Faculty of Sciences

Department of Biological Sciences and Biotechnology

SPATIO-TEMPORAL PATTERNS OF TERRESTRIAL MACROINVERTEBRATE ASSEMBLAGES AT GCWIHABA CAVE, BOTSWANA.

Kefeletswe Majoka

20100065

MSc Biological Sciences and Biotechnology (BIUST)

A Thesis Submitted to the Faculty of Science in Partial Fulfilment of the Requirements for the Award of the Degree of Master of Science in Biological Sciences and Biotechnology of the BIUST

Supervisor: Dr R. Mazebedi

Department of Biological Sciences and Biotechnology,

Faculty of Science, BIUST

mazebedir@biust.ac.bw

Co-Supervisor: Dr T. Hesselberg

Departmental Lecturer in Biological & Environmental Sciences

Oxford University Department for Continuing Education

Rewley House, 1 Wellington Square,Oxford, Oxfordshire, OX1 2JA, United Kingdom

thomas.hesselberg@biology.ox.ac.uk

DECLARATION REGARDING THE WORK AND COPYRIGHT

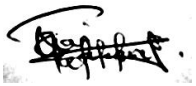
Candidate: KEFELETSWE MAJOKA

Student ID: 20100065

Thesis Titled: SPATIO-TEMPORAL PATTERNS OF TERRESTRIAL MACROINVERTEBRATE ASSEMBLAGES AT GCWIHABA CAVE, BOTSWANA.

I, **Kefeletswe Majoka**, certify that the Thesis is all my own original work and that I have not obtained a degree in this University or elsewhere on the basis of any of this work.

This dissertation/thesis is copyright material protected under the Berne Convention, the Copyright and Neighbouring Rights Act, Act. No. 8 of 2000 and other international and national enactments, in that behalf, on intellectual property. It must not be reproduced by any means, in full or in part, except for short extracts in fair dealing; for researcher private study, critical scholarly review or discourse with an acknowledgement, without the written permission of the office of the Postgraduate School, on behalf of both the author and the BIUST.

Signed:  Date: **01/04/25**

Primary Supervisor:

I, the Candidate's primary supervisor, hereby confirm that I have inspected the above titled thesis and, to the best of my knowledge, it is based on the original work of the candidate.

Signed: Date:

CERTIFICATION

The undersigned certifies that he/she has read and hereby recommends for acceptance by the Faculty of Science a dissertation/thesis titled: **SPATIO-TEMPORAL PATTERNS OF TERRESTRIAL MACROINVERTEBRATE ASSEMBLAGES AT GCWIHABA CAVE, BOTSWANA,** in fulfilment of the requirements for the degree of Master of Science *Biological Sciences and Biotechnology* of the BIUST.

Signature



Dr. Richard Mazebedi

(Supervisor)

Date: 03/04/2025

Dr. Thomas Hesselberg

(Co-Supervisor)

Date:

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to all those who supported me throughout the journey of completing this thesis. Despite the unexpected challenges and the moments of doubts, their unwavering support and belief in me pushed me beyond my limits and motivated me to persevere until the end.

I am deeply thankful to my supervisors, Dr Mazebedi and Dr Hesselberg, whose guidance, patience, and expertise were invaluable throughout this process. Their insightful feedback and constructive criticism have played a pivotal role in shaping this work.

I am also grateful for my family and friends for the love and understanding during this demanding time, colleagues and staff at the Department of Biology and Biotechnology (BIUST) for their enduring support and encouragement. My deepest gratitude also goes to the staff at Gcwihaba cave especially the cave guides who assisted me during my data collection trips, I could not have done it without them. Many thanks again to BIUST for sponsoring my project. Above it all I thank God for giving me the strength to endure everything.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION 12

1.1.Overview of the cave environment. 12

1.2. Ecosystem services from caves. 14

1.3. Cave macroinvertebrates diversity. 15

1.4.Classification of cave macroinvertebrates. 16

1.5. Factors affecting distribution of macroinvertebrates within caves. 17

1.6. Threats to cave ecosystems 18

1.7. Research problem 20

CHAPTER 2: MATERIALS AND METHODS 22

..... 22

2.1. Study area 22

2.1.1. Location 22

2.1.2. Climate 23

2.1.3. Formation process of Gcwihaba cave 23

2.1.4. Biodiversity of Gcwihaba area 23

2.2. Study design (Spatial and temporal design) 24

2.2.1. Spatial design 24

2.2.2. Temporal design 25

2.3. Environmental variables measurements 28

2.4. Data Analysis 28

CHAPTER 3: RESULTS 29

..... 29

3.1. Macroinvertebrates assemblage composition and diversity. 29

3.2 Variability of terrestrial macroinvertebrates across cave zones 33

3.3. Seasonal comparisons of terrestrial macroinvertebrates across seasons 35

CHAPTER 4: DISCUSSION 39

4.1. The diversity of Gcwihaba cave macroinvertebrates 39

4.2. Seasonal variation of Gcwihaba cave macroinvertebrates. 41

4.3. Study Limitations 43

4.4. Conclusion 44

References 45

LIST OF TABLES

Table 1. Taxonomic identification of taxa observed and their frequency of occurrence inside the cave. Un-unidentified. 30
Table 2. Hill numbers (effective numbers) of macroinvertebrate taxa across cave zones. 33
Table 3.Hill numbers (effective numbers) of macroinvertebrate taxa across seasons. A- hot and wet season, B-cold and dry season. 35

LIST OF FIGURES

Figure 1. Location of Gcwihaba hills in Botswana indicated by a red triangle. (Mazebedi & Hesselberg., 2020) 22
Figure 2. Approximate zoning of the cave. (Adapted and modified from Dandurand et al.,2019). 25
Figure 3. A live pitfall trap. 26
Figure 4.Hierarchical clustering of cave zones according to macroinvertebrate taxa. 34
Figure 5. Hierarchical clustering of cave zones according to macroinvertebrate taxa across seasons. A-hot and wet season, B- cold and dry season. 36
Figure 6. Relative abundance of macroinvertebrate taxa during cold and dry season and hot and wet season. 37
11Figure 7. Mao's Tau rarefaction curve. 38

LIST OF APPENDICES

Appendix A. Representatives of the 10 most abundant macroinvertebrate taxa in Gcwihaba caves 59

LIST OF ABBREVIATIONS

NW-SE North West – South East

ITFS - Integrated Taxonomic Information System

PAST - Paleontological Statistics Software

ABSTRACT

Cave ecosystems harbour diverse and specialized macroinvertebrate communities that provide vital ecosystem services. This study investigated the within cave and seasonal patterns of terrestrial macroinvertebrate assemblages in Gcwihaba cave, Botswana. Prior to this study, the macroinvertebrate assemblages of Gcwihaba cave were only superficially surveyed and the temporal patterns of the invertebrate communities were unknown. This study therefore aimed to improve knowledge on the macroinvertebrate taxa that occur in the cave, their distribution across the cave sectors and the influence of climatic seasons on the distribution patterns of the invertebrates. To examine the community patterns, terrestrial macroinvertebrates were collected within three cave zones; the light sector, the transition sector, and dark sector. These collections were undertaken during the rainy season (hot-wet) and the winter season (cold-dry) using pitfall traps and visual surveys. A total of 31 macroinvertebrate taxa were collected, including 18 new families. Cave cockroaches (Blaberidae) were the most abundant, followed by cave crickets (Rhaphidophoridae) and violin spiders (Sicarridae). Species richness was similar across zones, but diversity (Shannon and Simpson indices) decreased from the light zone to the dark zone, indicating a shift from a balanced community to one dominated by specialized taxa. The abundance of macroinvertebrates was significantly higher in the dark zone and during the hot-wet season, likely due to increased resource availability and favourable microclimates. Seasonal variations influenced community composition, with some taxa exhibiting distinct seasonal preferences. In terms of the spatial distribution of macroinvertebrate groups within the cave, the patterns are similar to those reported by previous research, the main contribution of this research is the recording of 16 additional macroinvertebrate taxa in the cave and a demonstration that there is high macroinvertebrate activity during the rainy season; which may benefit approaches to the cave management.

Keywords: Cave ecology, cave macroinvertebrates, species diversity, spatial distribution, temporal patterns, Gcwihaba cave, community dynamics, biodiversity conservation, seasonal variation

CHAPTER 1: INTRODUCTION

1.1.Overview of the cave environment.

Caves are underground, dark environments that humans can explore, containing air and/or water and inhabited by species that are adapted to underground life (Mammola, 2019). There is substantial amount of biodiversity supported by caves because caves are distributed throughout the world, with all continents having caves in most of their member countries (Culver & Pipan., 2009). Caves are diverse in terms of their size and internal morphology depending on factors leading to their formation (Moseley, 2009). These factors include tectonic activity, atmospheric impacts, rock weathering, glacier melting, chemical reactions of diverse compounds, rock disintegration, cave rock weathering and corrosion of rocks by water which shapes their morphological features (Engel, 2010). The deeper parts of caves show narrow fluctuations in terms of their annual internal temperatures and relative humidity, the latter often constantly close to saturation (Mammola, 2009). This makes the cave internal conditions uniquely stable though it is influenced by change in local weather conditions (Brunet & Medellin, 2001).

While caves may be morphologically diverse, they can be generally sub sectioned into distinct gradient zones based primarily on light availability which correspond with the composition of biological communities within the cave (Prous *et al.,* 2004; Engel, 2011). The entrance zone is illuminated, with some parts directly exposed to the sun at some point during the day. This zone can hold photosynthetic organisms like plants, lichen and algae depending on the amount of sunlight received (Mendes *et al.,* 2021). The zone also, owing to its proximity to the surface environment, fluctuates in environmental conditions such as temperature and humidity corresponding to the local weather conditions (Xiong *et al.,* 2023). The next zone into the cave, is the transition zone, which has relatively constant environmental conditions compared to the entrance. It is almost completely dark with variable humidity and temperature (Macud & Nuneza, 2014; Howarth & Moldovan, 2018). The deepest zone is the dark zone, the furthest away from the entrance with its characteristic total absence of light, humidity at or near saturation, relatively stable temperature, and very little air circulation (Biswas, 2010). Lunghi *et al*., (2014) highlighted species occurrence in caves is influenced by cave features like morphology and the cave's internal environmental parameters. Previous studies have shown that macroinvertebrate communities may vary between these cave zones (Lunghi *et al*.,2014; Mazebedi & Hesselberg, 2020).

The cave's local community and distribution is further determined by its internal ecological conditions like nutrient availability, light availability, stable temperatures and high humidity. Caves are typically energy-poor environments due to the lack of sunlight and primary production (Ravn *et al*., 2020). Macroinvertebrates therefore rely on organic matter that enters the cave from the surface, such as leaf litter and decaying wood (Ravn *et al*., 2020). The amount and type of these resources significantly influence the abundance and diversity of macroinvertebrates (Prous *et al*., 2004). The absence of light especially in the deeper parts of caves have also contributed to many cave dwelling macroinvertebrates evolving reduced or absent eyes and pigmentation while other senses like touch and chemoreception are enhanced (Culver & Pipan, 2019). This adaptation to darkness contributes to the unique biodiversity found in these environments. Stability of temperatures and relatively high humidity as another key feature of internal cave environments can favour species with narrow environmental tolerances and contribute to the evolution of specialised adaptations (Mammola, 2019). However, variations in these conditions such as proximity to cave entrances or presence of water bodies can create microhabitats and influence species distribution within the cave (Cuff *et al.,* 2021).

The above-mentioned features contribute to a unique and challenging environment that drives the evolution of highly specialised cave organisms (Mammola, 2019). Cave ecosystems therefore harbour a great diversity of specialized and endemic species with distinctive morphologies, physiologies and behaviours (Culver & Pipan, 2019), reflecting adaptive convergences (Wilkens & Strecker, 2003; Juan *et al.,* 2010; Mammola *et al.,* 2020) and unique evolutionary trajectories that largely match cave habitats (Christman *et al.,* 2005; Mammola *et al.,* 2018). Many cave macroinvertebrates are vulnerable because of their restricted distributions, high endemism and are specialised for living in the dark subterranean cave environment (Cardoso *et al*., 2021). The absence of light in caves has resulted in some taxa developing morphological adaptations which vary among species but are represented by having elongated appendages to aid in food and mate finding, depigmented skins due to absence of melanin to protect from sunlight rays and having no visual apparatus likely due to absence of light in their habitats. (Culver & Wilkens, 2000 ; Culver & Pipan, 2019). For example, about 192 species of the 512 known species of cave adapted arachnids show a reduction and /or total loss of eyes and pigmentation, elongated appendages, lower metabolic rates, production of fewer and larger eggs (Mammola *et al.*, 2022). For many other cave taxa, major life supporting activities like, resource location (food, mating partner and space), avoidance of competitors and predators all must be accomplished without vision (Horváth *et al.,* 2021). Through their diversity and specialised adaptations, cave organisms offer several benefits to human beings collectively referred to as ecosystem services (Canedoli *et al.,* 2022).

1.2. Ecosystem services from caves.

The continuity of ecosystem services that humans derive from caves rely on protection of cave environments and conservation of cave fauna (Medina, 2023). These services, while sometimes overlooked, are crucial for human well-being and include vital resources like clean water, regulation of biogeochemical cycles, and support for biodiversity (Culver & Pipan, 2019). The continued availability of ecosystem services from caves is dependent on the conservation of cave biodiversity, including cave macroinvertebrates. Ecosystem services can be categorised into four main categories as provision services, regulatory services, supporting services, cultural and recreational services (Kunz *et al*. 2011).

Provision services

One of the most significant ecosystem services provided by caves is the provision of groundwater. Caves often act as important aquifers, storing and filtering vast quantities of freshwater that are essential for human consumption, agriculture, and industry (Ford & Williams, 2007). More than half of the world's population relies on groundwater associated with caves for these purposes (Elshall *et al*., 2020). The unique geological structure of caves, with their interconnected networks of fissures and channels, facilitates the movement and storage of groundwater, making them crucial components of regional hydrological systems (White *et al*., 2019). Certain macroinvertebrates like, *Heptageniidae* and *Corydalidae,* are commonly used as bioindicators to assess water quality in subterranean aquatic ecosystems because of their sensitivity to environmental changes (Galindo- Pérez *et al*., 2017). Protecting cave environments from pollution and over-extraction is therefore essential for ensuring the continued availability of this vital resource (Gilli, 2015).

Regulatory services

Cave macroinvertebrates also play key roles in upholding ecological balance. As primary decomposers, they break down organic matter like leaves and bat guano, facilitating nutrient cycling within the cave environment (Mazebedi & Hesselberg, 2020). This decomposition process supports microbial communities and forms the foundation of the cave food web, sustaining various life forms. Bacterial genomic studies have revealed that a large number of bacterial communities that reside within the guano play a crucial role the nitrogen biogeochemical cycle (De Mandal *et al.,* 2015), making caves important sites for nutrient cycling which are processes essential for maintaining soil fertility and supporting plant growth, both within and outside of the cave environment (Cardoso *et al*.,2021).

Supporting services

Through macroinvertebrates diversity, caves also offer supporting services. Some insects, such as *Ceuthophilus pima*, a type of camel cricket native to North America, primarily serve as detritivores. They consume organic matter, fungi, and decomposing plant material, which positions them as significant players in the nutrient cycling process within caves and other underground ecosystems through their faecal deposits (Pape & O'Connor, 2014). This helps make nutrients available to other cave organisms. Camel crickets also play a crucial role as a primary food source for predatory organisms in caves (Pape & O'Connor, 2014), including cave spiders and cave salamanders, thereby supporting the balance of subterranean ecosystems. Additionally, other macroinvertebrates adapted to cave environments, such as the tropical cockroaches of the *Nocticola* genus, are preyed upon by arachnids like whip spiders (Lucanas & Lit, 2016).

Cultural and recreational services

Many caves hold considerable archaeological and paleontological significance, providing invaluable insights into human history and past environments (Faulkner, 2010). Caves also attract tourists, generating income and supporting local economies (Benedetto *et al*., 2022). The recognition and appreciation of these diverse ecosystem services, both tangible and intangible, are crucial for promoting the long-term conservation and sustainable stewardship of cave ecosystems (Gunn, 2004). Protecting caves ensures the continued delivery of these valuable services for present and future generations. Effective management strategies for caves must acknowledge and address the interconnectedness of these subterranean ecosystems with the broader surrounding environment.

1.3. Cave macroinvertebrates diversity.

Caves harbour a diverse array of macroinvertebrate life, playing crucial roles in the functioning of these subterranean ecosystems. Studies from tropical and temperate regions have demonstrated significant variation in cave biodiversity, with tropical caves generally exhibiting higher species richness due to greater organic input and stable climatic conditions. For instance, studies in Brazilian limestone caves in the neotropics have documented a wide range of troglobitic arthropods, including cave-adapted spiders, beetles, and crustaceans (Ferreira *et al*., 2023), while European caves, particularly in the Balkans peninsula situated in temperate region, are known for their high diversity of specialized subterranean fauna (Delić *et al*., 2023). The diversity of macroinvertebrates also varies with cave type; limestone and lava tube caves often support distinct communities due to differences in microclimate and nutrient availability. While this macroinvertebrate diversity is found globally, cave macroinvertebrates are better described in American and European countries

(Mazebedi & Hesselberg, 2020) with a handful of studies in Africa, such as (Ferreira *et al*., 2020; Mazebedi & Hesselberg, 2020;Harvey & Du Preez, 2014). Despite these efforts, considerable gaps persist, and African subterranean ecosystems have largely been understudied resulting in Africa ranking among the least explored continents concerning cave-restricted species and their habitats (Moutaoukil *et al*., 2024), thus a need for more studies to better understand local diversity.

Insects play various trophic roles that contribute to nutrient cycling within caves, including detritivore, predator, and parasite. Some like cave crickets bring organic matter into caves as they return from their foraging outside (Lavoie *et al.,* 2007). Their eggs are also eaten by several carabid beetle species (Kane & Ryan, 1983). Many of the cave species are guanophilic (Ferreira *et al.,* 2007). Some insects are also decomposers, and scavengers (Coleoptera). Different insect orders represented in caves include Thysanura (Zygentoma or silverfish), Orthoptera (Crickets, grasshoppers), Dermaptera, Isoptera (Termites), Blattodea (cockroaches), Hemiptera (True bugs, hoppers, aphids and cicadas), Phthiraptera (lice), Coleoptera (beetles), Hymenoptera (ants, bees and wasps), Lepidoptera (moths and butterflies) and Diptera (flies, mosquitos and midges) (Romero, 2009). Some specific insect species identified at Gcwihaba cave by Mazebedi & Hesselberg (2020) are darkling beetles and cave cockroaches. Class Arachnida is also well described; with nine cave orders representing 300 species (Mammola *et al.,* 2018) Their feeding habits include predation, consumption of detritus, and parasitism (Romero, 2009). Some of the orders in this class includes Acari (ticks and mites), Araneae (Spiders), Scorpionida (Scorpions) and Pseudoscorpionida (Pseudoscorpions) (Romero, 2009). Some of the specific mite species observed in Southern African caves include *Chiropturopoda coprohila*, *Laelaps sp*. and *Sancassania sp*. (Dippenaar- Schoeman & Myburgh, 2009). Some spiders and scorpion venoms have been found to contain neuroactive compounds that can help keep the human heart rhythm thus a potential application to human health. (Novak, 2001).

1.4.Classification of cave macroinvertebrates.

The Scheiner-Racovita system classifies cave species based on their ecology and morphological adaptations to the cave environment into three categories namely, a) troglobionts, b) troglophiles, and c) trogloxenes (Trajano & de Carvalho, 2017). Troglobionts are permanent cave dwellers, mostly found in the deeper parts of caves and they often exhibit adaptations to cave life such as, reduced or absent eyes, lack of pigmentation, elongated appendages for navigating in the dark, and slow metabolisms to conserve energy in resource-scarce environments (Howarth & Moldovan, 2018). Troglophiles are more flexible in their habitat preferences. These organisms use caves to complete their life cycle but can survive outside the cave (Hesselberg *et al.,* 2019,

Mammola & Isaia, 2017). They often venture into subterranean environments for shelter, or to escape harsh conditions on the surface. While they may exhibit some adaptations to cave life, they are not as specialised as troglobionts. Lastly, species that use caves occasionally are called trogloxenes. These organisms use caves for specific purposes such as hibernation, nesting or escaping predators. They do not complete their entire life cycle underground and rely on the surface environment for most of their needs (Howarth & Moldovan, 2018). Trogloxenes are usually found at the entrance zone or the twilight zone (Culver & Pipan, 2009), and they usually show no proper adaptive features to cave environments since they visit or use caves occasionally. Other studies (Sket, 2008; Novak *et al.,* 2012) have also classified troglobionts /troglobites as organisms that permanently reside in subterranean environments whereas trogloxenes were classified as organisms that are not able to establish sustainable populations in the subterranean environments.

1.5. Factors affecting distribution of macroinvertebrates within caves.

Environmental variability within caves has been postulated to be the main driver for distribution patterns of macroinvertebrates in cave ecosystems. Previous studies have demonstrated the importance of several environmental factors in influencing the occurrence and abundance of different cave macroinvertebrates groups including resource availability (Chertoprud *et al.,* 2020; Borisov *et al.,* 2021), microclimate variability (Mammola *et al.,* 2019; Cuff *et al.,* 2021), invasion history (Nicolosi *et al*., 2023), and species interactions (Cuff *et al.,* 2021). Some troglobionts like cave cockroaches may show a temporal variation in their distribution by burrowing beneath the guano for other sensitive phases of their life cycle like laying of eggs (Mammola *et al.,*2019). This varying usage of space within caves is due to different factors like specialization, through avoidance of inter- and intraspecific competition and nutrient limitation (Cuff *et al.*, 2021). Understanding these spatial variations is key as they determine the biological functions of individuals and species at large (Araújo *et al.,* 2010).

At a smaller scale, in response to climate change cave species could theoretically change their spatial distribution inside the cave system itself (Fejér & Moldovan, 2013; Lunghi *et al.*, 2017; Mammola & Isaia, 2017). Increasing temperatures due to climate change leads to internal cave temperatures especially in the shallow parts slightly rising leading to macroinvetebrates moving to cooler microhabitats within the caves where they can biologically function optimally (Mammola *et al*., 2019). Evidence suggests that several subterranean species tend to migrate toward greater depths and vice versa, from one season to the other (Mammola *et al.,* 2019: Tobin *et al.*, 2013). For instance, Bento *et al.,* (2016) found out that invertebrate communities showed differences in their structure according to the collection season, the rainy season having

the highest species diversity. Cave cricket's numbers have been found to vary around the cave entrance with time of day and season (Lavoie *et al.,* 2007). Short range endemics (organisms with limited geographic distribution due to their specialised adaptation to specific limited environmental conditions) may likely be pushed to extinction because of their inability to cope with environmental changes brought about by climate change. Most cave species have narrow thermal tolerances; therefore they may not be able to adapt to even slight temperature changes as such may die (Medina *et al*., 2023).

1.6. Threats to cave ecosystems

Despite harbouring important macroinvertebrate diversity, which provides essential ecosystem services, the cave ecosystems face key threats like climate change, invasion by alien species and a number of anthropogenic activities. Climate change forecasts using theoretical models have shown a possible impact in cave temperatures and humidity (Badino, 2004; Covington & Perne, 2015). Some model–data comparison studies have confirmed such forecasts (Domínguez-Villar *et al.,* 2015; Culver & Pipan., 2018). Cave environments are particularly sensitive to climatic altering activities carried out at the surface above and in the aquifers below (Gillieson, 2011). Throughout the course of evolution and adaptation, some cave species have lost their resistance to variation of temperature (Mermillod-Blondin *et al.,* 2013) thus with the onset of increased cave temperatures such species may be driven towards extinction. Caves exchange air masses and water with the external atmosphere and environment which are the main natural causation agents of disturbance in the sub-surface. (Mammola *et al.,* 2019; Chiarini *et al.,* 2022). This further exposes caves to climatic changes that occur on the surface. For instance, as global temperatures continue to rise due to increased carbon-dioxide concentration in the atmosphere, the amount of heat energy entering caves through exchange of air masses with the surface increases thus increasing internal cave temperatures which will directly affect cave animal species i.e. their metabolism rate (Chiarini *et al.,* 2022). Roth (2014) suggested that as global temperatures continue to rise, an increasing number of macroinvertebrate taxa may go extinct due to desiccation while nonnative alien species may retreat into caves for shade and establish populations which may outcompete the native species and colonize caves.

Another significant threat to cave ecosystems is the invasion by non-native or opportunistic invertebrate species, which can disrupt ecological balance. Invasive insects such as cockroaches, ants and flies often gets introduced into caves by human activities, climate change driven range expansions, or alterations in surrounding land use (Wagner & Van Driesche, 2010). These species can outcompete native cave adapted invertebrates for limited resources, introduce new pathogens or alter nutrient cycling by rapidly consuming organic matter that would otherwise sustain endemic species. Additionally, some insects such as invasive

predatory beetles or aggressive ant species, can directly prey on native cave fauna leading to population declines or local extinctions (Liebherr & Krushelnycky, 2007; Walker, 2006). The disruption caused by insect invasions can have cascading effects, threatening not only invertebrate biodiversity but also higher trophic levels, including bats and other cave dwelling vertebrates (Wagner & Van Driesche, 2010)

Tourism presents a significant threat to cave ecosystems through direct habitat modification and disturbance. For example, some caves such as the Kronio cave complex in Sicily, Italy (Badino and Torelli, 2014) and Lu Di Yan (Reed Flute Cave) in Guangxi, China (Cigna,2012) are developed as show caves and for tourist activities. To prepare caves for tourist activities, some modifications are done to the cave's internal environment including but not limited to modifying some cave sediments or speleothems (a structure formed in a cave by the deposition of minerals from water, e.g., a stalactite or stalagmite) thus destroying the natural morphologies and microhabitats for different cave organisms (Calaforra *et al.,* 2003). Nitzu (2021) demonstrated that species composition varies across different microhabitats, with certain species showing preference for specific microenvironments at different times. Therefore, tampering with natural morphologies inside caves will lead to a direct negative impact on macroinvertebrates compositions and distributions. Moreover, the frequent movement of tourists inside caves causes disturbances that ultimately negatively affect cave environments. The physical compaction of cave floor substrates (guano and soil) occurs as tourists step as they walk. Pacheco *et al.*, (2021) reported a significant difference between touristic and non-touristic areas of a cave due to the impacts of stepping on substrates like bat guano, fine sediment, cobble and boulders. It is therefore a necessity to establish well defined touristic routes and pathways in any subterranean environment to mitigate such threat to cave ecosystems (Pellegrini & Lopes Ferreira, 2012).

The introduction of artificial lighting to help illuminate the inside of caves for touristic purposes is also a threat as it disrupts the natural subterranean conditions and habitat use of cave adapted macroinvertebrate communities. For instance, Kurniawan *et al*., (2018) found clear differences in arthropod community structures between artificially lit show caves and unlit wild caves in the Gunungsewu karst area, suggesting that light influences where and how species are distributed within the cave environment. Nicolosi (2023) also assessed the impact of cave lighting by comparing two caves with similar conditions but differing touristic traffic. The results indicated that higher tourist numbers significantly increased lampenflora proliferation in Toirano cave as the caves are lit for prolonged periods. The growth of lampenflora such as algae and mosses further alters these habitats by modifying the substrate and microclimate, creating unsuitable conditions for some macroinvetebrates while providing niches for others. This can be observed in Cango caves, South Africa where deeper cave surfaces are covered by green algae after introduction of light inside the caves (Cigna, 2012).

Other human activities posing threats to cave ecosystems includes mining, pollution and deforestation, which leads to habitat degradation and biodiversity loss (Mammola *et al*., 2019). Mining particularly for limestone, phosphate and metals, alters cave morphology, disrupts subterranean water systems and destroys microhabitats essential for troglobitic species (Jaffe *et al*., 2016). Additionally, pollution from agricultural runoff, industrial waste and untreated sewage introduces heavy metals, pesticides, and nutrients into cave waters causing eutrophication and toxicity that threaten obligate cave organisms (Simon *et al*., 2007; Du Preez, 2014). Deforestation exacerbates these effects by reducing organic matter input which is crucial for cave detritivores and increasing soil erosion leading to sedimentation that clogs cave entrances and alters hydrological dynamics (Ladle & Whittaker, 2011). These disturbances not only threaten cave biodiversity and also compromise ecosystem services such as ground water filtration and paleoclimatic record preservation, thus the urgent need for conservation measures (Mammola *et al*., 2019).

1.7. Research problem

Despite the important ecosystem services provided by cave macroinvertebrates and the ecological roles they play in cave ecosystems, the inventory of Botswana's cave macroinvertebrates and other cave biodiversity is still in its infancy due to limited ecological studies in these kinds of subterranean ecosystems. The few ecological cave studies in Botswana have been undertaken at Gcwihaba cave, most likely because the cave is larger and easily explorable without cave equipment (Du Preez *et al.,* 2015). Cave macroinvertebrate diversity has only been quantitatively surveyed by (Mazebedi & Hesselberg 2020). Their preliminary survey did not look into seasonal patterns in the distribution of macroinvertebrates within the cave. A comprehensive study of cave macroinvertebrate assemblages was needed to improve the inventory of macroinvertebrates taxa and their community dynamics. To address the above knowledge gap about macroinvertebrates of Gcwihaba cave, the study set out to describe the spatio-temporal patterns of the cave's terrestrial macroinvertebrates communities. The hypothesis of this study was that macroinvertebrate communities of Gcwihaba cave undergo significant changes that are driven by weather seasons.

Study Objectives

The following objectives were drawn to address the aim above:

- To compare macroinvertebrate diversity and relative abundances across distinct cave zones within Gcwihaba cave during different weather seasons.

290 - To compare the similarity of macroinvertebrate assemblages of different cave zones across
291 different weather seasons.

CHAPTER 2: MATERIALS AND METHODS

2.1. Study area

2.1.1. Location

The Gcwihaba cave system is located within the Ngamiland district in the Okavango basin, to the north-western edge of Botswana (Figure 1) (Mbaiwa & Sakuze, 2009). The cave system is a complex underground tunnel network found underneath the low-lying dolomite Gcwihaba hills. The length of the cave is north-south oriented with corresponding north and south entrances (Dandurand *et al.,* 2019).

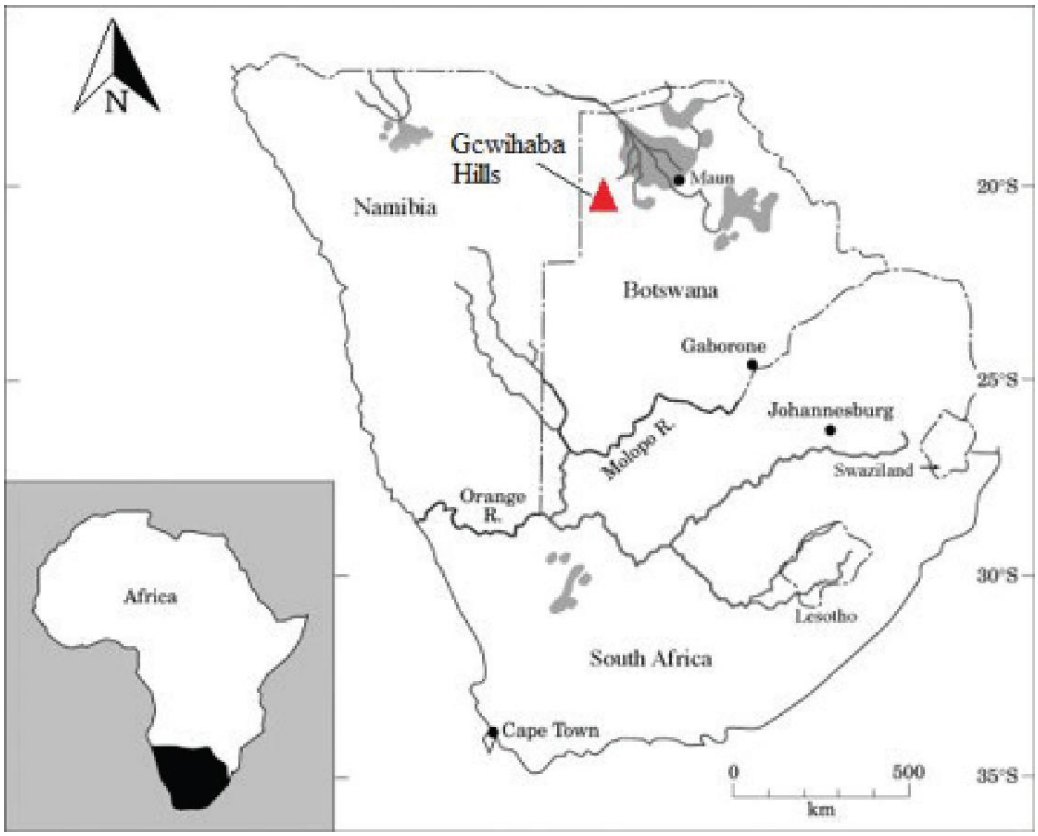


Figure 1. Location of Gcwihaba hills in Botswana indicated by a red triangle. (Mazebedi & Hesselberg., 2020)

2.1.2. Climate

The Gcwihaba hills are surrounded by a semi-arid, dense, shrub savanna, referred to as the Kalahari Thirstland (Thies & Lewis, 2015). The area receives an annual mean rainfall of 490 mm (Moses, 2019) with temperatures ranging from 8.5°C to 42.2°C (Thies & Lewis, 2015). Mean monthly maximum temperatures range between 32°C and 35°C in October and January, but daily maximums can be as high as 43°C. The lowest mean monthly temperature is about 10°C (recorded in July) (Moses, 2019).

2.1.3. Formation process of Gcwihaba cave

Gcwihaba cave was formed because of earth movements that occurred at the end of the pre-cambrian era (Cooke & Baillieul, 1974, Cooke 1975, Dandurand *et al.*, 2019). The earliest movements caused the elevation of the Gcwihaba hill mass above the earth surface. The second phase of earth movements involved a local up thrust of the dolomite part of the hill relative to the calcrete part resulting in a fault of about 3-5 m between the two rock types. The faults, which were in NW-SE direction, led to the formation of a river that flowed along the faults, resulting in the formation of Gcwihaba cave. The cave is likely formed by movement of acidic water across the fracture zones, which dissolved large volumes of the cave. The flow of the acidic water later formed a variety of secondary flowstone and dripstone formations including stalactites, stalagmites, massive bosses and pillars (Cooke 1975, Dandurand *et al.,* 2019). Some flowstones have been extensively eroded which Dandurand *et al.,* (2019) attributed to biogenic erosion. The base of some flowstones is covered by sandy material which could be either blown into the cave by wind or washed into the cave by water inflows from surface floods (Dandurand *et al.,* 2019).

2.1.4. Biodiversity of Gcwihaba area

The Gcwihaba hills and those around them, the Koanaka hills supports an assemblage of mammalian species, ranging from small rodents to large carnivores like cheetahs and flying ones like bats (Thies & Lewis, 2015). Previous studies together documented 43 mammalian species representing 7 orders. Three bat species (Chiroptera) which also occurs in Gcwihaba cave were documented in the area namely, *Rhinolophus denti* (Dent's horseshoe bat), *Hipposideros commersoni* (Commerson's leaf-nosed bat) and *Nycteris thebaica* (Egyptian

slit-faced) (Ferguson *et al.,*2010). Macroinvertebrate taxon like cave crickets, which have been found to be among the common taxa in Gcwihaba cave by Mazebedi & Hesselberg (2020) enhances biodiversity especially in caves like Gcwihaba cave. *C. secretus* cave cricket has been observed scavenging on mushrooms and other dead insects (Taylor *et al*., 2007). Beetles have also been observed to be common in Gcwihaba cave (Mazebedi & Hesselberg, 2020). Many of these cave beetles are predators preying on other invertebrates in the cave like the mites, collembola, and fly larvae in the guano. Some are specialized in terms of feeding, feeding either on eggs or larvae of a particular species (Culver & Pipan , 2009). Cave cockroaches (*Gyna sp*) also appear to be among the common taxa's in Gcwihaba cave (Mazebedi & Hesselberg, 2020). This taxon is typically found in association with guano, either burrowing in it or foraging. They are omnivores and consume fluids from rotting organic materials, i.e., from dead bats (Romero, 2009). The last observed common macroinvertebrate taxa in Gcwihaba cave is violin spiders (*Loxosceles sp*). These macroinvertebrates are top predators preying on a wide range of insects even those larger than them (Ponce-Saavedra *et al*., 2023). They also contribute food resources in the cave environment through laying of eggs and their dead bodies which may be eaten by other predators (Doran *et al.,* 2001). Predation by these macroinvertebrate taxa helps manage insect's populations thus balancing the cave ecosystem. This also helps ensure nutrients flow inside Gcwihaba cave.

2.2. Study design (Spatial and temporal design)

2.2.1. Spatial design

The cave zone was divided into three distinct zones based on light intensity, a key environmental gradient influencing cave ecosystems. Measurements were taken systematically at each sampling point along a transect extending from cave entrance to the deeper part of the cave (Lunghi *et al*., 2020). The light zone (0-20m) received direct sunlight and significant reflected light with average lux meter reading of 0.23 Klux. The transition zone (21-50m) exhibited diminishing light, with noticeable shadows and average meter reading of 0.01 Klux. The dark zone (51-130m) was characterised by complete darkness with light readings undetectable. These zones were established to represent a spatial gradient of light availability, a primary factor affecting macroinvertebrate distribution.

Given the cave's rugged and uneven terrain, establishing precise, continuous measurements throughout the entire cave length was logistically challenging. However, within each defined zone, pitfall traps were strategically placed along cave walls and across the floor to capture macroinvertebrates. Opportunistic visual surveys were conducted concurrently, with a hand held lamp to survey cave floor and walls and record macroinvertebrates observed. The number of individual macroinvertebrates of each species observed was recorded for each zone. This data augmented the pitfall trap data, providing a more comprehensive understanding of macroinvertebrates assemblages within each zone. The visual surveys were done by two people for 20 minutes per a 10 m interval. The same two visual surveyors carried out all collections for this study.

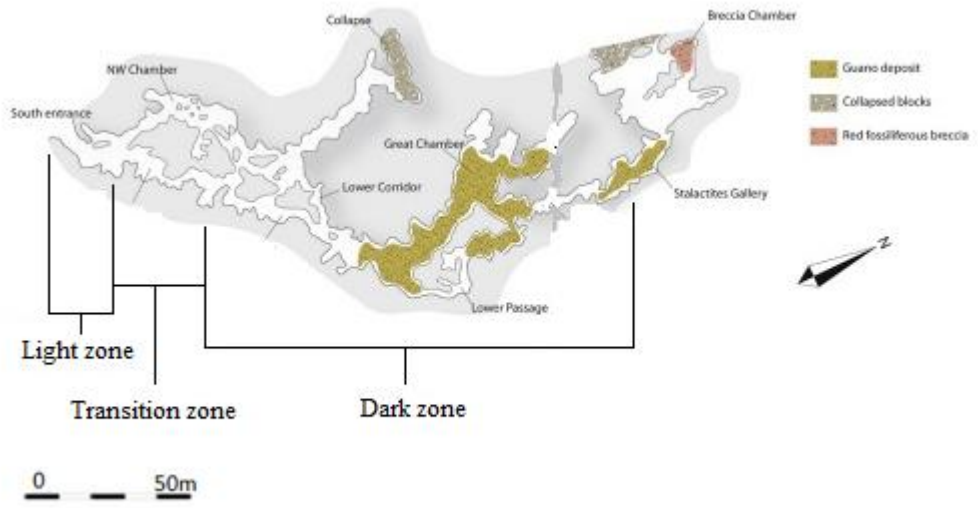


Figure 2. Approximate zoning of the cave. (Adapted and modified from Dandurand et al.,2019).

2.2.2. Temporal design

To determine macroinvertebrate abundance across seasons, collections were undertaken once in summer and once in winter. The winter collection was undertaken between the 24th of July 2021 and the 30th of July 2021, whereas the summer collection was undertaken between 2nd of February and the 11th of February 2022. The selected seasons present contrasting surface temperatures and humidity; hot and wet summers and cold and dry winters (as described in

2.1.2. Climate above). Although caves are stable environments, in highly fluctuating environments such as arid landscapes, they exhibit some degree of environmental fluctuations (Engel, 2011), which may influence seasonal variations in cave macroinvertebrate abundances.



Figure 3. A live pitfall trap.

Triplicate live pitfall traps made from 250 ml disposable cups were dug into the cave floor such that the mouth of each cup was on the same level as the floor surface (Figure 3). All the pitfall traps were retrieved after three days since it was observed that setting the traps longer led to death and cannibalism of trapped individuals. The use of live pitfall traps reduced disturbances on the fragile cave macroinvertebrate communities during installation and collection of samples after the three days, and allowed many macroinvertebrates to be caught during the sampling period with minimal effort.

Representative sample collections for each new species from the survey were taken to the Botswana International University of Science and Technology for identification. The sample collections were identified based on their morphological features using field guides prepared from the previous study by Mazebedi & Hesselberg (2020). The samples/specimens were

414 identified to meet necessary standards for scientific research purposes by experts.
415

2.3. Environmental variables measurements

To establish zones inside the cave (as described under 2.2.1.Spatial design), light intensity was measured using the Hanna HL97500 a portable lux meter. This instrument was selected for its sensitivity and ability to detect low light levels typical of cave environments. Measurements were taken at systematically at each sampling point along a transact extending from cave entrance to the deeper part of the cave (Lunghi *et al*., 2020). At each point, the lux meter sensor was held at ground level where macroinvertebrate activity was observed to capture light conditions directly influencing the macroinvetebrates. Readings were recorded in Klux, with the meter set to the 0.001-1.999 Klux range to allow for high resolution detection of low intensity light typical of caves.

2.4. Data Analysis

Diversity comparisons across cave zones and weather seasons were compared using Hill numbers computed for each cave zone using merged data for both seasons and separate seasons across zones. Hill numbers were used over the traditional diversity indices because they provide a unified and interpretable framework for comparing biodiversity while accounting for both species richness and evenness (Chao *et al*., 2014). These were used to compare macroinvertebrate diversity across cave zones. Hierarchical clustering with Bray-Curtis was used to measure similarity of cave zones based on species composition.

Comparison of macroinvertebrate diversity across seasons and cave zones was done by comparing data recorded during the two different seasons. A Poisson Generalized Linear Model was applied to analyse macroinvertebrate count data, as it is suitable for modelling discrete data that follow a Poisson distribution. The model used a log link function to examine the relationship between macroinvertebrate abundance and variables like season and cave zone, allowing for identification of statistically significant patterns. Furthermore, a PERMANOVA was used to assess statistical significance between macroinvertebrate composition of cave zones. A student t test was also used where appropriate. Statistical tests and graphs were performed using OriginPro 2022b software and Paleontological Statistics Software (PAST) version 5.02.

CHAPTER 3: RESULTS

3.1. Macroinvertebrates assemblage composition and diversity.

A total of 31 macroinvertebrate taxa belonging to 18 families were identified (Table 1). Cave cockroaches (*Blaberus*) were the most common with a total of 11078 observations, followed by cave crickets (Rhaphidophoridae) and violin spiders (*Loxosceles simillima)* with 1219 and 1183 observations, respectively. Only six taxa could not be identified to the family level. The majority of taxa were widely distributed throughout the cave, except for a few that occurred in only one or two zones. Out of the 31 taxa observed in this study, 18 had not been reported in previous surveys.

459 **Table 1. Taxonomic identification of taxa observed and their frequency of occurrence inside the cave. Un- unidentified.**

460

Taxa	Common name	Number of individuals	Class	Order	Family	Genus	Species	Cave zone(s) of occurrence
Blaberus	Cave cockroaches	11078	Insecta	Blattodea	Blaberidae	*Blaberus*	Un	L, T, D
Rhaphidophoridae	Cave crickets	1219	Insecta	Orthoptera	Rhaphidophoridae	Un	Un	L, T, D
Loxosceles simillima	Recluse/violin spiders	1183	Arachnida	Araneae	Sicarridae	*Loxosceles*	*Loxosceles simillima*	L, T, D
*Formicidae	Ants	1030	Insecta	Hymenoptera	Formicidae	Un	Un	L, T
*Carabidae	Ground beetles	630	Insecta	Coleoptera	Carabidae	Un	Un	L, T, D
*Periplaneta	Cave cockroaches	626	Insecta	Blattodea	Blattidae	Un	Un	L, T, D
**Paratachys*	Beetle	363	Insecta	Coleoptera	Carabidae	*Paratachys*	Un	L, T, D
**Cynaeus sp.*	Floor beetles	284	Insecta	Coleoptera	Tenebrionidae	*Cynaeus*	*Cynaeus sp*	L, T, D
Tenebrionidae	Darkling beetles	277	Insecta	Coleoptera	Tenebrionidae	Un	Un	L, T, D
Emesinae	Thread legged bugs	179	Insecta	Hemiptera	Reduviidae	Un	Un	L, T, D

Selenopidae	Flat spiders/wall spiders	107	Arachnida	Araneae	Selenopidae	Un	Un	L, T, D
*Trogidae	Hide beetles	97	Insecta	Coleoptera	Trogidae	Un	Un	L, T, D
**Strongylium*	Darkling beetles	87	Insecta	Coleoptera	Tenebrionidae	*Strongylium*	Un	L, T, D
Reduviidae	Assassin bugs	46	Insecta	Hemiptera	Reduviidae	*Emesinae*(subfamily)	Un	L, T, D
*Coccinellidae	Ladybug	40	Insecta	Coleoptera	Coccinellidae	Un	Un	T, D
*Isopoda	Pillbug	20	Malacostracans	Isopoda	Un	Un	Un	L, T, D
*Diplopoda	Millipede	19	Diplopoda	Un	Un	Un	Un	L
*Chilopoda	Centipede	14	Chilopoda	Un	Un	Un	Un	L
*Ixodida	Hard ticks	13	Ixodida	Ixodidae	Un	Un	Un	L, T, D
Lepidoptera	Butterflies/moths	13	Insecta	Lepidoptera	Un	Un	Un	L, T
Withiidae	Pseudoscorpion	11	Euchelicerata	Pseudoscorpiones	Withiidae	Un	Un	L, T, D
Physocyclus	Short bodied cellar spider	8	Euchelicerata	Araneae	Pholcidae	*Physocyclus*	Un	T, D
Pholcidae	Long bodied cellar spiders/dadd	5	Euchelicerata	Araneae	Pholcidae	Un	Un	L, T, D

	y long legs							
**Chlaenius tricolor*	Ground beetles	3	Insecta	Coleoptera	Carabidae	*Chlaenius*	*Chlaenius tricolor*	L
Vespidae	Wasps	3	Insecta	Hymenoptera	Vespidae	Un	Un	D
**Chlaenius dusaultii*	Ground beetles	2	Insecta	Coleoptera	Carabidae	*Chlaenius*	*Chlaenius dusaultii*	L
Gynnidomorpha alisman	Moths	2	Insecta	Lepidoptera	Tortricidae	*Gynnidomorpha*	*Gynnidomorpha alisman*	D
**Porcellionides*	Plum woodlouse	1	Malacostraca	Isopoda	Porcellionidae	*Porcellionides*	Un	L
*Cucujiformia	Beetle	1	Insecta	Coleoptera *Cucujiformia(infraorder)	Un	Un	Un	D
*Histeridae	Hister beetle	1	Insecta	Coleoptera	Histeridae	Un	Un	L
*Theriididae	Cobweb spider	1	Arachnida	Araneae	Theridiidae	*Theridion*	Un	D

461 *Shows taxa that were not recorded by previous surveys.

462 L – light zone, T – transition zone, D – dark zone

463 Un - Unknown

3.2 Variability of terrestrial macroinvertebrates across cave zones

The dark zone, transition zone and light zone had 15,13 and 14 number of species respectively (table 2). For Shannon diversity the light zone had the highest diversity(5.49),followed by transition and dark zone with 4.97 and 2.69 respectively. This indicates that the light zone and transition zones had a more balanced distribution of species compared to the dark zone. For Simpson diversity, the light zone still had the highest diversity (4.33) followed by transition and dark zones with 3.62 and 1.68 respectively. This indicates a few dominant species occupied the dark zone.

Table 2. Hill numbers (effective numbers) of macroinvertebrate taxa across cave zones.

Diversity order(q)	Light zone	Transition zone	Dark zone
q=0(Species richness)	14	13	15
q=1(Shannon diversity)	5.49	4.97	2.69
q=2(Simpson diversity)	4.33	3.62	1.68

According to figure 4, in terms of species composition, the light and transition zones are most similar as they merge at high similarity level (~ 0.7) while the dark zone is distinct from the rest as it joins the cluster at much lower similarity level meaning it has the most different species composition. A Poisson Generalized Linear Model (GLM) was conducted to examine the effect of cave zone (light zone, transition zone, dark zone) on macroinvertebrates abundance. The overall model was statistically significant (χ^2= 12.45, *df* = 2, *p* = 0.002), indicating that species abundance varied by cave zone. The dark zone had the highest macroinvertebrates abundance (*B* =6.45, *SE* =0.009, *z* = 688.98, *p* < 0.001). In comparison, macroinvertebrates abundance was significantly lower in the light zone (*B* =-1.45, *SE* = 0.021, *z* =-67.43, *p* <0.001) and the transition zone (*B* =-1.28, *SE* =0.020, *z* =-63.83, *p* < 0.001). These results suggest that macroinvertebrates are significantly more abundant in the dark zone compared to the other zones. A PERMANOVA was also conducted to assess whether there were statistically significant differences in the macroinvertebrate composition among the zones. The results indicated no significant differences (*F*= 1.19, *p* = 0.319).

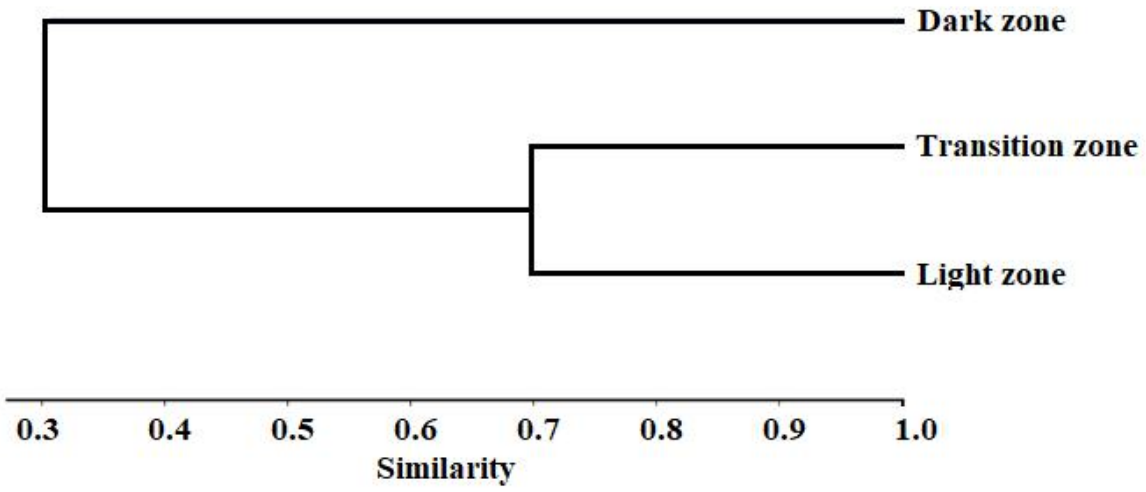


Figure 4. Hierarchical clustering of cave zones according to macroinvertebrate taxa.

3.3. Seasonal comparisons of terrestrial macroinvertebrates across seasons

According to table 3, species richness was higher across all zones during both seasons with a statistically significant difference of $t(2)= 5.00, p = 0.03$. Both Shannon and Simpson diversity decreased from light zone to dark zone with higher values recorded during the hot and wet season except in the light zone. However, the differences between seasons were not statistically significant for Shannon diversity ($t(2)= 1.18, p= 0.36$) and Simpson diversity ($t(2)= 0.85, p= 0.49$).

Table 3.Hill numbers (effective numbers) of macroinvertebrate taxa across seasons. A- hot and wet season, B- cold and dry season.

	Light zone		**Transition zone**		**Dark zone**	
Diversity order(q)	**A**	B	**A**	B	**A**	B
q=0(Species richness)	**10**	8	**10**	8	**9**	8
q=1(Shannon diversity)	**4.63**	4.67	**4.41**	3.90	**3.23**	3.12
q=2(Simpson diversity)	**5.48**	5.61	**5.17**	4.63	**3.82**	3.73

Hierarchical clustering shows the dark zone remained distinct across the two seasons, especially during the cold and dry season as it joined the cluster at very low similarity level (~0.1) (Figure 5). Light and transition zones were similar during both seasons as they merged at higher similarity levels. Paired student's t tests indicated no statistical difference of zones(in terms of abundance of macroinvertebrates) across the different seasons: transition zone (t(-1.59),p= 0.15) , dark zone (t(-1.28),p= 0.23). The light zone was close to significance, light zone (t(-2.06),p= 0.07).

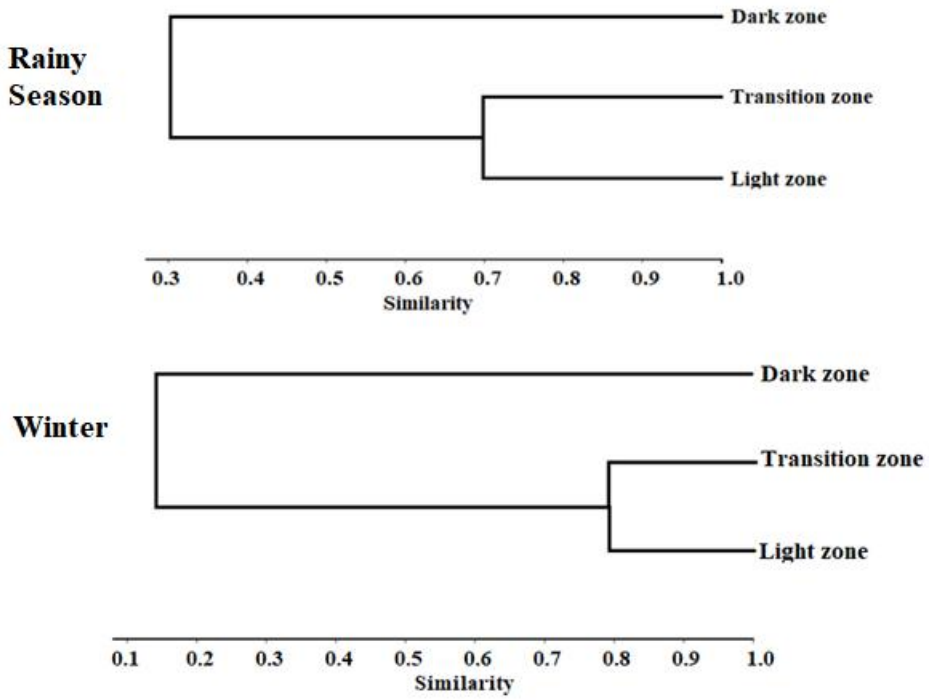


Figure 5. Hierarchical clustering of cave zones according to macroinvertebrate taxa across seasons. Rainy season-hot and wet season, Winter- cold and dry season.

Figure 6 illustrates the relative abundance (%) of various cave macroinvertebrates under two different seasonal conditions. *Histeridae*, *Pholcidae, Withiidae* and *Coccinellidae* show a higher relative abundance in the cold and dry season, suggesting that these groups thrive in colder and dry conditions. In contrast, taxa like *Porcellionidae*, *Trogidae* and *Tenebrionidae* exhibit a greater presence in the hot and wet season indicating adaptation to warmer environments. *Rhaphidophoridae, Sicarridae* and *Blaberidae* display a marked preference for the hot and wet season with minimal representation in the cold and dry season. A Poisson Generalized Linear Model revealed a significant effect of season on abundance, B = 1.23, SE = 0.018, z = 67.79, $p < 0.001$. Specifically, species abundance was significantly higher in the hot and wet season compared to the cold and dry season. These findings suggest that environmental conditions in the hot and wet season are more favourable for macroinvertebrates proliferation.

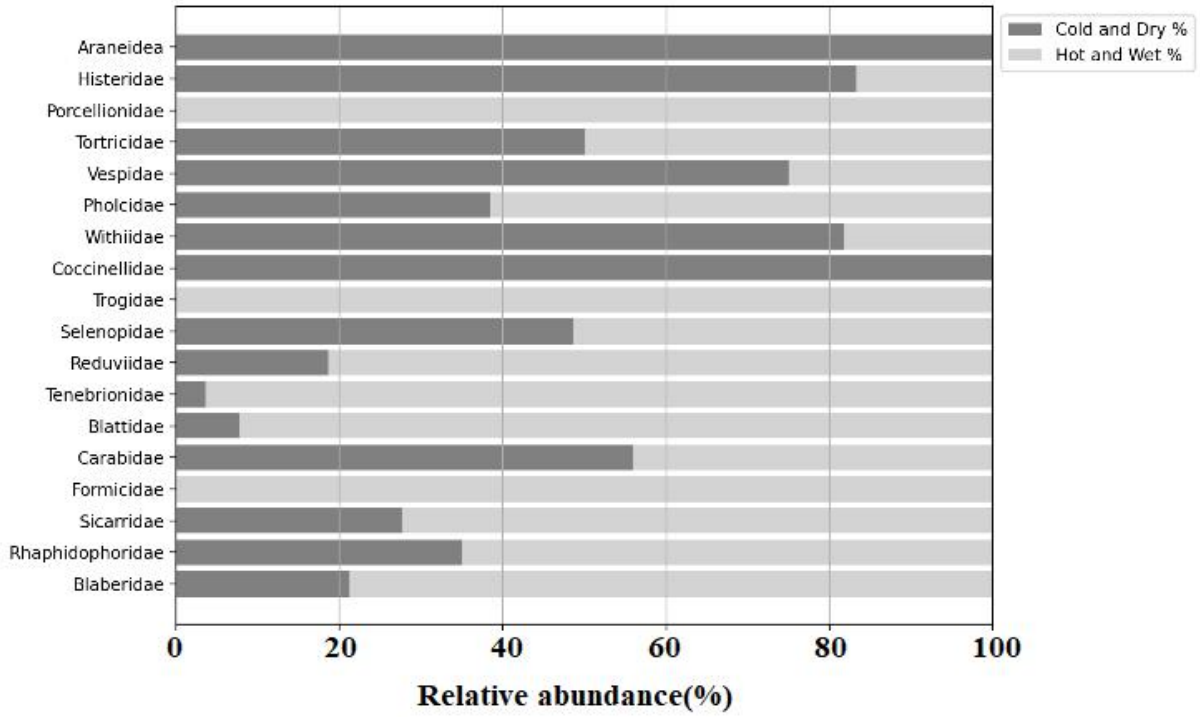


Figure 6. Relative abundance of macroinvertebrate taxa during cold and dry season and hot and wet season.

The estimated species richness (number of taxa) increases with sampling effort, suggesting that the actual number of species present is higher than what was recorded. The absence of a plateau in this rarefaction curve indicates that additional sampling would likely reveal more species (Figure 7).

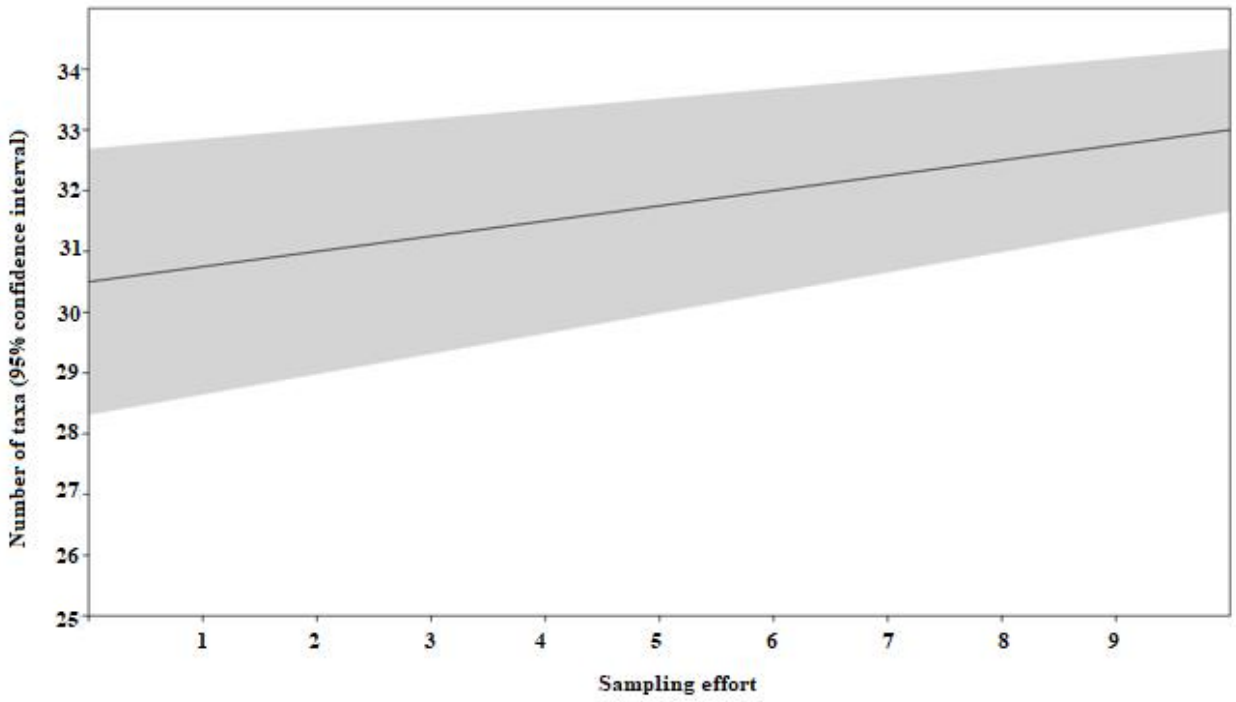


Figure 7. Estimated number of taxa to be observed with increase in sampling effort.

CHAPTER 4: DISCUSSION

4.1. The diversity of Gcwihaba cave macroinvertebrates

Our study identified a total of 31 macroinvertebrates taxa within Gcwihaba cave (table 1). This represents a substantial contribution to the known cave fauna, with 18 of these taxa not previously recorded by the previous study (Mazebedi & Hesselberg, 2020) in Gcwihaba cave. The newly recorded taxa were Formicidae found in light and transition zones only, Carabidae found across all three zones, *Periplaneta* found across all three zones, *Paratachys* found across all three zones, *Cynaeus sp.* found across all three zones, *Strongylium sp* found across all three zones, *Trogidae sp*. found across all three zones, Coccinellidae found in transition and dark zones only, Isopoda found across all three zones, Diplopoda found in light zone only, Chilopoda found in light zone only, Ixodida found across all three zones, *Chlaenius tricolor* found in light zone only, *Chlaenius dusaultii* found in light zone only, Theriididae found in dark zone only, Cucujiformia found in dark zone only, Histeridae found in light zone only, and *Porcellionides* found in light zone only. This highlights the importance of ongoing biodiversity surveys in understudied environments like caves. Dainelli *et al*., (2024) also recorded an equal number of taxa (31) in a guano-rich temperate cave. While 31 taxa were identified, the Mao's Tau rarefaction curve (Figure 7) continued to rise, suggesting that the true species richness of macroinvertebrates in Gcwihaba cave is likely higher and that further sampling efforts could reveal additional taxa.

The dominance of cave cockroaches (Blaberus) is notable, comprising a large proportion of the total individuals observed. The high abundance of cave crickets (Rhaphidophoridae) and violin spiders (*Loxosceles simillima)* (table 1) is another notable feature of this macroinvertebrate community. In temperate caves like Gcwihaba cave, cave crickets are considered as keystone species that can also be used as cave ecosystem bioindicators (Kurniawan *et al*., 2022) as they are the primary food sources for other cave species either as carcasses, their droppings or eggs (Yonder *et al*., 2010). Violin spiders act as natural predators in cave ecosystems preying on several cave dwellers (Silva & Ferreira., 2014). This

suggests the functional roles of these two taxa in Gcwihaba cave ecosystem, potentially regulating other invertebrate populations in this ecosystem.

The highest species richness was observed in the dark zone, closely followed by the light zone and transition zone (Table 2). However, when considering diversity indices, a different pattern emerges. Shannon diversity(q=1) and Simpson diversity(q=2) decrease from the light zone progressively with distance into the cave (Table 2). A similar pattern was observed by Mazebedi & Hesselberg (2020) and in other caves as well (Salaga, 2020). The light zone exhibited the highest Shannon diversity (H'= 5.49) and Simpson diversity (D= 4.33), indicating a more balanced distribution of species. The transition zone showed intermediate values (H'= 4.97, D= 3.62), while the dark zone had considerably lower diversity (H'=2.69, D=1.68). The lower diversity in the dark zone, despite similar species richness, suggests the dominance of a few species, as confirmed by the low Simpson diversity. This is consistent with expectations for cave ecosystems, where limited resources and environmental constraints often lead to communities dominated by highly adapted specialists (Milks *et al*., 2024). The cluster analysis (Figure 4) further supports these findings. The distinct separation of the dark zone from the other two zones highlights the distinct community composition in the deeper parts of the cave. The closer clustering of light and transition zones suggests a greater similarity in their macroinvertebrate communities, likely due to the shared influence of surface conditions and the observed similarities of the cave floor substrates. Gcwihaba cave is an important tourism attraction site in the area (Mbaiwa & Sakuze, 2009), and therefore tourists' movement within the cave could also play a role in the distribution of ground dwelling macroinvertebrates, but this needs to be confirmed. Other environmental factors like cave soil pH, electronic conductivity and moisture content could also be attributing factors to this pattern of diversity as shown in a previous study by Mazebedi & Hesselberg, (2020) in the same cave.

The species richness of macroinvertebrates for Gcwihaba cave was relatively high at 31 (Table 1) compared to 12 reported by the previous study (Mazebedi & Hesselberg, 2020). Even when compared with other caves globally, species richness reported in this study is relatively high. For example, Macud & Nuneza (2014) reported only ten species of macro-

invertebrates belonging to six orders in the Mighty Cave in Tagoloan, Lanao del Norte, Philippines. High species richness in cave ecosystems is often associated with fewer disturbances in the cave (Salaga, 2020). For example, caves that experience frequent human movements through tourism and other activities often have low macroinvertebrates abundance (Calaforra *et al*., 2003). However, other studies have reported more species richness, for instance, Pape & O'Connor, (2014) reported 98 species, including 16 species that were new to science in the Kartchner Caverns in Arizona. It is important to also note that other factors such as cave size, shape and type, sampling method, other environmental conditions play a vital role in species detection and diversity and could have led to the differences in diversity between the present study and the one mentioned above.

4.2. Seasonal variation of Gcwihaba cave macroinvertebrates.

The seasonal differences in macroinvertebrate diversity and similarity across zones reveal important ecological patterns within Gcwihaba cave. According to table 3 species richness (q=0) was generally higher in the hot and wet season across all zones, with a statistically significant difference (t (2) = 5.00, p = 0.03). Similarly, Shannon (q=1) and Simpson diversity (q=2) were higher during the hot and wet season compared to the cold and dry season, except in the light zone, where values remained relatively stable. These trends suggest that warmer temperatures and higher humidity levels during the wet season promotes increased biological activity and resource availability, which may explain the observed increase in species richness and evenness (Culver & Pipan, 2019). Figure 5 supports these observations by showing that cave zone similarity was higher during the hot and wet season (light and transition zones clustering at 0.7, dark zone diverging at 0.3) and lower during the cold and dry season (light and transition zones clustering at 0.8, dark zone diverging at 0.1). This suggests that during the hot and wet season, macroinvertebrates taxa were more evenly distributed across zones, leading to a more homogenous community structure, whereas during the cold and dry season, the dark zone became more distinct, indicating a relatively more specialized and isolated community. This is likely due to a slight drop in temperatures and humidity in the dark zone during the cold and dry season thus only a select few more adapted taxa could survive here. This is consistent with the decrease in species richness from hot and wet to cold and dry season across all zones (Table 3), particularly in the dark zone, where richness drops from 9 taxa to 8 taxa. Shannon and Simpson diversity also decline in the dark and transition zones during winter, further indicating that fewer species dominate the

community during the cold and dry season. These findings are supported by previous studies, which report that seasonal variations in temperature and humidity significantly influence subterranean species distributions (Tobin *et al*., 2013: Lunghi *et al*., 2017: Mammola *et al*., 2015).

Seasonal changes have shown to be influencing the overall abundance of macroinvertebrates in this cave. A general trend of higher macroinvertebrate abundance in the hot and wet season compared to the cold and dry was observed (Figure 6). This could be due to an increased influx of food resources during the hot and wet season from the outside environment in the form of tree leaves and rich guano from the insectivorous bats in the caves (Souza- Silva *et al.*, 2012). Another reason for the low abundance during cold and dry season could be due to overwintering mechanisms used by macroinvertebrates like hibernation to shield themselves from prevailing unfavourable conditions (Santos *et al.,* 2020; Day *et al.,* 2020). They may do this by migrating into even smaller cracks in cave walls and/or diapause, whereby some invertebrates discontinue their development and spend the winter season as either larvae or pupae until there are optimum conditions for them to develop (Farooq *et al.,* 2022). Thus, the observed fewer numbers during the cold and dry season.

Studies done by (Silva & Ferreira, 2014; Bento *et al.*, 2016) concurs with the present study as they reported an increase in abundance of invertebrates during hot, rainy seasons than in cold, dry seasons in caves. Poisson Generalized Linear Model revealed a significant effect of season on diversity of macroinvertebrates. This significant effect of seasonal variation is evidenced by the relative abundance of certain taxa like ants (Formicidae), ladybug (Coccinellidae), plum woodlouse (Porcellionidae) and hide beetles (Trogidae) as they were observed in only one of the seasons. These variations could be linked to specific life cycles, resource availability (Souza- Silva *et al.,* 2012) or microclimatic preferences. Specifically, the notable high relative abundance of ants (Formicidae) during the hot and wet season could be related to increased foraging activity (Roeder *et al*., 2022) or colony expansion (Wong *et al*., 2023) during warmer months. The dominance of cave cockroaches (Blaberidae), cave crickets (Rhaphidophoridae) and violin spiders (Sicarridae) across both seasons is a sign of their key roles and thus qualifying them to be considered as keystone species for any management plan for Gcwihaba cave. This could also be testament to their high degree of adaptability to this environment.

Cave cockroaches (Blaberidae) were the most abundant of all taxa recorded in this study (Table1) and were dominant during both seasons. Cave cockroaches (Blaberidae) were also observed scavenging on bat carcasses in the dark zone, which also could be a contributing factor to the observed abundance of cockroaches in both seasons (Bell *et al.,,* 2007). Similar studies (Macud & Nuneza, 2014; Kurniawan *et al.,* 2021; Mazebedi & Hesselberg., 2020) have also reported cave cockroaches as being among the most abundant taxa in caves. On the contrary, Marshall & Beehler (2007) have reported cave crickets to be the most abundant species in deep zone areas with thick guano deposits. They are usually considered as keystone species, owing to their large numbers (Weckerly, 2012). This could be as a result of cave cockroaches being the most adapted species in Gcwihaba cave as opposed to cave crickets as in the contrasting study. Despite this, cave crickets were the second most abundant species. Kurniawan *et al.,* (2022) also observed that cave cockroaches prefer the more humid dark zones.

One significant finding of particular interest was the sighting of reddish coloured ants (*Formicidae*) actively moving into the cave in the light and transition zones during the hot and wet season. Even though this ant taxa could not be identified to species level in the current study, its presence is a significant finding. Certain red ant species are known to be invasive and can pose a serious threat to cave ecosystems (Wagner & Van Driesche., 2010; Nicolosi *et al*., 2023). These ants can disrupt native invertebrate communities through predation, competition for the limited resources and alteration of the cave environment. Wagner & Van Driesche (2010) specifically highlight the negative impacts of these ants on cave beetles, a group that was also observed in Gcwihaba cave. This observation therefore provides compelling evidence that the cave fauna and fragile ecosystem here could be under serious threat and highlights an urgent need for management action to conserve this unique environment. The presence of Formicidae, together with other taxa like Carabidae, and Tenebrionidae, which are trogloxenes and troglophiles, may suggest that Gcwihaba cave acts as an ecotone between surface and subterranean habitats (Moseley, 2009).

4.3. Study Limitations

This study offers important insights into the spatio-temporal patterns of macroinvertebrate communities within Gcwihaba cave, however while efforts were made to identify all specimens to the lowest possible taxonomic level, limitations in taxonomic expertise and the

availability of identification resources may have resulted in some uncertainties. In particular, some specimens could only be identified to the family or genus level. Future research should utilize molecular techniques to improve the accuracy and resolution of species identifications.

4.4. Conclusion

In conclusion, this present study has expanded previous records by identifying 31 taxa,18 of which were not reported previously. While species richness remained relatively consistent across cave zones, a notable shift in community composition was observed, with diversity declining from light zone to the dark zone. This pattern suggests a transition from a more balanced community near the cave entrance to one dominated by specialised species adapted to harsher conditions in the deeper parts of the cave. Climatic seasons also influenced macroinvertebrate dynamics, with a greater macroinvertebrate abundance during the rainy season (hot and wet) season compared to the winter season (cold and dry). The seasonal differences were attributed to increased food resource availability and warmer temperatures with favoured high macroinvertebrate activity. While this study provided valuable knowledge on the macroinvertebrate taxa that are supported by Gcwihaba cave as well as their community dynamics, there is still needed to further our understanding especially the knowledge of the taxa at species level. Through well designed approaches, further research should also focus on explore the influence of environmental factors on individual taxa.

References

Araújo, C. S., Candido, D. M., de Araújo, H. F., Dias, S. C., & Vasconcellos, A. (2010). Seasonal variations in scorpion activities (Arachnida: Scorpiones) in an area of Caatinga vegetation in northeastern Brazil. Zoologia (Curitiba), 27, 372-376.

Badino, G. (2004). Clouds in caves. Speleogenesis and Evolution of Karst Aquifers, 2(2), 1-2.

Badino, G., & Torelli, L. (2014). The 'Progetto Kronio': history and problems of an extreme exploration in an intact archaeological deposit. *From Cave to Dolmen. Ritual and Symbolic Aspects in the Prehistory Between Sciacca, Sicily and the Central Mediterranean, Oxford, Archaeopress*, 31-42.

Bell, W. J., Roth, L. M., & Nalepa, C. A. (2007). *Cockroaches: ecology, behavior, and natural history*. JHU Press.

Benedetto, G., Madau, F. A., Carzedda, M., Marangon, F., & Troiano, S. (2022). Social economic benefits of an underground heritage: Measuring willingness to pay for karst caves in Italy. *Geoheritage*, *14*(2), 69.

Bento, D. D. M., Ferreira, R. L., Prous, X., Souza-Silva, M., Bellini, B. C., & Vasconcellos, A. (2016). Seasonal variations in cave invertebrate communities in the semiarid Caatinga, Brazil. Journal of Cave and Karst Studies, 78(2), 61-71.

Biswas, J. (2010). Kotumsar Cave biodiversity: a review of cavernicoles and their troglobiotic traits. Biodiversity and conservation, 19(1), 275-289.

Borisov, R. R., Chertoprud, E. S., Palatov, D. M., & Novichkova, A. A. (2021). Variability in macrozoobenthic assemblages along a gradient of environmental conditions in the stream water of karst caves (Lower Shakuranskaya Cave, western Caucasus). *Subterranean Biology*, *39*, 107-127.

Brunet, A. K., & Medellín, R. A. (2001). The species–area relationship in bat assemblages of tropical caves. Journal of Mammalogy, 82(4), 1114-1122.

Calaforra, J. M., Fernández-Cortés, A., Sánchez-Martos, F., Gisbert, J., & Pulido-Bosch, A. (2003). Environmental control for determining human impact and permanent visitor capacity in a potential show cave before tourist use. Environmental Conservation, 30(2), 160-167.

Canedoli, C., Ficetola, G. F., Corengia, D., Tognini, P., Ferrario, A., & Padoa-Schioppa, E. (2022). Integrating landscape ecology and the assessment of ecosystem services in the study of karst areas. *Landscape Ecology*, 1-19.

Cardoso, R. C., Ferreira, R. L., & Souza-Silva, M. (2021). Priorities for cave fauna conservation in the Iuiú karst landscape, northeastern Brazil: a t Cheeptham, N. (2013).

Advances and challenges in studying cave microbial diversity. Cave microbiomes: A novel resource for drug discovery, 1-34.threatened spot of troglobitic species diversity. Biodiversity and Conservation, 1-23.

Chagas-Jr, A., & Bichuette, M. E. (2018). A synopsis of centipedes in Brazilian caves: hidden species diversity that needs conservation (Myriapoda, Chilopoda). ZooKeys, (737), 13.

Chao, A., Gotelli, N. J., Hsieh, T. C., Sander, E. L., Ma, K. H., Colwell, R. K., & Ellison, A. M. (2014). Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecological monographs*, *84*(1), 45-67.

Chertoprud, E. S., Borisov, R. R., Palatov, D. M., Marinskiy, V. V., Krylenko, S. V., Kovacheva, N. P., & Pichkhaia, I. (2020). Aquatic macro-invertebrate fauna of caves of Samegrelo-Zemo Svaneti and Imereti, western Georgia, Caucasus: Troglobiotic and epigean species complexes. Зоологический журнал, 99(3), 275-289.

Chiarini, V., Duckeck, J., & De Waele, J. (2022). A global perspective on sustainable show cave tourism. *Geoheritage*, *14*(3), 82.

826 Christman, M. C., Culver, D. C., Madden, M. K., & White, D. (2005). Patterns of endemism
827 of the eastern North American cave fauna. Journal of Biogeography, 32(8), 1441-1452.
828
829 Cigna, A. A. (2012). The problem of lampenflora in show caves. Acta Carsologica Slovaca,
830 50(1), 5-10.
831
832 Cooke, H. (1975). The palaeoclimatic significance of caves and adjacent landforms in
833 western Ngamiland, Botswana. Geographical Journal, 430-444.
834
835 Cooke, HJ & Baillieul, T. (1974). The caves of Ngamiland: an interim report on explorations
836 and fieldwork 1972-74. Botswana Notes & Records, 6(1), 147-156.
837
838 Covington, M. D., & Perne, M. (2015). Consider a cylindrical cave: A physicist's view of
839 cave and karst science. Acta Carsologica, 44(3).
840
841 Cuff, J. P., Aharon, S., Steinpress, I. A., Seifan, M., Lubin, Y., & Gavish-Regev, E. (2021).
842 It's all about the zone: Spider assemblages in different ecological zones of levantine
843 caves. *Diversity, 13*(11), 576.
844
845 Culver, D., & Pipan, T. (2009). The Biology of Caves and Other Subterranean Habitats
846 (Biology of Habitats).
847
848 Culver, D. C., & Pipan, T. (2018). Insects in caves. Insect Biodiversity: Science and Society,
849 3, 123-152.
850
851 Culver, D. C., & Pipan, T. (2019). *The biology of caves and other subterranean habitats*.
852 Oxford University Press.
853
854 Culver, D. C., & Wilkens, H. (2000). Critical review of the relevant theories of the evolution
855 of subterranean animals. *Ecosystems of the World*, 381-398.
856
857 Dainelli, L., Mammola, S., Martínez, A., Langeneck, J., Serena, F., & Petroni, G. (2024). The
858 terrestrial macroinvertebrates of a guano-rich temperate cave–a system especially vulnerable
859 to climate change?. In *Our Symposium* (p. 38).

Dandurand, G., Duranthon, F., Jarry, M., Stratford, D. J., & Bruxelles, L. (2019). Biogenic corrosion caused by bats in Drotsky's Cave (the Gcwihaba Hills, NW Botswana). Geomorphology, 327, 284-296.

Day, C. A., Lewandowski, K., Vonesh, J. R., & Byrd, B. D. (2020). Phenology of Rock Pool Mosquitoes in the Southern Appalachian Mountains: Surveys Reveal Apparent Winter Hatching of Aedes japonicus and the Potential For Asymmetrical Stage-Specific Interactions. *Journal of the American Mosquito Control Association*, *36*(4), 216-226.

Deharveng, L., & Bedos, A. (2018). Diversity of terrestrial invertebrates in subterranean habitats. *Cave ecology*, 107-172.

De Mandal, S., Panda, A. K., Bisht, S. S., & Kumar, N. S. (2015). First report of bacterial community from a bat guano using Illumina next-generation sequencing. Genomics Data, 4, 99-101.

Dippenaar-Schoeman, A. S., & Myburgh, J. G. (2009). A review of the cave spiders (Arachnida: Araneae) from South Africa. *Transactions of the Royal Society of South Africa*, *64*(1), 53-61.

Domínguez-Villar, D., Lojen, S., Krklec, K., Baker, A., & Fairchild, I. J. (2015). Is global warming affecting cave temperatures? Experimental and model data from a paradigmatic case study. Climate Dynamics, 45, 569-581.

Doran, N. E., Richardson, A. M. M., & Swain, R. (2001). The reproductive behaviour of the Tasmanian cave spider Hickmania troglodytes (Araneae: Austrochilidae). *Journal of Zoology*, *253*(3), 405-418.

Du Preez, G. C. (2014). *Determining the effect of polluted mine water on the ecosystem health of a karstic cave environment in the Witwatersrand Basin* (Doctoral dissertation, North-West University).

Du Preez, G. C., Jacobs, G., Jordaan, A., Tiedt, L. R., & Forti, P. (2015). Hairy Stalagmites, a new biogenic root speleothem from Botswana.

Elshall, A. S., Arik, A. D., El-Kadi, A. I., Pierce, S., Ye, M., Burnett, K. M., ... & Chun, G. (2020). Groundwater sustainability: A review of the interactions between science and policy. *Environmental Research Letters*, *15*(9), 093004.

Engel, A. S. (2010). Microbial diversity of cave ecosystems. In *Geomicrobiology: molecular and environmental perspective*(pp. 219-238). Dordrecht: Springer Netherlands.

Engel, A. S. (2011). Karst ecosystems. Encyclopedia of Geobiology. Springer Encyclopedia of Earth Sciences Series (EESS, formerly Kluwer Edition), Berlin, 521-553.

Faimon, J., Troppová, D., Baldík, V., & Novotný, R. (2012). Air circulation and its impact on microclimatic variables in the Císařská Cave (Moravian Karst, Czech Republic). *International Journal of Climatology*, *32*(4), 599-623.

Farooq, M., Li, X., Li, Z., Yang, R., Tian, Z., Tan, L., ... & Xiao, W. (2022). The joint contributions of environmental filtering and spatial processes to macroinvertebrate metacommunity dynamics in the alpine stream environment of Baima Snow Mountain, Southwest China. *Diversity*, *14*(1), 28.

Faulkner, T. (2010). An external model of speleogenesis during Quaternary glacial cycles in the marbles of Central Scandinavia. *Cave and Karst Science*, *37*(3), 79-92.

Fejér, A., & Moldovan, O. (2013). Population size and dispersal patterns for a Drimeotus (Coleoptera, Leiodidae, Leptodirini) cave population. *Subterranean Biology*, *11*, 31-44.

Ferguson, A. W., McDonough, M. M., Thies, M. L., Lewis, P. J., & Gabadirwe, M. (2010). Mammals of the Koanaka Hills region in Ngamiland: A provisional checklist. Botswana Notes and Records, 42, 163-171.

Ferreira, R. L., Berbert-Born, M., & Souza-Silva, M. (2023). The Água Clara cave system in northeastern Brazil: the richest hotspot of subterranean biodiversity in South America. *Diversity*, *15*(6), 761.

928 Ferreira, R. L., Giribet, G., Du Preez, G., Ventouras, O., Janion, C., & Silva, M. S. (2020).
929 The Wynberg cave system, the most important site for cave fauna in South Africa at risk.
930 Subterranean Biology, 36, 73-81.
931
932 Ferreira, R. L., Prous, X., & Martins, R. P. (2007). Structure of bat guano communities in a
933 dry Brazilian cave. Tropical Zoology, 20(1), 55-74.
934
935 Ford, D., & Williams, P. D. (2007). *Karst hydrogeology and geomorphology*. John Wiley &
936 Sons.
937
938 Galindo-Pérez, E. J., Chávez-Sandoval, B. E., Espinoza-Graciano, E., Flores-Martínez, M. D.
939 C., Villeda-Callejas, M. D. P., Bhalli, J. A., ... & García-Franco, F. (2017). Cave
940 macroinvertebrates used as bioindicators of water quality. *Tecnología y ciencias del*
941 *agua*, *8*(5), 5-17.
942
943 Gilli, É. (2015). Karstology: Karsts, Caves and Springs: Elements of Fundamental and
944 Applied Karstology. CRC Press.
945
946 Gillieson, D. S. (2011). Management of caves. In Karst management (pp. 141-158). Springer,
947 Dordrecht.
948
949 Gunn, J. (2004). CONSERVATION: CAVE BIOTA. In *Encyclopedia of Caves and Karst*
950 *Science* (pp. 529-537). Routledge.
951
952 Harvey, M. S., & Du Preez, G. (2014). A new troglobitic ideoroncid pseudoscorpion
953 (Pseudoscorpiones: Ideoroncidae) from southern Africa. *The Journal of Arachnology*, *42*(1),
954 105-110.
955
956 Hesselberg, T., Simonsen, D., & Juan, C. (2019). Do cave orb spiders show unique
957 behavioural adaptations to subterranean life? A review of the evidence. *Behaviour*, *156*(10),
958 969-996.
959

Horváth, G., Sztruhala, S. S., Balázs, G., & Herczeg, G. (2021). Population divergence in aggregation and sheltering behaviour in surface-versus cave-adapted Asellus aquaticus (Crustacea: Isopoda). *Biological Journal of the Linnean Society*, *134*(3), 667-678.

Howarth, F. G. (2009). Cave insects. In Encyclopedia of insects (pp. 139-143). Academic Press

Howarth, F. G., & Moldovan, O. T. (2018). The ecological classification of cave animals and their adaptations. *Cave ecology*, 41-67.

Howarth, F. G., & Moldovan, O. T. (2018). Where cave animals live. *Cave ecology*, 23-37.

Jaffe, R., Prous, X., Zampaulo, R., Giannini, T. C., Imperatriz-Fonseca, V. L., Maurity, C., ... & Siqueira, J. O. (2016). Reconciling mining with the conservation of cave biodiversity: a quantitative baseline to help establish conservation priorities. *PloS one*, *11*(12), e0168348.

Jones, D. S., & Macalady, J. L. (2016). The snotty and the stringy: energy for subsurface life in caves. *Their world: A diversity of microbial environments*, 203-224.

Juan, C., Guzik, M. T., Jaume, D., & Cooper, S. J. (2010). Evolution in caves: Darwin's 'wrecks of ancient life'in the molecular era. Molecular Ecology, 19(18), 3865-3880.

Kane, T. C., & Ryan, T. (1983). Population ecology of carabid cave beetles. Oecologia, 60(1), 46-55.

Kunz, T. H., Braun de Torrez, E., Bauer, D., Lobova, T., & Fleming, T. H. (2011). Ecosystem services provided by bats. Annals of the New York academy of sciences, 1223(1), 1-38.

Kurniawan, I. D., Mustahiq Akbar, R. T., Ulfa, R. A., Wardani, M. K., & Satria, B. (2022). Population structure and habitat preference of cave crickets (Rhaphidophora sp.(Orthoptera: Rhaphidophoridae)) in Sanghyang Kenit cave, Citatah karst area, West Java. *Journal of Tropical Biodiversity & Biotechnology*, *7*(3).

Kurniawan, I. D., Soesilohadi, R. H., Rahmadi, C., Caraka, R. E., & Pardamean, B. (2018). The difference on Arthropod communities' structure within show caves and wild caves in Gunungsewu Karst area, Indonesia. *Ecol Environ Conserv*, *24*(1), 81-90.

Kurniawan, I., Susila, I., Kinasih, I., Rahman, I., & Nasrullah, R. (2021, January). Preliminary Study on Cavernicolous Insecta Community in Tasikmalaya Karst Area, West Java. In *Proceedings of the 1st International Conference on Islam, Science and Technology, ICONISTECH 2019, 11-12 July 2019, Bandung, Indonesia.*

Ladle, R. J., & Whittaker, R. J. (Eds.). (2011). *Conservation biogeography*. John Wiley & Sons.

Lavoie, K. H., Helf, K. L., & Poulson, T. L. (2007). The biology and ecology of North American cave crickets. Journal of Cave and Karst Studies, 69(1), 114-134.

Liebherr, J. K., & Krushelnycky, P. D. (2007). Unfortunate encounters? Novel interactions of native Mecyclothorax, alien Trechus obtusus (Coleoptera: Carabidae), and Argentine ant (Linepithema humile, Hymenoptera: Formicidae) across a Hawaiian landscape. *Beetle Conservation*, 61-73.

Lucanas, C. C., & Lit Jr, I. L. (2016). Cockroaches (Insecta, Blattodea) from caves of Polillo Island (Philippines), with description of a new species. *Subterranean Biology*, *19*, 51-64.

Lunghi, E., Corti, C., Mulargia, M., Zhao, Y., Manenti, R., Ficetola, G. F., & Veith, M. (2020). Cave morphology, microclimate and abundance of five cave predators from the Monte Albo (Sardinia, Italy). *Biodiversity Data Journal*, *8*, e48623.

Lunghi E, Manenti R, Ficetola GF (2014) Do cave features affect underground habitat exploitation by non-troglobite species? Acta Oecologica 55: 29–35.

Lunghi, E., Manenti, R., & Ficetola, G. F. (2017). Cave features, seasonality and subterranean distribution of non-obligate cave dwellers. *PeerJ*, *5*, e3169.

Macud, A. M., & Nuneza, O. M. (2014). Diversity of cave macro-invertebrates in Mighty cave, Tagoloan, Lanao del Norte, Philippines. Journal of Biodiversity and Environmental Sciences, 5(3), 276-386.

Mammola, S. (2019). Finding answers in the dark: caves as models in ecology fifty years after Poulson and White. Ecography, 42(7), 1331-1351.

Mammola, S., Cardoso, P., Culver, D. C., Deharveng, L., Ferreira, R. L., Fišer, C., ... & Zagmajster, M. (2019). Scientists' warning on the conservation of subterranean ecosystems. *BioScience*, *69*(8), 641-650.

Mammola, S., Piano, E., Cardoso, P., Vernon, P., Domínguez-Villar, D., Culver, D. C., ... & Isaia, M. (2019). Climate change going deep: The effects of global climatic alterations on cave ecosystems. *The Anthropocene Review*, *6*(1-2), 98-116.

Mammola, S., Elena, P., Mauro, G. P., & Isaia, M. (2015). Seasonal dynamics and micro-climatic preference of two Alpine endemic hypogean beetles. *International Journal of Speleology*, *44*(3), 239-249.

Mammola, S., & Isaia, M. (2017). Spiders in caves. Proceedings of the Royal Society B: Biological Sciences, 284(1853), 20170193.

Mammola, S., Cardoso, P., Ribera, C., Pavlek, M., & Isaia, M. (2018). A synthesis on cave-dwelling spiders in Europe. *Journal of Zoological Systematics and Evolutionary Research*, *56*(3), 301-316.

Mammola, S., Amorim, I. R., Bichuette, M. E., Borges, P. A., Cheeptham, N., Cooper, S. J., ... & Cardoso, P. (2020). Fundamental research questions in subterranean biology. *Biological Reviews*, *95*(6), 1855-1872.

Mammola, S., Pavlek, M., Huber, B. A., Isaia, M., Ballarin, F., Tolve, M., ... & Cardoso, P. (2022). A trait database and updated checklist for European subterranean spiders. Scientific data, 9(1), 236.

Marshall, A. J., & Beehler, B. M. (2007). The ecology of Papua. (No Title).

Mazebedi, R., & Hesselberg, T. (2020). A preliminary survey of the abundance, diversity and distribution of terrestrial macroinvertebrates of Gcwihaba cave, northwest Botswana. *Subterranean Biology*, *35*.

Mbaiwa, J. E., & Sakuze, L. K. (2009). Cultural tourism and livelihood diversification: The case of Gcwihaba Caves and XaiXai village in the Okavango Delta, Botswana. Journal of Tourism and Cultural Change, 7(1), 61-75.

Medina, M. J. A. L. D. O. (2023). *Climate change goes underground: temperature in caves and its effects on subterranean organisms* (Doctoral dissertation).

Medina, M. J., Antić, D., Borges, P. A., Borko, Š., Fišer, C., Lauritzen, S. E., ... & Reboleira, A. S. P. (2023). Temperature variation in caves and its significance for subterranean ecosystems. Scientific reports, 13(1), 20735.

Mendes Rabelo, L., Souza-Silva, M., & Lopes Ferreira, R. (2021). Epigean and hypogean drivers of Neotropical subterranean communities. Journal of Biogeography, 48(3), 662-675.

Mermillod-Blondin, F., Lefour, C., Lalouette, L., Renault, D., Malard, F., Simon, L., & Douady, C. J. (2013). Thermal tolerance breadths among groundwater crustaceans living in a thermally constant environment. Journal of Experimental Biology, 216(9), 1683-1694.

Milks, K., Cloud, F. B., & Morris, J. (2024). Cave Biodiversity: Speciation and Diversity of Subterranean Fauna. *The American Biology Teacher*, *86*(5), 323-325.

Moses, O. (2019). Weather systems influencing Botswana rainfall: the case of 9 December 2018 storm in Mahalapye, Botswana. Modeling Earth Systems and Environment, 5(4), 1473-1480.

Moutaouakil, S., Souza-Silva, M., Oliveira, L. F., Ghamizi, M., & Ferreira, R. L. (2024). A cave with remarkably high subterranean diversity in Africa and its significance for biodiversity conservation. *Subterranean Biology*, *50*, 1-28.

Nicolosi, G. (2023). The Effects of Human Impacts on Cave Ecosystems and Subterranean Biodiversity.

Nicolosi, G., Mammola, S., Verbrugge, L., & Isaia, M. (2023). Aliens in caves: the global dimension of biological invasions in subterranean ecosystems. *Biological Reviews*, *98*(3), 849-867.

Nitzu, E. (2021). Microhabitats-integrative environmental factors for species communities of Coleoptera in the karst landscape. *Biologia*, *76*(6), 1775-1783.

Novak, K. (2001). Spider venom helps hearts keep their rhythm. *Nature Medicine*, *7*(2), 155-155.

Novak, T., Perc, M., Lipovšek, S., & Janžekovič, F. (2012). Duality of terrestrial subterranean fauna. International Journal of Speleology, 41(2), 5.

Pacheco, G. S. M., de Oliveira, M. P. A., Cano, E., Souza Silva, M., & Ferreira, R. L. (2021). Tourism effects on the subterranean fauna in a Central American cave. *Insect Conservation and Diversity*, *14*(3), 294-306.

Pape, R., & O'Connor, B. (2014). Diversity and ecology of the macro-invertebrate fauna (Nemata and Arthropoda) of Kartchner Caverns, Cochise County, Arizona, United States of America. *Check List*, *10*(4), 761-794.

Pellegrini, T. G., & Lopes Ferreira, R. (2012). Management in a neotropical show cave: planning for invertebrates conservation. *International Journal of Speleology*, *41*(2), 27.

Ponce-Saavedra, J., Jiménez, M. L., Quijano-Ravell, A. F., Vargas-Sandoval, M., Chamé-Vázquez, D., Palacios-Cardiel, C., & Maldonado-Carrizales, J. (2023). The fauna of Arachnids in the Anthropocene of Mexico. In *Mexican Fauna in the Anthropocene* (pp. 17-46). Cham: Springer International Publishing.

Prous, X., Ferreira, R. L., & Martins, R. P. (2004). Ecotone delimitation: Epigean–hypogean transition in cave ecosystems. *Austral Ecology*, *29*(4), 374-382.

Ramírez-Fráncel, l. A., García-Herrera, l. V., Losada-Prado, S., Reinoso-Flórez, G., Sánchez-Hernández, A., Estrada-Villegas, S., ... & Guevara, G. (2022). Bats and their vital ecosystem services: a global review. Integrative Zoology, 17(1), 2-23.

Ravn, N. R., Michelsen, A., & Reboleira, A. S. P. (2020). Decomposition of organic matter in caves. *Frontiers in Ecology and Evolution*, *8*, 554651.

Reboleira, A. S., Bodawatta, K. H., Ravn, N. M., Lauritzen, S. E., Skoglund, R. Ø., Poulsen, M., ... & Jønsson, K. A. (2022). Nutrient-limited subarctic caves harbour more diverse and complex bacterial communities than their surface soil. *Environmental Microbiome*, *17*(1), 41.

Roeder, D. V., Paraskevopoulos, A. W., & Roeder, K. A. (2022). Thermal tolerance regulates foraging behaviour of ants. *Ecological Entomology*, *47*(3), 331-338.

Romero, A. (2009). *Cave biology: life in darkness*. Cambridge university press.

Roth, Z. U. (2014). A Phenological Study of Bat Communities in Southern Mississippi Caves.

Salaga, H. S. (2020). Cave macro-invertebrates in Linao, San Isidro, Davao del Norte, Philippines. *Extreme Life, Biospeology and Astrobiology*, *12*(1), 21-27.

Sánchez-Fernández D, Rizzo V, Bourdeau C, et al., (2018) The deep subterranean environment as a model system in ecological, biogeographical and evolutionary research. *Subterranean Biology* 25: 1–7.

Santos, J. S., Wolff, L. L., Baldan, L. T., & Guimarães, A. T. B. (2020). Seasonal records of benthic macroinvertebrates in a stream on the eastern edge of the Iguaçu National Park, Brazil. *Biodiversity Data Journal*, *8*.

Silva, M. S., & Ferreira, R. L. (2014). Loxosceles spiders (Araneae Sicariidae) preying on invertebrates in Brazilian caves. Speleobiology Notes, 6, 27-32.

Simon, K. S., Pipan, T., & Culver, D. C. (2007). A conceptual model of the flow and distribution of organic carbon in caves. *Journal of Cave and Karst Studies*, *69*(2), 279-284.

Sinclair, M. (2013). Heidegger, von Humboldt and the idea of the university. Intellectual History Review, 23(4), 499-515.

Sket, B. (2008). Can we agree on an ecological classification of subterranean animals?. Journal of Natural History, 42(21-22), 1549-1563.

Souza-Silva M, Bernardi LFO, Martins RP, Ferreira RL (2012) Transport and consumption of organic detritus in a neotropical limestone cave. Acta Carsologica 41: 139–150.

Taylor, S. J., Weckstein, J. D., Takiya, D. M., Krejca, J. K., Murdoch, J. D., Veni, G., ... & Reddell, J. R. (2007). Phylogeography of cave crickets (Ceuthophilus spp.) in central Texas: A keystone taxon for the conservation and management of federally listed endangered cave arthropods. *Technical Report INHS 2007 (58)*.

Thies, M. L., & Lewis, P. J. (2015). Effects of Fire on Small Mammals of the Koanaka Hills, Northwestern Botswana. Museum of Texas Tech University.

Tobin, B. W., Hutchins, B. T., & Schwartz, B. F. (2013). Spatial and temporal changes in invertebrate assemblage structure from the entrance to deep-cave zone of a temperate marble cave. *International Journal of Speleology*, *42*(3), 4.

Trajano, E., & de Carvalho, M. R. (2017). Towards a biologically meaningful classification of subterranean organisms: a critical analysis of the Schiner-Racovitza system from a historical perspective, difficulties of its application and implications for conservation. *Subterranean biology*, *22*, 1-26.

Voigtländer, K. (2011). 15 Chilopoda–Ecology. In Treatise on Zoology-Anatomy, Taxonomy, Biology. The Myriapoda, Volume 1 (pp. 309-325). Brill.

Wagner, D. L., & Van Driesche, R. G. (2010). Threats posed to rare or endangered insects by invasions of nonnative species. *Annual review of entomology*, *55*(1), 547-568.

Walker, K. L. (2006). Impact of the Little Fire Ant, Wasmannia auropunctata, on Native Forest Ants in Gabon 1. *Biotropica*, *38*(5), 666-673.

Weckerly, F. W. (2012). Cave cricket exit counts: environmental influences and duration of surveys.

White, A., Moravec, B., McIntosh, J., Olshansky, Y., Paras, B., Sanchez, R. A., ... & Chorover, J. (2019). Distinct stores and the routing of water in the deep critical zone of a snow-dominated volcanic catchment. *Hydrology and Earth System Sciences*, *23*(11), 4661-4683.

Wilkens, H., & Strecker, U. (2003). Convergent evolution of the cavefish Astyanax (Characidae, Teleostei): genetic evidence from reduced eye-size and pigmentation. Biological Journal of the Linnean Society, 80(4), 545-554.

Wong, M. K., Economo, E. P., & Guénard, B. (2023). The global spread and invasion capacities of alien ants. *Current Biology*, *33*(3), 566-571.

Xiong, Y., Zhou, Z., Ding, S., Zhang, H., Huang, J., Gong, X., & Su, D. (2023). Spatiotemporal Variation Characteristics and Influencing Factors of Karst Cave Microclimate Environments: A Case Study in Shuanghe Cave, Guizhou Province, China. *Atmosphere*, *14*(5), 813.

Yoder, J. A., Christensen, B. S., Croxall, T. J., Tank, J. L., & Hobbs III, H. H. (2010). The pheromone of the cave cricket, Hadenoecus cumberlandicus, causes cricket aggregation but does not attract the co-distributed predatory spider, Meta ovalis. *Journal of Insect Science*, *10*(1), 47.

Zepon, T., & Bichuette, M. E. (2017). Influence of substrate on the richness and composition of Neotropical cave fauna. *Anais da Academia Brasileira de Ciências*, *89*, 1615-1628.

Appendix A. Representatives of the top 10 most abundant macroinvertebrate taxa in Gcwihaba cave.



Carabidae(Ground beetles)



Rhaphidophoridae(Cave crickets)



Selenopidae(Flat spiders)



Blaberidae(Cave coakroaches)



Formicidae (Ants)



Tenebrionidae(Darkling beetle)



Reduviidae(Assasin bugs)



Sicariidae (Violin spiders)



Blattidae (Cave coakroaches)



Trogidae(Hide beetles)