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Front cover: Gypsum dissolution sinkholes, Turkey. See Gökkaya and Tunçel in this issue.

NATURAL AND HUMAN-INDUCED SUBSIDENCE DUE TO GYPSUM DISSOLUTION: A CASE STUDY FROM INANDIK, CENTRAL ANATOLIA, TURKEY

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Abstract

Gypsum dissolves relatively quickly and gypsum karst can evolve on a rapid time scale that may be accelerated by human-induced change, often resulting in severe subsidence damage. The area close to Inandık stream in Central Anatolia, Turkey, is affected by subsidence in two ways: formation of collapse sinkholes on agricultural land and progressive subsidence in Inandık village that has damaged buildings. This study focuses on these subsidence phenomena. Seven large sinkholes were formed on a terrace where episodic incision of the fluvial system has led to reduced thickness and mechanical strength of the cavity roof; a process that also increases the hydraulic gradient and enhances cavity development in the terrace area. These processes formed a sinkhole-prone terrace surface. The other subsidence phenomena, which have increased progressively in the last decade, relate to human activity in Inandık village. Water consumption increased after domestic water service systems were installed in houses in 2007, and in 2012 a sewer system was built in the village. Due to cracks and breakage, leaking water from buildings and sewer pipes infiltrated the gypsum substratum, resulting in dissolution of the bedrock and superficial cavity formation, as shown by GPR and borehole data. The process leading to subsidence caused severe damage to buildings. Consequently, it was decided to relocate the village three km south of its current location because of the high level of damage.

Introduction

Gypsum has a high dissolution rate that is greater than carbonate rocks (Klimchouk et al., 1996; Martinez et al., 1998); therefore, gypsum karst can evolve at a much faster rate (Benito et al., 1995; Cooper and Saunders, 2002; Gutiérrez and Cooper, 2013; Gutiérrez et al., 2014; De Waele et al., 2017) and be further accelerated by human-induced changes. Such high solubility leads to problems, the most frequent of which is subsidence, thereby threatening property. The subsidence hazard has been observed in various countries with gypsum karst terrain (Sauro, 1996; Jassim et al., 1997; Cooper, 1998; Paukštys et al., 1999; Cooper and Saunders, 2002; Klimchouk and Andrejchuk, 1996, 2002; Delle Rose et al., 2004; Johnson, 2005; Parise and Trocino, 2005; Gutiérrez et al., 2008; Koutepov et al., 2008; Parise et al., 2004, 2009; Thierry et al., 2009; Del Prete et al., 2010; Iovine et al., 2010; Cooper and Gutiérrez, 2013; Gutiérrez and Cooper, 2013; Gutiérrez, 2016; Calligaris et al., 2017). In Oviedo (Spain), subsidence due to gypsum dissolution led to the demolition of 362 flats at a loss of 18 million euro (Pando et al., 2012). Many buildings in Calatayud (Spain) have also been affected by subsidence and some of them had to be demolished due to the extent of the damage (Gutiérrez and Cooper, 2002; Gutiérrez, 2014).

Turkey has extensive gypsum outcrops in the provinces of Sivas and Çankırı (Fig. 1a). Despite the importance of gypsum karst, there are only a limited number of studies aimed at understanding the karstification of gypsum and related problems caused by subsidence. Although gypsum karst studies have been carried out in the Sivas region (Alagöz, 1967; Karacan and Yılmaz, 1997; Günay, 2002; Waltham, 2002; Doğan and Yeşilyurt, 2004; Doğan and Özel, 2005; Yilmaz, 2007; Keskin and Yılmaz, 2016) there has been no research on karst features and related hazards in the Çankırı region, except on some fossil subsidence sinkholes east of Çankırı (Doğan, 2002).

The area encompassing Inandık stream in the south of Çankırı province (Fig. 1b) has been influenced both by progressive subsidence in Inandık village, causing damage to buildings, and the formation of collapse sinkholes on agricultural land (Fig. 1c). This study deals with the triggering factors and their relationship with subsidence hazards in the area, as well as examining the origin of the collapse sinkholes. To our knowledge, the case of Inandık is the first published example of building damage due to gypsum dissolution in Turkey. The results of this study will contribute to understanding the controlling factors of subsidence mechanisms in this country and other gypsum karst areas.

Materials and Methods

The subsidence phenomena were analyzed by geological, geomorphological, and geophysical data. To detect buried collapse sinkholes and their period of generation, multiple sets of aerial photographs (1953, 1956, 1971, 1990, and 2008), orthophotos and satellite images from 2010 and 2016 were used. The importance of multi-temporal analysis for

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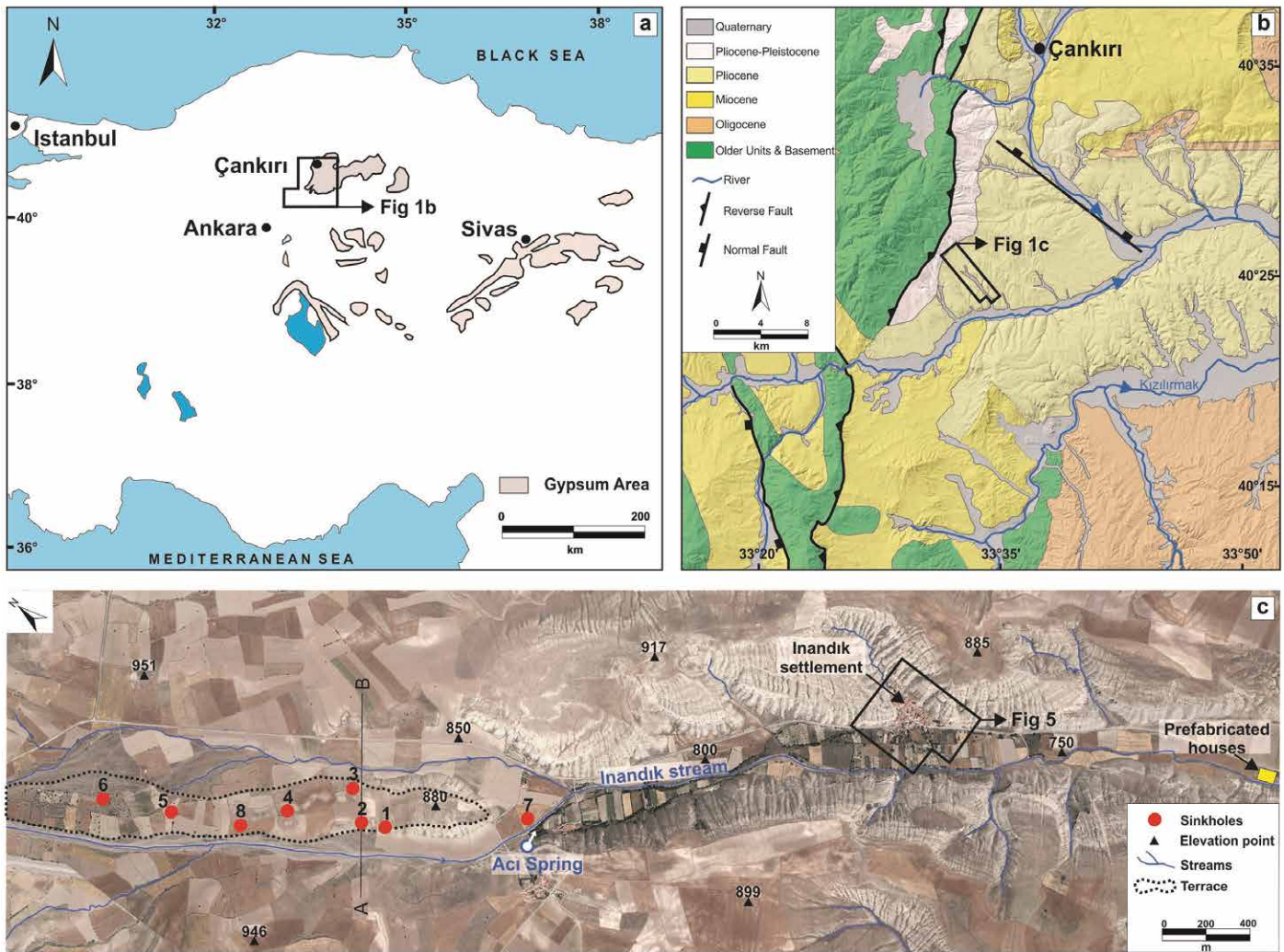
Figure 1. (a) Map showing location of study area. (b) Distribution of evaporite outcrops in study area and surroundings (modified from Karadenizli 2011). (c) Inandık village and vicinity.

understanding the evolution process of sinkholes has been stated on several occasions (Delle Rose and Parise, 2002; Festa et al., 2012; Basso et al., 2013; Calligaris et al., 2017).

Furthermore, the Turkish Disaster and Emergency Management Authority (AFAD) examined the village by digging boreholes and using high resolution near-subsurface imaging with ground penetrating radar (GPR). Images were obtained using a GSSI SIR 3000 portable device fitted with a 270 MHz antenna (Özçelik et al., 2016). For this study, we utilized the AFAD data and also produced a map showing the distribution of subsidence susceptibility. In addition, useful information on current subsidence in the settlement and sinkhole formation was obtained from local residents.

Background

The Çankırı region is located in the Çankırı-Çorum sedimentary basin (Fig. 1a). The basin experienced evaporite formation in the Oligocene, Late Miocene, and Pliocene reaching 7500 m in thickness (Karadenizli, 2011). Lacustrine Pliocene evaporites and the Bozkır Formation crop out around the study area (Fig. 1b). This evaporitic sequence has a thickness of up to 750 m and 60-100 m thick gypsum levels intercalated by mudstone, selenite, clayey limestone (Karadenizli, 2011) and salt, anhydrite and glauberite (Sönmez, 2014). Karst subsidence in the study area is linked to the dissolution of Pliocene evaporites.

The Kızılırmak drainage system developed in the basin at the beginning of the Quaternary, leading to closure of the basin. Karstification of gypsum and subsidence phenomena began when Pliocene evaporites were downcut by the Kızılırmak drainage system and overlain by loose alluvial deposits. The episodic downcutting of Inandık stream and its tributary in the study area generated a terrace 15 m above stream level and 25 m below adjacent ridges. The terrace is located northwest of Inandık village and its surface is overlain by alluvium 0 to 6 m thick (Fig. 1c).

The vicinity of Inandık has a semi-arid climate with a mean annual rainfall of 400 mm. Average temperatures range from -0.9°C in January to 23.1°C in July. Inandık stream, fed by karst springs, does not flow during the summer. The Acı (bitter) Spring, so named because it contains dissolved calcium sulfate, emerges from cavities in the gypsum at the point where the spring waters join Inandık stream.

The village was founded in the 19th century and contains 68 buildings that were mainly constructed in the first half of the 20th century. By 2015, most dwellings in the village were in poor condition due to damage from subsidence in the last decade. The most recent collapse sinkhole to be formed on agricultural land occurred in 2012. For these reasons, the inhabitants of Inandık applied to AFAD in 2015 for assistance to try and solve their problems.

After the investigation by AFAD was completed (Özçelik et al., 2016), the government decided to relocate the village three km south of its current location at a cost of 2 million euro because of the collapse threats. The provincial governorship has installed and allocated 72 prefabricated houses for temporary use until the village is moved (Fig. 1c). Once construction of the new village is complete, the residents will be permanently moved to the new location. Currently, the prefabricated houses are unoccupied and some residents of Inandık continue to live in the old village.

Sinkholes

Sinkholes are closed depressions typical of karst terrain. A significantly-sized cavity is necessary for the formation of bedrock collapse and caprock collapse sinkholes (e.g., Waltham et al., 2005; Gutiérrez et al., 2014, 2019). Other types of sinkholes, such as the cover-collapse sinkhole, may occur in the presence of limited-size cavities and voids (e.g., Gutiérrez, 2016). Cover-collapse sinkholes result from the upward propagation of cover failure, leading to the collapse of the overburden. This type of sinkhole is responsible for most sinkhole hazards around the world (Waltham et al., 2005; Parise, 2010; Gutiérrez et al., 2014). Their detection is made difficult because they are easily buried by farmers due to their relatively small size and depth. Analysis of historical documentation such as topographic maps, old pictures and images is therefore useful for detecting these sinkholes. Moreover, the data obtained can provide insights into their spatial and temporal distribution, as experienced with other types of natural hazards, such as landslides (Parise and Wasowski, 1999; Calcaterra and Parise, 2001; Pisano et al., 2017).

To detect the sinkholes in the current study, aerial and satellite images were compared and eight collapse sinkholes were identified northwest of Inandık. These were numbered according to their date of formation (Fig. 1c, Table 1). Five of them existed before 1953. Except for sinkhole 7, that is the smallest, all the sinkholes are clustered on the river terrace. Local residents reported that sinkholes smaller than a meter in diameter, similar to sinkhole 7, had been found in the valley bottom, but were buried. These cannot be detected in aerial photographs because of their size and having been filled in soon after formation.

Sinkhole 2 (Fig. 2a) is the largest with a diameter of 44 m and depth of 22.5 m at an altitude of 877 m a.s.l. It is the only bedrock collapse sinkhole and was formed before 1953. Blocks were observed in its bottom and many fractures are visible around the sinkhole, indicating that it has enlarged due to mass wasting. This sinkhole in particular shows that large cavities exist under the terrace.

Sinkhole 7 was formed between 1990 and 2008 at an altitude of 832 m a.s.l. This cover-collapse sinkhole was detected in the valley bottom and is relatively small and shallow, with a diameter of 1 m and depth of 1 m.

The most recent sinkhole is number 8 (Fig. 2b), formed in 2012. It is 13 m in diameter and somewhat enlarged since traces of erosion are visible at its margins, and is 10 m deep. The depth of the sinkhole is 5 m greater than the thickness of the alluvial deposit. It is classified as a cover-collapse sinkhole.

The other sinkholes can only be observed in some of the aerial photographs. They have now disappeared because farmers have filled them, canceling the surface features (Fig. 2c, d, e). These buried sinkholes can also be defined as

Table 1. Measurements, age, and type of sinkholes detected at Inandık.

Sinkhole Number	Formation Period	Altitude (m)	Diameter (m)	Depth (m)	Genetic Type
1	Before 1953	877	···	···	Buried sinkhole
2	Before 1953	882	44	22.5	Bedrock Collapse sinkhole
3	Before 1953	881	5	···	Buried sinkhole
4	Before 1953	886	12	···	Buried sinkhole
5	Before 1953	916	7	···	Buried sinkhole
6	1953–1971	927	13.5	···	Buried sinkhole
7	1990–2008	832	1	1	Cover collapse sinkhole
8	2012	898	13	10	Cover collapse sinkhole
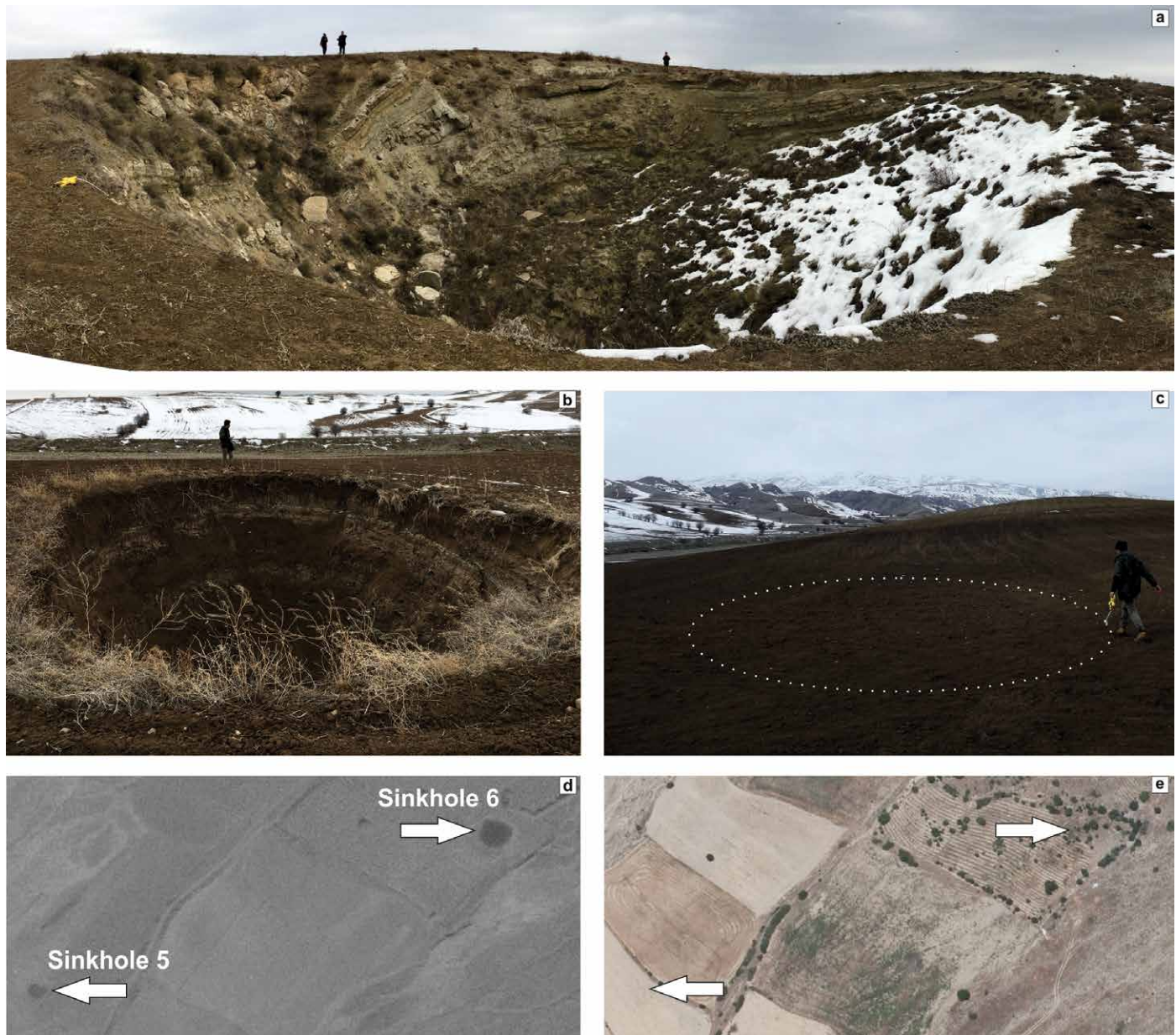
Figure 2. (a) Panoromic view of Sinkhole 2, the deepest and largest sinkhole in study area. Note separated blocks on the sides. (b) Sinkhole 8, most recent sinkhole in study area. (c) Sinkhole 4, buried by farmers. Soil containing small white pebbles brought from elsewhere mark the sinkhole within the boundary indicated. (d) Historical aerial photograph of sinkholes 5 and 6. (e) Recent view of buried sinkholes 5 and 6.

cover-collapse sinkhole because they formed in alluvial deposits.

Existing sinkholes are the predictors of future sinkhole formation. Regarding the spatial distribution of the eight sinkholes, they are clustered in a line of about 0.3 km^2 along the NW-SE 1.9 km alignment. This area offers the highest probability for formation of new sinkholes.

Subsidence in the village

Inandık village is situated in a tributary valley on the eastern flank of Inandık stream with the SW part of the village within the main valley (Fig. 1c). There is no streamflow in the valley bottom since precipitation falling in the area surrounding the village in the rainy season is drained underground. The recharge to Inandık stream includes discharge from Inandık settlement which, we believe, has enhanced dissolution of the bedrock, as documented on paleo-subsidence structures (Fig. 3) which can be observed in many gypsum bedrock outcrops in the village, providing us with data about recent subsidence events. They also demonstrate that subsidence phenomena have been occurring over a long period. In support of this, information from inhabitants and local authorities indicate that the village has been affected by subsidence and sinkholes for generations. Although some damage occurred in the past, the frequency of subsidence events has in-

Figure 3. Paleo-subsidence structures in gypsum bedrock. Cavities are filled with karstic residue and gypsum blocks. (Photograph taken on eastern slope of village).

creased progressively in the last decade. Inhabitants have heard noises originating underground, intermittently accompanying micro-seismic activity that was also detected by microtremor measurement (Özçelik et al., 2016), especially between 2012 and 2017, causing some residents to abandon their houses for fear of earthquakes. It is clear that the sounds frightening residents were generated underground by collapsing material in cavities. Although there is no big collapse sinkhole within the settlement, progressive deterioration is seen. Therefore, the subsidence mechanism most likely occurs when sagging is combined with a collapse, causing loss of support under Inandık settlement and has severely damaged nearly all buildings in the village, which show open fractures and tilted, cracked walls (Fig. 4). Moreover, some of the buildings are very old; therefore they are severely affected by even the slightest subsidence. Several have been demolished because the damage was beyond repair.

We received information from the local authority that the water consumption habits of the inhabitants had changed recently, which has most likely affected the karst system. There was limited water consumption previously in the village but usage increased significantly after in-house water was provided in 2007. The total annual water consumption of the inhabitants reached 118.7 tons in 2015. Around the same time, in 2012 a sewage system was installed in the village. However, some buildings still channel wastewater directly into the ground (Fig. 4b) due to badly-designed drains. Information from the local authority and inhabitants also suggests that the service and sewage pipes crack quite often. Such leaks result in a large amount of water seeping into the karst system in the village.

Spatial distribution of damage

Subsidence damage is caused by several factors including landslides, karst subsidence, and mining-induced subsidence. Land movement tends to be predominantly downward in coal mining and active karst subsidence areas, whilst a combination of downward and outward movement is typically observed in landslide areas (Cooper, 2008). Such movements cause damage to man-made structures and economic loss. Determining the degree and spatial distribution of the damage is necessary to understanding the subsidence mechanism. Therefore, several schemes have been generated to assess the damage to buildings (Cooper, 2008). The National Coal Board (NCB) (1975) in the UK created five categories for defining damage in coal mining areas (Cooper and Calow, 1998). Alexander (1986) and Chiocchio et al. (1997) developed a landslide damage classification of seven categories with two extra more severe damage categories but which otherwise closely resembles the NCB scheme. To determine the distribution of the subsidence hazard on gypsum karst, the NCB categories were adapted by Gutiérrez and Cooper (2002).

Buildings were assessed in the present study according to the categories of the NCB (1975) and Gutiérrez and Cooper (2002). Three category levels were used (Table 2) and a damage map showing the spatial distribution of the subsidence was generated (Fig. 5). This method has limitations because the internal damage to buildings was not assessed; moreover, the cause of damage could be related to other factors such as age or building materials. However, buildings in the village that are similar to each other exhibit no signs of damage. Relatively new structures constructed in the last 15 years also appear to be more resistant to subsidence and were evaluated separately on the map (Fig. 5).

Figure 4. (a) Displacement of house wall showing bulging and cracking caused by subsidence, and broken windows due to distortion. (b) Cracks on wall and pipes draining wastewater directly into ground. (c) Open joint in wall of building. (Photographs taken on February 26, 2017).

The map shows that most of the damage is concentrated at the bottom of small tributary valleys, whilst structures located in the main valley experienced no damage.

Ground penetrating radar (GPR) and borehole data

Five GPR profiles with a total length of 565 m (Fig. 5) were obtained from the settlement area (Özçelik et al., 2016). Profile 1 (Fig. 6a) mostly concentrates on the in-house and sewage water pipelines and shows shallow and relatively deep anomalies. Shallow anomalies between 20 and 32 m, and between 55 and 70 m are observed at a depth of 1 –

10 m. These reflections with different dips in this profile are likely related to sagging structures. Relatively deep anomalies are also observed 20 m and 40 m from the beginning

Table 2. Categorization of damage to structures in Inandık village (Gutiérrez and Cooper 2002, and references therein).

Category	Typical Damage
No Damage	...
Appreciable	Hairline cracks, several slight fractures (millimetric), doors and windows may stick slightly.
Severe	Open fractures, window and door frames distorted requiring partial or complete reconstruction, displaced tilted walls.

of the profile at a depth of 50-100 m. Profile 5 (Fig. 6b) focuses on the area northwest of the village. It shows anomalies located from the beginning of the profile at 10, 20, and 30 m and having a depth from 10 to 100 m. Shallow anomalies at 10 m and 25 m along the profile show a sagging structure. These anomalies seen on profiles 1 and 5 probably correspond to fractures, cavities, and sagging structures near the surface in the gypsum bedrock (Fig. 6a, b). Profile 1, located below the most damaged area, shows several anomalies more than profile 5, and both the anomalies and damaged buildings decrease towards the end of profile 5. These observations are compatible with the spatial distribution of the damage. Although the effect of subsidence was observed in structures near the other three profiles (Fig. 5), anomalies were not detected in these profiles.

Boreholes 1 and 2 were drilled in the valley bottom of Inandık village (Fig. 5). Both boreholes show dissolution cavities in the gypsum (Fig. 6c). According to this data, the thickness of the alluvium is 1.5-3 m. Borehole 3 was drilled on the bedrock northwest of the village. No void was detected in its log 3 (Özçelik et al., 2016).

Discussion

Sinkholes

Seven sinkholes are clustered on the terrace surface in the study area due to geomorphological and hydrologic factors. There is a relationship between the development of fluvial systems on a karst massif and the generation of sinkholes (Benito et al., 1998, 2000;
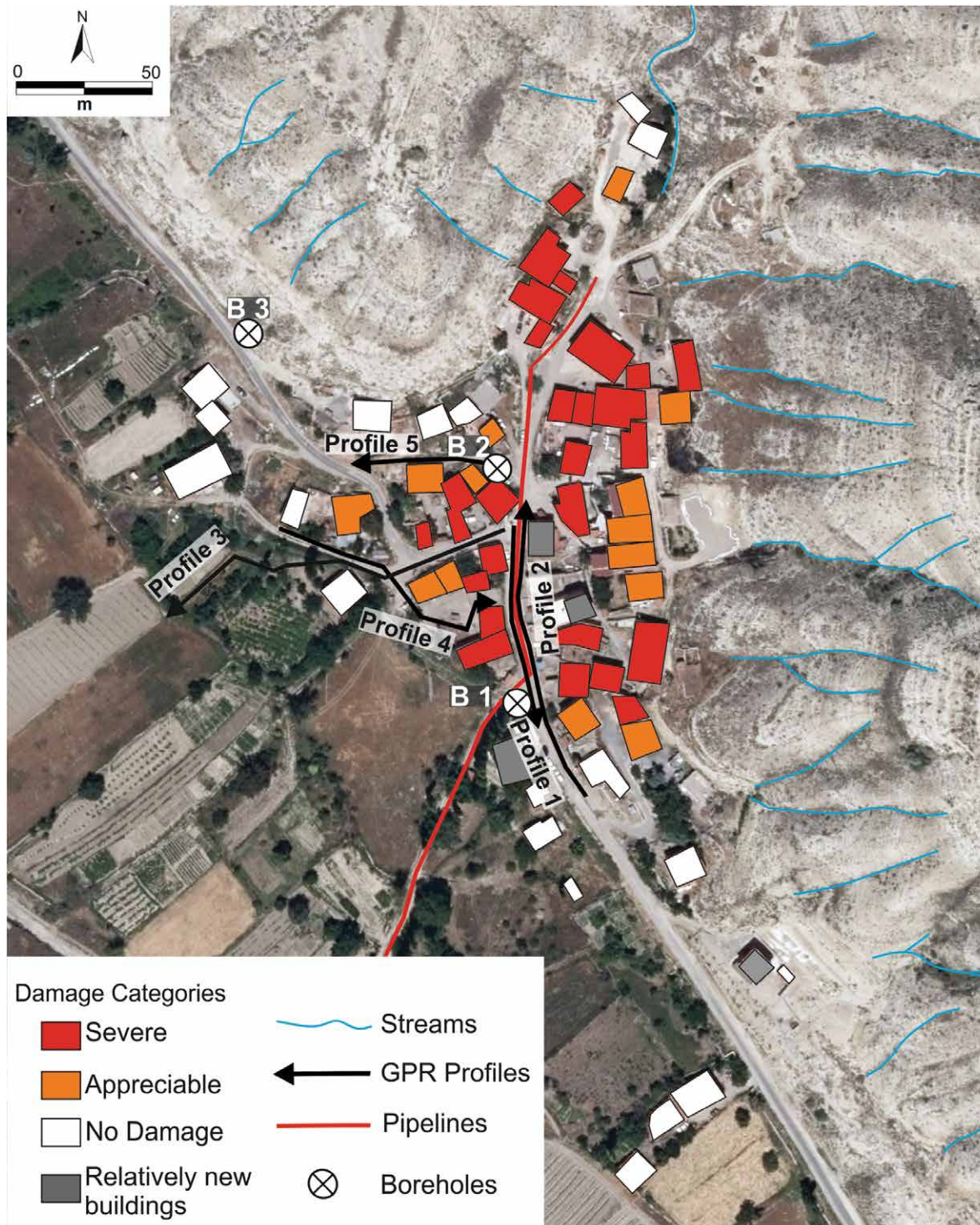

Figure 5. Map of damage distribution in March 2017 showing location of boreholes, GPR profiles (Özçelik et al. 2016) and pipeline.
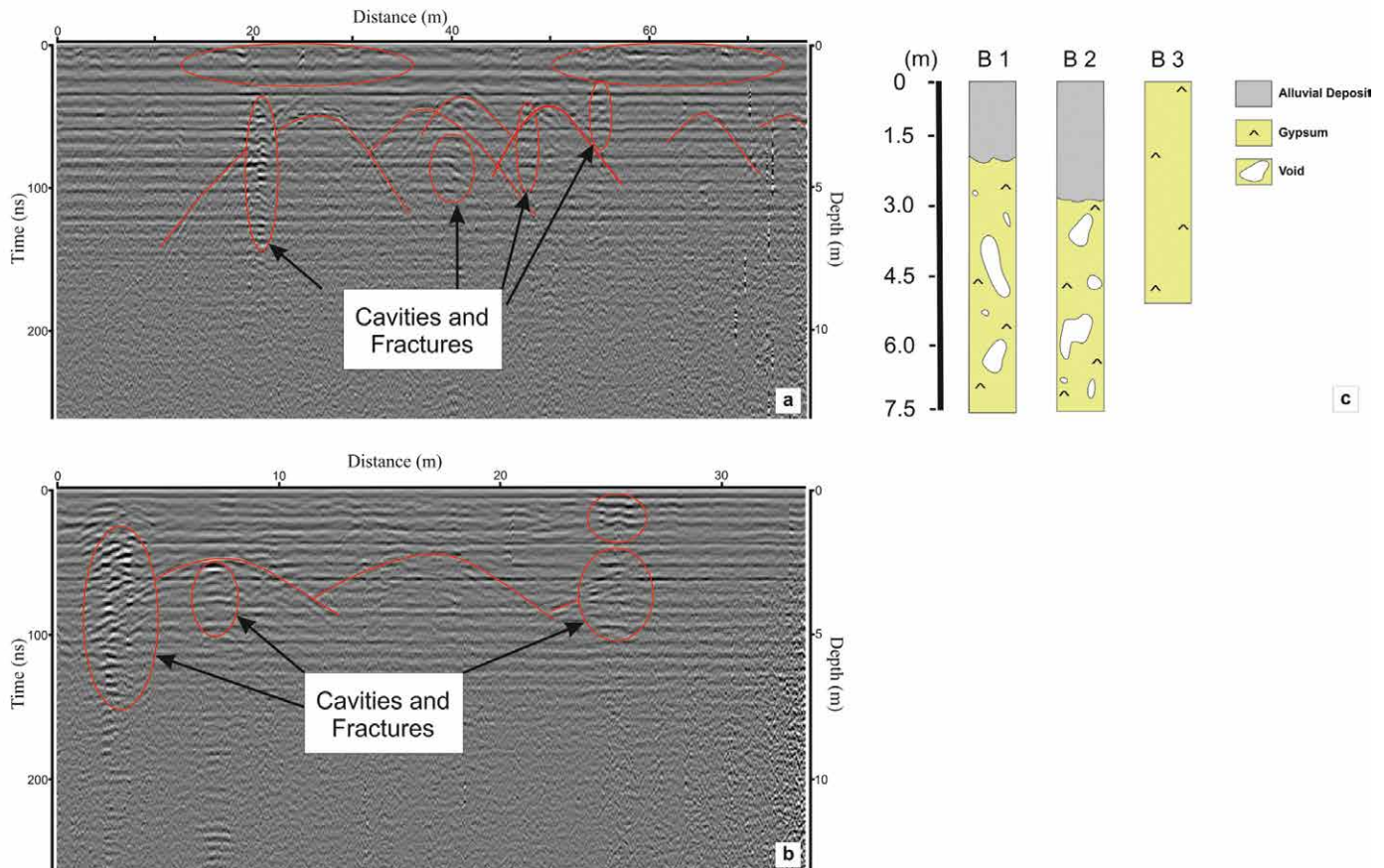
Figure 6. GPR profiles of (a) profile 1, and (b) profile 5. (c) Log of boreholes drilled in Inandık village (modified from Özçelik et al. 2016).

Doğan, 2005; Guerrero et al., 2008, 2013; Gutiérrez and Cooper, 2013; Gutiérrez, 2014). The incision of rivers forms a deep vadose zone and lower water table (Ortega et al., 2013). It also accelerates cavity development and internal erosion and gives rise to diminished thickness and mechanical strength of the cavity roofs.

The current geomorphological features of the area were formed when Inandık stream and its tributaries eroded the surface of the valley, giving rise to a terrace in which the gypsum cavities were less covered (Fig. 7). The streams incised the valley by more than 15 m forming a new, lower base level and a higher hydraulic gradient, thus accelerating cavity development, subsurface erosion, and upward migration of the cover deposits.

There was no sinkhole formation on the adjacent heights of the terrace (Fig. 7) because they have a relatively thick cavity roof. In the valley bottom, the alluvial cover is thicker and the hydraulic gradient is lower; therefore, only sinkholes 1 m wide or smaller were formed there.

The sinkholes we detected are due solely to natural processes. Human-induced factors such as the pumping up of groundwater or extra water input into the ground were not detected. Moreover, most sinkholes were formed before 1953 (Table 1) when human influence was far less. It is plausible that other sinkholes occurred in the Quaternary, but these would have been buried later by the stream system.

Subsidence in the village

In-house service and sewer pipes are more likely to cause subsidence (Fleury, 2009; Parise, 2015) and pipeline breakage gives rise to damage in karst areas (Jassim et al., 1997; Gutiérrez and Cooper, 2002; Cooper et al., 2011; Cooper and Gutiérrez, 2013; Parise et al., 2015). In Inandık, ground subsidence must have caused the abnormally frequent breaking of pipes, which caused extra water input into the ground and triggered progressive subsidence of the land, creating a vicious circle. Moreover, some household waste water flows directly into the ground, which causes localized subsidence. All these processes give rise to dissolution and the formation of new cavities (Fig. 8).

According to the GPR profiles, cavities were detected only on profiles 1 (Fig. 6a) and 5 (Fig. 6b). However, the distribution map of subsidence damage (Fig. 5) shows that it affects a wider area than the GPR result indicates. It is known that the clay content of alluvium is an obstacle influencing the depth that GPR systems can reach (Schrott and Sass, 2008; Pueyo-Anchuela et al., 2009; Nouioua et al., 2013); therefore, the penetration of the GPR system was not satis-
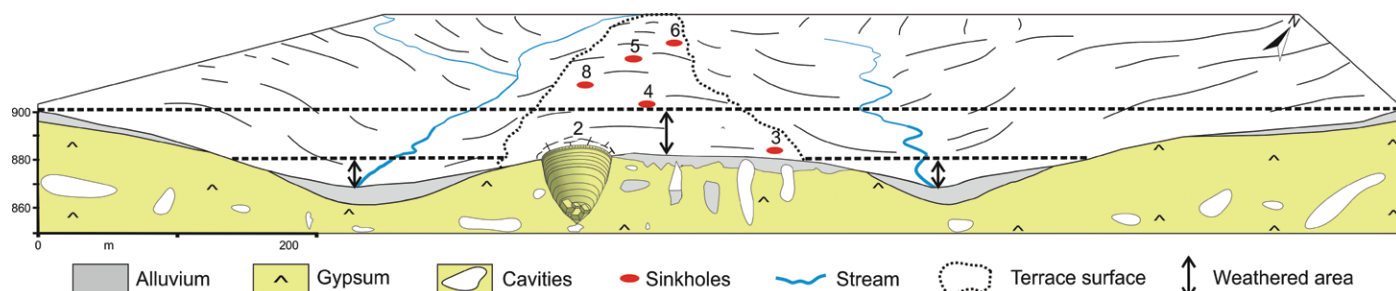
Figure 7. 3D Geomorphological scheme (A-B) of Inandık stream showing flow level differences and sinkholes (See Figure 1c).
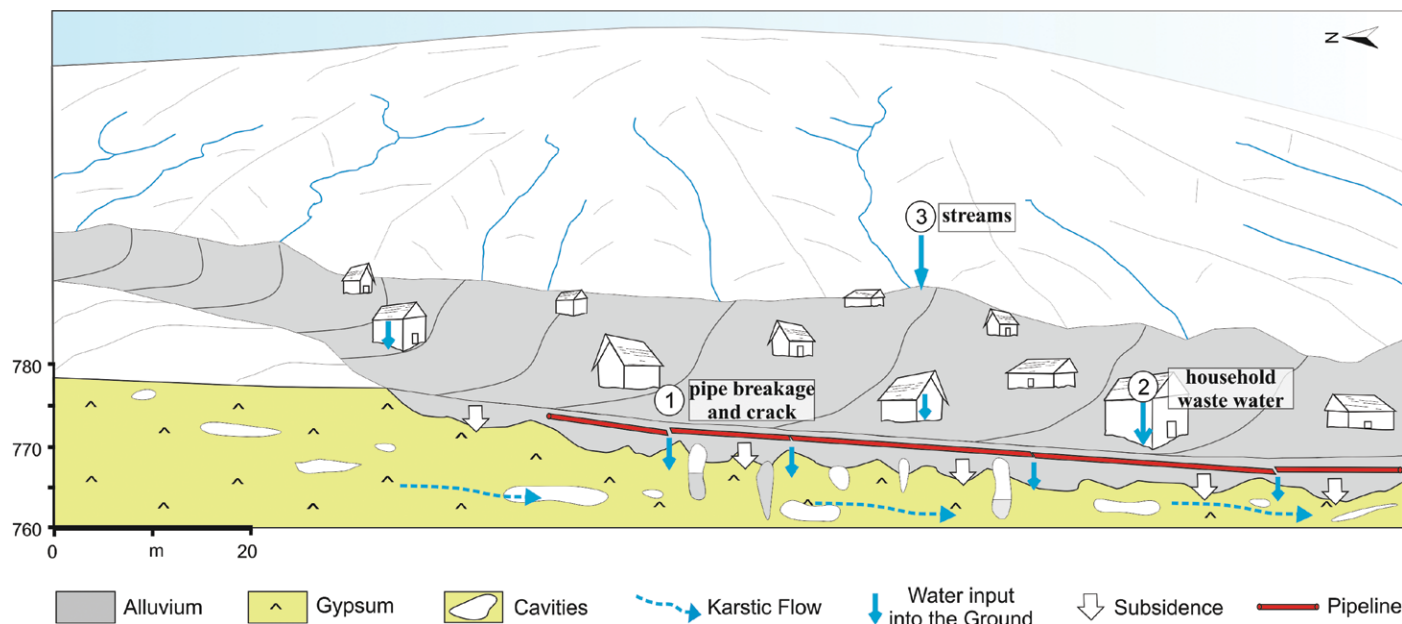

Figure 8. Cross-section of pipeline (Fig. 5) and 3D geomorphological scheme of Inandık village depicting effect of subsidence.

factory in several studies (Gutiérrez et al., 2011; Margiotta et al., 2012, 2016; Carbonel et al., 2014, 2015; Rodriguez, 2014; Zini et al., 2015). This limit to GPR penetration is probably the case in Inandık, explaining the apparent inconsistency between the results of profiles 1 and 2, which are on the same line and only two meters apart (Fig. 5). Therefore, the usefulness of GPR surveying for identification of possible sinkholes in alluvial karst areas may be limited due to high electrical conductivity values, whilst it remains appropriate for bare karst areas.

The Inandık settlement is situated on 1.5-3 m alluvial deposits overlying a layer of gypsum, according to the boreholes (Fig. 6c). These thin alluvial deposits caused the village to be favorable for subsidence. On the other hand, the main valley has relatively-thick alluvial deposits SW of the village and buildings here show no damage (Fig. 5). The center of the village is subject to greater internal erosion because of its thin alluvial cover.

From the borehole logs, registers, and GPR profiles 1 and 5, similar cavities to those in outcrops (Fig. 3) were deduced. They are very superficial, which explains the damage to single-story homes with smaller loads on their foundations.

Conclusion

The aim of this study was to examine the subsidence problems of Inandık village in Turkey. Gypsum dissolution is evident in the entire fluvial system of Inandık stream. A series of ground collapses was identified on a terrace located upstream from the settlement. On this terrace, the surface is more-than-usually prone to the formation of sinkholes because of the reduced thickness and mechanical strength of the cavity roof and the thinness of the alluvial deposits; hence, seven large sinkholes were formed.

In the settlement, in addition to circumstances favorable to the karst phenomena with houses located in an area subject to underground water flow and the presence of cavities (Fig. 3), the process has been accelerated by human action when a water distribution network was installed in homes in 2007, which increased water usage, and a new

sewage system was constructed in 2012. The pipes of these networks cracked and broke frequently and some homes still discharge water directly underground. This leakage has resulted in enhanced subsidence and progressive damage to buildings in the last decade. In consequence, the severity of the damage led to the decision to re-locate the village.

It is hoped that the results of this study will contribute to increasing the awareness among decision-makers who need to consider the karst system of the area they are responsible for in order to take preventative measures. To reduce or prevent hazards related to subsidence in a settlement which overlies gypsum, such as Inandık, extra water input to the subsurface should be minimized (Parise, 2015) and flexible pipes with a telescopic joint should be used to alleviate cracks and breakage.

Acknowledgments

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DESCRIPTION OF A NEW GENUS AND SPECIES AS THE FIRST GASTROPOD SPECIES FROM CAVES IN IRAN

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ABSTRACT

We report on a new stygobiont truncatelloid gastropod from the sulfidic ponds of Tashan Cave in the Zagros Mountains of Southwest Iran. The hydrogen sulfide habitat resembles those hitherto known for gastropods from sulfide-rich caves in Romania, Italy and Greece. The newly described genus *Trogloiranica* n. gen. with a newly described *T. tashanica* n. sp represent the first true stygobiont gastropods found in Iran. The phylogeny inferred from the mitochondrial (cytochrome oxidase subunit I) and the nuclear (histone 3) genomes, as well as anatomical evidence, place *T. tashanica* in the family Moitessieriidae Bourguignat, 1863. The new genus may represent an evolutionary relict from the post Miocene before the split of the families Moitessieriidae and Cochliopidae Tryon, 1866.

INTRODUCTION

The freshwater Mollusca of Iran have been a focus of research during past two decades (Mansoorian, 2001; Glöer and Pešić, 2009, 2012; Moghadam and Chegini, 2009; Mowlavi et al., 2009; Shahabuddin et al., 2012; Ektefa et al., 2013; Nouroozi, 2014), and the taxonomic position of the local Ponto-Caspian Hydrobiidae has been clarified only recently (Dellicado et al., 2016). Iran is situated at the interface of three zoo-geographic realms, Palaearctic, Oriental (Indo-Malayan), and Afrotropical (Ethiopian). The karstifiable carbonate formations cover around 11% of Iran's surface area with more than half (55.2%) being located at the Zagros Mountains in western Iran (Raeisi and Laumanns, 2012). Thus, it is not surprising that more than 90% of known Iranian troglobiont taxa are found in the Zagros karst formations (Malek-Hosseini and Zamani, 2017).

Tashan Cave, located near the Sarjooshar Village, harbors a unique ecosystem. The recent discovery of a blind fish, *Garra tashanensis* Mousavi-Sabet, Vatandoust, Fatemi and Eagderi, 2016, and of the isopod *Stenasellus tashanicus* Khalaji-Pirbalouty, Fatemi, Malek-Hosseini, Kuntner, 2018, has been followed by other discoveries including the new gastropod genus and species reported here. The Tashan Cave represents a specific hypogean habitat with several cave ponds containing groundwater rich in hydrogen sulfide. Earlier studies performed in sulfide-rich caves by Hose et al. (2000); Engel (2012); and Jones et al. (2014) have shown that chemoautotrophic sulfur-oxidizing microorganisms thrive in such environments using the redox interface between the sulfide in the water and the oxygen in the atmosphere to produce food *in situ*, independent of the external light energy. Sulfuric acid is a product of the bacterial sulfur oxidation and accelerates the dissolution of the limestone bedrock in the process called *sulfuric acid speleogenesis* (SAS), which results in the formation of *sulfide caves* (Engel et al., 2004; Porter et al., 2009). It is estimated that hydrogen sulfide-rich phreatic waters and microbially-generated sulfuric acid were involved in the speleogenesis of around 10% of worldwide known caves (Palmer, 2007). However, only a few of these caves remain active ducts containing hydrogen sulfide-rich groundwater. The sulfide caves host interesting ecosystems with unusually rich and diverse invertebrate communities dominated by crustaceans (Peterson et al., 2013; Por, 2014), insects (Tobler et al., 2013) and occasionally fishes (Reisch et al., 2010; Roach et al., 2011; Mousavi-Sabet at al., 2016). While sulfide-based ecosystems are usually well-recognized inside deep sea trenches (Deming and Baross, 1993), the groundwater sulfide ecosystems remain poorly studied (Engel, 2007). The sulfide cave ecosystems received attention with the studies of Sarbu et al. (1996). The first studied cave containing a sulfide ecosystem was Movile Cave in Romania (Sarbu and Popa, 1992; Sarbu et al., 1996; Sarbu, 2000; Engel, 2012), followed by the Frasassi caves and Grotto Azzurra in Italy (Macalady et al., 2007; Peterson et al., 2013), Tito Bustillo and Maltravieso caves in Spain (Schabereiter-Gurtner, 2002; Arrozo et al., 1997), Fiume Coperto Cave in Italy (Latella et al., 1999), Melissotrypa Cave in Greece (Falniowski and Sarbu, 2015), El Hamma in Tunisia (Por, 1963), Ayalon Cave and the Tabgha Spring in Israel (Por, 1963; 2007; 2011), Kugitangtou caves in Turkmenistan (Maltsev and Korshunov, 1998), Cueva de Villa Luz in Mexico (Hose et al., 2000; Engel, 2007), Bungonia and Nullarbor caves with Bunder Sinkhole in Australia (Holmes et al., 2001; Jaume et al., 2001), Lower Kane

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Cave in Wyoming, USA (Porter et al., 2002), Cesspool Cave in Virginia, USA (Engel et al., 2001), and the Mammoth and Parker Caves in North America (Hutchins et al., 2016).

Hydrogen sulfide is toxic for most organisms (Kelley et al., 2006), and only five of the around twenty so far known sulfide cave ecosystem sites host stygobiont gastropod species, as likely outcomes of extreme sulfide adaptation: *Heleobia dobrogica* (Grossu and Negrea, 1989) from Movile Cave (Falniowski et al., 2008), *Islamia sulfurea* Bodon and Cianfanelli, 2012 from the Frasassi caves (Bodon et al., 2009; Bodon and Cianfaneli, 2012), *Physella spelunca* Turner and Clench, 1974 from Lower Kane Cave in Wyoming (Porter, 2002; Wethington and Guralnick, 2004) as well as *Iglica hellenica* and *Daphiola magdalenae* (Falniowski and Sarbu, 2015) from Melissotrypa Cave in Greece. The discovery of a sixth sulfidic stygobiont gastropod species in the Tashan Cave of Iran, reported here, is thus of general ecological importance.

MATERIAL AND METHODS

Snails were collected from pools in Tashan Cave, located under a limestone plateau close to Sarjooshar Village in the south-eastern part of the Zagros Mountains, Tashan district, Behbahan County, Khuzestan Province, southwest Iran (Fig. 1 and 2). Specimens were collected by using dropper pipettes and forceps and preserved in 96 % ethanol. A few specimens were transferred into a small rimstone basin at the cave pool shelf for photographing and subsequently returned to the pool.

The sampled snails were fixed in 80% ethanol. The dissections were done under a NIKON SMZ18 microscope with dark field, and the structures were photographed with a CANON EOS 50D digital camera. Frontal, ventral, and lateral images of the holotype were made using a Nikon SMZ25 microscope with a Nikon D200 camera and an AF-S Micro NIKKOR 60 mm lens at the Vienna Natural History Museum (NHMW), Austria. Morphological terms follow Hershler and Ponder (1998).
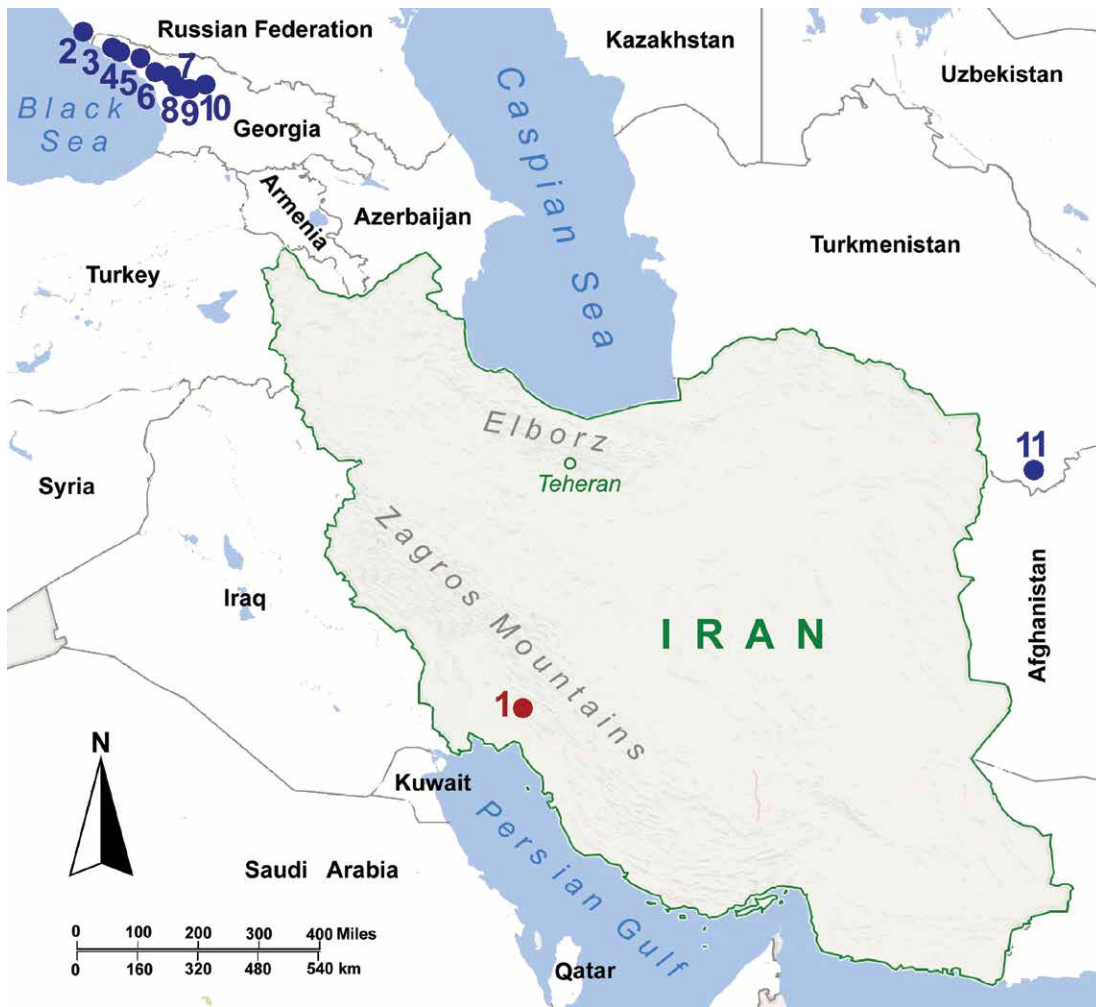
DNA was extracted from foot tissue, hydrated in Tris-EDTA (TE) buffer, using a Sherlock extraction kit (A&A Biotechnology), and dissolved in 20 mL of TE buffer. The extracted DNA was stored at −80 °C at the Department of Malacology of Institute of Zoology and Biomedical Research of the Jagiellonian University in Kraków. Our study targeted nucleotide data from the mitochondrial (cytochrome oxidase subunit I - COI) and the nuclear (histone 3 – H3) genomes, to produce phylogenetic inference from individual and combined gene datasets. Details of PCR conditions, primers used, and sequencing methods follow Szarowska et al. (2016). Sequences were aligned by MUSCLE (Edgar, 2004) implemented in MEGA 6

Figure 1. Map of Iran with the type locality of *Trogloiranica tashanica* n. sp.: Khuzestan Province, Behbahan County, Tashan district, Sarjooshar Village, Tashan Cave (**1** − Red ring) and geographically closest records of stygobiont gastropod species from SW Caucasus (**2-10** − Blue dots) and from Turkmenistan, Kushka, Shar-Arab spring (**11** – Blue dot).
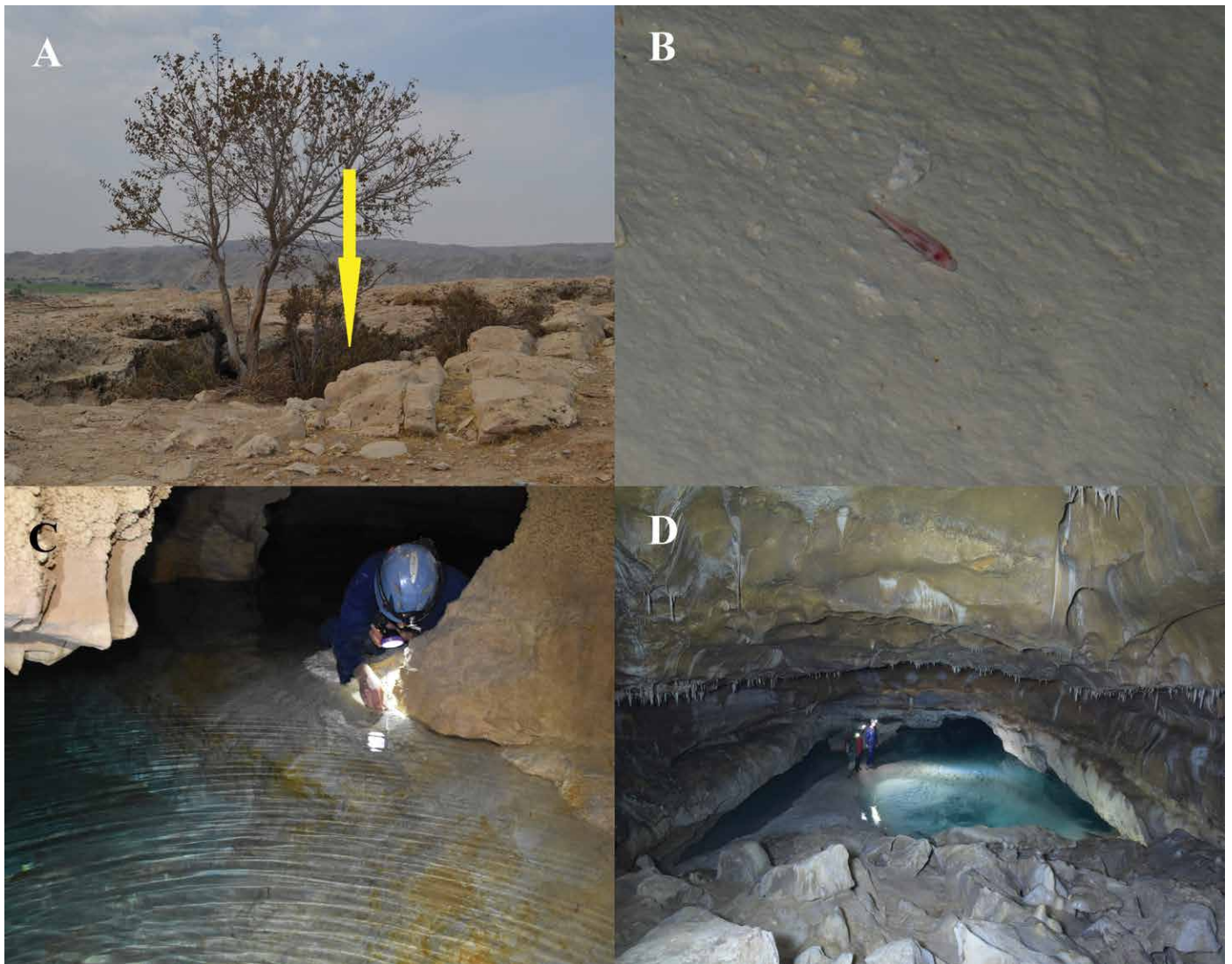
Figure 2. Photos of the type locality of *Trogloiranica tashanica* n. sp.: A: Tashan Cave entrance on the semi-desert limestone plateau; B hydrogen sulfide-rich pond inside the Tashan cave showing *Garra tashanensis*; C and D: sampling at the type locality (photo: Mohammad Javad Malek-Hosseini and Yaser Fatemi).

(Tamura et al., 2013) and in Bioedit 7.1.3.0 (Hall, 1999). The saturation test (Xia, 2000, Xia et al., 2003) was performed using DAMBE (Xia, 2013). Additional sequences from GenBank were used in the phylogenetic analysis (Table S1). The phylogenies were inferred using Bayesian inference (BI) and maximum likelihood (ML).

The Bayesian analyses were run using MrBayes v. 3.2.3 (Ronquist et al., 2012) with the default priors. Two simultaneous analyses were performed, each of which lasted 10,000,000 generations, with one cold chain and three heated chains, starting from random trees and sampling the trees every 1,000 generations. The first 25 % of trees were discarded as burn-in. The analyses were summarized as a 50 % majority-rule tree. The ML approach was applied with RAxML v. 8.0.24 (Stamatakis, 2014). We applied the GTR model, whose parameters were estimated by the RaxML (Stamatakis, 2014). One thousand searches were initiated with starting trees obtained through the randomized stepwise addition maximum parsimony method. The tree with the highest likelihood score was considered as the best representation of the phylogeny. Bootstrap support was calculated with 1,000 replicates and summarized on the best ML tree. RAxML analyses were done in CIPRES Science Gateway (Miller et al., 2010).

Abbreviations

NMBE	Naturhistorisches Museum, Bern, Switzerland
NHMUK	Natural History Museum, London, UK
NHMW	Natural History Museum, Vienna, Austria
ZMUH	Zoological Museum, University of Hormozgan, Iran

SMF	Senckenberg Museum, Frankfurt, Germany
H	Shell height
W	Shell width
BH	Height of the body whorl
BW	Width of the body whorl
AH	Aperture height
AW	Aperture width
LT	Type locality

RESULTS

The molecular and anatomical investigation of the live gastropods from the type locality in Tashan Cave revealed it to be a new species belonging to a new genus in Moitessieriidae Bourguignat, 1863.

Superfamily Truncatelloidea Gray, 1840
Family Moitessieriidae Bourguignat, 1863
Genus *Trogloiranica* n. gen.
Diagnosis

The diagnostic features of the genus are the same as those of the type species, *Trogloiranica tashanica* n. sp. The oval shell shape with low spire is unique within the Moitessieriidae. Nevertheless, similar shell shapes seem to have convergently evolved in other truncatelloid families such as Hydrobiidae Stimpson, 1865, Cochliopidae Tryon, 1866, Pomatiopsidae Stimpson, 1865, and Tateidae Sacco, 1896.

Etymology

The name derives from a prefix troglo- referring to the cave habitat of the new taxon and suffix -iranica referring to the country of origin.

***Trogloiranica tashanica* sp. n.**

Type locality

Iran, Khuzestan Province, Behbahan County, Tashan district, Sarjooshar Village, sulfidic freshwater ponds inside Tashan Cave, 30°51'54"N; 50°10'29"E (altitude 559 m a.s.l.).

Type material

Holotype: type locality: Yaser Fatemi and Mohammad Javad Malek-Hosseini leg. August 23, 2017 (holotype NMBE 558282).

Paratypes: type locality (same data) one paratype NMBE 558283; one paratype in each lot of ZMUH 123, 124 and 125; SMF 358125 one specimen, four specimens in coll. Grego; type locality Yaser Fatemi and Mohammad Javad Malek-Hosseini leg. August 27, 2017, two specimens in coll. Malek-Hosseini; type locality (same data); Yaser Fatemi and Mohammad Javad Malek-Hosseini leg. 17. March 2018 coll. 10 specimens in coll. Malek-Hosseini, two in coll. Jagiellonian University, Krakow.

Measurements

Holotype: H 2.39 mm; W 1.69 mm; BH 1.73 mm; NW 1.14; AH 1.69, AW 0.86 (holotype). Figure 3, A1-A6.

Diagnosis

The low spire with oval shell shape of the new species is rather unusual within the family Moitessieriidae, but a similar shape with more elevated spire is present in *Bythiospeum lamperti* (Geyer, 1907). Similar shell morphology can be found in representatives of other truncatelloid families such as: *Pseudamnicola hauffeni* Delicado and Ramos, 2012, Hydrobiidae from Spain; *Aroapyrgus pasionensis* Goodrich & Van der Schalie, 1937, Cochliopidae from Guatemala; *Tricula spelaea* Grego, 2018, Pomatiopsidae from Laos; *Pseudotricula auriforma* Ponder, Clark, Eberhard and Studer, 2005, Tateidae from Tasmania, and in the hydrobiid *Turkmenamnicola lindholmi* (Zhadin, 1952) from Turkmenistan. The cochliopid *Heleobia dobrogica* (Grossu & Negrea, 1989) found in similar sulfidic habitats in Movile Cave, Romania, has much more elongate shell shape. However, the molecular and anatomical data distinguish the new species from all other morphologically convergent species.

Description

Oval shell with conical spire with four rounded slightly convex whorls with a semi-deep suture and a blunt apex. Shell surface smooth with faint transverse growth lines covered by horny yellowish periostracum and partly by precipitated reddish–brown mineral crystals especially at the suture and marginal part of the body whorl. Aperture elongate-oval,
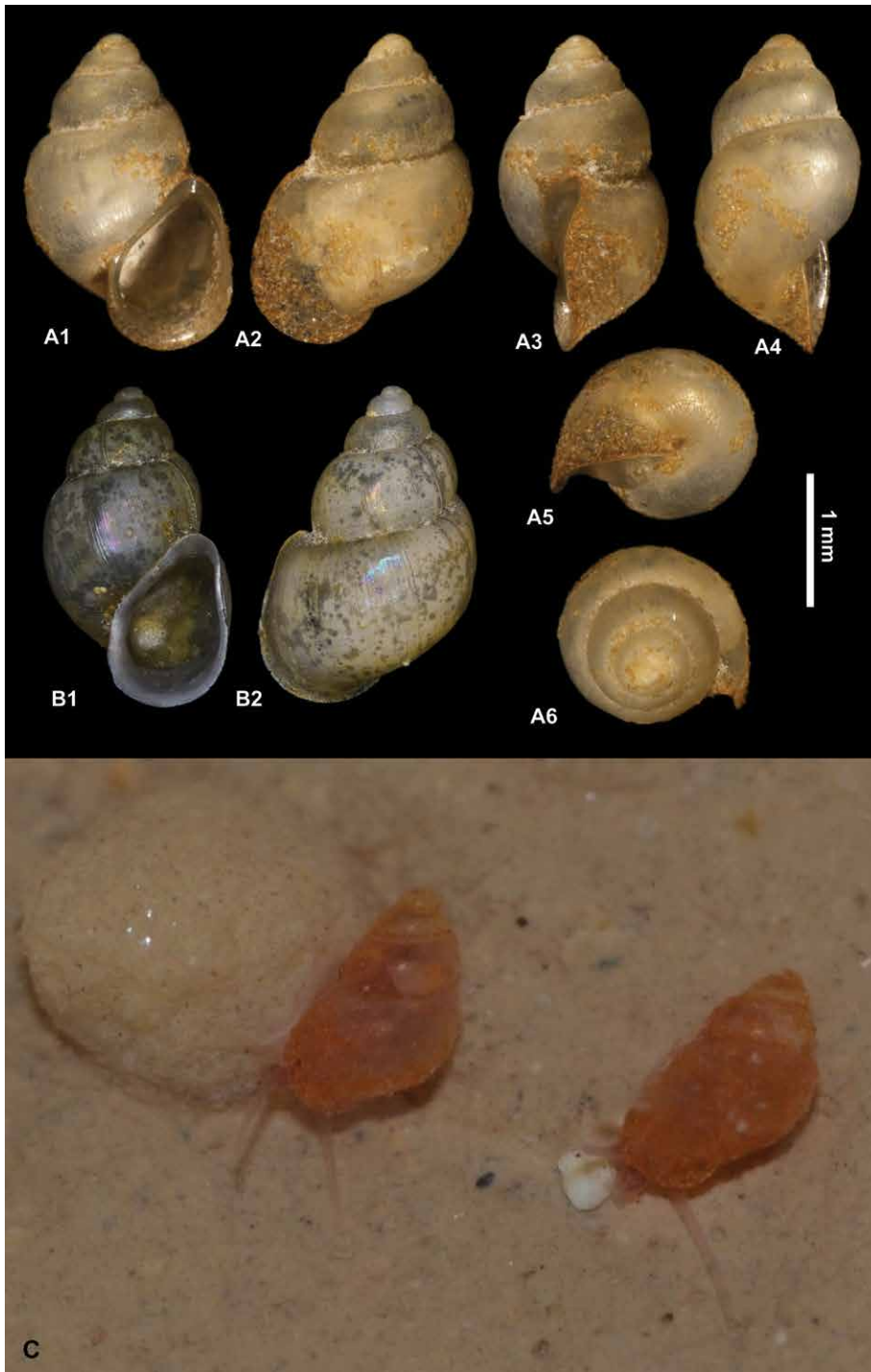
Figure 3. *Trogloiranica tashanica* n. sp., Iran, Khuzestan Province, Behbahan County, Tashan District, Sarjooshar Village, Tashan Cave. A1-A6: Holotype NMBE 558282; B1-B2: Paratype ZMUH 123; C: Live specimens of Trogloiranica tashanica n. sp., in their natural habitat within sulfide ponds of Tashan Cave (Specimens were transferred into a small stet basin at the pool shelf for photography) (photo: Jozef Grego, Yaser Fatemi and Mohammad Javad Malek-Hosseini).

pear-shaped and slightly depressed form labral side, adapically separated from the body whorl by a weak furrow. Peristome margin expanded especially at its lower side and blunt along its outline. The labral lip characteristically sinuous in lateral view, columellar lip straight. Umbilicus closed and obscure.

Anatomy

Animal without eyes and pigment. Female reproductive organs (Fig. 4) typical of the Moitessieriidae: with rather big bursa copulatrix and one small receptaculum seminis (distal one (i.e., in the position of rs_1 after Radoman 1983)). Penis (Fig. 5) simple, without any outgrowths. Loops of rectum (Fig. 5) with the faecal pellets arranged characteristically for the Moitessieriidae (Boeters & Gittenberger 1990), like a bandolier, not a chain.

Molecular phylogenetic relationships

We present two original COI (cytochrome oxidase subunit I) (552 bp, GenBank Accession numbers MK906039-MK906040) and two histone 3 sequences (283 bp, GenBank Accession numbers MK906041-MK906042). In both of these protein-coding loci the tests of Xia et al. (2003) revealed no saturation. Topology of the trees obtained with BI and ML analyses were identical. The obtained topologies using BI and ML were congruent. The phylogeny inferred with COI (Fig. 6) suggested a close relationship with the Moitessieriidae, but did not confirm the monophyly of the latter. Between the clade consisting of the *Bythiospeum* and the other grouping *Iglica* and *Paladilhiopsis* there were representatives of the family Cochliopidae, and *T. tashanica* between the Cochliopidae and *Iglica/Paladilhiopsis* clade. However, the low bootstrap supports are typical of deep nodes inferred with COI. On the other hand, the phylogeny inferred with nu-

clear H3 (Fig. 7) clearly demonstrated that *T. tashanica* belongs to the Moitessieriidae (Bootstrap support 86%). Likewise, the concatenated analysis supported this (Fig. 8).

Etymology

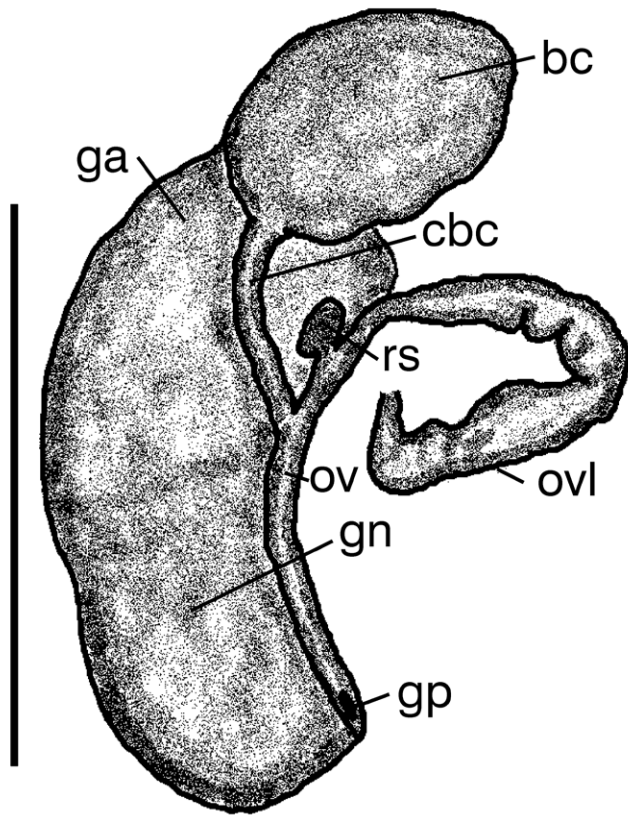The species is named after the type locality, the Tashan Cave.

Figure 4. Renal and pallial part of the female reproductive organs of *Trogloiranica tashanica* n. sp. ((bc−bursa copulatrix, cbc−duct of bursa copulatrix, ga−albumen gland, gn−capsule gland, gp−gonopore, ov−oviduct, ovl−loop of oviduct, rs−seminal receptacle); bar equals 0.5 mm.
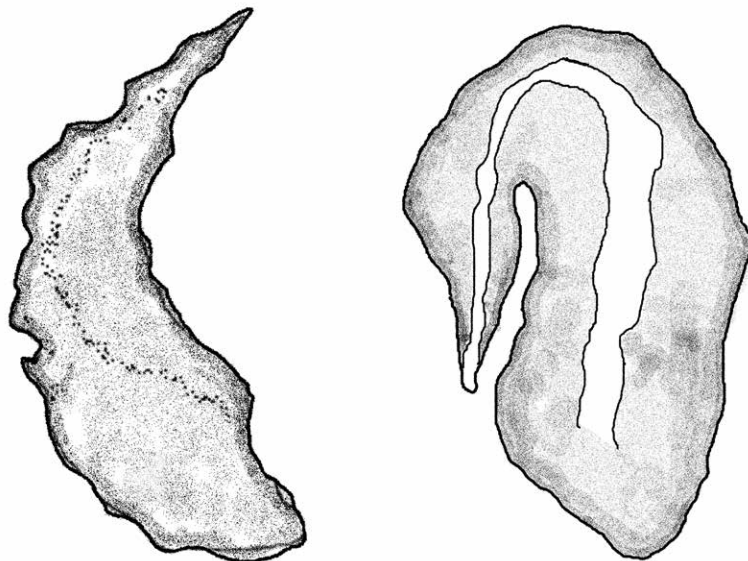

Figure 5. *Trogloiranica tashanica* n. sp., A. penis: at the left dorsally, in situ, in natural position, at the right ventrally, somewhat flattened, under the cover sleep. B. loops of the rectum, with the faecal pellets arranged characteristically for the Moitessieriidae, like a bandolier, not a chain.

Distribution

Known only from the type locality.

Ecology

Tashan Cave developed in Miocene limestones of Upper Red—Fars formation in the southeast part of the Zagros Mountain chain. The cave is situated under a semi-arid steppe plateau near the Sarjooshar village. The mostly horizontal cave with numerous passages and domes is several hundred meters long (detailed cave mapping is currently in progress). The morphology of the cave suggests a strong influence of sulfide-rich hydrothermal waters during the process of speleogenesis. Numerous pools and lakes inside the dark zone of the cave are rich in hydrogen sulfide, which is the main energy substrate for chemoautotrophic bacteria that utilize CO_2 and methane as a carbon source (Palmer, 2007). Preliminary stable isotope data (Sarbu, personal communication) suggest that the microbial biofilms that thrive in the cave pools represent the main source of food for the rich cave fauna including the *Trogloiranica tashanica* n. sp., the blind fish *Garra tashanensis* Mousavi-Sabet, Vatandoust, Fatemi and Eagderi, 2016, and the isopod *Stenasellus tashanicus* Khalaji-Pirbalouty, Fatemi, Malek-Hosseini, Kuntner, 2018 that were recently described from this cave. Specimens of *T. tashanica* n. sp. were found on the limestone walls, on the bottom rocks and on the mud covered by microbial biofilm in the cave pools. The water temperature was 25.4 °C and the pH was 7.8. The atmospheric conditions in the cave are almost constant throughout the year at 25.2 ° C − 25.4 °C, 530 ppm – 755 ppm CO_2 saturation and 99.9 % relative humidity.

DISCUSSION

Despite their ecological sensitivity and importance, subterranean ecosystems are very poorly studied worldwide and, except the areas of southern Europe, Balkans, southwestern Caucasus, eastern United States with northeast Mexico, Japan, Southeast Asia, New Zealand, and Tasmania, stygobiont gastropod records are extremely scarce (Bole and Velkovrh, 1986; Kabat and Hershler, 1993; Culver, 2012). This distribution represents only our present knowledge, while, most likely, the stygobiont Gastropoda inhabit all suitable habitats worldwide. The subterranean fauna of Iran is poorly known. Some cave-adapted groups of animals such as fishes (4 species), Crustacea (amphipods and isopods, more than 20 species), diplopods, and insects have been reported from Iran (Malek-Hosseini and Zamani, 2017). We suggest the putative presence of several groups of animals in hypogean environments of Iran, but a lot of field work is needed. *Trogloiranica tashanica* n. sp. is the first obligate cave-dwelling snail from Iran. The closest stygobiont gastropod records to this Iranian species can be found in the south-eastern part of Greater Caucasus in Georgia, in the vicinity of Sochi
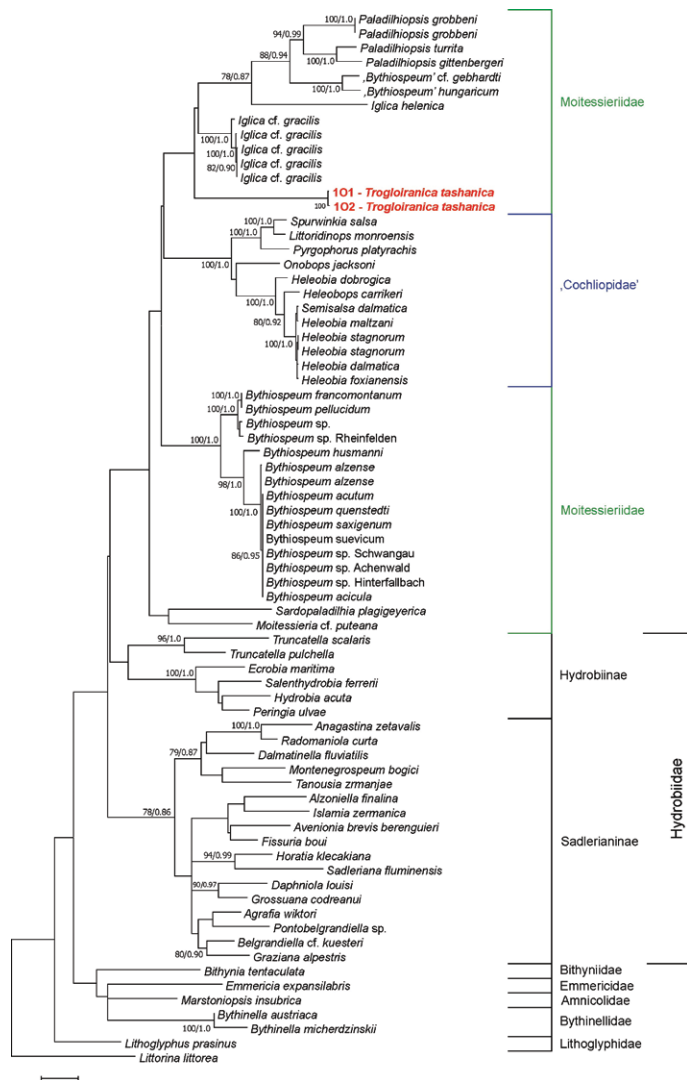
Figure 6. ML phylogeny based on COI. Bootstrap supports if > 70 % and Bayesian probabilities given. New sequences of *Trogloiranica tashanica* are indicated in bold red; for the other sequences, see Table S1.

Figure 7. ML phylogeny based on H3. Bootstrap supports if > 60 % and Bayesian probabilities are given. New sequences of *Trogloiranica tashanica* given in bold red for the other sequences see Table S1.

Figure 8. ML phylogeny based on concatenated data from COI and H3. Bootstrap supports if > 60 % and Bayesian probabilities are given. New sequences of *Trogloiranica tashanica* given in bold red.

in Russia (Starobogatov, 1962; Vinarsky et al., 2014), and southern Turkmenistan (Zhadin, 1952), where we could expect the closest relations. The records from the Balkans (Glöer and Grego, 2015, Grego et al., 2017) in west and southeast Asia (Grego, 2018) towards the East, are likely not at all closely related to the Ponto-Caspian stygobionts, as it is proven for the related freshwater crenobionts of the family Hydrobiidae (Delicado et al., 2016). Of the five other known sulfide stygobiont gastropods, *Heleobia dobrogica* from Movile Cave represents the closest known lineage to *T. tashanica* n. sp. not only geographically, but also ecologically and by its three million-year old separation from its surface relatives (Falniowski et al., 2008). It appears that the other four known sulfidic stygobionts from the Frasassi caves in Italy, Lower Kane Cave in Wyoming, and Melissotrypa Cave in Greece could represent species with much younger adaptation history (Wethington and Guralnick, 2004). The communication of *Islamia sulfurea* with their surface relatives in the Frasassi caves (Bodon et al., 2009) is indicated by the distribution of the same species in other habitats and by the presence of the recent invader *Potamopyrgus antipodarum* Gray, 1843 at the same locality. This invasive species was found for the first time in Europe in 1889, and in Italy in 1961. Considering the special and rich biodiversity encountered in sulfidic caves and the estimation that 10% of the world's caves were formed by sulfidic speleogenesis (in some regions such as Greece these estimates reach up to 20 % of the accessible caves (Vaxevanopoulos, 2009), we hypothesize that the sulfide-induced high subterranean diversity persisted in most of these ex-sulfidic caves after the input of hydrogen sulfide faded out. According to our experience, the ex-sulfidic cave Tahm Nam Dôn in Khammouane, Laos, hosted significantly higher stygobiontic gastropod diversity than any other neighboring cave without traces of the sulfide corrosion in its vicinity (Grego, 2018). If so, the importance
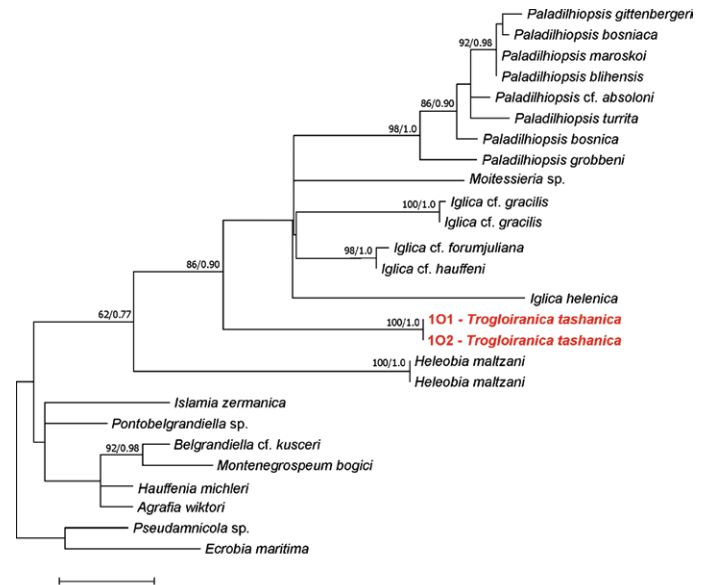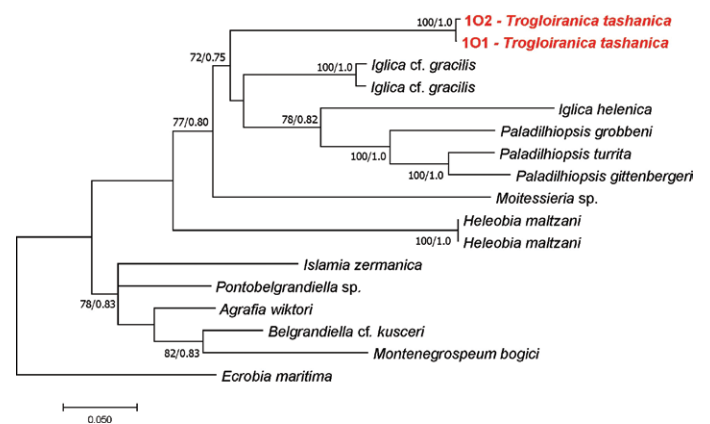
of the sulfidic processes in the formation of the recent stygobiont diversity would be significantly strengthened, and the high subterranean diversity of many isolated subterranean environment could be better explained.

CONCLUSIONS

Tashan Cave likely represents another chemoautotrophically based cave ecosystem. The new species record is the sixth worldwide known stygobiont gastropod inhabiting subterranean sulfide-rich waters and the first records of stygobiont gastropod from Iran, indicating the large potential of the country for the presence of additional subterranean life forms. Although the whole ecosystem has not been well understood yet, at least we know that the water contains sulfide. The long natural history leads the subterranean ecosystems towards narrow, sometimes extreme sulfide specialization by ecological adaptation to the stable underground environment. Such a narrow specialization and long-term stable conditions reduced the adaptability of all the single ecosystem components and made it extremely sensitive against all, even very small changes, in the environmental conditions. The resulting very fragile web could be to some extent regarded as a multisymbiotic system. The disappearance of one component can lead to the collapse of the entire ecosystem. That is the main reason why the protection and conservation of such unique habitats as the Tashan Cave ecosystem have worldwide importance. Most of the caves worldwide hosting such rich sulfide ecosystems are heavily protected with restricted access, reduced to a small number of researchers per year. We believe the new locality also deserves such strict protection and that with the help of local environmentalists and cavers it can be soon achieved. Unfortunately, due to the negligence of authorities, cavers, locals, and even the research groups, a lot of habitat destruction has already occurred in this recently-discovered cave.

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METABARCODING COMPARISON OF PROKARYOTIC MICROBIOMES FROM APPALACHIAN KARST CAVES TO SURFACE SOILS IN SOUTHWEST VIRGINIA, USA

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Abstract

Caves in carbonate rocks of the temperate Appalachian karst region of the United States host impressive ecosystems comprised of cave-adapted and endemic fauna. The primary source of biological energy to epigenic cave systems has been presumed to be surface-derived organic matter, however, the provenance of the prokaryotic microorganisms utilizing that organic matter is uncertain. Specifically, we sought to determine whether the microbes in these caves propagate from established subterranean communities, or rather, enter from the surface via infiltration and migration. This project addresses these overarching questions by determining a) significantly differing composition of prokaryotic communities inside the caves versus those found immediately outside; and b) similarity of the interior cave microbiomes between cave systems. Samples were collected from four caves in The Cedars area of Lee County VA and from two caves within Natural Tunnel State Park in Scott County, VA, and included cave sediments from streambeds, moist clay-sized deposits, and speleothems. Exterior soil samples were collected outside of the caves, with particular attention to sites of water infiltration into the caves below. Prokaryotic microbiomes were surveyed by Illumina sequencing the 16S rRNA barcode. Results detected 1894 unique operational taxonomic units (OTU), 21.4 % of which were shared between cave speleothems and surface soil, while 53.8 % of the OTU in cave sediment were also found in surface soil. The cave microbiomes had only small to moderate differences and were more similar to each other than to surface microbiomes. Of the phyla detected in this survey, two have not been reported in previous cave microbiome surveys, *Armatimonadetes* and *Parvarchaeota*. This survey demonstrates that the cave prokaryotic microbiomes in a temperate region with regular rainfall are primarily composed of cave-adapted microbes and that these microbes occur throughout independent cave systems in the same region.

INTRODUCTION

Caves are meteorologically stable environments with low temperature fluctuations, high humidity, and relatively neutral pH. These conditions would make caves attractive to many biological organisms except for the extreme lack of primary energy input (e.g., light) that limits these biomes to oligotrophs – organisms adapted for survival in environments with extremely low nutrient content. Cave-dwelling oligotrophic prokaryotes have been formally studied for decades (e.g., Caumartin 1963, Barton and Northup 2007), and a combination of culturing and environmental DNA studies have found a large variety of microbes living in subterranean environments (e.g., Tomczyk-Żak and Zielenkiewicz 2016). Due to the lack of energy input, cave and soil microbes often live in biofilm communities and survive using metal oxidation or a variety of strategies such as nitrogen fixation, acquisition of volatilized aromatic compounds, or utilization of dissolved organic matter introduced via infiltrating meteoric water (Northup and Lavoie 2001; Ortiz et al., 2014). Communal lifestyles promote recycling of limited resources, such as the use of waste products excreted from other bacteria containing proteins, lipids, and other biomolecules (Barton et al., 2004, Barton et al., 2007). Certain bacteria form notable structures in caves such as pool fingers and moon milk, as they break down organic calcium salts and excrete calcium ions as waste products (Barton and Northup 2007). Still other microorganisms play a role in cave formation by mediating carbonate dissolution (e.g., Engel et al., 2004).

Since cave microbes are adapted to survive at the cusp of starvation and utilize numerous alternative energy acquisition strategies, the majority of them are difficult to culture and identify using traditional microbiological techniques. The advent of molecular genetic techniques for the identification of environmental DNA (eDNA) provides the potential to distinguish many microorganisms that have been overlooked using other methods (Torsvik and Øvreås, 2002). The first generation of molecular methodologies included PCR amplification of a marker gene followed by a host of fingerprinting methods (e.g., DGGE, RFLP, RAPD, RISA) and allowed an estimation of diversity, but without the benefit of taxonomic identifications (Dorigo et al., 2005). A newer approach, metabarcoding, provide marker sequences from a single genetic locus that can taxonomically distinguish microbial species. The first successful applications of metabarcoding utilized clone libraries of PCR amplicons and Sanger sequencing (Handelsman, 2004). Most recently, metabarcoding analyses have

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been expanded to utilize next generation deep-sequencing technology enabling the identification of large numbers of microorganisms from many different environments (Wooley et al., 2010).

Metabarcoding coupled with next generation sequencing has been conducted in several caves, and operational taxonomic units OTU counts ranging from hundreds to thousands have been discovered on speleothems (Ortiz et al., 2013, Mendoza et al., 2016, Lavoie et al., 2017, Vardeh et al., 2018) and in sediment (De Mandal et al., 2017). There have been two notable outcomes from these studies. First, when cave interior microbes were compared to those of surface soils in two arid environments, very low overlapping OTU content was found – 16% from Kartchner Caverns, AZ (Ortiz et al., 2013) and 11.2 % from Lava Beds National Monument (Lavoie et al., 2017). Second, various cave micro-habitats harbored distinct microbiomes, irrelevant of their proximity to one another (Ortiz et al., 2013, Lavoie et al., 2017).

The purpose of this study was to perform a metabarcoding survey of the microbial communities from six caves found in the temperate Southern Appalachian Mountain range of North America with the intention of testing two hypotheses. Hypothesis 1 stated that caves in a temperate environment would share a larger proportion of microbes with the surface than caves in arid environments, and Hypothesis 2 stated that individual caves within a given karst ecosystem would exhibit insignificant differences in their microbiomes.

Materials and Methods

Study Area and Sampling

Samples were collected from 6 caves that developed in lower to middle Ordovician-aged carbonate rocks, located in southwestern Virginia (Miller and Brosge, 1954, Brent, 1963). Two of the caves, Bolling and Panel, are located within Natural Tunnel State Park in Scott County, VA. Both caves are gated and locked so human activity is limited to permitted researchers and guided tours conducted by park staff from May to October. Tours are conducted weekly during peak months (June-July) and monthly at non-peak times of year. Their entrances are 1.2 km apart and the two caves are hydrologically separated. Bolling Cave is located high on Copper Ridge on the western side of Stock Creek, roughly parallel to the north portal of Natural Tunnel. It has over 425 m of mapped passages. Infiltrating groundwater forms a small stream that exits the cave via a spring. Panel Cave is also situated in Copper Ridge, 1.2 km from the entrance of Bolling Cave, on the east side of Stock Creek, downstream from Natural Tunnel. It is a single meandering cavern approximately 190 m long. Panel Cave's stream is fed by infiltration from a sinking stream on the surface (unpublished dye trace data), and exits via a large spring. The remaining 4 caves, Smith's Milk, Gallohan 1 & 2, and Surgener, are located approximately 40 km from Natural Tunnel State Park in The Cedars Natural Preserve in Lee County, VA. This area was agricultural before its current designation as a natural preserve, and lands around the preserve are still farmed. These caves are not gated, but they occur on a combination of preserved and private land and are difficult to access. They are rarely visited, limited to sporadic tours by researchers and geology classes (once or twice a year), and the occasional trespasser. The four cave entrances occur within a 500 m radius and they are hydrologically connected with flow from Smith's Milk through Gallohan 2 and Gallohan 1, to Surgener (unpublished dye trace data). Recharge appears to be primarily, if not entirely, by surface infiltration of meteoric water. The cave stream exits Surgener through a large entrance and flows as a surface tributary to the Powell River. During periods of high river discharge, water can backflow to the entrance of Surgener Cave. Known animal occupants of all six caves are limited to several species of salamander, cave crickets, arachnids, and occasional bats during winter months (no large bat colonies have been observed). There is evidence of past bear activity in Bolling cave but no recent sightings.

Three types of samples were collected from each cave for microbiome analysis—speleothems or damp cave ceilings, cave sediments, and surface soils. Cave speleothems were sampled by rubbing sterile cotton swabs along the surfaces of damp formations, placing the cotton tip into a 1.5 mL microcentrifuge tube, and removing the wood handle with snips. Where no speleothems were present, samples were collected from damp cave ceilings. Sediments were collected by directly scooping substrate with an opened 15 mL sterile screw-cap conical tube and sealing the tube immediately after collection. The collected substrates ranged from small loose streambed gravel to sand or moist clay. Surface soils were collected from areas immediately outside of the cave entrances adjacent to the entrance pathway, as well as from areas overlying the caves wherever possible. Leaf litter was brushed away and top soil scooped into 15 mL sterile screw-cap tubes in a manner similar to the interior sediment sampling. All samples were transported in darkness to a laboratory at the University of Virginia's College at Wise and stored at −20 °C until further processing for DNA extraction. Three or more replicates of each type were collected from each site, and nitrile gloves were worn for all collections.

DNA Extraction and Sequencing

DNA was extracted from all soil and sediment samples using Qiagen's (Germantown, MD, USA) PowerSoil DNA Isolation kit following the manufacturer's instructions. DNA was extracted from the cotton swabs using the same kit with

the following modification—swabs were placed directly into the bead-beater tubes as if they were soil samples. DNA samples were stored at −20 °C. The 16S rDNA V4 regions from all the replicates were PCR amplified during a GCAT-SEEK workshop (Genome Consortium for Active Teaching: https://gcat-seek.weebly.com) held at Hampton University (Hampton, VA) July 2017. The V4 rDNA regions were PCR amplified and prepared for multiplexed paired-end Illumina sequencing following the protocol of Caporaso et al. (2011). Paired-end deep sequencing was completed by Wright Labs LLC (Huntingdon, PA) using Illumina's MiSeq technology (reagents kit v2). The number of successfully sequenced replicates from each location and type is included in Table S1.

OTU Clustering and Taxonomy Assignments

A total of 4,070,451 raw sequences were produced from 49 samples. These were paired, trimmed, and quality filtered (expected error of < 0.5 %) using USEARCH version 7 (Edgar, 2010) leaving 101,719 sequences, representing 1894 unique OTU. Reads were analyzed with QIIME 1.9.0 software package (Caporaso et al., 2010). OTU were chosen and grouped from the quality filtered reads using UCLUST and singletons were discarded. OTU was defined as a group of reads with ≥ 97 % similarity. Taxonomy was assigned using the Greengenes 16S rRNA gene database (release 13-5, DeSantis et al., 2006) and RDP Classifier (Wang et al., 2007).

Alpha Diversity Analyses

Replicate OTU data from each site and type were combined for all alpha diversity calculations (see Table S1 for number of completed replicates). Alpha diversity was estimated using Chao1 (Chao and Chiu 2016), Shannon (Smith and Wilson 1996), and Simpson (Pielou 1969) indexes calculated with the VEGAN package in R (Oksanen et al., 2018, R Core Team, 2018). Observed OTU represent the raw number of unique OTU from each site and type.

Beta Diversity Analyses

Comparisons between sample types and sites were conducted as multi-dimensional analyses. Principle Coordinates Analysis (PCoA) with scatter plots and Analysis of Similarity (ANOSIM) were conducted to compare the taxonomic compositions of each sample site. These were generated using identifiable OTU and their abundance with the software package PRIMER-E with the PERMANOVA add-on (Clark and Gorley, 2015). OTU counts were normalised with the square-root function and resemblance estimated using the Bray-Curtis index.

Results

Cave Versus Surface Alpha Diversity

A total of 1894 unique OTU were detected from the six sample sites; 1433 occurred within caves (482 from swabs and 1343 from sediment), and 1254 occurred in surface soil. Of these OTU, 1543 were taxonomically identifiable, with 1096 outside and 1174 inside; whereas, 640 OTU were found only in cave samples (Table S2).

Species richness and diversity estimates were calculated for the speleothems, cave sediments, and surface soil (Fig. 1, Supplemental Table S1). Surface soils and cave sediments exhibited the greatest richness according to observed values and Chao1 estimates, as well as the greatest diversities based on the Simpson and Shannon indices. Not only did cave sediments have the most abundant OTU, but the richness and diversity estimates demonstrated greater variability compared to soils outside the caves. Speleothem samples all had lower numbers of OTU than cave sediment or soil, but diversity estimates were much more variable, suggesting less consistency from sample to sample.

The OTU were organized by phylum, and relative abundance was calculated to determine differences between sample types (Table 1). The relative abundance of the most highly represented phyla was generally consistent between the exterior soil samples versus the cave sediments and speleothems. Differences were observed among the minor phyla; for example, *Tenericutes* was much less abundant in the speleothem samples versus outside soil and interior sediment, and seven phyla were not found on the speleothems. Two minor phyla were only observed inside the caves—NC10 and the Archaean, *Crenarchaeota*. The number of OTU with unassigned taxonomy was much larger among cave-specific sequences and several phyla had noticeably lower relative abundance in the cave-specific OTU. Two phyla, *Armatimonadetes* and the Archaean *Parvarchaeota* have not been previously reported to reside in caves.

Cave Versus Surface OTU Comparison

To determine the differences between the interiors and exterior environments of the six caves, we compared OTU observed on speleothems, exterior soil, and cave sediments (Fig. 2, Table S2). Each sample type was associated with unique OTU, and the cave sediments had the largest representation with 320 (Fig. 2A). Overall cave versus surface comparison shows that 1,133 of the 1894 OTU (59.8 %) were shared between the surface and subsurface. A comparison between the speleothem bacteria and surface soils show that 337 of 1574 OTU were shared (21.4 %). Comparisons between the cave sediments and exterior soils show that 904 OTU were shared (53.8 %) between the two environments.
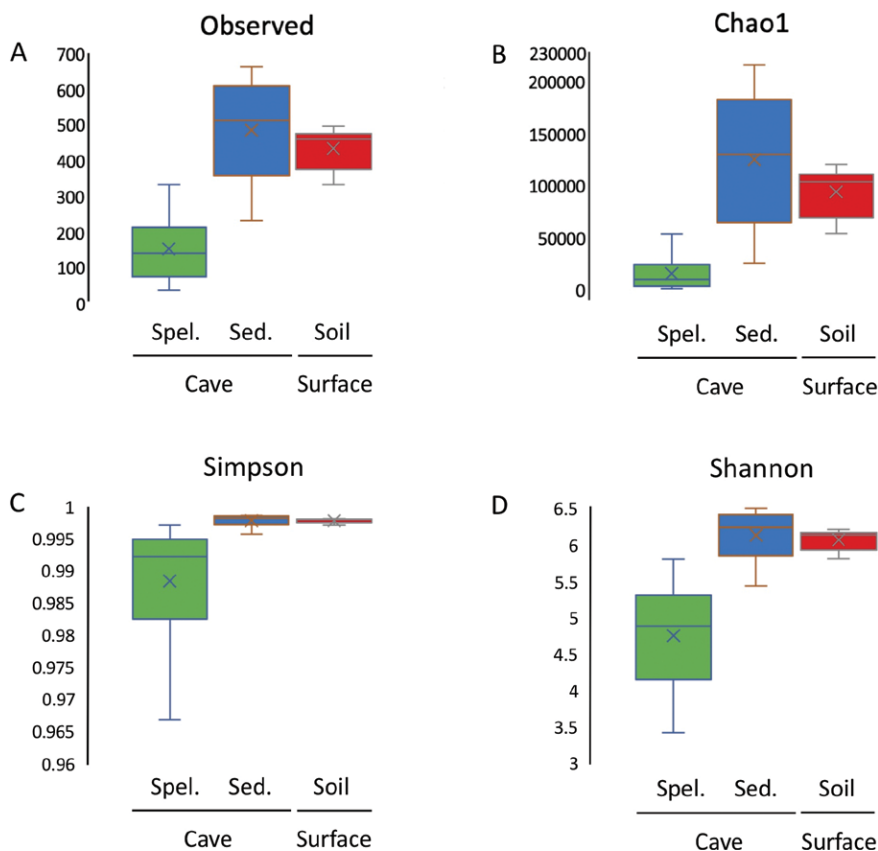
Figure 1. Box plots representing species richness and diversity estimates of the six caves sampled for this study. Species richness is represented as the number of unique observed operational taxonomic units (OTU) (A) and further estimated using the Chao1 (B), Simpson (C), and Shannon (D) indexes.

PCoA was completed for each replicate of each sample site and type (Fig. 2B). The exterior soil samples had the least amount of variation, with nearly all the replicates forming a distinct group. The interior sediment OTU were similar to exterior ones according to PCoA1 but dissimilar according to PCoA2. Cave sediment OTU were also much more variable than the exterior soil samples. Speleothem OTU were highly variable and overlapped very little with the sediment and soil samples.

Analysis of similarity (ANOSIM) was conducted to compare differences between the sample types. In overall comparisons, the largest significant R-value ($R = 0.726$, $P = 0.001$), and thus, the greatest difference occurred between speleothem bacteria versus exterior soil. The second most significant difference occurred between speleothem versus cave sediment bacteria ($R = 0.509$, $P = 0.001$), and third, the significant difference between cave sediments and exterior soils was $R = 0.23$ ($P = 0.001$).

Inter-cave Comparison

OTU from the interiors of the six caves were compared to determine the similarities of microbiomes within different caves residing in the same region. Of the 1,598 OTU identified from cave interiors, 544 were unique to one of the caves, leaving 1,054 that were found in more than one cave, including 109 found in all six (Fig. 3A, Supplemental Table S3).

To determine if these differences were significant, PCoA and ANOSIM analyses were performed. PCoA showed a noticeable delineation between speleothem and sediment OTU but not between caves, suggesting the microbiomes in the different caves are indistinct (Fig. 3B). Two-way ANOSIM analyses comparing the speleothem and sediment OTU between each cave demonstrated only small to moderate differences between them, and only 7/15 of the comparisons had a significant P value ≤ 0.05. Surgener Cave stands out as the only one whose microbiome showed significant differences from the other five. An R-value from the combined samples from all caves provided a significant R-value of 0.37, suggesting an overall low similarity of the microbiomes between the caves.

Discussion

Cave Microbiomes in a Temperate Environment Share Fewer than Expected OTU with the Surface Microbiome

A long-held assumption in cave ecology has been that cave microbiomes, representing a potential primary source of energy entering the food web, are composed of surface microbes and organic matter that have been transported in by flowing or dripping water, roots, or animal occupants (Laiz et al., 1999, Simon et al., 2003, Engel and Northup, 2008). The microbiomes of cave interiors have been studied for decades and an extensive list of troglobitic bacteria have been compiled (e.g., Tomcyzk-Zak and Zielenkiewicz, 2016), yet comparisons between interior and exterior microbial communities are sparse. Most recently, two metabarcoding surveys of cave systems in arid regions of North America compared cave speleothem and microbial mat microbiomes to those found in surface soils (Ortiz et al., 2013, Lavoie et al., 2017). Both studies found very low overlap between subsurface and exterior microbiomes; 16 % between speleothems in Kartchner Cavens, AZ and surface soil and 11.2 % between lava cave microbial mats and surface soil. These studies provide evidence that the majority of cave microbes may be cave-adapted endemics and not dormant or physiologically suppressed surface microbes that have washed in.

We surveyed the prokaryotic microbiomes from speleothems and soil from six caves in the temperate Appalachian region of Southwest Virginia and compared them to surface soil microbiomes to test the following hypothesis: caves in

Table 1. Relative amounts (%) of prokaryotic phyla found in each sample type.

Taxonomy	Surface Soil	Speleothems	Cave Sediment	Cave Specific
Phylum				
Actinobacteria	22.75	21.91	19.47	10.16
Proteobacteria	14.29	16.05	14.21	12.97
Acidobacteria	11.97	12.15	9.85	5.47
Planctomycetes	8.22	6.07	7.82	6.56
Gemmatimonadetes	7.26	7.16	7.52	5.63
Armatimonadetes	4.15	4.12	3.68	2.81
Bacteroidetes	3.03	3.90	3.61	3.44
Chloroflexi	3.03	3.04	3.08	2.81
TM7	1.92	1.52	1.95	2.81
BRC1	2.00	1.52	1.73	1.25
OD1	1.44	1.52	1.58	2.34
Tenericutes	1.68	0.22	1.13	nd
Verrucomicrobia	1.12	1.52	0.98	0.94
Chlamydiae	0.48	0.65	0.75	0.94
Cyanobacteria	0.72	0.43	0.53	0.47
GN04	0.16	0.87	0.68	1.41
WS3	0.56	0.65	0.68	0.63
Nitrospirae	0.32	nd	0.45	0.78
OP3	0.48	0.43	0.45	0.47
Firmicutes	nd	0.87	0.30	0.94
OP11	0.16	0.43	0.15	0.47
TM6	0.40	nd	0.30	nd
GOUTA4	0.08	0.22	0.15	0.31
AD3	0.08	nd	0.08	0.16
NC10	nd	nd	0.15	0.31
Chlorobi	0.08	nd	0.08	nd
Fibrobacteres	0.08	nd	nd	nd
GN02	0.08	nd	nd	nd
Archaea				
Parvarchaeota	0.88	1.30	2.26	4.53
Crenarchaeota	nd	0.22	nd	0.16
Unassigned				
...	12.61	13.23	16.39	30.31

a temperate environment would share a larger number of microbes with the surface than caves in an arid environment, based on the assumption that more surface microbes would enter caves in an environment with regular year-round rainfall than caves in arid environments that receive seasonal rainfall.

The Ortiz et al., (2013) and Lavoie et al., (2017) studies compared the microbiomes of cave speleothems to surface soils. When we compared speleothem+damp ceiling samples with surface soil we found a 21.4 % overlap, which demonstrated a higher incidence of overlapping microbes than was seen in the arid environments, but the overlap was still relatively low, suggesting over 78 % of the microbes living on speleothems and ceilings were cave-adapted. Based on these observations, we reject our first hypothesis.

We also included cave sediment microbes in our study and found that these had an OTU overlap of 53.8 %, much higher than speleothems but still lower than anticipated if surface soil and its accompanying microbes were being hydraulically transported into, and subsequently colonizing, caves. All six caves have perpetually-running streams that are presumably fed from a combination of surface water and groundwater sources. We believed this water could serve as a pathway for the entrance of microbes into the caves and that they would be more likely to be found in cave sediments than on speleothems. There was a much larger overlap between cave sediments and surface soils but it was still lower than expected, with a little over half the OTU found in both locations. These data suggest cave sediment microbiomes harbor large numbers of unique species and strains of microbes able to survive and propagate in the nutrient-poor subsurface environment, and that infiltrating groundwater and sinking streams are only minor contributing factors. Thus, we cannot comfortably reject or maintain our hypotheses without further comparisons.

The Microbiomes Among Caves are Similar

A comparison of three geographically distinct European caves discovered 474 distinct 16S OTU using a clonal approach and found very similar microbial profiles, leading the authors to posit that limestone caves harbor similar microbiota (Porca et al., 2012). In contrast, microbiome surveys of individual speleothems and microbial mats have demonstrated these features can have unique microbiomes, with no correlation between the number of shared OTU and

A.

B.

proximity (Legatzki, 2011,2012; Ortiz et al., 2013; Lavoie et al., 2017). These studies showed that a highly diverse microbial community could exist upon a single cave formation, and that community structure was variable from formation to formation, suggesting that microbial community composition may be site-specific at some scale.

Our second hypothesis, individual caves within an ecosystem would have insignificantly different microbiomes, was formed based on the observation that variation between speleothems and rock surfaces within caves is comparable to that found between geographically diverse caves (Ortiz et al., 2013). Considering the observations of Porca et al. (2012), we presumed the OTU found within the six sampled caves would be insignificantly different from cave to cave. The caves we sampled are located in different watersheds, but within a radius of 40 km, and four of the caves (Surgener, Gallohan 1, Gallohan 2, and Smith-Milk) represent separate entrances into a presumed single cave system. PCoA analyses demonstrated differences between cave samples, but according to ANOSIM, these were small to moderate differences, suggesting that the cave microbiomes are not unique to individual caves, which supports the observations of Porca et al. (2012), as well as our second hypothesis.

Cave-specific Microbial Taxa Unique to this Metagenomic Survey

The predominant cave-specific microbial phyla found in our study (Table 1) are similar to those described by previous studies (Vardeh et al., 2018; Lavoie et al., 2017; Mendoza et al., 2016; Ortiz et al., 2013): *Actinobacteria*, *Proteobacteria*, *Acidobacteria*, *Planctomycetes, Gemmatimonadetes*, and *Bacteroidetes*. We did, however, uncover cave-specific taxa from two phyla not mentioned in previous studies: *Armatimonadetes*, and *Parvarchaeota*.
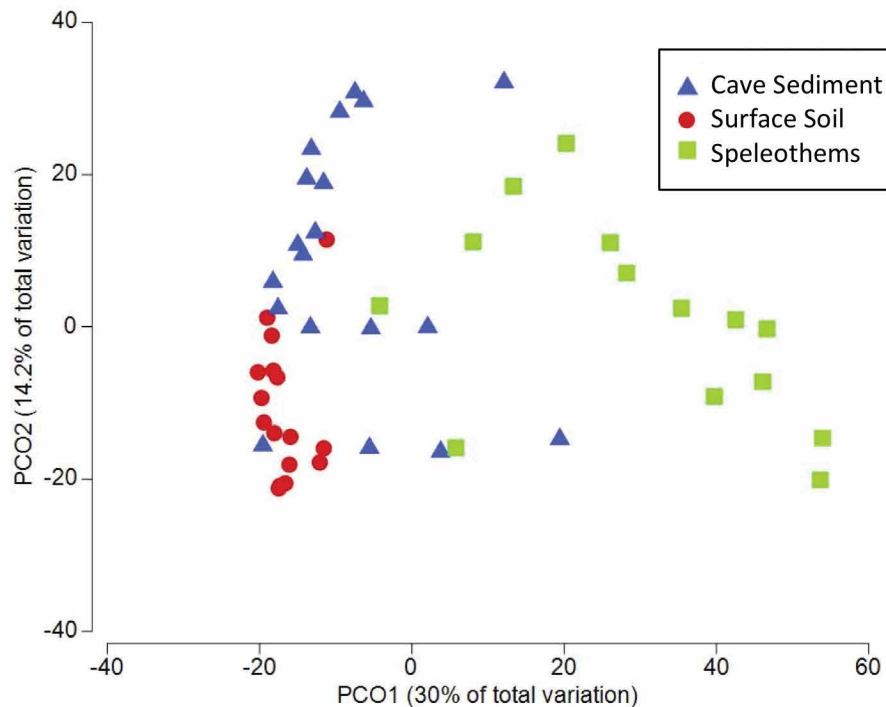
Figure 2. Comparisons of the prokaryotic microbiomes of cave interiors to surface soil. (A) Venn diagram showing the numbers of unique operational taxonomic units (OTU) shared between interior sample types and exterior soil. (B) Principle coordinates plot demonstrating the similarities between the sample types. Each symbol represents an independent sample from one of the six sample sites.
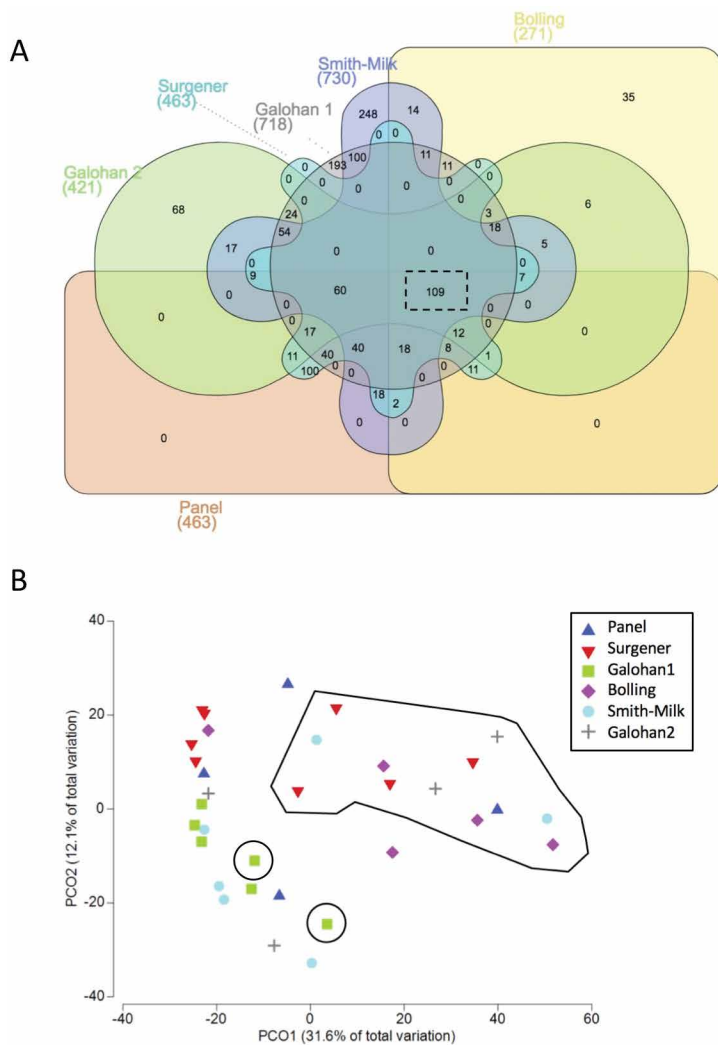
Figure 3. Comparisons of the microbiomes of the six caves sampled for this study. (A) Six-way Venn diagram showing the numbers of unique operational taxonomic units (OTU) shared between each cave. Speleothem and cave sediment OTU were combined for each cave. The diagram was generated using InteractiVenn (Heberle et al., 2015) (B) Principle coordinates plot demonstrating the similarities between the microbiomes of each cave. Each symbol represents an independent sample from one of the six caves. The speleothem samples are circled or enclosed within a freeform shape. The non-enclosed symbols represent cave sediment samples.

Armatimonadetes is a phylum first defined from 16S metagenomic surveys from a wide variety of environments including hot springs and geothermal soils (Hugenholtz et al., 1998; Stott et al., 2008), temperate soils (Dunbar et al., 2002; Lipson and Schmidt 2004), and freshwater (Crump and Hobbie 2005; Urbach et al., 2001; Wu et al., 2007). Three species from this phylum have been cultured and described as aerobic chemoheterotrophs. *Armatimonas rosea*, was isolated from the rhizophere of a reed from in a freshwater lake in Japan (Tamaki et al., 2011); *Fimbriimonas ginsengisoli* was collected from ginseng field soil in South Korea (Im et al., 2012); and, *Chthonomonas calidirosea* was cultured from geothermally-heated soil in New Zealand (Lee et al., 2011). Fifty-eight distinct cave-specific *Armatimonadetes* OTU were identified in our study and sixteen were identifiable as the genera *Armatimonas* and *Fimbriimonas* (Supplemental Table S3).

Parvarchaeota is an Archeaen with taxa first discovered in acid mine drainage (AMD) of Iron Mountain in Richmond, CA, USA, and are among the smallest known cells (Comolli et al., 2009; Baker et al., 2006). A survey of *Parvarchaeota* from 39 AMD and hot springs sites from around the world found them residing only in AMD sites. Follow-up culture plus genome analyses from the same study suggested they are iron-oxidizing anaerobes that must scavenge amino acids and nucleotides from the environment or other organisms (Chen et al., 2018).

Even though species from *Armatimonadetes* have not been reported from caves, this habitat is consistent with previous descriptions since they can live in a wide range of habitats. This is not the case for *Parvarchaeota* since the damp karst cave features do not offer a habitat similar to AMD.

Conclusions

Our data demonstrate a consistent distinction between the community structures of cave-dwelling microbes and surface microbes in a temperate environment with regular year-round rainfall. This study also demonstrates that the interiors of the six caves hosted relatively uniform microbiomes, suggesting these microbes have adapted to the cave environment. Mechanisms for communication between caves and the surface and between cave systems in the southern Appalachians are poorly understood and are presumably related to the complex geologic and evolutionary history of the region. These results suggest that further exploration of these ecosystems is warranted and could uncover unique microbiomes.

Acknowledgements

Table 2. One-way ANOSIM values comparing each sample type.

Cave	Speleothem vs. Cave Sediment		Cave Sediment vs. Surface Soil		Speleothem vs. Surface Soil		All Interior vs. Surface Soil	
	R-value	*P*-value	*R*-value	*P*-value	*R*-value	*P*-value	*R*-value	*P*-value
Surgener	0.889	0.029	0.889	0.029	0.771	0.029	0.677	0.001
Gallohan 1	0.833	0.1	0.537	0.029	0.714	0.067	0.615	0.001
Gallohan 2	1	0.1	0.583	0.1	1	0.333	0.725	0.01
Smith-Milk	0.75	0.1	0.093	0.314	0.607	0.133	0	0.536
Bolling	0.917	0.01	0	0.667	0.583	0.01	0.5	0.076
Panel	1	0.333	0	0.7	1	0.25	0.409	0.167
All[a]	0.726	0.001	0.23	0.001	0.509	0.001	0.43	0.001

[a] Combined data for all caves.

Table 3. Two-way ANOSIM TESTS cave vs cave (speleothems and sediment).

Cave	Panel		Bolling		Smith-Milk		Gallohan 2		Gallohan 1	
	R-value	P-value	*R*-value	P-value	*R*-value	P-value	*R*-value	P-value	*R*-value	P-value
Surgener	0.57	(0.011)	0.50	(.019)	0.558	(0.06)	0.607	(.04)	0.494	−0.002
Gallohan 1	0.343	(0.038)	0.586	(.027)	0.125	(0.152)	0.392	(0.089)	...	...
Gallohan 2	0	(0.60)	0.403	(0.067)	0	(0.778)	...	...	...	...
Smith-Milk	0.185	(0.162)	0.396	(0.080)	...	...	...	...	...	...
Bolling	0.048	(0.425)	...	...	...	...	...	...	...	...
All[a]	0.37	(.002)	...	...	...	...	...	...	...	...

[a] Combined data for all caves.

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EPILYTHIC CYANOBACTERIA AND ALGAE IN TWO GEOLOGICALLY DISTINCT CAVES IN SOUTH AFRICA

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Abstract

There is a lack of knowledge on cyanobacteria and algae living in caves in the southern hemisphere. As a result, a pioneer study was undertaken to investigate cyanobacterial and algal community composition in two morphologically and geologically distinct caves in South Africa. Skilpad Cave is characterized by a large sinkhole entrance in a dolomitic landscape. Three zones (light zone, twilight zone and dark zone) were identified based on differences in light intensity. Bushmen Cave, on the other hand, is a rockshelter overhang situated in a sandstone-dominated area and only presents a light and twilight zone. Cyanobacteria and algae were sampled twice, during the summer and winter of 2018 while abiotic factors of interest, i.e. light intensity, temperature and relative humidity, were also measured. A huge diversity of cyanobacteria (14 genera) and algae (48 genera) were identified in the two caves. While some genera were only present in one of the caves, other cosmopolitan genera were found in both caves. The most common genera encountered were *Phormidium*, *Oscillatoria* and *Nostoc* (cyanobacteria), *Pinnularia* and *Luticola* (diatoms), *Chlorella* and *Chlorococcum* (green algae). Cyanobacteria, green algae and diatoms were also the richest groups (taxa) in terms of the number of genera. More genera were present in the warm, wet season compared to the dry, cold season. Genus richness was positively correlated with light intensity in Skilpad Cave, but negatively in Bushmen Cave. *Petalonema alatum*, a cyanobacterium encountered in Bushmen Cave, represented a new record for Africa, and therefore, further research on cyanobacteria and algal assemblages in caves in the southern hemisphere is strongly recommended.

Introduction

Researching the biology of caves not only presents an opportunity to study unique and extreme ecosystems, it is also fundamental to our understanding of earth's delicate ecological balances (Lee et al., 2012). Caves typically represent extreme environments characterized by low nutrient availability (Pedersen, 2000), a limiting factor for many biota. However, some lifeforms, including cyanobacteria and algae, still find such environments suitable for colonization and growth (Mulec et al., 2008).

Cave-associated communities of cyanobacteria and algae are mainly influenced by three factors: light, temperature, and humidity (Hernández-Mariné and Canals, 1994; Ducarme et al., 2004; Poulîčková and Hašler, 2007; Lamprinou et al., 2009, 2012). Apart from these factors, water seepage, as well as subterranean characteristics such as cave dimensions, microclimate, morphology, substrate types, and nutrient availability, also influence microbial community colonization and composition (Golubić, 1967; Martinčič et al., 1981; Chang and Chang-Schneider, 1991; Czerwik-Marcinkowska, 2013; Popović et al., 2015).

Based on light intensity, a cave can be divided into three zones, namely the light, twilight and dark zones (Humphreys, 2000). The light zone, usually found at or near the entrance, is subjected to sunlight; and therefore, light intensity, temperature, and relative humidity vary throughout the day. The light zone typically supports various types of microorganisms, plants, and animals (Monro et al., 2018). The twilight zone, characterized by more stable environmental conditions, is exposed to indirect, low light conditions typically insufficient to support complex lifeforms, such as seed plants. Nonetheless, these conditions can sustain some cyanobacteria, algae, mosses, and ferns (Culver and Pipan, 2009). Finally, the dark zone is characterized by the absence of light; and therefore, photosynthetic life. However, specialized algae that utilize alternative metabolic pathways to produce energy have been reported on cave walls (Smarž et. al., 2013; Khan et al., 2016).

Cyanobacteria and algae growing on rock surfaces in aerophytic habitats are known as lithophytes. Of all lithophytes, cyanobacteria are the most adaptable to extreme environments, and together with algae, they usually play an important role in the early phases of colonization and succession (Mulec and Kosi, 2008). Later, these lifeforms may dominate in illuminated karstic cave walls and ceilings (Sambamurty, 2010; Popović et al., 2015).

Due to constant environmental parameters, Mulec et al. (2008) regarded caves as almost ideal natural laboratories. Stable temperature and relative humidity levels, together with the presence of water, particularly favor the growth of cyanobacteria, algae, fungi, mosses, and some ferns. Spores of such biota are carried by wind, migrating animals, and/or water runoff into caves (Falasco et al., 2014; Czerwik-Marcinkowska et al., 2019). Although organic matter can also be carried by water, especially during flooding (Simon et al., 2003; Souza Silva et al., 2011), or be deposited in bat guano

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(Wattez et al., 1990; Kasso and Balakrishnan, 2013), seed plants may be less common in caves due to the lack of sufficient light. They are, therefore, usually limited to the cave entrance (light zone), while the twilight and dark zones are typically marked by less diverse assemblages of cyanobacteria and algae. In general, as the amount of light decreases, the size and complexity of photosynthetic life also decrease (Grobbelaar, 2000; Abdullin, 2011; Lee et al., 2012).

According to Lee et al. (2012), the first microbiological study in caves was undertaken in the late 1940's. Since then, a large number of microbial studies have been undertaken in caves all over the world, especially in the northern hemisphere, and life forms of all kinds have been discovered in various cave systems. Most publications report on the microbial communities associated with caves from European countries, including Czech Republic (Poulîčková and Hašler, 2007), France (Borderie et al., 2011), Greece (Lamprinou et al., 2012), Italy (Cennamo et al., 2012), Poland (Czerwik-Marcinkowska and Mrozińska, 2011), Russia (Gainutdinov et al., 2017), Serbia (Popović et al., 2015, 2017), Slovenia (Mulec and Kosi, 2008), Spain (Busquets et al., 2014), and Turkey (Selvi and Altuner, 2007). In spite of the fact that rare and even new species are often found in caves (Hernandéz-Mariné and Canals 1994; Pipan 2005), a limited number of studies have been conducted on cyanobacterial and algal communities in caves located in the southern hemisphere. An in-depth literature review revealed that only a few phycological studies were documented in caves in South Africa, which were devoted to either cyanobacteria (Büdel et al., 1993; Wessels and Büdel, 1995; Büdel et al., 2004; Maree et al., 2018) or diatoms (Grobbelaar, 2000; Taylor and Lange-Bertalot, 2013).

The aim of this study was to investigate the composition of cyanobacterial and algal communities in different cave zones subjected to varying levels of light intensity, temperature and relative humidity levels. This study represents the first in Africa to include both cyanobacteria and different algal phyla present in caves.

Material and Methods

Study area

The study area included a karstic cave (Skilpad Cave) and rock shelter (Bushmen Cave), located 580 km apart (Fig. 1). These caves were studied during March and July 2018, representing the hot, wet and cold, dry seasons, respectively.

Skilpad Cave (26°11'9.04'' S; 27°11'46.15' E) is located at an altitude of 1633 m above sea level, 50 km north of the town Potchefstroom (North West province, South Africa), and is visited by a local caving club annually. The cave's entrance (facing South-East) is a sinkhole overgrown with cyanobacteria, algae, mosses, ferns, and trees, in contrast to the surrounding bioregion that consists of dry highland grassland classified as Carletonville Dolomite Grassland vegetation (Mucina et al., 2006). The geology of the surrounding area can be defined as dolomite, subordinate chert, minor carbonaceous shale, limestone, and quartzite (Burger, 2013). The average temperature and total precipitation for this region during March 2018 was 25°C and 177.7 mm, respectively. During July 2018 the average temperature and total precipitation decreased to 15°C and 7.6 mm, respectively (World Weather Online, 2018). Bats inhabit this cave and guano is present in deeper areas of the cave. Based on the light exposure classification provided by Lee et al. (2012), three zones of interest were identified, namely the light zone (entrance of sinkhole), the twilight zone (bottom of sinkhole) and the dark zone (first chamber of cave).

Bushmen Cave (28°34'28.07'' S; 28°26'15.42'' E) is located at an altitude of 1872 m above sea level, 9.5 km southeast of the town Clarens (Free State province, South Africa). It is named after the Bushmen (or San) people and their historic rock art that can be found on the shelter walls. Bushmen Cave is a rock shelter (open North-West facing overhang) used by hikers as an overnight shelter. It is located in the Drakensberg grassland bioregion, which is classified as Lesotho Highland Basalt Grassland vegetation (Mucina et al., 2006). The geology of the area is characterized as sandstone, pink-weathering granular or augen quartz-feldspar gneiss (Johnson 1991; Burger, 2013). The average temperature and total precipitation during March 2018 was 21°C and 270.9 mm, respectively. During July 2018, the average temperature decreased to 12°C and the total precipitation to 0.8 mm (World Weather Online, 2018). Following the same light classification by Lee et al. (2012), the entrance was identified as the light zone and the back wall of the shelter as the twilight zone. No dark zone exists in this rock shelter.

In Skilpad Cave a total of 13 sampling sites were selected: five sites were in the light and twilight zones, respectively, while three sites were in the dark zone. In Bushmen Cave, a total of 12 sites were sampled: five sites were situated in the light zone and seven in the twilight zone. Therefore, a total of 25 sites were sampled in the two caves.

Measurement of abiotic factors

At Skilpad and Bushmen caves, three abiotic factors (light intensity, temperature, and relative humidity) were measured at each site at hourly intervals (08:00 − 17:00) during both sampling intervals. Light intensity ($\mu mol\ m^2\ s^{-1}$) was measured using a calibrated LI-COR LI-250A light meter, while temperature (°C) and relative humidity (%) were measured using a TROTEC BL30 climate data logger.
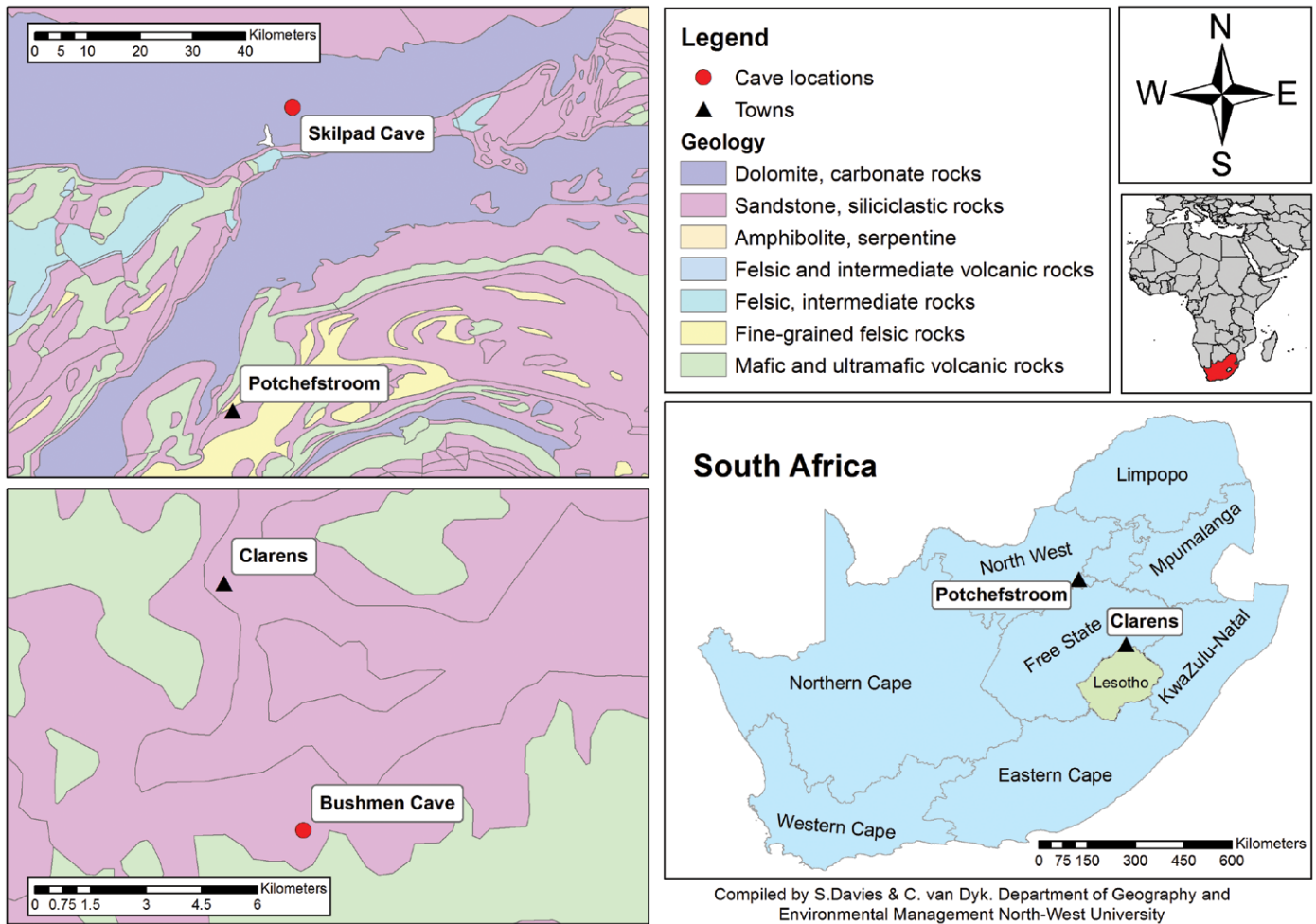
Figure 1. Map of the study areas situated near the towns of Potchefstroom and Clarens, South Africa. Cave locations and underlying geology are indicated on the map.

Sampling and analysis of cyanobacteria and algae

For cyanobacterial and algological studies, samples were scraped from the surface of cave walls using sterile scalpels and placed into labelled, sterile 60 ml polyethylene containers with airtight lids (Popović et al., 2017). The samples were refrigerated (4 °C) and transported to the North-West University (Potchefstroom campus) the following day. Each sample was divided in two sub-samples. One of the sub-samples was used to inoculate a GBG-11 liquid growth medium (Krüger, 1978) and an 1 % agar plate enriched with the same growth medium. Both the growth medium and agar plate were incubated in a growth chamber with a light intensity of 15 µmol m^{-2} s^{-1} and a temperature of 21 °C. Cyanobacteria and algae in the growth medium and on the agar were microscopically identified to genus level after a growth phase of two weeks. The second sub-sample was used for immediate cyanobacterial and algal identification to genus level. For both methods, identification was achieved using a Nikon Eclipse 80i light microscope equipped with a Nikon Digital Sight DS-U2 digital camera. Literature used for cyanobacterial and algal identification included John et al. (2002), Wehr and Sheath (2003), Janse van Vuuren et al. (2006), Taylor et al. (2007) and Hindák (2008).

Statistical analysis

The relationship between abiotic factors (light intensity, temperature, and relative humidity) and cyanobacterial and algal richness at the genus level for each study area (zone) was evaluated with a principal component analysis (PCA) using Canoco 5 software package. To assess the correlation between the respective abiotic factors and cyanobacterial and algal richness, Pearson's correlation coefficient and Spearman's rank correlation coefficient tests were used for parametric and non-parametric data, respectively. The distribution of the errors was considered using the D'Agostino and Pearson omnibus test. Univariate analyses were performed using Graphpad Prism 6 software package and statistical significance was regarded at $P < 0.05$.

Results and Discussion

Cyanobacterial and algal composition

In total, 62 epilithic cyanobacterial and algal genera were found in the two studied caves (Table 1). Microscopic investigations revealed that field samples were less diverse than those inoculated and grown in the growth medium and on the agar plates. Photosynthetic prokaryotic organisms were represented by cyanobacteria, also commonly known as blue-green bacteria and sometimes incorrectly referred to as blue-green algae.

Table 1. List of cyanobacteria and algae found in Skilpad and Bushmen caves during the wet (March 2018) and dry (July 2018) seasons. (LZ = light zone, TZ = twilight zone, DZ = dark zone; X = present).

Taxa and Authorities	Skilpad Cave March LZ	TZ	DZ	July LZ	TZ	DZ	Bushmen Cave March LZ	TZ	July LZ	TZ
Prokaryota:										
Cyanobacteria										
Anabaena Bory ex Bornet & Flahault, 1886	X	···	···	···	···	···	···	X	···	···
Aphanocapsa Nägeli, 1849	···	···	···	···	···	···	···	···	···	X
Aphanothece Nägeli, 1849	X	···	···	···	···	···	···	X	···	X
Calothrix Agardh ex Bornet et Flahault, 1886	X	···	···	···	···	···	X	X	···	X
Chroococcus Nägeli, 1849	X	X	···	X	X	···	X	X	X	X
Gloeocapsa Kützing, 1843	···	···	···	···	X	···	···	···	···	X
Komvophoron Anagnostidis et Komárek, 1988	X	X	···	···	···	···	···	X	X	···
Lyngbya Agardh ex Gomont, 1892	X	···	···	···	···	···	X	X	···	X
Microcoleus Desmaziéres ex Gomont, 1892	X	···	···	···	···	···	X	X	···	···
Nostoc Vaucher ex Bornet et Flahault, 1886	X	X	···	X	···	···	X	X	···	X
Oscillatoria Vaucher ex Gomont, 1892	X	X	···	X	X	···	X	X	X	X
Petalonema Berkeley ex Correns, 1889	···	···	···	···	···	···	···	X	···	X
Phormidium Kützing ex Gomont, 1892	X	X	···	X	X	X	X	X	X	X
Scytonema Agardh ex Bornet et Flahault, 1886	X	X	···	X	···	···	X	X	X	X
Eukaryota:										
Bacillariophyta										
Achnanthes Bory, 1822	···	···	···	···	···	···	···	···	···	X
Achnanthidium Kützing, 1844	···	···	···	···	···	···	···	X	···	···
Amphora Ehrenberg ex Kützing, 1844	···	···	···	···	···	···	···	X	···	X
Craticula Grunow, 1868	···	···	···	···	···	···	X	···	···	···
Cymbella Agardh, 1830	···	···	···	···	···	···	···	X	···	X
Diadesmis Kützing, 1844	···	···	···	···	X	···	···	X	···	···
Encyonema Kützing, 1834	···	···	···	···	···	···	···	···	···	X
Encyonopsis Krammer, 1997	···	···	···	···	···	···	···	X	X	X
Epithemia Kützing, 1844	···	X	···	···	···	···	···	X	···	X
Gomphonema Ehrenberg, 1832	···	···	···	···	···	···	···	X	···	···
Hantzschia Grunow, 1877	X	X	X	X	X	X	X	X	X	···
Luticola Mann, 1990	X	X	···	X	X	X	X	···	X	···
Melosira Agardh, 1824	···	···	···	···	···	···	···	X	···	···
Navicula Bory, 1822	X	···	···	···	···	···	X	X	X	X
Nitzschia Hassal, 1845	···	···	···	···	···	···	···	X	···	X
Orthoseira Thwaites, 1848	X	X	···	X	X	···	···	X	···	X
Pinnularia Ehrenberg, 1843	X	X	···	X	X	···	X	X	X	X

Table 1. (*Continued*).

Taxa and Authorities	Skilpad Cave March			July			Bushmen Cave March		July	
	LZ	TZ	DZ	LZ	TZ	DZ	LZ	TZ	LZ	TZ
Eukaryota:										
Bacillariophyta										
Planothidium Round & Bukhtiyarova, 1996	···	···	X	···	···	···	···	···	X	···
Rhopalodia Müller, 1895	···	···	···	···	···	···	···	···	···	X
Charophyta										
Actinotaenium (Nägeli) Teiling, 1954	···	···	···	···	···	···	···	X	···	···
Cosmarium Corda ex Ralfs, 1848	···	···	···	···	···	···	X	X	···	···
Gonatozygon De Bary, 1858	···	···	···	···	···	···	···	X	···	···
Hyalotheca Ehrenberg ex Ralfs, 1848	···	···	···	···	···	···	···	X	···	···
Klebsormidium Silva, Mattox & Blackwell, 1972	X	X	···	X	···	···	X	X	X	···
Mesotaenium Nägeli, 1849	···	···	···	···	···	···	···	X	···	X
Mougeotia Agardh, 1824	···	···	···	···	···	···	···	X	···	X
Spirogyra Link, 1820	···	···	···	···	···	···	···	X	···	X
Chlorophyta										
Bracteacoccus Tereg, 1922	···	X	···	···	···	···	···	X	X	···
Chlamydomonas Ehrenberg, 1833	X	X	···	···	···	···	···	X	X	···
Chlorella Beyerinck, 1890	X	X	X	X	X	X	X	X	X	X
Chlorococcum Meneghini, 1842	X	···	X	X	X	X	X	X	X	X
Chlorosarcinopsis Herndon, 1958	···	···	···	X	X	···	···	···	···	···
Desmococcus Brand, 1925	···	X	···	X	···	···	···	···	···	···
Leptosira Borzi, 1883	···	···	···	X	···	···	···	···	···	···
Microspora Thuret, 1850	···	···	···	···	···	···	···	X	···	···
Oedogonium Link ex Hirn, 1900	···	X	···	···	···	···	···	X	···	···
Oocystis Nägeli ex Braun, 1855	···	···	···	···	···	···	···	X	···	···
Scenedesmus Meyen, 1829	···	···	···	···	···	···	X	···	···	···
Scotiellopsis Vinatzer, 1975	···	X	···	···	···	···	···	···	···	···
Stichococcus Nägeli, 1849	···	X	···	···	···	···	···	···	···	···
Tetracystis Brown & Bold, 1964	···	···	···	···	···	···	···	···	X	···
Trebouxia Puymaly, 1924	···	X	···	···	···	···	X	···	···	X
Trentepohlia Martius, 1817	X	X	···	···	···	···	···	X	···	···
Ulotrix Kützing, 1833	···	···	···							
Ochrophyta										
Botrydium Wallroth, 1815	···	X	···	···	···	···	···	···	···	···
Eustigmatos Hibberd, 1981	···	···	···	X	···	···	···	···	X	···
Monodopsis Hibberd, 1981	X	···	···	···	···	···	···	···	···	···
Rhodophyta										
Porphyridium Nägeli, 1849	···	X	···	···	···	···	···	···	···	···

A total of 14 cyanobacteria genera were found in Bushmen Cave, of which 12 were also present in Skilpad Cave. *Aphanocapsa* and *Petalonema* were found in Bushmen Cave, but not Skilpad Cave. The presence of *Petalonema alatum* in Bushmen Cave represented a first record of this species in Africa (Maree et al., 2018). The most prevalent cyanobacteria included *Phormidium* (28 sites), *Oscillatoria* (21 sites), and *Nostoc* (20 sites). *Phormidium*, a filamentous cyanobacterium commonly found in caves, was the most frequently encountered genus both in Skilpad and Bushmen caves. *Phormidium* has long cylindrical filaments with uniseriate trichomes and its dark green to brown sheaths are thin and firm (Czerwik-Marcinkowska and Mrozińska, 2011). It was described from caves throughout Europe, e.g. in Greece (Lamprinou et al., 2013), Spain (Asencio and Aboal, 2000; 2004), Italy (Giordano et al., 2000), Hungary (Claus, 1964), Serbia (Popović et al., 2017) and Poland (Czerwik-Marcinkowska et al., 2015). The second most abundant cyanobacterium was *Oscillatoria,* regularly found in sub-aerial habitats (Mulec and Kosi, 2008; Sethi et al., 2012). Distribution records of *Oscillatoria* include caves in Hungary (Claus, 1964), Serbia (Popović et al., 2017) and Slovenia (Mulec and Kosi, 2008). *Nostoc,* characterized by a gelatinous thallus, was previously found in caves amongst others, in the Czech Republic (Poulíčková and Hašler 2007), Greece (Lamprinou et al., 2012), Israel (Vinogradova et al., 2004), Serbia (Popović et al., 2015), Poland (Czerwik-Marcinkowska et al., 2015), Hungary (Claus, 1964), Turkey (Selvi and Altuner 2007), Slovenia (Mulec and Kosi, 2008) and South Africa (Taylor and Lange-Bertalot, 2013).

Eukaryotic phyla comprised of Bacillariophyta (diatoms; 19 genera), Charophyta (8 genera), Chlorophyta (green algae; 17 genera), Ochrophyta (3 genera), and Rhodophyta (red algae; 1 genus). All five these phyla were identified in Skilpad Cave, while four were present in Bushmen Cave. Chlorophyta and Bacillariophyta represented the richest phyla, in terms of the number of genera, in Skilpad and Bushmen caves, respectively. Czerwik-Marcinkowska and Mrozińska (2011) reported Chlorophyta as one of the dominant phyla in a Polish limestone cave, while Bacillariophyta (diatoms) was also observed by Czerwik-Marcinkowska et al. (2015) in similar environments. The diatom *Pinnularia* (Phylum Bacillariophyta) was identified at 20 sites and is well-adapted to colonize a wide range of substrates (Macedo et al., 2009). A Polish study reported *Pinnularia* in five caves (Czerwik-Marcinkowska and Mrozińska, 2011) and it was also found in Israeli caves (Vinogradova et al., 2009). *Luticola*, another diatom, occurred at 15 sites in Skilpad and Bushmen caves and was previously reported growing on wet rocks and mosses in Slovenian (Mulec and Kosi, 2008), Turkish (Selvi and Altuner 2007), Israeli (Vinogradova et al., 2009) and Serbian (Popović et al., 2017) caves. *Chlorella* and *Chlorococcum* are green algae (Phylum Chlorophyta) and both were collected at 17 sites. *Chlorella* was previously reported from Germany (Claus, 1964; Chang and Chang-Schneider 1991), Czech Republic (Poulíčková and Hašler 2007), Hungary (Claus, 1964), Slovenia (Mulec and Kosi, 2008) and Turkey (Selvi and Altuner 2007), while *Chlorococcum* have been reported from Polish caves (Czerwik-Marcinkowska and Mrozińska, 2011).

In accordance to the results of this study, Czerwik-Marcinkowska et al. (2015) regarded cyanobacteria, green algae, and diatoms as essential components of cave microflora. Our results clearly indicate that some cyanobacterial and algal genera were singular to Skilpad Cave, while others were only found in Bushmen Cave (Table 1). Although it was not investigated, the distribution of genera may have been influenced by different rock types in the two caves.
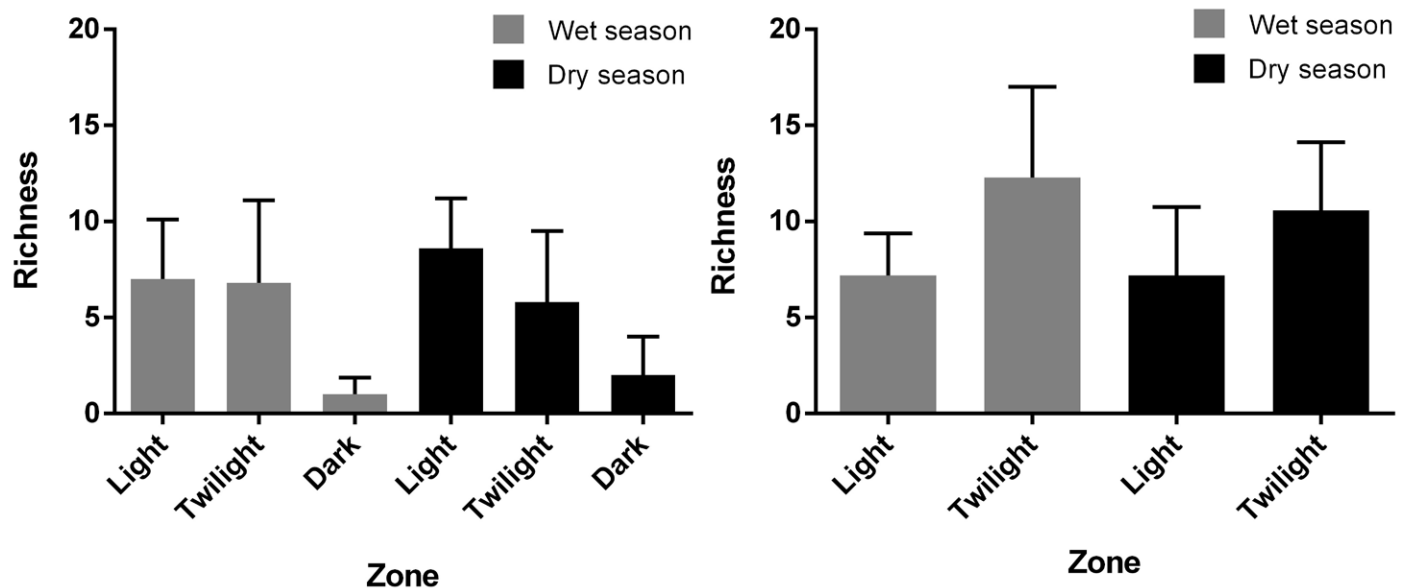

Figure 2. Richness in Skilpad Cave (A) and Bushmen Cave (B). Bars represent the richness in each cave zone during each season. Error bars denote standard deviatation.

Table 2. Abiotic factors measured in Skilpad and Bushmen Caves during the wet (March 2018) and dry (July 2018) seasons (LZ= light zone, TZ = twilight zone, DZ = dark zone).

	Skilpad Cave									Bushman Cave					
	Light Intensity (µmole m² s⁻¹)			Temperature (°C)			Relative Humidity (%)			Light Intensity (µmole m² s⁻¹)		Temperature (°C)		Relative Humidity (%)	
	LZ	TZ	DZ	LZ	TZ	DZ	LZ	TZ	DZ	LZ	TZ	LZ	TZ	LZ	TZ
March 2018															
Minimum	2.3	0.5	0	19.5	21.9	21.1	54.4	59.7	63.4	235.5	10.6	21.6	20.8	39.0	41.3
Maximum	27.4	3.5	0	25.1	24.3	23.2	79.2	69.0	73.3	718.3	30.8	33.5	28.2	74.1	78.4
Average	9.1 ± 7.3	1.9 ± 1.1	0	22.5 ± 1.7	23.2 ± 0.8	22.2 ± 0.7	63.9 ± 7.6	63.9 ± 2.3	67.2 ± 3.2	517.0 ± 181.3	16.8 ± 8.3	28.1 ± 3.5	22.7 ± 8.2	51.3 ± 11.3	49.2 ± 20.4
July 2018															
Minimum	2.0	0.4	0	8.9	11.3	11.6	38.8	43.2	60.4	341.72	13.7	6.2	9.4	11.4	28.5
Maximum	15.5	2.6	0	16.7	14.9	14.8	56.2	57.5	72.3	1015.8	99.6	22.8	16.6	53.4	52.4
Average	8.8 ± 5.3	1.2 ± 0.7	0	12.6 ± 2.3	13.4 ± 1.0	13.3 ± 1.1	50.8 ± 5.0	49.8 ± 3.9	64.4 ± 3.9	606.1 ± 298.0	31.5 ± 28.3	15.2 ± 7.1	10.9 ± 4.4	22.6 ± 14.1	33.8 ± 14.0

During the wet season, 54 cyanobacterial and algal genera were identified in both caves. This number decreased to 41 during the dry season. When considering genus richness per cave zone per season (Fig. 2), it is evident that the richness decreased from the light zone to the dark zone during both seasons in Skilpad Cave. However, in Bushmen Cave the twilight zone consistently presented the greatest richness. To investigate these findings, the abiotic conditions, in relation to genus richness per cave zone per season, was considered.

Abiotic conditions in relation to epilithic richness

Results (minimum, maximum, and average values) of the abiotic factors measured during this study are reported in Table 2. During the wet season (March 2018) variation in Skilpad Cave was observed for light intensity with average values ranging from 9.1 ± 7.3 µmol m² s⁻¹ (light zone) to 0 µmol m² s⁻¹ (dark zone). The same was evidenced for the dry season (July 2018) as average light intensity values ranged from 8.8 ± 5.3 µmol m² s⁻¹ (light zone) to 0 µmol m² s⁻¹ (dark zone). However, variation between the two sampling intervals was especially evident for temperature and relative humidity levels. Bushmen Cave, on the other hand, does not have a dark zone and was exposed to substantially higher light intensity levels. Maximum light intensity levels in Bushmen Cave were 718.3 µmol m² s⁻¹ and 1015.8 µmol m² s⁻¹ during the wet and dry seasons, respectively, which is likely the result of Bushmen Cave being an overhang rock-shelter characterized by a large North-West facing opening. To the contrary, Skilpad Cave has a narrower sinkhole opening with rock walls, which together with the vegetation surrounding the sinkhole entrance, prevent direct exposure to sunlight. Figure S1 (supplementary material) further illustrates light intensity variation between cave zones and seasons, as well as the difference in exposure between the two studied cave systems.

In order to relate the observed cyanobacterial and algal richness to the abiotic conditions, principal component analysis (PCA) biplots were created. With 90.9 % of the variation explained on the X (57.4 %) and Y (33.5 %) axes, Figure 3A illustrates that richness in Skilpad Cave was positively related to light intensity and therefore also the light zones during both sampling intervals. The significance of this relationship was further investigated using a correlation coefficient test, which confirmed that a strong, positive correlation ($r = 0.7$; $P < 0.001$) existed between richness and light intensity. Furthermore, a moderate, negative correlation (-0.4; $P < 0.05$) was evidenced between richness and relative humidity. No significant correlation was observed between richness and temperature in Skilpad Cave. The PCA biplot (Fig. 3B) for Bushmen Cave, with 98 % of the variation explained on the X (58.2 %) and Y (39.8 %) axes, illustrated that a negative relationship existed between richness and light intensity. In support of this, a correlation coefficient test revealed that a moderate, negative correlation (-0.43; $P < 0.05$) existed between richness and light intensity. No other significant correlations were evidenced in Bushmen Cave.

It is evident that light intensity played a major role in cyanobacterial and algal richness in the studied caves. The positive correlations between richness, light intensity, and relative humidity, as evidenced in Skilpad Cave, have been well documented (Asencio and Aboal, 2000, Poulíčková and Hašler, 2007; Vinogradova et al., 2009). Vinogradova et al. (2009) found that the taxonomic composition of algae is influenced by the gradient of illumination as the number of species drastically decreased from the entrance (46 species) to the deeper reaches (26 species) of an Israeli cave. However, the reason for the negative
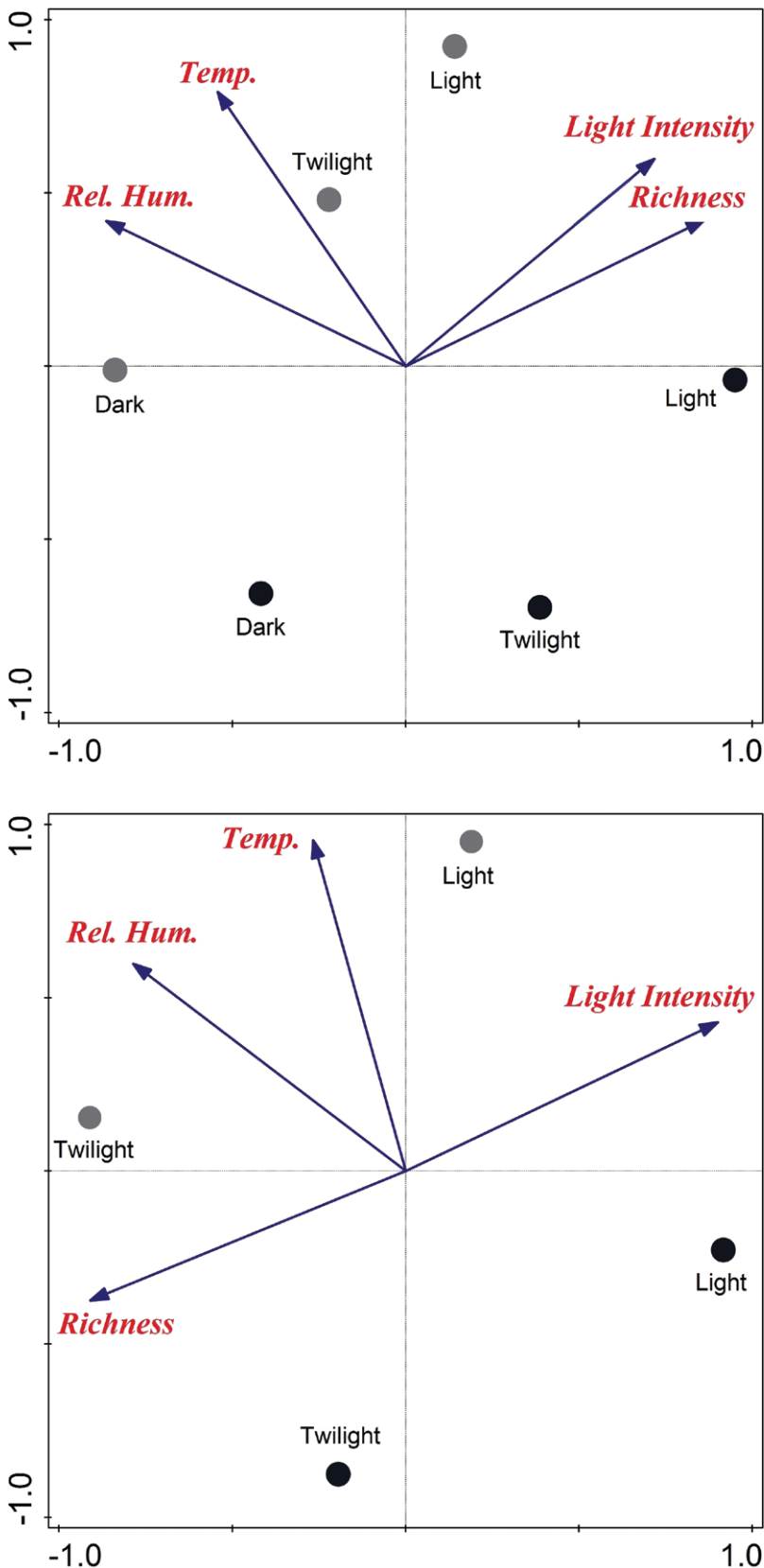
Figure 3. PCA biplot for Skilpad Cave (A) and Bushman Cave (B). Three arrows represent abiotic factors (light intensity, temperature, and relative humidity) and the other arrow represents the cyanobacterial and algal richness. The grey and the black dots represent the cave zones in the wet and dry season respectively.

correlation evidenced between richness and light intensity in Bushmen Cave remains unknown. It may, however, be due to huge differences in the light zones of the two caves in terms of morphology and abiotic conditions.

Conclusions

Limited studies have focused on cyanobacteria and algae in caves located in the southern hemisphere and especially in the southern parts of Africa. Furthermore, only a few scientific works have been published on either cyanobacteria or diatoms associated with South African caves.

During this study two geologically distinct caves in South Africa were studied in terms of their cyanobacterial and algal composition in relation to abiotic environmental variables (light intensity, temperature and relative humidity) considered relevant to the survival of photosynthetic cave microorganisms. A total of 14 cyanobacteria genera were identified from Bushmen Cave, a rock shelter overhang with no dark zone. Twelve of these cyanobacteria genera were identified from Skilpad Cave, a sinkhole entrance cave with a dark zone. The cyanobacterium *Petalonema alatum* was found in Bushmen Cave and represented the first record of this species in Africa. *Phormidium*, *Oscillatoria* and *Nostoc* were the most common cyanobacteria and occurred in both caves. All three of these genera are also commonly found and widely distributed in northern hemisphere caves.

Algae belonging to five phyla were identified in Skilpad Cave, while representatives of four phyla were found in Bushmen Cave. Chlorophyta (green algae) and Bacillariophyta (diatoms) were represented by a large number of genera in both caves. The most common algae encountered included the diatoms *Pinnularia* and *Luticola* and the green algae *Chlorella* and *Chlorococcum*. Results of this study corresponded to findings in the northern hemisphere that cyanobacteria, green algae and diatoms are usually the dominant photosynthetic microorganisms found in caves. A higher richness (based on the number of genera) was recorded in the warm, wet season compared to the cold, dry season.

Considering the abiotic conditions, higher light intensities were recorded in Bushmen Cave than Skilpad Cave due to differences in the physical morphology and orientation of the caves. Temperature and relative humidity values also differed between the caves and the

two sampling intervals. In Skilpad Cave, the genus richness was positively correlated with light intensity, a well-documented relationship. However, an inverse tendency prevailed in Bushmen Cave, a phenomenon that could not be explained.

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A MINIMAL MODEL FOR PREDICTING VENTILATION RATES OF SUBTERRANEAN CAVES

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ABSTRACT

The ventilation of carbon dioxide within subterranean caves regulates the growth of speleothems: mineral deposits found in caves that provide important clues about past climate. While previous studies have used internal temperature measurements to predict ventilation rates, such data would not be available for the task of climate reconstruction. Here, we develop a parsimonious model to predict ventilation rates from knowledge of outside temperatures and the cave's physical dimensions only. In the model, ventilation arises from buoyancy-driven flows created in passageways that connect to the outside. A few key simplifications lead to a system amenable to perturbation analysis, resulting in explicit expressions for ventilation rates. We compare these predictions to time-resolved, *in-situ* measurements of transported cave gases (carbon dioxide and radon). The theory accurately accounts for the timing and magnitude of seasonal and synoptic variations of these gases, and is, therefore, diagnostic of potential seasonal biases in speleothem growth. With proper *in situ* geochemical calibrations, this model may be used to establish a first-order estimate of atmospheric paleotemperatures.

Introduction

Subterranean caves contain calcium-carbonate ($CaCO_3$) mineral deposits, known as speleothems, which record important clues about past climate. Much like tree-rings and ice cores, these deposits can be used to infer past conditions and identify major climatic shifts (Wang et al., 2008). Speleothem records, however, can be difficult to interpret due to a number of competing factors that influence chemical and isotopic composition, mineral fabric, and timing of deposition (Frisia et al., 2000; Fairchild et al., 2006; Lachniet, 2009; Fairchild and Baker, 2012; Wong and Breecker, 2015). Early cave researchers realized the potential importance of internal cave conditions (Hendy, 1971), but only within the past twenty years has *in situ* monitoring technology become sufficiently advanced to record microclimate data with which to calibrate speleothem records (Perrier et al., 2005; Spötl et al., 2005; Baldini et al., 2006; Banner et al., 2007; Kowalczk and Froelich, 2010; Tremaine et al., 2016). Recent laboratory studies and modeling efforts have further suggested that strong ventilation impacts processes such as evaporation and gas exchange across the thin water-film atop stalagmites, and therefore, the rate, timing, and isotopic composition of speleothems (Wackerbarth et al., 2010; Deininger et al., 2012; Dreybrodt et al., 2016; Hansen et al., 2017, 2019). These studies highlighted the fact that to fully understand a speleothem paleoclimate record, it is necessary to have a holistic understanding of the site-specific parameters that control stalagmite growth. These include, but are not limited to, rainfall amount, hydrologic saturation, vegetation and soil productivity, dripwater residence time and water-rock interaction, drip rates, and ventilation (Fairchild and Baker, 2012).

Speleothems are the end product of a series of chemical reactions: (1) rainwater combines with soil-zone carbon dioxide to form carbonic acid: $H_2O + CO_{2\,(g)} \rightarrow H_2CO_3$, (2) carbonic acid percolates downward and slowly dissolves carbonate bedrock, creating a high pCO_2 solution of calcium and bicarbonate: $H_2CO_3 + CaCO_3 \rightarrow Ca^{2+}_{(aq)} + 2HCO^-_{3\,(aq)}$, and (3) the drip reaches a cave void, where the gradient between low-pCO_2 cave air and high-pCO_2 dripwater causes the drip to degas CO_2, driving the pH of the solution up and causing re-precipitation of calcium carbonate: $Ca^{2+}_{(aq)} + 2HCO^-_{3\,(aq)} \rightarrow CO_{2\,(g)} + H_2O + CaCO_3$. Cave ventilation modulates cave air CO_2 concentration and thus the gradient between air and drip, driving both the timing and the vigor of speleothem growth. Without ventilation, cave air CO_2 would come to chemical equilibrium with dripwater CO_2, and speleothem formation would cease.

In addition to controlling the rate and timing of deposition, ventilation has been shown in several long-term cave monitoring studies to have significant impacts on the isotopic composition of dripwater and subsequent speleothem calcite (Spötl et al., 2005; Frisia et al., 2011; Lambert and Aharon, 2011; Tremaine et al., 2011; Feng et al., 2012). Results from other field, theoretical, and laboratory studies support the hypothesis that under strong ventilation regimes where CO_2 is quickly removed from dripwater, rapid precipitation may induce kinetic isotope fractionation, causing a shift in oxygen and carbon isotopes within the speleothem and making paleoclimatic interpretation of the record much more difficult (Scholz et al., 2009; Polag et al., 2010; Watkins et al., 2013, 2014; Stoll et al., 2015). A study by Daëron et al. (2019)

recently demonstrated that even at extremely slow precipitation rates, such as those of subaqueous mammillary calcite in Devils Hole Cave (Nevada, USA), inorganic calcite suffers from kinetic fractionation effects in natural earth-surface systems. Indeed, these effects make the study of ventilation imperative for interpretation of speleothem oxygen and carbon isotopes.

Because ventilation controls the rate of calcite precipitation, it not only influences when a speleothem forms (which seasons), but also where the speleothem forms (i.e., on or within the cave ceiling (stalactite) or on the cave floor (stalagmite)). Wherever dripwater encounters low-CO_2 cave air, there is a potential for calcite precipitation up-stream of the stalagmite. This phenomenon is known as Prior-Calcite-Precipitation, or PCP (Fairchild et al., 2000). When PCP occurs, dripwater cation-to-calcium (X/Ca) ratios change as Ca is preferentially removed from solution, and minor elements such as Sr and Mg become more concentrated (Fairchild and Baker, 2012). PCP is often associated with climate-driven water balance above the cave, where more/less rainfall and thus hydrologic saturation above the cave results in higher/lower drip rates, lower/higher PCP, and lower/higher speleothem X/Ca ratios (Karmann et al., 2007; McDonald et al., 2007; Tremaine and Froelich, 2013; Tadros et al., 2016). Additionally, strong ventilation and low cave-air CO_2 promotes PCP and can exert control on variations in speleothem X/Ca ratios (Sherwin and Baldini, 2011; Wong et al., 2011). It is this seasonal variation in PCP, along with enhanced kinetic isotope effects during stronger ventilation, that will allow our ventilation model to be run in reverse to estimate the seasonality of atmospheric temperature differences.

Given that the process of cave ventilation can have significant impacts on the isotopic and trace element composition of modern calcite, we must endeavor to understand the ventilation of each cave that contains speleothems of paleoclimatic importance. This is especially true in light of recent work that recognized how cave ventilation varies seasonally with well-defined global patterns (James et al., 2015). While past studies have quantified cave ventilation in terms of internal temperature measurements (De Freitas and Littlejohn, 1987; Christoforou et al., 1996; Gregorič et al., 2014), such data would not be available for the task of climate reconstruction. Instead, predictive models must be developed.

Here, we construct a theoretical framework to predict ventilation rates from a minimal set of external information. Building on previous work (Wigley and Brown, 1971; De Freitas and Littlejohn, 1987; Christoforou et al., 1996; Kowalczk and Froelich, 2010), we model ventilation as arising from buoyancy-driven flows created by the internal-external temperature difference. Unlike previous studies, our model does not rely on any internal measurements. It only requires basic physical parameters of the cave itself and outside temperature data. To close the model, we require the flow to be critical via the composite Froude number, a condition borrowed from the field of oceanography (Armi, 1986; Dalziel, 1991; Pratt, 2008). Analysis of the relevant scales, along with a few key simplifications, produces a system that can be solved through perturbation methods, ultimately giving explicit formulas for how ventilation depends on the system's physical parameters.

To test our ventilation model, we use two tracers common to all limestone caves; carbon dioxide (CO_2) and radon-222 (^{222}Rn). As previously discussed, cave air CO_2 is derived from microbial decay and plant-root respiration in the soil zone, as well as dissolved limestone bedrock. Cave air CO_2 concentrations are modulated by a combination of input from both dripwaters and gaseous transport through cracks and fissures above the cave, import of atmospheric CO_2 via ventilation, and removal via condensation corrosion (Sánchez-Moral et al., 1999; Gabrovšek and Dreybrodt, 2000; Mattey et al., 2016). ^{222}Rn is a daughter isotope in a radioactive decay chain that begins with ^{238}U and ends with ^{206}Pb. Limestone contains an average 1.3 ppm ^{238}U to 2.5 ppm ^{238}U, and therefore, acts as a continuous source of radon production and advection into cave chambers through dripwaters, surfaces, and cracks (Fernández et al., 1986; Hakl et al., 1997). ^{222}Rn is an ideal tracer for cave ventilation because atmospheric concentrations of radon are negligible and it is an inert gas with a 3.82-day half-life, which is sufficiently long when compared with most cave-air exchange rates (Fernández et al., 1986; Perrier et al., 2005; Richon et al., 2005; Kowalczk and Froelich, 2010). We report a set of time-resolved measurements of cave air gas concentrations from a cave located in Florida Caverns State Park. The theory accounts for qualitative trends in the measurements throughout the year, and, over certain periods, even shows quantitative agreement with weekly-scale fluctuations. This comparison also allows the production rates of CO_2 and ^{222}Rn to be estimated, which could complement other, more invasive, estimation techniques (Faimon et al., 2006; Kowalczk and Froelich, 2010).

Study Site and Micrometeorological Station

Dragon's Tooth Cave (DTC) is a pristine, karst-hosted dry cave located in Florida Caverns State Park, Marianna, northwest Florida (Fig. 1a-b). The cave formed within a carbonate bluff along the nearby Chipola River, and has two primary passages that trend northwest to southeast in alignment with regional bedrock fractures. The two large passages are intersected by a third east-west trending corridor that dips at approximately 45 degrees from west to east, serving as an entrance to both sections. The northern section was first surveyed and mapped in 1978. The floor of the northern section lies at 18 meters above sea level (ASL) and is composed of mud-clay, which is typical of caves in Marianna that are subjected to seasonal groundwater inundation when Chipola River levels exceed 3−4 meters above the
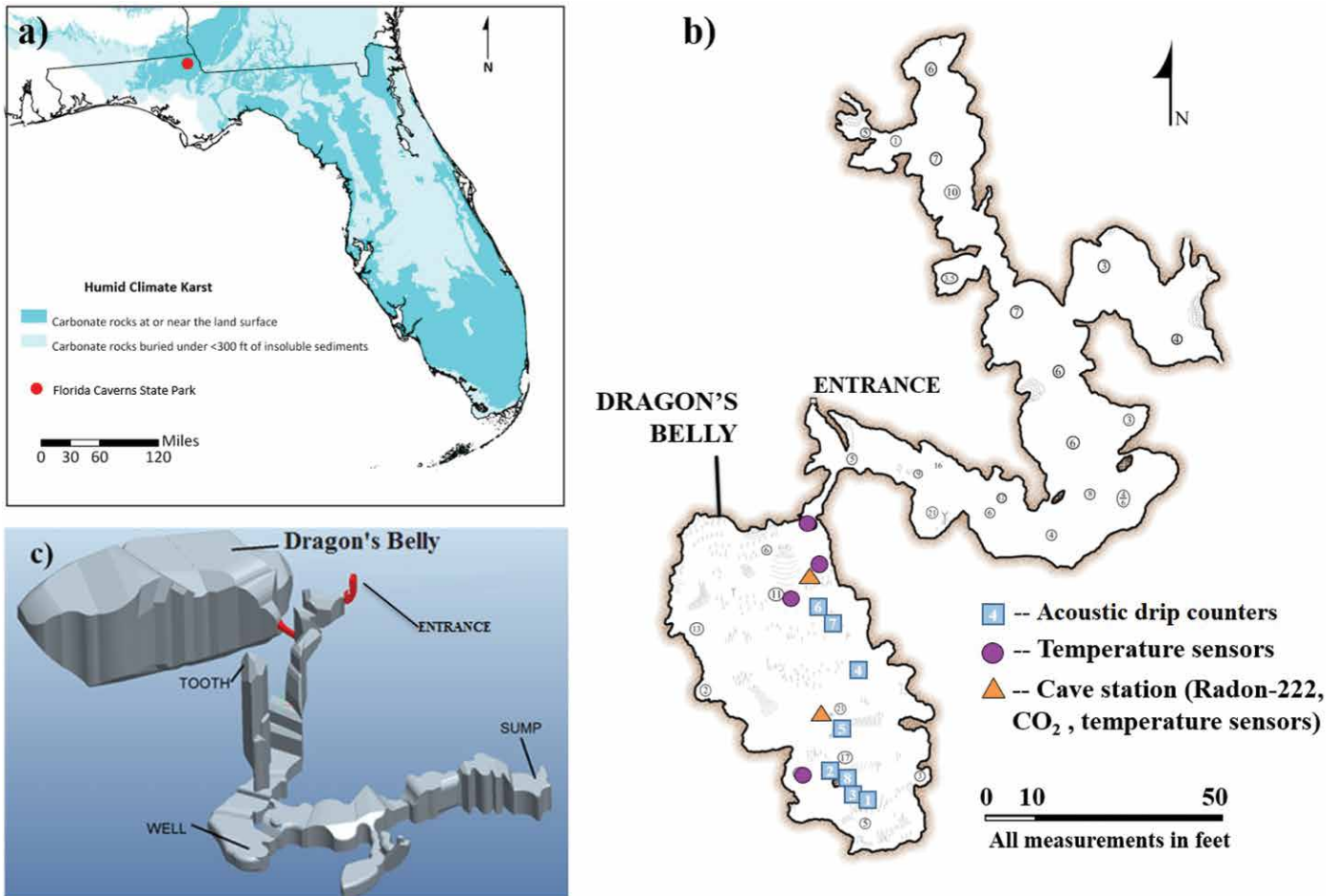
Figure 1. Study site: (a) Karst areas in Florida, USA (data is obtained from https://pubs.usgs.gov/of/2014/1156/downloads/). (b) Dragon's Tooth is a system of caves which includes the main study site, the Dragon's Belly. Also shown are the locations of our sensors within the Dragon's Belly (colored symbols) and elevations (circled numbers). (c) A 3D rendering of the Dragon's Tooth Cave system shows the approximately rectangular geometry of the Dragon's Belly and the narrow passageway that connects to the entrance of the cave system (highlighted in red).

riverbed. Passages within DTC are 2−3 meters tall, thus floodwaters tend to fill the passages and remove any stalactites or soda straws that may have formed, minimizing the potential of flowstone decoration. In 1984, cavers observed airflow through a pile of breakdown near the entrance and dug a narrow tunnel making a connection to the southern section of the cave, finding a previously unexplored room that was highly decorated with beautiful speleothem formations. This room later came to be known as the Dragon's Belly (DB). The floor of the DB lies at 21 meters ASL; groundwater slowly fills the DB from below only during Chipola flooding events that exceed 5 meters above riverbed. Topography above the cave suggests that the DB is the remains of a large diameter NW-to-SE conduit that has collapsed at both ends of the room. In 2012, our group mapped the DTC using Leica ScanStationP20 to obtain highly accurate measurements of its dimensions. The main chamber of the DB is nearly rectangular, 41 m long, 12 m wide, and 6 m high with 45° slopes at each end of the room that pinch off at the ceiling, and it connects to the entrance of the cave system via a single, narrow passageway (0.6 m high, 0.3 m wide and 3 m long). These geometrically simple features make the DB an ideal site for testing basic ventilation models. Once a firm theoretical foundation is established, future studies could examine more complicated cave systems (e.g., complex geometries and/or multiple openings).

Beginning in November 2011, we established a micrometeorological monitoring program inside the DB to continuously collect spatial and temporal variations of ^{222}Rn, CO_2, temperature, and drip rates. ^{222}Rn and CO_2 levels were measured via two cave stations deployed 15-meters apart (Fig. 1b). These stations were equipped with Durridge RAD7 detectors to measure radon, LiCor-820 gas analyzers to measure CO_2, as well as Vaisala HMP45C temperature and relative humidity sensors. Four additional Onset HOBO Pro v2 External Temperature Data Loggers were also deployed throughout the DB. In May 2012, eight acoustic drip counters mounted on tripods were deployed under stalactites in the DB to measure drip rate (Fig. 1b). All data were recorded hourly, with the exception of occasional outages due to flooding events or power failures. Meanwhile, we obtained external temperature data from daily measurements taken at Marianna Municipal Airport, located 8 km from the study site.

The Ventilation Model

We model the ventilation in DB's room as a well-mixed compartment with a single opening that exchanges heat with the surrounding surfaces and the outside environment. Because the internal cave temperature T_c is typically different from the external temperature T_e, a buoyancy-driven flow is created in the connecting passageway (Hunt and Linden, 1999; Pratt, 2008). During the summer, warm outside air flows into the DB through the top half of the opening, is cooled by contact with the cave walls, and exits through the lower half of the same opening. The airflow direction reverses during the winter (see Figs. 2a–b for a schematic). Faimon et al. (2012) showed that temperature accounted for more than 99 % of variations in cave air density. Therefore, we neglect secondary effects such as humidity and ambient pressure.

As long as vertical mixing and viscous effects are weak (i.e., large Peclet and Reynolds numbers), the flow in the opening is essentially that of two layers: a top layer of depth H_1 and speed U_1, and a bottom layer with values H_2 and U_2. In accordance with the Boussinesq approximation, we only take density variations into account insofar as they affect buoyancy (Boussinesq, 1897). Enforcing the Boussinesq approximation and conservation of volume inside the cave results in a symmetric exchange flow (i.e., both the depths and speeds are the same in the two layers) $H_1 = H_2 = H$ and $U_1 = U_2 = U$. Clearly, H must be the half-height of the passageway ($H = 0.3$ m), but U must be determined through other considerations, starting with a balance of the cave's total thermal energy.

Thermal energy balance and closure condition

Under the assumptions of (1) well-mixed air inside the DB room, (2) the Boussinesq approximation, and (3) a symmetric two-layer exchange flow in the cave opening, the rate of change of the cave's thermal energy takes the form (Christoforou et al., 1996):

$$\rho_o c_v V \dot{T}_c = \rho_o c_p A U (T_e - T_c) + \int_S q\, dS \tag{1}$$

The main unknowns in this ordinary differential equation (ODE) are the cave's internal temperature, T_c, and the exchange speed, U. Meanwhile, the outside temperatures, T_e, will be taken from the measurements at Marianna Airport. The parameters are the mean air density $\rho_0 = 1.205$ kg/m^3, the specific heat of air at constant pressure $c_p = 1005$ J/(kgK) and at constant volume $c_v = 717$ J/(kgK), the total volume of the cave chamber $V = 2952$ m^3, and the half-area of the cave passageway $A = 0.09$ m^2.

The first term on the right of Equation (1) represents thermal exchange with the outside via the two-layer flow, while the second represents exchange with the cave walls through surface integration of the local heat-transfer-per-unit-area q [J/(m^{2}s)]. Temperature differences between these surfaces and the enclosed air give rise to convective flows that can promote heat transfer greatly. The Nusselt number, Nu (the ratio of convective to conductive heat transfer), quantifies this increase and can be estimated through knowledge of the Rayleigh number, . Here, T_w is the wall temperature and l is a length scale of the surface under consideration, while $g = 9.8$ m/s^2 is the gravitational acceleration, $\nu = 15.1 \times 10^{-6}$ m^2/s and $\alpha = 2.12 \times 10^{-5}$ m^2/s are the viscosity and thermal diffusivity of air, and $T_0 = 291.9$ K is the mean annual temperature in Marianna, Florida. To estimate the Nusselt number, we use the well-established scaling law (Holman, 2002; Bergman and Incropera, 2011)

$$\text{Nu} = \chi \text{Ra}^{1/3}, \tag{2}$$

where χ is a constant that depends on the orientation of the surface and the direction of the thermal gradient. The sidewalls produce convection regardless of the sign of the temperature gradient, whereas the floor and ceiling only produce convection in the presence of an unstable temperature gradient (see Appendix A for further details). The above law holds for Ra $> 10^7$, and inside the DB's we typically have Ra $\sim 10^{10}$.

The local heat transfer on a particular surface is given by $q = \rho_0 c_p \alpha N_u (T_w - T_c)/l$ (Holman, 2002), and insertion of the Nu-Ra relationship gives

$$\int_s q\, dS = \rho_0 c_p \left(\frac{g\alpha^2}{\nu T_0} \right)^{1/3} \int_s \chi (T_w - T_c)^{4/3}\, dS \tag{3}$$

Here, we have adopted the convention $X^p = |X|^p \, \text{sgn}\, X$ for any variable X in order to shorten expressions. We will take the wall temperature, T_w, to be a different constant on each surface (i.e., the ceiling, the floor, and the four sidewalls) so that the integral in Equation (3) reduces to a summation over these six faces. We note that the length scale l has dropped out of Equation (3) as a consequence of the Nu $\sim$ Ra$^{1/3}$ law. Though easily overlooked, this cancellation simplifies the analysis considerably and avoids certain ambiguities in the definition of l (see Appendix A). This is one factor that will ultimately allow us to obtain closed-form expressions for the ventilation rate.

Both T_c and U are still unknown at this stage, and so a second relationship is required to close the model. Because the DB's is a single-opening room, we can leverage fundamental results from the study of two-layer exchange flows

(Armi, 1986; Dalziel, 1991; Pratt, 2008). Such flows have been found to spontaneously organize into a critical state, characterized in this context by a composite Froude number equal to one. Imposing this condition gives the speed of the exchange flow as

$$U = \sqrt{\frac{1}{2} \frac{Hg|T_e \, 2 \, T_c|}{T_0}} \tag{4}$$

We note that other closure conditions could be used for caves with multiple openings, which we will explore in a future manuscript (Christoforou et al., 1996; Flynn and Caulfield, 2006).

Estimating internal-surface temperatures

Our main goal is to solve Equations (1) – (4) for the cave temperature, T_c, and the exchange speed, U, but first it is necessary to estimate the cave-wall temperatures, T_w, for insertion into Equation (3). Here, we will use temperature measurements from inside the cave to guide and validate certain approximations made in our model. Once constructed, though, the model will not rely on these internal measurements, and the same framework could be applied to other caves without any recalibration.

We begin with a formula often used in the design of wine cellars to estimate the underground temperature at a depth z (Pinchover and Rubinstein, 2005; Tinti et al., 2014),

$$T(z,t) = T_0 + \Delta T_e^{-z/z_0} \cos(\omega(t - t_0) - z/z_0) \tag{5}$$

Here, $\omega = 1.99 \times 10^{-7}$ rad/s is the annual frequency, $\Delta T = 9.3$ K is the amplitude of the seasonal temperature variation (illustrated by the dashed curve in Figure 2d), $\alpha_{lim} = (5.6 \pm 1.6) \times 1027$ m²/s is the thermal diffusivity of the limestone medium, $z_0 = \sqrt{2\alpha_{lim}/\omega} = 2.4 \pm 1.3$ m is the attenuation depth, and t_0 simply sets an initial time. This formula neglects higher modes, for example diurnal and synoptic variations, since they attenuate much more rapidly with depth. The attenuation depth, z_0, depends on the physical properties of the local geology, thus, by using appropriate values for thermal diffusivity, temperatures of surfaces within caves formed in bedrock other than limestone can be calculated.

Equation (5) is an exact solution to a diffusion problem in a homogeneous domain with a periodically driven upper surface. Although the presence of the cave violates the homogeneity assumption, the much lower density of the enclosed air implies that a very large change in air temperature would be required to modify the limestone temperature
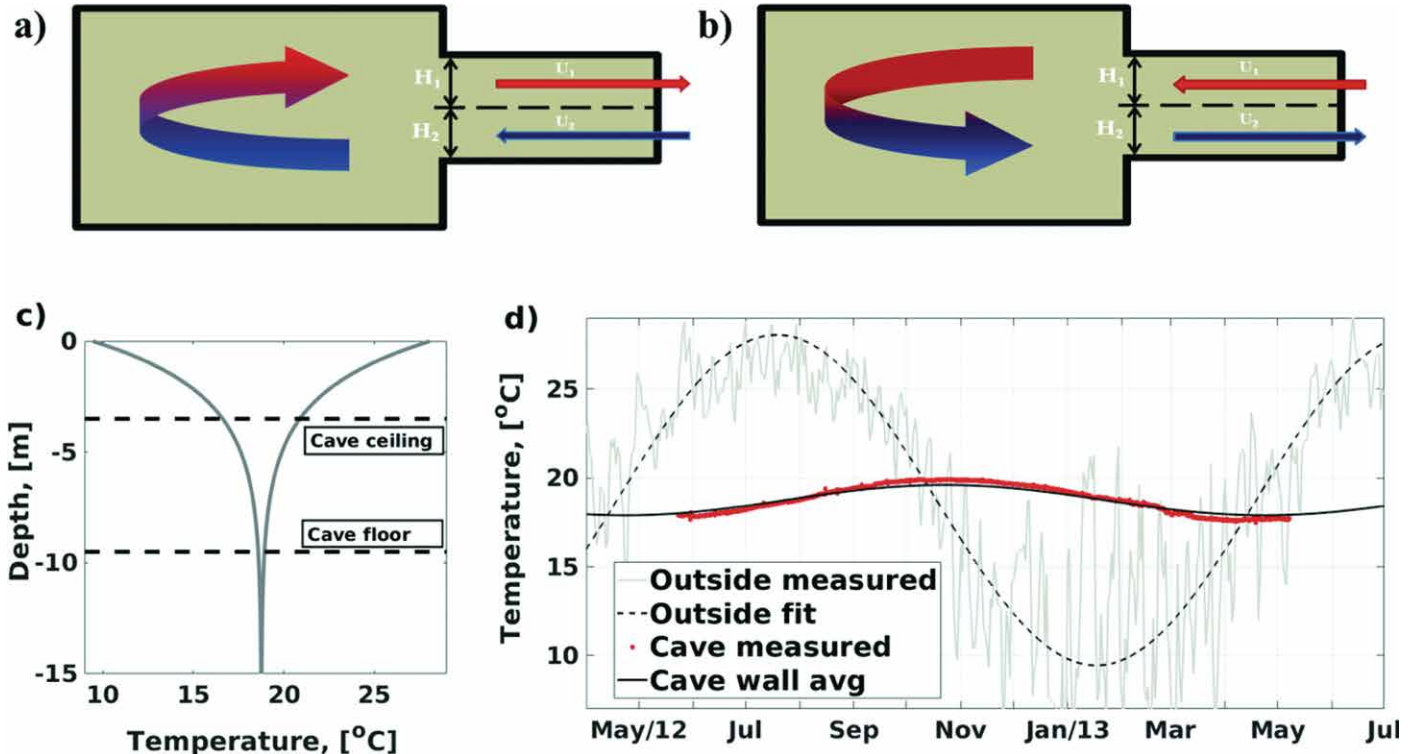

Figure 2. The ventilation model: (a) – (b) The temperature difference between the cave and the outside creates an exchange flow, regardless of whether the cave is (a) warmer or (b) cooler than the outside. (c) The temperature range at various depths as predicted by the wine-cellar formula, Equation (5), with the ceiling and floor of the Dragon's Belly indicated. (d) The cave-wall temperatures can be estimated by inserting the outside temperatures (measurements in faint gray and seasonal fit in dashed black) into Equation (5). The solid black line shows the predicted average wall temperature, which agrees well with internal measurements (red).

appreciably. We therefore apply the formula to estimate the temperature of each cave surface. For the sidewalls, we estimate an average temperature by integrating the formula over the height of the wall with a 3-point trapezoid rule. Figure 2c shows the temperature range predicted by the wine-cellar formula as it varies with depth. Note that at approximately 12 m depth, the temperature of the ground becomes equal to the annual mean of the outside temperature, and there are no seasonal variations below 12 m. That 12 m depth depends on the physical properties of the local geology and varies depending on where the cave is formed. Notably, the temperature of the DB's ceiling varies over a much wider range than does the floor (by a factor of 12).

We next compare these predictions with temperature measurements taken inside the cave. Internal convective flows tend to homogenize the cave-air temperature, and so regardless of where the sensors are placed, the measurements are influenced by all of the surrounding surfaces. We therefore define an average wall temperature, T_{wa}, which incorporates the temperatures of the ceiling, floor, and sidewalls as weighted by surface area

$$T_{wa} = \frac{1}{S} \sum_{i=1}^{6} S_i T_i \tag{6}$$

Here, T_i and S_i are the temperature and area of surface i, and S is the total surface area (1620 m^2). Figure 2d shows the estimated T_{wa} (black) as it compares with measurements (red). The prediction captures the phase of the measurements very accurately and under-predicts the amplitude slightly. The under-predicted amplitude is of minor consequence when compared to the scale of the outside-temperature fluctuations, shown by the faint gray data in Figure 2d. Due to their larger magnitude, the outside-temperature fluctuations are primarily responsible for creating the inside/outside temperature difference that drives exchange.

Figure 2d provides one last insight. The predicted T_{wa} agrees well with internal measurements, even though it does not take ventilation into account. This suggests that the cave's thermal exchange is a wall-dominated process, a concept that is supported by the scaling analysis in Appendix C. It may seem paradoxical that, while ventilation is the main quantity we aim to predict, observations suggest it to be a secondary effect. The resolution is that, while ventilation is indeed secondary for thermal exchange, it is the primary transport mechanism for cave gases such as carbon dioxide and radon. By using simple internal-temperature estimates to predict ventilation rates, our model can accurately describe the transport of these gases.

Predicting exchange rates

Having obtained reliable wall-temperature estimates, it is now possible to solve Equations (1) – (4) for the cave air temperature, T_c, and the speed of the exchange flow, U. Once U is known, the main quantity we seek is the ventilation rate

$$\lambda_v = AU/V, \tag{7}$$

which allows us to determine how cave gases are exchanged with the outside. We solve Equations (1) – (4) with two different approaches. The first is a numerical strategy that tracks the temperature of each individual cave surface and dynamically switches thermal convection on or off depending on the direction of the local temperature gradient. This model thus represents the complex physics of thermal convection with spatial and temporal resolution (see Appendix B for further details). The second approach is a course-grained model that assumes spatially uniform convection to arrive at a system amenable to perturbation analysis. This analysis ultimately gives an explicit expression for how the ventilation rate depends on cave and climatic parameters (see Appendix C for details). With these two approaches, we have both the physical fidelity offered by a computational model and the transparency offered by a purely analytical model. Comparison between the two will allow us to test the additional assumptions made in the perturbation model.

As described in Appendix C, analysis of the relevant physical scales identifies two dimensionless parameters, π_1 and π_2. Briefly, π_1 represents the timescale of wall-thermal exchange compared to the timescale of the outside temperature variation (i.e., a year), while π_2 represents the relative strength of ventilation-induced versus wall-induced thermal exchange. In terms of these parameters, perturbation analysis gives the ventilation rate as

$$\lambda_v \sim \frac{A}{v} \sqrt{\frac{Hg}{2}} \left| \frac{T_{wa} - T_e}{T_0} \right|^{1/2} \left(1 - 0.5 \left(\frac{\pi_2}{x_0} \right)^{3/4} \left| \frac{T_{wa} - T_e}{T_0} \right|^{1/8} \right), \quad \pi_2 \ll 1 \tag{8}$$

This formula shows that the ventilation rate depends primarily on the absolute difference between the cave-wall temperature, T_{wa}, and the outside temperature, T_e. The term involving π_2 represents nonlinear damping of ventilation due to the internal air adjusting to the outside temperature. For the DB's room, this term only makes a 1 % contribution. However, caves with less idealized features may experience a larger contribution from this term, and future studies on such caves could allow us to examine it more closely.

Once λ_v is known (from either the numerical or perturbation model), we can determine how cave gases, namely carbon dioxide and ^{222}Rn, are exchanged with the outside environment. The transport of either quantity can be described by the ODE

$$\dot{C} = \frac{S}{V} \Phi - \lambda_v (C - C_e) - \lambda_r C \qquad (9)$$

where C is the concentration of CO_2 [ppmv] or activity of ^{222}Rn [Bq/m^3] inside the cave (Kowalczk and Froelich, 2010). The first term on the right represents the flux of CO_2 or radon into the cave through internal surfaces. We assume the production term of either gas, Φ, to be constant and will first estimate its value from measurements and then tune the production term to minimize errors. The second term on the right represents exchange with the outside via ventilation. Here, C_e is the external concentration, where C_e = 392 ppmv for CO_2 and C_e is negligible for ^{222}Rn. The last term on the right represents decay of ^{222}Rn, where λ_r = 2.1 × 10^{-6} s^{-1} (this term is zero for CO_2).

Results: Field Measurements and Theoretical Predictions

We now discuss the measurements taken at the DB's site, shown by the red curves in Figures 3a-c for CO_2 and Figures 3d-f for ^{222}Rn. Cave air gas concentrations in the DB were collected at 30-minute intervals over a 14-month period, with occasional interruptions due to flooding and/or power outages. Both the perturbation model and the numerical model were performed with a 24-hour resolution. Models and measured data are plotted together in Figure 3. The highest concentrations of cave gases occur during fall and spring, while the lowest concentrations occur during summer, followed by winter. This result is counter to those in caves under predominantly chimney-driven airflow regimes that promote strong winter-time ventilation (e.g., Obir Cave (Spötl et al., 2005), Inner Space Caverns (Banner et al., 2007), and Hollow Ridge Cave (Kowalczk and Froelich, 2010)).

During the winter, carbon-dioxide levels in the DB are near their minimum, while radon levels are intermediate. Qualitatively, these trends suggest ventilation to be lowest during fall and spring, when daily average inside-outside temperature differences are small, and highest during summer and winter, when the temperature differences are maximal. The observations support the idea of ventilation being caused primarily by buoyancy-driven flows.

Predictions of CO_2 and ^{222}Rn are shown by the dark curves in Figure 3. The gray curves represent the numerical model, with convection switched on/off for each surface individually, while the black curves show the perturbation solution given by Equation (8). The two predictions are nearly indistinguishable, providing validation for the additional assumptions made in the perturbation model. We estimate the source term, Φ, by minimizing the difference between model simulations and measurements, giving Φ = 3.1 × 10^{-9} kg/(m^{2}s) for CO_2 and Φ = 25×10^{-6} kBq/(m^{2}s) for ^{222}Rn. We note that our estimated source terms for ^{222}Rn and CO_2 are comparable to those found in the literature (e.g., for ^{222}Rn: Φ = 9.9 × 10^{-6} kBq/(m^{2}s) (Faimon et al., 2006) and Φ = 13 ~ 61 × 10^{-6} kBq/(m^{2}s) (Kowalczk and Froelich, 2010); for CO_2: Φ = 1.989 × 10^{-10} kg/(m^{2}s) (Baldini et al., 2006) and Φ = 1.56 × 10^{-9} kg/(m^{2}s) (Faimon et al., 2006)).
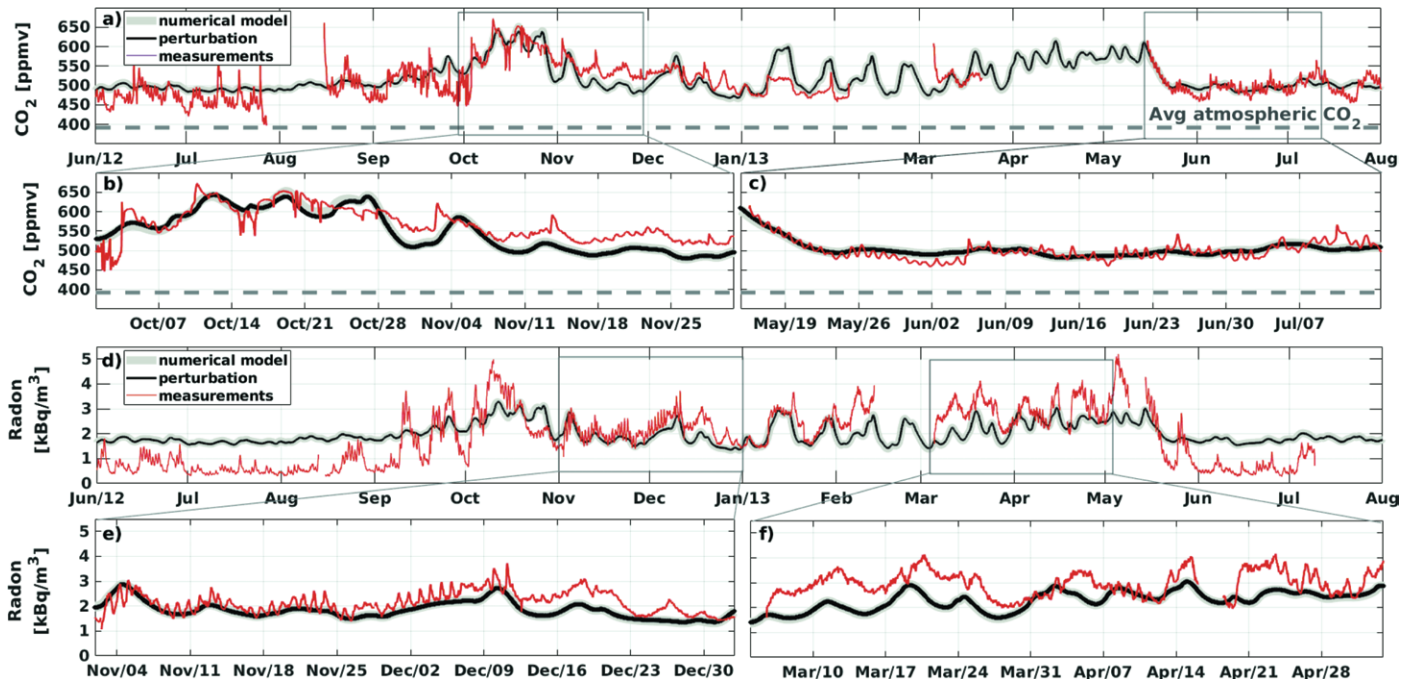

Figure 3. Variations of *in situ* gas concentrations. (a) The measured levels of CO_2 (red) over the full 14-month period, along with predicted values from the numerical model (gray) and the perturbation solution (black), the two of which are nearly identical. (b) – (c) Zoom of selected date ranges offers a closer comparison. (d) – (f) Similar plots for ^{222}Rn. Notice both gases show prominent variations on a near-weekly timescale, and the theory exhibits similar fluctuations.

With the source term as the only fitted parameter, both the CO_2 and radon predictions correspond well with measurements over the majority of the 14-month study. In particular, the model accounts for the higher levels of cave gases in the fall/spring and lower levels in the summer/winter. The only obvious discrepancy is in comparing radon levels in the summer, where the theory predicts a significantly higher level than was measured. Carbon dioxide shows good agreement during this same period, though, suggesting the discrepancy to be specific to radon.

While not a focus of this study, hydrologic conditions within the rock above the DB may have a significant influence on agreement between modeled and measured ^{222}Rn over seasonal time periods. Andrews and Wood (1972) demonstrated that radon emanation from limestone occurs from within a mineral surface layer only a few microns thick, and that under dry conditions, alpha-recoil causes radon gas to be ejected from the crystal lattice into inter-crystal cracks and imperfections, eventually to find its way out of the limestone through relatively slow diffusion in air at the rate of approximately 10^{-2} cm^2/s. Conversely, under wet conditions within the epikarst, alpha-recoil can cause radon particles to be ejected from the crystal lattice directly into pore water. As dripwater percolates downward, it has a scavenging effect, driving a significantly higher flux of radon during periods when the epikarst is wetter. An excellent example of this phenomenon was illustrated by Kowalczk and Froelich (2010) at Hollow Ridge Cave, when a tropical storm delivered 83 mm of rainfall, wetting the epikarst and causing a nearby river to flood and plug up the lower entrances of the cave, temporarily ceasing ventilation. Directly following this rainfall, ^{222}Rn emanation rates increased from normal 48−222 Bq m^{-2} hr^{-1} to approximately 1200 Bq m^{-2} hr^{-1}. Unpublished data from laboratory testing of limestone from both DTC and Hollow Ridge Cave confirms that wet transport can increase ^{222}Rn emanation by 1.6~2.4 times dry transport (William Burnett − FSU, personal communication to Khazmutdinova and Tremaine, 2015).

Figure 4 illustrates that prior to August 2012, most of the monitored drip rates were essentially zero. During August, a 120 mm rainfall and several successive ~40 mm rainfalls prompted increased pore water within the overburden. Approximately 1−2 weeks after each event, an increase in ^{222}Rn was observed, followed by a decay to background levels. In September 2012, all monitored sites rapidly increased in drip rate as the epikarst reached some saturation threshold. These conditions persisted until May 2013, when lower rainfall and increased evapotranspiration reduced pore water storage below the saturation threshold, causing measured radon to again fall below values predicted by our model.

In addition to the seasonal variation, the CO_2 and ^{222}Rn measurements show more rapid fluctuations on a few characteristic scales. Figures 3b-c and Figures 3e-f zoom on selected date ranges to highlight the rapid fluctuations. First, the measurements show very rapid, diurnal fluctuations, which resulted from the different day/night temperatures. In the model, we only input daily outside temperatures, and so these very fast fluctuations are not present. Presumably they could be captured with more temporal resolution (e.g., hourly readings). Interestingly, there is a second, intermediate
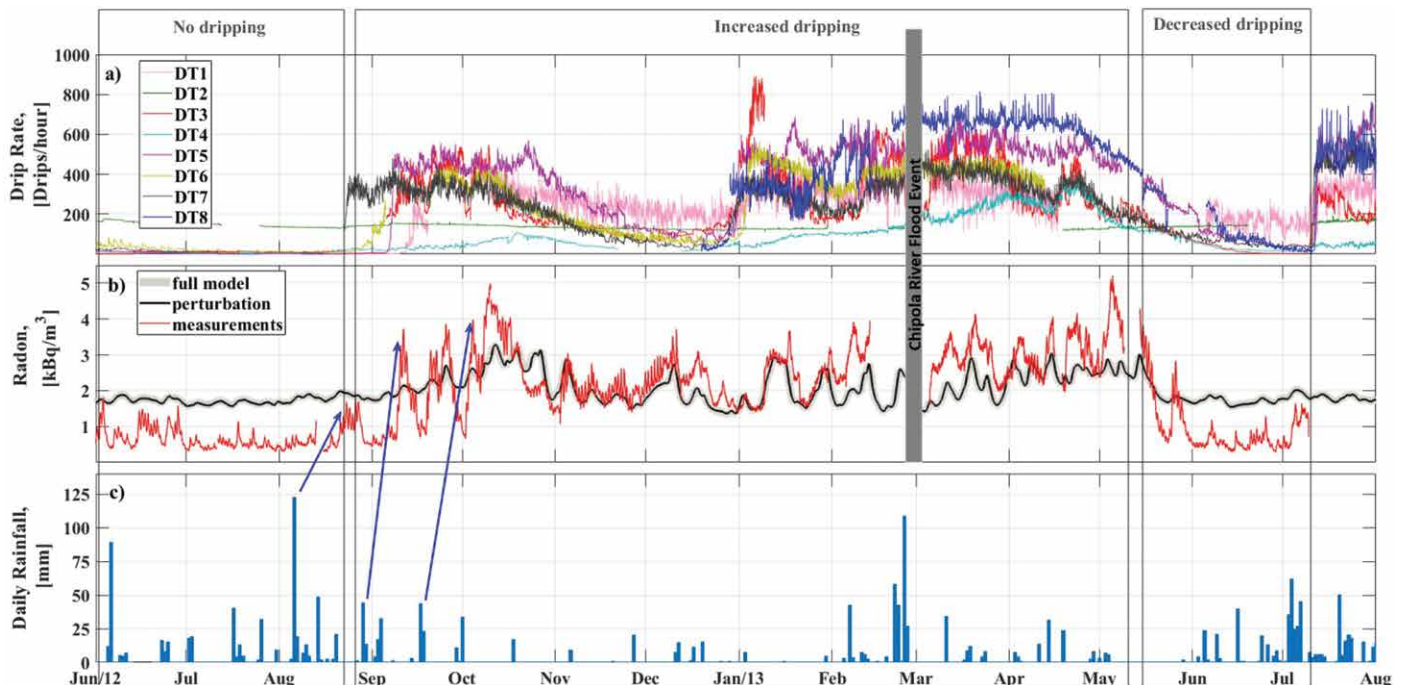

Figure 4. Time series of measured drip rate, ^{222}Rn concentration, and daily rainfall. (a) The measured drip rates at eight locations within the Dragon's Belly. (b) The measured levels of radon (red) over the full 14-month period, along with predicted values from the numerical model (grey) and the perturbation solution (black). (c) Daily rainfall above Dragon's Belly. Blue arrows indicate radon response to several successive rainfalls.

timescale (on the order of 5–9 days) that is also evident in the CO_2 and ^{222}Rn data. This nearly weekly cycle is likely influenced by synoptic-scale meteorology (Holton and Hakim, 2012) although human activity may play a role too (Daniel et al., 2012; Earl et al., 2016). Regardless, the model shows variations over exactly the same scale. This implies that the weekly cycle in cave-gas levels is ultimately tied to a weekly cycle in outside temperatures that drives an exchange flow as described by our theory. Over certain periods, for example those shown in Figure 3e, the theory even shows quantitative agreement with tracer measurements.

Discussion: Implications for Paleotemperature Modeling

Here we develop the logical framework for combining this ventilation modeling approach with several important *in-situ* geochemical calibrations that parameterize variations in modern isotope and X/Ca ratios in calcite. This synthesis of modeling and empirical field data engenders a first-order estimation of monthly and annual atmospheric air temperature, as well as length of seasons.

Until recently, subsampling a speleothem for powders from individual growth bands was restricted to the technical limits of physically machining the mineral surface with a drill press or micromill to produce powders. Because the average speleothem precipitates at a rate of 100−500 μm/y, it would be necessary to mill at approximately 8−41 μm (monthly resolution) to investigate seasonal ventilation, well below the limits of moderately priced technology. Advances in Inductively Coupled Plasma Mass Spectrometry (ICP-MS) during the last ten years has vastly increased sample resolution, specifically in carbon and oxygen isotopes. Using ion-microprobe, investigators can determine precise oxygen isotope ratios in $CaCO_3$ speleothems and foraminifera at sample sizes as small as 6−10 μm (Kozdon et al., 2009; Orland et al, 2012). Chen et al. (2017) developed Laser Ablation (LA) ICP-MS techniques to measure carbon isotope ratios in magmatic and hydrothermal calcites, achieving results that agreed well with conventional Isotope Ratio (IR)-MS methods, but on spot sizes of only 33 μm. Together, these advancements allow for measurements of speleothem calcite isotopes at the same resolution as trace-elemental composition (via LA-ICP-MS), opening the door to using carbon, oxygen, and X/Ca ratios as concomitant proxies for seasonal ventilation strength and timing.

It is now well established that monitoring individual drip site chemistry is a necessary first step toward interpreting the speleothem forming from that drip (Fairchild and Baker, 2012). In order to calibrate modern ventilation to speleothem calcite, it is necessary to monitor the drip for incoming isotopes, as well as the partial pressure of CO_2 (pCO_2), pH, and X/Ca ratios, while simultaneously farming calcite under that drip and analyzing it to observe seasonal patterns in isotopes and X/Ca ratios (Mickler et al., 2004; Spötl et al., 2005; Banner et al., 2007; Tremaine, 2010, 2015). The cave site must be chosen such that calcite precipitates (1) year-round, and, (2) rapidly enough to precipitate sufficient mass of calcite to exchange farming plates on a monthly basis. Cave air CO_2 and ^{222}Rn production rates (Φ; Equation (9)) should be measured or closely estimated. Finally, the cave site must ventilate seasonally with sufficient strength to induce measurable variations in oxygen and carbon isotopes and X/Ca ratios within farmed calcite (see Tremaine et al., 2011; Wong et al., 2011), and be monitored at bi-weekly intervals for cave air CO_2, relative-humidity, temperature, and ^{222}Rn. With all of these conditions met in an ideal cave site, it would be straightforward to create a calibration between modern seasonal ventilation strength, seasonal atmospheric temperatures, and seasonal changes in coeval farmed calcite isotopes and X/Ca ratios. These relationships may then be parameterized and incorporated into the ventilation model as ventilation (λ_v; Equation (8)) and CO_2 (C; Equation (9)).

By tuning our model to the specific dimensions of the studied cave site, these quantitative relationships can be applied backwards in time. First, the speleothem would be analyzed for isotopes and trace elements over small sections at monthly resolution (33 μm spot size for an axial extension of $\sim$ 400 μm/y). The model would be inverted and tuned to match the strength of ventilation, with the output being the difference in temperature between the cave and the atmosphere during ventilation season. By knowing the relationships between seasonal changes in modern ventilation strength and concomitant changes in calcite isotopes and X/Ca ratios, this approach would allow a first order estimation of the temperature difference between atmosphere and cave air temperature. By comparing monthly atmospheric temperature profiles, one could infer average annual temperature, and length of seasons. We acknowledge that this approach requires the assumption that no major changes in cave morphology have occurred during the life-span of the speleothem under analysis, which is never guaranteed, and therefore, this approach may give insight that is only semi-quantitative.

CONCLUSIONS

In this study, we constructed a theoretical framework to predict cave ventilation rates from a minimal set of external information, namely knowledge of the outside temperature and the physical dimensions of the cave. The major advantage is that external temperatures are much more easily accessible than microclimate conditions inside the cave. Through scaling analysis and a few key modeling assumptions, we obtained explicit relationships for how ventilation depends on system parameters given by Equation (8). Comparison with time-resolved, *in-situ* measurements demonstrates that the theory accurately describes seasonal and synoptic-scale fluctuations of transported cave gases. As an

immediate application, this theory could be used to improve estimates for production rates of cave gases such as CO_2 and ^{222}Rn. Currently-used field techniques can be destructive to the cave's fragile environment, but our methodology is completely non-intrusive as it relies only on knowledge of outside temperatures.

A longer-term application is to use these results to improve speleothem interpretation for climate reconstruction. Of course, in this application, neither the internal microclimatic conditions nor the external ones would be known *a priori*. Rather, inferring past climate is inherently an inverse problem, typically treated by representing the climate's influence on the proxy (e.g., speleothem, tree ring, etc.) via a transfer function (i.e., a forward model like ours), and then inverting this transfer function through inverse modeling for climate reconstruction. While it has been recognized that ventilation plays a critical role in speleothem growth, such effects have not yet been quantitatively incorporated into paleoclimate inference models, likely due to the complexity involved. The transparency afforded by our model, however, could prove useful in obtaining tractable transfer functions for climate reconstruction. In particular, we have shown that the ventilation rate can be accurately estimated from knowledge of outside temperature, without requiring internal temperature and climatic variables as additional inputs. Incorporating this theory into existing paleoclimate models, and the subsequent inversion of the transfer function, may allow for better estimates of the corresponding external temperatures (i.e., reconstruct the past climate). In essence, the simpler the forward model, the more feasible inversion becomes.

Acknowledgments

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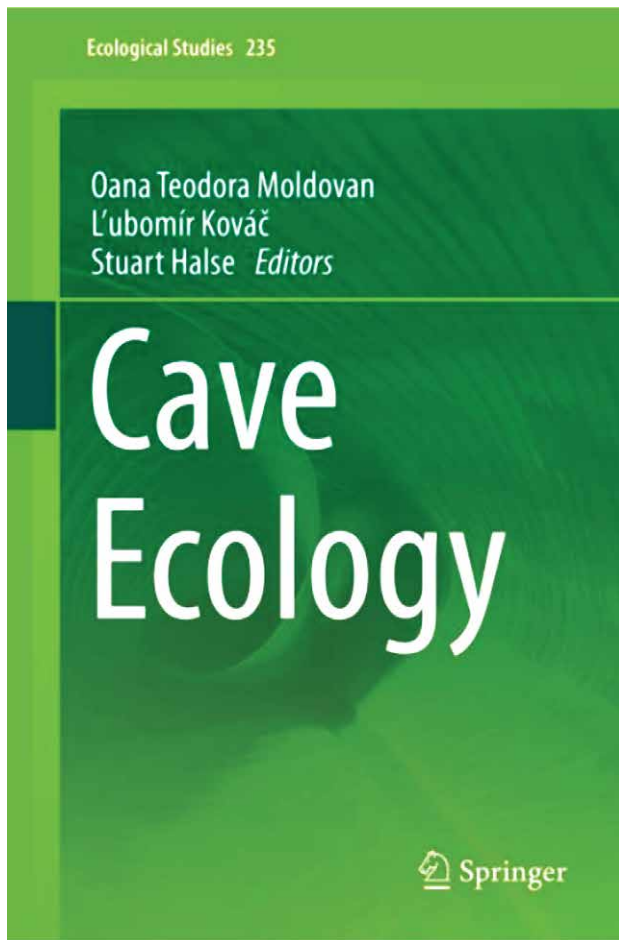
Cave Ecology

Oana Teodora Moldovan, Ľubomír Kováč, and Stuart Halse, editors, 2018, Springer, Cham, Switzerland, 545 p., 8.8 X 9.4 inches, hardbound $219.00, ISBN 978-3-319-98850-4 e-book $199.70, ISBN 978-3-319-98852-8

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The field of subterranean biology is a rapidly expanding one, an expansion nowhere more in evidence than in book-length publications. A recent addition to this library is the volume under review. The editors are all closely associated with the International Society for Subterranean Biology including the current president (Halse) and journal editor (Moldovan), and the authorship of individual chapters reflects the international membership of the society, with first authors from ten different countries. While the editors wisely do not claim completeness of coverage of cave ecology, there are a number of topics, ranging from very general (i.e., "Where Cave Animals Live") to the highly specific (i.e., "Volcanic Anchialine Habitats of Lanzarote"). As perhaps befits the title, evolution and adaptation of subterranean animals is given short shrift, with almost no coverage of the recent work on the mechanisms of evolution in the Mexican cavefish, *Astyanax mexicanus*, itself the subject to two recent books (Keene et al. 2015 and Wilkens and Strecker 2017) and articles in premier journals such as Nature, Science, and Proceedings of the National Academy of Sciences. More surprising is the very strong focus on caves rather than non-cave subterranean habitats, such as the hyporheic, the milieu souterrain superficiel (MSS), and epikarst, even though in the preface the authors claim a direct connection with the classic "Essai sur les problèmes biospéologiques" of the Romanian biologist Emil Racovitza, published in 1907. Racovitza, much admired by European speleobiologists, emphasized the importance of non-cave habitats such as cracks and crevices, which led to the discovery of the hyporheic, the MSS and other habitats. Of the 24 chapters in the book, only one focuses on non-cave habitats (chapter 20 on calcrete aquifers). Other chapters touch on these habitats, and the editors suggest using the word "cave" for any subterranean habitat, but this is not the same as a Racovitzan focus on them. The focus on caves per se is more in line with American work on cave biology, even though Americans were the first authors of only six of the 24 chapters. Implicit in some of the chapters is the idea that non-cave habitats are staging grounds for the eventual colonization of caves, and that it is in caves that the species most highly modified for subterranean life are found, a view which we do not hold (see Culver and Pipan 2014).

The chapters are divided into six groups:
- Introduction to karst, caves and cave habitats (2 chapters)
- There is rich and diverse life in caves (7 chapters)
- About communities, populations, and food (4 chapters)
- Particular cave ecosystems (7 chapters)
- Conservation and protection of cave habitats and cave fauna (2 chapters)
- Cave ecology for the twenty-first century (1 chapter).

Several chapters are excellent and unique summaries of sub-disciplines. Deharveng and Bedos' chapter on terrestrial invertebrates in subterranean habitats, the longest chapter at 67 pages, is a welcome and thorough update of compilations in Encylopaedia Biospeologica (1994-2001) of the obligate terrestrial fauna of all subterranean habitats. With their knowledge of both temperate and tropic subterranean habitats, they are uniquely qualified to write this chapter. The chapter on microbial life by Hershey and Barton, is a good, if brief introduction to microbial diversity and how it is assessed, but does not really cover function. Mulec provides a surprisingly interesting account of phototrophs and their ecology, even though of course they are not found in zones of total darkness. Zagmajster et al. provide an up to date summary of what we know about biodiversity patterns, from global to local scales. The growing availability

of geo-referenced locality data, as well as the development of general and testable models of biodiversity make this a rapidly growing field. By way of full disclosure, Culver is one of the co-authors of this chapter. Ribera et al., in chapter 10, provide a very lucid summary of the major issues of colonization of caves—the evolution of troglomorphy, multiple versus single origins, the extent of dispersal, and the relation between age and troglomorphism. Trontelj, in chapter 12, provides a thoughtful discussion of the structure and genetics of subterranean populations, focusing on dispersal versus vicariance (i.e., geographic separation of populations, as by physical barriers), and the distinction between processes that take place on the surface (which he terms exogenous) and in the subterranean realm (which he terms endogenous). In the final chapter of the section, "About Communities, Populations and Food", Venarsky and Huntsman summarize the dynamics of both detritus-based and chemoautotrophic cave systems. They point out similarities and differences with surface ecosystems, but unfortunately, do not include any quantitative data. The final chapter on future developments in cave ecology, the eminent Australian speleobiologist William Humphreys looks at how technical advances in sampling, molecular sequencing, etc. are likely to move the field forward. Implicit in this argument is that it is not ideas but technology that is holding us back. In addition, Humphreys makes the argument that many paradigms stemming from the Northern Hemisphere are misleading in terms of the Australian fauna and have actually been an impediment to research. Well over 100 pages of the book are devoted to the description of particular subterranean ecosystems. This emphasis on individual cases is typical of books on subterranean biology, and probably reflects both the diversity of subterranean habitats and the lack of a thorough synthesis of subterranean biology. Noteworthy among the chapters in this section are those by Oromí on lava tubes and Ferreira et al. on ferruginous caves in Brazil. The most diverse lava tube fauna is not in Hawaii but in the Canary Islands (by more than two-fold), and Oromí provides a welcome summary of the Canarian lava tube, both for species composition and the geological and environmental context of their occurrence. Ferreira et al. summarize the remarkable fauna found in caves developed in iron ore, among which the Brazilian caves are best known. The fauna is very rich in species, and occurs in very short, shallow caves, often without a dark zone. Niemiller et al. provide a very useful summary of protections, from the local to international level, available for the subterranean fauna, for Europe and the Americas. They also provide a succinct summary of the threats to the subterranean fauna. While there are a number of excellent chapters as we have summarized above, the question remains as to whether the book is complete enough to serve as the primary text for the field. The ecological coverage is broad but the evolutionary coverage is not. The depth of coverage of ecological topics varies greatly. For example, 67 pages are devoted to the terrestrial fauna while only 21 are devoted to the aquatic fauna. Nevertheless, it is a book that should on the shelf of every speleobiological researcher, or at least in the institutional library. While the price of the book is prohibitively high for almost all students and many researchers, individual chapters are available as pdf files from Springer at a cost of approximately $33.

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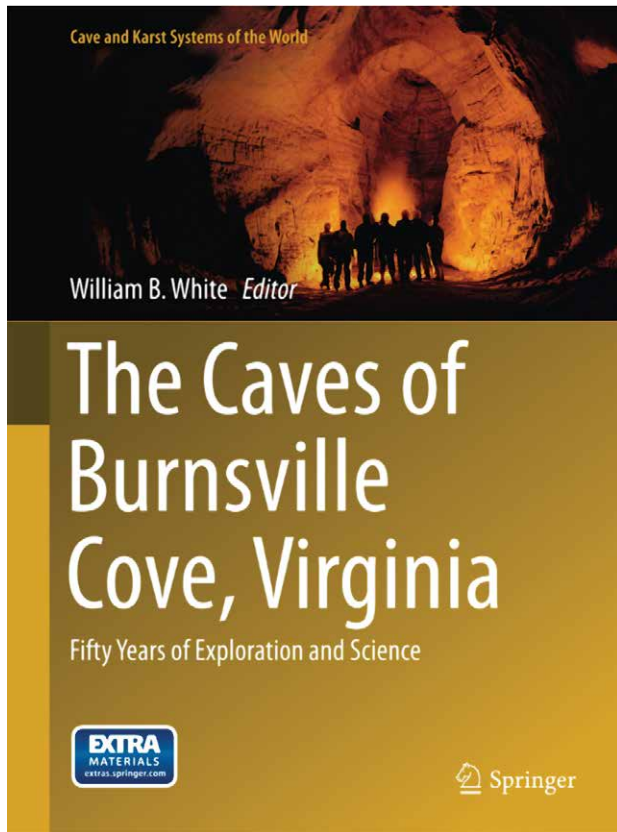
The Caves of Burnsville Cove, Virginia: Fifty Years of Exploration and Science

Reviewed by: David C. Culver, Department of Environmental Science, American University, 4400 Massachusetts Ave. NW, Washington DC 20016.

Burnsville Cove is a small limestone valley in west-central Virginia, at the border of Highland and Bath Counties. It occupies an area of approximately 50 km^2 and as of 2013 it contained 97 surveyed caves with a total combined length of more than 112 km. Three caves are more than 10 km long: Butler-Sinking Creek System, Chestnut Ridge Cave System, and Helictite Cave. For more than 40 years the caves in the cove have been explored and mapped by cavers of the Butler Cave Conservation Society, many of whom live in the area and own property there. This is their story, assembled and edited by William B. White, who has done a remarkable job of bringing together the stories of exploration and many scientific studies in Burnsville Cove.

This book presents a detailed summary of the exploration history of the caves, maps and descriptions of the cave passages, and the scientific research produced in the Cove over the last 60 years. Roughly half of the book (11 of 24 chapters) is devoted to exploration, which rightly begins with Breathing Cave, a classic maze cave (9914 m long as of 2013), the only large cave known in the cove prior to 1958. Exploration and surveying began in the 1940s, with major discoveries found in the late 1950s. Its geology and development were the subjects of one of the earliest cave geology theses, written by George Deike, then a graduate student at the University of Missouri. Chapter 18 contains a compressed version of this pioneering study. Butler Cave, discovered in 1958, figured more prominently in both the history of the Butler Cave Conservation Society and the developing understanding of the caves and their hydrology. Butler Cave itself is much more linear than Breathing Cave, and consists of a major trunk passage containing an underground stream, which follows the axis of a syncline.

The exploration of all of the major caves, and some of the minor ones found after the discovery of Butler Cave, are chronicled. For many of the caves, the stories of exploration and major discoveries follow in roughly chronological order. Especially informative and riveting is Gregg Clemmer's description of the initial exploration of the Chestnut Ridge System (nearly 34 km as of 2008) and the value of persistence in exploration. The difficulty of the cave made multi-day trips the norm, and a short description of the techniques used is included. Many of the cave entrances in Burnsville Cove were accessible only by digging, and nowhere was this more the case than in Barberry Cave, where three entrances were excavated, including construction of a 20 m deep shaft – Big Bucks Pit. In a trip-by-trip account, even more detail is provided by Lucas for several caves, including Helictite Cave (11.7 km long as of 2008). How useful such details are, for readers interested in topics beyond exploration, remains to be seen; but taken together, the eleven exploration chapters compose a remarkable chronicle.

An interesting aspect of Burnsville Cove is the large number of cavers who have moved there, which led to the founding of the Butler Cave Conservation Society in 1968, with the initial goal of managing and conserving the Butler Cave Sinking Creek System. A chapter by Fred Wefer and Keith Wheeland outlines its history. Membership in the BCCS is by invitation, and it is arguably the most exclusive caver organization in the USA. In a separate publication, Maria Pérez and John Wilson (2019) use BCCS as an example of what they call "caver villages" and explore their sociological aspects. One of the important activities of BCCS is land acquisition, as described in a chapter by Wheeland. BCCS owns a 25 hectare parcel of land that contains the only known entrance to Butler Cave, as well as a 33 hectare parcel containing one of the entrances to the Chestnut Ridge System. Other parcels are owned by individual BCCS members. The Society has been fortunate in that the land has clearly been purchased both to maintain access and to

protect the land. These two goals can come into conflict, as in the case for bat protection, but in Burnsville Cove there has no such conflict.

The remaining ten chapters are devoted to scientific studies in Burnsville Cove. An overview covers a range of topics not included in the other chapters. There are five chapters on geology, and one each on hydrogeology, meteorology, mineralogy and geomorphic evolution. It is important to keep in mind that all of this research was done without the benefit of government funding. This was not by choice, although such a choice would have been made by some authors; but it was a necessity based on the general lack of respect given to speleological studies of any kind. White reviews a number of interesting scientific topics that arose from exploration of the Burnsville Cove caves. Among them are paleoclimate, the origin of moonmilk, and the cause of the "breathing" phenomenon in certain caves, which was investigated using reversing fans at entrances. Noticeably absent is any discussion of biology, beyond the sentence that dismisses Burnsville Cove as not being a biological hotspot. This is in part because no BCCS members have a strong interest in the topic, and because the fauna appears to be rather depauperate. Nonetheless, there are cave-limited species, including the amphipod *Stygobromus conradi,* whose type locality is Breathing Cave.

One important fact that emerges from this study is that the caves are developed in the Silurian Tonoloway Limestone rather than the Silurian-Devonian Keyser Limestone, as was thought to have been the case for decades.

Nevin Davis provides a thorough review of the hydrogeology of the cove, and delineates the four major subsurface basins. These included some of the earliest systematic tracer tests conducted in the USA. Although a bit difficult to read, it is the best overview map in the book, as it also includes line drawings of the major caves. His map of the drainage basins is also reproduced in the supplemental material available at the publicly available website (***extra.springer.com***).

The chapter on meteorology, by Fred Wefer and Phil Lucas, is one where the absence of modern data and equipment is most apparent. The description of temperature variability is fine, but it was done in the absence of data-loggers such as those now available (e.g., the ubiquitous Hobo™ instruments), which were not available at the time.

The chapter on minerals and speleothems is especially good, reflecting White's long-standing interest in the topic. The final chapter on geomorphic evolution of the cove is very thought provoking, and White includes the recent ideas on deep-seated phreatic origin of the system, as developed by Dan Doctor and Benjamin Schwartz.

This volume is perhaps the most comprehensive study of any limestone cave system. However, I have one overall criticism, that of the quality of maps. The copious use of original cave maps adds a great deal, but historical accuracy would not have been compromised by the inclusion of area maps. This is especially true in introductory chapters where there is over-use of poorly reproduced topographic maps lacking annotation. Wonderful digital maps are available these days, but do not appear in this volume. Nevertheless, it is a remarkable publication and belongs on your bookshelf, notwithstanding its high price.

This is among the first volumes in the series of books on Cave and Karst Systems of the World, edited by James Lamoreaux, and the first one about a U.S. cave system. While the price is high, the series does make literature on cave exploration and research more readily available, at least for those with access to a research library. PDFs of individual chapters can be also be purchased at the Springer website.

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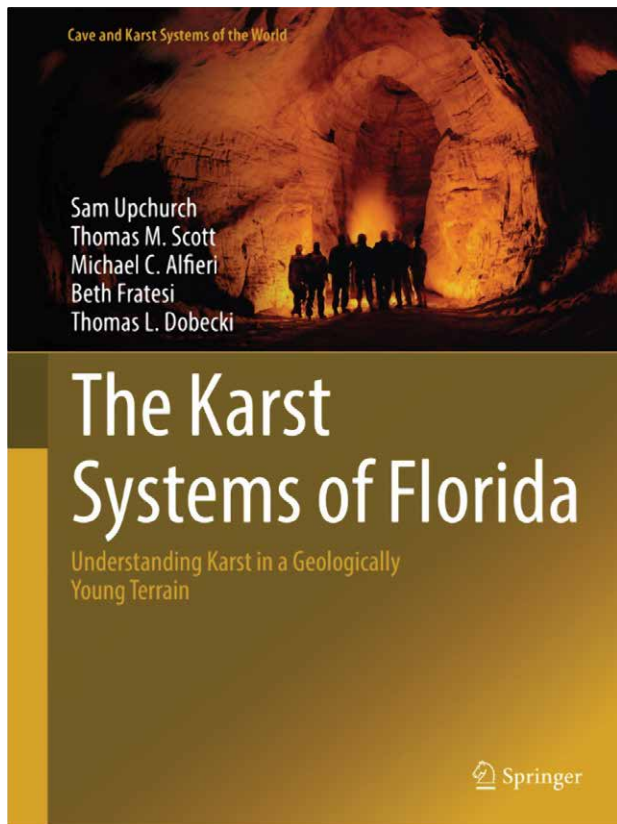
THE KARST SYSTEMS OF FLORIDA. UNDERSTANDING KARST IN A GEOLOGICALLY YOUNG TERRAIN

Sam Upchurch, Thomas M. Scott, Michael C. Alfieri, Beth Fratesi, and Thomas L. Dobecki, 2019, Springer, Cham, Switzerland, 479 p., 8.6 X 11.2 inches, hardbound $189.00, ISBN 978-3-319-69634-8, e-book $169.00, ISBN 978-3-319-69635-5.

Reviewed by: David C. Culver, Department of Environmental Science, American University, 4400 Massachusetts Ave. NW, Washington DC 20016

The Florida karst differs from most karst areas in the United States and throughout the world in that it is eogenetic—karst in geologically young limestone that is still undergoing diagenesis and consolidation, and has not been subject to deep burial and the effects of heat and pressure. Eogenetic karst, in contrast with telogenetic karst, has higher primary porosity, typically higher sinkhole density, and large springs. The authors list a number of persuasive reasons why the Florida karst should be of broad interest, including:

- The most extensive expanse of geologically young (Paleogene and younger), eogenetic karst in North America
- It includes two of the most productive aquifers in the world—the Floridan aquifer system and the Biscayne Aquifer
- Because of population pressure, it is an excellent laboratory for studying karstic aquifers under stress
- The landscape is remarkably flat, and
- It has been dramatically affected by sea level changes.

Because of the huge economic importance of the Florida karst (including a water supply and an engineering hazard as the result of sinkhole formation), it is appropriate that four of the five authors are from consulting firms. This is not to say the book lacks a strong academic focus—it doesn't lack it.

There are seven chapters. The first gives a general background, and the next two set the scene to the Florida karst—basic geology (chapter 2) and geomorphic characterization (chapter 3). Chapter two is noteworthy for its inclusion of extensive discussion of sediments, a topic not always found in books on karst. Of course, any karst region has a unique geological context and the authors do a good job in chapter three describing the components of the Florida Platform, with carbonate rocks ranging in age from the Cretaceous to the Pleistocene. As the authors caution readers, the terms highlands, lowlands, valleys, etc. must be seen in the context of a maximum land topographic relief of 105 m. Noteworthy are the very clear maps of physiographic provinces for each the karst geomorphic districts.

Chapters four and five focus on the water supply. In chapter four, after an overview of the factors influencing any hydrological cycle, the authors describe the three main aquifer systems in detail—the largely Pleistocene Surficial Aquifer System, including the Biscayne Aquifer, the Miocene Intermediate Aquifer System, and the older Floridan Aquifer System. A topic of particular interest in Florida is saltwater intrusion and the authors discuss the saltwater freshwater transition zone at length. Chapter five is really a continuation of chapter four and features both a general discussion of water quality trends and a detailed look at water quality trends aquifer by aquifer. The point out that water quality declines both because of land use activities and groundwater withdrawal.

Chapter six covers the controls on karst development, including denudation rates, fractures, time, depth, etc. The emphasis is on surface karst features, not surprising given their ubiquity and economic importance. The authors also describe the controls on karst landforms (e.g. fractures, epigenetic vs. hypogenetic development, time available).

Chapters seven and eight may be of most interest to speleologists. Chapter seven discusses cave development and sinkhole formation. The discussion of the mechanisms of sinkhole development and risk is especially thorough. They conclude that qualitative estimates of sinkhole development risk are good, but quantitative estimates are not. Much more problematic are his use of terms for karst features in mountainous terrains, especially poljes. There are a number of definitions of poljes (a Slavic word for field), but the strict definition is *a large, flat floored depression in karst limestone, whose long axis is developed parallel to major structural trends and can reach tens of kilometers in length.*

Superficial deposits tend to accumulate on the floor. (Field 2003). Anyone familiar with these very large landscape features first described from the Balkans, will be surprised to see features like Payne's Prairie described as a polje. It is surely a karst wetland and an intermittent lake, but if it is a polje, it is a very different type.

Chapter eight is a review of the major karst landforms found in Florida, including natural bridges, sandhill lakes, fluviokarst features (e.g., swallets and resurgences), karst escarpments, and somewhat uncommon features such as phytokarst and karren. The chapter (and the book) closes with a discussion of hypogene karst features. Hypogene karst is a very hot topic with some advocates, such as Alexander Klimchouk (Klimchouk et al. 2017) holding it to be of major importance). Even its definition is in dispute[1], and the authors take a rather cautious approach in identifying hypogene karst features. They suggest that they can be found at the mixing zone of freshwater and saltwater, and in lower strata, but also point out there is little direct evidence for their existence.

The book is remarkably self-contained in the sense that one does not necessarily need a prior background in karst to read the book. Very fundamental concepts such as carbonate dissolution are covered, and there is extensive background material in the first three chapters. In this way it is reminiscent of the book, **Speleothem Science**. The authors have also attempted to make each chapter self-contained, with separate bibliographies for each chapter, presumably in response to the availability of pdfs of individual chapters from Springer, at a cost of $29.95. While still expensive, it makes the book more accessible to more readers.

Overall the book is very well written, in generally accessible language. It is well illustrated throughout, both with photos and many excellent maps. There are always ways to make a book better, especially when one is not doing the writing. A glossary would have been nice, especially for the non-specialist. While the authors make a convincing case that the Florida karst is special and unique, a comparison with other areas with flat lying eogenetic karst, especially the Yucatan Peninsula of Mexico would have been interesting. Nevertheless, this volume belongs in the library of every karst researchers and at least the most relevant chapters should be in the library of students and young researchers.

This is among the first volumes about a U.S. cave system in the series of books on Cave and Karst Systems of the World, edited by James Lamoreaux.

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GUIDE TO AUTHORS

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