpions (Scorpiones) were reported from three caves, and short-tailed whip scorpions (Schizomida) were observed in five caves in the Puntarenas region.

Records from 27 caves and subterranean sites mentioned Amblypygi, and there is a cave named after this animal (the Amblypigio cave). Cave-dwelling Amblypygi were reported to belong to the family Phrynidae, with one record of Tarantulidae. The second family was not mentioned in other sources and may refer to *Phrynus parvulus* (Pocock, 1902) of the family Phrynidae, previously known as *Tarantula marginemaculatus*. All Amblypygi in Costa Rica belong to the family Phrynidae, within the genera *Paraphrynus* (Moreno, 1940) and *Phrynus* (Harvey 2019). The identified species of cave-dwelling whip scorpions was *Paraphrynus laevifrons* (Pocock, 1894).



Figure 4 Arachnids in Costa Rican caves. A wandering spider (*Ancylopetes bogotensis*) with an egg sack B tarantula (*Theraphosidae*) C tailless whip scorpions (*Phrynidae*).

Myriapoda

Millipedes (Diplopoda) and centipedes (Chilopoda) were observed in at least 19 caves, but there were almost no data on their taxonomy, except for one record mentioning the family Polyxenidae. Garden centipedes from the class Symphyla were recorded from two caves.

Insecta

The reported species of cockroaches (Blattodea) were *Blaberus giganteus* (Linnaeus, 1758) and *Megaloblatta blaberoides* (Walker, 1871). A noteworthy refuge for cockroaches was the Hediondo cave, which harbors a large number of cockroaches from the *Blaberus* genus. Beetles (Coleoptera) from the families Alleculidae (Tenebrionidae), Bostrichidae, Carabidae, Cerambycidae, Curculionidae, Cleridae, Passalidae, Scarabaeidae, Scolytidae, Staphylinidae, and Tenebrionidae were observed inside Costa Rican caves and near their entrances. The Lamiinae subfamily and Clytini tribe of the Cerambycidae family, *Temnocheila* sp. (Trogossitidae), *Pyrophorus* sp. (Elateridae), and *Zophobas atratus* (Blanchard, 1845) (Tenebrionidae) were the only beetles classified at a lower taxonomic level. Cave crickets were observed on cave walls, but the available records only refer to them by their common names.

Bugs (Auchenorrhyncha) belonging to the families Fulgoridae and Cicadellidae were documented in Barra Honda National Park. True bugs (Heteroptera) from the families Reduviidae, Pentatomidae, Lygaeidae, Coreidae, Corixidae, and Cydnidae were reported to inhabit caves. Based on the specimens we collected during our field trips, we present the first record of the true bug *Amnestus subferrugineus* (Westwood 1837) (Heteroptera: Cydnidae) for the fauna of Costa Rica (Figure 5). This is the first record of the genus *Amnestus* Dallas, 1851 in Costa Rican caves.

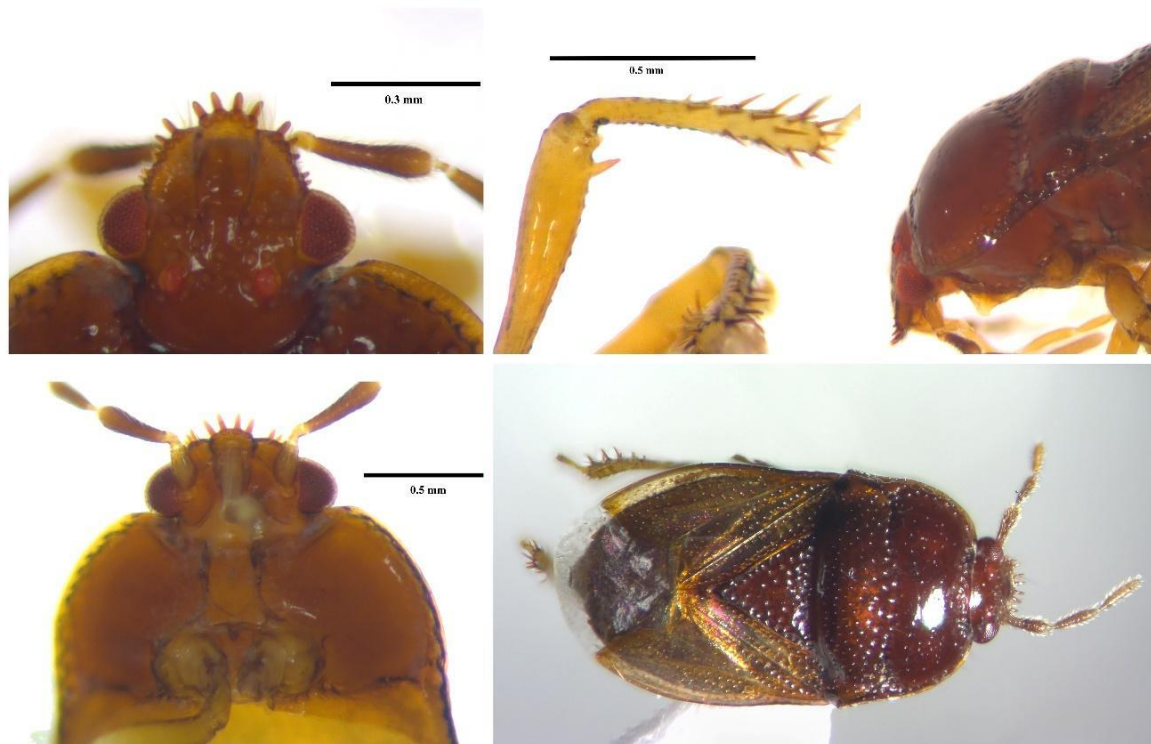


Figure 5 Morphological characteristics of the true bug (Amnestus subferrugineus) found in Costa Rican caves.

A few earwigs (Dermaptera) were mentioned in expedition reports from the Puntarenas province. Flies (Diptera), including but not limited to the families Streblidae, Tabanidae, Tachinidae, and Heleomyzidae, were reported in caves. Parasitic wingless flies *Strebla wiedemanni* Kolenati, 1856 and *Trichobius parasiticus* Gervais, 1844 were collected from vampire bats in various parts of the country. A single record of Ephemeroptera was reported from the Corredores cave. Ants (Formicidae) were observed in at least nine caves. Other Hymenoptera included the families Eumenidae, Ichneumonidae, Mutillidae, Pompilidae, Sphecidae, Tenthredinidae, and the wasp *Polistes instabilis* de Saussure, 1853 (Vespidae). All of the observed insect orders are listed in Table 1.

Entognatha

Springtails (Collembola) have also been observed in Costa Rican caves, but there have only been a few mentions of lower taxa. *Megalothorax minimus* Willem, 1900, *Isotomiella minor*

(Schaeffer, 1896), *Folsomina onychiurina* (Denis, 1931), *Folsomides* sp., *Isotomurus* sp. *Trogolaphysa* sp. Bristletails (Diplura) were found in caves, but no specific information regarding their taxonomy was available.

Other invertebrates

Reports exist regarding worms belonging to Turbellaria, Oligochaeta, and Clitellata. The term “worm” was also used as a general morphological descriptor for invertebrates observed within caves.

Table 1 Classes of invertebrates in Costa Rican caves. The first column represents the taxon. The second column (CS) presents the conservation status of the species: 1. IUCN Red List - “Least Concern” (LC), “Not Evaluated” (NE), “Data Deficient” (DD), “Vulnerable” (VU), 2. Included in the CITES convention: CITES, 3. Included in the annexes of the Costa Rican Biodiversity law (LEY) – “Vulnerable” (VU), “Reduced or threatened population” (TR). The third column (N) represents the number of individual sites where the taxon was present. The last column presents the sources of information regarding the taxa.

Taxon	CS	N	References
TURBELLARIA			
Turbellaria indet.		1	(Peacock and Hempel 1993)
POLYCHAETA			
Phyllodocida			
Nereididae			
<i>Lycastopsis</i> sp.		1	(Peacock and Hempel 1993)
OLIGOCHAETA			
Haplotaxida		1	(Peacock and Hempel 1993)
CLITELLATA			
Clitellata indet.		1	(Graening 2004)

Hirudinea

Hirudinea indet. 1 (Lips and Lips 2008)

BIVALVIA

Bivalvia indet. 1 ND[†]

GASTROPODA**Heterobranchia****Stylommatophora**

Achatinidae

Subulininae 8 ND

Subulina octona (Bruguière, 1789) 1 ND

Gastropoda indet. 10 (Lips and Lips 2008, Palacios 1994, Peacock and Hempel 1993), ND

THECOSTRACA

Pollicipedidae

Pollicipes elegans (Lesson, 1831) 1 ND

COPEPODA

Copepoda indet. 1 (Peacock and Hempel 1993)

MALACOSTRACA

Bathynellacea PS[§] (Peacock and Hempel 1993)

Decapoda

Palaemonidae

Macrobrachium carcinus (Linnaeus, 1758) IUCN-LC 5 (Hobbs 1994, Peacock and Hempel 1993), ND

Pseudothelphusidae

Potamocarcinus magnus (Rathbun, 1896) IUCN-LC PS[§] (Hobbs 1994)

Pseudothelphusa puntarenas Hobbs 1991[‡] IUCN-DD 1 (Hobbs 1991, Hobbs 1994, Peacock and Hempel 1993), ND

Pseudothelphusa sp. 1 (Gonzalez 2012)

Ptychophallus montanus (Rathbun, 1898) 1 (Hobbs 1994, Peacock and Hempel 1993)

Pseudothelphusidae indet.	7	(Hobbs 1994, Lips and Lips 2008, Peacock and Hempel 1993b, Quesada 2016), ND
Isopoda		
Oniscidea	11	(Graening 2004, Hempel 1989, Lips and Lips 2008, Palacios 1994, Peacock and Hempel 1993, Strinati et al. 1987), ND
Armadillidae	1	ND
ARACHNIDA		
Opiliones		
Phalangodidae	4	(Peacock and Hempel 1993)
Zalmoxidae		
<i>Pachylicus hispidus</i> Goodnight & Goodnight, 1983	1	(Goodnight and Goodnight 1983, Juberthie and Strinati 1994)
<i>Panopiliops inops</i> Goodnight & Goodnight, 1983	1	(Goodnight and Goodnight 1983, Juberthie and Strinati 1994)
Indet.	9	(Graening 2004, Hempel 1989, Lips and Lips 2008, Peacock and Hempel 1993), ND
Acari		
Acariformes		
Pyemotidae	1	(Palacios 1994)
Ixodida	1	(Hempel 1989)
Mesostigmata		
Ascidae	1	(Palacios 1994)
Dinychidae		
<i>Urodiaspis</i> sp.	1	(Palacios 1994)
Macronyssidae		
<i>Radfordiella desmodi</i> Radovsky, 1967	1	(Rojas et al. 2008)
Spinturnicidae		

<i>Periglischrus herrerae</i> Machado-Allison, 1965	1	(Rojas et al. 2008)
Uropodidae		
<i>Neodiscopoma</i> sp.	1	(Palacios 1994)
Indet.	1	(Palacios 1994)
Oribatida		
Carabodidae		
	1	(Palacios 1994)
Galunmidae		
	1	(Lips and Lips 2008)
Indet.	1	(Lips and Lips 2008)
Trombidiformes		
Arrenuridae		
<i>Arrenurus golfitensis</i> Cook, 1980	1	(Juberthie and Strinati 1994)
<i>Arrenurus plevamus</i> Cook, 1980	1	(Juberthie and Strinati 1994)
<i>Arrenurus zukovus</i> Cook, 1980	1	(Juberthie and Strinati 1994)
Hydrachnellae		
<i>Protolimnesia mesoamericana</i> Cook, 1980	1	(Juberthie and Strinati 1994)
Limnesiidae		
<i>Neomamersa costarriquensis</i> Cook, 1980	PS [§]	(Juberthie and Strinati 1994)
<i>Neomamersa decussa</i> Cook, 1980	PS [§]	(Juberthie and Strinati 1994)
<i>Psammolimnesia costarriquena</i> Cook, 1980	PS [§]	(Juberthie and Strinati 1994)
Omartacaridae		
<i>Omartacarus motasi</i> Cook, 1980	NC [§]	(Juberthie and Strinati 1994)
Rhagidiidae		
	1	(Palacios 1994)
Torrenticolidae		
<i>Torrenticola amala</i> Cook, 1980	1	(Juberthie and Strinati 1994)
<i>Frontipodopsis mesoamericana</i> Cook, 1980	1	(Juberthie and Strinati 1994)
<i>Frontipodopsis staheli</i> Walter, 1919	1	(Juberthie and Strinati 1994)
<i>Maharashtracarus neotropicus</i> Cook, 1980	1	(Juberthie and Strinati 1994)

Acari indet.		14	(Graening 2004, Hempel 1989, Lips and Lips 2008, Peacock and Hempel 1993, Strinati et al. 1987), ND
Pseudoscorpiones		5	(Lips and Lips 2008, Palacios 1994, Peacock and Hempel 1993, Strinati et al. 1987), ND
Scorpiones		3	(Hempel 1989, Peacock and Hempel 1993, Strinati et al. 1987)
Araneae			
Attidae		1	ND
Ctenidae			
<i>Ctenus</i> sp.		1	ND
<i>Ancylometes bogotensis</i> (Keyserling, 1877)		1	ND
Dysderidae		1	(Alpizar et al. 2006)
Lycosidae		1	ND
Segestriidae			
<i>Ariadna isthmica</i> Beatty, 1970		1	(Alpizar et al. 2006)
Theraphosidae		2	(Alpizar et al. 2006), ND
<i>Sericopelma upala</i> Valerio, 1980	LEY-RTP	1	(Alpizar et al. 2006)
Theridiidae		1	(Alpizar et al. 2006)
Theridiosomatidae		1	(Alpizar et al. 2006)
Trechaleidae			
<i>Trechalea</i> sp.		1	ND
Araneae indet.		20	(Graening 2004, Hapka et al. 1992, Hempel 1989, Lips and Lips 2008, Quesada and Deleva 2016, Palacios 1994, Peacock and Hempel 1993, Strinati et al. 1987)
Amblypygi			
Phrynidae			
<i>Paraphrynus laevifrons</i> (Pocock, 1894)		1	(Mullinex 1975, Juberthie and Strinati 1994, Alpizar et al. 2006)
<i>Paraphrynus viridiceps</i> (Pocock, 1894) ¹		7	(Peacock and Hempel 1993)

<i>Paraphrynus</i> sp.	7	ND
Phrynidae indet.	20	(Graening 2004, Debeljak 1988, Hapka et al. 1992, Hempel 1989, Lips and Lips 2008, Quesada 2018, Peacock and Hempel 1993, Strinati et al. 1987), ND
Schizomida	5	(Lips and Lips 2008, Juberthie and Strinati 1994, Strinati et al. 1987)
SYMPHYLA		
Symphyla indet.	2	(Lips and Lips 2008, Strinati et al. 1987)
CHILOPODA		
Chilopoda indet.	3	(Lips and Lips 2008, Strinati et al. 1987), ND
DIPLOPODA		
Polyxenidae	1	(Palacios 1994)
Diplopoda indet.	17	(Graening 2004, Hempel 1989, Lips and Lips 2008, Palacios 1994, Peacock and Hempel 1993, Strinati et al. 1987), ND
ENTOGNATHA		
Collembola		
Neelipleona		
Neelidae		
<i>Megalothorax cf. minimus</i> Willem, 1900	1	(Palacios 1994)
<i>Megalothorax</i> sp.	1	(Palacios 1994)
Entomobryomorpha		
Paronellidae		
<i>Cyphodeus</i> sp.	1	(Juberthie and Strinati 1994)
<i>Trogolaphysa</i> sp.	1	(Palacios 1994)
Isotomidae		
<i>Folsomides</i> sp.	1	(Palacios 1994)
<i>Folsomina onychiurina</i> Denis, 1931	1	(Palacios 1994)
<i>Isotomurus minor</i> UN	1	(Palacios 1994)

<i>Isotomiella cf. minor</i> (Schäffer, 1896)	1	(Palacios 1994)
Collembola indet.	1	(Hapka et al. 1992, Quesada and Deleva 2016, Lips and Lips 2008, Peacock and Hempel 1993, Strinati et al. 1987), ND
Diplura		
Japygidae	1	(Strinati et al. 1987)
Diplura indet.	4	(Graening 2004, Hempel 1989, Lips and Lips 2008), ND
INSECTA		
Archaeognatha		
Meinertellidae		
<i>Grasiella</i> sp.	1	(Juberthie and Strinati 1994)
Zygentoma		
Nicoletiidae		
<i>Nicoletia cf. phytophile</i> Gervais, 1844	1	(Juberthie and Strinati 1994)
Zygentoma (reported as Thysanura)	1	(Strinati et al. 1987)
Ephemeroptera		
Heptageniidae	1	ND
Odonata		
	2	(Hempel 1989, Peacock and Hempel 1993)
Orthoptera		
Acrididae	1	(Hempel 1989)
Gryllacrididae	1	(Hempel 1989)
Gryllidae	1	(Hempel 1989)
Phalangopsidae	11	(Ulloa and Quesada 2010), ND
Tettigoniidae	1	(Hempel 1989)
Orthoptera indet.	12	(Quesada and Deleva 2016, Goicoechea 2010, Graening 2004, Hapka et al. 1992, Lips and Lips 2008, Peacock and Hempel 1993, Strinati et al. 1987)
Neuroptera		

Chrysopidae	1	(Hempel 1989)
Myrmeleontidae	2	(Graening 2004, Hempel 1989)
Dermaptera	7	(Lips and Lips 2008, Peacock and Hempel 1993)
Mantodea		
Mantidae	1	(Hempel 1989)
Blattodea		
Blaberidae		
<i>Blaberus giganteus</i> (Linnaeus, 1758)	3	(Graening 2004), ND
<i>Blaberus</i> sp.	1	(Ulloa and Quesada 2010)
Ectobiidae		
<i>Megaloblatta blaberoidea</i> (Walker, 1871) (as <i>M. rufipes</i> Dohrn, 1887)	1	(Palacios 1994)
Blattodea indet.	16	(Hapka et al. 1992, Hempel 1989, Lips and Lips 2008, Peacock and Hempel 1993), ND
Isoptera	1	(Hempel 1989)
Hemiptera		
Coreidae	1	(Hempel 1989)
Cicadellidae	1	(Hempel 1989)
Corixidae	1	(Hempel 1989)
Cydnidae		
<i>Amnestus subferrugineus</i> (Westwood, 1837)	1	ND
Fulgoridae	1	(Hempel 1989)
Lygaeidae	1	(Hempel 1989)
Pentatomidae	1	(Hapka et al. 1992, Hempel 1989)
Reduviidae		
<i>Triatoma</i> sp.	1	(Hempel 1989)
Hemiptera indet.	9	(Graening 2004, Hapka et al. 1992, Lips and Lips 2008, Peacock and Hempel 1993)

Hymenoptera

Eumenidae	1	(Hempel 1989)
Formicidae	10	(Graening 2004, Hapka et al. 1992, Hempel 1989, Peacock and Hempel 1993), ND
Ichneumonidae	1	(Hempel 1989)
Mutillidae	1	(Graening 2004, Hempel 1989)
Pompilidae	1	(Hempel 1989)
Sphecidae	1	(Graening 2004)
Tenthredinidae	1	(Hempel 1989)
Vespidae	1	(Hempel 1989)
<i>Polistes instabilis</i> de Saussure, 1853	1	(Graening 2004)
Hymenoptera indet.	4	(Hempel 1989, Lips and Lips 2008, Peacock and Hempel 1993)

Coleoptera

Alleculidae	1	(Hempel 1989)
Bostrichidae	1	(Hempel 1989)
Carabidae	2	(Graening 2004)
Cerambycidae	2	(Hempel 1989)
Cleridae	1	(Hempel 1989)
Curculionidae	1	(Hempel 1989)
Elateridae		
<i>Pyrophorus</i> sp.	1	(Hempel 1989)
Passalidae	1	(Hempel 1989)
Scarabaeidae	1	(Graening 2004)
Scolytidae	1	(Hempel 1989)
Staphylinidae	1	ND
Tenebrionidae	1	(Graening 2004)
<i>Zophobas atratus</i> (Fabricius, 1775)	2	(Tschinkel 1984, Juberthie and Strinati 1994)

Trogossitidae		
<i>Temnoscheila</i> (as <i>Temnochila</i>) sp.	1	(Hempel 1989)
Coleoptera indet.	12	(Hapka et al. 1992, Lips and Lips 2008, Palacios 1994, Peacock and Hempel 1993)
Trichoptera	1	(Hempel 1989)
Lepidoptera		
Nymphalidae	1	(Hempel 1989)
(as Brassolidae)		
Hesperiidae	1	(Hempel 1989)
Lycaenidae	1	(Hempel 1989)
Noctuidae	1	(Hempel 1989)
Nymphalidae	1	(Hempel 1989)
Tineidae	1	(Lips and Lips 2008)
Lepidoptera indet.	3	(Hapka et al. 1992, Lips and Lips 2008, Peacock and Hempel 1993, (Strinati et al. 1987)
Diptera		
Heleomyzidae	1	(Graening 2004)
Streblidae		
<i>Exastinion clovisi</i> (Pessõa & Guimarães, 1937)	1	(Mitchell et al. 2018)
<i>Megistopoda aranea</i> (Coquillett, 1899)	1	(Mitchell et al. 2018)
<i>Strebla wiedemanni</i> Kolenati, 1863	1	(Rojas et al. 2008)
<i>Trichobius lionycteridis</i> Wenzel, 1966	1	(Mitchell et al. 2018)
<i>Trichobius pallidus</i> (Curran, 1934)	3	(Mitchell et al. 2018)
<i>Trichobius parasiticus</i> Gervais, 1844	1	(Rojas et al. 2008)
Indet.	1	(Hempel 1989)
Tabanidae	1	(Hempel 1989)
Tachinidae	1	(Hempel 1989)

Insecta indet.	15	(Debeljak 1988, Goicoechea 2010a, Lips and Lips 2008), ND
Unknown arthropods	4	(Lips and Lips 2008)
Unknown invertebrates	5	(Lips and Lips 2008)

† - ND - new data: original contribution to this paper, ‡ - stygobiont, § - some sources report a region instead of a single site: PS – Puntarenas, NC – Nicoya, / - species is most likely misidentified.

Vertebrates

Actinopterygii

Two species of Costa Rican fish (Actinopterygii) display adaptations to cave life. These species are the three-barbed catfish from the *Rhamdia* genus and the characid Mexican tetra (*Psalidodon fasciatus* (De Filippi, 1853)). Pale-colored individuals of the catfish species *Rhamdia guatemalensis* (Günther, 1864) were observed in the Corredores and Bananal cave systems as well as in other adjacent caves (Figure 6). Furthermore, pale-colored individuals of the same genus have been reported in an artificial tunnel near Arenal volcano. The Mexican tetra, also known as the blind cave fish, was studied in a karstic spring in Guanacaste. Livebearing fishes from the *Brachyrhaphis* genus, rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)), Nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758)), and various unidentified characids (Characidae), cyprinids (Cyprinidae), and catfish (Heptapteridae) were reported from caves and springs (Table 2, Supplementary Material 1).



Figure 6 Pale-colored catfish (*Rhamdia guatemalensis*) in the Corredores cave.

Amphibia

Frogs and toads (Anura) were observed both at the entrances and deep inside the caves (Figure 7). We found records of at least eight species belonging to five frog and toad families (Table 2). These included poison dart frogs, *Dendrobates auratus* (Girard, 1855), *Oophaga granulifera* (Taylor, 1958), thin-toed frogs (*Leptodactylus savagei* Heyer, 2005), grass frogs (*Lithobates forreri* (Boulenger, 1883)), *L. warszewitschii* (Schmidt, 1857)), toads (*Rhinella horribilis* (Wiegmann, 1833)), and *Incilius aucoinae* (O'Neill & Mendelson, 2004). Cane toads (*Rhinella horribilis*) were observed at the bottom vertical shafts on several occasions. There were two observations of tadpoles from the Carma and Corredores caves. The cave “Pozo Sapo Gordo” (“Fat Toad Abyss”) received its name because of the presence of a large cane toad.



Figure 7 Frogs and toads found in Costa Rican caves. A Forrers grass frog (*Lithobates forreri*) B green and black poison dart frog (*Dendrobates auratus*) C and E Fitzinger's Robber Frog (*Craugastor fitzingeri*) D rainforest toad (*Incilius aucoinae*) F thin-toed frog (*Leptodactylus savagei*) G cane toad (*Rhinella horribilis*).

Reptilia

The South American snapping turtle (*Chelydra acutirostris* (Peters, 1862)) and the white-lipped mud turtle (*Kinosternon leucostomum* (Duméril, Bibron & Duméril, 1851)) were observed on multiple occasions deep inside a flooded artificial tunnel (Figure 8 A and B). The fer-de-lance (*Bothrops asper* (Garman, 1883)) was observed both at the entrances and in narrow passages inside the caves (Figure 8C). The caves “Serpiente Dormida,” “Pozo del Chispero,” “Terciopelo,” and “Pozo Oropel” were named after encounters between snakes and cave explorers. The aquatic prawn snake (*Hydromorphus concolor* (Peters, 1859)) was observed in an artificial tunnel near Arenal volcano (Figure 8E). Additionally, there were sightings of a boa (Boaidae) and an unknown species of snake, solely identified based on visual characteristics. The night lizard (*Lepidophyma reticulatum* (Taylor, 1955)) was

observed in at least three caves (Figure 8D). Reports also mentioned the presence of geckos (Geckota) within a cave.

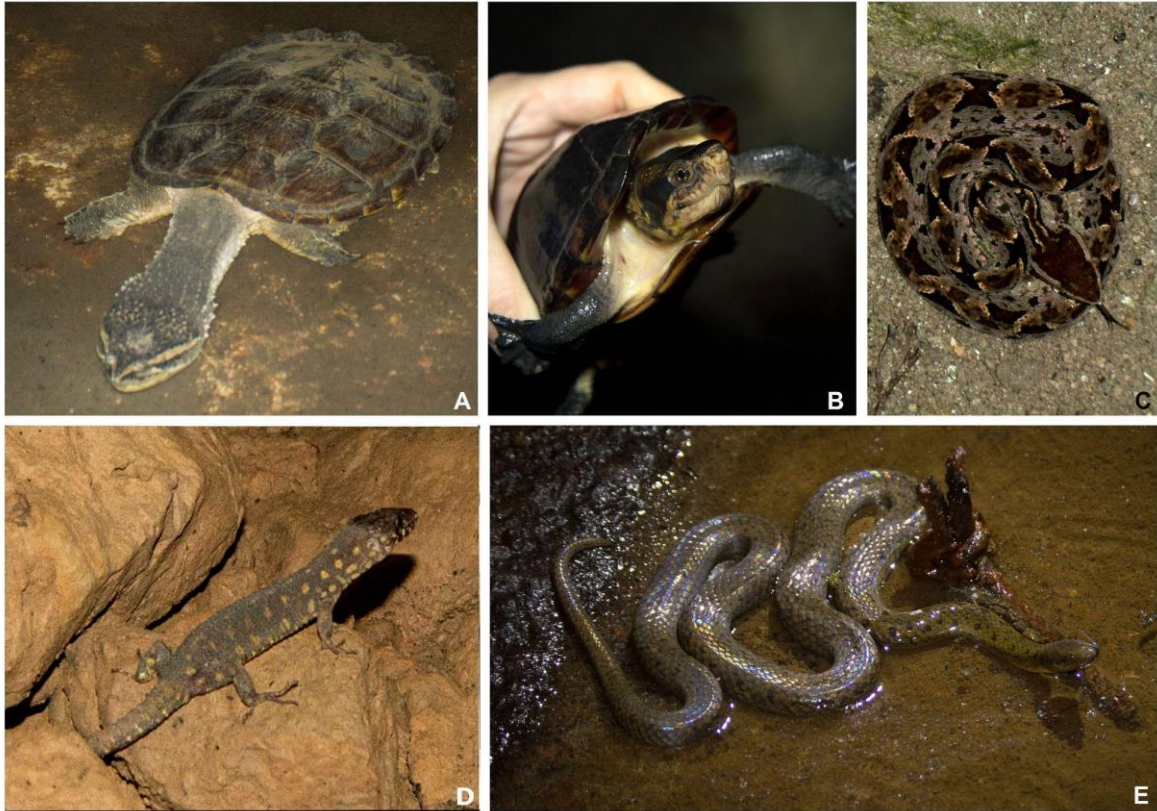


Figure 8 Reptiles living in caves. A South American snapping turtle (*Chelydra acutirostris*) B white-lipped mud turtle (*Kinosternon leucostomum*) C fer-de-lance - (*Bothrops asper*) D Costa Rican tropical night lizard (*Lepidophyma reticulatum*) E prawn snake (*Hydromorphus concolor*).

Aves

Information regarding birds residing in and around caves was limited. However, there were a few noteworthy observations. The entrance of an artificial tunnel near Rio Terraba served as a nesting site for a black vulture (*Coragyps atratus* (Bechstein, 1793)). Additionally, sightings near cave entrances included a great tinamou (*Tinamus major* (Gmelin, 1789)) and a wood rail (*Aramides cajaneus* Müller, 1776).

Mammalia

Non-volant mammals in the subterranean ecosystem predominantly comprise of small predators and rodents. Various tracks attributed to carnivorous mammals such as cats and mustelids have been observed in different caves. In Palo Verde National Park, the “Tigre cave” presumably served as a roosting site for a large cat, possibly a jaguar or puma. An ocelot (*Leopardus pardalis* (Linnaeus, 1758)) was sighted in an artificial tunnel, and bones of kinkajou (*Potos flavus* (Schreber, 1774)) were discovered in Trampa vertical cave. Opossums (Didelphidae) of at least four species were observed within the caves. Caves in Barra Honda yielded bones from various mammals, including peccary (*Dicotyles tajacu* (Linnaeus, 1758)), cottontail rabbit (*Sylvilagus* sp.), white-tailed deer (*Odocoileus virginianus* (Zimmermann, 1780)), armadillo (*Dasypus novemcinctus* (Linnaeus, 1758)), porcupine (*Coendou mexicanus*), and several species of rodents (Rodentia). It remains unclear whether these mammals entered the caves or whether their carcasses were brought in by predators.

Table 2 Vertebrate fauna in Costa Rican caves. The first column represents the taxon. The second column (CS) presents the conservation status of the species: 1. IUCN Red List - “Least Concern” (LC), “Not Evaluated” (NE), “Data Deficient” (DD), “Vulnerable” (VU), 2. Included in the CITES convention: CITES, 3. Included in the annexes of the Costa Rican Biodiversity law (LEY) – “Vulnerable” (VU), “Reduced or threatened population” (TR). The third column (N) represents the number of individual sites where the taxon was present. The last column presents the source of the information on the taxa.

Taxon	CS	N	Reference(s)
ACTINOPTERYGII			
Cypriniformes			
Cyprinidae		1	(Peacock and Hempel 1993)
Characiformes			
Characidae			
<i>Psalidodon fasciatus</i> (De Filippi, 1853) (as <i>Astyanax fasciatus</i>) [§]	IUCN-LC	1	(Romero 1985)
Characidae indet.		1	ND
Siluriformes			
Heptapteridae			
<i>Rhamdia guatemalensis</i> (Günther, 1864) [§]	IUCN-LC	5	(Debeljak 1988, Juberthie and Strinati 1994, Grupo Espeleológico Anthros 2023), ND
<i>Rhamdia nicaraguensis</i> (Günther, 1864)	IUCN-LC	1	(Gonzalez 2012)
<i>Rhamdia</i> sp.		4	(Strinati et al. 1987), ND

Heptapteridae indet.		3	(Quesada and Deleva 2016, Peacock and Hempel 1993)
Salmoniformes			
Salmonidae			
<i>Oncorhynchus mykiss</i> (Walbaum, 1792)		1	(González 2010)
Cichliformes			
Cichlidae			
<i>Oreochromis niloticus</i> (Linnaeus, 1758)	IUCN-LC	1	(Gonzalez 2012)
Cyprinodontiformes			
Poeciliidae			
<i>Brachyrhaphis rhabdophora</i> (Regan, 1908)	IUCN-VU	1	(Romero 1985)
<i>Brachyrhaphis olomina</i> (Meek, 1914)	IUCN-DD	1	(Gonzalez 2012)
Indet. ‡		1	(Woodman 1988)
AMPHIBIA			
Anura			
Craugastoridae			
<i>Craugastor fitzingeri</i> (Schmidt, 1857)	IUCN-LC	2	(Quesada 2018), ND
Bufonidae			
<i>Rhinella horribilis</i> (Wiegmann, 1833)	IUCN-LC	5	(Gonzalez 2012, Graening 2004), ND
<i>Incilius aucoinae</i> (O'Neill & Mendelson, 2004)	IUCN-LC	1	ND
Bufonidae indet.		2	(Hapka et al. 1992), ND
Dendrobatidae			
<i>Dendrobates auratus</i> (Girard, 1855)	IUCN-LC, CITES-II, LEY-RTP	1	(Quesada 2018), ND
<i>Oophaga granulifera</i> (Taylor, 1958)	IUCN-VU, CITES-II, LEY-RTP	1	ND
Leptodactylidae			
<i>Leptodactylus savagei</i> Heyer, 2005	IUCN-LC	2	(Quesada and Deleva 2016), ND
Ranidae			
<i>Lithobates warszewitschii</i> (Schmidt, 1857)	IUCN-LC	1	(Ulloa and Quesada 2010)
<i>Lithobates forreri</i> (Boulenger, 1883)	IUCN-LC	1	ND
Ranidae indet.		1	(Lips and Lips 2008, Peacock and Hempel 1993, Strinati et al. 1987), ND
Anura indet.		1	(Quesada 2009b)
Amphibia indet. ‡		8	(Woodman 1988)
REPTILIA			
Testudines			
Chelydridae			
<i>Chelydra acutirostris</i> Peters, 1862	CITES-II	1	(Gonzalez 2012), ND

Kinosternidae				
<i>Kinostemon leucostomum</i> (Duméril, Bibron & Duméril, 1851)	CITES-II	1	(Gonzalez 2012), ND	
Squamata				
Boaidae				
		2	(Lyon et al. 2004), Vicente-Santos 2019)	
Colubridae				
<i>Hydromorphus concolor</i> Peters, 1859	IUCN-LC	1	ND	
Viperidae				
<i>Bothrops asper</i> (Garman, 1883)	IUCN-LC	2	(Quesada 2009a), ND	
<i>Bothriechis schlegelii</i> (Berthold, 1846)	IUCN-LC	1	(Hapka et al. 1992)	
Serpentes				
		2	(Hapka et al. 1992)	
Xantusiidae				
<i>Lepidophyma reticulatum</i> Taylor, 1955	IUCN-LC	2	(Ulloa 2009b), ND	
Gekkota				
		1	(Graening 2004)	
Reptilia indet. ‡				
		1	(Goicoechea 2010, Graening 2004, Hapka et al. 1992, Woodman 1988)	
AVES				
Tinamiformes				
Tinamidae				
<i>Tinamus major</i> (Gmelin, 1789)	IUCN-LC	1	ND	
Gruiformes				
Rallidae				
<i>Aramides cajaneus</i> (Müller, 1776)	IUCN-LC	1	(Gonzalez 2012)	
Cathartiformes				
Cathartidae				
<i>Coragyps atratus</i> (Bechstein, 1793)	IUCN-LC	1	(Quesada 2018), ND	
MAMMALIA				
Cingulata				
Dasypodidae				
<i>Dasypus novemcinctus</i> Linnaeus, 1758 ‡	IUCN-LC	2	(Hempel 1989, Woodman 1988)	
Didelphimorphia				
Didelphidae				
<i>Caluromys derbianus</i> (Waterhouse, 1841)	IUCN-LC	1	(Trescott and Vicente-Santos 2019)	
<i>Didelphis marsupialis</i> Linnaeus, 1758	IUCN-LC	1	(Hempel 1989)	
<i>Didelphis</i> sp. ‡		1	(Woodman 1988)	
<i>Marmosa mexicana</i> Merriam, 1897	IUCN-LC	1	(Hempel 1989)	
<i>Philander opossum</i> (Linnaeus, 1758)	IUCN-LC	1	(Gonzalez 2012)	
Didelphidae indet.				
		2	ND	

Lagomorpha

Leporidae

Sylvilagus sp. 2 (Hempel 1989)

Rodentia

Cricetidae

Oryzomys sp. 1 (Hempel 1989)

Ototylomys phyllotis Merriam, 1901 ‡ IUCN-LC 1 (Hempel 1989, Woodman 1988)

Peromyscus stirtoni Dickey, 1928 ‡ IUCN-LC 1 (Woodman 1988)

Peromyscus sp. ‡ 1 (Woodman 1988)

Reithrodontomys sp. ‡ 1 (Woodman 1988)

Sigmodon hispidus Say & Ord, 1825 ‡ IUCN-LC 3 (Hempel 1989, Woodman 1988)

Cuniculidae

Cuniculus paca (Linnaeus, 1766) IUCN-LC, LEY-RTP 3 (Hempel 1989, Woodman 1988)

Dasyproctidae

Dasyprocta punctata (Gray, 1842) ‡ IUCN-LC 4 (Hempel 1989, Lips and Lips 2008, Woodman 1988)

Erethizontidae

Coendou mexicanus (Kerr, 1792) ‡ IUCN-LC 2 (Hempel 1989, Woodman 1988)

Geomyidae

Orthogeomys sp. 1 (Hempel 1989)

Heteromyidae

Liomys salvini (Thomas, 1893) ‡ IUCN-LC, LEY-RTP 2 (Hempel 1989, Woodman 1988)

Carnivora

Procyonidae

Potos flavus (Schreber, 1774) ‡ IUCN-LC 1 (Hempel 1989)

Felidae

Leopardus pardalis (Linnaeus, 1758) IUCN-LC, CITES-I, LEY-VU 1 (Gonzalez 2012)

Indet. 2 (Graening 2004, Hapka et al. 1992)

Indet. 1 ND

Perissodactyla

Equidae

Equus ferus caballus Linnaeus, 1758 ‡ 1 ND

Artiodactyla

Cervidae

Odocoileus virginianus (Zimmermann, 1780) ‡ IUCN-LC 1 (Hempel 1989)

Tayassuidae

Dicotyles tajacu (Linnaeus, 1758) ‡ IUCN-LC 2 (Hempel 1989, Woodman 1988)

† - ND - new data: original contribution to this paper, ‡ - bones, § - observed individuals with morphological adaptations toward cave life.

Chiroptera

Bats were documented at least in 97 subterranean sites throughout the country (Table 3). Thirty-six bat species from the families Emballonuridae, Mormoopidae, Natalidae, Noctilionidae, Phyllostomidae, and Vespertilionidae have been reported inside caves or at their entrances (Figure 9). The most frequently observed species was Seba's short-tailed bat (*Carollia perspicillata* (Linnaeus, 1758)), found in 44 locations, followed by the common vampire bat (*Desmodus rotundus* (Geoffroy, 1810)) (34 locations), greater dog-like bat (*Peropteryx kappleri* (Peters, 1867)) (22 locations), greater sac-winged bat (*Saccopteryx bilineata* (Temminck, 1838)) (17 locations), Pallas's long-tongued bat (*Glossophaga soricina* (Pallas, 1766)) (12 locations), and Tomes' sword-nosed bat (*Lonchorhina aurita* Tomes, 1863 (10 locations)). Parnell's mustached bat was reported as either *Pteronotus parnellii* or *P. mesoamericanus* at 17 locations. The funnel-eared bat, found in 10 locations, was identified as *Natalus mexicanus* in some sources and either *Natalus stramineus* or *Natalus lanatus* in others. However, these scientific names are currently accepted as synonyms, suggesting that they likely represent the same species (Solari, 2019). Regarding the importance of caves as bat roosts, eight species (*Glossophaga leachii*, *Lonchophylla robusta*, *Lonchorhina aurita*, *Natalus mexicanus*, *Pteronotus davyi*, *Pteronotus gymnonotus*, *Pteronotus mesoamericanus*, and *Pteronotus personatus*) were considered cave-dependant. Among the caves with the highest reported bat species richness were La Trampa (13 species), Corredores (11 species), Gabinarraca (8 species), Emus (8 species), Damas (7 species) Pozo Hediondo (6 species), and an artificial tunnel near Arenal volcano (6 species) (Supplementary Material 1). The global conservation status of all the observed cave-dwelling bats was Least Concern. The Costa Rican Law for the protection of wildlife included four species (*Anoura cultrata*, *Chrotopterus auritus*, *Lonchophylla concava* and *Trinycteris nicefori*) in the category "Reduced or Threatened population" (Table 3).



Figure 9 Cave-dwelling bats in Costa Rica. A Mexican greater funnel-eared bat (*Natalus mexicanus*) B a group of common vampire bats (*Desmodus rotundus*) with pups C sword-nosed bats (*Lonchorhina aurita*) D Parnell's mustached bats (*Pteronotus parnellii*) E Jamaican fruit bat (*Artibeus jamaicensis*) F greater sac-winged bat (*Saccopteryx bilineata*) G Goldman's nectar bat (*Lonchophylla concava*) H a group of Seba's short-tailed bats (*Carollia perspicillata*) with an albino pup I hairy-legged vampire bat (*Diphylla ecaudata*) with a pup J orange nectar bat (*Lonchophylla robusta*) K fringe-lipped bat (*Trachops cirrhosus*) L greater spear-nosed bat (*Phyllostomus hastatus*).

Table 3 Bats (Chiroptera) in Costa Rican caves. The first column represents the taxon. The second column (CD) represents the species dependence on caves. The third column (CS) shows the species conservation status, according to the IUCN Red List (IUCN) and the Costa Rican Biodiversity law (LEY). The abbreviations are "Least concern: (LC) and "reduced or threatened population" (RTP). The fourth column (N_s) represents the number of individual sites where the taxon was present. The last column presents the source of the information on the taxa.

Taxon	CD	CS	N _s	References
Emballonuridae				
<i>Balantiopteryx plicata</i> (Peters, 1867)		IUCN-LC	2	(Timm and McClearn 2007, Graening 2004)
<i>Peropteryx kappleri</i> (Peters, 1867)		IUCN-LC	22	(Deleva and Chaverri 2018, Goicoechea and Quesada 2019, Lips and Lips 2008, Quesada 2009b), ND [†]
<i>Peropteryx macrotis</i> (Wagner, 1843)		IUCN-LC	2	(Deleva and Chaverri 2018)

<i>Peropteryx</i> sp.		IUCN-LC	1	(Lips and Lips 2008),
<i>Saccopteryx bilineata</i> (Temminck, 1838)		IUCN-LC	17	(Deleva and Chaverri 2018, Goicoechea and Quesada 2019, Gonzalez 2012, Quesada 2018, Quesada and Deleva 2016, Timm and McClearn 2007)
Mormoopidae				
<i>Pteronotus davyi</i> (Gray, 1838)	+	IUCN-LC	4	(Cubero and Artavia 2016, Flemming 2003, Hempel 1989)
<i>Pteronotus gymnotus</i> (Natterer, 1843)	+	IUCN-LC	5	(Cubero and Artavia 2016, Deleva and Chaverri 2018), ND
<i>Pteronotus parnellii</i> (= <i>mesoamericanus</i>) (Gray, 1843)	+	IUCN-LC	17	(Cubero and Artavia 2016, Deleva and Chaverri 2018, Gonzalez 2012, Heithaus and Fleming 1978, Hempel 1989, Mitchell et al. 2018, Quesada 2018, Quesada and Deleva 2016, Trescott and Vicente-Santos 2019), ND
<i>Pteronotus personatus</i> (Wagner, 1843)	+	IUCN-LC	3	(Cubero and Artavia 2016), Deleva and Chaverri 2018, Lips and Lips 2008)
<i>Pteronotus</i> sp.			2	(Hempel 1989, Ulloa and Quesada 2010), ND
Natalidae				
<i>Natalus mexicanus</i> (= <i>lanatus</i> or <i>stramineus</i>) (Wagner, 1843)	+	IUCN-LC	10	(Cubero and Artavia 2016, Deleva and Chaverri 2018, Hempel 1989, Flemming 2003, Rodríguez-Herrera et al. 2011, Trescott and Vicente-Santos 2019), ND
Noctilionidae				
<i>Noctilio leporinus</i> (Linnaeus, 1758)		IUCN-LC	1	(Romero 1985)
Phyllostomidae				
<i>Anoura cultrata</i> Handley, 1960		IUCN-LC, LEY-RTP	1	(Mitchell et al. 2018)
<i>Anoura</i> sp.			2	(Deleva and Chaverri 2018)
<i>Artibeus jamaicensis</i> Leach, 1821		IUCN-LC	10	(Cubero and Artavia 2016, Deleva and Chaverri 2018, Goicoechea and Quesada 2019, Hempel 1989, Mitchell et al. 2018, Quesada 2018), ND
<i>Artibeus lituratus</i> (Olfers, 1818)		IUCN-LC	1	(Cubero and Artavia 2016)
<i>Artibeus</i> sp.			3	(Hempel 1989)
<i>Carollia perspicillata</i> (Linnaeus, 1758)		IUCN-LC	44	(Cubero and Artavia 2016, Deleva and Chaverri 2018, Fleming and Heithaus 1986, Flemming 2003, Gonzalez 2012, Goicoechea and Quesada 2019, Heithaus and Fleming 1978, Hempel 1989, Lips and Lips 2008, Mitchell et al. 2018, Ulloa 2009b, Quesada and Deleva 2016, Quesada 2018, Trescott and Vicente-Santos 2019, Villalobos-Chaves et al. 2016), ND
<i>Carollia sowellii</i> Baker, Solari & Hoffmann, 2002		IUCN-LC	2	(Deleva and Chaverri 2018, Villalobos-Chaves et al. 2016)
<i>Carollia subrufa</i> (Hahn, 1905)		IUCN-LC	1	(Heithaus and Fleming 1978)
<i>Chrotopterus auritus</i> Peters, 1856	+	IUCN-LC, LEY-RTP	2	(Deleva and Chaverri 2018, Graening 2004)
<i>Dermanura phaeotis</i> Miller, 1902		IUCN-LC	1	(Cubero and Artavia 2016)

<i>Desmodus rotundus</i> (Geoffroy, 1810)		IUCN- LC	34	(Cubero and Artavia 2016, Deleva and Chaverri 2018, Gonzalez 2012, Goicoechea and Quesada 2019, Graening 2004, Hapka et al. 1992, Heithaus and Fleming 1978, Hempel 1989, Lips and Lips 2008, Peacock and Hempel 1993, Quesada 2013, Quesada 2015, Quesada and Deleva 2016, Trescott and Vicente-Santos 2019, Trimm and McClearn 2007, Ulloa 2009b, Villalobos-Chaves et al. 2016, Vicente-Santos 2019), ND
<i>Diphylla ecaudata</i> Spix, 1823		IUCN- LC	4	(Cubero and Artavia 2016, Hempel 1989, Trescott and Vicente-Santos 2019), ND
<i>Glossophaga commissarisi</i> Gardner, 1962		IUCN- LC	1	(Cubero and Artavia 2016)
<i>Glossophaga leachii</i> Gray, 1844	+	IUCN- LC	1	(Cubero and Artavia 2016)
<i>Glossophaga soricina</i> (Pallas, 1766)		IUCN- LC	12	(Cubero and Artavia 2016, Deleva and Chaverri 2018, Gonzalez 2012, Flemming 2003, Hempel 1989, Lips and Lips 2008), ND
<i>Lamproncyteris brachyotis</i> (Dobson, 1879)		IUCN- LC	3	(Cubero and Artavia 2016)
<i>Lonchophylla concava</i> Goldman, 1914		IUCN- LC, LEY- RTP	3	(Deleva and Chaverri 2018)
<i>Lonchophylla robusta</i> Miller, 1912	+	IUCN- LC	7	(Armstrong 1969, Deleva and Chaverri 2018, Goicoechea and Quesada 2019, Lips and Lips 2008, Trescott and Vicente-Santos 2019), ND
<i>Lonchorhina aurita</i> Tomes, 1863	+	IUCN- LC	10	(Deleva and Chaverri 2018, Mitchell et al. 2018, Nelson 1965, Trescott and Deleva 2016, Trescott and Vicente-Santos 2019, Villalobos-Chaves et al. 2016, Vicente-Santos 2019), ND
<i>Micronycteris megalotis</i> (Gray, 1842)		IUCN- LC	2	(Hempel 1989, Peacock and Hempel 1993)
<i>Micronycteris microtis</i> (Miller, 1898)		IUCN- LC	1	(Villalobos-Chaves et al. 2016)
<i>Micronycteris schmidtorum</i> Sanborn, 1935		IUCN- LC	1	(Woodman 1988)
<i>Phyllostomus discolor</i> Wagner, 1843		IUCN- LC	1	(Deleva and Chaverri 2018)
<i>Phyllostomus hastatus</i> (Pallas, 1767)		IUCN- LC	6	(Deleva and Chaverri 2018, Goicoechea and Quesada 2019, Gonzalez 2012, Hempel 1989, Quesada and Deleva 2016, Timm and McClearn 2007), ND
<i>Phyllostomus</i> sp.			1	(Peacock and Hempel 1993)
<i>Tonatia saurophila</i> Koopman & Williams, 1951		IUCN- LC	1	(Trescott and Vicente-Santos 2019)
<i>Trachops cirrhosus</i> (Spix, 1823)		IUCN- LC	5	(Deleva and Chaverri 2018, Trescott and Vicente-Santos 2019, Vicente-Santos 2019), ND
<i>Trinycteris nicefori</i> Sanborn, 1949		IUCN- LC, LEY- RTP	2	(Vásquez and Artavia 2017)
Phyllostomidae indet.			10	(Aguilar 2010, Brizuela et al. 2015, Goicoechea 2019, Lips and Lips 2008, Madrigal 2010, Quesada and Alfaro 2005, Quesada 2015, Quesada and Deleva 2016), ND

Vespertilionidae

<i>Rhogessa bickhami</i> Baird, Marchán- Rivadeneira, Pérez & Baker, 2012	IUCN- LC	1	(Cubero and Artavia 2016)
Chiroptera indet.		16	(Carvajal 2014, Hapka et al. 1992, Goicoechea 2018, Hempel 1989, Lips and Lips 2008, Peacock and Hempel 1993, Quesada 2010, Strinati et al. 1987, Trescott 2012, Ulloa 2012, Woodman 1988)

† - ND - new data: original contribution to this paper.

Discussion

Our literature review and field observations of cave-dwelling fauna in Costa Rica yielded a database of 959 records encompassing 123 species, with the remainder mentioning higher taxonomic levels. Some literature records are expedition reports that introduce the possibility of misidentification, particularly in cases involving closely related species. However, the information gathered undoubtedly represents a valuable depiction of the current state of knowledge regarding cave-dwelling fauna in Costa Rica. There are a few noteworthy records of possible “trogllobites,” which are typical cave-dwelling organisms morphologically adapted to subterranean life. Notably, a freshwater crab from Southern Costa Rica, *Pseudothelphusa puntarenas*, has been described as a cave dweller (Hobbs, 1994). Furthermore, various specimens of springtails (*Trogolaphysa sp.*) and mites (Rhagidiidae) from Barra Honda (Palacios 1994) exhibit morphological adaptations that are indicative of cave life. Similarly, a single harvestman species from Southern Costa Rica (Goodnight and Goodnight 1983) displayed morphological changes that were attributed to cave adaptation.

Although only long-term studies could confirm their exact categorization, it is likely that most vertebrates in Costa Rican subterranean ecosystems fall under the category of troglophiles, referring to species that find suitable living conditions within caves but still rely on surface access for activities, such as feeding or reproduction. During our field observations, we frequently encountered cave-dwelling cane toads (*Rhinella horribilis*) thriving on abundant invertebrate prey as well as numerous frog species located near the entrances. A thin-toed frog (*Leptodactylus savage*) was also noted in the caves. However, these species cannot be considered as true cave dwellers if they are unable to reproduce underground. Nonetheless, live tadpoles have been documented in subterranean lakes in Southern Costa Rica (Peacock and Hempel 1993), suggesting the potential for the long-term survival of cave-dwelling populations of amphibians. We confirm the recent commentary of

Sperandei et al. (2023) that neotropical frogs should not be considered accidentals in caves and that more attention should be given to their monitoring in subterranean habitats. Reptiles such as night lizards (*Lepidophyma reticulatum*) and turtles (*Chelydra acutirostris*) inhabit deep subterranean passages. Additionally, two distinct fish species, the three-barbed catfish *Rhamdia guatemalensis* and the Mexican tetra *Psalidodon fasciatus*, exhibited signs of adaptation to subterranean life, such as pale coloration and reduced eye size (Romero 1985, Juberthie and Strinati 1994). Their biology, adaptations, and taxonomy have yet to be studied in detail.

Our study highlights that the number of taxa recorded in Costa Rican caves is relatively low compared to the country's enormous potential as a biodiversity hotspot (Avalos 2019). We argue that this is due to the lack of detailed research and low sampling effort rather than the true scarcity of biodiversity. It is also important to note that some subterranean sites in Costa Rica have not been studied and most have limited biospeleological records. For comparison purposes, the cave fauna of Venezuela, which has received considerable research attention, includes over 350 identified invertebrate species, 46 of which are classified as troglobites (Galán and Herrera 2006). A detailed review of Central American subterranean aquatic fauna revealed rich biodiversity in a relatively small geographic area (Reddell 1981; Mejía-Ortíz et al. 2021). Recent studies on a limited number of caves in Belize (Wynne and Pleytez 2005; Taylor et al. 2011) have resulted in the discovery of at least 80 unique taxa with possible new species for science. Several studies have focused on the diversity and ecology of cave invertebrates in the Guatemala (Pacheco et al. 2020, Pacheco et al. 2021). These studies are encouraging and could hint at the results expected in the Costa Rican caves if we apply a more systematic approach to biospeleological research.

Costa Rican caves and artificial subterranean sites are crucial habitats for bats, as most of them (72%, n=97) were occupied by these mammals. The existing literature shows that at least 52 bat species that occur in Costa Rica dwell in caves across their geographic ranges (Sagot and Chaverri 2015, Oliveira et al. 2018, IUCN 2023). For example, studies conducted in Brazil have identified 81 species that inhabit caves (Oliveira et al. 2018, Barros and Bernard 2023). For Costa Rica, the studies we found focused primarily on quantifying the diversity of cave-dwelling bats, yet there is limited information on the abundance and the

seasonal dynamics of populations (Peacock and Hempel 1993, Gonzalez 2012, Cubero and Artavia 2016, Villalobos-Chaves et al. 2016, Deleva and Chaverri 2018). Since some species of bats that rely on caves as their roosting sites are highly specialized and may be more vulnerable to disturbance, it is crucial to identify and prioritize the conservation of important underground bat roosts in the country (Sagot and Chaverri 2015, Tanalgo et al. 2022). Long-term monitoring of cave-dwelling bats should be a high priority for local authorities, as it would provide a valuable contribution to research and conservation efforts in the country and decision-making for sustainable activities within caves, most notably tourism (Deleva and Chaverri 2018). Other research questions worth pursuing in future studies on Costa Rican bats are related to their ecology and behavior. Special attention must be paid to the importance of artificial subterranean sites such as roosts, as they can provide excellent conditions for bats and other animals (Gonzalez 2012, Deleva and Chaverri 2018). However, these are often overlooked in monitoring and conservation measures (Weigand et al. 2022, Deleva et al. 2023).

The relatively low number of species discovered in caves suggests the need to expand research on the subterranean fauna of Costa Rica. For example, there are considerable gaps in fundamental knowledge about whole taxonomic groups, such as Amphipoda, Schizomida, Gastropoda, and Diplura, and there are no studies on the ecology or behavior of cave organisms. A promising research topic would be to study in detail the adaptations of pale catfish toward cave life (Perdices et al. 2002) and more studies on the stygofauna. With the use of more advanced methods, such as environmental DNA (Saccò et al. 2022), acoustic monitoring, different trapping techniques, and citizen science, we believe that there is enormous potential for discovering new species and gaining a better understanding of the ecology and diversity of Costa Rica's subterranean ecosystems. In particular, we suggest that future studies focus on long-term investigations of cave invertebrate communities and compare them with other habitats in the country (Smith et al. 2023). Future studies should also include soil-dwelling organisms, particularly the Mesovoid Shallow Substratum (MSS). The study of MSS is very promising, as this habitat is an integral part of the subterranean environment (Gers 1998) and has been proven to bring valuable discoveries in other parts of the world (Langourov et al. 2014, Mammola et al. 2016, Ortuño et al. 2023). Apart from undoubtedly critical taxonomic studies, some fundamental questions in subterranean biology

worth pursuing are related to the ecology of cave organisms and their adaptation to the environment. Particularly interesting topics could be related to the ecosystem services of subterranean communities and their functional diversity (Mammola et al. 2020).

The Barra Honda National Park is an excellent example of successful cave and karst conservation in Costa Rica. This national park was created primarily to protect the unique karstic landscape (Goicoechea 2015), and the subterranean sites within its borders were well preserved. However, most of the subterranean sites in the country are located outside protected areas, indicating severe challenges to their conservation. The Zona Sur karst area, which consists of an extensive karst surface with the most significant number of caves in the country (Ulloa 2011), lacks state protection. In addition, most cave-dwelling species lack legal protection. For example, all bat species in our database are stated as Least Concern in the global IUCN red list because of their wide distribution. However, they may be locally rare, particularly because cave-dependent species are vulnerable to disturbances in their roosts. Some of the country's most crucial subterranean bat roosts, Corredores, Gabinarraca, Damas, and Emus, lack any state of protection. Cave ecosystems are particularly vulnerable to anthropogenic threats such as pollution, disturbance due to tourist activities, and climate change (Mammola et al. 2019). With the rapid development of tourism and speleological activities (Ulloa and Goicoechea 2013), it is essential to preserve and protect the subterranean habitats and unique species assemblages that inhabit these sites. Understanding the unique adaptations and survival strategies of subterranean organisms will provide crucial data for developing effective conservation strategies to preserve fragile ecosystems.

Conclusions

Although the Costa Rican subterranean fauna has been the subject of a limited number of studies, our review and research have shown the current state of knowledge on the biodiversity of one-third of the known subterranean sites in the country. However, compared with cave-dwelling fauna from cave systems in other countries in the region, such as Belize, Guatemala, and Venezuela, we can infer that it is likely that a large number of Costa Rican subterranean organisms are yet to be described and reported within Costa Rican caves.

Finally, with the current work, we hope to inspire and encourage future studies to focus on the exploration and documentation of new species in the underground habitats of the country.

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Supplementary Material

Dataset of cave-dwelling animals in Costa Rica. The dataset includes information on taxonomic diversity, site, location, type of roost, protected areas, conservation status, and references. Available online at <https://doi.org/10.3897/subtbiol.47.113219.suppl1>

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Chapter 2. Important underground roosts for bats in Bulgaria: current state and priorities for conservation

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Abstract: Bulgaria has a very rich bat fauna and large colonies of bats can be found in caves, mines and other underground roosts. Respectively, there are more than 107 underground roosts that are listed as important bat sites, most of which are protected by statutory laws and are of national or international importance. Despite the existence of formal protection, many roosts face anthropogenic disturbances due to the popularity of outdoor activities, such as caving and the lack of actual regulation. Currently, the evaluation was only based on the size of the colony and the presence of protected species. However, this approach is limited to roosts that contain high diversity and neglects the ones that contain high biotic importance that are highly threatened by various threats. Here, we evaluated conservation priorities and identified the most vulnerable underground bat roosts in Bulgaria, using the Bat Cave Vulnerability Index and proposed measures to adequately protect sites. We found that 32% of the Bulgarian bat roosts assessed are at a "high priority" level for conservation and protection, while 39% are at a "medium priority" that may require constant monitoring. This novel and integrative approach applied to bat roost prioritization in the country enabled the detection of sites that need urgent conservation attention and is the first step in establishing better strategies for the bat monitoring network in Bulgaria.

Introduction

With more than 6000 caves (Bulgarian Federation of Speleology 2022), large areas of well-preserved natural habitats, an abundance of abandoned structures and a mild climate, Bulgaria is a suitable place for bats. Of the 47 species inhabiting Europe (IUCN 2022b), 33 are recorded in the country (Benda et al. 2003, Schunger et al. 2004, Niermann et al. 2007, Popov and Lakovski 2019, IUCN 2022a). All bat species in Bulgaria are protected by law (Republic of Bulgaria 2022). All of the 12 species listed as a priority for conservation by the Habitats Directive inhabit caves, 10 of them being considered cave-dwelling and two species are using caves during periods of swarming and hibernation (EU 1992, Ivanova 2005). Considering the enormous diversity of bats and the numerous underground roosts in the country, priority for monitoring and conserving is given to a limited number of sites that are listed as Important Bat Underground Habitats (Ivanova 2005).

The important bat underground roosts in Bulgaria were first classified by Ivanova (2005). The criteria initially used were according to the guidelines for the selection of Biological Sites of Special Scientific Interest (SSSIs) of the Nature Conservation Council in Great Britain: “4 or more species and 50 or more individuals; 3 or more species and 100 or more individuals; 2 or more species and 150 or more individuals” (Walsh et al. 2019). The previous list has included 92 underground roosts, with some of the underground roosts sheltering significant diversity of bats with regional, national or international importance (Ivanova 2005, EUROBATS 2022). The list includes caves and artificial roosts - buildings, bunkers and mines. This list was gradually updated and now consists of 107 sites, most of which are subject to regular monitoring according to the National Biodiversity Monitoring System at the Ministry of Environment and Waters of Bulgaria (Petrov 2015a, Petrov 2015b, MOEW 2022c, Toshkova and Deleva 2022). Although most of the important bat roosts are included in some form of a protected area, not all are specifically protected (e.g. the establishment of physical protection) due to the presence of important and vulnerable bat colonies in the cave site. For example, some roosts are considered natural landmarks or archaeological sites and, hence, the restrictions represent their cultural or aesthetic importance and do not necessarily consider the conservation of the biodiversity present. In addition to caves and mines, the important bat roosts in Bulgaria include several buildings and structures with environmental conditions, suitable for cave-dwelling bats, i.e. overground bat sites. Although the presence

of protected bat species should guarantee the preservation of every roost (Republic of Bulgaria 2022), the conservation state of buildings, particularly those structures which are abandoned, is often uncertain. In some cases, this leaves some bat roosts more vulnerable to anthropogenic pressures than others.

Bat populations in Bulgaria are threatened by continuous habitat loss, pollution, climate change, wind turbines and disturbance and are particularly vulnerable in their roosts (Popov 2018). The existing protection of important underground roosts considers only the diversity and abundance of bats, but their susceptibility to threats and human pressures are widely neglected. In this way, there are some roosts that are mismatched with protection and persistently threatened due to their high accessibility and popularity amongst cave visitors. Other roosts, located in remote areas, are equipped with gates and signboards despite being only visited sporadically by speleologists and researchers (SFN 2020). Although often inhabited by large bat colonies, artificial roosts, such as abandoned buildings, bunkers or mines, are overlooked during conservation planning. Therefore, there is a need to establish urgent and more practical protection measures for the most vulnerable underground roosts to ensure the preservation of bat populations and their ecosystem services in the country. The Bat Cave Vulnerability Index (Tanalgo et al. 2018) is a practical tool to identify the most vulnerable caves and set priorities for future conservation. The Index integrates several important factors, such as species diversity, presence of rare species, size of colonies and morphological characteristics of caves and their approach. It was already successfully applied in several countries and artificial roosts (Deleva and Chaverri 2018, Tanalgo et al. 2022b). In this study, we applied this approach to determine the levels of conservation priorities for bat roosts in Bulgaria and to guide our focus on sites that require additional protection and urgent conservation actions. We have proposed key conservation actions for each roost, in accordance with the Conservation Evidence Initiative (Berthinussen et al. 2021). Consequently, we hope that this work would be relevant to developing effective policy-making related to the protection and conservation of important bat roosts in Bulgaria.

Material and methods

The study was carried out on underground roosts and overground structures with large bat colonies located in the Republic of Bulgaria (Figure 1). We built a dataset that includes all important underground bat roosts, following Ivanova (2005) (Suppl. material 1). Our sources are from the period between 2003 and 2022, with most of the data obtained before 2017. We obtained data for the distribution of each bat species amongst roosts and the location of each roost from the available literature, such as published research articles (Benda et al. 2003, Ivanova 2005), official monitoring reports (Petrov 2010, Petrov 2015a, Toshkova and Deleva 2022), the database of the Natura 2000 network in Bulgaria available at the website of the Ministry of Environment and Waters, i.e. MOEW (2022a) and the national database of the National Biodiversity Monitoring System (available upon request at MOEW (2022c)). We checked the conservation state of each roost using the information on protected areas of Bulgaria (MOEW 2022b). We checked if a roost is located within one or more protected areas using the spatial data provided by the Ministry of Environment and Waters (MOEW 2022d). When a roost was located in overlapping protected areas, for example - a Natural landmark and a Natura 2000 zone, we took into account the higher level of protection or the one with restrictions on visits.

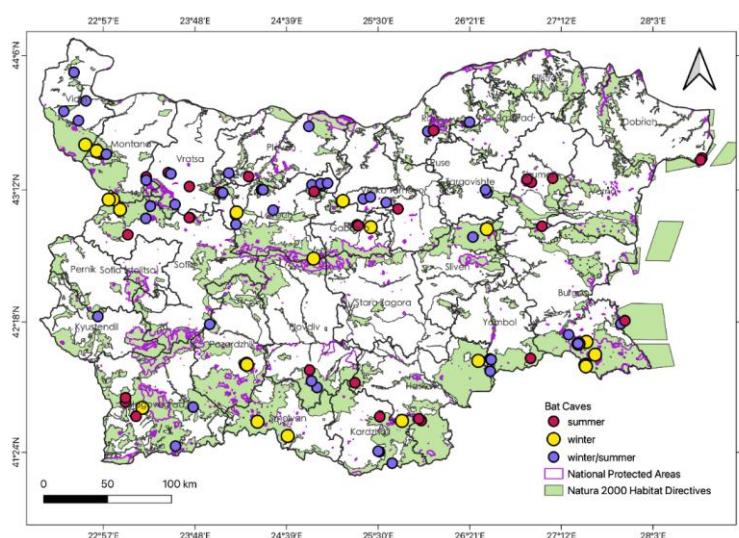


Figure 1 Important bat underground roosts and protected areas in Bulgaria.

Assessing conservation priority using BCVI

We assessed cave priorities using the Bat Cave Vulnerability Index (BCVI) (see Tanalgo et al. (2018) for a complete prioritisation scheme). The index is composed of two components: Biotic Potential (BP) and Biotic Vulnerability (BV). The Biotic Potential (BP) takes into account the bat roost species richness, abundance, relative abundance, endemism and conservation status. We report the abundance of each species as the maximum number of individuals observed at each roost. The Biotic Vulnerability (BV) assesses the characteristics of the cave landscape feature and threats, such as cave morphology, visitation and land use in the surrounding areas. As the Index was originally developed for tropical caves, we adapted new criteria to assess the Biotic Vulnerability (BV) score that is contextualised in the Bulgarian environment. For example, in Bulgaria, cave temples are rare, but some of our caves shelter industrial structures, such as dairy farms, places to grow mushrooms, fuel repositories and wine cellars (Bulgarian Federation of Speleology 2022). We consequently changed the category from “temples” to “temples and structures” and included the following categories: 4 = no structures are present, 3 = old and abandoned structures are present, 2 = structures may be present, but rarely used (e.g. water-capturing structures, that are maintained several times a year), 1 = functioning and frequently-used structures (e.g. operating dairies, mushroom gardens, temples, wine cellars etc.) are present. The BP Index has a value between 1 and 4, with 1 being the highest level of priority. The BV Index has values of A, B, C and D, with A being the most vulnerable to disturbances. The sub-indices (BP and BV) are integrated to obtain the BCVI status and determine the overall priority of the caves. We used the latest IUCN Red List (version 2022-1) for the assessment of each species' global conservation and endemism status. In addition to the BCVI, we present new data on the importance status of each roost, following the methodology used in Bulgaria up to now, described by Ivanova (2005). The categories of importance are based on the presence of the total number of individuals and the number of species in each roost: Regional (25 to 100 individuals of ≥ 4 species), National (100 to 500 individuals of ≥ 3 species or 500 to 1000 individuals of ≥ 2 species) and International (1000 or more individuals of ≥ 2 species). We did all calculations in Excel 2021 for Windows (Microsoft corporation 2021). We mapped

caves and their conservation status using the software QGIS v. 3.26 (QGIS 2022) and visualized data using R Studio (R Studio Team 2021).

Assessing suitable conservation actions

In addition to the Vulnerability Index, we assessed the condition and existing potential threats to each roost, based on the physical signs present, for example, collapsed entrances, household waste, graffiti and broken infrastructure (Petrov 2015b). We used the latest monitoring reports and the database of the National Museum of Natural History as a source of information (Petrov 2010, Petrov 2015a, Toshkova and Deleva 2022). We conducted an intensive literature search to effectively develop and propose appropriate conservation actions for each specific site. We used the available data from the Conservation Evidence initiative (Berthinussen et al. 2021) and considered the general assessment of each conservation action, the individual study used in its evaluation in combination with all the guidelines and recommendations provided by the Eurobats working groups. Then we measured their relevance for our specific cases and species. We selected only effective bat conservation actions with high-quality evidence and no undesirable effects.

Results

All the 33 bat species and six species complex groups found in Bulgaria were evaluated for all underground sites. According to the IUCN Red List (IUCN 2022a), the majority of the species are considered as Least Concern ($n = 27$), and six are Near Threatened ($n = 6$). There are three (3) species under the threatened category (Vulnerable) and a single data-deficient species. The Bulgarian Red Data Book (Golemanski et al. 2015) lists as Least Concern 11 species, as Near Threatened four species, 10 species are listed as Vulnerable, two species are data-deficient and six species do not have an assigned category. The most common species include *Rhinolophus ferrumequinum*, which occurs in at least 89% ($n = 96$ sites) of cave sites, followed by *Rhinolophus hipposideros* (73%, $n = 75$ sites) and *Miniopterus schreibersii* (70%, $n = 75$ sites). Several species were not observed in underground roosts or were very rare as they are not considered cave-dwelling ($n = 7$). We present the species of bats, their conservation status and distribution in roosts in Table 1.

Table 1 Cave-dwelling bats recorded in Bulgaria, their roost distribution and conservation status. The cave-dwelling species are marked with *. No: the number of roosts from the current dataset in which the species is observed. RO: the relative occurrence of the bat species in all caves assessed in the study. IUCN: Conservation status according to IUCN Red List (Global). BG RB: Conservation status, according to the Bulgarian Red Data Book (Golemanski et al. 2015). BBA: Appendices of the Bulgarian Biodiversity Act. 92/43 EEC: Appendices of the COUNCIL DIRECTIVE 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. BERN: Berne Convention on the Conservation of European Wildlife and Natural Habitats. BON: Appendices of the Convention on the Conservation of Migratory Species of Wild Animals. EU: the species is listed in the EUROBATS agreement for the conservation of the populations of the European bats.

Code	Species	No	RO	IUCN	BG RB	BB A	92/4 3	BER N	BO N	E U
Rhifer	<i>Rhinolophus ferrumequinum</i> *	96	89.72	LC	NT	2, 3	2, 4	II	II	+
Rhihip	<i>Rhinolophus hipposideros</i> *	78	72.897	LC	LC	2, 3	2, 4	II	II	+
Minsch	<i>Miniopterus schreibersii</i> *	75	70.093	NT	VU	2, 3	2, 4	II	II	+
Rhieur	<i>Rhinolophus euryale</i>	74	69.159	NT	VU	2, 3	2, 4	II	II	+
Myomyo/bl y	<i>Myotis myotis</i> / <i>M. blythii</i> *	66	61.682	LC	NT	3	4	II	II	+
Myobra	<i>Myotis brandtii</i>	55	51.402	LC	LC	3	4	II	II	+
Myocap	<i>Myotis capaccinii</i> *	54	50.467	VU	VU	2, 3	2, 4	II	II	+
Myobly	<i>Myotis blythii</i> *	45	42.056	LC	NT	2, 3	2, 4	II	II	+
Myomyo	<i>Myotis myotis</i> *	44	41.121	LC	NT	2, 3	2, 4	II	II	+
Myoema	<i>Myotis emarginatus</i> *	41	38.318	LC	VU	2, 3	2, 4	II	II	+
Rhimeh	<i>Rhinolophus mehelyi</i> *	26	24.299	VU	VU	2, 3	2, 4	II	II	+
				LC/N						
Rhimed	<i>Rhinolophus media complex</i> *	25	23.364	T	VU	2, 3	2, 4	II	II	+
Pleaus	<i>Plecotus austriacus</i>	25	23.364	LC	LC	3	4	II	II	+
Rhi sp.	<i>Rhinolophus sp.</i> *	24	22.43	N/A		2, 3	2, 4	II	II	+
Myobec	<i>Myotis bechsteinii</i>	24	22.43	NT	VU	2, 3	2, 4	II	II	+
Rhibla	<i>Rhinolophus blasii</i> *	23	21.495	LC	VU	2, 3	2, 4	II	II	+
Myodau	<i>Myotis daubentonii</i>	23	21.495	LC		3	4	II	II	+
Eptser	<i>Eptesicus serotinus</i>	21	19.626	LC	LC	3	4	II	II	+
Nycnoc	<i>Nyctalus noctula</i>	16	14.953	LC	LC	3	4	II	II	+
Hipsav	<i>Hypsugo savii</i>	16	14.953	LC	LC	3	4	II	II	+
Pippip	<i>Pipistrellus pipistrellus</i>	14	13.084	LC	LC	3	4	II	II	+
Myonat	<i>Myotis nattereri</i>	13	12.15	LC	LC	3	4	II	II	+
Barbar	<i>Barbastella barbastellus</i>	12	11.21	NT	VU	2, 3	2, 4	II	II	+
Myo sp.	<i>Myotis sp.</i>	9	8.4112	N/A	N/A	3	4	II	II	+
Pleaur	<i>Plecotus auritus</i>	8	7.4766	LC	NT	3	4	II	II	+
Myomys	<i>Myotis mystacinus</i>	8	7.4766	LC	LC	3	4	II	II	+
Vesmur	<i>Vespertilio murinus</i>	6	5.6075	LC	LC	3	4	II	II	+
Myoalc	<i>Myotis alcahoae</i>	5	4.6729	DD		3		II	II	+
Nyclei	<i>Nyctalus leisleri</i>	5	4.6729	LC	VU	3	4	II	II	+
Pippyg	<i>Pipistrellus pygmaeus</i>	5	4.6729	LC		3	4	II	II	+

Rhimeh/eur	<i>Rhinolophus mehelyi/ euryale*</i>	4	3.7383	N/A	VU	2, 3	2, 4	II	II	+
Myoaur	<i>Myotis aurascens</i>	4	3.7383	LC		3	4	II	II	+
Pip sp.	<i>Pipistrellus sp.</i>	3	2.8037	N/A		3	4	II	II	+
Pipkuh/nat	<i>Pippistrillus kuhlii/ P. nathusii</i>	3	2.8037	LC		3	4	II	II	+
Pipkuh	<i>Pipistrellus kuhlii</i>	3	2.8037	LC		3	4	II	II	+
Pipnat	<i>Pipistrellus nathusii</i>	3	2.8037	LC	LC	3	4	II	II	+
Tadten	<i>Tadarida teniotis</i>	3	2.8037	LC	DD	3	4	II	II	+
Eptnil	<i>Eptesicus nilssonii</i>	0	0	LC	DD	3	4	II	II	+
Myodas	<i>Myotis dasycneme</i>	0	0	NT		3	2, 4	II	II	+
Nyclas	<i>Nyctalus lasiopterus</i>	0	0	VU	VU	3	4	II	II	+
Ple sp.	<i>Plecotus sp.</i>	0	0	N/A	N/A	3	4	II	II	+

We assessed a total of 107 underground sites for this current prioritization analysis. We obtained data for 92 bat roosts from previous records (Ivanova 2005). We included an additional 15 sites recently added to the list, with 96 (90%) natural caves, six (6%) overground sites (buildings, Figure 2), three (3%) mine sites and two (2%) bunkers. We included information on location, occupancy (summer, winter or both), protected areas, importance, threats and species diversity. The exact coordinates of the roosts could not be shared publicly as the locations contain the presence of sensitive to disturbance species and habitats, for which visitation, even for research purposes, could be harmful. We present the low-resolution coordinates of the roosts in Suppl. material 1, following the recommendations of the Best Practices for Generalising Sensitive Species Occurrence Data (Chapman 2020). The exact locations will be made available upon request. Regarding the level of protection, most of the sites (n = 64) received legal protection in the form of visitation prohibition by the Natura 2000 network (Habitats Directive), 31 cave sites are located in protected natural landmarks, nine caves within protected areas and two within natural reserves (Suppl. material 1). A single cave (Tangarachkata) does not have legal protection. Almost all the roosts were subjected to some form of visitation regulations. Visitation is prohibited during the breeding season of bats (from 1 March to 30 June) in 54 sites, a single site during the hibernation period (from 1 December to 31 March) and both breeding and hibernation periods in three sites. Visitation is prohibited all year round in 28 sites and three caves are restricted for camping or group visits. There are no visitation restrictions for 13 sites. Physical conservation actions and restrictions present in Bulgaria include gates, fences, signs and some security regulations. There are 18 sites currently equipped with gates and seven have a fence around

the entrance. Signboards with information about bats are placed on 37 sites. There are six show caves with more strict protection due to their economic value (e.g. entrance gate, opening hours, personnel and signaling security equipment) (Suppl. material 1). The disturbance is by far the main concern for the majority of the sites ($n = 98$), followed by the threat of roost destruction ($n = 4$) and improper gate design ($n = 4$, Figure 3). Only one site did not face any conservation concerns, as the bat colony is located in a heavily guarded area. The main target groups, which might cause disturbance are tourists ($n = 35$), cavers ($n = 55$, Figure 4), rock climbers ($n = 1$) and occasional visitors ($n = 1$). In eight of the sites, the main disturbing factors were cave and bat researchers, who were the most frequent visitors. Our suggested conservation actions include restriction of visitation, modification of cave gates, placement of signboards and actions, specifically aimed at cavers. In the case of the Tangarachkata cave, we propose that the site should be declared a protected area.



Figure 2 Artificial structures such as Perla 2 (1A and 1B), Abandoned residency (2A and 2B) and an abandoned mushroom greenhouse (3A and 3B) are sheltering large colonies of cave-dwelling bats. Some natural caves in Bulgaria are adapted for human use: Mandrata in the village of Mikre (4) has a whole house built at the entrance, the cave with the same name nearby - Mandrata at Alexandrovo, is accessible with an automobile (5). The Karangin Cave, located in the Rhodope Mountain is turned into a sheepfold (6). Photo credit: S. Deleva.



Figure 3 Improper gate design: A - the gate at the Musinska Cave allows bats to fly in and out, but it is not optimal. B - the cave at the entrance of Kalna Matnitsa Cave remains open to allow bat access. C - The Kaletto Cave entrance is equipped with a gate, that might stop bats, but does not stop visitors. D - The gate at the Uhlovitsa show Cave is still waiting for its renovation. E - Although the intention of the gate at the Bratanova Cave entrance is to protect bats, it is built without consulting with the EUROBATS recommendations. F - The gate at one of the entrances of the Magurata show cave. Photo credit: S. Deleva

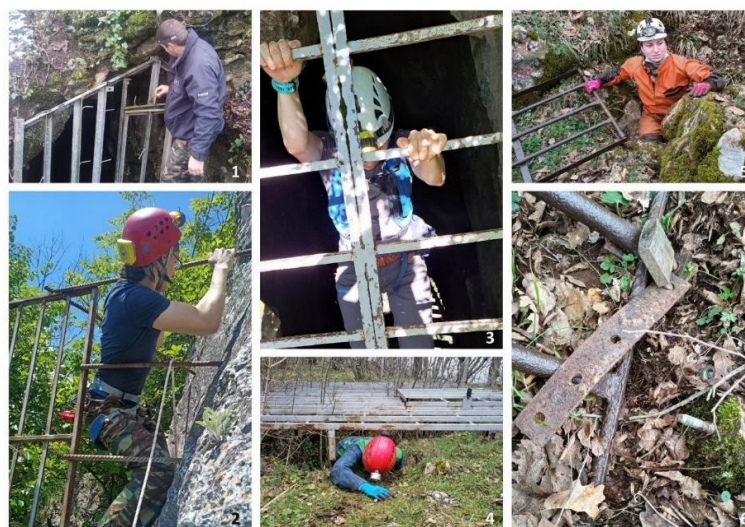


Figure 4 Examples of ineffective cave gates: 1 - Parts of the gate of the Bratanovata Cave are twisted to allow easier access. 2. The fence at the Divdyadovski Zandan Cave cannot stop visitors. 3 - Access to the Derventskata Cave is officially restricted, but cannot stop visitors. 3 - Access to the Derventskata Cave is officially restricted, but cavers are freely passing through the gate. 4 - Kaletto Cave has a locked gate, but cavers have created a shortcut under it. 5 and 6 - the Elenina dupka Cave has a very strong gate, but cavers have unscrewed the bolts holding the padlock. Photo credits: S. Deleva (1, 2, 4 and 6), M. Kolev (3), S. Markova (5).

We used data generated from a previous cave assessment over a period of time for our BCVI prioritisation (Table 2). Therefore, our results for species diversity and abundance represent the maximum population estimates of each roost rather than the current state of the populations. Amongst the assessed caves in terms of Biotic Potential (BP), 47 (44%) of the caves have the highest BP (Level 1), while five (5, 5%) caves at mid-high (Level 2), 13 (12%) caves at mid-low (Level 3) and 42 (39%) roost at the lowest level (Level 4). In terms of Biotic Vulnerability (BV), 20 (19%) of the sites were the most vulnerable to threats (Status A), 56 (52%) are in the mid-high vulnerability (Status B) and 31 (29%) are in the mid-low level. No cave sites were recorded in Status D (i.e. the lowest level of vulnerability). Of the roosts with the highest BP, 43 are natural caves and four are buildings and infrastructures. Five of the most vulnerable bat roosts (Status A) are show caves, but three are not, yet they are as easy to explore and even more accessible than a show cave. There were 31 roosts that scored as low conservation priority. Most of them are vertical caves, located in remote areas with restricted access (Table 2). At the provincial level, BP levels did not show a significant difference ($\chi^2 = 77.41$, $p = 0.1591$) with four ($n = 4$) and two ($n = 2$) provinces having all its roosts considered in high and low levels in terms of BP, respectively. Similarly, BV did not show a significant difference at the provincial level ($\chi^2 = 45.10$, $p = 0.426$). Only a single province has all its caves falling within high vulnerability. Overall, combining BP and BV, we identified 34 (32%) high-priority caves that require the highest and most urgent need of conservation protection and 42 (39%) bat caves mid- priority that may need monitoring to ensure the existing population continues to thrive, while there are 31 (29%) at low priority, which can be potentially considered for other cave use and activities due to the absence of important or vulnerable bat populations (Figure 5). When compared at the provincial level ($\chi^2 = 249.515$, $p = 0.083$), three Provinces (Kardzhali, Pleven and Varna) have all caves assessed as high-priority for conservation, while single provinces have all caves in medium-priority (Dobrich) and low-priority (Yambol) (Figure 6). All threats and conservation actions are presented in Suppl. material 1. When we used the criteria, described by Ivanova (2005), the importance status of the roosts was the following: International - 61 roosts, National - 33 roosts, regional - 6 roosts, no status - 7 roosts (Suppl. material 1).

*Table 2 Important bat underground roosts in Bulgaria and the Bat Cave Vulnerability Index. The show caves are marked with *.*

B P	B V	Type	Name	Occupancy	Legal visitation restrictions	Main concern	Target group	Immediate conservation actions
			Devetashkata					Daily security.
1	A	Cave	Peshtera*	Winter/Summer	Show cave	Disturbance	Tourists	Signboards. Fines.
			Dyavolskoto					
1	A	Cave	Garlo*	Winter	Show cave		Tourists	Not needed
			Emenskata					Physical restriction of access to the cave
1	A	Cave	Peshtera	Winter/Summer	Breeding	Disturbance	Tourists	entrance.
1	A	Cave	Karangin	Summer	Year-round	Disturbance	Tourists	Signboards
1	A	Cave	Magurata*	Winter/Summer	Show cave	Disturbance	Tourists	Light reduction
			Mandrata					Physical restriction of access to the cave
1	A	Cave	(Chavdarci)	Winter/summer	Breeding	Disturbance	Tourists	entrance.
			Orlova Chuka*					Limitation of bat
1	A	Cave	Orlova Chuka*	Winter/Summer	Show cave	Disturbance	Researchers	capturing
		Buildin						Immediate
1	A	g	Perla 2	Winter/Summer	No	Destruction	Owners	protection
			Bunker Gara Peyo					
3	A	Bunker	Yavorov	Summer	Breeding	Disturbance	Tourists	
			Musina					
3	A	Cave	(Musinskata)	Winter/Summer	No	Disturbance	Tourists	
3	A	Cave	Suhi Pech	Winter/Summer	No	Disturbance	Cavers	Targeted at cavers
			Bacho Kiro					Improper gate design
4	A	Cave	Bacho Kiro	Winter	Year-round	Disturbance	Tourists	Modification of the gate
4	A	Cave	Futiovata Peshtera	Winter/Summer	Breeding	Disturbance	Tourists	Signboards
4	A	Cave	Leyarnitsite	Winter/Summer	Camping	Disturbance	Tourists	
			Razkoprkite					Physical restriction of access to the cave
4	A	Cave	Razkoprkite	Summer	Breeding	Disturbance	Tourists	entrance.
		Buildin	Rezidentsia					Immediate
4	A	g	Shumen	Summer	Breeding	Destruction	Owners	protection
4	A	Cave	Saeva Dupka	Winter	Year-round	Disturbance	Tourists	
4	A	Cave	Snezhanka*	Winter	Show cave	Disturbance	Tourists	
			Uhlovica*					Improper gate design
4	A	Cave	Uhlovica*	Winter	Year-round	Disturbance	Local authorities	Modification of the gate
4	A	Cave	Vodnata	Winter/Summer	Breeding	Disturbance	Cavers	Targeted at cavers
			Aina Ini					Restriction of visitations by the
1	B	Cave	Aina Ini	Winter/Summer	Year-round	Disturbance	Researchers	local RIEW
1	B	Cave	Andaka	Winter/Summer	Breeding	Disturbance	Cavers	Targeted at cavers

1	B	Cave	Bilernicite Biserna	Summer	Breeding	Disturbance	Tourists	Daily security. Signboards. Fines.
1	B	Cave	(Zandana)*	Winter/Summer	Breeding	Disturbance	Tourists	Not needed
1	B	Cave	Elenina Dupka	Winter	Year-round	Disturbance	Cavers	Targeted at cavers Physical restriction
1	B	Buildin g	Gabarnitsi Bachkovo	Summer	Breeding Breeding and	Collapse	Occasional visitors	of access to the cave entrance - Fence
1	B	Cave	Gargina Dupka Golashkata	Winter/Summer	hibernation	Disturbance	Cavers	Targeted at cavers Immediate
1	B	Mine	Peshtera Haydushkata Peshtera	Winter/Summer	Year-round	Disturbance	Researchers	protection
1	B	Cave	(Devenci) Kresnenski	Winter/Summer	Breeding	Disturbance	Cavers	Targeted at cavers
1	B	Buildin g	Prolom - Zhp Kanton	Summer	Breeding	Disturbance	Tourists	Physical restriction of access to the cave
1	B	Cave	Mandrata (Mikre)	Winter/Summer	Breeding Breeding and	Disturbance	Tourists	entrance. Immediate
1	B	Cave	Nanin Kamak Parnicite -	Winter/Summer	hibernation	Disturbance	Tourists	protection
1	B	Cave	Dolniya Parnik Parnicite -	Winter/Summer	Year-round	Disturbance	Cavers	Targeted at cavers
1	B	Cave	Gorniya Parnik	Winter/Summer	Year-round	Disturbance	Cavers	Targeted at cavers
1	B	Cave	Ponora	Winter/Summer	Year-round	Disturbance	Cavers	Targeted at cavers
1	B	Buildin g	Rezervoari Madzharovo	Summer	Breeding	Destruction	Owners	Immediate protection
1	B	Cave	Samara	Winter/Summer	Year-round	Disturbance	Tourists	
1	B	Cave	Sedlarkata	Summer	Year-round	Disturbance	Cavers	Targeted at cavers
1	B	Cave	Skoka	Summer	Breeding	Disturbance	Cavers	Targeted at cavers
1	B	Cave	Tauk Liman Troevratca	Summer	Breeding	Disturbance	Tourists	
1	B	Cave	(Zidanka)	Winter/Summer	Breeding	Disturbance	Cavers	Targeted at cavers
1	B	Cave	Varkan	Winter/Summer	Year-round	Disturbance	Cavers	Targeted at cavers
1	B	Cave	Vodnite Dupki	Winter	Year-round	Disturbance	Tourists	
1	B	Cave	Yarasa Ini	Summer	Breeding	Disturbance	Researchers	Limitation of visits
1	B	Cave	Urushka Maara	Winter/Summer	Breeding	Disturbance	Tourists	
1	B	Cave	Zorovica	Summer	Breeding	Disturbance	Researchers	Limitation of visits
2	B	Cave	Dinevata Pesht	Winter	Year-round	Disturbance	Cavers	Targeted at cavers
2	B	Cave	Morovica	Winter/Summer	Year-round	Disturbance	Cavers	Targeted at cavers
2	B	Cave	Razhishkata	Winter/Summer	Breeding	Disturbance	Tourists	

			Tunnels in the wall of Al.					
		Buildin	Stamboliyski					
2	B	g	Reservoir	Winter	No	None	None	Not needed
3	B	Cave	Bozhkova Dupka	Winter/Summer	Year-round	Disturbance	Cavers	Targeted at cavers
3	B	Cave	Chelovechata	Summer	Breeding	Disturbance	Cavers	Targeted at cavers
3	B	Cave	Marina Dupka	Winter/Summer	Year-round	Disturbance	Cavers	Targeted at cavers
			Tyulenovata					
3	B	Cave	Peshtera (Sv.N)	Summer	Breeding	Disturbance	Cavers	Targeted at cavers
					Group activities			
4	B	Cave	Bashovichki Pec	Winter/Summer	only	Disturbance	Cavers	Targeted at cavers
4	B	Cave	Bozhiyat Most	Summer	Year-round	Disturbance	Tourists	Better signboards
4	B	Cave	Bozkite	Winter	Breeding	Disturbance	Tourists	Remove the gate
			Golyamata					Physical restriction of access to the cave entrance.
4	B	Cave	Mitrovska	Summer	Breeding	Disturbance	Tourists	
			Golyamata					
4	B	Cave	Prilepna	Summer		Disturbance	Tourists	
4	B	Cave	Kolibata	Summer	Breeding	Disturbance	Cavers	Targeted at cavers
								Physical restriction of access to the cave entrance.
4	B	Cave	Kozarnika	Summer	Breeding	Disturbance	Tourists	
			Kresnenski					
4	B	Bunker	Prolom Bunker	Summer	Breeding	Disturbance	Tourists	
4	B	Mine	Lesovo Galerii	Winter/Summer	No	Disturbance	Tourists	
			Minna Galeria					
4	B	Mine	Aida	Summer	Breeding	Disturbance	Tourists	
4	B	Cave	Mishin Kamak	Winter	Year-round	Disturbance	Cavers	Targeted at cavers
			Novata (Starata)					
4	B	Cave	Peshtera	Winter	Breeding	Disturbance	Cavers	Targeted at cavers
4	B	Cave	Orlovata Peshtera	Winter/Summer	Breeding	Disturbance	Tourists	
			Padaloto (Izvorat					
4	B	Cave	Na Yantra)	Summer	No	Disturbance	Cavers	Targeted at cavers
4	B	Cave	Prileparnika	Winter/Summer	No	Disturbance	Cavers	Targeted at cavers
			Sharaliyska		Hibernatio			
4	B	Cave	Peshtera	Winter	n	Disturbance	Cavers	Targeted at cavers
4	B	Cave	Starshelitsa	Winter/Summer	Breeding	Disturbance	Cavers	Targeted at cavers
4	B	Cave	Subatta	Winter	Year-round	Disturbance	Cavers	Targeted at cavers
			Temnata Dupka					
4	B	Cave	(S. Milanovo)	Winter/Summer	Year-round	Disturbance	Cavers	Targeted at cavers
4	B	Cave	Tsarskata	Winter/Summer	Breeding	Disturbance	Cavers	Targeted at cavers
4	B	Cave	Vodni Pech	Winter	Breeding	Disturbance	Cavers	Targeted at cavers
						Improper gate design	Local authorities	Modification of the gate
4	B	Cave	Yubileyna*	Winter	Show cave			
			Bratanovata			Improper gate design	Local authorities	Modification of the gate
1	C	Cave	Peshtera	Winter	Year-round			

			Derventskata						
1	C	Cave	Peshtera	Winter/Summer	Year-round	Disturbance	Cavers	Targeted at cavers	
			Divdyadovski					Signboard inside the	
1	C	Cave	Zandan	Summer	Breeding	Disturbance	Climbers	cave	
								Immediate	
1	C	Cave	Gabarnika	Winter/Summer	Breeding	Disturbance	Researchers	protection	
			Golyamata						
1	C	Cave	Balabanova	Winter	Breeding	Disturbance	Cavers	Targeted at cavers	
1	C	Cave	Golyamata Vapa	Winter	No	Disturbance	Cavers	Targeted at cavers	
1	C	Cave	Gyurgen Dere	Summer	Breeding	Disturbance	Cavers	Not needed	
1	C	Cave	Ivanova Voda	Winter/Summer	Breeding	Disturbance	Cavers	Targeted at cavers	
1	C	Cave	Kalna Matnica	Winter/Summer	Breeding	Disturbance	Cavers	Targeted at cavers	
1	C	Cave	Lednika (Kotel)	Winter/Summer	Year-round	Disturbance	Cavers	Targeted at cavers	
1	C	Cave	Manuilovata	Winter/Summer	Breeding	Disturbance	Cavers	Targeted at cavers	
					Breeding				
			Serapionovata		and				
1	C	Cave	Peshtera	Winter/Summer	hibernation	Disturbance	Cavers	Targeted at cavers	
			Tyulenovata						
1	C	Cave	Peshtera (M. Nos)	Summer	No	Disturbance	Cavers	Targeted at cavers	
			Parasinskata						
2	C	Cave	Propast	Winter/Summer	Breeding	Disturbance	Cavers	Targeted at cavers	
			Hilyadite					Restriction of access	
3	C	Cave	Ochichki	Summer	Year-round	Disturbance	Researchers	to the bat colony.	
3	C	Cave	Kaleto	Winter	Camping	Disturbance	Cavers	Targeted at cavers	
3	C	Cave	Rupata	Winter/Summer	Breeding	Disturbance	Cavers	Targeted at cavers	
3	C	Cave	Shamaka	Winter	Breeding	Disturbance	Cavers	Targeted at cavers	
			Tangarachkata						
3	C	Cave	Dupka	Winter/Summer	No	Disturbance	Cavers	Targeted at cavers	
3	C	Cave	Zandana	Winter	Breeding	Disturbance	Researchers	Limitation of visits	
4	C	Cave	Asandeliya	Winter/Summer	No	Disturbance	Cavers	Targeted at cavers	
4	C	Cave	Dranchi Dupka	Winter/Summer	Breeding	Disturbance	Cavers	Signboard	
			Genchovata						
4	C	Cave	Peshtera	Summer	Breeding	Disturbance	Cavers	Targeted at cavers	
			Golyamata						
4	C	Cave	Vitanovska	Winter	No	Disturbance	Cavers	Targeted at cavers	
4	C	Cave	Kalenska Peshtera	Summer	No	Disturbance	Tourists		
4	C	Cave	Kanchova Varpina	Summer	Breeding	Disturbance	Cavers	Targeted at cavers	
4	C	Cave	Kesedzhiisa	Winter/Summer	Breeding	Disturbance	Cavers	Targeted at cavers	
					Breeding				
			Lyastovicata		and				
4	C	Cave	(Glozhene)	Winter/Summer	hibernation	Disturbance	Cavers	Targeted at cavers	
4	C	Cave	Vodnata Pesht	Summer	Breeding	Disturbance	Cavers	Targeted at cavers	
			Zandana						
4	C	Cave	(Ilindentsi)	Winter/Summer	Breeding	Disturbance	Tourists	Limitation of visits	
			Zandana						
4	C	Cave	(Ruykova)	Summer	Breeding	Disturbance	Cavers	Targeted at cavers	

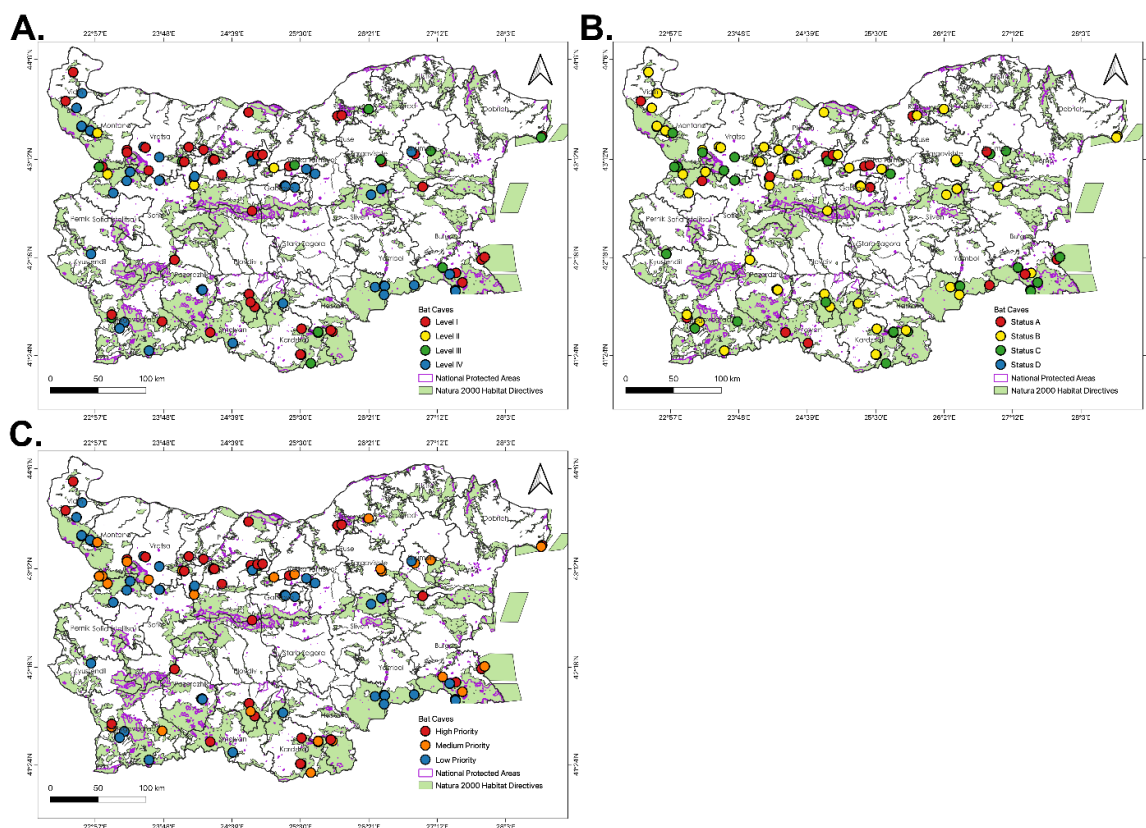


Figure 5 Underground roosts priorities according to (A) Biotic Potential (BP), (B) Biotic Vulnerability (BV) and (C) BCVI priorities.

Discussion

Caves and underground habitats are important for at least 48% of global bat species, with 28% of bat caves being threatened, but information on key priority caves for conservation at the national level remains limited (Tanalgo et al. 2022a). This work is the first effort to comprehensively assess the vulnerability and conservation priorities of important bat roosts in Bulgaria and their protection. Since its implementation as part of the integration of the country into the European Union, the Natura 2000 network covers more than 30.3% of the territory of Bulgaria and caves are listed as habitats of community interest (code 8310) (EU 1992). Most important bat roosts are included either as separate protected zones, i.e. declared for the protection of a single cave or as a part of a larger protected area. All bat species in Bulgaria are legally protected, with 12 species, most of which are cave-dwelling, listed in Annex II of the Habitats Directive. Any form of visitation, including touristic activities and

speleological exploration are restricted in most protected areas where habitat 8310 - "Caves not open to the public" or bat species are listed as objects of conservation priority for the Habitats Directive. The Natura 2000 network is proven effective at covering territories with natural caves and the presence of cave-dwelling bats (Lisón et al. 2013), but implementing its regulations is not optimal in Bulgaria as we often observe threats, such as unregulated visitation in protected sites. In reality, most of the important bat roost sites face disturbance and other anthropogenic threats, such as pollution and vandalism (Toshkova and Deleva 2022).

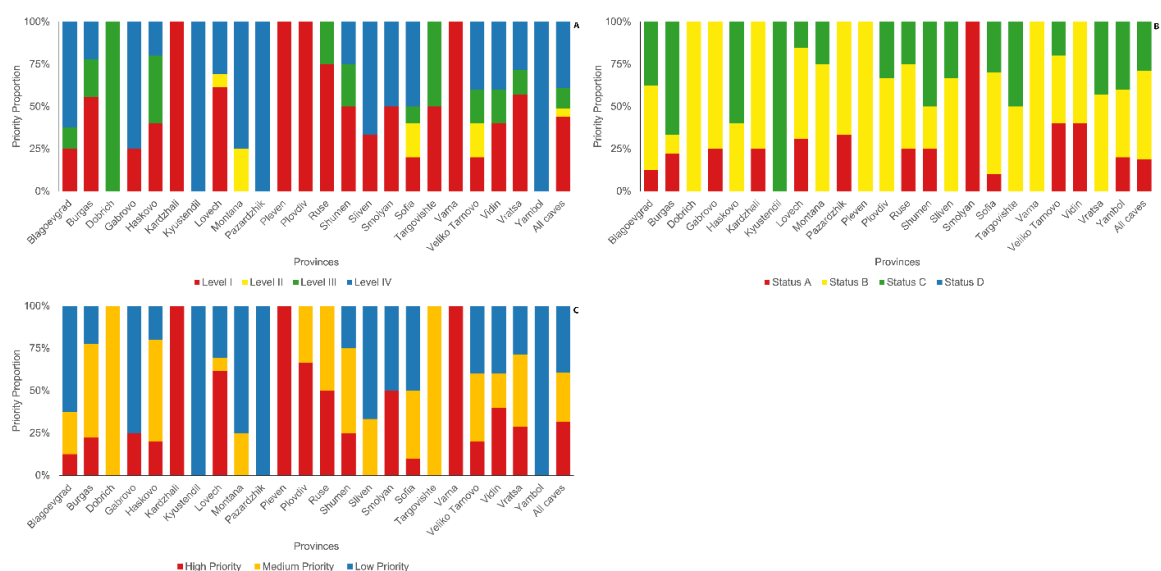


Figure 6 Comparison of (A) Biotic Potential (BP), (B) Biotic Vulnerability (BV) and (C) BCVI priorities, across provinces.

Cave visitation restriction is by far the most effective conservation action, but its application in Bulgaria has proven to be very difficult. The proposed period of visitation restriction for most of the sites (1 March to 30 June) does not match the period of the actual breeding season for bats and their occupancy in the roost and this is concerning, especially in the conditions of the changing climate that might affect the roosting patterns amongst bats (Festa et al. 2022). Visitation restrictions, especially the regimens of the Natura 2000 network, are not enforced in practice and often cavers and tourists are unaware of or ignore the existing

regulations. The most vulnerable roosts (Status A) need urgent conservation actions with individually chosen conservation interventions. The most extreme restriction measures, such as cave gating, may have mixed and even negative effects on bat populations (Mitchell-Jones et al. 2007, Berthinussen et al. 2021) and should be applied with caution and after carefully considering all existing evidence. If physical restrictions to the entrance are needed, we recommend a fence around a large perimeter and not a gate, in accordance with the recommendations of the EUROBATS agreement (Mitchell-Jones et al. 2007). Moreover, the blocking of cave entrances with objects or vegetation should be avoided and actions for clearing entrances should be a priority in future conservation projects.

One of the issues in conservation is that the nature protection legislation prioritizes the natural caves (EU 1992). However, due to different factors, such as habitat loss, many bats are increasingly roosting in artificial structures, whose conservation status remains unclear or absent. Some of the most important roosts for bats in Bulgaria are artificial structures, for example, buildings, mines and even one operating structure in a dam. These structures have already been established as important habitats for bats and some of them have been occupied for many years (Ivanova 2005). In some countries, the establishment of artificial underground roosts for bats is practiced as part of conservation initiatives. Still, in Bulgaria, artificial structures are often neglected by decision-makers. To our knowledge, there are no buildings declared as protected sites due to the presence of bats in Bulgaria. Moreover, those roosts are particularly vulnerable as they could naturally collapse or face destruction by the owners. For example, the abandoned hotel "Perla 2" is currently sheltering thousands of bats from 11 different species. However, this building is a private property and there are projects for demolition (Melteva 2013). The MOEW should consider these abandoned buildings as overground bat roosts and adopt the accepted conservation measures of underground sites for their protection.

Show caves are important bat roosts, but are excluded from the 8310 habitat and from monitoring obligations. Attention and efforts towards show caves as important bat roosts and their inclusion as a habitat of importance when considering monitoring and conservation initiatives should be necessary (Weigand et al. 2022). In our assessed caves, often only part of the show cave is accessible to tourists. For example, less than a kilometer of the area of

Orlova Chuka Cave is open for visitors, but the only entrance is locked due to its show cave status. This leads to a limited access to the rest of the cave - more than 13 km of galleries are protected from disturbance. A positive example of a show cave in Bulgaria that considers bats is the Biserna (Zandana) Cave in the Shumensko Plato Nature Park. The Cave is open for controlled visitation only during spring and autumn and the entrance is locked during the hibernation and breeding seasons. Cave tourism is often a double-edged sword in a way that it could affect bat cave biodiversity by disrupting bat behaviour and their roosting habitat (Furey and Racey 2016). Still, properly-managed cave tourism could potentially promote bat conservation and cave protection (Debata 2020, Tanalgo and Hughes 2021).

Caves that were considered less vulnerable using the index (BV, Status B and C), are caves that require effort to access, such as special equipment, high exploration efforts, permits or are located in remote areas. Cavers, researchers and, in rare cases, tomb raiders, are often the key factors contributing to the disturbance in these roost sites. Our assessment shows that the efforts to physically protect caves, located in remote areas are likely ineffective in protecting bat colonies. We observed that reinforcement of regulations is often ignored by many visitors, evident by the removal of signboards and damage to existing gates (Toshkova and Deleva 2022, Figure 4). Speleology is popular in Bulgaria and imposing rules that are impossible to enforce would only lead to conflicts. However, when properly trained, cavers could potentially be part of effective conservation measures by engaging them in bat conservation and monitoring (Bücs 2020). Anthropogenic disturbance to cave bats is not the only pressure that threatens cave biodiversity, but may potentially be exacerbated by other threatening processes, such as habitat loss, pesticides and climate change. Concentrating efforts on increasing awareness amongst cavers and local people should be prioritised and integrated with conservation initiatives in cave protection in the country. As the climate in Bulgaria has been changing in recent years (Marinova et al. 2017, Dale and Zhekova 2019), bat colonies are expected to move to more suitable roosts; thus, the need to focus efforts towards identifying and monitoring vulnerable sites are equally significant initiatives. Speleologists in Bulgaria could contribute significantly to filling the knowledge gaps in bat distribution if given the opportunity. A solution to minimize disturbance would be to provide an evidence-based visitation protocol.

The Bat Cave Vulnerability Index (BCVI) was originally developed for the prioritization of bat caves in the tropical region (e.g. in Deleva and Chaverri (2018)). Using appropriate metrics and components to assess cave priorities, the Vulnerability Index enables identifying areas with high conservation importance. This is the first extensive application of the approach outside the tropical realm and has shown effectiveness in identifying underground sites for conservation, including artificial ones. This Index provides an alternative to the approach to identify the roost importance, based solely on diversity and abundance (Ivanova 2005) and the two methods could be compared and their reliability evaluated once we have more data. The prioritization has certain limitations, for example, the influence of seasonality on bat abundances and species cave occupation, sampling methods and efforts, which definitely would alter the biotic potential of caves and the overall priorities of caves. Although these caveats require future validation and testing, our current work provides a useful overview of bat cave conservation in Bulgarian subterranean habitats. In our analysis, we found that 18% of the important bat roosts are currently facing a severe level of threat that requires immediate action. These caves are a high priority for both the research effort and monitoring, while mid-priority caves need to be monitored to ensure that remaining populations are protected from further declines. Consequently, the priorities set for caves will be relevant to inform policy-makers to effectively protect bats and other organisms dependent on healthy underground ecosystems.

Conclusions

Our current work has demonstrated the prioritization of important underground roosts for conservation and protection and has discussed key issues and threats in them. Here, we found that anthropogenic activities, such as widespread caving activities and tourism, are the main concerns for bat roosts in the country, particularly in sites such as Mandrata (Alexandrovo), Emenskata, Perla 2, Rezidentsia Shumen, Karangin and Suhi Pec. We urge decision-makers to prioritize the sites that require urgent conservation attention to preserve important bat populations. We have also found that, while the Natura 2000 network is effective in covering the important bat roosts, the regulations are not well enforced on many sites. Most of the important bat sites in Bulgaria are legally protected by the Natura 2000 network and their

visitation is prohibited all year or during specific periods. Yet, most of the sites are imperiled by severe disturbance combined with other threats. The existing restrictions, especially in the case of the national protected area network, need to be updated to specifically address bats and to reflect the current state of the roosts. Furthermore, using a novel integrative approach for prioritization, we were able to identify vulnerable and important underground roosts for conservation in Bulgaria. We have also shown the feasibility and effective use of such an approach in the European context, which may be a useful step forward to the application of the Index in European caves through the adaptation of conservation organizations (e.g. Eurobats). We hope that our current work would inspire more effort by developing policies to protect cave-dwelling bats and their roosts in the country, especially in the face of the changing human environment.

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Supplementary material

Dataset: Important underground bat roosts in Bulgaria. Available online at: <https://bdj.pensoft.net/article/download/suppl/8289827/>

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Chapter 3. Diversity and Conservation of Cave-Dwelling Bats in the Brunca Region of Costa Rica

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Abstract: The Brunca region in Costa Rica contains the largest number of caves in the country, yet the diversity and distribution of bat species within those caves is currently unknown. Without this information, it is not possible to assess changes in populations and assemblages that may indicate severe damages to these critical roosting habitats, and to take evidence-based conservation actions. We present the first study to describe the diversity of cave-dwelling bat species in the Brunca region of Costa Rica in a large number of caves. We collected data of bat species diversity by direct observation and capturing bats inside roosts. Bats were observed in 38 of the 44 surveyed caves, representing 20 species from 4 families, with colony sizes ranging from a few individuals to >7500. In addition, we collected information about the human activities carried out in and around the roosts to assess potential threats that these sites face. Data indicate that caves suffer mostly from unregulated tourist visitation and that one of the most visited caves is also the one with the most species-rich bat assemblages. Our study determined the most important and vulnerable bat roosts in the region and shows the need for urgent conservation actions to protect them.

Introduction

Caves are broadly defined as natural openings in solid rock [1], and as such, they serve as the “windows” in which we glance into the underground (citation). Caves have been attracting people’s interest from prehistoric times by serving as shelters, sacred places, or sources of artistic expression [2], but present a significant challenge for scientific research because they are largely hidden from view and hardly accessible for exploration. Most caves do not appear on topographic maps or satellite images and are neglected by mainstream scientists, making cave research a priority for only a small number of highly dedicated individuals [3]. Their unique features, the complete absence of light, almost constant temperature, and high air humidity make caves a suitable habitat for a large variety of highly specialized organisms such as cave crickets [1,4], and some vertebrates including the blind salamander and angel fish [1,5]. These organisms are so well adapted to the specific conditions in caves that they would not survive in a surface habitat.

Caves are dependent on energy sources brought by several organisms that forage at the surface and which use caves as shelter, such as oilbirds, swiftlets, and bats [1,2,6]. Bats, in fact, are so tightly associated with caves and are often so abundant that they can significantly modify these ecosystems by altering their microclimatic conditions and providing significant amounts of guano, the essential food source and base of the food chain in most caves [2,7]. In turn, caves provide bats a refuge from predators and inclement weather, and a critical venue for social interactions [8–10]. Caves are so important for bats worldwide that the majority of species, including many that are vulnerable and rare, are either specialized cave-dwellers or use caves temporarily [11]. Also, the largest aggregations of bats are found in caves, with numbers reaching several millions of individuals [12–14], which makes them critical roosts for species that form such large colonies. Costa Rica is a bat diversity hotspot with 114 species, most of which are well represented in the southwestern Brunca region [15,16]. The Brunca region has the largest karst region (185 km²) and the largest number of caves (n = 156) in the country [17]. While many of the species that inhabit the region are known to roost in caves in other parts of their range [15,18,19], there is still no baseline information about the populations of cave-dwelling bats in this region. Cave-dwelling bats are extremely important for the local ecosystems, playing the role of pollinators, seed-dispersers, and pest-suppressors [20]. Cave-related tourism activities are also becoming

popular in the Brunca region [17], which makes the caves potential sources of local income, but also endangers cave-dwelling bats, which are often vulnerable to disturbance. Other threats that bats are facing include habitat loss and direct killing at their roosts. To the best of our knowledge, there is no information on distribution and the ecology of cave-dwelling bats in this region, nor information about which roosts contain large colonies or rare species of bats that need to be considered a conservation priority [18], or even the types of threats these roosts are facing. This study is the first to assess the diversity and distribution of cave-dwelling bats species in a large number of the caves in the country. Our study provides valuable information about underground roosts of conservation importance.

Monitoring cave roosts is a highly accurate method for estimating colony size, species composition, and seasonal changes in the populations of cave-dwelling bats [21,22], and in the temperate zones, it is a widely distributed activity with well-established traditions [23–25]. The regular monitoring of bat roosts is of particular importance to assess population decline in vulnerable species and for the identification of potential threats (e.g., disturbance or dangerous diseases) [26,27]. Roost monitoring is, however, uncommon in Costa Rica, where most of the research on bat diversity and distribution is conducted around research stations and with the method of mist-netting [28–30], and most recently also with acoustic monitoring at feeding or commuting sites. Our efforts are aimed at establishing roosts of national monitoring priority, which will help us assess population trends and serve as a base for conservation activities in the future.

Cave-dwelling bats are facing threats worldwide such as habitat loss, pollution, disturbance, quarrying and mining, guano extraction, and vandalism [31–33]. Bats that roost in caves are particularly vulnerable to human activities due to their tendency to aggregate in large colonies in a single roost, as a single disturbance event could lead to the eradication of an entire colony [10]. In the Neotropics, cave-dwelling bats face additional threats due to direct killing, as local residents attempt to eradicate species that are considered pests, specifically vampire bats (*Desmodus rotundus*) [34]. These bats are chased by cattle farmers and killed inside the caves where they roost, leading to the decline of other species as well [35]. The efforts against vampire bats threaten all of the cave-roosting bats, as people aiming to kill vampire bats do not distinguish the different species and often destroy all bats in a single roost [36]. It is

crucial to obtain information about the distribution of cave-dwelling bats in Costa Rica, so we can take conservation actions before important bat colonies are lost.

Materials and Methods

Study Region

We investigated caves and artificial tunnels in the Southwestern (Brunca) region of Costa Rica (N 9.23643, W 82.84233) at sites ranging in altitude from 0 to 520 masl. The Brunca region contains the largest karst area of the country, with 185 km² of karst surface and 156 caves [17]. The region has an average annual temperature of 26.2 °C and an average annual rainfall of 4398 mm [37]. The dominant habitat of the region is evergreen moist lowland forest, including large areas of well-preserved rainforests in the Corcovado and Amistad national parks [38]. For a better visual portrayal of the field sites, we roughly divided them into 5 areas (Figure 1): area A—Ballena, area B—Boruca, area C—Osa, area D—Rio Claro, and area E—Corredores. We do not provide the exact locations of the field sites to protect them from further disturbance; however, these data are available upon request.

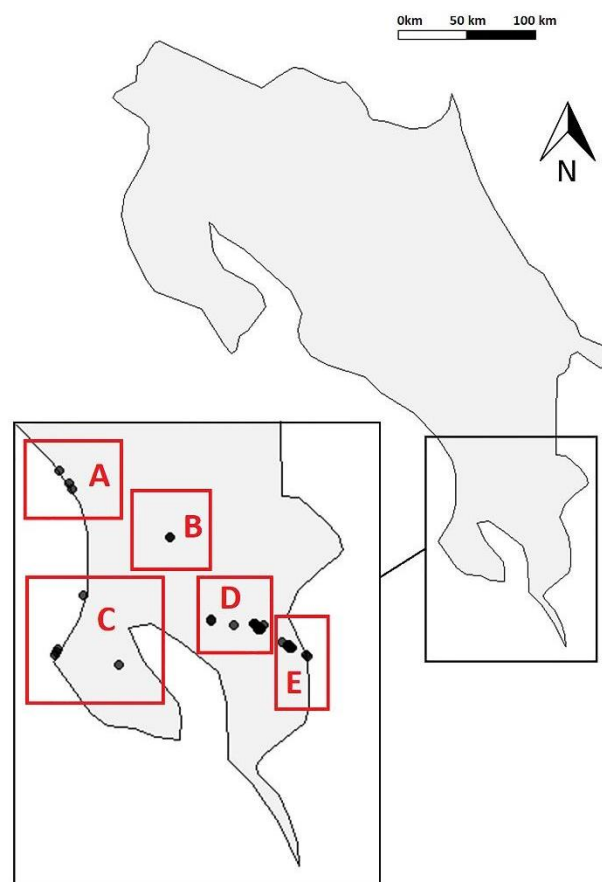


Figure 1 The Brunca region on the map of Costa Rica and the general location of the field work sites (marked by black dots), divided into 5 areas: (A) Ballena, (B) Boruca, (C) Osa, (D) Rio Claro, and (E) Corredores.

Cave Surveys

We obtained information about the existing caves in the Brunca region by consulting with the members of the only caving organization in Costa Rica—Anthros Speleological Group. The organization manages a national cave database, containing maps and descriptions of more than 340 caves in the country [39], which we used to select caves for research. Our selection of caves for this study was based on their size (in favor of greater length and depth) and available information about the presence of bat colonies in expedition reports [40]. We selected for research 14 caves from the national database and searched for additional caves by interviewing local people and performing transects in karst areas. Our research took place during the periods from December 2015–May 2016, October 2016–May 2017, and December 2017–February 2018. We recorded the location and altitude of each cave using a GPS unit (Garmin, KS, USA). We entered the caves during daylight hours in groups ranging from two to four people using standard caving equipment (i.e., helmet, two independent light

sources, and protective clothing). To survey the caves with vertical passages we used technical equipment and followed the approach of the single-rope technique [41]. We used the available cave maps to aid our movement inside the caves and to facilitate our bat surveys. If maps were not available, we created them using standard cave survey methods [42]. The number of visits varied between 1 and 4 times, depending on the complexity of the roost and the presence of bats during our first visit (Appendix A, Table A1, column D). If we observed only a few (<30) bats and low species richness (1 or 2 species), we visited the roost only once. When a large number of individuals or indirect traces of presence (e.g., guano, food remains) was observed during our first visit, we performed additional monitoring and tried to obtain data in both the rainy and dry seasons if possible. Due to high water levels and technical difficulties, some caves were inaccessible during the rainy season, so we visited them in the intermediate period between seasons (December–January and May–June). In addition to the field surveys, we interviewed local people about the activities conducted in caves. We included questions about visitation, hunting, other uses of the caves, and awareness about bat populations. We focused on farmers and landowners living near the locations of the caves. We also used direct observations in and around roosts to assess additional anthropogenic activities. For example, we recorded evidence of graffiti, waste, broken speleothems, footprints, entrance blocking or traces of resource extraction inside the roosts. In proximity to the roosts (radius of 1 km), we recorded the land use activities (agriculture, deforestation, quarrying). Depending on the dimension of the activities, we evaluated them on a scale from 1 to 4, 1 being the highest and 4 the lowest disturbance.

Bat Surveys

We used flashlights and binoculars to search visually for roosting bats or indirect traces of their presence (i.e., skulls, guano or food remains) [43]. Small clusters of bats (up to 50 individuals) were counted directly at the cave. We photographed larger groups of bats using a digital DSLR camera (D3200, Nikon, Tokyo, Japan) and used ImageJ software [44] to estimate the number of individuals. We determined the species of observed bats using the available literature [15,16,45] as a reference. To confirm species identification and obtain biometric data, we used a custom-made hand-held 2 m mist net to capture a few individuals within the roost. We measured forearm length, sex, age, and reproductive status of the captured bats [21]. In one vertical cave, we used a harp trap at the entrance to capture bats

during their evening emerge. After taking biometric data, we released all individuals without further disturbance. No voucher specimens were collected during this study. We operated under the research permit INV-ACOSA-018-16.

Assessing Conservation Priority

We evaluated the conservation priority of each cave using the Bat Cave Vulnerability Index (BCVI), based on the bat species diversity and presence of human-induced threats in the caves [46]. The index is a novel approach for conservation prioritization of bat caves and it was developed with a focus on tropical regions. It contains two components: Biotic Potential Index (BP) and Biotic Vulnerability Index (BV). The Biotic Potential Index includes several species diversity and rarity measurements, including species richness, abundance, relative abundance, endemism, conservation status [47], and rarity index. The BP index has a value between 1 and 4, with level 1 being the highest and 4 the lowest biotic potential. The Biotic Vulnerability Index includes information on cave accessibility, morphology, visitation, and land use in adjacent areas. The BV index has a value of A, B, C, and D, with A being the highest vulnerability to disturbance and D no disturbance. We classified all roosts based on the combined values of BP and BV. The roosts with indicated values of 1A and 1B were considered of highest conservation priority. The roosts with values between 1C and 3D were considered as medium conservation priority, and the roosts in category 4 of low priority.

Results

In the study period we visited 44 underground roosts, including 40 caves, two artificial tunnels, and two abandoned gold mines. From the researched caves, 30 were described for the first time during this study and included in the National Cave Database, and named after geographical or morphological features in their respective locations (Appendix A, Table A1). From all caves, 22 were horizontal and 22 were vertical (Appendix A, Figure A1). We observed bats in 38 of the 44 roosts. We identified 20 species of bats from the families Phyllostomidae, Emballonuridae, Natalidae and Mormoopidae (Table 1, Figure 2). The most frequently observed species was *Carollia perspicillata*, which occurred in 25 roosts. Other common species were *Peropteryx kappleri* (found in 18 roosts) and *Saccopteryx bilineata* (14 roosts). We observed relatively large ($n > 100$ ind.) colonies of bats (*Anoura* sp., *Artibeus*

jamaicensis, *C. perspicillata*, *Desmodus rotundus*, *Natalus mexicanus*, *Pteronotus gymnonotus*, *Pteronotus parnellii*, and *Pteronotus personatus*) in 11 caves and very large (>500 ind.) (*C. perspicillata*, *P. gymnonotus*, *P. parnellii*, and *P. personatus*) in 6 caves (Appendix A, Table A1). The largest colony of bats was observed in the Campanario cave, estimated at around 7600 individuals, and included three species of the genus *Pteronotus* (Appendix A, Table A1). Other large colonies are those found in the Laguna Perdida (ca. 2000 individuals) and Corredores (ca. 1500 individuals) caves. The roost with the greater species richness was Corredores, with 8 species, followed by Emus and Laguna Perdida with 7 species (Appendix A, Table A1). Three caves were inhabited by mixed colonies of *Pteronotus* spp.: Tortuga, Campanario, and Corredores; the first two are considered maternity colonies since we observed hundreds of pups. Both Tortuga and Campanario are very similar littoral caves, which have a single entrance, leading to a simple chamber, and are partially filled with sea water during high tides. In the Campanario cave, we observed a third species of *Pteronotus*, *P. personatus*, which is so far the only known location of the species during this study. A single bat species occurred in 14 caves, specifically the greater dog-like bat (*Peropteryx kappleri*) and Seba's short-tailed bat (*C. perspicillata*), observed in 6 roosts each.

Table 1 Observed species of bats and their respective locations.

Family	Species	Roosts
	<i>Anoura</i> sp.	Laguna Perdida, Piedras Blancas 2
	<i>Artibeus jamaicensis</i>	Arelis, Carma, Corredores, Gran Galería, Túnel ICE 2, San Pedrillo Afrodiziaco Pozo, Alma, Árbol Caido, Bananal, Bombasa, Buena Cueva, Caballo
	<i>Carollia perspicillata</i>	Muerto, Cinco Millones, Corredores, Dos Brazos, Emús, Final 7 Pozo, Gran Galería, Gran Madre, Túnel ICE 1, Túnel ICE 2, San Josecito, Laguna Perdida, Los Sueños, Miramar Pozo, San Pedrillo, Sapo Gordo Pozo, Titi Mono, Tortuga
	<i>Carollia sowelli</i>	Miramar
Phyllostomidae	<i>Chrotopterus auritus</i>	Corredores
	<i>Desmodus rotundus</i>	Alma, Bombasa, Buena Cueva, Cinco Millones, Emús, Gran Madre, Túnel ICE 2, San Josecito, Laguna Perdida, Los Sueños, Miramar
	<i>Glossophaga soricina</i>	Alma, Bombasa, Corredores, Dos Brazos
	<i>Lonchophylla concava</i>	San Josecito, Miramar Pozo, San Pedrillo
	<i>Lonchophylla robusta</i>	Bombasa, Laguna Perdida
	<i>Lonchorhina aurita</i>	Gran Madre, Miramar
	<i>Phyllostomus discolor</i>	Arelis
	<i>Phyllostomus hastatus</i>	Laguna Perdida

	<i>Trachops cirrhosus</i>	Bombasa, San Pedrillo Alma, Arbol Caido, Arelis, Bamboo Pozo, Banano Quemado, Caballo Muerto, Castillo Real, Cinco Millones, Cueva 1 No Name, Cueva Cerca Cor, Emús, Gran Galería, Gran Madre, La Troja, Metros 12, Monteadores, Rectángulo, Serpiente Dormida
Emballonuridae	<i>Peropteryx kappleri</i>	Emús, Gran Galería
	<i>Peropteryx macrotis</i>	Alma, Arelis, Bamboo Pozo, Cinco Millones, Corredores, Emús, Gran Galería, Gran Madre, Túnel ICE 2, Laguna Perdida, Los Sueños, Monteadores, Rectángulo, San Pedrillo
	<i>Saccopteryx bilineata</i>	Corredores, Emus
Natalidae	<i>Natalus mexicanus</i>	Campanario, Corredores, Tortuga
	<i>Pteronotus gymnotus</i>	Bombasa, Campanario, Corredores, Emus, Túnel ICE 2, Laguna Perdida, Los Sueños, Tortuga
Mormoopidae	<i>Pteronotus parnellii</i>	Campanario
	<i>Pteronotus personatus</i>	

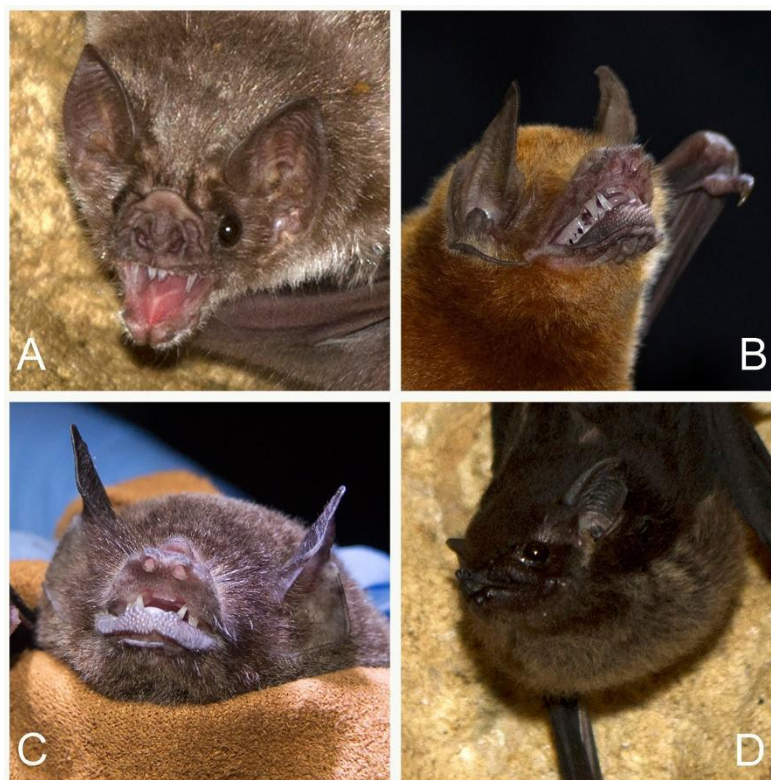


Figure 2 Some of the species, observed during our study: (A) Common vampire bat (*D. rotundus*), (B) Big naked-backed bat (*P. gymnotus*), (C) Parnell's mustached bat (*P. parnellii*), and (D) Greater sac-winged bat (*S. bilineata*). Photo credit: S. Deleva.

We interviewed 10 groups of people, mainly farmers and landowners around the locations of the field sites. There were reports of hunting activities in two of the caves, in both of which we observed blocked entrances. Most of the farmers were not interested in the caves on their land and were not visiting them, but in two cases the owners were collecting entrance fees for their caves without offering other services, such as a guided tour or providing safety equipment. During our visits, six of the landowners showed us new caves and assisted in the explorations. From our observations inside the roosts, we recorded traces of uncontrolled visitation (footprints and waste) in 16 of the 44 roosts, vandalism (broken speleothems and graffiti) in two caves, and disposal of large quantities of household waste in one cave. The activities observed around the caves included small-scale agriculture and deforestation. Only three caves were located in a relatively undisturbed habitat (rainforest without agriculture activities in a 1 km radius around the cave entrance). We evaluated the morphological features of the roosts, difficulty of approach (distance to urbanized areas), cave use (tourism, hunting, littering, etc.), and the land-use activities around the roosts to calculate the Biotic Vulnerability Index (BV). According to the BV index, three caves had the highest level of vulnerability (Level A), and 13 caves showed high vulnerability (Level B). The rest of the caves ($n = 27$) had a lower level of vulnerability (Level C). Only one cave showed no disturbance (Level D) (Table 2). Based on the Biotic Potential Index (BP), 11 caves had high diversity with large bat populations and high species richness, including rare species (Level 1). Only one cave was classified as Level 2, with relatively large bat populations, and three caves were classified as level 3, with few species, mainly common, widespread, and small populations of bats. The other 29 caves were classified as level 4, as they showed very low species richness, represented by only a few individuals (Table 2). We combined the two indexes to determine the roosts with the highest conservation priority (Bat Cave Vulnerability Index). Our results showed that nine caves have the highest conservation priority. The Tortuga cave received the highest BCVI (1A) due to the large bat colony and vulnerability. In addition, eight other caves (i.e., Arelis, Bombasa, Corredores, Dos Brazos, Emus, Túnel Ice 2, Laguna Perdida, and San Pedrillo) had a BCVI result of 1B—highest species diversity and very high vulnerability. Medium conservation priority caves were evaluated with BCVI values between 1C (High diversity and low vulnerability) and 3B (low diversity and high

disturbance). The roosts with category 4A, 4B, and 4C were evaluated as low conservation priority due to their low bat diversity (Table 2).

Table 2 Bat Cave Vulnerability Index (BCVI) with description of each observed category and the roosts.

BCVI		Priority	Description *	Roosts
B P	B V			
1	A	High	Large population, highest site accessibility, highly prone to disturbance.	Tortuga
1	B	High	Large population, high species diversity, high site accessibility, highly prone to disturbance.	Arelis, Bombasa, Corredores, Dos Brazos, Emus, Túnel Ice 2, Laguna Perdida, San Pedrillo
1	C	Medium	Large population, high species diversity, low site accessibility and less prone to disturbance.	Campanario, Miramar
2	C	Medium	Relatively high population, low species diversity, low site accessibility and less prone to disturbance.	Carma
3	B	Medium	Small populations, relatively high species diversity, high site accessibility, highly prone to disturbance.	Alma, Gran Galeria
3	D	Medium	Relatively large population, low species diversity, rare species present, low site accessibility, not prone to disturbance.	Piedras Blancas 2
4	A	Low	No bats present, highest site accessibility, highly prone to disturbance.	Arco, Ventana
4	B	Low	Small populations, relatively high species diversity, high site accessibility.	San Jocesito Cataratas, Los Sueños, Gran Madre Afrodiziaco, Aprendizaje, Arbol Caido, Bamboo, Bananal, Banano Quemado, Buena Cueva, Caballo Muerto, Castillo Real, Cinco millones, Cueva 1, Cueva 3, Cueva 5, Cueva cerca Corredores., Final 7, Túnel ICE 2, La Troja, Lagrima, Metros 12, Monteadores, Rectangulo, Sapo Gordo, Serpiente Dormida, Titi Mono.
4	C	Low	Very small population, low species diversity, lower site accessibility and less prone to disturbance.	

Discussion

This study represents the first significant effort to characterize bat diversity in a large number of caves in Costa Rica. We provide new data on some additional roosting resources that are available to many bat species in the region, and our study will serve as a baseline for further research on the cave-dwelling bats in the Brunca region and in the country (Appendix A, Table A1). With our results, we have identified caves with a large number of species that

potentially may require strong initiatives to protect, such as the Corredores and Laguna Perdida caves [46]. We have also identified colonies of a few species that are extremely rare and were only found in one or two caves, including Corredores and Emus (*N. mexicanus* in both, *C. auritus* in the former, and *Peropteryx macrotis* in the latter), Miramar (*L. aurita*), and Campanario (*P. personatus*), which points to the need for establishing strict visitation controls for all visitors (including speleologists) to secure the persistence of these colonies, as some of these species may be considered of high extinction risk [48]. These caves were not categorized as high-priority by the BCVI given that they do not suffer, yet, from human disturbance, as the caves are fairly inaccessible. Furthermore, we have also identified two maternity colonies that harbor large numbers of individuals from three species of the genus *Pteronotus*, primarily in the Campanario cave, but also in Tortuga (Figure 3). To secure the long-term persistence of these populations, visitation of these sites should never be allowed during lactation (February—May) as during this period, a single event of disturbance could lead to the detachment and fall (and possibly death) of hundreds of pups [25].

From the 44 studied caves, 38 (86.36%) were occupied by bats, which represents a higher occupation rate than similar studies reported in other countries in Latin America. In Puerto Rico, for example, only 31% of the caves in the National Speleology database are used as roosts [49]. A similar study in Brazil shows that only about half of the observed caves had bats [50]. We also found that in combination, all caves harbor a total of 20 species. However, we know that at least 46 species in the Brunca region of Costa Rica are considered cave-dwellers [15], and therefore, wonder why none of these other species were recorded in our study (Appendix A, Table A2). One possibility is that other roosting resources are more readily available to bats that are not cave-specialists, such as several species within the tribe Micronycterini (e.g., *Micronycteris microtis* and *Lampronnycteris brachyotis*), which may often roost in hollow trees [51], and *Artibeus lituratus*, which is typically found roosting under foliage [52]. Alternatively, more vulnerable and rare species, such as the carnivorous bat *Vampyrum spectrum* and *Macrophyllum macrophyllum* [53–55], may have already disappeared from the area where the majority of study caves were located, as surrounding natural habitats face severe loss and/or significant degradation [38]. However, we hope that some of these other species will be recorded as we continue to monitor the same and

additional cave roosts with a combination of other research techniques, including captures at the cave entrance and the use of ultrasound detectors [56].



Figure 3 A nursery colony of *Pteronotus* spp. in the Tortuga cave. Photo credit: S. Deleva.

From the 44 caves we visited, only two (i.e., Laguna Perdida and Piedras Blancas 2) are under some level of protection, as they are located in the Piedras Blancas National Park. There is no law protecting caves in Costa Rica, and most are located on private properties, which makes their protection a decision of the landowners. From the 114 species of bats in Costa Rica, at least 48 are cave-dwellers and depend on caves as roosts in one degree or another [15,57]. This makes the protection of caves and other underground roosts such as artificial tunnels and gold mines a matter of the highest priority. The most effective way to preserve the caves is to propose the roosts categorized as of high conservation priority as important sites for the conservation of bats (Sitio Importante para la Conservación de los Murciélagos), based on the documentation of the Latin American Bat Conservation Network [58]. Two of the caves with the highest conservation priority, Corredores and Emus, are also the most heavily affected by anthropogenic activities, such as uncontrolled visitation and vandalism, and both are readily accessible. Due to the frequent disturbance, the bat colonies in these caves are facing a dire future unless conservation groups, tour guides, and landowners join forces to set limits to visitation rates and enforce proper visitation guidelines [59].

The Bat Cave Vulnerability Index (BCVI) has further allowed us to identify roosts with high species diversity that are currently subject to anthropogenic pressure, thus urgent actions are needed to prevent further disturbance. Some roosts with high bat diversity are excluded from the list due to their difficult approach, which makes them less vulnerable to anthropogenic pressure. Others, which are highly vulnerable or already affected by anthropogenic activities, were excluded from the list due to low species diversity. We propose that if limited resources are available, we should focus our conservation efforts on the roosts of category 1A and 1B, but the other categories need further monitoring, as new visits can detect new bat species or new threats that could be prevented before extensive damage is caused to the colonies [46].

Conclusions

With this study, we now have the tools to develop conservation strategies to protect the most important and vulnerable roosts and baseline information to start long-term monitoring programs of the bat colonies that inhabit these caves. Previous data of cave-dwelling bats in the Brunca region are available from a few expedition reports made in 1993, conducted mainly by non-specialists, that present general information about the presence of just a few bat species without information on their abundance [40]. As such, this report does not provide reliable baseline data to gauge changes in bat colony size and species assemblages that might allow us to determine if caves are suffering from human activities conducted in them, most notably uncontrolled visitation.

Our data confirm that caves in the Brunca region of Costa Rica are inhabited by a rich bat fauna and we must take urgent conservation efforts to protect them. Many of the bats in Costa Rica are of the lowest conservation priority (least concern) because of their wide distribution, but some species may be locally rare or declining. We propose cave surveys be included in the national priorities for bat research and an annual monitoring scheme for roosts to be set up. In this way, we will be able to trace the change in populations and to take actions if certain species are declining.

Acknowledgments

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Supplementary materials



Figure A1 Some of the field work sites, mentioned in this study: (A) San Pedrillo cave, photo: S. Deleva; (B) Emus cave, photo: S. Trescott; (C) Bamboo cave, photo: C. Castillo Salazar; (D) Gran Madre cave, photo: S. Deleva.

Appendix A

Table A1. All field sites in the Brunca region, their location (see Figure 1), visit dates, the Bat Cave Vulnerability Index (BCVI), and the Number of individuals of each species.

Cave	Area	Visits/mm/yy	BCVI	No of Species	A sp. **	A jam	C per	C sow	C aur	D rot	G sor	L con	L rob	L aur	N mex	P kap	P mac	P dis	P has	P gym	P par	P per	S bil	T cir	
AFRODIZIACO POZO *	D	03/16	4 C	1	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
ALMA	E	02/16, 05/16, 02/17, 12/17	3 B	5	0	0	10	0	0	6	3	0	0	0	0	59	0	0	0	0	0	0	0	15	0
APRENDIZAJE POZO *	D	03/16	4 C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ARBOL CAIDO *	D	03/16	4 C	2	0	0	14	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0
ARCO *	A	02/16, 05/16	4 A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ARELIS *	D	01/16; 04/16	1 B	4	0	22	0	0	0	0	0	0	0	0	45	0	36	0	0	0	0	0	55	0	0
BAMBOO POZO *	D	03/17	4 C	2	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	14	0	0
BANANAL	E	01/17	4 C	1	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BANANO QUEMADO	E	03/16	4 C	2	0	0	0	3	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
BOMBASA *	C	02/18	1 B	6	0	0	350	0	0	1	40	0	20	0	0	0	0	0	0	0	100	0	0	1	0
BUENA CUEVA *	D	03/16	4 C	2	0	0	74	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CABALLO MUERTO *	D	01/16, 03/16	4 C	2	0	0	3	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
CAMPANARIO *	C	05/17, 02/18	1 C	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2000	5000	600	0	0	0	0
CARMA	E	02/16	2 C	1	0	179	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CASTILLO REAL	E	04/16	4 C	1	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0
CINCO MILLIONES *	D	03/16	4 C	4	0	0	15	0	0	4	0	0	0	0	26	0	0	0	0	0	0	0	20	0	0
CORREDORES	E	01/16, 03.16, 02/16, 12/17	1 B	8	0	14	49	0	1	0	1	0	0	0	235	0	0	0	0	500	700	0	8	0	0
CUEVA 1 NO NAME *	D	01/16	4 C	1	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0
CUEVA 3 NO NAME *	D	01/16	4 C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CUEVA 5 NO NAME *	D	01/16	4 C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CUEVA CERCA COR	E	02/16	4 C	1	0	0	0	0	0	0	0	0	0	0	22	0	0	0	0	0	0	0	0	0	0
DOS BRAZOS *	C	03/17, 02/18	1 B	2	0	0	663	0	0	0	90	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EMUS	D	01/16, 04/16, 12/17	1 B	7	0	0	813	0	0	44	0	0	0	0	10	9	2	0	0	0	200	0	26	0	0
FINAL 7 POZO *	D	03/16	4 C	1	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GRAN GALERIA	E	03/16, 10/16, 12/17	3 B	5	0	49	60	0	0	0	0	0	0	0	15	1	0	0	0	0	0	0	24	0	0
GRAN MADRE *	D	03/16, 03/16	4 C	5	0	0	16	0	0	4	0	0	0	1	62	0	0	0	0	0	0	0	31	0	0
ICE 1 TUNNEL	B	02/17	4 C	1	0	0	200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ICE 2 TUNNEL	B	02/17, 12/17	1 B	5	0	320	70	0	0	39	0	0	0	0	0	0	0	0	0	0	150	0	12	0	0
LA TROJA	E	04/16	4 C	1	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0
LAGRIMA POZO *	D	03/16	4 C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LAGUNA PERDIDA *	D	10/16, 10/16, 12/17	1 B	7	100	0	1239	0	0	176	0	0	118	0	0	0	0	57	0	350	0	1	0	0	0
LOS SUEÑOS *	D	11/16, 02/18	4 B	4	0	0	250	0	0	25	0	0	0	0	0	0	0	0	0	0	4	0	65	0	0
METROS 12 NO NAME *	D	01/16	4 C	1	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0
MIRAMAR POZO *	E	12/15, 01/16	1 C	5	0	0	134	1	0	1	0	11	0	108	0	0	0	0	0	0	0	0	0	0	0
MONTEADORES *	E	01/16	4 C	2	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	2	0	0
PIEDRAS BLANCAS 2 *	D	10/17	3 D	1	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RECTANGULO	E	04/16	4 C	2	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0	4	0	0
SAN JOSECITO *	C	05/17, 02/18	4 B	3	0	0	35	0	0	19	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0
SAN PEDRILLO *	C	05/17	1 B	5	0	34	5	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	1	1	0
SAPO GORDO POZO *	D	03/16	4 C	1	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SERPIENTE DORMIDA	E	03/17	4 C	1	0	0	0	0	0	0	0	0	0	0	28	0	0	0	0	0	0	0	0	0	0
TITI MONO *	D	03/16	4 C	1	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TORTUGA *	A	05/16, 10/16, 02/17	1 A	3	0	0	6	0	0	0	0	0	0	0	0	0	0	0	427	400	0	0	0	0	0
VENTANA *	A	05/16	4 A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* New caves, according to the National Cave database. ** Species abbreviations: *Anoura* sp., *Artibeus jamaicensis*, *Carollia perspicillata*, *Carollia sowelli*, *Chrotopterus auritus*, *Desmodus rotundus*, *Glossophaga soricina*, *Lonchophylla concava*, *Lonchophylla robusta*, *Lonchorhina aurita*, *Natalus mexicanus*, *Peropteryx kappleri*, *Peropteryx macrotis*, *Phyllostomus discolor*, *Phyllostomus hastatus*, *Pteronotus gymnotus*, *Pteronotus parnellii*, *Pteronotus personatus*, *Saccopteryx bilineata*, *Trachops cirrhosus*.

Table A2 Bat species in the Brunca region, their conservation status, population trend, presence in caves, and cave dependence.

Common Name	Latin Name	IUCN Status	Population Trend	Cave-Dwelling	Cave-Dependent
Handley's tailless bat	<i>Anoura cultrata</i>	LC	decreasing	Yes	No
Geoffroy's tailless bat	<i>Anoura geoffroyi</i>	LC	stable	Yes	No
Jamaican fruit bat	<i>Artibeus jamaicensis</i>	LC	stable	Yes	No
Great fruit-eating bat	<i>Artibeus lituratus</i>	LC	stable	Yes	No
Chestnut short-tailed bat	<i>Carollia castanea</i>	LC	stable	Yes	No
Seba's short-tailed bat	<i>Carollia perspicillata</i>	LC	stable	Yes	No
Sowell's short-tailed bat	<i>Carollia sowelli</i>	LC	stable	Yes	No
Shaggy bat	<i>Centronycteris centralis</i>	LC	unknown	No	No
Wrinkle-faced bat	<i>Centurio senex</i>	LC	stable	No	No
Salvin's big-eyed bat	<i>Chiroderma salvini</i>	LC	stable	No data	No
Hairy big-eyed bat	<i>Chiroderma villosum</i>	LC	stable	No	No
Godman's long-tailed bat	<i>Choeroniscus godmani</i>	LC	unknown	No data	No data
Big-eared wooly bat	<i>Chrotopterus auritus</i>	LC	stable	Yes	No
Wagner's sac-winged bat	<i>Cormura brevirostris</i>	LC	unknown	No	No
Aztec fruit-eating bat	<i>Dermanura azteca</i>	LC	unknown	Yes	No
Toltec fruit-eating bat	<i>Dermanura tolteca</i>	LC	unknown	Yes	No
Thomas' fruit eating bat	<i>Dermanura watsoni</i>	LC	stable	No	No
Common vampire bat	<i>Desmodus rotundus</i>	LC	stable	Yes	No
White-winged vampire bat	<i>Diaemus youngi</i>	LC	unknown	Yes	No
Northern ghost bat	<i>Diclidurus albus</i>	LC	unknown	No	No
Hairy-legged vampire bat	<i>Diphylla ecaudata</i>	LC	stable	Yes	No
Velvety fruit-eating bat	<i>Enchisthenes hartii</i>	LC	unknown	No data	No data
Brazilian brown bat	<i>Eptesicus brasiliensis</i>	LC	unknown	No	No
Chirqui brown bat	<i>Eptesicus chiriquinus</i>	LC	unknown	No	No
Argentine brown bat	<i>Eptesicus furinalis</i>	LC	unknown	Yes	No
Big brown bat	<i>Eptesicus fuscus</i>	LC	increasing	Yes	No
Black bonneted bat	<i>Eumops auripendulus</i>	LC	unknown	No	No
Sanborn's bonneted bat	<i>Eumops hansae</i>	LC	unknown	No data	No data
Commissaris's long-tongued bat	<i>Glossophaga commissarisi</i>	LC	stable	Yes	No
Pallas's long-tongued bat	<i>Glossophaga soricina</i>	LC	stable	Yes	No
Underwood's long-tongued bat	<i>Hylonycteris underwoodi</i>	LC	stable	Yes	Yes
Yellow-throated big-eared bat	<i>Lampronnycteris brachyotis</i>	LC	stable	Yes	No
Desert red bat	<i>Lasiurus blossevillii</i>	LC	unknown	No	No
Southern yellow bat	<i>Lasiurus ega</i>	LC	unknown	No	No
Dark long-tongued bat	<i>Lichonycteris obscura</i>	LC	unknown	No data	No data
Goldman's nectar bat	<i>Lonchophylla concava</i>	LC	unknown	Yes	No data
Orange nectar bat	<i>Lonchophylla robusta</i>	LC	unknown	Yes	Yes
Tomes's sword-nosed bat	<i>Lonchorhina aurita</i>	LC	stable	Yes	Yes
Pygmy round-eared bat	<i>Lophostoma brasiliense</i>	LC	stable	No	No
White-throated round-eared bat	<i>Lophostoma silvicolium</i>	LC	unknown	No	No
Long-legged bat	<i>Macrophyllum macrophyllum</i>	LC	unknown	Yes	No data
Hairy big-eared bat	<i>Micronycteris hirsuta</i>	LC	unknown	No	No

Common Name	Latin Name	IUCN Status	Population Trend	Cave-Dwelling	Cave-Dependent
Common big-eared bat	<i>Micronycteris microtis</i>	LC	stable	Yes	No
White-bellied big-eared bat	<i>Micronycteris minuta</i>	LC	unknown	Yes	No
Schmidts's big-eared bat	<i>Micronycteris schmidtorum</i>	LC	stable	No	No
Striped hairy-nosed bat	<i>Mimon crenulatum</i>	LC	stable	No	No
Coiban Mastiff Bat	<i>Molossus coibensis</i>	LC	unknown	No	No
Velvety free-tailed bat	<i>Molossus molossus</i>	LC	unknown	No	No
Miller's mastiff bat	<i>Molossus pretiosus</i>	LC	unknown	Yes	No data
Black mastiff bat	<i>Molossus rufus</i>	LC	stable	No	No
Sinaloa mastiff bat	<i>Molossus sinaloae</i>	LC	stable	Yes	No
Silver-tipped myotis	<i>Myotis albescens</i>	LC	stable	Yes	No
Hairy-legged myotis	<i>Myotis keaysi</i>	LC	unknown	Yes	No
Black myotis	<i>Myotis nigricans</i>	LC	stable	Yes	No
Montane myotis	<i>Myotis oxyotus</i>	LC	unknown	No data	No data
Riparian myotis	<i>Myotis riparius</i>	LC	stable	No data	No data
Mexican funnel-eared bat	<i>Natalus mexicanus</i>	LC	unknown	Yes	Yes
Lesser bulldog bat	<i>Noctilio albiventris</i>	LC	stable	No	No
Greater bulldog bat	<i>Noctilio leporinus</i>	LC	unknown	Yes	No
Greater dog-like bat	<i>Peropteryx kappleri</i>	LC	unknown	Yes	No
Lesser doglike bat	<i>Peropteryx macrotis</i>	LC	stable	Yes	No
Pale spear-nosed bat	<i>Phyllostomus discolor</i>	LC	stable	Yes	No
Greater spear-nosed bat	<i>Phyllostomus hastatus</i>	LC	stable	Yes	No
Heller's broad-nosed bat	<i>Platyrrhinus helleri</i>	LC	stable	Yes	No
Greater broad-nosed bat	<i>Platyrrhinus vittatus</i>	LC	unknown	Yes	No data
Naked-backed bat	<i>Pteronotus davyi</i>	LC	stable	Yes	Yes
Big naked-backed bat	<i>Pteronotus gymnonotus</i>	LC	stable	Yes	Yes
Parnell's mustached bat	<i>Pteronotus mesoamericanus</i>	LC	unknown	Yes	Yes
Wagner's mustached bat	<i>Pteronotus personatus</i>	LC	stable	Yes	Yes
Thomas' yellow bat	<i>Rhogeessa io</i>	LC	unknown	No	No
Proboscis bat	<i>Rhynchonycteris naso</i>	LC	unknown	No	No
Greater sac-winged bat	<i>Saccopteryx bilineata</i>	LC	unknown	Yes	No
Lesser sac-winged bat	<i>Saccopteryx leptura</i>	LC	unknown	No	No
Talamancan yellow-shouldered bat	<i>Sturmira mordax</i>	NT	stable	No data	No data
Mexican free-tailed bat	<i>Tadarida brasiliensis</i>	LC	stable	Yes	No
Spix's disk-winged bat	<i>Thyroptera tricolor</i>	LC	unknown	No	No
Stripe-headed round-eared bat	<i>Tonatia saurophila</i>	LC	stable	No	No
Fringe-lipped bat	<i>Trachops cirrhosus</i>	LC	stable	Yes	No
Niceforo's big-eared bat	<i>Trinycteris nicefori</i>	LC	unknown	No	No
Tent-making bat	<i>Uroderma bilobatum</i>	LC	stable	No	No
Striped yellow-eared bat	<i>Vampyriscus nymphaea</i>	LC	unknown	No	No
Northern little yellow-eared bat	<i>Vampyressa thuyone</i>	LC	unknown	No	No
Great stripe-faced bat	<i>Vampyrodes major</i>	LC	unknown	No	No
Spectral bat	<i>Vampyrum spectrum</i>	NT	decreasing	Yes	No

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Chapter 4. Factors affecting the functional diversity of cave-dwelling bats in a neotropical region and its significance for conservation

The manuscript is not published yet.

Abstract: While understanding abundance and species richness is important for conservation purposes for caves and other vital habitats, an increasing number of studies show that functional diversity may be more meaningful for the effective assessment of ecosystem health. Our study aims to evaluate which anthropogenic and environmental factors, both underground and on the surface, influence the bat functional diversity in a large number of caves in a tropical region – the Brunca region of Costa Rica. We surveyed 44 roosts and assessed anthropogenic and environmental factors such as cave size, complexity, and deforestation. Our results confirm that cave size is the most important factor affecting bat diversity, influencing species richness and functional divergence. Contrary to expectations, visitation did not negatively impact bat diversity in caves but instead showed a positive correlation with diversity indices. The latter could be explained by a greater tendency of tourists to visit larger caves, and not by tourism increasing bat diversity.

Introduction

Habitat loss is considered the greatest threat to biodiversity worldwide (Moorcroft, 2014). For example, 62% of endangered species are considered as such primarily due to significant habitat changes, rendering them unsuitable to sustain healthy populations of many native species. These changes include the complete or partial loss of feeding resources, increased potential predators and parasites, and loss of roosts or shelter (Maxwell et al., 2016). Because of the severity of the situation and the continuously increasing human population, which causes further encroachment of pristine natural habitats, it is unfeasible to consider protecting all species. Therefore, effective conservation practices need to establish priorities; for example, governments and conservation agencies could prioritize preserving the most endangered species (Buckley, 1985), maximize the conservation of phylogenetic diversity, i.e., protect as many species as possible (Myers et al., 2000), or efforts could be focused at protecting species for which conservation actions would have the broadest co-benefits for other species, habitats, and ecosystem services (Roberge & Angelstam, 2004).

Caves are unique and fragile ecosystems that harbor many rare and endemic species (Lauritzen, 2018). However, they are threatened by numerous anthropogenic factors, including pollution, destruction by quarries, climate change, and disturbance by tourists and visitors (Niemi et al., 2018). Many cave-dwelling species remain poorly studied and will likely go extinct due to high anthropogenic pressure. For example, bats that use caves for roosting are severely affected by cave visitation; many species, including rare and endangered, suffer from the loss or deterioration of important cave roosts (Sagot & Chaverri, 2015). Bats are known to play a critical role in underground ecosystems as their guano often is the primary source of nutrients and the base for fragile food chains within caves (Gunn, 2004); therefore, bats may play a role as a flagship species and their presence in caves could help protect the whole underground ecosystem (Roberge & Angelstam, 2004). Some cave-roosting bats are also known to play essential ecosystem services, including the reduction of potential insect pests, pollination, and seed dispersal (Kunz et al., 2011), so the protection of bat colonies could help preserve the functionality of ecosystems surrounding caves.

Multiple studies have addressed whether bat presence and species richness within a cave depend on the roost's characteristics (Phelps et al., 2016, 2018; Vargas-Mena et al., 2020).

For example, it is considered that bats generally prefer large and more complex caves (Peñuela-Salgado & Perez-Tores, 2015). Such roosts receive more attention when making conservation decisions. In some cases, however, small caves are just as important as large ones, especially during the period of swarming and hibernation (Dixon, 2011). Anthropogenic pressures in and around caves are also considered important correlates of bat presence, and caves in urbanized areas have less diverse bat populations than the ones in pristine areas (Phelps et al., 2016). This is especially true for insectivorous bats and rare or indicator species. Microclimatic factors such as temperature and humidity are also important determinants of abundance and diversity (Avila-Flores & Medellín, 2004). Certain bat species are also highly tolerant of extreme conditions, including very high temperatures, which might prove unbearable for other species (Ladle et al., 2012). In addition, other factors, such as interspecific interactions, could also play a role in bat presence in roosts (Kelm et al., 2021; Salinas-Ramos et al., 2020).

While understanding abundance and species richness is important for conservation purposes for caves and other vital habitats, an increasing number of studies show that functional diversity may be more meaningful for the effective assessment of ecosystem health (Cadotte et al., 2011; Goswami et al., 2017; McGill et al., 2006). Functional diversity is the measure of functional traits of an organism that influences one or more aspects of diversity traits in ecosystems (Díaz & Cabido, 2001; Goswami et al., 2017). Functional diversity would be more helpful in guiding conservation efforts in caves because its indices may show the actual state of an ecosystem. The widely used species richness might not be accurate as it does not address the function of the organisms, which means that some sites with an equal number of species would not be equal regarding conservation priority (Martínez-Ferreira et al., 2020). With the current rapid loss of habitats and the limited resources for conservation available, priority should be given to the conservation of areas or roosts that could provide shelter to a larger number of species with diverse ecological roles (Tanalgo et al., 2018). By focusing our protection on greater functional diversity, we may help slow down or stop the effect of anthropogenic disturbance on ecosystem fitness while decreasing our investment in protecting roosts that may not be providing the most significant number of ecological roles.

Our study aims to evaluate which anthropogenic and environmental factors, both underground and on the surface, contribute to an increase in bat functional diversity in a large number of caves in a tropical region – the Brunca region of Costa Rica. The area has numerous caves with many bat species, some of which are affected by anthropogenic activities and urgently need state protection (Deleva & Chaverri, 2018). Based on results from previous studies, we predict that disturbance and deforestation will negatively impact diversity estimates (Farneda et al. 2020), whereas cave size will have a positive impact on diversity (Phelps et al., 2016). With this study, we want to address conservation priorities beyond the number of species in each roost and apply the results to future conservation initiatives. The results of this and other similar studies could provide an important tool to ensure proper conservation actions aimed primarily at preserving ecosystem functionality. One key implication of our research is recognizing the need to protect caves that may be less attractive for tourism but harbor a larger functional diversity. By doing so, we can ensure the preservation of vital ecological functions and contribute to the overall health of these cave ecosystems.

Materials and methods

Study region

We surveyed subterranean sites in the Southern parts of the Puntarenas province of Costa Rica, also called the Brunca region (N 8.64, W 83.11). The location of the sites ranged in altitude from 0 to 520 m asl. The region has an average annual temperature of 23.6°C and an average annual rainfall of 414.5 mm (Climate Data, 2021). The dominant habitat in the region is evergreen moist lowland forest, including large areas of well-preserved rainforests in the Corcovado and La Amistad national parks (Gilbert et al., 2016). Other habitats include pastures, plantations, agricultural land, urbanized areas, coastal areas, and rivers (Silva, 2001). We visited underground sites with different morphology and origin: caves with horizontal or vertical development (with different origin such as karstic or marine-erosive), pits, marine caves, and tunnels (abandoned mines and exploration galleries). We are addressing all the studied subterranean sites as caves, although some were of anthropogenic origin. Our fieldwork took place between May 2016 and February 2018. Inside the roosts,

we used standardized methods to assess bat diversity and colony size (Hayes et. al. 2009). The methodology is described in detail in Deleva & Chaverri (2018) and Deleva (2023).

Surface factors

The surface level disturbance was calculated as the percentage of degraded habitat in the form of deforestation and urbanized zones in an area within a 1 km radius around the site. Fragmented landscape and human settlements in this radius around a roost pose a significant threat to cave-dwelling bats (Gorresen & Willig, 2004, Kingston, 2013). We used the software Google Earth Pro (satellite images, obtained in 2019) to create circular buffers of 1 km radius around the cave entrances and calculate the area of each degraded habitat.

Characterizing caves

During each visit, we measured air temperature (°C) and relative humidity (%) at several points in each cave, specifically at the entrance, the natural end of the cave, and under the location of bat colonies. We used a digital thermometer/hygrometer with a precision of $\pm 0.5^{\circ}\text{C}/\pm 4,0\% \text{ H}$ (Peak Meter, PM6508). The average temperature in each roost was used in subsequent analyses. Maps of the caves were obtained from the National Karst Registry (Peacock & Hempel, 1993, Grupo Espeleológico Anthros, 2023). We used the maps to assess the cave size and complexity of the roosts (see next section). We calculated the total surface area of the sites using the ImageJ software (Schneider et al., 2012). The human disturbance inside the roosts was evaluated through direct observations and surveys among speleologists and local residents (Deleva & Chaverri 2018).

We analyzed parameters considered important for bat occupancy (Oliveira et al., 2018; Phelps et al., 2016). The parameters include size (area in m^2 and length in m), depth, and altitude of the entrance. In addition, we developed a scale to assign a numeric value to qualitative variables such as the presence of water, the complexity of the cave, visitation, the size of entrance parts, and the presence of a dark zone (Table 1). We gathered data on several factors of human disturbance inside the roosts, such as household waste, graffiti, hunting, and killing of bats, as they were reported as relevant to bat distribution (Phelps et al., 2016). As we did not observe any of those factors, or in very isolated cases, we excluded them from the analysis. For disturbance inside the roosts, we took into account human visitation, as this was the only significant observed threat.

Table 1 Evaluating the factors, affecting bat distribution inside the roosts. The first column (F) represents the factors: water, complexity, entrance parts development, presence of dark zone and visitation frequency. The second column (Score) shows the assigned value which varies between 1 and 5. The last column is the description of each value.

Factor	Score	Description
Water	0	No water, roost is completely dry.
	1	One or several puddles, small drippings. Periodically wet.
	2	A small river, periodically drying out. Water present in the wet season.
	3	Water covers up to 50% of the surface. River is active all-year round.
	4	Water covers more than 50% of the surface of the cave. Water is present all-year round.
	5	Water cave (more than 80% lakes or river). Water is present all year round.
Complexity	0	Gallery or abyss, not complex. No chambers, only one gallery.
	1	One tunnel with a single chamber. Single big chamber with small chamber.
	2	A main gallery with one or two chambers.
	3	A main gallery with several chambers of the same size.
	4	Several entrances, main gallery with different sized chambers.
	5	Multiple levels, chambers of different sizes. More than one entrance.
Entrance parts	0	Small entrance (less than 50 cm diameter). Closed by vegetation. No vegetation.
	1	Single entrance less than 1 m in diameter. Humans need to crawl in/squeeze. Some vegetation.
	2	Single entrance up to 2 m. Vegetation and rock-dwelling animals.
	3	Entrance big enough for a person to walk in comfortably. Entrance open and surrounded by vegetation. Several entrances even if small in size.
	4	Entrance large, open and more than 2 m in height. Microhabitats around the entrance zone.
	5	Entrance is large (higher than the main height of the galleries of the cave) or more than 5 m in height. Entrance has an arch/overhang and vegetation.
Dark zone	0	Cave does not have a dark zone with stable conditions at all.
	1	Cave has a dark zone. Less than 20% of the cave is a dark zone with stable conditions
	2	Up to 40% of the total cave length is a dark zone with stable conditions.
	3	Up to 60% of the total cave length is a dark zone with stable conditions.
	4	Up to 80% of the total cave length is a dark zone with stable conditions.
	5	More than 80% of the cave is a dark zone with stable environmental conditions.
Visitation	0	Roost is visited sporadically every few years.
	1	Roost is visited once a year
	2	Roost is visited once in 3 to 6 months.
	3	Roost is visited monthly
	4	Roost is visited weekly
	5	Roost is visited daily

Functional diversity

We created a dataset with the functional attributes of the observed bat species 1) adult body mass, 2) forearm length, 3) head and body length, 4) foraging guild, and 5) diet. The guild

was based on the foraging strategy of bat species, as described in Kalko et al. (1996): (1) Uncluttered space/aerial insectivore; (2) background cluttered space/aerial insectivore; (3) highly cluttered space/aerial insectivore; (4) highly cluttered space/gleaning insectivore; (5) highly cluttered space/gleaning carnivore; (6) highly cluttered space/gleaning piscivore; (7) highly cluttered space/gleaning sanguivore, (8) highly cluttered space/gleaning frugivore; (9) highly cluttered space/gleaning nectarivore; and (10) highly cluttered space/gleaning omnivore. The diet was identified based on the existing database of functional traits of bats (Kalko 1996, Jones et al. 2009, Gessinger et al. 2019). We selected the functional diversity indices: 1) Rao's quadratic entropy (Rao) – measure of diversity within a set of entities based on their proportion of abundance and dissimilarity to each other, 2) Functional Richness (FRic) - the number of unique functional traits or strategies present in a community, regardless of the abundance of each strategy, 3) Functional Divergence (FDiv) - the degree to which different entities within a community or system differ in their functional traits, and 4) Functional Dissimilarity (FDis) - the degree of difference in functional traits or strategies between two entities. In addition, we included the standard diversity indices 5) species richness - the number of species for each site, 6) Shannon - the richness and evenness within a community, and 7) Simpson diversity index – the probability that two randomly chosen individuals from the community will belong to different species.

Analysis

As explanatory variables, we considered the parameters 1) water, 2) complexity, 3) entrance parts development, 4) dark zone and anthropogenic factors, 5) visitation frequency, 6) deforestation, and 7) urbanization (Table 1). We performed a principal component analysis (PCA) to eliminate correlations among the explanatory variables. We used eigenvalues greater than 1 to determine the number of components retained. The number of retained components was 3; the first component explained 64.43% of the variance, the second explained 23.92% and the third explained 9.20%. In the selection, we included loadings greater than 0.40 to determine which variables contributed to each principal component (Kassambara, 2017). The variables that quantify cave size, including complexity, length, and area, contributed primarily to the first principal component (PC1, Table 2); we used the results of PC1 to summarize cave size for subsequent analyses. Altitude and temperature contributed, in opposite directions (as expected), to the second principal component (PC2);

given the latter, we opted to leave altitude as an explanatory variable rather than use the results of PC2. We decided to use other relevant explanatory variables separately, including deforestation and visitation.

Table 2 Principal component analysis of the explanatory variables. The highlighted loadings are used in the analysis.

Parameter	PC 1	PC 2	PC 3
Deforestation	0.0412	0.3495	0.3897
Visitation	0.3089	-0.3234	0.1014
Water	0.3401	-0.2948	-0.0196
Complexity	0.4464	0.1291	-0.1215
Entrance parts	0.0717	-0.2472	-0.6707
Dark zone	0.3642	0.1815	0.3461
Length	0.4659	0.0958	-0.0795
Depth	0.2160	0.3699	-0.0206
Area	0.4086	0.0079	-0.1003
Altitude	-0.1348	0.4787	-0.2485
Temperature	-0.0369	-0.4476	0.4178

We tested the effect of the components on the dependent variables, i.e., the seven diversity indices. For this, we created a Generalized Linear Mixed-Effects Model, in which we used the principal component 1 (cave size), visitation, altitude, and deforestation as explanatory variables with the different functional and diversity indices as dependent variables. Following a beta distribution, we normalized the data using the *fitdist* function of the *fitdistplus* package (Package *fitdistrplus* version 1.1-6). Since the caves close to one another might have similar bat assemblages, we included geographical distance as a random variable in the model. We did a cluster analysis to check for similarities between the location of different roosts. We used a distance matrix based on geographical coordinates. Then, we did a hierarchical cluster analysis on dissimilarities with the *hclust* function of the "cluster" package in R studio (version 1.4.1717). The cluster group was used as the random variable to account for the effect of geographic similarities. We performed the functional diversity analysis with the *FDiversity* software (Casanoves et al. 2010).

Results

Bat diversity

We observed 20 bat species from the families Phyllostomidae, Emballonuridae, Natalidae and Mormoopidae. The most frequently observed species was *Carollia perspicillata* (25 sites). We identified representatives of almost all foraging guilds with the only exception being guild (6) piscivore (Figure 1). The most numerous bat colonies were in the Campanario cave, where we observed ca. 7600 individuals, followed by the Laguna Perdida (ca. 2000 individuals) and Corredores (ca. 1500 individuals) caves. All diversity and abundance results are presented in Supplementary Material 1.

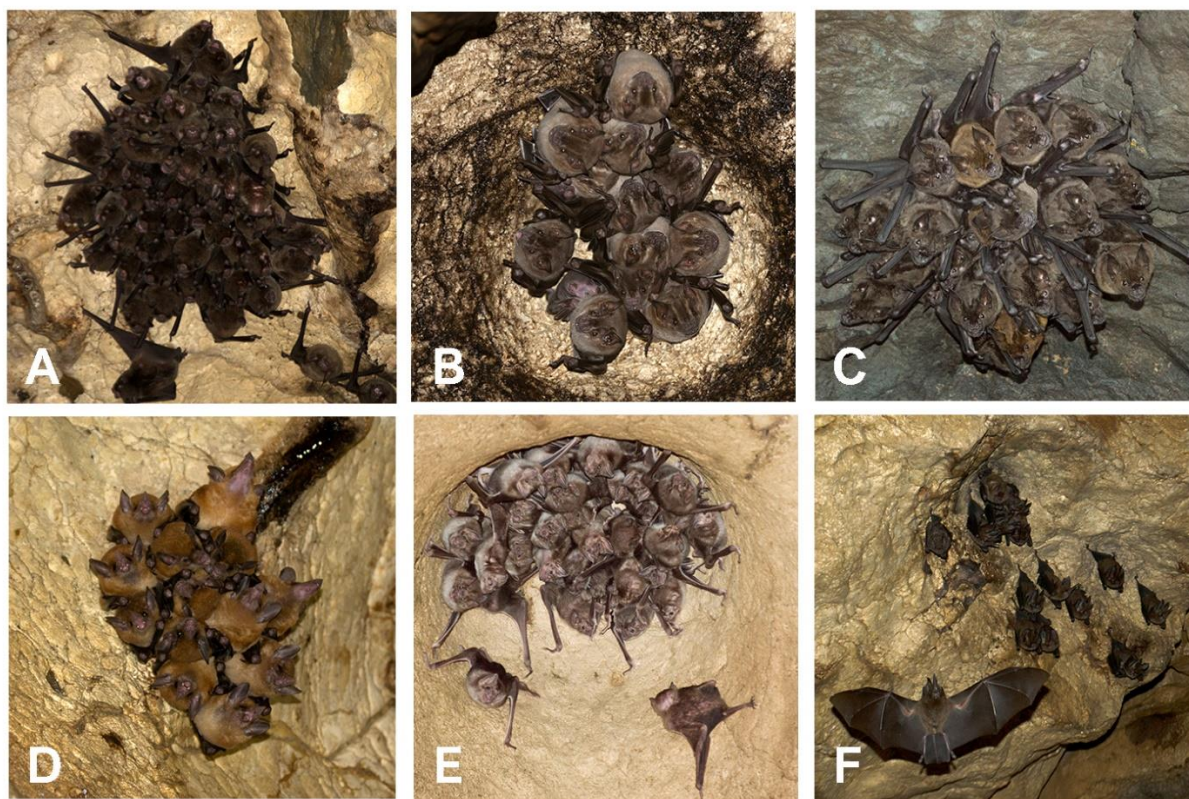


Figure 2 Cave roosting bat species found in the studied sites, representing different functional groups: A – *Pteronotus mesoamericanus* – cluttered space/aerial insectivore, B. *Artibeus jamaicensis* - highly cluttered space/gleaning frugivore, C - *Phyllostomus hastatus* - highly cluttered space/gleaning omnivore, D - *Lonchophylla robusta* - highly cluttered space/gleaning nectarivore, E - *Desmodus rotundus*- highly cluttered space/gleaning sanguivore, and F *Lonchorhina aurita* - Uncluttered space/aerial insectivore

Caves and environmental factors

We surveyed 44 roosts – 40 caves and four tunnels. We observed bats in 38 of them. The cave length varied between 5 m (Cueva 5) and 1624 m (Corredores). The deepest caves were Serpiente Dormida (-157.27), Carma (-97.84), and the Bananal system (-92 m). The Emus cave had the highest positive development (difference between entrance and the end of the cave) of 22.86 m. The deforestation percentage ranged between 3 and 55, with an average of 15.3%. The most deforested areas were observed near the Abrojo village (Serpiente Dormida, Miramar de Abrojo), where caves were located close to the populated areas and on agricultural land. The caves located in the most pristine areas were in the Osa Peninsula (Campanario, San Pedrillo), around the town of Uvita (Arco), and near the Corredor district in the town of Neily (Carma). The caves most affected by visitation were the marine caves at the Ventanas and Arcos beaches, followed by the Corredores and Emus cave systems. The least affected by visitation were the vertical shafts in the Quebrada Roman valley near Rio Claro, which were discovered by us and thus never visited before.

The average mean temperature in the observed roosts was 25.8° C. There were two caves diverging from the rest of the sites with an average temperature above 30° C – the Tortuga (30.5°C) and Campanario (36°C) caves. Those caves were similar in their morphology and origin - both were marine caves with a similar size located on the Pacific coast. The two caves harbor maternity colonies of bats from the genus *Pteronotus*. The humidity inside the roosts had minimal variation (less than 15%) and averaged 92.9%. The dimensions and characteristics of each cave are presented in Supplementary Material 1.

Based on the distance matrix, we identified eight different cluster groups (Figure 2). The most numerous group (Group 1) contained 19 caves, located in the Quebrada Roman valley near the Rio Claro karstic area (Ulloa et al., 2011). The second cluster contained 12 caves, located in the Rio Corredor and Abrojo karst area. The third cluster combined four volcanic and marine caves, located on the shore of the Pacific Ocean in the Osa Peninsula. The fourth cluster with three sites included two volcanic caves and an artificial gold mine, located in Isla Violin and the Uvita area of the Pacific Ocean. The fifth group contained the single known underground roost located in the internal parts of the Osa Peninsula. The sixth group was represented by two artificial tunnels, located at the Rio Terraba shore. The seventh group

has two caves in the Piedras Blancas National Park. The last cluster group had a single cave, located north of Rio Claro town (Figure 2).

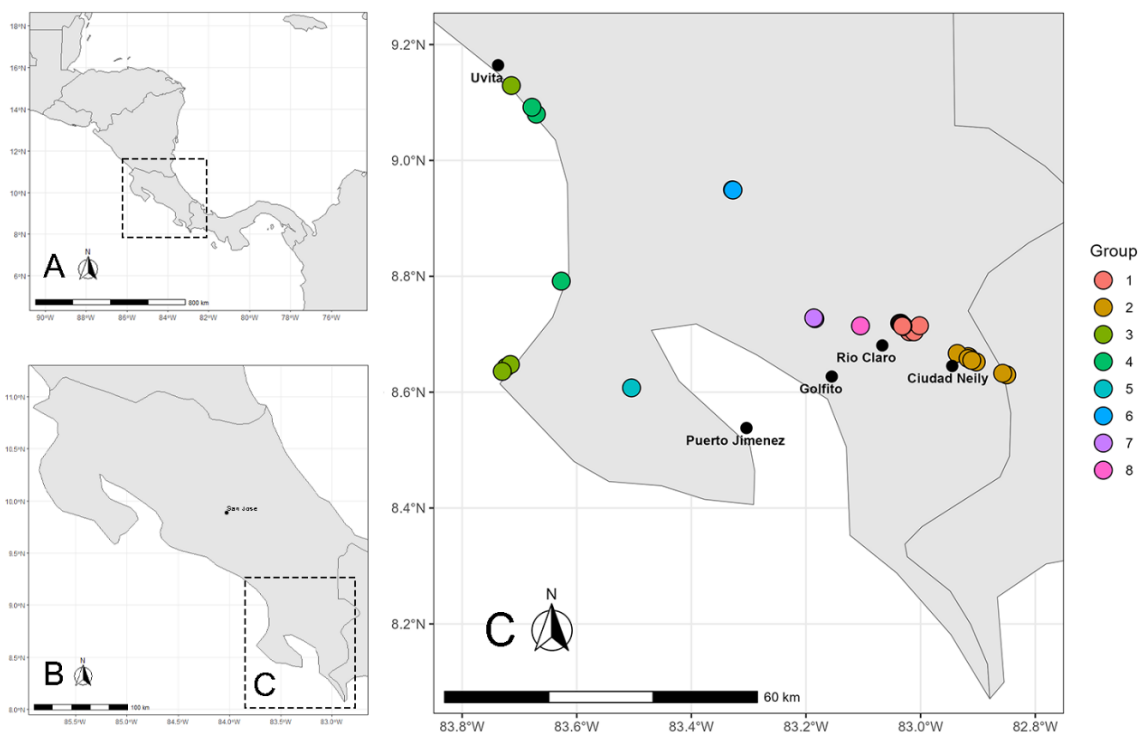


Figure 3 Map of the region (A), Costa Rica (B), the Brunca Region (C) and the research sites in this study. The zoomed area (C) shows the field research sites. The sites are colored according to the cluster groups in which they are distributed, based on the distance matrix.

Diversity indices

The caves exhibiting the highest species richness were Corredores ($n = 8$), Emus ($n = 7$), and Laguna Perdida ($n = 7$), followed by Bombasa ($n = 6$), Alma, Gran Galeria, Gran Madre, Ice 2, Miramar, and San Pedrillo, each with 5 species. The roosts with the highest scores on the Rao entropy index included Arelis, San Pedrillo, Gran Galeria, and Laguna Perdida. Functional richness values were most significant in Cinco Milliones, Laguna Perdida, Corredores, Los Sueños, and Arelis. The highest values for functional divergence were observed in the roosts Ice 2, Arbol Caido, Sapo Gordo, Bananal, and Mono Titi. Functional dissimilarity was most prominent in the roosts Arelis, San Pedrillo, Gran Galeria, Ice 2, and Cataratas San Josecito. Shannon index values peaked in Arelis, Gran Galeria, Cinco

Millones, Laguna Perdida, and Corredores. The Simpson index had the highest values in the roosts Arelis, Gran Galeria, Cinco Millones, Corredores, and Ice 2 (Supplementary material 1).

Cave size was significantly, and positively, correlated with both functional divergence and species richness (Figure 3, Table 3). Visitation was positively correlated to the Rao entropy index and the Shannon index. Species richness and the Shannon index showed a negative correlation with altitude. Surprisingly, our study did not find that deforestation significantly influenced any diversity indices (Figure 3).

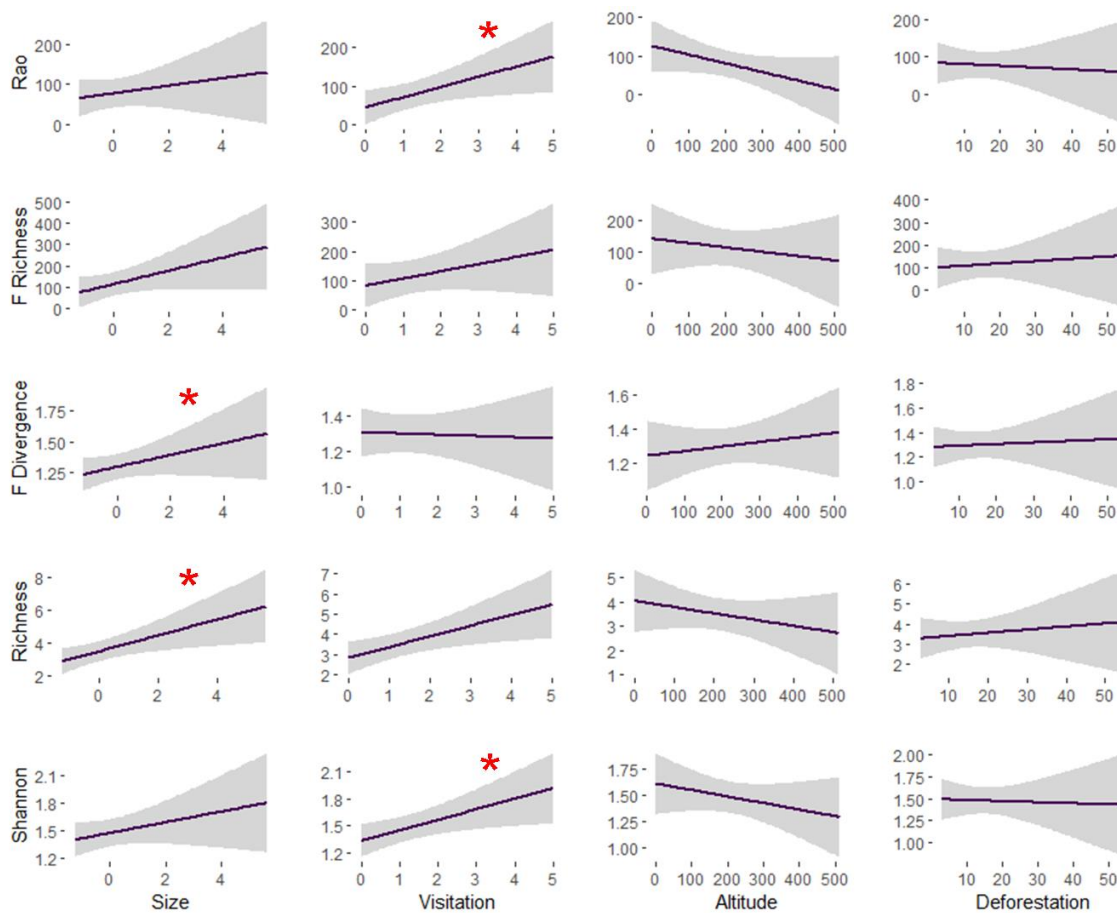


Figure 2 Correlations between functional diversity indices and variables. The diversity indices are Rao's quadratic entropy (Rao), Functional Richness (F Richness), Functional Divergence (F Divergence), Species Richness (Richness) and the Shannon diversity Index. The values marked with * are statistically significant.

Table 3 Functional diversity and variables. The first column (Var), represents the tested variables cave size, visitation, altitude and deforestation. The second column (Index) states the diversity indices - Rao's quadratic entropy (Rao), Functional Richness (F Richness), Functional Divergence (F Divergence), Species Richness (Richness) and the Shannon diversity Index (Shannon). The third column represents the estimated value of the index, the fourth column (SE) represents the standard error. The fifth column presents the t-value, and the last column is the probability value. The highlighted rows exhibit values of statistical significance.

Var	Index	Estimate	SE	t value	p-value
Cave size	Rao	-0.0692	0.23455	-0.295	0.768
	F Richness	0.25183	0.30895	0.815	0.415
	F divergence	0.10311	0.04352	2.369	0.01782
	Richness	0.1366	0.07617	1.793	0.0729
	Shannon	0.02198	0.05174	0.425	0.671
Visitation	Rao	0.53429	0.27838	1.919	0.055
	F Richness	0.20954	0.36669	0.571	0.568
	F divergence	-0.0068	0.04804	-0.141	0.88781
	Richness	0.16358	0.10027	1.631	0.1028
	Shannon	0.11706	0.06141	1.906	0.0566
Altitude	Rao	-0.2479	0.27694	-0.895	0.371
	F Richness	-0.0543	0.36479	-0.149	0.882
	F divergence	0.01192	0.05327	0.224	0.8229
	Richness	-0.0189	0.10393	-0.182	0.8556
	Shannon	0.01011	0.06109	0.165	0.8686
Deforestation	Rao	0.06585	0.23196	0.284	0.777
	F Richness	0.17392	0.30555	0.569	0.569
	F divergence	-0.0296	0.04011	-0.737	0.46118
	Richness	0.05007	0.08126	0.616	0.5378
	Shannon	-0.0037	0.05117	-0.073	0.9416

Discussion

This study represents a comprehensive effort to characterize how a number of environmental and anthropogenic parameters affect bat functional diversity in a large number of caves in southern Costa Rica. Our aim was to thoroughly evaluate which roosts in the studied area are hosting more diverse and significant bat communities in order to make better-informed decisions regarding the conservation and sustainable use of caves. Our results showed the importance of certain roosts not necessarily being represented by species number and abundance. Similar to previous studies (Vargas-Mena et al., 2020) our results confirmed the prediction that the cave size is significantly positively correlated with phylogenetic (i.e.,

species richness) and functional diversity. However, a large part of the results were not correlated to either of the explanatory variables. Therefore, our data and analyses seem to provide a somewhat incomplete picture of the factors that explain which bat species occupy which cave roosts.

In our prior study (Deleva & Chaverri, 2018), we assessed roost diversity using the Bat Caves Vulnerability Index (Tanalgo et al., 2018). The 11 roosts with the highest biotic potential were identified as significant for bat conservation, prompting suggested conservation actions. However, our current analysis indicates a need to expand this list to include roosts with the highest functional diversity, such as Arelis, San Pedrillo, Cinco Milliones, and Gran Galeria. Although smaller and hosting fewer bat colonies, these caves consistently scored high on multiple functional diversity indices and harbor species not well represented elsewhere. While our previous study emphasized prioritizing Arelis and San Pedrillo, it overlooked Cinco Milliones and Gran Galeria, the latter being used for touristic activities. Future initiatives should recognize the importance of Gran Galeria in bat conservation efforts.

Contrary to our initial expectation, we found no negative correlation between disturbance in the form of visitation rates and bat diversity within caves; rather, a positive correlation was observed with the selected diversity indices, specifically Rao and Shannon. This unexpected outcome may be explained by the fact that the region's largest and most complex caves are sheltering large and diverse bat colonies. Remarkably, these same roosts are highly attractive for touristic activities due to their size and complexity.

Our study did not find a significant impact of deforestation on functional diversity. A number of studies have shown a negative correlation between deforestation, habitat fragmentation, and functional diversity (García-Morales et al., 2016; Farneda et al., 2020). Other studies however, suggest that habitat fragmentation may actually support higher bat diversity. This could be because fragmented habitats create complex mosaics that offer greater habitat and resource diversity for bats compared to large continuous mature forests (BakwoFils et al., 2021; Presley et al., 2018). Our results align with the latter perspective. Additionally, most studies on functional diversity have relied on capturing foraging bats as a method to assess bat diversity. In contrast, our study observes bats in their roosts. The availability of roosts such as caves may be an important limiting factor for some bat species, particularly

mormoopids (Díaz-B et al., 2023). As our study design did not allow us to explore the diversity of habitats around the roosts and their potential influence on bat communities (Martínez-Ferreira et al., 2020), further research should delve into these aspects to enhance our understanding of bat diversity and functional roles.

The absence of discernible variations in outcomes with regard to altitude, temperature, and humidity may be accounted for by the limited geographical range of the roosts or the predominant placement of caves at a uniform altitude, thereby resulting in comparable microclimatic conditions and surrounding habitats near the roosts. Notably, littoral caves Tortuga and Campanario stood out as exceptions, where the average roost temperatures were 30.5°C and 36.0°C, respectively, reaching even higher values in close proximity to the bat colonies. Despite the observed general formation of mixed-species colonies by *Pteronotus* sp. within roosts, consistent with previous studies (Barros & Bernard, 2023), we note distinctive conditions specifically within maternity roosts. We can regard the two roosts as “neotropical hot caves” (Ladle et al., 2012). The presence of significant bat colonies appeared to influence the microclimatic conditions, and notably – the temperature and the presence of ammonia and other gases in the cave atmosphere due to the large accumulations of guano. We did not observe other bat species in the maternity chambers. It is plausible to assume that the presence of *Pteronotus* maternity colonies may act as a deterrent, preventing other bat species from occupying the roost.

Our study is deemed successful in its primary aim of addressing and identifying cryptic roosts that merit conservation attention, as previous studies were focused primarily on the species richness and abundance. While we have made significant progress in clarifying the factors influencing the distribution of bats among roosts, lingering questions warrant further exploration. Specifically, studies have yet to unravel additional factors that might influence the distribution of bats among roosts, with a particular focus on interspecific relations and symbiotic interactions within bat communities (Barros & Bernard, 2023). Moreover, the impact of seasonal variations and migrations on population dynamics remains an unanswered aspect that requires consideration. In the field of conservation, our findings underscore the necessity of adopting an individualized approach for each roost. Acknowledging each site's unique characteristics and ecological nuances is important for formulating effective

conservation strategies tailored to the specific needs and challenges posed by diverse roost environments.

Conclusion

In conclusion, we confirm that the functional diversity indices proved helpful in determining priority sites for conservation that were previously overlooked based only on species richness and abundance. While cave size was correlated with diversity, we found that smaller sites could be considered equally important for bat conservation. We recommend that in the future, we should stop focusing on species diversity and abundance alone and consistently include functional diversity in projects involving conservation decisions.

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Supplementary material

Dataset containing site names, location, diversity indices, environmental parameters, presence, and abundance of bat species. We present the low-resolution coordinates of the roosts in Suppl. material 1, following the recommendations of the Best Practices for Generalizing Sensitive Species Occurrence Data (Chapman, 2020). The exact locations will be made available upon request.

Available online at <https://figshare.com/s/7a2495af676c2bf6d2d1>

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Chapter 5. Exploring the nutrient input of cave-dwelling bats to the surface ecosystems as a potential ecosystem service

The manuscript is not published yet.

Abstract

Bat guano is rich in nutrients such as nitrogen (N) and phosphorus (P), which are essential for plant growth. Bat guano is the base of the food chains in subterranean ecosystems. In caves with subterranean rivers, the water could transfer guano deposits to surface aquatic ecosystems. With large aggregations of bats in caves, their nutrient contribution could be significant, especially for highly weathered, nutrient-poor tropical soils. We investigated the contribution of cave-roosting bats to nutrient input to aquatic ecosystems in caves with subterranean streams. To make our results globally relevant, we sampled sites in two different countries - Costa Rica and Bulgaria. We measured the concentrations of N in the form of nitrates, nitrites, and ammonium, and P in the form of phosphates in water before and after contact with the guano source and again at the contact point with the stream surface. To further test if the nutrients from bat guano are assimilated by plants, we compared the $\delta^{15}\text{N}$ isotopic signature of guano with that of moss (Bryophyta) and other types of vegetation growing near the river banks, in distances of up to 128 m downstream from the cave entrances on selected sites. Our results show that water exiting caves with large bat colonies has higher concentration of N and P than the same water before contact with the bat guano. Furthermore, we showed that the size of bat colony was positively correlated with the nutrient concentration in the water. We further established that the nutrients from guano are assimilated by vegetation in increasing distance from the cave entrances. Our study shows that cave bats provide an additional and as yet ignored ecosystem service, namely providing nutrients to surface aquatic ecosystems. These results could be used as an argument for bat and cave conservation.

Introduction

Nutrients such as nitrogen (N) and phosphorus (P) are essential for life on Earth, as both elements constitute nucleic acids, proteins, and other universally occurring molecules in organisms (Metzler, 2003). However, in many ecosystems, N and P are scarce, limiting the growth and reproduction of plants and influencing the primary production of both freshwater and terrestrial ecosystems (Elser et al., 2007). Several tropical ecosystems, for example, have undisturbed soils that are strongly nutrient-limited, particularly in P (Elser et al., 2007; Vitousek, 1984). Additionally, many tropical freshwater habitats experience either N (Flecker et al., 2002) or P (Pringle & Triska, 1991) limitation. The simultaneous addition of N and P can have strong positive synergistic effects on terrestrial and aquatic net primary production (Elser et al., 2007), potentially influencing species composition and biodiversity by altering the competitiveness of different species (Glover, 2021).

Karstic caves, formed by the activity of running water (Gunn, 2004), have streams that transport nutrients such as N and P before their utilization. These nutrients enter streams from upstream runoff or atmospheric inputs and are incorporated into organic forms through the decomposition of organic matter and excretion (Allan & Castillo, 2007). Primary producers, like periphyton and aquatic plants, rely on surrounding water to supply the necessary nutrients. Bacteria and fungi, while able to receive nutrients from the substrate, also depend on water as a source of nutrients. In some systems, animals, such as birds (Ligeza & Smal, 2003) and herbivorous mammals (Wolf et al., 2013), may contribute nutrients or facilitate nutrient flux within their habitat. The importance of seabirds in nutrient contribution is evident in some places, with their sudden removal shown to alter plant community structure (Fukami et al., 2006). Nitrogen input by whales and seals may also positively influence marine ecosystem primary production (Smith et al., 2013). Another group of mammals with significant potential nutrient contributions to ecosystems is bats.

Bats play various ecological roles, such as controlling insect pests, pollinating plant crops, and dispersing seeds to foster the recovery of degraded tropical ecosystems (Kasso & Balakrishnan, 2013; Kunz et al., 2011). However, bats may also contribute nutrients to terrestrial and aquatic habitats surrounding their roost sites (Culver & Pipan, 2009). Bat guano, with its high concentrations of N and P, serves as an excellent fertilizer (Shetty et al.,

2013; Studier et al., 1994). Studies indicate that single bats or bat colonies not only provide a significant input of N into the soil, but also incorporate these nutrients into plant biomass (Schöner et al., 2017; Voigt et al., 2015).

Some of the largest bat concentrations are found in caves, with some caves harboring colonies of several thousand to millions of bats (Hristov et al., 2010; McFarlane et al., 2015). The N nitrogen accumulation in caves with the largest bat colonies was estimated to reach up to 51146 kg y⁻¹ (Lundberg et al., 2022). In caverns with underground streams, water constantly removes guano deposits from the cave floor into adjacent aquatic systems, and could transport significant amounts of nutrients, especially when very large bat colonies are present (Lundberg et al., 2022).

This study aims to explore the nutrient input of bat guano into surface ecosystems as a potential ecosystem service. Our hypothesis is that bat guano will contribute to the nutrient content of water emerging from caves and this effect will increase with the size of the bat colonies. Specifically, we predict higher concentrations of N and P in underground and aboveground rivers after water has had contact with guano deposits from bat colonies. Furthermore, we hypothesized that river vegetation close to cave entrances with water running out of the cave effectively assimilates nutrients from bat guano. As bat guano is naturally enriched in the heavier isotope of N - $\delta^{15}\text{N}$ (Studier et al., 1994), we used it as a natural marker to trace for bat-derived nutrient influx. We predicted that plants located closer to cave entrances would be enriched with $\delta^{15}\text{N}$ relative to $\delta^{14}\text{N}$ stable isotopes, compared to plants growing further downstream, suggesting guano assimilation (Grafe et al., 2011; Voigt et al., 2015). This study will highlight the value of underground bat colonies for the surface ecosystems adjacent to cave entrances.

Materials and methods

Study sites and bat surveys

We sampled caves and tunnels in Costa Rica and Bulgaria with streams flowing underground and emerging at the surface as springs. All of the sites hosted bat colonies of at least 300 individuals. We specifically selected sites where prior exploration and surveys had been conducted (Deleva et al., 2023). Cave maps were obtained from local speleological databases (BFSp, 2023; Grupo Espeleológico Anthros, 2023). The diversity and abundance of bats were assessed during each visit using standard methodology for surveying and monitoring underground bat roosts (Kunz et al., 2009, Deleva & Chaverri, 2018, Deleva 2023). We measured the distance between sampling points on the cave maps and the area of guano deposits, using the software imageJ (Schneider et al., 2012).

Sampling

For each site, we established three water sampling points. The first sampling point was positioned within the roost, at the origin of the subterranean river, and in an area without any presence of bats (Figure 1, Point 1). The second sampling point was set up downstream from the place of the largest guano accumulation, or at the location of the bat colony (Figure 1, Point 2). The third sampling point was situated at the cave's entrance, where the underground river surfaces (Figure 1, Point 3). While we collected additional samples from locations potentially indicative of non-guano derived N and P with surface origin such as water dripping from stalactites, other underground streams, or springs—we did not include them in the current statistical analysis. We used 50 ml acid-washed polypropylene tubes to collect water samples slightly below the water's surface, and away from the stream's shore, thereby minimizing disturbance to sediments and organic materials. The collection process began at the point closest to the cave entrance, progressing upstream to avoid contamination. At each point, we gathered two 45 ml samples, each filtered through a 0.22 μm membrane filter (Membrane Solutions, nylon syringe filter). For preservation, water samples were kept on ice during transport and subsequently frozen at -20°C until analysis.

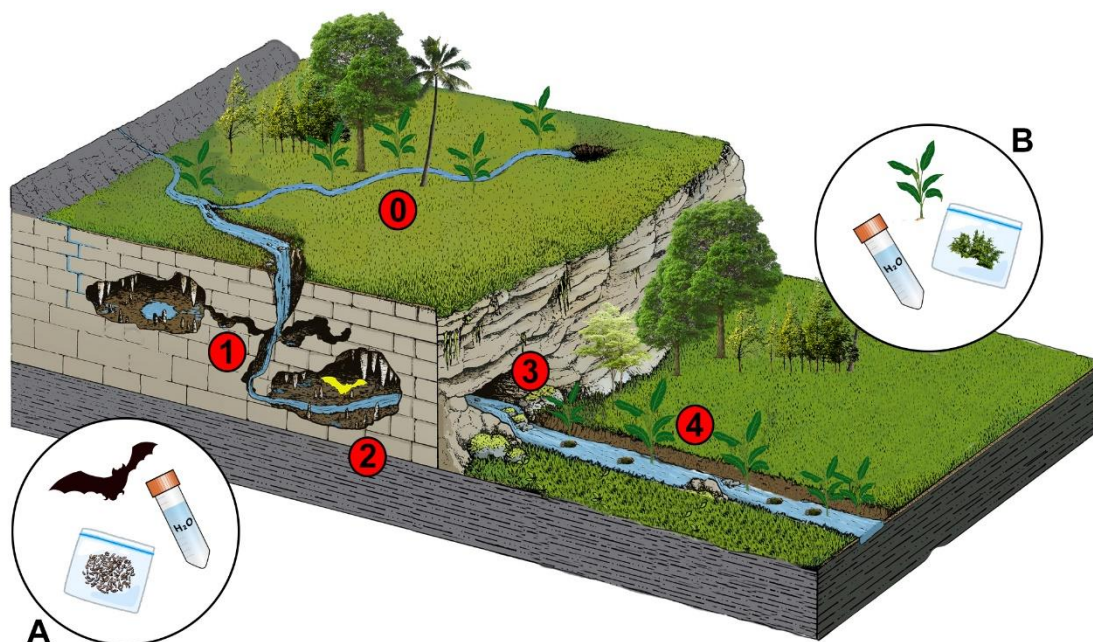


Figure 1 Schematic representation of a typical research site along with its sampling locations: Point 0 – control, Point 1 - clean water, Point 2 - water carrying dissolved bat guano, Point 3 - surface, Point 4 – sampling points for vegetation and moss, taken within distance of the cave entrance. The collected samples underground (A) included water (Points 1, 2 and 3), and bat guano (Point 2). On the surface (B) we collected samples of water and plant tissue (Points 0 and 4).

Moss was collected from rocks completely covered in stream water, emerging from the cave (Figure 1, Point 3). We conducted initial visual identifications of moss, using field guide (Gladstein et al., 2001) followed by the collection of voucher specimens, which were preserved for subsequent detailed taxonomic verification. The identification of moss species in the field posed significant challenges; however, the risk of collecting erroneous samples was mitigated through site-specific isotopic signature analyses. At one research site, we collected samples from moss submerged in water and above water level for comparative analysis. Vegetation was collected in the form of the second fully developed leaf from *Heliconia latispatha* plants, growing on the banks of the stream whose roots were either completely submerged or in full contact with the water (Figure 1, Point 4). Heliconia and moss samples were taken where available in a geometric progression (meters 1, 8, 16, 32, 64 etc.), extending up to 128 meters downstream from the cave entrance or until the stream joined a larger river or disappears underground. We collected composite samples of bat guano for each cave for stable isotope analysis. In addition, on each site we collected control samples of moss and vegetation from places with similar environmental conditions, but

without contact with the sampled rivers (Figure 1, Point 0). We used satellite images depicting agricultural use and urbanization in a radius of 5 km around the cave entrances with a focus on sinkholes and karstic streams flowing through agricultural land that could provide water to the underground rivers of our research sites and thus pollute the control (Figure 1 Point 1) sample (Manenti et al., 2021).

Laboratory analyses

Reactive nitrogen, in the form of nitrate-N $\text{NO}_3\text{-N}$, nitrite $\text{NO}_2\text{-N}$, and ammonium $\text{NH}_4\text{-N}$ and phosphorus in the form of phosphate PO_4^- were measured directly via a copperized cadmium column (Lachat QuickChem 8500 method 12-107-04-1-B). The water samples from Costa Rica were analyzed at the Chemical Oceanography Laboratory at the Center for Research in Marine Sciences and Limnology at the University of Costa Rica (CIMAR, UCR). The samples from Bulgaria were analyzed at the certified private laboratory LEMNA. Both laboratories used the same standard methodology and equipment.

Samples of guano, moss, and vegetation were dried on silica gel, ground into powder using mortar and pestle and weighed into 0.7 mg tin capsules. The samples were sent to the specialized isotope laboratory in the Centre for Stable Isotope Research and Analysis at the Technical University, Munich, Germany for analysis with mass spectrometry. In the laboratory, the samples were tested for total N, total C, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$. The isotope, used to trace bat guano into plant tissue and used for for the statistical analysis was $\delta^{15}\text{N}$.

Statistical analysis

We analyzed the concentrations ($\mu\text{mol l}^{-1}$) of nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), and phosphate (PO_4^-) in water. The values for each element were compared between the sampling points 1, 2 and 3 (Figure 1). We considered sampling point 1 as our control group. To aid the statistical analysis, we divided the amount of guano entering the underground streams, based on the bat colony size into three categories, depending on the number of individuals permanently roosting directly above the underground river: “category 1” - low (less than 500 individuals), “category 2” - middle (between 500 and 1000 individuals), and “category 3” - high (more than 1000 individuals). We applied a General Linear Model (GLM), with nutrient concentration as the dependent variable and colony size and sampling positions as explanatory variables. We used Analysis of Variance (ANOVA)

to identify significant differences in nutrient concentrations across the sampling points, followed by post-hoc test. Results were deemed significant at p-values lower than 0.05.

The contribution of bat-derived N to total foliar N in moss was estimated using a Linear Model, with $\delta^{15}\text{N}$ values as the dependent variable and distance from the cave entrance as an explanatory variable. We used the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of plant and moss tissue to visualize and create descriptive statistics. Data analysis and visualization were conducted using R Software (R Studio Team, 2023). All data are presented in the Supplementary material 1.

In addition, we calculated a two-source isotopic mixing model of $\delta^{15}\text{N}$, drawing on methodologies from similar studies (Cecchetti et al., 2020; Gaiotto et al., 2022, Mariotti et al., 1981, Phillips et al., 2014). The mixing model calculates guano-derived nitrogen percentages as

$$\%GDN = [(SAMPLE - CONTROL)/(GUANO - CONTROL)] \times 100$$

where % GDN is the percentage of guano-derived nitrogen in a given sample, SAMPLE is the observed $\delta^{15}\text{N}$ of the sample, GUANO is the guano sample for the site, which represents 100% of the mix, CONTROL is the $\delta^{15}\text{N}$ of the control sample, which does not have nitrogen input from bat guano (representing 0%). The model assumes that isotopic fractionation associated with N uptake is negligible. We also performed the mixing models in the MixSIAR package in R (Stock & Semmens, 2016). The two-source mixing model that we calculated however, was possibly biased due to the subpar selection of the control samples and thus we further focused solely on the direct examination and analysis of the $\delta^{15}\text{N}$ values to ensure objective interpretation of the results.

Results

Between 2018 and 2022, we collected and analyzed 54 water, 83 plant, and 13 guano samples from 15 research sites. In Costa Rica, we studied eight subterranean sites, comprising five natural caves and three artificial tunnels (Table 1). The artificial tunnels investigated were Aguacate, Arenal, and Colón, while the natural caves studied were Corredores, Emus, Huecos, Los Sueños, and Venado (Figure 2A). In Bulgaria, we investigated six natural cave systems: Devetashkata, Gargina Dupka, Yurushka Maara, Kalenskata, Parnitsite, and

Sedlarkata (Figure 2B). Two of the research sites – Parnitsite and Venado, had complex morphology with more than one underground stream. Water from these sites were sampled separately.

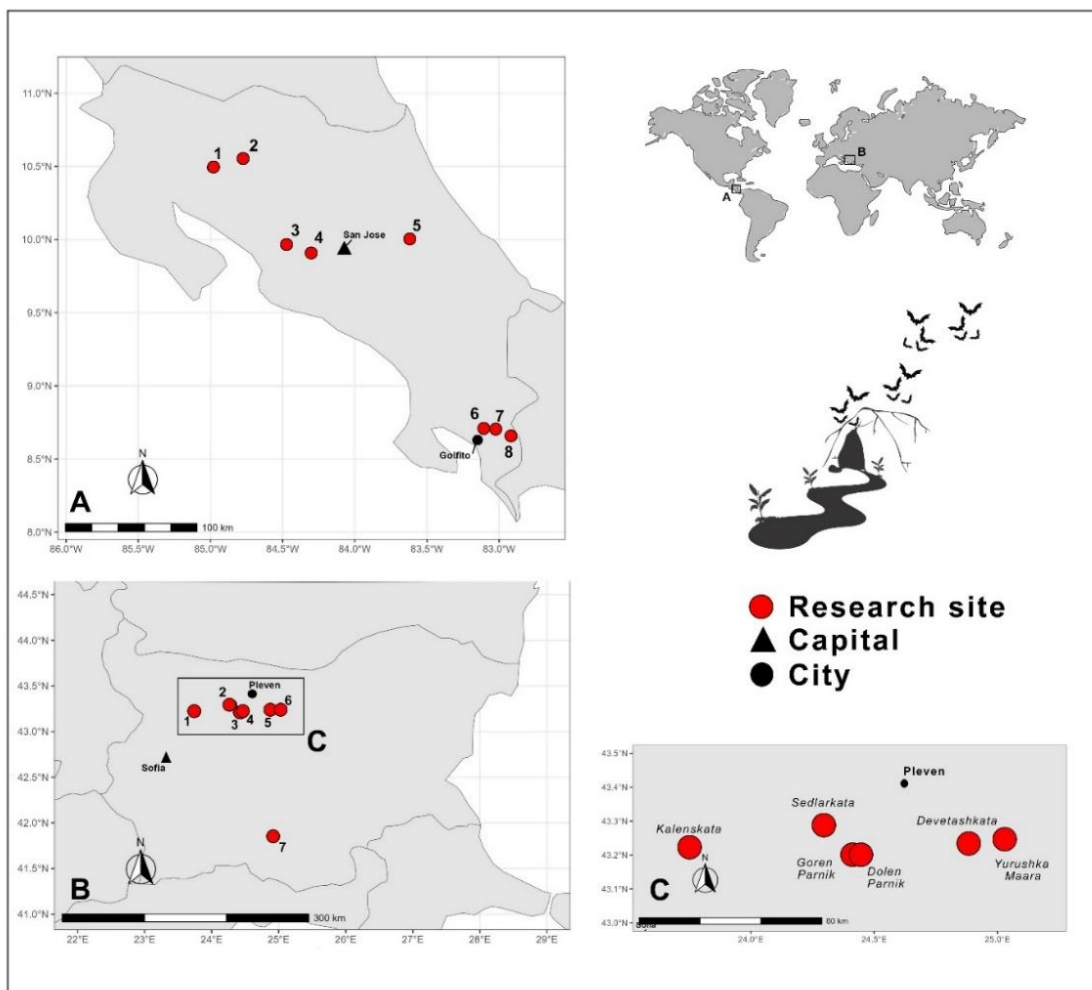


Figure 2 Locations of sampling sites during our study. The sites in Costa Rica (A) are: 1. Arenal, 2. Venado (Gabinarraca), 3. Aguacate, 4. Mina Colón, 5. Huecos, 6. Los Sueños, 7. Emus, 8. Corredores. The sites in Bulgaria (B) are 1. Kalenskata, 2. Sedlarkata 3. Goren Parnik, 4. Dolen Parnik, 5. Devetashkata, 6. Yurushka Maara, 7. Gargina Dupka. Section C represents an area in Bulgaria with a high density of research sites.

Table 1 Research sites, location, number of samples for each site and colony size of bats. The first column (Site) represents the site name and country. The second column (Location) is the low resolution location of the research sites. The third and fourth columns (Samples) show the number of water and isotope samples from each site, used in the statistical analysis. The fifth column (№ of individuals - cave) represents the colony size in the whole roost. The sixth column (№ of individuals - water) is the number of bats, roosting directly above the water. The seventh column (Guano category) refers to the assigned category of guano or the amount, transported by water in the roost. The last column refers to notes on the roost.

	Site	Location		Samples		№ of individuals		Guano category	Notes
		N	E	Water	Isotopes	cave	water		
Bulgaria	Devetashkata peshtera	43.23	24.89	4	-	95000	94000	3	Hibernation roost.
	Gargina dupka	41.85	24.93	5	11	11500	5700	3	
	Kalenska	43.22	23.75	-	9	1200	1000	3	
	Parnitsite dolen parnik	43.20	24.42	3	-	58000	55000	3	Cave system with two separate bat colonies.
	Parnitsite goren parnik	43.20	24.43	3	-	4500	4000	3	
	Sedlarkata	43.29	24.30	3	7	300	300	1	
	Yurushka Maara	43.25	25.03	-	7	6600	600	2	
Costa Rica	Aguacate*	9.97	-84.47	3	10	1200	600	2	
	Arenal*	10.49	-84.99	6	9	3500	3000	3	Samples from <i>Heliconia latispatha</i> .
	Corredores	8.66	-82.91	3	-	1600	200	1	
	Emus	8.70	-83.02	3	-	1200	600	2	
	Huecos	10.01	-83.62	3	6	1300	700	2	
	Los Sueños	8.71	-83.10	6	8	700	600	2	
	Mina Colón*	9.91	-84.30	3	14	800	800	2	
	Venado left (waterfall)	10.56	-84.77	3	15	500	200	1	Two different rivers, merging near the cave entrance.
	Venado main gallery	10.56	-84.77	6		2000	850	2	

* Tunnels.

Water

Our results across all sites consistently showed elevated levels of all nutrients between sampling positions 1 and 3. However, there were no significant differences in nutrient concentrations before and after bat colonies when data from all bat colony sizes were combined (Figure 3). The concentrations did not yield statistically significant results, indicating that sampling position alone does not account for major differences in nutrient concentrations (Table 2).

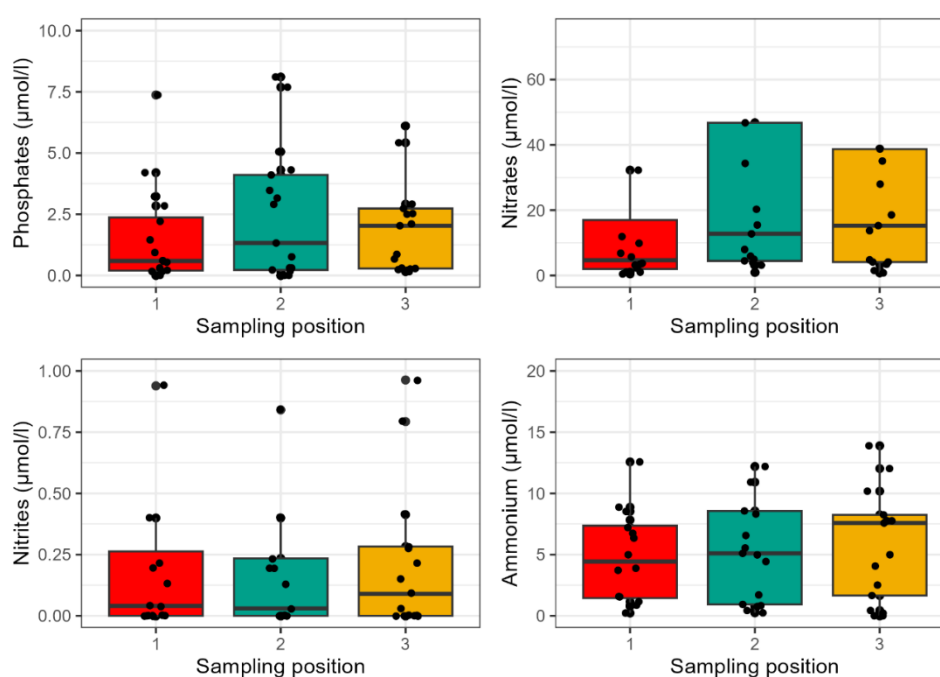


Figure 3 Values of the different studied nutrients ($\mu\text{mol/l}$) distributed among sampling positions: 1: before the bat colonies, 2: inside the cave and after the bat colony, 3: at the exit of the cave).

Table 2 ANOVA results for the impact of sampling position on concentrations of various nutrients in water samples. The first column refers to the analyzed nutrient. The second column (Df) is the degrees of freedom. The third column Sum Sq is the Sum of Squares. The fourth column (Mean Sq) is the Mean Square. The fifth column (f value) measures the significance of the model. The last column (p-value) indicates the statistical significance.

Variable	Df	Sum Sq	Mean Sq	f value	p-value
Phosphates (PO_4^-)	2	8.7	4.360	0.633	0.535
Nitrates (NO_3^-)	2	86982	43491	0.354	0.703
Nitrites (NO_2^-)	2	0.164	0.082	0.126	0.882
Ammonium (NH_4^+)	2	1036	517.8	1.09	0.344

The General Linear Model indicated that the guano category significantly impacts phosphate and nitrate concentrations, with p-values of 0.003 and 0.001, respectively. This suggests that varying amounts of guano can affect the levels of PO_4 and NO_3 . For the phosphates, the p-value for the interaction was relatively low (Table 3), and post-hoc tests revealed a significant combined effect of sampling position and guano category (Table 4), so we will present these results. Specifically, the post-hoc test reveals a significant increase in phosphate concentrations at sampling points 2 and 3 compared to sampling point 1 in caves with the highest guano concentration - category 3 ($p < 0.05$, Figure 4A, Table 4). For nitrates, significant differences were observed between medium (category 2) and high (category 3) guano concentrations ($p = 0.0016$, Figure 4B, Table 4). The analysis of the other nutrients did not yield statistically significant results, as detailed in Table 2.

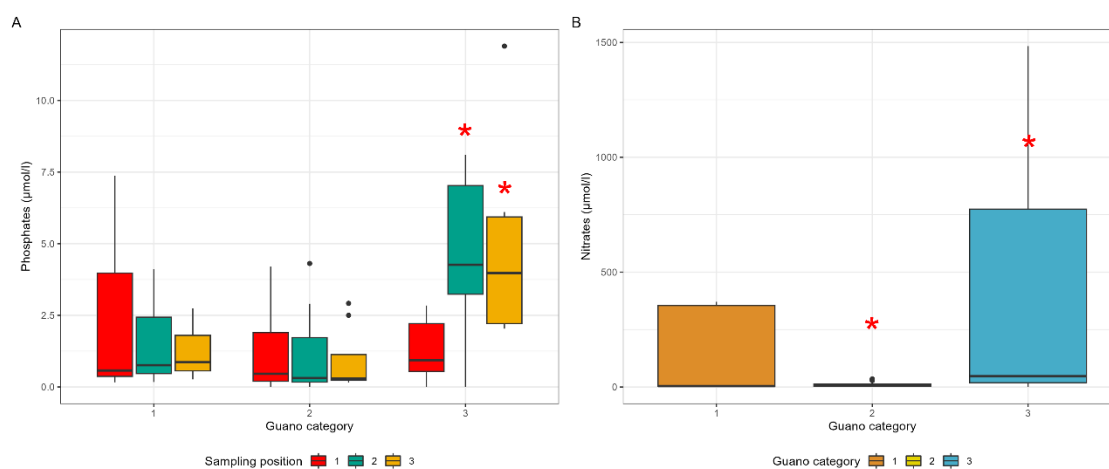


Figure 4 Concentrations of nutrients distributed among guano categories. A: Concentration of phosphates distributed among sampling positions: 1: before the bat colonies, 2: inside the cave and after the bat colony, 3: at the exit of the cave. B: Concentration of nitrates among guano categories. The amount of guano refers to the assigned category of guano, present at the site: 1: low, 2: middle, 3: high. The values, marked with a red * have statistically significant differences from the other categories.

Table 3 ANOVA results for the linear model for the impact of guano category on concentrations of various nutrients in water samples. The first column (Nt) refers to the analyzed nutrient. The second column (Variable) refers to the variable – Sampling position, Guano category (amount of guano) and relationship between the two variables. The third column (Df) is the degree of freedom. The fourth column Sum Sq is the Sum of Squares. The fifth column (Mean Sq) is the Mean Square. The sixth column (f value) measures the significance of the model. The last column(p-value) indicates the statistical significance. The highlighted rows, marked with * exhibit values of statistical significance.

Nt	Variable	Df	Sum Sq	Mean Sq	f value	p-value
PO ₄	Sampling position	2	8.720	4.360	0.8343	0.44140
	Guano Category	2	69.036	34.518	6.6058	0.00326*
	Guano Category * Sampling Position	4	40.446	10.112	1.9351	0.12283
NO ₃	Guano Category	2	1507876	753938	7.4007	0.001802*
	Sampling position	2	61374	30687	0.3012	0.741533
	Guano Category * Sampling Position	4	109565	27391	0.2689	0.896304
NO ₂	Guano Category	2	2.9754	1.48771	2.2638	0.1168
	Sampling position	2	0.1198	0.05988	0.0911	0.9131
	Guano Category * Sampling Position	4	0.6285	0.15714	0.2391	0.9146
NH ₄	Guano Category	2	342.8	171.41	0.3320	0.7194
	Sampling position	2	1031.6	515.81	0.9990	0.3770
	Guano Category * Sampling Position	4	812.3	203.08	0.3933	0.8122

Table 4 Post-hoc test on the results for the linear model for the impact of guano category on concentrations of various nutrients in water samples. The first column (Nt) refers to the analyzed nutrient. The second column (Contrast) indicates the interaction between different guano categories and sampling position. The column third column (Estimate) shows the estimated difference in concentration between the guano categories being compared. The fourth column (SE) provides the standard error. The Degrees of Freedom (df) column reflects the sample size used in the analysis, impacting the critical values for significance testing. The sixth column (t ratio) column contains the calculated t-statistic, indicating the magnitude of the difference relative to the variability observed in the data. The last column (p-value) presents the statistical significance. The highlighted rows, marked with * exhibit values of statistical significance.

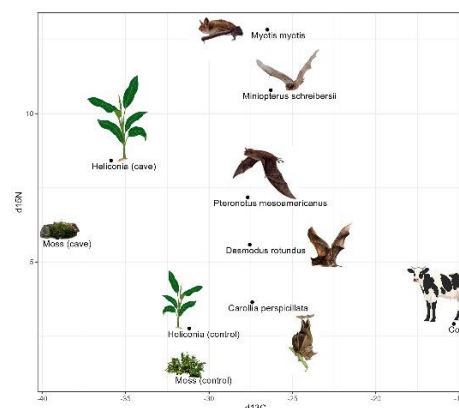
Nt	Contrast	Estimate	SE	df	T ratio	p-value
PO ₄	Guano category 1					
	Sampling position 1 -2	1.0233	1.87	41	0.548	0.8479
	Sampling position 1- 3	1.4156	1.87	41	0.758	0.7303
	Sampling position 2 – 3	0.3923	1.87	41	0.210	0.9760
	Guano category 2					
	Sampling position 1 -2	0.0989	1.14	41	0.087	0.9959
	Sampling position 1- 3	0.3613	1.14	41	0.316	0.9465
	Sampling position 2 – 3	0.2624	1.14	41	0.230	0.9714
	Guano category 3					
	Sampling position 1 -2	-3.2747	1.38	41	-2.366	0.0580
	Sampling position 1- 3	-3.7088	1.38	41	-2.679	0.0279*
	Sampling position 2 – 3	-0.4342	1.32	41	-0.329	0.9422
	categories 1 - 2	0.772	0.893	41	0.864	0.6660
	categories 1 - 3	-1.745	0.944	41	-1.849	0.1667
	categories 2 - 3	-2.516	0.726	41	-3.465	0.0035*
NO ₃	categories 1 - 2	113	125	41	0.906	0.6394
	categories 1 - 3	-265	132	41	-2.015	0.1213
	categories 2 - 3	-379	101	41	-3.733	0.0016*

Stable isotope analysis

The average $\delta^{15}\text{N}$ values for bat guano were +8.24, and -26.77 for $\delta^{13}\text{C}$. Composite guano samples, representing entire bat colonies, were collected at most sites. In areas with a single dominant species or where different species of bats roost separately, it was possible to identify the isotopic signature of guano from different species. The guano from insectivorous bats, such as *Miniopterus schreibersii* and *Pteronotus mesoamericanus*, showed higher $\delta^{15}\text{N}$ values and nitrogen content than those from frugivorous bats. Additionally, cow manure samples from the vicinity of the subterranean sites' entrances were collected for reference (Table 5).

Table 5 Examples of nutrient and isotope values for sources used in our analysis.

Source	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	%N	%C
<i>Myotis myotis</i>	-26.5	12.83	8.65	31.75
<i>Miniopterus schreibersii</i>	-26.3	10.79	14.18	35.22
<i>Heliconia (cave)</i>	-35.87	8.41	3.96	44.82
<i>Pteronotus mesoamericanus</i>	-27.68	7.18	5.02	38.86
<i>Moss (cave)</i>	-39.09	5.80	1.73	22.60
<i>Desmodus rotundus</i>	-27.56	5.58	3.15	39.41
<i>Carollia perspicillata</i>	-27.41	3.64	3.63	39.31
Cow	-15.33	2.91	1.3	26.8
<i>Heliconia (control)</i>	-31.18	2.75	3.07	44.28
<i>Moss (control)</i>	-31.00	1.19	1.87	41.10



The analyses consistently found that the $\delta^{15}\text{N}$ isotopic signature of moss tissues near the cave entrances is more similar to bat guano, with the concentrations decreasing with an increased distance from the cave entrance (Table 6). The linear model revealed that distance significantly influences $\delta^{15}\text{N}$ levels ($p = 0.001$). Specifically, we see a negative relationship between distance and $\delta^{15}\text{N}$ levels (Figure 5A). Figure 5B represents the isotopic values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in bat guano and moss samples, illustrating variations among sites.

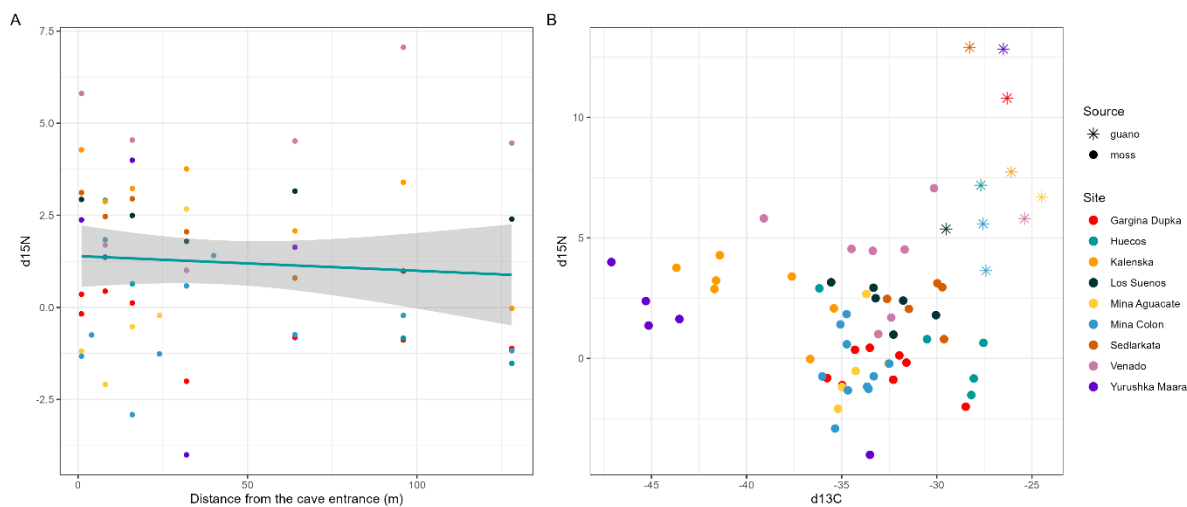


Figure 5 A. Relationship between $\delta^{15}\text{N}$ levels in moss samples and their distance from cave entrances. B. Isotopic values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ across the same moss samples, illustrating variations among sites. The isotope values of bat guano for each site are shown on Figure 5B with an asterisk shape.

Table 6 Values of $\delta^{15}\text{N}$ levels in moss samples and their distance from cave entrances. The first column is the name of the site and the country. The second column (Gua) represents the isotopic values of bat guano. The third column presents the $\delta^{15}\text{N}$ values over distance. The last column (Con) represents the $\delta^{15}\text{N}$ values of control samples, taken away from the cave entrances.

		Distance (m)								
	Site	Gua	1	8	16	32	64	96	128	Con
Bulgaria	Gargina Dupka	10.80	0.36	0.44	0.12	-2.01	-0.82	-0.89	-1.11	-2.07
	Kalenskata	7.73	4.28	2.88	3.23	3.76	2.08	3.40	-0.03	-4.13
	Sedlarkata	12.91	3.12	2.47	2.95	2.05	0.80			4.36
	Yurushka Maara	12.83	2.38	1.36	4.00	-4.00	1.63			5.16
	Huecos	7.18		2.90	0.64		0.80	-0.84		-1.52
Costa Rica	Los Sueños	5.37	2.93		2.50	1.79	3.16	0.99	2.40	-0.16
	Mina Aguacate	6.68	-1.19	-2.09	-0.52	2.67				-4.81
	Mina Colon	3.64	-1.32	1.84	-2.91	0.58	-0.74	-0.22	-1.17	1.38
	Venado	5.80	5.81	1.69	4.54	1.01	4.52	7.06	4.46	1.12

The isotopic signature of the *Heliconia latispatha* plant tissue showed a strong influence from bat guano, with $\delta^{15}\text{N}$ values decreasing over distance (Figure 6). A linear model testing the relationship between $\delta^{15}\text{N}$ levels and distance from the cave's entrance revealed a significant negative trend (p-value 0.004). This further shows, albeit with a very small sample of only one cave, that $\delta^{15}\text{N}$ levels in *Heliconia* tissues decrease with increasing distances from the cave's entrance.

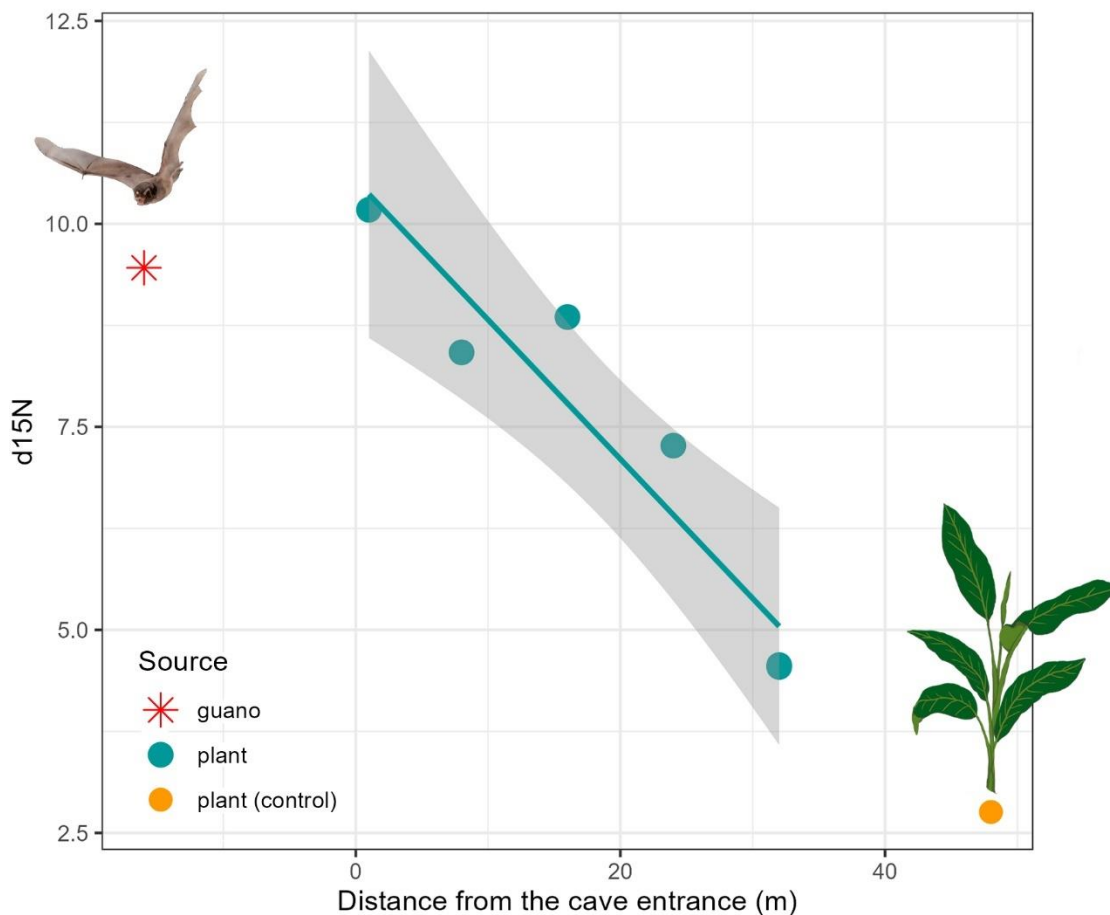


Figure 6 Isotopic signature of $\delta^{15}N$ from bat guano and plant tissue of *Heliconia latispatha*, over distance from the cave's entrance at the Arenal research site. The $\delta^{15}N$ isotope value of bat guano is shown on with a red asterisk shape. The control sample of *Heliconia* tissue (yellow) is taken 50 m away from the entrance, growing away from the river.

Discussion

In this study, we investigated the impact of bat colonies on the nutrient enrichment in riparian vegetation growing adjacent to caves with subterranean streams. Our findings reveal an increase in the levels of nitrogen and phosphorus in the water emerging from caves inhabited by large bat populations, attributable to the interaction of the water with bat guano. Notably, the enhancement in phosphate and nitrate levels was statistically significant, underscoring the role of bat guano as a source of this nutrient. As expected, the size of bat colonies was an important factor for nutrient enrichment. Additionally, our research demonstrates that the assimilation of nitrogen derived from guano by vegetation changes over distance from the source. Our results support our hypothesis that in caves sheltering bat colonies, the

underground streams are transporting nutrients from bat guano to the surface, where they get assimilated by plant tissue in the vegetation around the cave entrances. This novel insight unveils an overlooked ecosystem service provided by cave bats, wherein they contribute to the nutrient dynamics of adjacent surface aquatic ecosystems.

Bats contribute to ecosystems by controlling insect pests, pollinating crops, dispersing seeds to rejuvenate degraded tropical areas (Kasso & Balakrishnan, 2013; Kunz et al., 2011), supporting the subterranean ecosystems with their guano (Culver & Pipan, 2009). Bats are already known to influence the composition of plant communities around their roosts by dispersing seeds (Kunz et al., 2011). Their impact can also influence the fitness and growth of plants in laboratory settings (Shetty et al., 2013). The economic and ecological services provided by bats are frequently cited as crucial arguments for their conservation (Maas et al., 2013; Medellin et al., 2017; Tuneu-Corral et al., 2023). Echoing studies on the nutrient contributions of salmon (Helfield et al., 2001; Quinn et al., 2018) and sea birds (Gaiotto et al., 2022) to terrestrial and riparian ecosystems, our research confirms that bats also play a similarly vital role in plant nutrition. However, our study expands this understanding by demonstrating how underground streams facilitate the transfer of nutrients across different systems.

This study confirms the role of vampire bats in ecosystem nutrient cycles, as we found evidence that nitrogen from their guano is incorporated into the tissue of plants growing near their roosts, similar to previous studies which studied trees used as roosts (Voigt et al., 2015). Often labeled as parasites and disease vectors, vampire bats are culled in their roosts often indiscriminately, which results in mortality and loss of roosts for other bat species (Frick et al., 2020). Our new findings show that the nutrient-rich feces of vampire bats are important for supporting plant communities, and removing these bats from their roosts could have detrimental effects on the environment. This illustrates that every organism holds a unique position within the ecosystem and should be preserved. We believe that the ecological role and importance of bats are yet to be fully understood.

The size of bat colonies was an important factor for nutrient enrichment, with statistically significant results for the phosphates. Our study however, used caves with similar bat colonies, mostly varying between 500 and 2000 individuals. Given that the largest colonies

of cave-dwelling bats may reach millions of individuals (Hristov et al., 2010; McFarlane et al., 2015), we expect the contribution to be significant for the ecosystems surrounding caves. Future studies should also include caves without bat colonies, so we can fully understand the impact of the removal of bats from a roost, as it could happen after a disturbance event. In investigating the impact of colony size on nitrates, nitrites and ammonium levels within sampling positions, our models demonstrated limited explanatory abilities. Our findings underscore the complexity of factors influencing nutrient levels in subterranean waters and suggest that further research is needed to fully understand these dynamics.

Our study's methodology harbors certain limitations that hinder our ability to precisely quantify the nutrient influx attributable to bat guano. A notable constraint was the inconsistent presence of vegetation types, such as *Heliconia* and other plants, across our research sites; this variability, with the exception of moss, did not allow us to perform a comprehensive comparative analysis. The two-source mixing model suggested that bat guano is a primary nutrient source for moss and *Heliconia* near cave entrances, with some sites arguably almost entirely dependent on it. The isotopic signature of the *Heliconia* plant tissue showed a strong influence from bat guano, with $\delta^{15}\text{N}$ values of the plants growing in immediate proximity to the cave entrance even higher than the signature of bat guano samples. This could be explained by the fact that several bat species roosted in the cave, and our guano sample might have been collected from a spot where the majority of bats were frugivorous and thus having lower $\delta^{15}\text{N}$ values. However, we approach the results of this model with extreme caution due to the model's potential bias, the high complexity of the studied systems, and our inability to account for all potential nitrogen sources for each site (Cecchetti et al., 2020). This is why in the results we focused solely on the direct examination and analysis of the raw $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values to ensure objectivity.

Additional challenges were presented during the collection of water samples by their remote locations, resulting in the acquisition of very small quantities. The dynamic nature of underground ecosystems, coupled with our study's temporal limitations and the examination of multiple caves, further complicates the comparison between sites. Our methodology did not fully account for the impact of environmental variables, such as flash floods following rainfall, which can significantly extend the distribution of nutrients. By investigating various

sites rather than concentrating on a singular system, our study offers only a fragmented insight into the complex interplay between bat guano and plant life. This approach unveils pressing questions regarding nutrient input estimations, nutrient dispersion, and its effects on surface invertebrate populations, highlighting the necessity for future research to delve into how guano-derived nutrients bolster vegetation and, by extension, influence higher trophic levels.

Our study shows that the significance of bat guano extends beyond its immediate nutrient contribution in subterranean habitats, serving as a fundamental building block in the construction and maintenance of complex ecosystem dynamics on the surface. Its role in enriching aquatic ecosystems might extend beyond plants, providing nutrients for water invertebrates, which are crucial to the aquatic food webs. Therefore, understanding the nuanced effects of guano on these ecosystems can reveal critical insights into biodiversity conservation and ecosystem resilience. We recommend that future studies adopt a methodologically robust and continuous monitoring approach to unveil the intricate relationships between bat guano, nutrient levels, and broader ecological impacts.

Conclusions

In conclusion, this exploration of the nutrient contribution of bat colonies within caves as a potential ecosystem service underscores the importance of bats in nutrient cycling and prompts further investigation into their ecological contributions and the broader implications for both cave and surface ecosystems. Since cave systems are very dynamic and the nutrient input is influenced by various factors, more variables should be measured for future studies. Our research is highly relevant for bat conservation because the main arguments for the protection of bats are always centered on the ecosystem services they provide, and our findings add another one to the list.

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Supplementary material

Dataset 1 contains raw data of nitrates, nitrites, ammonium, and phosphates from underground water sources. Dataset 2 contains raw data of stable isotopes from bat guano and vegetation. Dataset 3 contains values from the statistical analysis. Available online at <https://figshare.com/s/3757b6b13f447965fa2d>

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GENERAL DISCUSSION

This dissertation researched the diversity and conservation issues of cave-dwelling bats in Costa Rica and Bulgaria and addressed relevant questions about the conservation of important underground roosts for bats. It notably filled knowledge gaps by presenting the first comprehensive details on Costa Rica's cave-dwelling fauna, cataloging bat species, assessing conservation status, and identifying prevalent threats (Deleva et al., 2023a). Additionally, it compiled the initial list of important subterranean bat sites in Costa Rica, evaluating conservation priorities and highlighting the most vulnerable roosts. In Bulgaria, the research investigated bat species diversity, and anthropogenic cave disturbances, and proposed protective measures. In both countries, the Bat Cave Vulnerability Index was used to identify vulnerable roosts (Deleva et al., 2023b).

Despite geographical and climatic differences, upon closer examination, similarities emerge between Costa Rica and Bulgaria. Both countries are of similar size, are located on a crossroad between continents and housing a high biodiversity amidst a variety of habitats. While Bulgarian caves and bat fauna have undergone extensive study for over a century, Costa Rica's underground roosts required fundamental initial exploration, which was hereby provided. Artificial roosts hold equal significance to natural caves in both Costa Rica and Bulgaria. In both regions, man-made structures play a pivotal role in supporting bat populations. While natural caves offer traditional roosting habitats, artificial roosts, such as tunnels or abandoned buildings, serve as crucial alternatives, supplementing the limited availability of natural roosts.

Contrasting the level of protection and conservation measures in both countries reveals significant differences. Bulgaria exhibits extensive legal protection for numerous sites, notably under Natura 2000 network regulations, emphasizing visitation restrictions and seasonal constraints during bat breeding and hibernation (MOEW, 2023). Conservation efforts, including gates, fences, and informative signboards, are prevalent, with select caves receiving heightened protection due to economic value (Deleva et al., 2023b). In contrast, Costa Rica shows a considerable number of sites outside protected zones with no specified conservation actions identified (Deleva et al., 2023a). Comparisons are somewhat limited due to the used methodology – all known caves were studied in Costa Rica while in Bulgaria

the exclusive focus was on important bat roosts. Nonetheless, Bulgaria's successful establishment and ongoing monitoring of key bat roosts could serve as a valuable example for Costa Rica's conservation initiatives. The optimal course of action would be the establishment of protected status for the most important and vulnerable underground bat roosts in Costa Rica.

Disturbances stemming from uncontrolled visitation, vandalism, and improper waste disposal are significant concerns in both countries. Proposed conservation strategies, encompassing visitor restrictions, informational campaigns, and entrance fencing, are universally applicable. In Bulgaria, targeted actions aimed at cavers are necessary to minimize disturbances, while in Costa Rica, the small but well-informed caving community actively engages in cave conservation. This might be attributed to the past popularity of caving in Bulgaria, which attracts diverse groups, whereas Costa Rica's caving community is smaller, well-educated and more receptive to information. A promising initiative involves engaging Costa Rican speleologists in a sustainable, long-term citizen science-based monitoring project.

The environmental and human factors influencing bat functional diversity were studied in numerous caves in southern Costa Rica. Findings highlighted that certain roosts' importance may not be fully represented by species number and abundance alone, echoing previous research that cave size correlates positively with phylogenetic and functional diversity. Despite these insights, many results did not correlate with the explanatory variables, suggesting alternative explanations of the factors determining bat species distribution across caves. The study expanded on previous work by recommending conservation attention for caves with high functional diversity, noting the overlooked significance of some caves used for tourism.

Surprisingly, bat diversity showed a positive correlation with cave visitation rates, challenging initial expectations. Additionally, the study contradicted the anticipated negative impact of deforestation on functional diversity, aligning with views that habitat fragmentation may offer diverse habitats supporting bat diversity. Observations from roosts, rather than capturing foraging bats, offer a unique perspective on diversity, underscoring the need for further research into habitat influences on bat communities. The study successfully

highlighted conservation-worthy cryptic roosts, suggesting a need for tailored conservation strategies acknowledging each site's unique ecological characteristics. Next studies should repeat this research in Bulgaria, where data are collected over a longer period of time and the results might provide more complete understanding of the factors, affecting bat diversity and distribution among roosts.

This study of the impact on bat guano on riparian ecosystems was simultaneously conducted in both Costa Rica and Bulgaria, which yielded globally relevant results. The research supports the initial hypothesis that underground streams transport nutrients from large bat colonies to surface vegetation, revealing a vital ecosystem service provided by cave-dwelling bats in nutrient dynamics of adjacent aquatic ecosystems. While bats are recognized for their roles in insect control, pollination, and seed dispersal, this study adds to their ecological importance by demonstrating their contribution to plant nutrition via guano-enriched stream water, offering a sustainable fertilizer alternative that leverages bat-derived nutrients without damaging cave ecosystems. However, the study acknowledges limitations in quantifying nutrient influx and calls for future research to delve into the comprehensive effects of guano-derived nutrients on vegetation and higher trophic levels, underscoring the complex dynamics between bats, their guano, and ecosystem health.

This dissertation has made significant contributions to understanding the diversity and conservation of cave-dwelling bats in Costa Rica and Bulgaria, providing critical insights into the conservation of vital underground roosts. The study underscores the universal challenges and solutions in bat conservation, despite geographical and climatic differences. Additionally, the research highlights the potential of bat guano as a sustainable nutrient source for riparian ecosystems, offering new perspectives on the ecological roles of bats. The findings advocate for a tailored approach to conservation, emphasizing the importance of both natural and artificial roosts, and suggest that engaging local communities in conservation efforts can enhance outcomes. Future research should focus on further investigation on the factors affecting bat diversity and distribution, as well as the broader ecological impacts of bat guano on ecosystems, to inform more effective conservation strategies and underscore the integral role of bats in maintaining biodiversity and ecosystem health.

CONCLUSIONS AND CONTRIBUTIONS

This dissertation represents a significant milestone in the study of cave-dwelling fauna in Costa Rica, offering the first comprehensive review of this unique ecosystem and the first in-depth analysis of cave-dwelling bats in the country. In the course of the study, more than 30 new caves were discovered and surveyed in Costa Rica, adding fundamental knowledge to the natural history of the country. Through extensive fieldwork, the list of important underground bat roosts in Bulgaria was updated, adding new sites and emphasizing on the ones in urgent need of conservation actions. Environmental factors influencing the functional diversity of bats in Costa Rica were examined, alongside a pioneering investigation into the nutrient contributions of cave-dwelling bats.

On the conservation front, this dissertation proposes the first monitoring program for cave-dwelling bats in Costa Rica and identified underground roosts crucial for their preservation. Moreover, during the fieldwork for the study we performed a capacity building among biologists in Costa Rica, launched an informative website about caves and bats (<http://bruncabats.info>), and presented a report on crucial underground roosts to SINAC in the Puntarenas province. The updated list of important underground bat roosts in Bulgaria and suggested conservation actions was shared with the responsible authorities. Another conservation contribution is the study of the ecosystem services provided by bats to bolster the argument for their conservation. These conclusions collectively underscore the valuable contributions of this research to the understanding and protection of cave ecosystems and their inhabitants in Costa Rica and Bulgaria.

Future directions include the implementation of a long-term monitoring program to track changes in cave-dwelling bat populations in Costa Rica. This program could focus on assessing the impact of environmental changes, human activities, and potential conservation efforts on these species and should include local communities as citizen scientists. The investigation into the ecosystem services provided by bat guano to aquatic ecosystems is worth expanding further. This could involve a more comprehensive study on nutrient dynamics, food webs, and the broader impact of bat guano on surface ecosystems.

Scientific contributions

- First extensive review of cave-dwelling fauna of Costa Rica.
- Reported new species of true bug for the fauna of Costa Rica.
- First review on cave-dwelling bats in Costa Rica.
- Updated list of Important bat roosts in Bulgaria and their conservation priority.
- Discovered and surveyed more than 30 new caves in Costa Rica, adding to the fundamental knowledge on the natural history of the country.
- Study on the factors affecting functional diversity of bats in Costa Rica.
- First study of nutrients from cave-dwelling bats and the potential ecosystem service from bat guano to aquatic ecosystems.

Conservation contributions

- Developed the first monitoring program for cave-dwelling bats in Costa Rica.
- Established underground roosts with conservation importance for Costa Rica.
- New underground roosts added to the Important Bat Roosts in Bulgaria.
- Priorities for conservation of underground roosts established for Bulgaria.
- Capacity building for future cave biologists in Costa Rica.
- Created website about caves and bats in Costa Rica: <http://bruncabats.info>
- Report about important bat roosts in Puntarenas province presented to SINAC.
- Explored ecosystem service of bats as an argument for their conservation.

Future directions

- Implement a long-term monitoring program in Costa Rica.
- Extend the investigation into the ecosystem services provided by bat guano to aquatic ecosystems.
- Develop educational programs and citizen science workshops aimed at collecting data on cave ecology and conservation of bats.

FUNDING

Year	Grant	Amount (\$)
2022	Bulgarian National Science Fund, project КП-06-H51/9 "Caves as a reservoir for novel and reoccurring zoonoses - ecological monitoring and metagenomic analysis in real-time."	Expenses for fieldwork in Bulgaria.
2022	Project "Field observation of bats according to the National Biodiversity Monitoring Scheme" Contract № 14838.	Article processing charges for one publication. Expenses for fieldwork in Bulgaria.
2021	EU Framework Horizon 2020 through the COST Action CA18107 "Climate change and bats: from science to conservation – ClimBats" (https://climbats.eu/).	Expenses for fieldwork in Bulgaria.
2022	Program "Young scientists and postdoctoral fellows", Ministry of Education and Science. Duration – 12 months.	Salary for 12 months
2019	The Explorers club Mammont Scholar grant. Project: "Nutrient contribution of cave bat colonies to surface ecosystems in the Paleotropics"	1,500
2019	Bulgarian Speleology Federation, Funding for expeditions. Project: Expedition "Mulu 2019" Malaysia.	800
2019	Bat Conservation International's Student scholarship. Project: "Nutrient contribution of cave bat colonies to surface ecosystems in the Paleotropics".	3600
2019	Universidad de Costa Rica, Scholarship for a three-month internship in Panama.	2700
2019	The explorers Club Fjallraven Grant. Project Nutrient contribution of cave bat colonies to surface ecosystems in the Paleotropics.	1500
2018	Technical University of Munich. Analyzing 100 isotope samples free of charge.	Analysis of 100 isotope samples
2018	Early researchers grant of National Geographic society. Project EC-194R-18: "Exploring a new ecosystem service provided by bats as a tool to promote their conservation".	4780
2018	B8A65 Contribución en nutrientes de las colonias de murciélagos a los ecosistemas acuáticos.	Expenses for fieldwork and laboratory analysis in Costa Rica.
2018, 2019	the Alvaro Ugalde Grant of Osa Conservation. Project: "The hidden treasures of Osa". Continuation in 2019	3666
2017	Idea Wild scholarship to purchase equipment. Project: Research, Ecology and Conservation of cave-dwelling bats in the Brunca Region of Costa Rica.	600
2016	Universidad de Costa Rica, Project "Diversity and conservation of cave-dwelling bats in the Brunca region of Costa Rica"	Expenses for fieldwork in Costa Rica.
2015	Rufford Small Grant for nature protection. Project No 16923-1: Protecting unique underground systems using bats as flagship taxa.	6,098

SUPPLEMENTARY MATERIAL**Guidelines for survey and monitoring of cave-dwelling bats in Costa Rica****By:** Stanimira Deleva

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Abstract: Regular monitoring of bats is the most reliable method for assessing their current conservation status and population trends. Cave-dwelling bats, given their tendency to form large aggregations in caves and other subterranean sites, are excellent subjects for research. A set of standard methodologies and guidelines for researching and monitoring cave-dwelling bats in Costa Rica is presented in this document. Established methodologies have been adhered to, with modifications made to suit the specific conditions of our country. The target species were highlighted, and a list of important underground sites for monitoring was proposed.

Given the popularity of caving as an outdoor activity, bat monitoring can be conducted not only by researchers but also by speleologists, tour guides, and other enthusiasts, provided that the described basic guidelines are followed. In addition to the methodology, useful information on best practices and safety protocols is offered. Periodic updates to this document are recommended to incorporate new findings and research methods, ensuring its continued relevance and effectiveness in bat conservation efforts in Costa Rica.

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