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Cave monitoring and the potential for palaeoclimate reconstruction from Cueva de Asiul, Cantabria (N. Spain)

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Abstract: Palaeoclimate records from northern Iberia are becoming increasingly sought after as this region is one of the most southerly terrestrial locations in Europe to have its climate dictated principally by the North Atlantic. Terrestrial records therefore have the potential to offer insights into changing oceanic and atmospheric circulation in the wider North Atlantic region. Cave speleothems offer one of the most promising archives from northern Iberia due to their wide geographic distribution and potential for accurately dated climate reconstruction. Cueva de Asiul, situated in Cantabria (N. Iberia; 43°19'0.63''N, 3°35'28.32''W; 285 m a.s.l) within the Matienzo karst depression is one such site that offers the potential for palaeoclimate reconstructions. Here we present three years of climate and cave monitoring from Cueva de Asiul, giving detailed insight into local meteorology, hydrology and cave ventilation dynamics. In doing so, this paper presents a background to high resolution, Holocene duration speleothem records which have been extracted from this cave. Annual average cave temperatures are +13.7°C, with a maximum range of 1°C, reflecting the seasonality of external air temperature (average external temperature +13.8°C). Cave ventilation is controlled by changes in external air temperature and variations in external air pressure during low pressure events. Local rainfall measurements show an average of 1400 mm/year with the majority of rainfall occurring during the winter, with periods of water excess between October and April. Speleothem drip rates are characterised by summer lows and a rapid transition to higher rates at the onset of the winter season. Stable isotope analysis (δ¹⁸O, δ²H) indicate that aquifer water is derived predominantly from the previous year’s rainfall and the rainfall feeding the karst system is controlled by a strong amount effect. Speleothems from this site are potentially suited to preserving extended records of rainfall amount in northern Spain and therefore have the potential to inform more clearly about Holocene scale changes in the rainfall source region, the North Atlantic.

Keywords: Cueva de Asiul, cave monitoring, palaeoclimate, speleothem, northern Spain

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INTRODUCTION

Cueva de Asiul is located in the Matienzo karst depression, Cantabria (N. Spain). The northern coastline of Spain is one of the most southerly locations in Europe to be predominately influenced by North Atlantic sourced weather systems (Gimeno et al., 2010). This region is therefore ideally placed to record variations in competing air masses and is of critical significance for understanding the role of the North Atlantic in controlling Europe’s climate (Baldini et al., 2015). Current palaeoclimate records from this region are principally derived from lake (Gonzalez-Samperiz et al., 2008; Morellón et al., 2009; Roberts et al., 2012) or ocean sediments (Martínez-Garcia et al., 2014, 2015; Mojtahid et al., 2013), cave speleothems (Dominguez-Villar et al., 2009; Moreno et al., 2010; Stoll et al., 2013; Martinez-Pillado et al., 2014; Baldini et al., 2015) and from archaeological evidence (Lopez-Merino et al., 2010). However, few of these records replicate at high resolution over long periods of time. Local sensitivity to atmospheric processes, orographic barriers and at some sites human interference, likely cause discrepancies within the records from this

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our main research goal, to present high-resolution characterisation of Matienzo climate and the cave from Cueva de Asiul, which allows for the components of the cave climate system: weather patterns, this work is focused on two major site basis. In addition to understanding regional cave monitoring must be undertaken on a site by the growth and chemical uptake of speleothems, specific karst/cave processes which may govern throughout the Quaternary. extraordinary repository of palaeoclimate archives from likely contains speleothem material which is an of active phreatic levels within the cave systems to the present day. Collectively, the Matienzo depression likely contains speleothem material which is an extraordinary repository of palaeoclimate archives from throughout the Quaternary. However, to fully understand the climate and site specific karst/cave processes which may govern the growth and chemical uptake of speleothems, cave monitoring must be undertaken on a site by site basis. In addition to understanding regional weather patterns, this work is focused on two major components of the cave climate system:

1) Soil and karst hydrology: the soil and karst zones facilitate or delay the transfer of rainfall into cave systems and by doing so regulate the timing and delivery of calcium carbonate rich water to actively growing speleothem deposits (Baker et al., 1997; Fairchild et al., 2006; Fairchild & Baker, 2012). Hydrological changes therefore have the potential to influence both the rate of speleothem deposition and the extent to which speleothems incorporate accurate changes in external climate proxies (Dreybrodt, 1999; McDermott et al., 2004).

2) Cave ventilation: variations in air exchange between the karst, cave and external environments govern both cave air temperature and the carbon dioxide composition of cave air (e.g., Spöl et al., 2005; Smith et al., 2015). Whilst caves normally have a relatively stable air temperature, which reflects an average annual external value (Fairchild & Baker, 2012) the CO2 composition of cave air can vary dramatically, both between sites but also at the same site during different times of the year (Mattey et al., 2010). Cave air CO2 content regulates the degassing of CO2 from drip waters and in doing so influences the rate of speleothem growth and potentially the uptake of chemical proxies into the carbonate lattice (Baldini et al., 2010; Mattey et al., 2010). It is therefore vital that we understand the dynamics of cave air temperature, CO2 and hydrology before assessing the suitability of speleothem deposits from any given cave as archives of palaeoclimatic information.

We present three years of detailed cave monitoring from Cueva de Asiul, which allows for the characterisation of Matienzo climate and the cave climate system. This monitoring study underpins our main research goal, to present high-resolution speleothem records, offering information about climate change in northern Iberia and the role of North Atlantic weather systems in controlling European climate through the Holocene.

REGIONAL SETTING AND SITE DESCRIPTION

The production of palaeoclimate records through the analysis of speleothem deposits is becoming increasingly common in north western Iberia, especially so on the northern coastline. This interest has developed due to the regions close proximity to the North Atlantic and the ability of speleothems to record subtle changes in Holocene climatic conditions, linked to major variations in oceanic and atmospheric systems (Baldini et al., 2015). Currently, these reconstructions are somewhat restricted however, due to regional aridity in the mid Holocene when a large number of sites show a synchronous stop in growth of most speleothem deposits (Stoll et al., 2013). This has resulted in more focused studies which reconstruct smaller sections of the Holocene at high resolution (e.g. Dominguez-Villar et al., 2008; Dominguez-Villar et al., 2009; Martin-Chivelet et al., 2011; Baldini et al., 2015). Further work is required to find a cave site which may produce a more complete Holocene speleothem record from this region.

Cueva de Asiul setting

The Matienzo valley (43°19'0''N, 3°35'28''W) is a ~26 km2 closed karstic depression located in the Cantabrian Cordillera; within 40 km inland of the northern Iberian coastline in the province of Cantabrica (Fig. 1). This mountainous region is mainly composed of uplifted Cretaceous sediments with E-W trending fault lines. The exploitation of these fault lines by chemical weathering has led to the formation of large karstic depressions (Quin, 2010). Cueva de Asiul (285 m.a.s.l.), located on the northern slope of the La Vega arm of the depression (Fig. 1), is developed within thinly bedded Aptian limestone, broken by shallow sandstone lenses (Quin, 2010). The hydrological recharge area above the cave system is home to a small range of shrub and grass communities, only controlled by periodic grazing activities. Soil depths of 50 cm are common, with sections of bedrock exposed across the hillside.

Cave description

Cueva de Asiul is a small cave system, with a cave volume of close to 2.7 x 10^3 litres. The cave is horizontal, extending 75 m into the hillside in a north westerly direction. Access is via a small entrance (<1.5 m²), leading to a single passage with several high avens. The cave ends at 75 m at a calcited boulder choke (Fig. 2). No streams exist within the cave, but drip water fed pools are found at 40 and 60 m depth into the system and remain year round. The karst above the cave is relatively shallow (10-40 m rock overburden), with the most limited overburden toward the entrance and where high avens are observed.
CAVE AND CLIMATE MONITORING METHODS

To characterise the contemporary cave environment in which these speleothems are currently growing, a three-year climate and cave monitoring programme was initiated in 2010. This programme included high resolution logging of cave and external air temperature, cave air CO₂ content, cave air pressure, speleothem drip rate and drip water electrical conductivity (EC). In addition, sampling of event based rainfall allowed for the measurement of rainfall amount and isotopic (δ¹⁸O and δ²H) composition on a monthly scale. Monthly monitoring was also undertaken at the cave, including the measurement of soil air CO₂ content, bulk drip water collection and the analysis of this water for pH, EC, temperature and isotope composition (δ¹⁸O and δ²H).

External and cave air temperature
External and cave air temperatures were measured from April 2010 using a TinyTag Plus2 logger recording every 30 minutes (measurement uncertainly ± 0.02°C). External monitoring was undertaken within 250 m of Cueva de Asiul entrance, at 20 m higher altitude. Internal logging took place at 65 m depth into the cave system (Fig. 2). One logger was suspended 1 m above the ground free of any rock contact and a second was in direct contact with the cave wall. These loggers showed very little difference in temperature (within logger error); data presented here comes from the suspended logger.

Rainfall and speleothem drip rates
Measurements of rainfall amount were undertaken during rainfall collection in the village of Matienzo (1 km from the cave site and at 70 m lower altitude) from February 2011. δ¹⁸O and δ²H analysis of rainwater was undertaken via Isotope Ratio Mass Spectrometry (IRMS) at the NERC Isotope Geosciences Facilities using an Isoprime ratio mass spectrometer. Analytical errors reported at one standard deviation are 0.06‰ for δ¹⁸O and for δ²H are 1‰, and are reported relative to VSMOW.
Speleothem drip rate was measured at each of the main three speleothem sites (ASF, ASM and ASR). Drip rates were logged every ten minutes using acoustic Stalagmate drip loggers (Smith et al., 2015) from April 2010 (ASF) and May 2013 (ASR and ASM). Drip waters were also collected at each speleothem site as monthly bulk collections (a single container collecting all of one speleothem drip water for one month) for δ18O and δD analysis.

Cave, soil and external air CO2

Cave air CO2 was logged in the rear chamber of Cueva de Asiul every hour from February 2011 to November 2013 with one break in the record during the winter of 2011 due to logger malfunction. Soil CO2 was also measured from October 2011 to November 2013 at 50 cm soil depth (believed to be the junction between the soil and epikarst zones) using a right angled tube with a ventilated base section buried beneath the soil surface and vertical access port to enable gas samples to be extracted or CO2 levels to be measured (Fig. 3). The soil tube was installed into a trench in January 2011 and completely covered with soil and the pre-existing vegetation; visual recovery of the area was complete by the end of the summer season. Monthly and automated CO2 measurements of soil and cave air were taken using the same Vaisala Carbocap GM70 probe (working sensitivity of ± 10% of the reading).

Fig. 3. Image of the soil sampling device (a) showing both the perforated lower section which enables air exchange between the soil and the tube (b) and the silicon tube used for extracting gas samples from the base of the soil zone (c).

Carbon isotope (δ13C) analysis of soil and cave air was undertaken between December 2011 and October 2013 on a monthly basis. Soil air samples were extracted from the base of the soil sampling device via a small diameter silicon tube which was pre-fixed into the sampler (Fig. 3). Air was then extracted using a 20 ml syringe and injected into a 12 ml pre-evacuated glass exetainer. Cave and external air was sampled in a similar fashion using a 20 ml syringe, injecting air directly into the pre-evacuated glass exetainer. External air samples from immediately adjacent to the cave entrance were collected on five occasions between 2011 and 2013 to give a baseline external δ13C in CO2. δ13C in all samples were analysed at the NERC Life Sciences Mass Spectrometry Facility, Lancaster node, CEH Lancaster, using a GV Instruments Tracegas Pre-concentrator coupled to an IsoPrime IRMS. Analytical errors were reported at one standard deviation as 0.16‰, and are reported relative to VPDB.

Modern carbonate deposits

Modern carbonate samples were collected by removing the top 100 µm of two actively depositing speleothems (ASM and ASR, Fig. 2), and also using a glass plate to collect modern carbonate deposition integrated over a full annual cycle on the top of a third actively growing speleothem (ASF, Fig. 2). This carbonate was subsequently analysed for carbon and oxygen isotopes at the Stable Isotope Facilities, British Geological Survey, using an IsoPrime isotope ratio mass spectrometer with Multiprep device; average 2σ uncertainty is 0.07‰. Isotope values are reported relative to the international VPDB standard.

RESULTS

Rainfall dynamics in Matienzo

North western Iberian climate is dominated by its close proximity to the North Atlantic Ocean, setting this region apart from more southerly areas of Spain, which are also influenced by Mediterranean sourced precipitation. During this study, approximately 80% of Matienzo’s prevailing air masses tracked over the North Atlantic (Fig. 4), associated with westerly frontal systems (calculated via HYSPLIT modelling; Baldini et al., 2010; Draxler & Rolph, 2010). The air mass history of each rainfall event was calculated using five-day (120-h) kinematic back trajectories originating from Matienzo (43°31’N, -3°58’W), for more detailed methodology see Baldini et al. (2010). These models originate at the end of each rainfall collection day to encapsulate the whole rainfall event, this is essential as the peak rainfall period is unknown. Rainfall for three atmospheric levels was computed (850, 700 and 500 hecto-pascals, hPa) approximately 1500, 3015 and 5575 masl (Baldini et al., 2010). As suggested by the Air Resources Laboratory (ARL) [part of National Oceanographic and Atmospheric Administration (NOAA)], atmospheric levels of 850 and 700 hPa were used for modelling to give a good approximation of where frontal and synoptic rainfall is delivered (Baldini et al., 2010). The en-route uptake of moisture over the North Atlantic has been shown to be a major source of precipitation for sites in Western Europe (Baldini et al., 2010), as expected in Matienzo (Fig. 4). However, moisture delivery from the North Atlantic source region varies on a range of temporal scales, often influenced by atmospheric modes such as the North Atlantic Oscillation (NAO), Scandinavian pattern and a local expression of the East Atlantic/Western Russian pattern (Baldini et al., 2008; Roberts et al., 2012; Baldini et al., 2015) and changes in North Atlantic conditions.

During the monitoring program, the Matienzo depression received approximately 1400 mm/ year of precipitation. This is slightly higher than the 1050 mm/ year received at the closest MET station in

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Peak monthly rainfall was measured in February 2013 (300 mm/month) and a monthly low of 20 mm recorded in August 2012. Precipitation mainly fell during the winter, with water excess being dominant (Thornthwaite, 1948) during November – April each year. Rainfall stable isotope monitoring shows $\delta^{18}O$ values which range from −16.5 to +4.5‰ (mean = −4.9‰; 2σ = 6.1‰; n = 198) and $\delta^2H$ ranging from −130.7 to +12.8‰ (mean = −27.4‰; 2σ = 43.8‰; n = 195). Producing a local meteoric water line of rainfall collected in Matienzo with a slope of 6.86 and an intercept of 5.8, slightly lower than the global meteoric water line (slope = 8.0; intercept = 10). On the monthly scale, the $\delta^{18}O$ composition of rainfall in the Matienzo depression (monthly weighted mean) was strongly regulated by a rainfall amount effect (Pearson correlation; $r^2 = 0.51$; p < 0.01), with more negative isotopic values related to higher rainfall amounts (Fig. 5) and to a lesser extent by temperature (Pearson correlation; $r^2 = 0.33$; p < 0.01), where lower temperatures were related to more negative isotope values.

### Air temperature and carbon dioxide dynamics

Average annual cave air temperature at 65 m underground in Cueva de Asiul (+13.7 ± 0.5°C) was very similar to the average external air temperature (+13.8°C), but with a temperature range of 1°C at 65 m depth. Within this range, a clear seasonality of temperature was observed (Fig. 6). Summer (April to November) cave air temperatures were characterised by a gradual, stable increase from +13 to +14°C. Under winter conditions rapid reductions in cave temperature were observed, by up to 0.7°C (Fig. 6).

$CO_2$ does not replicate with the same intensity of seasonality compared to that observed in the temperature records. Peak values of $CO_2$ were measured in June 2013 (2090 ppm) and low values prevail during October 2012 (360 ppm; Fig. 6). Much stronger seasonality was observed in soil air $CO_2$ measured on a monthly basis, demonstrating peak concentrations (4283 ppm) during the summer and low values (647 ppm) during the winter (Fig. 6).
**Speleothem drip waters**

All speleothem drip sites displayed constant dripping throughout the study years and a distinct seasonality in drip discharge. Peak discharge amounts varied between the speleothems but discharge maxima (0.91 l/day at ASF based upon an average drip volume = 0.076 ml) occurred at all sites during the winter. The summers were characterised by gradually decreasing drip rates (min 0.14 l/day at ASF) under dryer karst conditions at all sites, followed by a very rapid return to higher rates at the onset of seasonal rainfall. Superimposed upon this seasonal signal are discrete spikes in speleothem discharge which can range up to an additional 70 drips per hour (an additional <5.5 ml/hour). δ¹⁸O analysis of speleothem ASF drip waters showed a range between ~5.3 and ~6.9‰ with an average value of ~6.08‰ (n = 155).

**DISCUSSION**

**Cave air temperature and ventilation dynamics**

In Cueva de Asiul, patterns of cave air temperature closely resemble the seasonality of external temperature cycles (Fig. 6), indicating a dominant air density control upon ventilation regime (Wigley & Brown, 1976; Smith et al., 2015). During the summer this temperature record is buffered by bedrock heating, resulting in a steady and uninterrupted rise in cave air temperatures (Fig. 6). Under winter conditions, external air temperature drops significantly, and therefore transgresses more frequently below the cave internal temperature threshold, meaning colder external air can flow into the cave system over more of the diurnal cycle. Cold external air overwhelms the effect of bedrock heating, forcing cave air temperatures to remain low throughout the coldest part of the winter season (Fig. 6). At the end of the winter, cave air temperatures begin to rise several weeks before any observed increase in external temperature (Fig. 6) (Smith et al., 2015). This process is driven by a combination of cave air stagnation, as external air temperatures become very close to those internal to the cave environment, and the conduction of heat through surrounding bedrock, warming the cave air (Smith et al., 2015).

CO₂ levels remain low within the cave during the summer, indicating a lack of CO₂ build up. CO₂ build up is thwarted in Cueva de Asiul by diurnal ventilation of the cave (Smith et al., 2015). External diurnal temperature transgressions over the cave internal temperature threshold thereby keep CO₂ levels within the cave low throughout the summer.

High resolution CO₂ and cave air pressure monitoring have also identified an event scale ventilation process which can occur at any point of the year, independent of the dominant temperature driven ventilation. This secondary ventilation regime is driven by changes in external air pressure (Genty & Deflandre, 1998) which create a pressure gradient between the cave and external air (Smith et al., 2015). When external pressure is low, this imbalance causes air to flow rapidly out of the cave system, aiding the drawdown of karst and soil air on an event (hourly) scale. This pressure driven process causes small scale perturbations to cave air CO₂ content due to the high pCO₂ content of air within the soil zone being drawn into the cave.

To further assess the sources of CO₂ in Cueva de Asiul, monitoring for δ¹³C in CO₂ was undertaken on a monthly basis, both within the cave but also in the soil zone (Fig. 6) and using external air samples. The highest cave air δ¹³C measurements (~10.9‰) are similar to those taken in the open atmosphere (~8.9‰) highlighting the importance of external air ventilation even at 65 m depth into the cave (Fig. 7). The lowest δ¹³C in cave air CO₂ (~16‰) indicates a CO₂ source enriched in ¹³C, with mixing model end member values calculated as low as ~46‰ (Fig. 7). This calculated end member is lower than either the measured soil δ¹³C (~21‰) or the calculated soil zone end members (~34‰). The very low δ¹³C values calculated in this mixing model potentially indicate microbial oxidation of methane within the soil, upper karst or a ground air component (Mattey et al., 2013; Mattey et al., 2010).

δ¹³C in CO₂ and cave temperature highlight the importance of two key processes in controlling the composition of cave air. These are 1. ventilation of the cave chamber with external air, and 2. draw down of ‘ground air’ through the karst. These two processes combine, creating a relatively low pCO₂ environment year round, meaning that speleothem deposition is not systematically limited for long periods during any one season.

**Karst recharge dynamics**

Rainfall isotope monitoring highlights a strong amount effect in modern precipitation at this site (Fig. 5). However, the degree to which external rainfall dynamics are preserved within speleothem calcite demands an understanding of the karst hydrological system.

Drip monitoring studies in Cueva de Asiul show that speleothems are constantly supplied with supersaturated karst water, facilitating year round carbonate deposition. Water excess calculations indicate that these karst waters are sourced primarily...
from winter rainfall, with evapotranspiration limiting the ingress of summer rainfall to only the largest events (Fig. 8). Significant homogenisation of rainfall within the karst is however thought to partially obscure the $\delta^{18}$O of the summer events.

Drip water $\delta^{18}$O collected within the cave ($\delta^{18}$O[drip] range = -5.5 to -7.0‰) offer a muted range of isotope values from those observed in annual rainfall $\delta^{18}$O[rainfall] (range = +4.5 to -16.5‰), indicating that karst water mixing was sufficient to homogenise water isotope values. When compared to the local winter meteoric water line for Matienzo (Fig. 9), cave drip water $\delta^{18}$O[drip] cluster around the average winter rainfall $\delta^{18}$O values of the preceding year (2010-11 rainfall $\delta^{18}$O = -5.9‰, drip water = -6.4‰; 2011-12 rainfall $\delta^{18}$O = -5.5‰, drip water = -5.8‰; 2012-2013 rainfall $\delta^{18}$O = -6.6‰, drip water -6.2‰). This suggests that water percolation through the karst preserves external rainfall $\delta^{18}$O, and that speleothem $\delta^{18}$O should offer an accurate proxy of variable rainfall amounts in northern Iberia. Longer term monitoring of cave drip waters is required to confirm the preservation of annual rainfall trends on drip water $\delta^{18}$O over decadal or longer scales. Palaeoclimate records from this cave should therefore be ideally suited to record longer term changes in rainfall amount to this region, possibly regulated by changes within the major rainfall source region, the North Atlantic.

Transfer of climate signals into modern carbonate deposits

Karst processes are shown to effectively transfer an average $\delta^{18}$O signal to the site of speleothem growth (Fig. 9). To ensure that this signal is accurately incorporated into depositing carbonate, we analyse the $\delta^{18}$O in modern carbonate from both the very top of speleothem deposits (ASR and ASM) and calcite grown on artificial substrates (on top of ASF). Average drip water $\delta^{18}$O (-6.08‰, 1 stdv = 0.4‰) is compared to modern speleothem carbonate $\delta^{18}$O (-5.1 ± 0.32‰) and calcite plate carbonate $\delta^{18}$O (-5.12 ± 0.34‰), at the average cave temperature of 13.7ºC, using the experimentally derived fractionation factor of Tremaine et al. (2011):

$$1000 \ln \alpha = (16.1 \pm 0.65) \left(10^3 T^{-1}\right) (-24.6 \pm 2.2)$$

This fractionation factor has proved more effective at calculating real in cave $\delta^{18}$O fractionation between drip water and speleothem calcite than laboratory based calculations and presents a range of $\delta^{18}$O fractionation at which palaeoclimate reconstructions may be made (Tremaine et al., 2011). $\delta^{18}$O of speleothems from Cueva de Asiul fall within error of this fractionation coefficient, indicating that the oxygen isotope deposition environment in Cueva de Asiul is similar to other cave sites from around the World (Tremaine et al., 2011). This suggests
that calcite deposition in Cueva de Asiul is ideal for accurate palaeoclimate reconstruction.

**CONCLUSION**

Cave monitoring in Cueva de Asiul focused on understanding the growth environment for speleothems that have been extracted for palaeoclimate reconstruction. The cave has a complex ventilation system driven by diurnal changes in external temperature and air pressure, resulting in relatively low cave air pCO₂, which promotes the growth of speleothems year round. Super-saturated waters entering the cave preserve a smoothed rainfall δ²⁸O signature and precipitate speleothem carbonate under similar fractionation conditions as other cave sites. Speleothems from this cave are therefore thought ideal for preserving records of rainfall amount and may potentially offer insights into changes in the Cave's major rainfall source region, the North Atlantic.

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**REFERENCES**


**INTRODUCTION**

Since 2010 we have been studying several caves formed in crystalline limestones and metasomatic silicites of the Štiavnické vrchy Mountains. These caves are located in the Inner Western Carpathians of central Slovakia (Fig. 1). This paper presents evidence that these caves are of hydrothermal origin, linked to several phases of the evolution of the Štiavnica stratovolcano. Hydrothermal caves belong to a larger group of caves of hypogene origin identified on the basis of regional paleo-hydrogeological analysis, morphogenetic analysis of caves, cave sediments and minerals, and geochemical alteration of the host rock during hypogene speleogenesis (Dubljanskij, 1990; Dublyansky, 2000; Onac, 2002; Klimchouk, 2007; Spötl et al., 2009; and others). The caves described here contribute to a larger view of genetic variability of caves in Slovakia, which has a varied and complex geological setting. This study presents information about hydrothermal speleogenesis, including hydrothermal caves formed in metasomatic silicites.

**STUDIED CAVES AND THE SURROUNDING AREA**

The area of investigation is near Banská Štiavnica, a well-known historic mining town included in the World Heritage list. The area is in the central part of the Štiavnica stratovolcano, the largest volcano in the Carpathian volcanic arc, which is part of the Alpine-Balkan-Carpathian-Dinaride orogen (Konečný et al., 1995, 2001; Konečný & Lexa, 2001; Lexa & Koděra, 2010). The Miocene Štiavnica stratovolcano evolved during several stages from Badenian to Sarmatian, 15.0 to 10.7 Ma (Chernyshev et al., 2013). Early hydrothermal systems, related to emplacement of subvolcanic magmatic bodies in the pre-caldera stage of stratovolcanic evolution, formed mainly skarn and porphyry types of mineralisation. Vein mineralisation developed during later hydrothermal
events. Early type of veins developed during the pre-caldera stage and it is related to faults activated during subsidence of the caldera. Later type of veins developed in the post-caldera stage and they are related to faults associated with long-lasting horst uplift in the center of the caldera (Lexa et al., 1999; Lexa, 2001; Koděra et al., 2005). The mountains also host several lenses of Middle to Late Triassic carbonates that are locally metamorphosed to marble in contact with magmatic intrusions. The intrusions were emplaced during early stages of stratovolcano evolution (Konečný et al., 1998a,b) (Fig. 2).

The central zone of the Štiavnica stratovolcano hosts caves in limestones and in andesites altered to metasomatic silicites. Some caves have morphologic and mineralogic features of hypogene or hydrothermal origin.

The First Karst Cave

The First Karst Cave is located in the northern part of the Štiavnické vrchy Mountains, about 3 km south of the Sklené Teplice Spa on the southwest side of the Teplá Stream Valley (Figs. 1 and 2). Middle Triassic limestones and dolomites crop out in the area (Konečný & Lexa, 1984; Konečný et al., 1998a,b). Based on our observations, these formations are Gutenstein dolomites and limestones and Steinalm limestones of the Hronicum Unit. Hydrothermal alteration occurred in each of these carbonate types, mainly along veins and stockworks. Steinalm limestones have been recrystallized to marble. The entrance shaft leading to the cave was dug by miners 200 to 300 years ago (Fig. 3A). A natural cave opening to the surface is unknown. Original cave passages and several smaller geode-like cavities were encountered at the base of the shaft. Two blind adits were dug along ore veins that intersect the cave. The southern adit is 20 m long, and the shorter eastern adit is 16 m long. The length of the cave and adits is approximately 100 m with a vertical range of 21 m. The total depth from the entrance of the artificial shaft is 31 m (Ivan, 1991) (Figs. 3B and 3C). From the bottom of the shaft, the cave is a down-sloping passage with several stair steps. In the upper segment, the passage is mostly 2 to 2.5 m high and 3 to 4 m wide. The cave pattern is controlled by faults of E-W and N-S directions (Bella et al., 2011a,b).

The Šobov caves

The Šobov Cave and the Šobov Chimney are located in the Šobov silicate quarry on the slope of the Malý Šobov Mount (836 m), approximately 1 km north of the town of Banská Štiavnica and 1 km south of the Banská Belá village (Figs. 1, 2, 4A, and 4B). Both caves were encountered when an exploratory mine adit was dug between 700 m and 715 m a.s.l. Here, metasomatic silicate is the result of acid leaching and silification of andesite porphyries by magmatic fluids associated with the emplacement of a subvolcanic diorite intrusion during the pre-caldera stage of the Štiavnica stratovolcano in Early to Upper Badenian (Štohl et al., 1994; Lexa et al., 1999; Konečný & Lexa, 2001; Lexa, 2001). The silicate is 95 to 98% SiO₂, with pyrite, anatase and rutile accessory minerals (Polák, 1961, 1963). Silicate is monotonous, composed almost entirely of fine-crystalline quartz accompanied by quartz veinlets (Oružinský, 1989). In the upper part of the quarry, the silicates contain rare caverns. Laterally, silicates grade in to argillites that contain sericite, pyrophyllite and pyrite.

The Šobov Cave is a steeply inclined oval cavity with several ceiling hollows and small cupolas. Its origin was controlled by a WNW-oriented zone of weakness. The cave is 3.5 m long with a vertical extent of 3 m (Figs. 4C and 4D). The cavity is a fragment of a larger cave, but the upper part was destroyed during exploitation of the overlying quarry. The lower part of the cave was probably removed during excavation of the exploration mine adit (Bella et al., 2010, 2011a).

The Šobov Chimney, located 7 m south-east of Šobov Cave, is a vertical fissure cavity with a height of 6.3 m and width of 0.8 to 2.1 m (Fig. 4C). It is controlled...
by a steep to near-vertical NNE trending fault. Blind cupolas occur in the upper part of the cave, and small quartz crystals are found in the fissure exposed by the cave. Quartz crystals several centimeters in size are also known from other fissures in the metasomatic silicites of this quarry (Bella et al., 2010).

**METHODS**

Since 2010 our research in the Štiavnické vrchy Mountains has focused on documenting morphological and mineralogical evidence for the hydrothermal origin of the caves (Bella et al., 2010, 2011a,b). The orientations of structural-tectonic discontinuities in caves were compared to the pattern of faults and epithermal veins in the vicinity. Based on detailed observations and surveys, we have compared cave morphologies with indicators of hydrothermal speleogenesis (in sense of Bakalowicz et al., 1987; Ford & Williams, 1989, 2007; Dubljanskij, 1990; Palmer, 1991, 2007; Dublyansky, 2000; Klimchouk, 2007; Audra et al., 2009b and others).

Sample sets of hard, altered and crumbled rocks and minerals were collected in the caves. In the limestone First Karst Cave, samples of clays were obtained from filled fissures in the walls, and from partly filled passages. All of the samples were processed in a laboratory. The samples were treated...
with sodium acetate, hydrogen peroxide and sodium dithionite, and the clay fraction <2 μm was separated by sedimentation. Samples sedimented on glass slides were analyzed with a Philips PW 1710 diffractometer (Cu-Kα radiation with a graphite monochromator) examining oriented and non-oriented preparates, as well as saturated with ethylene glycol to identify swelling layers. Oriented preparates allow clear identification of layered silicates (Šucha, 2000).

Fluid inclusions in samples from the limestone First Karst Cave were studied using doubly polished wafers (~200 μm thick) prepared from calcite samples taken from geode-like cavities and cave walls. The observed inclusions were assigned a primary or secondary origin according to the criteria of Roedder (1984). For microthermometric measurements, inclusions grouped into assemblages of primary fluid inclusions (in the sense of Goldstein & Reynolds, 1994) have
Hydrothermal speleogenesis in carbonates and metasomatic silicites

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Fig. 4. Caves and channels in the Šobov silicite quarry: A) Šobov quarry (arrow points to the opening of exploration mine adit); B) exploration mine adit with openings of Šobov Cave (I) and Šobov Chimney (II); C) plan of Šobov Cave and Šobov Chimney; D) rising chain of cupola-like forms of the Šobov Cave; E) wall asymmetrical depressions in the lower part of the Šobov Cave; F) small channels deepened into metasomatic silicites along fractures (all photos: P. Bella).

Stable carbon and oxygen isotope composition of carbonates was measured on an isotope ratio mass spectrometer MAT253 (Thermo) coupled through a continuous flow interface using the automated gas preparation and introduction system Gasbench. Samples of carbonate were micro-drilled from polished rock sections, sealed in borosilicate glass vials, flushed by helium and reacted for 24 hours with phosphoric acid at 26°C, using the method of McCrea (1950). Each analytical run was calibrated using series of two working standards regularly scattered between samples. Measured δ¹⁸O and δ¹³C values of carbonates are reported in permil relative to PDB, while calculated δ¹⁸O of water are relative to SMOW.

RESULTS

Structural-tectonic control and morphology of caves

Two main linear segments of the First Karst Cave (in ground plan; Fig. 3B) are developed along east- and north-oriented, steeply-dipping, crossing faults. These faults were fundamental controlling factors for epithermal mineralization and speleogenesis in basement carbonates of the Štiavnica stratovolcano. Both the northern and western side adits (Fig. 3B) were dug along mineralized veins that intersect the cave passage. These are mainly carbonate veins dipped to 280°-285° at 60°-70°. In some places, the veins are filled with Fe-oxyhydroxides and goethite (ochre). The orientation of faults and mineralized veins in the cave corresponds to the orientation of faults of the Hodruša-Štiavnica horst.

Probably, the First Karst Cave is the fragment of a larger three-dimensional cave system. Several smaller spherical hollows that were encountered during the excavation of the northern adit are geode-like cavities (in sense of Audra et al., 2009a). Other geodes and geode-like cavities, visible in some cave walls, were intersected and integrated into the main cave passage during the main development phase of the cave. Numerous small cupolas, ceiling pockets and holes, upward-convex arches, and oval chimneys (Figs. 3C to 3F) occur in the main cave passage. The diameter of the spherical cavities in the ceiling mostly ranges from 30 to 50 cm. The chain of coalesced cupola-like forms and upward-convex arches that extends from the lower to upper part of the cave indicates that water circulated upward in the past. Rising wall channels with asymmetrical dissolution depressions are on some steep cave walls and below some ceiling spherical forms, and indicate ascending water flow. The vertical conduit in the lowest part of the cave resembles a feeder (in sense of Klimchouk, 2007).
through which fluids had been rising into the cave. These features are indentical with morphological indicators of hypogene or hydrothermal caves (see Klímcouk, 2007; Audra et al., 2009b; and others). The original outlet from the upward-leading main cave passage onto the surface was probably enlarged by miners (remnants of solutional cavities are visible in the lower part of the entrance artificial shaft). Besides, several small natural cavities and caves with spigework morphology (e.g. Voštínová Cave), probably genetically connected with the studied cave, occur near the upper edge of the entrance artificial shaft. Other possible cave openings to the surface were filled and covered by slope sediments or destroyed by slope processes during younger landform development.

The Šobov Cave is a steeply inclined chimney with side cavities that are blind cupolas. Ascending flow of thermal fluids is indicated by a rising chain of cupola-like forms and large asymmetrical depressions in silicite walls (Fig. 4D and 4E). These speleogens are similar to transitional wall and ceiling features seen in hypogene limestone caves. The Šobov Chimney is controlled by an obvious NNE-oriented vertical tectonic fracture. Steep to vertical fractures in both Šobov caves controlled the ascending flow of thermal fluids. Smaller channels deepened into metasomatic silicites along fractures have been observed in other parts of the Šobov quarry (Fig. 4F). The morphology of these caves indicates their hypogene or hydrothermal speleogenesis.

**Cave deposits and their mineralogy**

Distinct macroscopic features of hydrothermal alteration as a silicified bedrock and carbonate rocks replaced by high Fe silicites occur in the First Karst Cave (Fig. 5A). Porous silicites of red color are common in cave passages and small side cavities in recrystallized host carbonates. Cave floors are mostly covered by brown clay sediments (Fig. 5B). The host rock of the cave is massive pinkish-gray marble, correlative with Middle Triassic limestones and dolostones of the Fatricum unit. The isotopic composition of the host rock is homogenous across dimensions of the cave, with \( \delta^{13}C \) ranging between 1.1 and 2.8‰ and \( \delta^{18}O \) between -23.1 and -18.7‰.

Intensively hydrothermally altered, light yellow-green friable rock also occurs on cave walls (Fig. 5D). Fissures and side cavities in the lower part of the cave are partially filled by very fine-grained sediments with alternating layers of yellow-ochre, brown and black color (Fig. 5E). These sediments were locally redistributed and deposited in stagnant water conditions within the karstified carbonates. There are no stratigraphic or morphologic indicators of fluvial erosion and transport in these accumulations.

Results of the XRD analysis show that brown clayey material (Sample no. 1, Fig. 5B) contains relatively high content of quartz and small quantity of carbonates. In the clay fraction we detected several layered silicates, mostly smectite and kaolinite (Fig. 6A). Minerals of the illite group (for simplicity illite) are present in trace amounts. The light yellow-green friable rock (Sample no. 2, Fig. 5D) contains predominately illite with minor kaolinite (Fig. 6B), and there is a relatively high content of calcite and quartz. Illite does not contain expandable layers (no change in XRD maxima after a saturation with ethylene glycol). The half-width of the first basal reflection is very low (about 0.2° 20), indicating a high ordering in relatively coarse illite crystals (Fig. 6C). According to the dominant coloration, fine-grained layered sediments (Sample no. 3, Fig. 5E) can be separated into three subsamples consisting of yellow-ochre, brown and black material. The subsamples were analyzed separately. The yellow-ochre and black colored samples have the same mineral composition; they are dominantly formed by goethite (Fig. 6D). The black coloration of some thin goethite layers cannot be explained by differences in mineral composition, but may be caused by an admixture of manganese. Fine-grained sediments of brown color have a mineral composition almost the same as brown clayed sediments on the floor of cave passages.

Three generations of secondary carbonate deposits were recognized:

1) Geode cavities filled with druses of scalenohedral calcite of pale brown, pinkish to white colour up to 4 cm long (Fig. 5C). The drusy aggregates are grown on an Mn-pigmented dark brown rim...
of host marble, representing an alteration zone formed by mineralizing fluid. Typical thickness of this transition zone is from 5 to 20 mm.

II) White laminated crust, locally overgrowing scarce calcite rhombs, patched by nests of prismatic aragonite needles. It covers present walls of main cave passage, truncating geodes and their mineralization (generation I). The thickness of the calcite crust ranges from 1 to 8 mm. Aragonite needles are up to 40 mm in length. The crust is usually grown on a pale, 3-10 mm thick alteration zone in host marble or geode mineralization. The generation II is believed to be coeval with main cave formation, its secondary carbonates have been deposited towards the termination of cave formation.

III) Dripstones, flowstones and soda straws of white color, deposited as the latest carbonate mineralization in the cave and oldest adit. Superposition and cross-cutting relationships of these three generations are shown on Fig. 7.

Fluid inclusion microthermometry was performed on two samples of calcite, representing generations I and II of the First Karst Cave. Generation III did not contain fluid inclusions suitable for this method.

Microthermometry of primary fluid inclusions hosted in generation I calcite showed a relatively broad range of homogenization temperatures (Th) from 115 to 279°C (Figs. 8 and 9), however most of the data were in range from 115 to 168°C (mean value 137°C). Higher values of homogenization temperatures of some inclusions probably result from necking down of inclusions that was observed to affect some other inclusions in the analysed sample. All analysed fluid inclusions showed ice melting temperatures (Tm) in range from -0.1 to +0.2°C, indicative of predominance of meteoric component in the source water. The positive values of ice melting temperatures are most likely related to the limited accuracy of stage calibration (± 0.2°C) rather than metastability of ice on heating.

Primary fluid inclusions in generation II calcite also showed a broad range of Th values from 112 to 199°C (Fig. 9), with most of the data in range from 143 to 169°C (mean value 153°C). Again, the highest Th values are most likely related to stretching of some inclusions on heating. Most measured Tm values were close to 0°C (-0.1 to +0.2°C), indicative of pure water (~0 wt% NaCl eq.), but still some inclusions showed...
decreased values down to -1.3°C, corresponding to salinities of 2.2 wt% NaCl eq. Increased salinities show slight positive correlation with corresponding homogenization temperatures (Fig. 9), which can be interpreted as mixing with a hydrothermal fluid containing small amount of magmatic component.

Stable C and O isotope measurements focused on three generations of secondary carbonate fills. Figure 7 shows the relationship of carbonate fills to the host rock and the alteration zones. Both oxygen and carbon isotope delta values show very large spans. Most notable are distinct isotope shifts across both alteration zones, towards lighter carbon in generations I and II, and heavier oxygen in generation II. Ranges of isotope compositions are summarized in Table 1 and graphically represented in Fig. 7.

![Fig. 7. Schematic sketch (a) and close-up photographs (b, c) of secondary carbonate fills. I, II, and III labels indicate generations/alteration events as in Table 1. Stable C and O isotope values are plotted in profiles from intact host rock through alteration zones to subsequent mineral fills. Relative chronology of the stages I, II and III is established by their truncation and superposition.](image)

![Fig. 8. Typical primary fluid inclusion hosted in a calcite crystal from the First Karst Cave (photo: P. Koděra). Inclusion contains liquid water with contraction bubble (salinity 0 wt% NaCl eq., homogenization temperature 165°C).](image)

![Fig. 9. A) Histograms of homogenization temperatures (Th) and B) salinities of primary fluid inclusions in two generations of calcite from the First Karst Cave; C) Salinity vs. Th diagram, showing input of hydrothermal fluids other than pure meteoric origin, corresponding to major range of Th values of generation II calcite.](image)
Table 1. Ranges of isotope compositions of the host rock and three generations of secondary cave carbonates (labelling corresponds to zones sketched in Fig. 7).

<table>
<thead>
<tr>
<th>Zone</th>
<th>Host rock, cave deposits</th>
<th>δ¹³C (%)</th>
<th>δ¹⁸O (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>host marble</td>
<td>1.0 to 2.9</td>
<td>-24.0 to -20.4</td>
</tr>
<tr>
<td>Ia</td>
<td>outer brown alteration zone of host marble</td>
<td>+1.3 → -4.4</td>
<td>-18.4 to -20.7</td>
</tr>
<tr>
<td>Ib</td>
<td>pinkish drusy calcite</td>
<td>-3.9 to -1.5</td>
<td>-24.1 to -19.6</td>
</tr>
<tr>
<td>Ic</td>
<td>white drusy calcite</td>
<td>-3.1 to -5.5</td>
<td>-19.6 to -27.3</td>
</tr>
<tr>
<td>Id</td>
<td>brown drusy calcite</td>
<td>-4.0 to -5.5</td>
<td>-16.2 to -26.1</td>
</tr>
<tr>
<td>Ie</td>
<td>latest white drusy calcite</td>
<td>-2.6</td>
<td>-17.2</td>
</tr>
<tr>
<td>IIa</td>
<td>pale alteration zone in host marble</td>
<td>1.5 to -3.2</td>
<td>-19.9 to -14.6</td>
</tr>
<tr>
<td>IIb</td>
<td>calcite rhombs covered by laminated crust</td>
<td>-7.7 to -8.5</td>
<td>-7.6 to -5.6</td>
</tr>
<tr>
<td>IIc</td>
<td>needle crystals of aragonite</td>
<td>-7.5 to -8.3</td>
<td>-8.0 to -7.0</td>
</tr>
<tr>
<td>III</td>
<td>late flowstones and soda straws</td>
<td>-7.9 to -10.4</td>
<td>-8.4 to -10.4</td>
</tr>
</tbody>
</table>

Note: Ranges of isotope delta values are indicated by “→” where a distinct trend exists from older to younger laminae, or by “to” where a trend is absent.

**INTERPRETATION AND DISCUSSION**

**Hydrothermal speleogenesis in basement carbonates of the stratovolcano**

The carbonate host rocks of the First Karst Cave correlate with marine Middle Triassic limestones. Marine isotope signature of carbon is roughly preserved (1.0 to 2.9‰) while that of oxygen is totally obliterated, being more than 20‰ depleted against Middle Triassic values (-5 to 0‰) (Veizer et al., 1999; Korte et al., 2005). Apparent recrystallization of rock and large scale alteration of the original oxygen isotope composition suggest equilibration with a large and penetrative water reservoir at elevated temperatures. Unfortunately the isotope composition of the circulating water remains unknown due to lack of indicators of metamorphic grade in the marble.

Isotope compositions of waters depositing calcite of both generations I and II may be calculated from isotope compositions of calcites at homogenization temperatures of their fluid inclusions, using fractionation factors of O’Neil et al. (1969) and Ohimoto and Rye (1979) for fractionation of oxygen between calcite and H₂O and carbon between calcite-CO₂, respectively. At given temperatures, CO₂ is assumed as dominant carbon bearing species in the fluid.

For generation I, with calcite composition δ¹³C = -27.3 to -16.2‰ and δ¹⁸O = -5.5 to -1.5‰, and temperatures between 115 and 168°C with mean at 137°C, equilibrium water has δ¹³C = -13.4 to +2.5‰ and equilibrium CO₂ has δ¹⁸O = -8.7 to -2.3‰. Using mean temperature yields ranges δ¹³C = -11.3 to 0.2‰ and δ¹⁸O = -7.6 to -3.6‰. Light oxygen isotope values probably reflect substantial meteoric component in fluids. However, the presence of heavier oxygen of some analyses may reflect participation of a minor magmatic component in hydrothermal fluids and/or oxygen isotope exchange of heated meteoric waters with volcanic rocks in the vicinity of the cave.

In generation II calcite rhombs and crusts have composition δ¹³C = -7.5 to -5.6‰ and δ¹⁸O = -8.5 to -7.7‰, and fluid inclusion temperature range from 143 to 169°C with median at 153°C. Water in equilibrium with calcite at these conditions would have δ¹³C = +9.6 to +13.4‰ and δ¹⁸O = -10.3 to -8.5‰, and using mean temperature the ranges are δ¹³C = +10.3 to +12.3‰ and δ¹⁸O = -9.9 to -9.1‰. The source of fluid may be meteoric water strongly modified by isotope exchange with rock-forming minerals during deep circulation.

Solutional shapes of cavities – both small-scale geodes and large-scale main passage – with secondary carbonates suggest, that the fluids at certain point in their evolution turned from corrosive to depositing, probably due to pH increase. Close after this “switch”, the temperature and isotope composition of depositing fluids were probably not far from former corrosive fluids, responsible for cavity formation.

The hydrothermal origin of the First Karst Cave is evidenced by irregular spherical morphologies sculptured by rising thermal water, fluid inclusions in calcite crystals, hydrothermal alteration of host rocks and clays of hydrothermal origin composed variably of smectite-kaolinite and goethite.

Mineral association and paleotemperature data show that hydrothermal processes were probably active in several phases (Table 2). A multiphase dissolution of carbonates is documented by the remains of several partially denuded fills in older geodes, geode-like cavities and ceiling hollows that are now integrated into larger, younger cupola-like cavities and main cave passage.

Hydrothermal phases of cave development consists of dissolution of carbonates by fluids of variable temperatures. Fluid inclusions hosted in large calcite crystals of the zone Ic-Id, also in geode-like cavities (Fig. 6), indicate crystallization temperatures in range from 115 to 170°C. Primary fluid inclusions in generation II calcite indicate crystallization temperatures in range from 112 to 180°C. The smectite-kaolinite and goethite mineral associations probably formed at temperatures less than 100 to 150°C (see McDowell & Elders, 1980; Reyes, 1990; Sucha et al., 1993; Sucha, 2000). The occurrence of smectite indicates a low-acid to neutral environment with pH 5-7, while the occurrence of kaolinite is linked mainly with solutions with low pH values between 2-4 (Giese, 1988; Lahodny-Sarc et al., 1993).

Quartz crystals in fissures, exposed by the cave, originated from hot fluids with temperatures probably of at least 200°C, as indicated by fluid inclusion data from vein quartz in this ore field (Julényová, 1996; Lexa & Koděra, 2010). The illite mineral association, contained in the light yellow-green friable rock on cave walls, is a typical product of hydrothermal alteration of...
Hydrothermal processes responsible for the origin of the First Karst Cave were most likely part of a complex extensive hydrothermal system forming hydrothermal ore deposits 3 km from the cave. The hydrothermal deposits evolved during various stage of the development of the Štiavnica stratovolcano in Badenian to Sarmatian time (Lexa et al., 1999; Lexa, 2001). The deposits include pre-caldera stage skarn-porphyry systems (Sklené Teplice – Vydišová Valley and Kozi Hill) and post-caldera stage Ag-Au epithermal vein systems (Windischleuten vein). The Sklené Teplice system is believed to have caused silicification of carbonates north of the cave at Bukovec, while the Windischleuten vein system south of the cave is related to the horst uplift in the center of the caldera. The northwest part of the Windischleuten vein encroaches into Triassic limestones, dolomites and siliceous sediments of the Veľký bok Group (Lexa et al., 1997), in which the First Karst Cave is developed.

Fluid inclusion petrography and microthermometry and clay mineralogy of both systems are similar and partially overlap with data obtained for the cave. Silicified zones from Bukovec contain fluid inclusions with salinities from 1.0 to 5.6 wt% NaCl eq. and two different ranges of homogenization temperatures (Julényová, 1996). The upper range (200 to 240°C) corresponds to major crystallization of quartz, followed by a lower temperature, less significant crystallization stage (150 to 180°C). Paleofluids from the Windischleuten vein system have not yet been studied. However, there exist fluid inclusion data from a similar Au-Ag vein systems in the neighboring areas of Hodruša and Vyhne (Lexa & Kodéra, 2010). Here, fluids precipitated vein filling minerals during several stages, while salinities did not significantly exceed 5 wt% NaCl eq. and homogenization temperatures ranged from 150 to 310°C, depending on the stage.

Calculated oxygen isotope data of water in equilibrium with generations I and II calcite from the cave can be also compared to published isotope data from skarn-porphyry and Ag-Au epithermal systems in the central zone of the Štiavnica stratovolcano. Data from Sklené Teplice and from Windischleuten are not available, however, data can be compared to similar coeval systems in this ore district. Hydrothermal fluids at the skarn-porphyry system Vysoká-Zlatno showed strongly contrasting chemistry (in sense of Bögli, 1964), CO₂-rich fluids with δ¹⁸O close to +12.5‰, while Ag-Au epithermal system at Vysoká-Zlatno showed mostly strong magmatic signature (δ¹⁸O close to +3‰) (Kodéra et al., 2010), but Ag-Au epithermal system at Všechnvštých Ag-Au vein showed significantly lighter oxygen isotope signature (δ¹⁸O close to 1.1‰), indicative of significant meteoric component in the fluids (Kodéra et al., 2007). Data from calcites from the First Karst Cave (-13.4 to +2.5‰) δ¹⁸Ofluid for generation I and +9.6 to +13.4‰δ¹⁸Ofluid for generation II) overlap with data from both neighbouring systems.

In conclusion, neither fluid inclusion nor stable isotope data made clear which of the two neighboring systems was responsible for the origin of the First Karst Cave. However, the presence of vein mineralisation intersecting the cave indicates that the post-caldera epithermal system is slightly more likely.

In both hydrothermal environments the low salinity of fluids can be attributed to dilution of magmatic-hydrothermal fluids by mixing with meteoric waters during their ascent from depth (Julényová, 1996; Lexa & Kodéra, 2010). Thermal waters rising along deep faults were saturated with CO₂ from magmatic and volcanic activity. Their solutional aggressiveness could be maintained, renewed or enhanced by decreasing temperature (the inverse relationship between calcite solubility and temperature, even at constant CO₂ levels; see Palmer, 1991) and mixing of waters of contrasting chemistry (in sense of Bögli, 1964), mainly at shallow depths (see Dublyansky, 2000 and others). The post-magmatic hydrothermal dissolution of carbonates was responsible for the development the First Karst Cave. During the formation of this
cave Middle Triassic host carbonates in the area were covered by fractured volcanic or volcanoclastic rocks of unknown thickness.

After hydrothermal phases with the most intense dissolution of carbonates and the dominant enlargement of underground passages and cavities, older carbonate deposits in the cave were truncated and partially dissolved in phreatic conditions. Clayey sediments were redeposited and deposited in slowly moving to stagnant water (Table 2). Finally, downcutting of the stream valley extended below the elevation of the cave and the current vadose development phase began. During the youngest vadose phase, water from precipitation that seeps into the cave has not altered older corrosion forms on the bedrock walls and ceilings.

In the area surrounding the First Karst Cave, hydrothermal processes are still active. Hot geothermal waters (33 to 35°C) within the hydrogeological structure near Sklené Teplice Spa are connected to Triassic carbonates and ascend along two crossing faults. One fault is NNE-oriented and bounds pre-Tertiary rocks of the Hodruša-Štiavnica horst from the west. The second is a transverse fault oriented WNW that bounds the Bukovec block (Remšík et al., 2001 and others).

Analogous examples of hydrothermal speleogenesis associated with metallogenesis of ore deposits (caves originated by a post-magmatic dissolution of metamorphosed carbonates) are known from the Romanian Carpathians (Mărza & Silvestru, 1988; Onac, 2002; Onac & Damm, 2002; and others).

Hydrothermal or mixed hydrothermal/vadose origin of skarn hosted caves of the Băiţa metallogenic district in the Bihor Mountains is related to a deep-seated Upper Cretaceous granitic pluton (Onac, 2002). In the NE part of Greece, the early genesis of Maronia Cave is related to an andesite intrusion during Miocene that generated ascending thermal fluid circulation in Middle Eocene numulitic limestones causing development of hypogenic solution voids (Vaxevanopoulos & Melfo, 2010).

Hydrothermal speleogenesis in metasomatic silicites

In the two investigated caves in the Šobov silicite quarry, spherical morphology sculptured by ascending fluids, hydrothermal alteration of host rocks and the occurrence of quartz crystals are distinct indicators of hydrothermal origin. Speleogenesis in the Šobov silicites was caused by hydrothermal processes during the pre-caldera phase of development of the Štiavnica stratovolcano in Upper Badenian.

The Šobov paleohydrothermal system is a typical hydrothermal system associated with high temperature (> 200°C) magmatic fluids of high oxidation state (well known in Economic Geology as „high sulfidation“ or „acid sulfate“ systems), related here to the emplacement of a diorite intrusion in the footwall of the altered andesite (Lexa et al., 1999) (Fig. 10). Hydrothermal systems of this type form in two stages (Sillitoe, 1989; Hedenquist & Arribas, 1999). The first (major) stage is related to upflow of magmatic fluids containing HCl, SO₂ and HF that within the central upflow column condense into the hydrothermal system, forming H₂S and H₂SO₄ and a very acidic (pH 1-3) solution (Rye et al., 1992). This acidic fluid is capable of leaching most of the major elements from the host rock, resulting in vuggy textures. Highly siliceous rock is either porous and vuggy or massive and dense, the latter largely due to silicification of the residual quartz developed due to leaching (Simmons et al., 2005). Cavernous and metasomatic silicites occur in the center of the system, surrounded by argillized rocks affected by advanced argillic style of alteration (including alunite, pyrophylite, dickite and quartz). The origin of caverns and caves in the Šobov area is probably related to this early stage of leaching. Acids reacted here with andesite, altering it to metasomatic silicite that is locally cavernous, especially in its upper part. The mushroom-shaped body of metasomatic silicite is surrounded by a zone rich in illite, pyrophyllite, and pyrite (Uhlík & Šucha, 1997; Uhlík et al., 2001). The presence of pyrophyllite indicates a temperature of more than 270°C, while the
amount of pyrophyllite and pyrite decreases with the increasing distance from the silicate body.

The second stage is caused by penetration of more neutral fluids, possibly with a meteoric component. In the Sobov hydrothermal system these fluids were responsible for precipitation of quartz crystals in cavities and local opal/chalcedony veins in upper part of the system. Oružinský and Hurai (1985) published data on microthermometry of fluid inclusions in quartz crystals from several cavities in the quarry. Homogenization temperatures ranged here from 200 to 300°C (with a maximum between 250 to 270°C) and salinities were lower than 2.7 wt% NaCl eq., with major components NaCl and KCl. These data indicate a minimum depth of origin 660 m beneath the paleosurface (Oružinský & Hurai, 1985). One independent low-temperature pulse at 150°C was also detected by Oružinský and Hurai (1985). In deeper parts, these more neutral fluids may be responsible for minor disseminated metal mineralization (Lexa et al., 1999). Cavities in metasomatic silicites were filled by minerals precipitated from flowing and cooling fluids. Later, these fills were washed out by post-hydrothermal waters.

From a genetic and geochemical viewpoint the described deep-seated speleogenesis in metasomatic silicites of the Sobov area differs from the cave origin in siliceous rocks by weathering dissolution process called “arenisation” and subsequent mechanical erosion caused by underground flowing waters. This lithologically specific cave origin is initiated by hydration of quartz or by incongruent dissolution of silicate minerals controlled by the pH of the associated solution. The dissolution of quartz by meteoric waters along joints in silicates, or along crystal boundaries, leads to weathering of silicate into soft “neosandstone”, and eventually into sand (Martini, 1979, 2000, 2004; Jennings, 1983; Piccini & Mecchia, 2009; Wray, 2010; Mecchia et al., 2014; and others). The presence of gypsum and pyrophyllite led Zawidzki et al. (1976) to propose that some caves in quartz-sandstones of the “tepui” table mountains in Venezuela were the result of a low-grade metamorphic event with related rising hydrothermal fluids possibly enriched in H₂S. But local minor variations of the S isotopic signature may be related to additional minor sources of reduced sulphur from peat bogs and decomposed vegetation. In these settings, there is no need to invoke hydrothermal events or oxidation of sulphides in the host rock for the formation of gypsum (Sauro et al., 2014).

CONCLUSION

The First Karst Cave is a remarkable example of a hydrothermal limestone cave associated with Miocene volcanic and magmatic processes in central Slovakia. The cave originated in hydrothermally altered rocks, characterized by cupolas, ceiling pockets and holes, chimneys, geode-like cavities with idiomorphic calcite crystals and clays of hydrothermal origin. Mineral associations indicate two phases of hydrothermal activity within the cave formation when smectite, kaolinite, goethite and secondary carbonates of generations I and II were formed. The origin and development of this cave was multiphase and multiprocess, related to multiphase dissolution of carbonates. This conclusion is based on remains of partially denuded fills in older geodes and geode-like cavities cut by larger and younger ceiling hollows and main cave passage. The main phases of intensive dissolution of carbonates by hydrothermal fluids resulted in development of hypogene cavities and passages. This was followed by a post-hydrothermal phreatic phase, and finally vadose phase that did not made striking changes in the original cave morphology.

The caves in the Sobov quarry are associated with magmatic-hydrothermal activity. They are a rare occurrence of hydrothermal caves in metasomatic silicites and are the only known hydrothermal silicate caves in Slovakia. Their origin is related to deep-seated hydrothermal processes related to a diorite intrusion in the northern part of central zone of the Štiavnica stratovolcano during its pre-caldera evolution phase in Upper Badenian.

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REFERENCES


Hypothermal speleogenesis in carbonates and metasomatic silicates


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Hydrothermal speleogenesis in carbonates and metasomatic silicites

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INTRODUCTION

Work related conditions in a cave such as exposure to heat, chemicals, dust, and poor lighting could influence the integrity of the visual system and predispose the eye to diseases that eventually affect vision (Ovenseri-Ogbomo et al., 2012). Poor lighting conditions cause a variety of symptoms of visual discomfort and may increase the risk of accidents (Veitch, 2001; Van Bommel, 2006; Reinhold & Tint, 2009; Pais & Melo, 2011). Visual discomfort results in signs and symptoms such as eyestrain, blurred vision, visual irritability, headaches, muscle aches and stress (Boyce et al., 1997; Kerkhof, 1999; Van Bommel, 2006; Pais & Melo, 2011). There are also other symptoms caused by the lack of lighting: tired eyes and watery and itchy eyes. Other specific disorders include degeneration of vision sharpness (blurred and diplopia) and slowness in changing focus (Woodside & Kocurek, 1997; Blehm et., 2005; Reinhold & Tint, 2009).

Contrast sensitivity and visual acuity are fundamental descriptors of the human visual system playing a key role in the quality of vision (Kohnen et al., 2005; Kang et al., 2009). Good visual acuity is crucial for several professions (Johnson & Casson, 1995) regarding safety purposes. Visual performance is critical in miner’s ability to judge the speed or direction of a machine (Reyes et al., 2013) and for cavers to explore, map or perform research in caves. Visual acuity typically measured under optimal viewing conditions with appropriate refractive correction will be altered by different environmental conditions and refractive properties in the work environment (Johnson & Casson, 1995).

There are some professional activities involving visual tasks with resolution of detail that must be performed under conditions of reduced illumination and contrast. Speleologists perform their activity in demanding visual conditions of low illumination, as
cave environment has similar conditions to night vision. LED light systems could help to improve the lighting conditions. The visual system is able to operate effectively from starlight to bright sunlight; over a change in illumination by more than a factor of $10^{11}$ (Stockman & Sharpe, 2006). However, changes in visual function occur under reduced illumination: reductions in visual acuity in central and peripheral locations, as well as reduced contrast sensitivity for all spatial frequencies (Wood & Owens, 2005). Visual contrast sensitivity is an indicator of visual pattern-detection ability for stimuli of various sizes. Visual stimuli encountered in everyday life activities are often of much lower contrast, due to various conditions such as inclement weather or darkness (Zavod, 2004).

Filters are currently used by eye care practitioners to assist people with low vision in maximising use of residual vision, improve visual function, control glare and improve orientation and mobility skills (Eperjesi et al., 2002). It could also be used in some tasks to improve contrast sensitivity, selectively absorbing light on the short wavelength end of the visible spectrum, where the rods are most sensitive, and transmitting light on the long end of the spectrum (Thomas et al., 2010).

The visual function during speleological activities has not been documented. The aim of this study is to evaluate the lighting conditions and speleologists’ visual performance using optical filters when exposed to the lighting conditions of cave environments. The specific objectives of this study are to (1) evaluate visual function of speleologists who were directly involved in caving and/or are exposed to the cave environment, (2) evaluate lighting conditions for those doing exploration and research in caves, (3) evaluate visual performance in the cave environment, and (4) evaluate visual performance with filters in the cave environment.

**METHODS**

A cross-sectional study was conducted between December 2013 and January 2014. Twenty-three volunteer speleologists participated in the present study. Examination procedures were thoroughly explained and informed consent was obtained prior to participation. Lisbon School of Health Technology (ESTeSL) Ethics Committee has approved the protocol for the research project. This study adhered to the tenets of the Declaration of Helsinki in 1995 (as revised in Edinburgh 2000).

A questionnaire was administered to participants to describe their socio demographic data, previous and current work history, detailed medical and ocular history, current use and type of medication, the use of protective eye wear, lighting conditions, activities performed, accidents, time and length of stay in the cave.

**Visual function tests**

All participants underwent a visual examination conducted by 2 Orthoptists at ESTeSL Clinical Orthoptic Laboratory and in two Portuguese caves lacking of natural lighting. All subjects who normally wore corrective lenses were asked to wear them during vision testing. The first step involved a visual function examination at ESTeSL. Twenty-three volunteer speleologists were submitted to an evaluation of visual acuity, contrast sensitivity, stereovisual, refractive error (auto-refraction), intraocular pressure (tonometry), ocular alignment and near convergence point. The second step involved a visual function examination (visual acuity, contrast sensitivity and stereovisual) in the cave environment. Sixteen speleologists agreed to spend half a day in the cave and evaluations of flashlight levels were also undertaken. In this step seven subjects were excluded from the study because they were not available during the study period.

Two organic filters (450 nm and 550 nm) were used to measure and compare visual function (distance visual acuity, near visual acuity and contrast sensitivity) with and without filters in the cave environment.

The visual acuity and contrast sensitivity tests were administered monocularly to each eye in the lab (an eye occluder was held over one eye while the other eye was tested) to identify visual impairment and binocularly in the cave environment to evaluate functional vision.

**Distance visual acuity** (VA) was assessed in mesopic conditions in the lab at a distance of 2.5 m with an Early Treatment Diabetic Retinopathy Study (ETDRS) CSV 1000 Vector Vision Chart. Visual acuity was recorded as the last line on which at least 3 of the 5 letters were identified correctly. Pinhole acuity was assessed in eyes presenting VA higher than 0.1 LogMAR.

**Near visual acuity** was assessed in photopic conditions at a distance of 40 cm with an ETDRS Good-Lite chart. Visual acuity was recorded as the last line on which at least 3 of the 5 letters were identified correctly.

**Contrast sensitivity** was assessed in mesopic conditions with the Vector Vision – CSV 1000 E. The test contained a matrix of circles filled with sinusoidal gratings (dark and light bars). Spatial frequency (3, 6, 12, and 18 cycles/degree) increased from top to bottom, and contrast decreased from left to right. The grating bars were oriented vertically. The contrast level of the last test patch correctly identified on each row was recorded as the contrast sensitivity score for that row (log units). The procedure was repeated for each row in descending order. Distance visual acuity of 0.5 LogMAR or better was a criterion to perform this test in order to avoid confounding the results by excessive optical refraction error (Hudnell et al., 2001).

**Ocular alignment** was assessed only in the lab with a cover test (CT) at distance and near (6 m and 33 cm, respectively) to test the presence of heterotropias and heterophorias. The CT was performed with the head held straight and a black paddle occluder as a cover. Detailed fixation objects were used as targets. Manifest strabismus was defined as constant or intermittent tropia of any magnitude at distance or near fixation (Friedman et al., 2009). A prism cover test was employed to assess the magnitude of the deviation present.
Subjects

Filter condition and the two filters (450/550) for each significant differences in performance between the no were analysed using the related-samples Wilcoxon.

in contrast sensitivity of the right and left eyes was accepted as significant. Significant differences in performance between the no filter condition and the two filters (450/550) for each visual task (visual acuity and contrast sensitivity) were analysed using the related-samples Kruskal-Wallis test.

RESULTS

Subjects

The mean age of the speleologists was 40.65 (±10.93) years. The majority of the participants were males (65.2%). The mean number of years of experience in caving was 15.20 ± 11.20 (median = 14.00) years. Speleologists performed this activity during the daytime in approximately 12 days per year. The mean time spent in the cave was 4.30 ± 2.49 hours per day during the daytime. Accidents during this activity were reported by 21.7% of the participants. For the type of activity performed, the developed tasks consisted in: walking (vertical progression in galleries, sub-vertical and horizontal), topography, cartography, bats observation and photography.

Visual function

The majority of the subjects (n = 13) have been observed in the last two years by an ophthalmologist (56.5%), 7 subjects (30.4%) had been observed at 4 years or more and 1 subject never had an ophthalmic observation. Optical correction was found in 14 subjects with 4 subjects using glasses just for near due to presbyopia.

We detected 26.1% (n = 6) participants with visual impairment (decreased visual acuity) of which refractive error (17.4%) was the major cause. Two subjects had a medical past history of a visual pathology, one had a retinal detachment and other had a keratoconus. The majority of the subjects had best uncorrected or corrected visual acuities LogMAR of 0.3 or better. Three subjects had a monocular visual acuity superior to LogMAR 0.3, two because of an uncorrected refractive error (both subjects reach LogMAR 0.2 with a pinhole) and one because of a keratoconus. One subject had also an intra-ocular pressure superior to 20 mmHg. There were no cases of manifest strabismus and the near convergence point was normal in all subjects (<10 cm).

The average values of contrast sensitivity were similar to the population norms (Table 1). There were not significant differences between the contrast sensitivity of the right (RE) and left eyes (LE) for the 3 cpd (p = 0.917), 6 cpd (p = 0.108), 12 cpd (p = 0.503) and 18 cpd (p = 0.634) spatial frequencies.

Binocular visual acuity in the cave environment was -0.05 ± 0.15 LogMAR (20/18) and all subjects had best uncorrected or corrected visual acuities LogMAR of 0.3 or better (Table 2). Only two participants had a reduced binocular visual acuity due to the presence of a refractive error.

All subjects had a normal near visual acuity of 1M. Median value of stereoscopic was of 50° in the lab and of 40° in the cave but the differences were not statistically significant (p = 0.119).

During the cave activity blur vision was the most referred visual symptom (62.5%, n = 10). However, in 3 subjects we found out that these symptoms could be related with the presence of a refractive error and
in 2 subjects we detected a past medical history of retinal detachment and keratoconus. The second most common symptom was visual irritability (43.8%, n = 7). In this group one subject had a refractive error and other previous retinal detachment.

Binocular visual acuity for distance without filter was not statistically different from the visual acuity with the 550 or 450 filters (p = 0.093). Improved contrast sensitivity for the 4 spatial frequencies was observed with the use of 450 nm optical filters, but this difference was only statistically significant for the 6 cpd (p = 0.034) and for the 18 cpd (p = 0.026) spatial frequencies (Table 3). For 3 cpd (p = 0.093) and 12 cpd (p = 0.368) spatial frequencies the differences were not statistically significant. Pairwise comparison for 6 cpd and 18 cpd spatial frequencies did not show significant differences between the three conditions (without filter, with 550 filters and 450 filters). However, speleologists preferred the 450 nm filters (68.8%) when compared with the 550 nm filters (6.3%) or without filter (25.0%).

**Illuminance levels**

All vision tests in the laboratory were administered under artificial lighting mounted at ceiling level (21 fluorescent lamps with diffuser grilles, 18 W each, with a correlated color temperature of 4000 K and color rendering index of 82) with an illuminance ranging from 443 lux to 568 lux. For near vision tests, additional local lighting was used with a total illuminance ranging from 1552 lux on the lower plane to 2390 lux in the upper plane. In the cave environment the tests were administered under artificial lighting with an illuminance extending from 37 lux to 100 lux at 40 cm and at 2.5 m, respectively. Daylight was not available either in the laboratory or in the cave.

In the cave environment the majority of the speleologists used a flashlight on the helmet or head at level 2 (26.1%) without diffuser (47.8%). In this position the mean illuminance values were 451.0 ± 305.7 lux (Table 4).

Some speleologists used the headlamp with diffuser and only one used a hand flashlight, which had a very low value of illuminance (28 lux). The use of the diffuser filter resulted in lower illuminance values for all levels of light intensity (1 to 4) when comparing to headlamps without diffuser.

**DISCUSSION**

In the first part of the study (laboratory evaluation of visual performance) it was found a decreased monocular visual acuity in 6 speleologists, of which refractive error was the major cause. This finding must be analysed with caution, because in visual tasks involving detection of low contrast levels, a degraded visual acuity could have significant impact in terms of performance (Johnson & Casson, 1995). Visual acuity screening for various occupations had been typically performed under near-optimal visual conditions (Johnson & Casson, 1995). Nevertheless, many visual tasks are performed under low luminance or contrast.

We also observed two subjects with eye pathology (retinal detachment and keratoconus). These reported pathologies did not seem to be related with their caving activities. In this study, there were no apparent visual signs or symptoms of visual pathologies related to the exposure to the cave environment. This fact points to different work conditions compared to mines, which have an enormous impact on miners’ health (Ovenseri-Ogbomo et al., 2012). Also, none of the speleologists had previous history of acute or chronic conjunctivitis, which leads the authors to conclude that occupationally associated eye diseases/conditions were not obvious among this group of speleologists.
The present study reports visual acuity and contrast sensitivity of speleologists performing activities in caves. In this environment, the binocular visual performance was not impaired. Binocular visual acuity in the cave was normal in the majority participants as well as binocular contrast sensitivity for all spatial frequencies. Only two subjects had binocular visual acuity of > 0.1 LogMAR. These two subjects were not impaired in their work tasks, because visual acuity was sufficient for their activities (cave guide and bat observation). For example, a night security guard to recognize faces from a distance of 6 m under low illumination needs to have a visual acuity of 0.5 LogMAR (20/60) (Johnson & Casson, 1995). The visibility conditions in the cave differ according to the level of light intensity used by cavers on their head/helmet sources. Binocular contrast sensitivity in the cave was normal for all spatial frequencies. It has been found that under mesopic (i.e., twilight) conditions, sensitivity to the lowest spatial frequencies is the same as that found under normal (photopic) conditions, but under scotopic (i.e., night-time) conditions, sensitivity functions are dramatically lowered across the entire spectrum from the normal contrast sensitivity function (Sekuler & Blake, 1994). However, the findings of this study could be explained because of the light conditions used by cavers. All flashlights had a diffuser filter. However, cavers did not use it because the focused beam has increasingly higher values of illuminance from level 1 to 4.

Visual acuity measurements were made in the cave with the speleologist in a static position and the helmet light set to a medium intensity (451.0 ± 305.7 lux), which was the most commonly used by the cavers. Under this setting, the light conditions were very similar to a photopic environment, thus explaining the good visual performance in the cave. However, in some of the activities, like walking or during bats observation, the

<table>
<thead>
<tr>
<th>Visual function</th>
<th>Mean ± Std. Deviation</th>
<th>Median</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual acuity without filter</td>
<td>-0.05 ± 0.15 (20/18)</td>
<td>-0.10 (20/16)</td>
<td>0.093</td>
</tr>
<tr>
<td>Visual acuity with 550 filter</td>
<td>-0.04 ± 0.16 (20/18)</td>
<td>-0.10 (20/16)</td>
<td></td>
</tr>
<tr>
<td>Visual acuity with 450 filter</td>
<td>-0.07 ± 0.15 (20/17)</td>
<td>-0.10 (20/16)</td>
<td></td>
</tr>
<tr>
<td>Contrast sensitivity (3 cpd) without filter</td>
<td>1.73±0.17</td>
<td>1.63</td>
<td></td>
</tr>
<tr>
<td>Contrast sensitivity (3 cpd) with 550 filter</td>
<td>1.76±0.17</td>
<td>1.78</td>
<td></td>
</tr>
<tr>
<td>Contrast sensitivity (3 cpd) with 450 filter</td>
<td>1.84±0.12</td>
<td>1.78</td>
<td></td>
</tr>
<tr>
<td>Contrast sensitivity (6 cpd) without filter</td>
<td>1.99±0.24</td>
<td>1.92</td>
<td></td>
</tr>
<tr>
<td>Contrast sensitivity (6 cpd) with 550 filter</td>
<td>1.92±0.16</td>
<td>1.84</td>
<td>0.034*</td>
</tr>
<tr>
<td>Contrast sensitivity (6 cpd) with 450 filter</td>
<td>2.04±0.16</td>
<td>2.07</td>
<td></td>
</tr>
<tr>
<td>Contrast sensitivity (12 cpd) without filter</td>
<td>1.56±0.33</td>
<td>1.54</td>
<td>0.368</td>
</tr>
<tr>
<td>Contrast sensitivity (12 cpd) with 550 filter</td>
<td>1.58±0.37</td>
<td>1.62</td>
<td></td>
</tr>
<tr>
<td>Contrast sensitivity (12 cpd) with 450 filter</td>
<td>1.64±0.39</td>
<td>1.69</td>
<td></td>
</tr>
<tr>
<td>Contrast sensitivity (18 cpd) without filter</td>
<td>1.05±0.34</td>
<td>1.03</td>
<td>0.026*</td>
</tr>
<tr>
<td>Contrast sensitivity (18 cpd) with 550 filter</td>
<td>0.97±0.25</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>Contrast sensitivity (18 cpd) with 450 filter</td>
<td>1.18±0.39</td>
<td>1.25</td>
<td></td>
</tr>
</tbody>
</table>

Visual acuities are reported in LogMAR units with the Snellen equivalent of the mean in parenthesis. Contrast sensitivity values are reported in log units.

*Significant difference (p<0.05).

<table>
<thead>
<tr>
<th>Flashlight in level 1 without diffuser (n=15)</th>
<th>Flashlight in level 2 without diffuser (n=14)</th>
<th>Flashlight in level 3 without diffuser (n=11)</th>
<th>Flashlight in level 4 without diffuser (n=7)</th>
<th>Flashlight in level 1 with diffuser (n=3)</th>
<th>Flashlight in level 2 with diffuser (n=2)</th>
<th>Hand flashlight (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>250.2</td>
<td>451.0</td>
<td>1551.3</td>
<td>1670.2</td>
<td>134.0</td>
<td>210.0</td>
</tr>
<tr>
<td>Median</td>
<td>100.0</td>
<td>400.0</td>
<td>840.0</td>
<td>2000.0</td>
<td>170.0</td>
<td>210.0</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>414.3</td>
<td>305.7</td>
<td>1165.9</td>
<td>1275.4</td>
<td>108.6</td>
<td>127.3</td>
</tr>
<tr>
<td>Minimum</td>
<td>16.8</td>
<td>50.0</td>
<td>365.0</td>
<td>92.0</td>
<td>12.0</td>
<td>120.0</td>
</tr>
<tr>
<td>Maximum</td>
<td>1600.0</td>
<td>1300.0</td>
<td>3000.0</td>
<td>4000.0</td>
<td>220.0</td>
<td>300.0</td>
</tr>
</tbody>
</table>
light conditions could be very low and similar to the scotopic environment.

The use of optical filters did not decrease visual acuity. The improvement in contrast sensitivity with the 450 nm filters could be beneficial to the cavers’ activities. It is important that eye care practitioners are able to provide accurate advice on whether filters will provide a long-term benefit, prior to their recommendation to cavers.

Good lighting includes quantity and quality requirements, and should necessarily be appropriate to the activity/task being carried out, bearing in mind the comfort and visual efficiency of the worker (Piccoli et al., 2004; Pais & Melo, 2011). According to the Artificial Light norm (DIN 5035-2, 1990), the level of illuminance that should be used for normal visual tasks with medium details is 500-750 lux, which is in accordance with most of the tasks performed by the cavers. For tasks with slight visual requirements and high contrast, 120-250 lux is required (analogly with the referred example of the mines) while for normal visual tasks with medium details the requirement is 500-750 lux. Visual demanding tasks with small details need illuminance values of 1000 to 1500 lux and very demanding with very small details visual tasks need levels of 2000-3000 lux (DIN 5035-2, 1990).

In this study, only 7 cavers had normalized illuminance values for the activities/task performed in the cave (headlamp without diffuser with 4 levels of intensity). Some of the cavers need to acquire appropriate equipment with adjustable lighting settings that are suitable for different activities, mainly for visual tasks with medium details.

CONCLUSIONS

This study was able to quantify the visual performance of speleologists in their natural environment of activity, the cave. There were no signs and symptoms of visual pathologies related to the cave low light exposure on these speleologists. Also, none of the cavers had previous history of acute or chronic conjunctivitis, which leads us to conclude that occupational associated eye diseases and disorders were not present among the speleologists. Most of the visual symptoms referred by the participants during the cave activity seemed to be related with the presence of a refractive error or a previous diagnosed ophthalmic pathology.

In the cave environment, binocular visual performance was not impaired by the existent lighting conditions (flashlights). The illuminance levels for the cavers who used the intensity light level 2 (without diffuser) on their headlamps were adequate to the majority of the activities/tasks performed.

The enhancement in contrast sensitivity when using the 450 nm filters could be beneficial to cavers or other researchers to potentially improve activities in the cave. Filters could be correlated with operational use after being tested in a larger sample and applied to different cave activities. Although this was an exploratory study, it is important to recognize the potential effects of using optical filters in activities that require medium to high detail observations (e.g., bat or mineral research). Further research is therefore needed to better understand the influence of lighting conditions in the visual symptoms for those carrying activities in caves. It is also important to observe the improvement in contrast sensitivity, when cavers or researchers spend longer time in the cave.

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REFERENCES


DiN 5035-2, 1990 – Artificial lighting, lighting of work places.


Genetic analyses determine connectivity among cave and surface populations of the Jamaican endemic freshwater crab Sesarma fossarum in the Cockpit Country

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Abstract: The Jamaican freshwater crab Sesarma fossarum (Decapoda: Brachyura: Sesarmidae) is endemic to western central Jamaica where it occurs in cave and surface streams of karst regions. In the present study, we examine the population genetic structure of the species, providing evidence for intraspecific differentiation and genetic substructure among twelve sampled populations. Interestingly, crabs from caves appear genetically undistinguishable from representatives of nearby surface waters, despite previously observed and described morphometric differentiation. In contrast, genetic isolation takes place among populations from rivers and caves belonging to different watersheds. In one case, even populations from different tributaries of the same river were characterized by different genotypes. Overall, the species shows low haplotype and nucleotide diversities, which indicates a high homogeneity and point towards a relatively recent intraspecific radiation and diversification. Our results on the genetic diversification of S. fossarum helps to reconstruct unknown subterranean water flow and cave connections in its native range, allowing prediction of its further dispersal and differentiation potential. Unfortunately, its natural habitat of Jamaican cockpit karst, which also is home to several other endemic species and is a globally-recognized Key Biodiversity Area, is under imminent threat of intensive bauxite mining.

Keywords: endemism; biodiversity; troglophile; conservation; karst

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INTRODUCTION

The Cockpit Country in western central Jamaica is a typical example of polygonal karst (Sweeting, 1958; Fincham, 1997; Chenoweth et al., 2001). This special environment provides enormous habitat richness and is the key for the very high biodiversity that can be found associated to it (Hamilton-Smith, 2001; Clements et al., 2006; Anadon-Irizary, 2012), also because of its complex freshwater flux. This system is composed of many sinkholes in the geometric centre of the karst hills, which connect surface runoff with mostly subterranean rivers that emerge along the periphery of the Cockpit Country (Fincham, 1997). In many instances, it is not obvious to where the subterranean water drains, because accessibility is often limited. This unique ecosystem is under constant change, due to the relatively easy erosion of the limestone, which may result in the change of drainage systems and subterranean connections (White, 2002).

Here we investigate a freshwater species endemic to the Cockpit Country and give evidence about its genetic isolation patterns. We studied genetic variation within the trogophilic freshwater crab Sesarma fossarum (Schubart et al., 1997) showing that there is a marked trend for local endemism due to restricted gene flow throughout its distribution range. The studied species thrives in mountain creeks and small rivers, but also occurs subterraneously in caves along the western slopes of the Cockpit Country and in adjacent lowlands (Schubart et al., 1997). It belongs to the crab family Sesarmidae that experienced a marked adaptive radiation within the island’s freshwaters, caves and rainforests (Schubart et al., 1998). Similar radiations were found for Jamaican lizards and frogs (Hedges, 1996; Butler et al., 2007; Losos, 2010). As these crabs depend on freshwater rivers and streams, water divides must present a serious biogeographic barrier for dispersal (Reimer & Schubart, 1998). As Jamaica has often changed...
Tissue extraction, PCR and sequencing

DNA extraction and isolation was carried out following a modified Puregene DNA Extraction Method (Gentra Systems: Minneapolis, MN55447, USA). Genomic DNA was stored in 20μl TE buffer [10 mM Tris/HCL, 1 mM EDTA (pH 8.0)] at -20°C. Cytochrome oxidase subunit I (Cox1) fragments of 661 to 790 base pairs (bp) length (including the entire “Palumbi region”) were amplified by means of PCR-reaction (25μl total volume) with the reverse primer COH1b (5’-TGTATARGCRTCTGGRTARTC-3’ and as forward primer either COL8 (5’GAYCAAATACCTTTATTTGT-3’, overall 790 basepairs (bp), including the 3’ end of the “Folmer region”) or COL1b (5’-CCWGCTGGDGGWGGDGAYCC-3’ for a shorter fragment of 661 bp) (Schubart, 2009). This mitochondrial gene is relatively variable and is commonly used for population genetics, and more recently also for animal species identification within the barcoding approach (Hebert et al., 2003). PCR parameters consisted of denaturing at 95°C for 15 s, annealing at 51°C for 30 s, and elongation at 72°C for 1 min for 40 cycles, with a final elongation of 10min. Sequencing was mainly outsourced to GATC Biotech AG, Konstanz, Germany or run on a ABI PrismTM 310 Genetic Analyzer (Applied Biosystems) at the University of Regensburg.

Computational analysis

Sequences were aligned with MEGA 5.0 (http://www.megasoftware.net/) and up to 745 bp per specimen were used for computational analysis. In order to graphically depict the genetic distance between the mitochondrial genotypes, a haplotype network was computed with TCS 1.21 (http://darwin.uvigo.es/software/tcs.html) (calculated maximum connection steps at 95%). Haplotype and nucleotide diversities were calculated with DnaSP v5 (http://www.ub.es/geomorphological shape and thus hydrology during its history, “speciation could have occurred frequently among closely related populations” (Schubart et al., 1997: p. 417). The outcome of these events can be seen in the high current diversity of the endemic Sesarmidae on Jamaica (Schubart et al., 2010; Schubart & Santl, 2014). Along with geographic separation, additional ecological specialization may have led to cladogenesis. Schubart et al. (1998: p. 364) suggested “that two or more radiations took place simultaneously in distinct geographic regions of Jamaica” and as evolution is in constant flux, there may be some evidence for an ongoing adaptive radiation among the species of the genus Sesarma.

In this study we examine if there is genetic differentiation and ongoing geographic differentiation in Sesarma fossarum. Furthermore, we use genetic data from several cave populations throughout the distribution range of S. fossarum to infer unknown subterranean water flow in the sampling area and predict the connectivity of S. fossarum throughout the Cockpit Country. The corresponding results should help to understand whether cave individuals are more closely related to each other than they are to surface individuals and thus if geographic isolation, or isolation by distance, is a greater contributor to population structure than habitat.

METHODS

Specimen collection

Between 1994 and 2009, 77 specimens of Sesarma fossarum were collected from twelve sampling sites (Fig. 1). Whenever possible, a minimum of ten specimens per population was gathered for statistical analyses (Table 1). Animals were killed on ice or by freezing and then preserved in 95% ethanol.

Fig. 1. Sampling sites of S. fossarum in western Jamaica (Cockpit Country). Yellow circles represent the locations of the different sampling sites of the Jamaican freshwater crabs. The hydrological map represents rivers and creeks as well as the up to date known watersheds (WRAMIS, Hydrologic Database).
RESULTS

The Cox1 gene fragment was amplified and sequenced from each of the collected animals. The individual sequences were aligned and did not include any gaps, and translation into amino-acid sequences did not reveal any stop codons so that no potential co-amplification of pseudogenes could be detected. In total, nine polymorphic sites were identified (five at position-1 of the open reading frame and four at position-3. Three of the former mutations lead to changes of amino-acids. The corresponding haplotype network was computed in order to visualize the genetic differences. Single mismatches in the alignment are depicted as connecting lines between groups of animals with the same haplotype. The haplotype network revealed ten different haplotypes based on nine polymorphic sites among the 77 specimens of the Jamaican endemic freshwater crab *Sesarma fossarum* (Fig. 2). The haplotypes were separated by a maximum of five mutational steps. Haplotype 1 is defined as the most common in the dataset and was found in 37 specimens from four populations, including all Lottery (10) and Jamaran Cave (9) individuals, as well as nine out of ten samples from both Jackson Young Cave and Lemy River. Haplotype 4 is formed by nine of the ten Windsor animals and haplotype 2 consists of all ten samples from Hastings and the only Spring Vale specimen. Five animals of the Great River population belong to haplotype 7 that is also found in all four Ginger Hill and one of the Peterkin Cave specimens. Another Great River animal carried haplotype 9, which was shared with three Maggotty samples and the other available Peterkin Cave animal. Private haplotypes are found in one animal from Great River (haplotype 8), Lemy River (haplotype 6), Windsor (haplotype 5), and Jackson Young Cave (haplotype 3), all in close connection (one basepair difference) to the corresponding common haplotype of the respective population. The only animal from Flamstead Rise Cave does not share any other haplotype and has its own (haplotype 10).

In order to quantify genetic differences within the individual populations of *S. fossarum*, nucleotide and haplotype diversities were calculated. Overall, high genetic homogeneity can be recognized in most populations: not more than three haplotypes were found per sampling site, and most populations consisted of a single haplotype (Table 2). The populations Peterkin Cave (h = 1, N = 2) and Great River (h = 0.52, N = 7) seem to be characterized by high haplotype diversities, but the respective sample sizes, especially of Peterkin Cave, are too low.

Based on haplotype network and the nucleotide and haplotype diversities, *Φst*-values and the respective *p*-values were calculated (Table 3). The *Φst*-values are an indirect measure for the degree of gene flow between two different populations and the *p*-value expresses the significance of the corresponding data. The higher the *Φst*-value (up to 1), the lower is potential gene flow between two populations and thus the higher genetic isolation. The populations of Maggotty, Peterkin Cave,
Flamstead Rise Cave and Spring Vale were excluded from the analyses, due to small sample sizes. The headwaters of Great River and Ginger Hill are in close geographic vicinity (Fig. 1) and the respective samples mostly shared haplotype 7 (Fig. 2). The samples were thus pooled for the AMOVA to obtain a sample size exceeding 10. The significance level was defined at a p-value smaller than 0.05. The Windsor population reveals a strong lack of gene flow to all the other populations. The same was observed for Hastings and the pooled Great River/Ginger Hill populations. In contrast, Jackson Young Cave, Lemy River, Lottery and Jarman Cave populations are not restricted in gene flow among each other. In order to address gene flow between the northern and southern populations, we pooled the respective populations (south: Great River, Ginger Hill and Maggotty; north: remaining populations without Peterkin Cave, Flamstead Rise Cave and Spring Vale). As already recognizable in the phylogenetic tree (Fig. S1) the southern populations seem to be separated from the northern group, although it is not statistically significant (Table 3).

Although there is genetic structuring in the studied species, there are not more than five basepair mutations between the most distant Cox1 haplotypes (Fig. 2), which is also reflected in the low nucleotide and haplotype diversities within the populations and in the total counts (Table 2). This indicates a high homogeneity among the populations and strengthens the evidence that the lineage of the Sesarmidae on Jamaica is relatively young, diversifying into the current species not earlier than 3.5 Myr ago, and the separations of the sister species Sesarma dolphinum versus Sesarma abeokuta, and of their ancestor versus Sesarma fossarum is probably much younger (Schubart et al., 1998; Schubart et al., 2010; Schubart & Santl, 2014). A phylogenetic tree of the sampled populations of S. fossarum (see Fig. S1 in the supplementary material) and the AMOVA (Table 3) indicated a separation of the “northern” to the “southern” populations, the latter including Great River, Ginger Hill and Maggotty. Reimer et al. (1998) suggested that changing watersheds could also be responsible for the isolation of a population. It seems to be that there is at least one water divide, which maintains the isolation of the sampled populations. As S. fossarum is restricted to rivers, it is strongly dependent on water and its immediate surroundings. Taking into account the results from the haplotype network (Fig. 2) and the AMOVA (Table 3), the restriction of gene flow between the sampled populations can be plotted on a map. Hence, one hypothetical water divide can be drawn somewhere between Jarman Cave and Great River (Table 3, Fig. 3). Interestingly, the studied cave populations do not differ genetically from nearby surface populations, despite the observed morphometric differences (Stemmer & Schubart, 2013). The Hastings population obviously drains over the Roaring River to the east populations lie and to predict their connectivity. This way, we can infer subterranean water flow from the presented data.

Table 2. Haplotype (h) and nucleotide (Π) diversities for each sampled population of the Jamaican freshwater crab Sesarma fossarum. The 77 collected specimens are distributed over 10 distinct haplotypes.

<table>
<thead>
<tr>
<th>Population</th>
<th>Specimens</th>
<th>Haplotypes</th>
<th>h</th>
<th>Π</th>
</tr>
</thead>
<tbody>
<tr>
<td>Windsor</td>
<td>10</td>
<td>2</td>
<td>0.20</td>
<td>0.00027</td>
</tr>
<tr>
<td>Hastings</td>
<td>10</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Jackson Young Cave</td>
<td>10</td>
<td>2</td>
<td>0.20</td>
<td>0.00027</td>
</tr>
<tr>
<td>Lemy River</td>
<td>10</td>
<td>2</td>
<td>0.20</td>
<td>0.00027</td>
</tr>
<tr>
<td>Lottery</td>
<td>10</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Jarman Cave</td>
<td>9</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Great River</td>
<td>7</td>
<td>3</td>
<td>0.52</td>
<td>0.00077</td>
</tr>
<tr>
<td>Ginger Hill</td>
<td>4</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Maggotty</td>
<td>3</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Peterkin Cave</td>
<td>2</td>
<td>2</td>
<td>1.00</td>
<td>0.00134</td>
</tr>
<tr>
<td>Flamstead Rise Cave</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spring Vale</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>(10)</td>
<td>0.72</td>
<td>0.00197</td>
</tr>
</tbody>
</table>

DISCUSSION

The present study provides new evidence for subterranean connections among caves in western Jamaica by means of genetic similarities in the troglobophilic freshwater crab S. fossarum. This species can be subdivided into several populations, with complex interconnections. Relating our new data from population genetics to a hydrological map of the native range of S. fossarum allows to discern where the boundaries between the sampled
and shares the drainage system with the Windsor population, but nevertheless the two populations surprisingly do not share haplotypes and thus lack gene flow between them (Table 3, Fig. 3). Spring Vale belongs to the Hastings population according to the haplotype network (Fig. 2). This was already assumed (Fincham, 1997) because water from this cave was predicted to exit as the spring of the Roaring River in Hastings. The Φst-values reveal that the populations of Jarman Cave, Jackson Young Cave, Lemy River and Lottery are not restricted in gene flow and as they show no gene flow to all the other populations, it can be proposed that the streams from which these animals were collected drain towards the north into the Montego River. The Great River/Ginger Hill group is located in the southwest and is restricted in gene flow to all other populations. These two locations are geographically very close to each other, but belong to two different rivers (Fig. 3). The current data suggest that Ginger Hill and Great River animals are genetically identical (Fig. 2) and were therefore pooled for the AMOVA analyses. An explanation for this observation could be that the headwaters of both rivers are in close vicinity or were even connected in the past. The Maggotty population has probably no gene flow to any other population (Fig. 2), but this was not tested with AMOVA, due to the small sample size. This observation appears obvious as this population is also geographically separated from the rest and the corresponding stream drains from the northeast into the Black River (Fig. 3). Nevertheless, the haplotype network shows that Maggotty and one Great River specimens share the same haplotype, suggesting another recent connection or translocation. Furthermore, the relation of Spring Vale, Flamstead Rise Cave and Peterkin Cave to the others can only be guessed, but more material would provide some interesting additional information about the substructure and the water divides in this region. Furthermore, additional genetic markers need to be included in order to fully resolve the isolation patterns of *S. fossarum*.

Overall, it appears that the underwater system is more complex than thought (Fincham, 1997), connecting some parts of the area underground. Hence, destruction of one side of that ecosystem can lead to the loss of unique evolutionary units and/or influence the connected sites as well (Beckford & Bailey, 2009). Unfortunately, the Cockpit Country, which was shaped over millions of years, is attracting commercial interests and foreign investors, especially for bauxite mining that has already destroyed some parts of it (Chenoweth et al., 2001; Day & Chenoweth, 2009). Moreover, an expansion of the mining area is planned deeper into the Cockpit Country that within a few decades could provoke the disappearance of endemic species or populations, associated with this habitat (Watson et al., 1997; Hamilton-Smith, 2001). For example, the populations in the south (Great River, Ginger Hill and Maggotty) could already be affected by these mining operations, which have the potential to ultimately change watersheds and hence the dispersal of water-dependent species in this area.

### Table 3. Gene flow between sampling sites. Given are Φst-values and their corresponding p-values of the Jamaican freshwater crab *Sesarma fossarum* of all sampling sites compared with each other; +: p<0.001 (very significant). Southern sampling sites include Great River, Ginger Hill, and Maggotty.

<table>
<thead>
<tr>
<th>p-value</th>
<th>WIN</th>
<th>HAS</th>
<th>JYC</th>
<th>LEM</th>
<th>LOT</th>
<th>JAR</th>
<th>GRE/GIN</th>
<th>N</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Φst-value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Windsor</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hastings</td>
<td>0.9091</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jackson Young Cave</td>
<td>0.8333</td>
<td>0.9524</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lemy River</td>
<td>0.8333</td>
<td>0.9524</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lottery</td>
<td>0.9091</td>
<td>1.0000</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jarman Cave</td>
<td>0.9042</td>
<td>1.0000</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Great River/Ginger Hill</td>
<td>0.8640</td>
<td>0.9364</td>
<td>0.9078</td>
<td>0.9078</td>
<td>0.9364</td>
<td>0.9330</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>South</td>
<td>0.7775</td>
<td>0.8784</td>
<td>0.8538</td>
<td>0.8538</td>
<td>0.8784</td>
<td>0.8732</td>
<td>0.6660</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 3.** Genetic isolation among *S. fossarum* populations, shown on a hydrological map of western Jamaica (Cockpit Country). Black bars represent the hypothesized water divides isolating populations of freshwater crabs based on the current results. The red arrows show the postulated direction of dispersal of these populations, following the big river systems (blue): 1) Windsor; 2) Hastings; 3) Jackson Young Cave; 4) Lemy River (referred to Leme in Stemmer & Schubart 2013); 5) Lottery; 6) Jarman Cave; 7) Great River; 8) Ginger Hill; 9) Maggotty; 10) Peterkin Cave; 11) Flamstead Rise Cave; 12) Spring Vale.
CONCLUSIONS

The geological history and karst structure of the Cockpit Country are major factors for the complex structuring of the species Sesarma fossarum and probably also for many other species. The results show that S. fossarum has a relatively high intraspecific diversity and geographic substructure similar to Sesarma dolphiniun (see Schubart et al., 2010; Schubart & Santi, 2014) and that this is probably a general feature of the adaptive radiation of the Sesarmidae on Jamaica and seems to differ from freshwater crabs in the other Caribbean islands (Schubart et al., 2011). The endemic species of Jamaica are excellent models to show how evolution is driving diversification within a geomorphologically diverse environment and will allow even more insights in future studies.

The previously identified watersheds (WRAMIS) are clearly oversimplified and most probably do not represent the actual drainage system and may need to be revised. The here presented study can serve as an example about how population genetics can be utilised to refine hydrological and topographic maps. In order to do so, more samples need to be collected, more sites need to be taken into account and additional genetic markers need to be incorporated. In the future, population genetic data from several species could be combined in order to get an even more accurate map of ecological and geological interconnections.

ACKNOWLEDGEMENTS

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REFERENCES


Beckford C.L. & Bailey S.W., 2009 – Vulnerability, constraints and survival on small-scale food farms in St Elizabeth, Jamaica: Strengthening local food production systems. Global Change and Caribbean Vulnerability: Environment, economy and society at risk, 218-236


Connectivity of populations of *S. fossarum* in the Cockpit Country


White W.B., 2002 – *Karst hydrology: Recent developments and open questions*. Engineering Geology, **65**: 85-105. [http://dx.doi.org/10.1016/S0013-7952(01)00116-8](http://dx.doi.org/10.1016/S0013-7952(01)00116-8)
INTRODUCTION

The role of the microbiome in immunity and disease progression has only recently been recognized, and has mainly focused on bacteria (Cui et al., 2013; Huffnagle & Noverr, 2013; Lauer & Hernandez, 2015). White-nose syndrome (WNS) is a fungal disease of hibernating bats that has rapidly spread through the eastern United States and Canada, killing > 6.7 million bats since it was first reported in 2006 in Albany, New York (U.S. Fish and Wildlife Service, 2012). The fungus that causes WNS, *Pseudogymnoascus destructans* (*Pd*), is also found in Europe, but for reasons that are unclear, does not cause significant mortality or morbidity of European bats (Wibbelt et al., 2010; Lorch et al., 2011; Puechmaille et al., 2011). Hypotheses for this include behavioral or physiological adaptations in European bats, or environmental differences between Europe and North America (Wibbelt et al., 2010). European strains of *Pd* are lethal to North American bats, demonstrating that the presence of different strains of *Pd* does not explain the lack of bat mortality in Europe (Warnecke et al., 2012). Whether *Pd* interacts with fungi and bacteria naturally occurring on bats, or whether the microbiome on bats or in hibernacula differs between North America and Europe, is unknown. The few studies on mycobiota present on hibernating bats have shown the composition of fungal taxa is...
similar between Europe and North America, although sample sizes are generally small and differences in methodology make comparison difficult (Larcher et al., 2003; Voyron et al., 2011; Johnson et al., 2013; Vanderwolf et al., 2013a).

In the United States, Johnson et al. (2013) sampled both Pd-positive and Pd-negative bats and found that the fungal diversity on the former was much lower than on the latter. This led these authors to hypothesize that Pd was responsible for suppressing the normal fungal diversity present on hibernating bats. Here we address this hypothesis by drawing on relatively large datasets describing fungal diversity on pre- and post-WNS Myotis spp. bats in eastern Canada.

**METHODS**

The principal source for our pre-WNS information concerning fungal diversity on bats is a 2010 dataset consisting of 117 fungal species isolated from the external surface of apparently healthy hibernating bats (Myotis lucifugus LeConte 1831 and M. septentrionalis Trouessart 1897) in eight caves and mines in New Brunswick, Canada (Vanderwolf et al., 2013a). No Pd was cultured in 2010 and visible signs of WNS (fungal growth on bats and behavioral changes of bats) were not seen in the province until March 2011. We replicated our 2010 study during the winter of 2012 in some of the same caves where bats showed obvious signs of WNS (i.e. visible growth of Pd on exposed skin), with Pd presence confirmed by histology and qPCR at the Canadian Wildlife Health Cooperative. To gain an understanding of inter-year variation in fungal assemblages on bats at specific sites, we re-sampled hibernacula in 2012 that were sampled in 2010 and where WNS presence remained unconfirmed (i.e. no visible evidence of WNS-infected bats in 2012). However, we acknowledge that lack of visible WNS does not necessarily equate with lack of Pd presence (Verant et al., 2014). In an effort to track the source of some of the fungal spores on live hibernating bats we also cultured fungi from bat carcasses remaining in one of our study sites (Berriton Cave) 12-24 months following initial WNS mortality and compared this to fungal assemblages on live bats.

Of the 8 New Brunswick hibernacula sampled in 2010, 5 were re-sampled in 2012. In one excluded hibernaculum the bat population had decreased to < 10 bats due to WNS infection, below our prescribed sample size, while in another bats had changed roosting positions so as to no longer be accessible to us. The 5 hibernacula sampled included 3 limestone caves (one with WNS-positive bats), one gypsum cave (with WNS-positive bats), and one long-abandoned manganese mine (with WNS-positive bats). Data on physical characteristics of study sites can be found in Vanderwolf et al. (2012). We followed the protocol of the United States Fish and Wildlife Service (2009) for minimizing the spread of WNS during all visits to caves.

**Field Sampling**

During February - March 2012, swabs (n = 202) were taken from the skin and dorsal fur of live Myotis lucifugus (n = 34) and M. septentrionalis (n = 16; 10 bats/cave, 50 bats total) with a sterile, dry, cotton-tipped applicator. ‘Skin’ includes the face, ears, patagium, and/or uropatagium, depending on which skin surfaces were accessible to us. Bats were swabbed while roosting and were not removed from the walls, which precluded determining sex. In Pd-positive hibernacula, only Myotis spp. with visible Pd growth were sampled. Methods were identical to Vanderwolf et al. (2013a). Swabs were cultured on either dextrose-peptone-yeast extract (DPYA) agar or Sabouraud-Dextrose (SAB) agar, both of which contained the antibiotics chlorotetracycline (30 mg/liter) and streptomycin (30 mg/liter). Four swabs were taken per bat using all combinations of fur or skin on either SAB or DPYA. A new applicator was used for each swab. After swabbing, the applicator was immediately streaked across an agar surface in a petri plate. Diluting streaks were completed in the hibernaculum within 3 h of the initial streak, after which plates were sealed in situ with parafilm (Pechiney Plastic Packaging, Chicago, IL).

In 2012 three dead M. lucifugus heavily colonized with fungal hyphae were collected from Berriton Cave and samples of hyphae cultured on DPYA. In 2013 fungal hyphae were removed from a dead M. lucifugus in situ in Berriton Cave using sterile technique and placed on 2 DPYA plates.

**Laboratory Processing**

All plates were incubated, inverted in the dark at 7°C, and monitored over 4 months. Pure cultures of each distinct colony were maintained on DPYA without oxgall and sodium propionate. Identifications were carried out by comparing the micro- and macromorphological characteristics of the microfungi to the taxonomic literature and compendia. We had access to reference collections of cultures from Myotis spp. collected in 2010 and identified previously using a mix of sequencing and morphological features (Vanderwolf et al., 2013a). Some isolates were sent to taxonomic specialists for confirmation of identification, usually through a combination of morphological and molecular genetic techniques. Identifications of Pd were confirmed by sequencing as part of another study (Khankhet et al., 2014). Permanent cultures are housed in the University of Alberta Microfungus Collection and Herbarium (UAMH 11721, 11723-11725, 11732-11734, 11813, 11815-11817) and desiccant-dried samples are vouchered in the New Brunswick Museum (NBMM# F-04812-04823, 04840, 04844-04870, 04883-04915, 04942-04948).

**Statistical Analysis**

The number of fungal taxa per bat in 2012 was not normally distributed, even with transformation. Therefore a Kruskal-Wallis test was used to determine if the number of fungal taxa per bat differed between hibernacula in 2012. A Mann-Whitney test was used to determine if the number of fungal taxa per bat in 2012 differed between M. lucifugus and M. septentrionalis and Pd-positive versus Pd-negative Myotis spp. When comparing 2012 results to the 2010 study, only data from the United States Fish and Wildlife Service (2009) for Vanderwolf et al. (2013a). Swabs were cultured on either dextrose-peptone-yeast extract (DPYA) agar or Sabouraud-Dextrose (SAB) agar, both of which contained the antibiotics chlorotetracycline (30 mg/liter) and streptomycin (30 mg/liter). Four swabs were taken per bat using all combinations of fur or skin on either SAB or DPYA. A new applicator was used for each swab. After swabbing, the applicator was immediately streaked across an agar surface in a petri plate. Diluting streaks were completed in the hibernaculum within 3 h of the initial streak, after which plates were sealed in situ with parafilm (Pechiney Plastic Packaging, Chicago, IL).

In 2012 three dead M. lucifugus heavily colonized with fungal hyphae were collected from Berriton Cave and samples of hyphae cultured on DPYA. In 2013 fungal hyphae were removed from a dead M. lucifugus in situ in Berriton Cave using sterile technique and placed on 2 DPYA plates.

**Statistical Analysis**

The number of fungal taxa per bat in 2012 was not normally distributed, even with transformation. Therefore a Kruskal-Wallis test was used to determine if the number of fungal taxa per bat differed between hibernacula in 2012. A Mann-Whitney test was used to determine if the number of fungal taxa per bat in 2012 differed between M. lucifugus and M. septentrionalis and Pd-positive versus Pd-negative Myotis spp. When comparing 2012 results to the 2010 study, only data from
the 5 hibernacula sampled in both years were used. A Wilcoxon signed ranks test was used to determine if the number of fungal taxa per bat among the hibernacula in 2012 was significantly different from that in 2010. The average number of fungal taxa isolated from each hibernaculum was normally distributed and therefore a paired t-test was used to compare 2012 data to 2010. Minitab Statistical Software (https://www.minitab.com/en-us) was used for all tests.

RESULTS

Post-WNS Fungal Diversity

Fungi were successfully cultured from all 50 bats sampled in 2012 and from 187 of 202 (92.6%) swabs collected, producing a total of 885 isolates. Swabs that did not produce fungi generally produced unidentified bacteria and yeast, which were also present on some swabs with fungi. A mean of 8.2 ± 4.3 SD fungal taxa (range: 2-14) were isolated from each bat (Table 1). Berryton Cave was notable for its high mean number of fungal taxa per bat compared to other sites we examined. The number of fungal taxa per bat was significantly different among hibernacula (Table 1), both when including Berryton (H₂,₅₀ = 20.82, P<0.00) and excluding Berryton (H₃,₄₀ = 8.66, P = 0.03). The number of fungal taxa per bat was also significantly higher on *M. lucifugus* vs. *M. septentrionalis* (W₂,₅₀ = 1016.5, p = 0.008) with a mean of 9.3 ± 4.1 SD fungal taxa per *M. lucifugus* (n = 34) and 6.0 ± 4.0 SD per *M. septentrionalis* (n = 16).

Eighty fungal taxa in 56 genera were isolated in 2012 (Table 2). Thirty-seven (46.3%) of the 80 identified taxa were found on a single bat only. The fungal taxa most commonly isolated were: *Pseudogymnoascus pannorum* sensu lato (68.6% of bats), *Pd* (66.7%), *Mucor* spp. (52.9%), *Mortierella* spp. (49.0%), *Penicillium* spp. (45.1%), *Leuconoeurospora* spp. (33.3%), *Oidiodendron truncatum* (33.3%), *Cladosporium* spp. (25.5%), *Humicola* cf. *UAMH 11595* sp. (23.5%), and *Cephalotrichum stemonitis* (21.6%) (Table 2). Some fungal taxa were isolated more frequently using *DPYA* than SAB, such as *Arachniotus* spp. (10 positive *DPYA* plates, 0 SAB), *Chrysosporium* spp. (18 *DPYA*, 4 SAB), and *Microascus* spp. (33 *DPYA*, 13 SAB), while *Mortierella* spp. were isolated more frequently on SAB (37 SAB, 1 *DPYA*). Excluding sterile cultures, 51 fungal taxa were isolated using SAB (n = 102 plates), 59 using *DPYA* (n = 100), 60 from fur (n = 102), and 59 from skin (n = 100).

<table>
<thead>
<tr>
<th>2010</th>
<th>2012</th>
<th># of <em>M. lucifugus</em> sampled 2012</th>
<th># of <em>M. septentrionalis</em> sampled 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berryton Cave</td>
<td>6.5 ± 1.9</td>
<td>13.1 ± 2.6</td>
<td>10</td>
</tr>
<tr>
<td>Harbell’s Cave</td>
<td>6.1 ± 1.7</td>
<td>4.7 ± 3.9</td>
<td>0</td>
</tr>
<tr>
<td>Howes Cave</td>
<td>6.8 ± 2.5</td>
<td>7.0 ± 3.0</td>
<td>5</td>
</tr>
<tr>
<td>Markhamville Mine</td>
<td>8.5 ± 1.7</td>
<td>9.1 ± 3.6</td>
<td>9</td>
</tr>
<tr>
<td>White Cave</td>
<td>7.7 ± 3.8</td>
<td>7.4 ± 3.8</td>
<td>10</td>
</tr>
<tr>
<td>Overall</td>
<td>7.1 ± 2.6</td>
<td>8.2 ± 4.3</td>
<td>34</td>
</tr>
</tbody>
</table>

Table 2. Identification and frequency of fungal taxa isolated in 2012 from the skin and fur of hibernating bats (L = *M. lucifugus*, n = 34; S = *M. septentrionalis*, n = 16) in 5 hibernacula in New Brunswick, Canada. Taxa marked with an asterisk (*) were not isolated in 2010.
<table>
<thead>
<tr>
<th>Species</th>
<th>L</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Cladorrhinum sp.</td>
<td>2</td>
<td>2 L</td>
</tr>
<tr>
<td>Cladosporium sp.</td>
<td>5</td>
<td>8 L, 5 S</td>
</tr>
<tr>
<td>*Clonostachys sp.</td>
<td>1</td>
<td>1 S</td>
</tr>
<tr>
<td>*C. rosea (Link) Schroers, Samuels, Seifert &amp; W. Gams</td>
<td>1</td>
<td>1 S</td>
</tr>
<tr>
<td>Fusarium sp.</td>
<td>1</td>
<td>1 S</td>
</tr>
<tr>
<td>Gymnoascus reessii Baran.</td>
<td>2</td>
<td>2 L</td>
</tr>
<tr>
<td>*Humicola sp.</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>Humicola cf. UAMH 11595</td>
<td>4</td>
<td>10 L, 2 S</td>
</tr>
<tr>
<td>Isaria farinosa (Holmsk.) Fr.</td>
<td>4</td>
<td>3 L, 2 S</td>
</tr>
<tr>
<td>*Kickxella alabastrina Coem.</td>
<td>2</td>
<td>2 L</td>
</tr>
<tr>
<td>*Lecythophora sp.</td>
<td>1</td>
<td>1 S</td>
</tr>
<tr>
<td>Leuconeurospora polyaeoioides Malloch, Sigler and Hambleton</td>
<td>3</td>
<td>9 L, 1 S</td>
</tr>
<tr>
<td>Leuconeurospora capsici (J.F.H. Beyma) Malloch, Sigler &amp; Hambleton</td>
<td>3</td>
<td>10 L</td>
</tr>
<tr>
<td>*Malbranchea sp.</td>
<td>1</td>
<td>2 S</td>
</tr>
<tr>
<td>Mammaria sp.</td>
<td>2</td>
<td>2 L</td>
</tr>
<tr>
<td>Microascus sp.</td>
<td>2</td>
<td>2 L</td>
</tr>
<tr>
<td>Microascus caviariformis Malloch &amp; Hubart</td>
<td>3</td>
<td>10 L, 2 S</td>
</tr>
<tr>
<td>Microascus type 2</td>
<td>1</td>
<td>10 L</td>
</tr>
<tr>
<td>*Monodictys sp.</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>*Myceliophthora sp.</td>
<td>1</td>
<td>1 S</td>
</tr>
<tr>
<td>Oidiodendron sp.</td>
<td>3</td>
<td>2 L, 1 S</td>
</tr>
<tr>
<td>Oidiodendron type 2</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>O. myxotrichoides M. Calduch, Gené &amp; Guarro</td>
<td>2</td>
<td>1 L, 1 S</td>
</tr>
<tr>
<td>O. truncatum G.L. Barron</td>
<td>5</td>
<td>14 L, 2 S</td>
</tr>
<tr>
<td>Paecilomyces carneus (Duché &amp; R. Heim) A.H.S. Br. &amp; G. Sm.</td>
<td>1</td>
<td>1 S</td>
</tr>
<tr>
<td>P. inflatus (Burnside) J.W. Carmich.</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>5</td>
<td>17 L, 6 S</td>
</tr>
<tr>
<td>P. commune Thom</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>P. decumbens Thom</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>P. miczynskii K.M. Zalessky</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>P. solitum Westling</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>P. thomii Maire</td>
<td>2</td>
<td>1 L, 3 S</td>
</tr>
<tr>
<td>P. vulpinum (Cooke &amp; Massee) Seifert &amp; Samson</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>*Phaeoacremonium sp.</td>
<td>1</td>
<td>1 S</td>
</tr>
<tr>
<td>Phialophora sp.</td>
<td>1</td>
<td>3 S</td>
</tr>
<tr>
<td>*Polytolypa sp.</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>Preussia sp.</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>Pseudoarachniotus ruber (Tiegh.) G.F. Orr, G.R. Ghosh &amp; K. Roy</td>
<td>2</td>
<td>2 L</td>
</tr>
<tr>
<td>Pseudogymnoascus pannorum sensu lato (Link) Minnis &amp; D.L. Lindner</td>
<td>5</td>
<td>32 L, 2 S</td>
</tr>
<tr>
<td>*P. destructans (Blehart &amp; Gargas) Minnis &amp; D.L. Lindner</td>
<td>5</td>
<td>30 L, 3 S</td>
</tr>
<tr>
<td>P. roseus Raillo</td>
<td>3</td>
<td>3 L, 1 S</td>
</tr>
<tr>
<td>*cf. Rhinocladiella sp.</td>
<td>1</td>
<td>1 S</td>
</tr>
<tr>
<td>Simplicillium sp.</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>*Sporendonema sp.</td>
<td>1</td>
<td>1 S</td>
</tr>
<tr>
<td>*Stachylium sp.</td>
<td>1</td>
<td>1 S</td>
</tr>
<tr>
<td>*Thamnidi um elegans Link</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>Thelebolus crustaceus (Fuctel) Kimbr.</td>
<td>4</td>
<td>2 L, 3 S</td>
</tr>
<tr>
<td>Thysanophora penicillioides (Roum.) W.B. Kendr.</td>
<td>2</td>
<td>2 S</td>
</tr>
<tr>
<td>*Torula sp.</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>Trichoderma sp.</td>
<td>5</td>
<td>6 L, 3 S</td>
</tr>
<tr>
<td>Trichosporiella sp.</td>
<td>3</td>
<td>5 L, 1 S</td>
</tr>
<tr>
<td>Wardomyces humicola Hennebert &amp; G.L. Barron</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>W. inflatus (Marchal) Hennebert</td>
<td>1</td>
<td>2 L</td>
</tr>
</tbody>
</table>

**Basidiomycota**

<table>
<thead>
<tr>
<th>Species</th>
<th>L</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asterotremella sp.</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>Unidentified Basidiomycete</td>
<td>3</td>
<td>3 L, 3 S</td>
</tr>
<tr>
<td>Cystofilobasidium sp.</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>Trichosporon sp.</td>
<td>2</td>
<td>2 L, 2 S</td>
</tr>
<tr>
<td>T. dulcitum (Berkhout) Weijman</td>
<td>4</td>
<td>3 L, 4 S</td>
</tr>
<tr>
<td>*cf. Tubulicrinis sp.</td>
<td>1</td>
<td>1 S</td>
</tr>
</tbody>
</table>
Pre- vs. post- WNS Fungal Diversity

The mean number of fungal taxa isolated per hibernaculum was not significantly different in 2012 (32.4 ± 4.3 SD) when compared to 2010 (29.6 ± 6.1 SD; T_{1,4} = 0.66, P = 0.543). Neither was the mean number of fungal taxa per bat in 2012 significantly different from that recorded in 2010 among the five hibernacula re-examined (Table 1; Wilcoxon statistic_{1.4,9} = 677, P = 0.137). However, the mean number of fungal taxa per bat in Berryton Cave was significantly higher in 2012 (13.1 ± 2.6 SD) than 2010 (6.5 ± 1.9 SD; Wilcoxon statistic_{1.0} = 55, P = 0.006). This increase in the number of fungal taxa per bat in Berryton Cave between 2010 and 2012 was not reflected evenly across all fungal taxa, but rather in the number of bats from which Microasucs type 2, Microasucs caviariformis, Chrysosporium sp., Chrysosporium merdarium, Pd. Leuconeurospora capsici, Arachniotus sp., Arthroderma silverae, and O. truncatum were cultured.

Pseudogymnoascus destructans

Pd was cultured from 100% of bats with visible Pd growth (n = 30 bats), and from 19.0% of bats without visible Pd growth (n = 21 bats). Bats with visible Pd growth comprised 1 M. septentrionalis and 29 M. lucifugus. Bats without visible Pd growth included 16 M. septentrionalis (3 culturing positive for Pd) and 5 M. lucifugus (1 culturing positive for Pd). All Myotis spp. sampled without visible Pd growth were located in hibernacula considered Pd-negative. Pd-positive Myotis spp. had significantly higher numbers of fungal taxa per bat (9.9 ± 4.0 SD) than Pd-negative Myotis spp. (6.9 ± 4.0 SD; W_{1,58} = 698, P = 0.0089). Among bats with visible Pd growth, culturing Pd was marginally more successful from skin (93.1% positive swabs, n = 58 swabs) than fur (80.0%, n = 60) and on DPYA (89.7%, n = 58) than SAB (83.3%, n = 60). For bats without visible Pd growth, culturing Pd was marginally more successful from skin (14.3% positive swabs, n = 42 swabs) than fur (11.9%, n = 42) and on DPYA (14.3%, n = 42) than SAB (11.9%, n = 42). Pd co-occurred with other Pseudogymnoascus spp.; 28 bats harbored both Pd and Pseudogymnoascus spp. while 6 bats cultured positive for Pd but with no other Pseudogymnoascus spp. Although two of our study sites were identified as Pd-negative based on the lack of visible fungal growth on bats, culture data proved both to be Pd-positive. In Howes Cave, Pd was cultured from 1 of 10 M. lucifugus sampled in February 2012, one month before visible Pd growth on bats was observed. In Harbell’s Cave, Pd was cultured from 3 of 10 M. septentrionalis (clustered together) sampled thirteen months before visible Pd growth on bats was observed. Pd was not detected prior to the visible appearance of fungal growth at other sites, in part because no culturing was done in 2011.

Fungi on dead bats

In 2012 Mucor sp., Mortierella sp., P. pannorum sensu lato, Pd. Arachniotus sp., Acremonium spp., and C. merdarium were cultured from dead M. lucifugus in Berryton Cave (n = 3 bats; Fig. 1). The composition of fungi on dead bats changed as carcass decomposition progressed. Fast growing Zygomyctes such as Mucor sp. and Mortierella sp. were commonly seen on freshly dead bats in Berryton Cave (Fig. 1A). As carcasses decomposed fungi that grow more slowly, such as Trichoderma sp., Arachniotus sp., P. pannorum sensu lato, and Chrysosporium sp., became evident (Fig. 1B). Carcasses at an advanced stage of decomposition, consisting only of remnant fur and bone, were often covered with ascomata of M. caviariformis (Fig. 1C). In 2013, P. pannorum sensu lato, M. caviariformis, Fusarium sp., Trichosporon dalcitum, L. capsici, A. silverae, Pd. O. truncatum, Mucor sp., Mortierella sp., and C. stemonitis were cultured from a long-dead M. lucifugus in Berryton Cave. All fungi isolated from dead bats were also cultured in 2010 and 2012 from live bats, with the exception that no Pd was cultured in 2010. Most notably, those fungi contributing to the elevated diversity found on live bats in Berryton Cave in 2012 were also isolated from dead bats remaining after a 2011 WNS mortality event in this hibernaculum.

DISCUSSION

Post-WNS Fungal Diversity

The greater number of fungal taxa isolated from M. lucifugus versus M. septentrionalis in 2012 may reflect environmental features of individual hibernacula rather than a biological difference between the bat species. Ten of 34 M. lucifugus sampled were roosting in Berryton Cave, the hibernaculum with the highest number of fungal taxa per bat. Conversely, ten of the 16 M. septentrionalis sampled were roosting in a hibernaculum with near the lowest fungal diversity among all sites in both 2010 and 2012 (Harbell’s Cave). Vanderwolf et al. (2013a) found that hibernacula with substantial flowing or standing water bodies (n = 3 sites) had lower fungal diversity compared to those lacking such features (n = 5 sites). Harbell’s Cave was the only site with substantial water (flowing) sampled in 2012. There was no significant difference in the number of fungal taxa isolated

<table>
<thead>
<tr>
<th>Fungal Diversity on WNS bats in Canada</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Zygomyctota</td>
<td>M. verticillata Linnem.</td>
<td>1</td>
</tr>
<tr>
<td>Mucor sp.</td>
<td>5</td>
<td>22 L, 5 S</td>
</tr>
<tr>
<td>Oomycota</td>
<td><em>Pythium</em> sp.</td>
<td>2</td>
</tr>
<tr>
<td>unidentified yeast</td>
<td>1</td>
<td>1 L</td>
</tr>
<tr>
<td>Sterile</td>
<td>5</td>
<td>8 L, 9 S</td>
</tr>
</tbody>
</table>

International Journal of Speleology, 45 (1), 43-50. Tampa, FL (USA) January 2016
Fig. 1. All pictures taken in Berryton Cave. A) Mucor sp. growing from the head of a freshy dead Myotis sp. 15 March 2011 (carcass <3 months old); B) yellow Chrysosporium merdarium and red Arachniotus sp., among other species, growing on a dead Myotis sp. 14 December 2011 (carcass <10 months old); C) Microascus caviariformis (ascomata shown) growing on a long dead Myotis sp. 14 December 2011 (carcass ~12 months old). Age of carcasses are rough estimates.

from M. lucifugus versus M. septentrionalis in 2010 (Vanderwolf et al., 2013a).

Berryton Cave had a significantly higher mean number of fungal taxa per bat than any other site sampled in 2012. Pre-WNS, Berryton Cave hosted the greatest number of hibernating bats among known hibernacula in New Brunswick (Vanderwolf et al., 2012). The arrival of WNS led to mass mortality of bats in this cave, with thousands of carcasses on the cave floor and walls. Although the majority of carcasses were removed by scavenging raccoons or for research purposes (McAlpine et al., 2011), we estimate <100 remained in the cave. During repeated visits over 3 years, we observed these carcasses being colonized by a succession of fungi. The fungal taxa that increased in frequency on live bats in Berryton Cave between 2010 and 2012 were identical to those cultured from dead bats. It appears that spores originating from carcasses settled on live bats, elevating over-all fungal diversity at this site. Few or no bat carcasses were found in other Pd-positive hibernacula in the province. It appears Pd-infected bats present at these sites exited hibernacula and died on the landscape. This varying pattern of mortality events at Pd-infected bat hibernacula may therefore influence the diversity of fungi that can be cultured from live bats in the immediate aftermath of WNS infection.

Johnson et al. (2013) found a total of 31 fungal taxa on M. septentrionalis in two Pd-negative hibernacula in Illinois, with a mean of 9.75 ± 1.49 SD fungal taxa per bat (n = 8 bats) and 5.17 ± 4.92 SD (n = 6 bats) respectively. They found 8 fungal taxa on M. sodalis (n = 1 bat) in a Pd-negative Illinois hibernaculum. Our results, both in Pd-negative hibernacula in 2010 and Pd-positive hibernacula in 2012, are very similar in terms of number and composition of fungal taxa (43 fungal taxa on M. septentrionalis in 2012 with x = 6.0 ± 4.0 SD fungal taxa/bat, n = 16 bats). Nineteen of 44 fungal taxa and 9 of the 10 most common taxa isolated from 3 bat species by Johnson et al. (2013) were also found on bats in New Brunswick in 2012 or 2010 (Vanderwolf et al. 2013a). Penicillium spp., Cladosporium spp., Pseudogymnoascus pannorum sensu lato, and Mortierella spp. are reported among the most common fungal taxa isolated from live bats in both New Brunswick and Illinois.

Pre- vs. post- WNS Fungal Diversity

In 2010, 83 fungal taxa in 57 genera (n = 50 bats) were isolated (subset of data from Vanderwolf et al. 2013a) while 80 fungal taxa in 56 genera (n = 50 bats) were isolated from the same hibernacula in 2012. In 2010, 24 genera were isolated that were not found in 2012. In 2012, 25 genera were isolated that were not found in 2010. These unique genera were isolated from a mean of 1.5 ± 1.0 SD bats in 2010 (range 1-4) and 1.3 ± 0.7 SD bats in 2012 (range 1-4). These genera are either naturally rare in the cave environment or infrequently encountered by bats above ground. Excluding Pd, 9 of the 10 fungal taxa most commonly isolated were identical in 2010 and 2012. The 30 most common taxa isolated in 2010 were also isolated in 2012. Clearly, the composition and number of the most common and widespread fungal taxa on hibernating Myotis spp. did not change with the introduction of Pd to New Brunswick hibernacula.

Pseudogymnoascus destructans

Johnson et al. (2013) found that Pd-negative bats (n = 25) sampled April-May in Illinois had a much greater diversity of fungi than Pd-positive bats (n = 5) sampled in June in Indiana. Although Johnson et al. (2013) suggested that it was presence of Pd that lead to this reduced fungal diversity, we believe
the pattern they report is the result of unbalanced sample sizes, fungal assemblages sampled from bats during and outside the hibernation period, and the comparison of assemblages from different geographic regions. If the hypothesis that Pd is widespread and native to Europe is correct (Puechmaille et al., 2011), then the relatively larger numbers of studies on cave fungi carried out in Europe have been conducted in the presence of Pd. These studies report diversities of fungal taxa similar to those reported for fungal studies from North American caves (Vanderwolf et al., 2013b). Our data suggest that environmental and ecological characteristics of individual caves are the principal variables influencing the fungal assemblages that can be cultured from hibernating bats at specific hibernacula.

We detected no difference in fungal diversity on the fur or skin of hibernating bats when comparing pre- (2010) and post- (2012) WNS data. However, Pd-positive bats had a significantly higher number of fungal taxa per bat than Pd-negative bats sampled in 2012. As noted, we believe this difference was due to environmental conditions in individual hibernacula (Pd-positive Berryton and Pd-negative Harbell’s Cave). We found no evidence to suggest that in nature Pd interacts with other bat-associated fungi when present on the external surface of bats, even among closely related species of the same genus. As stated in Vanderwolf et al. (2013a), bats appear to acquire a diversity of spores from the environment and may be important fungal dispersers. However with the exception of Trichophyton reddelli (Lorch et al., 2015), Pd, and an unidentified ascomycete recently reported on Eptesicus fuscus (McAlpine et al., 2016), fungi are not known to actively grow on bat skin or fur. This would seem to preclude competition between Pd and other fungi present on WNS-infected bats, although it would not preclude competition between fungal species on other substrates in the cave environment. Hoyt et al. (2015) found that a bacterium isolated from bats inhibits the growth of Pd in vitro. It is therefore possible that Pd may interact with other microbiota present on hibernating bats such as bacteria, viruses, or fungi that actively grow on bats that we did not culture. Regardless, the fungal assemblage found on cave walls and in cave sediments (Zhang et al., 2014) is similar to that on hibernating bats, demonstrating further that the ectomycota cultured from bats is a sub-sample of fungi present in the environment.

**Fungi on dead bats**

Previous studies of fungi that grow on dead bats have found many of the same or related taxa that we encountered in New Brunswick. Mucor sp. and Cladosporium sp. were identified from two dead Myotis sp. in Europe (Puechmaille et al., 2011). Penicillium sp. was found growing on two dead Rhinolophus ferrumequinum in the United Kingdom (Wibbelt et al., 2010), and Chrysosporium merdarium was isolated from a dead bat in Hungary (Zeller, 1966). In Italy, Mucor hiemalis f. hiemalis, M. racemosus, M. plumbeus, Mortierella polycephala, Mortierella gamsii, Fusarium dimerum, F. equiseti, Trichosporon chioretorum, Chrysosporium sp., C. merdarium, Alternaria sp., Aspergillus sp., Candida palmioleophila, Thielavia sp., Lecanicillium lecanii, Penicillium sp., Penicillium griseofulvum, and Cladosporium cladosporioides were isolated from 17 dead bats of various species (Voyron et al., 2011). Some of these fungi were also isolated from live bats in the same cave (Voyron et al., 2011). All fungi we isolated from dead bats in New Brunswick were also cultured from live bats, both in 2010 and 2012 (Vanderwolf et al., 2013a). Cave fungi appear to be opportunistic and proliferate when sources of nutrition become available in the oligotrophic cave habitat (Cubbon, 1976; Minn, 1988). In the absence of vertebrate and insect scavengers, fungi are important decomposers of organic matter in cave environments. Most fungi present in caves seem unable to use live bats as a food source. However, multiple fungal species proliferate on dead bats, with the spores these fungi produce dispersing into the surrounding cave environment. Where there is an influx of substrates into a cave on which fungi may grow (e.g. dead bats as a result of WNS mortality), the frequency of fungal taxa that can be cultured from live bats in hibernacula appears to shift. At one of our study sites in New Brunswick (Berryton Cave), we found that those fungal taxa growing on dead bats were cultured with increased frequency from live bats following the mortality of thousands of Pd-infected Myotis spp.

**CONCLUSION**

The fungal assemblage cultured from hibernating bats was not affected by the presence of Pd. Differences in fungal assemblages on M. lucifugus and M. septentrionalis appear to be related to environmental and ecological characteristics of individual caves. The increase in the number of fungal taxa per live bat in one hibernaculum from 2010 to 2012 is likely due to the growth of fungi on dead bats in the cave associated with WNS mortality. Currently lacking are detailed data on changes in fungal diversity over time as fungal substrates decompose in caves and how these changes might influence fungal assemblages in the surrounding environment. It appears that fungal assemblages on live bats may be sensitive to sporadic or rare introductions of fungal substrates to the cave environment.

**ACKNOWLEDGEMENTS**

Access to hibernacula located on private lands was generously provided by David Roberts, Joan Chown, and Tony Gilchrist. Greg Thorn helped with identification of Basidiomycetes. Sampling at Underground Lake Cave, a Class 1 New Brunswick Protected Natural Area, was made possible through a scientific research permit provided by the New Brunswick Department of Natural Resources Protected Natural Areas Program. Scientific permits for handling bats and/or entering sites was provided by the New Brunswick Department of Natural Resources Species-at-Risk Program and the New Brunswick Protected Natural Areas Program. Research funding was provided by the Canadian Wildlife Federation, Crabtree Foundation, New Brunswick Protected Natural Areas Program. Scientific data on changes in fungal diversity over time as fungal substrates decompose in caves and how these changes might influence fungal assemblages in the surrounding environment. It appears that fungal assemblages on live bats may be sensitive to sporadic or rare introductions of fungal substrates to the cave environment.

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INTRODUCTION

The Deccan Volcanic Province (DVP) basalts spread over half a million square kilometres and cover major parts of western, central India and sub-central parts of Peninsular India. The basalts and associated volcanic suite of rocks were emplaced over a time span from 69 to 63 Ma (Hooper et al., 2010). The bulk of the DVP is made up of pāhoehoe and ´ā´a flows of varying thicknesses (5 – 30 m). The stratigraphic classification of the western DVP has been established based on lithological, geochemical and palaeomagnetic studies (Godbole et al., 1996; Subbarao and Hooper, 1988).

The present study area forms a part of western Deccan Volcanic Province (DVP). Considering the thickness (around 1.5 km) and the vast areal extent of the DVP, it is concurred that very large volumes of lava were emplaced during this period.

Emplacement of lava over such a large area requires transport of the lava over great distances and that too in a short time span. Studies from active volcanoes in other places suggest that an insulated and thermally efficient lava transport system involving inflated lava sheets, lava tubes and lava channels, can emplace lava flows across great distances. (Keszthelyi and Self, 1998). Identification of a lava transport system and related features is easier in recent and younger
volcanic terrains but in the highly dissected Palaeocene Deccan volcanic Province (DVP), the task becomes difficult due to rarity of good outcrops. Nonetheless, remnants of lava tubes and channels have been identified from the pāhoehoe flows of western DVP, by various workers (see Thorat, 1996; Sharma and Vaddadi, 1996; Misra, 2002; Duraishwami et al., 2004; Sen et al., 2012). In all these reported occurrences, the lava tubes are filled with solidified lava and display a conspicuous sinuous shape with reddened amygdular basalt forming lateral accretionary levees. Small openings with a flat base, convex top and limited extension, observed in and around Pune, have been described as lava tunnels by earlier workers (see Kulkarni and Gaikwad, 1984 and Phadke and Ghate, 1984). Similar features of small dimension are seen in the hill adjoining Fergusson College, Pune; which are comparable to blister caves described by Grimes (2002).

Lava caves have been reported from modern lava flows and younger volcanic terrains. World over, lava caves that occur in pāhoehoe lava flows are called ‘pyroduct’ (Coan, 1844) or ‘lava tubes and lava tunnels’ as these are believed to have been formed by the conduits that drain the lava down slope (Lockwood and Hazlett, 2010). Lava tube caves or pyroducts have been identified from modern lava flows and younger volcanic terrains of Hawaii, Australia, and Korea, etc. The world’s largest and deepest lava caves are found in the geologically young Hawaii islands. Some of the more commonly known ones are the Kazumura cave, the Blair cave, the John Martins cave. Lave caves in pāhoehoe flows are also known from Bend, Oregon (Greeley, 1971), Undara volcano, W. Queensland, Australia (Atkinson et al., 1975), Mammoth crater, USA (Waters et al., 1990) and Chyulu hills, Kenya (Simons, 1998), Cheju Island, Korea (Waltham and Park, 2002). Small caves with shallow roofed chambers are reported from Victoria, Australia (Grimes, 2002) and from Hawaii (Halliday, 2002).

The mechanism of formation of lava caves in ancient lava flows can be better interpreted by comparison with modern lava flows. Observations of active lava flows confirm that most pyroducts are formed at the tip of the lava flow by a continual process of advance (moving ahead) and inflation. Inflation and cratering over of channels, as mechanisms in the formation of pyroduct; have been discussed and compared (Kempe et al., 2010; Lockwood and Hazlett, 2010); the inflationary mode being more prevalent. In the inflationary mode, a gas space exists above the internal lava river and hence a cave can form even if the lava does not drain off completely (Kempe et al., 2010). Mode of formation of pyroduct in pāhoehoe flows is dependent on the slope available. On steeper slopes, cratering over of channels lead to formation of pyroducts and on gentler slopes; pyroducts develop beneath inflating crusts (Lockwood and Hazlett, 2010). Pyroducts also formed from partial draining of flow lobes as reported from Kiluaea volcano (Peterson and Swanson, 1974; Greeley, 1987; Hon et al., 1994; and Mount Eccles, Victoria (Grimes, 2002). These according to Kempe et al. (2010) could deepen by thermal and mechanical erosion of their floor. Caves formed by draining of lobes and flows below the crust are generally smaller than those formed by roofing of channel (Grimes, 2002; Grimes, 2005) and caves have been designated as sub-crustal caves.

With this background, the present paper attempts to understand the mode of formation of a small lava cave found in the compound pāhoehoe basalt flows exposed in Ghoradeshwar hill near Pune. This is the first reported volcano - speleological work from the DVP.

GEOLOGY

Ghoradeshwar hill, located in the Pune district, western Maharashtra is an eastward extension of an offshoot ridge from the Sahyadri mountain range and forms interfluves between the Indrayani and Pauna rivers, which drain the northern and southern parts respectively (Fig. 1). On the southern flank of the hill at an elevation of about 790 m, there is a cave opening facing east having a height of 148 cm and width of 310 cm (Fig. 1). The hill slopes have a steep gradient with a thin (0 - 30 cm) veneer of regolith.

The cave occurs in flows belonging to the Karla Formation of the Lonavala Sub group. The flows of Karla Formation are older than the Diveghat Formation of the Wai Sub group, which has been dated by ⁴⁰Ar/³⁹Ar, indicating an age range of 66.2 to 65.3 Ma (Hooper et al., 2010). Hence, it is evident that the flows of Karla Formation which host the lava caves predate 66.2 Ma, but must be younger than the onset of the DVP eruptions 69 Ma.

The Deccan basalts have undergone considerable erosion since their eruption, with major uplift affecting the western Indian margin throughout the Tertiary period (Widdowson, 1997). This uplift and extensive erosion of the Deccan plateau, in the Tertiary times has resulted in the development and evolution of Deccan palaeosurfaces. More recent erosion of the resultant upland plateau with isolated hills and hill ranges has exposed the lava cave. Weathering and solution activity due to seepage of water has modified the caves further.

The pāhoehoe flows are made up of several small flow units/lobes varying in thickness from a few centimetres to a maximum of 5 m. Two compound pāhoehoe flows can be demarcated, based on the presence of a thin, interflow horizon, a red bole (Fig. 2). The lower compound flow is exposed from the base of the hill to about 800 m above msl. It is a very sparsely phryic flow with more or less rounded vesicles and is highly vesicular and amygdular in nature. A few vesicular units resemble spongy pāhoehoe flow. The cave occurs in the lower flow. At the cave entrance, fine grained, highly vesicular pāhoehoe units are exposed. The upper compound flow is sparsely phryic, massive in nature and jointed. Joint sets trend NW-SE and so do the major fractures. A doleritic dyke, trending NW-SE, occupying one such a fracture, intrudes the lava pile.

The pāhoehoe flows are thus made up of laterally extensive inter-fingering flow units or lobes, resulting in an irregular micro-topography. These flows resemble
Genesis of lava cave (Deccan Plateau, India)
International Journal of Speleology, 45 (1), 51-58. Tampa, FL (USA) January 2016

Fig. 1. A) and B) Location map of the study area; C) 3-D view of Ghoradeshwar hill (Google Earth); D) White arrows indicates the location of man-made Buddhist caves towards the south-west side; E) Location of the natural lava cave towards the eastern side.

Fig. 2. Litho section of the flows exposed at Ghoradeshwar hill.
the sheet lobes described by Lockwood and Hazlett (2010). Breakouts leading to smaller pāhoehoe toes are also seen. The individual units are recognized by the presence of pipe amygdales at the base and a zone of spherical vesicles towards the top. Pipe vesicles at the base of the flow units are vertical or inclined. The vesicles in the upper vesicular zone, show alignment, parallel to the flow unit tops. The top surface of the units form reddened glassy crusts due to chilling. Crudely developed ropes and cords are seen on the reddened crust (RC) at places. Vesicle cylinders, indicating escape of stream of volatiles are also seen at places. In a vertical section near the Buddhist cave, four small pāhoehoe flow units can be delineated (Fig. 3A) clearly. The internal characteristics of the individual flow units indicate endogenous growth by inflation.

Numerous small scale features like squeeze-ups occupying the inflation clefts and connecting the different lobes and units are also seen at places, especially near the Buddhist cave temple (Fig. 3B). This results when the semi-solid lava inside squeezes up through the fractures that have resulted from the stretching of the upper solidified crust. Hence, they generally occur as wedge shaped features interconnecting lobes. The abundance of these features in this area is interesting since it indicates the beginning of growth by sequential lobe-by-lobed emplacement.

![Fig. 3. A) Four flow units in Pāhoehoe (red line marks the reddened crust); B) Squeeze-up (shown by an arrow) in the pāhoehoe flow; length of the pen is 13.5 cm; C) Close up of the chilled reddened crust marking the unit contact. HVZ- Horizontal Vesicle Zone, UVZ – Upper Vesicular Zone, PVZ- Pipe vesicle zone.](image)

**OBSERVATION**

The cave entrance is semi-circular, with a flat floor, which leads into the central part of the cave. This consists of a chamber along with some smaller openings. The gradient of the cave is from south-west to north with total examined passage length of about 20 m. The cross sections in Fig. 4 show the morphological variation along the passage. The passage to the north is nearly circular while the passage to the south-west is semi-circular and oval shaped. The entrance to the passage in the north is large enough for a person to stand. These passages have a twilight zone characterised by decreasing light level. The entrance were highly vesicular and amygdular. The lower flow lobe exposed at the cave entrance is vesicular and amygdular with small spherical vesicles and amygdules. Within the vesicular and amygdular zone a couple of thin (less than a millimetre) light coloured bands are seen, which represent fractures developed parallel to cooling and inflating surface. The roof of the entrance exposes a reddened glassy crust that separates two small flow units or sheet lobes (Fig. 5A). Three seepage points (Fig. 5B) were observed in the narrower south-western passage which is ~8 m in length. The wall of the cave joins the floor at a right angle. At the top, a curved wall overhangs the passage, while a shelf (Fig. 5C) is observed at the top of the wall. The roof of the cave towards the northern end has an angular gothic shape with a median fracture in the ceiling (Fig. 5D). From the morphological variation along the passage it is evident that the gothic cross section is restricted to only a small portion.
During the monsoon season infiltration of the rainfall is observed along the seepage points; joint planes and fractures along the ceiling/roof. This water trickles along the sides of the cave as well. This leaves a thin calcium carbonate coating on the cave walls. The water flows towards the northern part, where the cave terminates. As there is no further movement of the water, the accumulation forms a stagnant water body in the cave. On the walls of the cave, water level markings due to slowly receding water level can be clearly observed. During the monsoon, the rate of infiltration is accelerated due to seasonal leaching and the presence of grass and shrub type vegetation on the hilltop (Kale and Kulkarni, 1992; Verachtert et al., 2010). Such vegetation facilitates the seepage process through the soil and the rocks beneath.

Cave fill material covers the floor of the cave. In order to understand the depth and nature of this cave fill material, core samples were collected. Small trenches were also dug to examine the sediments. The examined depth of the sediments varied from a maximum of 65 cm in the central part of the cave (near the entrance), gradually decreasing towards the northern end. Different layers in the sediment fill could be discerned; a top silt and mud layer (top soil) overlying silt embedded with rock fragments with a lower sandy silt layer resting on the lava floor. The size of the rock fragments ranged from boulder to pebble and clearly represent the collapsed part of ceiling. The silty and clayey nature of the sediment is suggestive of deposition in still or very slow moving water. Due to the high silt and mud content, the sediments exhibit cohesive properties.

A few small bone fragments were collected from the fine-grained cave sediments at 40 cm and 47 cm depth respectively. To ascertain the relative age of these bones, fluorine dating technique; a standard procedure in archaeological chemistry (Joshi, 2006-07) was carried out (Table 1). The procedure for this technique was developed by Carnot in 1892. He observed that there is a disparity in the concentration of the fluorine in the bones obtained from different environments. Fluorine has a strong affinity for the hydroxyapatite $[Ca_{10}(PO_4)_{6}(OH)_2]$, an inorganic phosphate material found in the bones. The hydroxyapatite acts as a natural trap for wandering fluoride ions occurring as traces in the groundwater and soils. The exchange of hydroxyl ions of hydroxyapatite with fluoride ions leads to the formation of Fluorapatite $[Ca_{10}(PO_4)_{6}(F)_2]$, a stable mineral.

During the fossilization process, along with the fluorapatite, the agglomeration of other mineral matters may vary according to the respective environment. The phosphate measurement reveals the extent of contamination from the soil or other extraneous matter in the bone. The formula for fluorine dating is:

$$\text{Ratio} = \frac{100 \times \% \text{ Fluorine}}{\% \text{ Phosphorous pentoxide}}$$

The fluorine/phosphate ratio is independent of the density of bone and therefore contamination problem is minimised. The percentage of phosphorous pentoxide ($P_2O_5$) was determined from the percentage
of phosphorous. The value of obtained phosphorous when multiplied with 2.21 gives the percentage of phosphorous pentoxide. The antiquity of the fossils can be determined by the high values of ratio while low values of ratio reveal contemporary fresh bones. The theoretical saturation value of this ratio is 8.92 (Table 2).

The ratios obtained for the present samples are 0.508 and 1.099. Based on the fluorine-phosphorous ratio it can be ascertained that the bone fragments range from mid to late Holocene period.

The cave cricket (Ceuthophilus) and bats (Rousettus leschenaulti) are the only fauna found in the cave. The cave crickets forage on the surface at night, returning to roost during the day. The bat species prefers to live in caves, where water is in the vicinity (Korad et al., 2007). Lichens are observed on the bare speleothem surfaces near the entrance zone.

### Table 1. Result of Fluorine dating.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Depth</th>
<th>Material</th>
<th>% Phosphate</th>
<th>% P₂O₅</th>
<th>% Fluorine</th>
<th>Ratio (F/P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>40 cm</td>
<td>Bone</td>
<td>10.5</td>
<td>23.62</td>
<td>0.12</td>
<td>0.508</td>
</tr>
<tr>
<td>2.</td>
<td>47 cm</td>
<td>Bone</td>
<td>9.7</td>
<td>21.82</td>
<td>0.24</td>
<td>1.099</td>
</tr>
</tbody>
</table>

### Table 2. Fluorine /Phosphate ratio in fossilized bones from sites of different periods/ cultures and various region giving their approximate ages (Joshi 2006-07).

<table>
<thead>
<tr>
<th>Site/ Region</th>
<th>Period/ Culture</th>
<th>Approximate age</th>
<th>100F/ P₂O₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site from Jammu and Kashmir, Andhra Pradesh, Karnataka, Madhya Pradesh and Maharashtra</td>
<td>late Holocene</td>
<td>500 – 2000 B.C.</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Moti Pipali (Gujarat) and Phalodi (Rajasthan)</td>
<td>mid Holocene to terminal Pleistocene</td>
<td>2500 – 6000 B.C.</td>
<td>1-2</td>
</tr>
<tr>
<td>Mundur (Karnataka) and Kurnool caves (Andhra Pradesh)</td>
<td>early Holocene to upper Palaolithic</td>
<td>7 ka – 10 ka</td>
<td>2.10-3.70</td>
</tr>
<tr>
<td>Kalpi (Yamuna cliff), Manjra and Ghod (Maharashtra)</td>
<td>upper Palaolithic/ upper Pleistocene</td>
<td>10 ka – 20 ka</td>
<td>3 - 5</td>
</tr>
<tr>
<td>Belghat (Narmada valley), Kalegaon and Nevasa (Maharashtra)</td>
<td>upper Pleistocene/ middle Palaolithic</td>
<td>20 ka – 40 ka</td>
<td>4.24 - 6</td>
</tr>
<tr>
<td>Hathnora and Gowarighat (Narmada valley)</td>
<td>late middle Pleistocene/ middle and lower Palaolithic</td>
<td>&gt; 40 ka</td>
<td>7.70 – 8.15</td>
</tr>
<tr>
<td>Hunsgi valley, Chirki, Nevasa</td>
<td>mid Pleistocene/ acheulian</td>
<td>0.1 Ma – 0.2 Ma</td>
<td>6.73 – 8.39</td>
</tr>
<tr>
<td>Bor, Siwalik fossils</td>
<td>lower Pleistocene/ acheulian</td>
<td>0.7 Ma – 1.8 Ma</td>
<td>6.66 – 8.45</td>
</tr>
<tr>
<td>Kutch (Tertiary fossil)</td>
<td>Tertiary period</td>
<td>-</td>
<td>8.15 - 9.42</td>
</tr>
<tr>
<td>Theoretical saturation value</td>
<td>-</td>
<td>-</td>
<td>8.92</td>
</tr>
</tbody>
</table>

**DISCUSSION**

When discussing the genesis of lava caves in DVP, it is important to consider the nature of the flow in which it is observed and also its emplacement mechanism. Besides, in a 65 Ma dissected lava provinces, the extent of exhumation should also be taken into consideration. The lava cave reported from Ghoradeshwar, occurs in a small isolated hill. The flow units exposed in other isolated hills in the adjacent area belong to the same litho-stratigraphic unit, but extension of the cave is not seen. The lava flow which hosts the natural cave is a compound pāhoehoe flow with small toes, lobes and sheet lobes that exhibit laterally inter-fingerling relationship with small scale features like squeeze-ups. Flow lobes and toes develop gradually from flow sheet lobes by continuous supply of lava to the advancing front (Lockwood and Hazlett, 2010). These are comparable to the inflation layers described by Kempe (2013). Importance of inflated pāhoehoe sheets in emplacement of DVP have been discussed by a few workers (e.g., Keszthelyi et al., 1999; Bondre et al., 2000). Based on the surface features and comparing it with other compound pāhoehoe flows from Maharashtra, we presume that the emplacement of these pāhoehoe flows has been by inflation and continual budding of the advancing lava lobes, similar to the mechanism described by Duraiswami et al. (2004).

As described earlier, the lava cave has a broad sinuous/meandering outline with openings to the north and south that open into the central chamber, where the present cave entrance is seen. Downward erosion or thermal erosion was not observed in the lava cave. Towards the upper part of the cave, near the ceiling, the reddened glassy crust that separates the older (flow unit) flow lobe is seen. The cave is thus seen only in the lower pāhoehoe flow (sheet lobe), within a single (flow unit) flow lobe. Covered conduits can form within a single thick flow unit (Harter, 2009). Pyroducts are known to develop beneath inflating crusts on gentler slopes (Lockwood and Hazlett, 2010). The importance of pyroducts for the transport of lava where topography is gentle is well demonstrated by the Hashemite University cave, found in the Quaternary flow field of the Qais/Makais Volcanoes (Al-Malabeh and Kempe, 2012). The dimensions of the cave explored at Ghoradeshevar, are small compared to
the major conduits/pyroducts described from other volcanic fields, but are morphologically comparable with inflated lobes. The small blister caves reported as tunnels by earlier workers (Kulkarni and Gaikwad, 1984; Phadke and Ghate, 1984) are also from Pune. From this, it is apparent that the gentle topography around Pune controlled the progression of lava in this lava field. Further, the gentle micro-topography at Ghoradeshwar controlled the advancement of pāhoehoe lobes and toes within the sheet lobe. It is possible that the progression of flow was from the east, where the cave opening is presently seen. The moderate gradients controlled the lava flow which advanced in the southern and northern direction. The portion of the cave, near the entrance where cave fill material is seen, could have developed a small plunge pool.

The cross sectional pattern of the lava cave along the 20 m passage mostly varies from circular to semi-circular, with a small portion exhibiting a gothic shape. The gothic shape could have resulted from the escape of gas leading to modification of the ceiling along the median part when the lava had not completely solidified. From the dimensions and related morphology it would be appropriate to categorize the present cave as a small sub-crustal cave, formed by draining of an inflated pāhoehoe lava lobe (flow unit). The drained flow lobes may have interconnected forming a conduit of smaller dimension as seen in the area.

According to Rajaguru and Misra (1997), the last glacial period is well documented in many parts of India and many of the rivers were aggrading between 12-18 ka. The early Holocene (9-6 ka) was a period of good monsoon in India, when black soil developed over the aggraded alluvial plains in the Deccan plateau. The speleothem deposits seen in the cave can be related to the early Holocene humid phase, when intensification of monsoons took place. Fluorine dating of the bones retrieved from the sediments indicates mid to late Holocene age of the sediments. Archaeologically, the cave contents are young, but it is difficult to ascertain the exact age of exposure of the cave opening.

CULTURAL ASPECT

In India, natural caves have been occupied by Stone Age man in Madhya Pradesh, Andhra Pradesh and Maharashtra. There are nearly 1550 rock cut caves in India, of which ~1200 are located in Maharashtra. The tradition of carving temple in the rocks began in India, of which ~1200 are located in Maharashtra. The fundamental objective of these caves was to serve as Buddhist chapel and monasteries (Patel, 2007). The fundamental objective of these caves was to serve as Buddhist chapel and monasteries (Patel, 2007). The fundamental objective of these caves was to serve as Buddhist chapel and monasteries (Patel, 2007). The fundamental objective of these caves was to serve as Buddhist chapel and monasteries (Patel, 2007).

At Ghoradeshwar, there are a few Buddhist caves carved in pāhoehoe lava flows at an elevation of about 760 m and those occur in the same flows in which the lava cave is observed. These caves belonged to Hinayana doctrine of Buddhism, in which the Buddha was worshipped only through symbols such as stupa. It is interesting to note that most of the rock cut caves reported from Maharashtra, in Deccan Volcanic Province are from pāhoehoe flows. This includes the rock cut caves of world heritage sites of Ajanta and Ellora. At Ellora, Ansari et al. (2014) have indicated the presence of a lava conduit very close to the cave 32. This in all probability indicates that early man took advantage of the existing openings in pāhoehoe flows and sculpted the caves to suit their requirements. Lava caves are known to have served as homes and temporary shelters throughout history and are significant archaeological sites.

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REFERENCES


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Apparent glacially induced structural controls on limestone conduit development in Ohio Caverns, United States

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Abstract: Rock discontinuities such as bedding planes and joints are important controls on the form that caves take. We examined structural controls on the development of Ohio Caverns. The cave formed in Devonian limestone underlying a small bedrock knob (Mt. Tabor) within the Interior Lowland province, United States. The area has been overridden by continental glaciation multiple times. The bedrock is pervasively fractured, with many curved and wavy near-vertical fractures showing many different orientations. In the case of Ohio Caverns, it appears that the controlling fractures in map view may not be joints sensu stricto, but rather some combination of tensile and shear (mode-1 and mode-2) fractures, probably forming in the regime transition between tensile and shear fracturing. This is easy to envision in a situation with ice advancing over this topographic high, and would result in the curved fractures that are observed in many places in the cave. It can also explain the numerous fracture directions. However, not all fractures are conduit-significant. The cave initiated on or near a single bedding plane, and the cave passages exhibit strong keyhole or plus-sign cross sections. Passage and fracture orientations are inconsistent with regionally expected directions. It is likely that mechanical, hydraulic, and thermal stresses related to glaciation caused the fracturing in Mt. Tabor. The cave then developed on this template according to local hydrologic conditions. This presents a newly documented structural template sub-type for cave development.

Keywords: fractures; Ohio; glacial; thermal; speleogenesis

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INTRODUCTION

The overall form that a cave takes, as well as its position in the landscape, results from the dominant initial porosity present in the host rock and the mode of recharge to the limestone mass during speleogenesis (Palmer, 1991). For most caves, dominant rock porosity is in the form of discontinuities (joints, bedding planes) that serve as a template upon which cave growth occurs. Consequently, for relict caves the morphology of the passages, along with other information, can be used to infer cave genesis and to also shed light upon paleohydrologic conditions. Cave passages developed along joints tend to be straight or angular and passages developed along bedding planes tend to be sinuous. If a cave system is composed predominantly of one of these passage types, then the overall cave may be categorized as joint-controlled or bedding-plane controlled (White 1988, p. 69). Stemming from these come two major cave conduit topologies: branchworks and mazes.

Branchwork caves (Fig. 1) are distinguished by stream passages that connect and merge into larger, fewer passages. The conduit arrangement mimics the behavior of surface streams, except that they are underground. Their passages usually do not branch out downstream, and few closed loops exist. Those loops that do form develop by sequential diversion to lower levels of the cave, where they merge with original passages farther downstream.

A maze is defined as one whose passages contain many closed loops that form simultaneously. One type is an anastomotic maze (Fig. 1), which is composed of curving tubes that intersect one another and form many closed loops to create the appearance of being braided. They are formed by regular floodwaters that are supplied by sinking streams or by water fed through a karst surface of bare bedrock. Whereas anastomotic caves have curving tubes and form braided patterns, network maze caves have very angular intersections and make a grid-like pattern. They can be formed various ways, for example...
by water seeping through overlying or underlying insoluble rock, regular flooding, waters with different chemistries mixing, or the oxidation of hydrogen sulfide producing sulfuric acid (Palmer, 1991).

We investigated a relict cave, Ohio Caverns, that is present within the Bellefontaine Upland area of the Interior Lowland physiographic province (ODNR 1998). This region is an interlobate outlier within the Till Plains of the Central Lowlands province of North America (Fig. 2, inset). Previous workers (Hills, 1916; Hoy, 1993; Hoy, et al., 1995; Brayman, 2002; Codispoti, 2011) have inferred a relationship between the cave and glacial activity, as reflected in the chemical and clastic sediments found within the cave. Here, we examine the cave morphology for additional evidence of the influence of glacial activity. Preliminary comparison of overall cave pattern (Fig. 3) to Palmer’s (2007) matrix (Fig. 1) does not show a clear match for genetic type. The present study seeks to identify the structural controls on development of this cave within the context of glacially overridden terrain.

With respect to nomenclature, it is probably correct that most “joint” controlled cave passages throughout the world are formed along mode-1 (tensile, opening) fractures, which are properly termed “joints”. These form nearly vertically, and normal to horizontal bedding. Such cave passages are linear in map view, or angular if they make use of multiple joint directions. For clarity within this paper we will use the term “fracture” when the mode of origin for a discontinuity is unclear.
There has long been discussion in the literature as to the effects of glaciation on karst (e.g., Ford et al., 1983; Ford & Williams, 2007; Smart, 2004), and the ways in which earlier karst landforms may “survive” glaciation. Recent work (Cooper 2014; Cooper & Mylroie 2014, 2015; Faulkner, 2007, 2010) using morphometric, hydrologic, and other approaches has shown that caves may form earlier, or may form post glaciation. Localized conditions are very important (Mylroie, 1984). Sediment infilling is common.

Many caves in alpine settings have been affected by glaciation, and have been studied with regard to their genesis (e.g., Hauselmann, 2002). These steep terrain settings generate classical stair-step shaft-and-gallery morphologies. Less commonly examined are those caves that have been impacted by continental glaciation. Castleguard Cave (Alberta, Canada), part of which lies under the Columbia Icefields, may serve as a potential analog. It has been the subject of extensive hydrologic and geologic study (Ford, et al., 1983; Smart, 1983). Seasonal flooding due to melting shifts the cave from a vadose to phreatic state annually. In New York State there is extensive cave development in the flat-lying Helderberg Limestone, and evidence of past glaciation in the form of sediments is seen in some caves (Palmer et al., 1991; Cooper & Mylroie, 2015). Changes in drainage due to removal of ice-blockage are also recognized in these karst systems (Palmer et al., 1991). A generalized linkage between circum-glacial sedimentation and paragenetic (upward) growth of cave passages has been observed from many locations (Farrant & Smart, 2011). The effects of ice margin hydrology have also been indicated as speleogenetic factors (Murphy et al., 2015).

Overall, the genesis of caves formed in circum-glacial conditions is one of substantial complexity. This results from polyphase growth, extremely variable hydraulic gradients, changing boundary conditions, and possible mechanical effects of ice-contact. The present study adds to the body of knowledge by investigating the structural controls on cave development in an isolated knob that has been overridden multiple times (Quinn & Goldthwait, 1979) by continental glaciation. The two competing hypotheses being evaluated are: a) The cave has formed along regionally oriented tectonic fractures, and b) the cave has formed along fractures that are of local, possibly glaciogenic, origin.

**STUDY SITE AND REVIEW OF PROBLEM**

Ohio Caverns (Fig. 3) is an extensive, mostly horizontal, multi-conduit cave system formed in the Devonian Columbus Limestone, Champaign County, Ohio. This area is on the eastern limb of the Cincinnati Arch, with rocks having a <1° dip (Stout et al., 1943). The cave has a mapped length of over 1.5 km, an elevation of ~390 meters, and is located at North 40.24° West 83.70°. This landmark has been known and open to the public for many years (Hills, 1916; White, 1926). Numerous maps of the cave have been prepared (Hills, 1916; Richey, 1921, White, 1926; Grissom, 1997; Ohio Caverns, 2013), though none can be considered a complete representation of the karst system because many sediment plugged (unexcavated) conduits are present and therefore unmapped.

The cave is located in an area of gentle relief. The bedrock knob (Mt. Tabor) in which the cave is found rises 30 m above broad valleys infilled with glacial outwash. A thin capping of till and Devonian Ohio Shale are present above the limestone on Mt. Tabor (Fig. 4). Glaciers have overridden the knob under which Ohio Caverns lies at least twice in the past (Quinn & Goldthwait, 1979). About 19,000 years ago, a thinner sheet of ice split at Mt. Tabor, creating the Scioto Sublobe and Miami Sublobe (Quinn & Goldthwait, 1979).

The shale/limestone contact is exposed at the northeastern entrance to the cave (recently excavated) and is irregular. Observed conduit (passage) development is focused along a single bedding plane, although some lower level passages, mostly sediment filled, are known. Many of the passages in the main level were almost completely filled by sediment, which was excavated during development of trails in the cave for visitors. Individual conduit profiles are cross- or keyhole-shaped, with enlargement along the horizontal bedding plane as well as upward and downward into fractures (Figs. 5, 6). The keyhole morphology suggests initial formation under phreatic conditions, with subsequent lowering of the water table and vadose incision. The scallop studies of Brayman (2002) show that flow was primarily from east to west, and he determined a velocity of about 1 cm/s. Lower portions of the cross-section are filled with 6 m or more of clastic sediments (Brayman, 2002).
The precise age of the cave is not known, though some attempts at dating have been made. The cave is noted for its speleothems, which vary from dark red (earliest phase) to pure white (latest phase). The red speleothems are small, mostly microgour coatings. The white may commonly be 0.5 m in length, and at least one stalactite exceeds 1 m (the “Crystal King”). No dating of speleothems has been permitted.

Clastic sediment dating usually provides an age estimate closest to the age of the growth of the cave, but must be considered a minimum (Sasowsky, 1998). The clastic sediments have been examined by Hoy (1993), Brayman (2002), and Codispoti (2011). Hoy (1993) compared the cave sediment characteristics to those of the nearby glacial till, and concluded that they were similar overall, but that the cave sediments had been water lain. He ruled out limestone insoluble residue as a source. Brayman (2002) collected samples from “Mother-in-law-hole/Overlook Hole” in the cave, and identified 5 phases of fluvial deposition in a 5+ m section. Sediment texture varied from clay to sand, with minor larger clasts. The sand size fraction was dominated by carbonates, but had up to 50% sedimentary rock clasts and even up to 100% igneous rock clasts. He also determined that all were laid down in a normal magnetic chron. Because the cave is at the same elevation as current base level (adjacent valley floor), it is highly likely that the cave fill was deposited in Chron 1n (Brunhes), Middle to Late Quaternary, <780 ka.

Codispoti (2011) performed a limited analysis of 3 small sediment samples from the same location. Her attempt to use carbon dating was not successful,
because the sediments contained negligible organic matter. Likewise, no significant pollen was identified.

Two studies have examined the structural features or controls with respect to the cave. Hills (1916) noted the apparent effect of joints for localized passage segments (i.e., that the segments seem to follow joints). Codispoti (2011) was mainly concerned with speleothem deposition in the cave, but made a broad survey (n=62) of all joints in the part of the cave open to the public. The fractures measured by Codispoti show a dominant joint direction of N-NW and a secondary of NE (Fig. 7A). She concluded that these joint directions are a combined result of continental collisions during the Paleozoic and current stress regimes. She attributed the NE jointing that she measured in the cave to Paleozoic tectonism, whereas the NW jointing was attributed to regional erosional unroofing from the modern stress regime.

**METHODS**

The goal of the research was to test the hypotheses mentioned in the introduction by examining fractures that relate to conduit development in the cave. This was accomplished in 3 phases: 1) Compilation of a base map by merging existing cave surveys; 2) Observation/measurement of the fracture orientations, morphology, and nature in the cave; 3) Analysis of data en masse and in relation to position in the cave and in the landscape.

In order to focus on those fractures most important to cave forming process, we only measured fractures associated with main or side cave passage development. We refer to these as “conduit-significant fractures”. This excluded many fractures that were present in the cave ceiling at a variety of orientations, but which did not show significant lateral (dissolutional) enlargement; these we refer to as “conduit-insignificant fractures”.

Fracture strike was measured to within a few degrees accuracy by using tripod, laser pointers, Suunto compass, and Brunton pocket transit. A declination correction of 005.51°W was applied to orientation data collected in the field. If the fracture was clear on the cave ceiling, a person was positioned at a distance, and then the angle was shot towards them. In more difficult settings, a tripod upon which opposing red lasers were affixed was employed. The lasers were aligned with the fracture direction and the azimuth was measured on a straight part of the tripod head (aluminium). The southwestern branch is closed for conservation reasons by management, so no direct measurements or observations could be made there.

The cave survey data were extracted from a printed lineplot because the raw data were not available. The relationship of the cave to the landscape was analyzed by georeferencing the cave entrances to a topographic map created from Lidar data (via GlobalMapper v. 16). Azimuthal (rose plot) diagrams were created using Rockworks software.

**RESULTS**

To understand the structural controls on conduit development we examined and measured select fractures within the cave (Fig. 8) and compared them to conduit segments, local topographic aspect, and regional fracturing. We found that: 1) There were copious fractures but no predominant orientation existed; 2) The direction of fracturing varied between beds, as shown in peeled away sections of ceiling; 3) Passages in some beds did not seem to be controlled by a fracture, or were originally controlled by a fracture which had since been completely dissolved away; 4) Many of the “major” fractures which were controlling passages were very curvilinear; 5) No fresh joint faces were exposed—all had been dissolutionally weathered. Summary results can be seen in Fig. 7.
fractures along narrow ceiling apexes. Many of these exhibit a wavy/undulating form on a wavelength of 0.1 to 1 m (Figs. 9, 10). There are also passages that are straight in which no fractures are apparent in the ceiling. In some places, a thin bed (a few centimeters) is seen at ceiling level that does not have a fracture, but the overlying bed has a fracture that the conduit follows. There are also instances of en-echelon fracturing where the conduit seems to “shift” from one fracture to the other.

The comprehensive survey of conduit-significant fractures in the cave covered all portions of the cave that were open (Fig. 8). The data do not clearly reveal isolated dominant directions (Fig. 7C). The major direction class centers on 340 degrees, but there are substantial peaks at 045, 075, and also 285. We also plotted cave survey data azimuths (Fig. 7B) to see if differences emerged between the 2 data sets. The cave survey data show a peak at 345 degrees, which correlates well with the 340 peak in conduit-significant fracture orientations. The cave survey data also show lesser peaks at 035, 085, and 295 degrees, which have less compelling overlap with the joint data.

The data collected for the present study show no concordance with the fracture data (Fig. 7A) of Codispoti (2011). This is because the present work measured fractures associated with conduit enlargement, and Codispoti measured wall and ceiling joints throughout the cave, regardless of dissolutional enlargement. Therefore, her set includes the full range of discontinuities in the rock mass, as opposed to just those used in cave development.

**DISCUSSION**

The fractures observed in the cave could originate from a variety of conditions. However, the most likely causes to consider are:

i) Joints from regional (tectonic) stress fields

ii) Unloading (glacial or other)

iii) Stress relief (topographic) fracturing

iv) Glacial shear or other influences
Glacially induced structural controls

The four immediately following subsections explore the data in the context of these possible causes. The final discussion subsection synthesizes the origin of the cave in the context of the local geologic history.

Joints from regional stress fields

In many studies of caves, a clear relation between conduit orientation and regional joint sets is seen. Conjugate passage orientations, especially in maze caves, are strong indications of this. The Mt. Tabor area is in the Central Lowland physiographic province, and has not been subjected to extensive folding and faulting. But, it has been affected by wide field stresses related to the Alleghenian and other orogenies. The present state of stress in Ohio is reported (Evans, 1994) to result in joints with orientation 010 to 040 degrees. An additional regional trend for fractures in Ohio is 060 to 070 degrees (Ver Steeg, 1944) that Engelder (1982) indicates is the same orientation as the contemporary stress field. A cave that is truly controlled by regional joints will take on a network cave pattern in map view, will resemble a grid, and will have sharp, angular passage patterns (Fig. 1). In map view, Ohio Caverns does not exhibit the pattern of a network cave. Fractures created due to regional stresses are no doubt present in Mt. Tabor, but the conduit-significant data from Ohio Caverns do not show primary correlation with them (Fig. 7).

Stress relief (topographic) fractures

There are examples in the literature of caves following fractures that are not associated with regional stress fields. Along valleys in the Appalachian Plateau, there are many examples of valley parallel caves that make use of relatively young stress-relief fractures (Sasowsky and White, 1994). However, the relationship of overall cave passages of Ohio Caverns (Fig. 2) does not show such topographic concordance. If anything, the major passages of the cave seem to cut crosswise to the main valley walls.

Unloading fractures

The removal of overlying materials is recognized to produce surface parallel (unloading) fractures. The most well known unloading fractures are curved exfoliation features, usually in non-layered, homogeneous materials such as the granites of the Sierra Nevada batholith (e.g., Bahat et al., 1999) and other locations (e.g., Jahns, 1943). These have classically been ascribed to removal of confining stress, although recent work provides an alternative explanation involving near-surface compressive stresses (Martel, 2011). At Mt. Tabor, formerly overlying material could include both rock and glaciers. The effect of unloading has potential influence at Ohio Caverns due to the known factor of ice retreat. Additionally, unloading could potentially provide an explanation for the curved fractures that are seen in the cave. However, the orientation of the curved fractures in Mt. Tabor do not show concordance with the present day land surface, and in fact the map-view fractures seem to be curved in strike primarily, not in dip. Therefore, it does not seem feasible that unloading controls the morphology of the cave in map view.

It is possible, however, that the main bedding plane along which the cave formed has been “opened” by unloading. 0 to 30 m of rock overlies this plane, and it is quite likely that horizontal permeability along the plane would be enhanced by the reduced vertical stresses associated with deglaciation. This would control the vertical position along which the cave originated, but not the overall topology of the connections in map view.

Glacially-related fractures

Mt. Tabor has been overridden by ice at least twice, during MIS 6 and MIS 2 (Quinn & Goldthwait, 1979; Szabo & Chanda, 2004; Ehlers & Gibbard, 2007). Clark et al. (1994) report maximum ice thicknesses of 250 to 500 m for this area of Ohio. This process undoubtedly exerted significant mechanical, hydraulic, and thermal stresses upon this relatively
small rock mass. With an upland area of 1.18 km², and hill volume above valley floor of 7.4 x 10⁶ m³ (calculated from digital elevation model) all three effects may have been profound in the fractures they could produce. Both glacial and periglacial processes were in play.

Mechanical stresses include the loading and unloading by the ice sheets, as well as shear stresses and push imposed by the moving ice stream. Because the area is at the confluence of two lobes, we would also expect differential stresses through time as the various ice streams waxed and waned. The damaging effects of glacial stresses on bedrock are well known (Aber & Ber, 2007). In fact, masses of rock larger than Mt. Tabor, called megablocks, frequently exhibit significant shearing and faulting caused by ice push (Aber & Ber, 2007).

The forces of glacial movement upon underlying landscapes are well recognized in features such as drumlins, roches moutonnées, grooves, and so forth. During continental glaciations existing landscapes tend to be modified, but not obliterated. When the ice sheets overrode Mt. Tabor, it is easy to envision differential stresses being imposed on this topographic high as ice traversed the sides and top of the hill. Limits upon the possible shear stress are placed by the yield strength of ice, which is thought to be around 100 kPa (Menzies, 1995). However, the actual stresses applied to the landscape depend upon many factors such as speed of movement of the ice, fluid conditions at the land-ice contact, sediment content of the basal ice, configuration of the flow, etc.

Basic thermal stress was also applied to the rock mass from cooling and heating on a variety of time scales. Rutqvist et al. (2008) found that 30% of thermally induced fractures were random in direction, and this is accordant with the observed distribution of fractures. Additionally, freezing of water in the rock mass is a well-known cause of rock disintegration, as are other freezing related processes (i.e., Murton et al., 2006; Oberender & Plan, 2015). Finally, the interaction of the solid rock with glacial meltwaters from valley margin streams or moulins could potentially open up pathways in essence by “hydrofracturing”.

Synthesis of cave origin and controls

Glaciation of this region has resulted in significant changes to surface and groundwater flow systems. These changes are important to consider in the context of cave development. The area is presently drained by the southwest flowing Mad River, a tributary of the Ohio River that lies only a few km west of Ohio Caverns. Prior to 2 Ma, an ancestral Mad River served as local base level, draining to the pre-glacial Teays River, which flowed westward through central Ohio (Norris & Spicer, 1958; Melhorn & Kempton, 1991). This preglacial river was deeply incised. The present day course of the Mad River follows this now “buried” valley that holds up to 100 meters of glacio-alluvial fill. In terms of groundwater flow, Codispoti (2011) hypothesized that the local change in base level caused by the valley filling could have profoundly affected groundwater flow paths, and consequently cave development might have initially occurred along steeper gradients that were present > 2 Ma. This is possible, but the lower parts of the cave are completely filled by sediments at present, and so nothing is known of their topology and character. The currently traversable extent of the cave is almost exclusively horizontal, and at grade with the current valley floor. This supports the concept that the portion of the cave we see is genetically related to hydrologic regimes associated with the latest stages of glaciation and retreat.

The characteristics of the cave sediment infill support that the sediment is derived from adjacent drift, and that they were water transported. There is not, however, sufficient evidence to determine the age or specific linkage to surface events. The simplest interpretation would emplace the sediments at or near the same time as the valley fill deposits that surround Mt. Tabor. The valley fill directly adjacent consists of sand and gravel of Wisconsinan age (Pavey et al., 2013), but there are also tills interbedded with sand and gravel not far away. This sedimentation episode would have likely been during the Wisconsinan retreat 20 ka to 13 ka, but it is also probable that the cave sediments significantly post-date the main phase of speleogenesis. This is indicated by the substantial thickness of fill that is present, suggesting a “backing up” of existing relatively tall passages.

With regard to structural controls, the overall rock mass hosting the cave is fractured in many directions and with varying fracture density. The pervasively fractured ceiling (Fig. 11) is suggestive of some carbonate units in the Appalachians. For example the Elbrook Fm. (Cambrian) and Newala Fm.
(Ordovician; Feder, 1964) exhibit an extremely fractured nature, which expresses as a “butcher-block” pattern (Waynesboro Fm., Ordovician; Rader & Henika, 1978). This has been attributed to multiple stress fields throughout the lifetime of the units. However, the study area is far to the west, and would not be expected to suffer the same level of stresses as found closer to the orogen.

In the case of Ohio Caverns, it appears that the controlling fractures in map view may not be joints sensu stricto, but rather some combination of tensile and shear (mode-1 and mode-2) fractures, probably forming in the regime transition between tensile and shear fracturing. This is easy to envision in a situation with ice advancing over this topographic asperity, and would result in the curved fractures which are observed in many places in the cave. It can also explain the numerous fracture directions.

**CONCLUSIONS**

Conduit growth in Ohio Caverns made use of near-vertical fractures and a bedding plane as an initial template for development. However, the fractures so used do not match orientations expected from regional stress fields. The highly fractured nature of the rock indicates changing stresses that were applied tectonically, topographically, and glacially. Most of the fractures are curved and wavy, indicating anisotropy of stress or material properties. The curved fractures are likely related to stresses induced by glacial override of this small knob. Such curved joints do not develop in all glaciated limestone areas. For example, in Schoharie County, New York, many caves are developed along systematic or localized valley-parallel stress relief joints (Cooper & Mylroie, 2015). But, Ohio Caverns formed in a relatively unique setting where a small limestone knob has been overridden by a continental glacier. This setting appears to have generated an unusual structural template upon which speleogenesis could occur.

The presence of numerous fracture directions at Ohio Caverns suggests that the cave could have formed in virtually any direction, and the measured conduit directions (Fig. 7B) support this; there is no preferred orientation. The specific fractures upon which conduit development did occur appears to have been controlled by the paleohydrology of the site, because water being driven from areas of higher head to areas of lower head would follow the path of least resistance within the carbonate mass.

Normally, the regional stress regime affects the development of passages due to systematic fracturing. However, in map view it is clear that Ohio Caverns does not match the regional pattern well enough to consider it to be a template for the development of the cave. Likewise, Ohio Caverns cuts across topography, so it does not appear to be developed along topographically controlled stress-relief fractures. Furthermore, it is evident from inside the cave that the passages themselves are fracture-controlled due to their development along the near-vertical fracture planes. Overall, the cave is relatively confined to one bedding plane parting, but passage directions are influenced by the near-vertical fracturing in the cave.

The knob was most certainly subjected to unloading and stress relief during times of glacial movement and retreat. We interpret that this, along with the fact that the glaciers sliding over it were applying shear, caused the fractures to straddle the boundary between modes 1 and 2. The pattern shown by the relict cave today reflects a long and complex structural and hydrologic history. It also demonstrates a new class of discontinuities, albeit rare, that can serve as a basis for cave development.

Although available data do not allow firm correlation of the speleogenetic history to glacial and interglacial events, we can make some educated inferences. Pre-Illinoian (MIS 22+) drift deposits are not known directly from this area, but are known to the south along the Ohio River. So, initial override of Mt. Tabor by ice during this time is possible (circa 1 Ma). Ice override certainly occurred during the Wisconsinan and Illinoian (MIS 2 & 6) Stages, based upon the maximum extents of glaciation (terminal moraines, Szabo et al., 2011). So, it is possible that Mt. Tabor has been subjected to 3 episodes of glacial override, and the accompanying stresses discussed earlier in the paper. Working backwards in time from present, the sedimentary fills in the cave are similar to, and at approximately the same elevation as, the Wisconsinan drift in the adjacent valleys. This implies that sedimentation occurred during Wisconsinan deglaciation, and that the cave probably initiated during one of the earlier interglacials. The timing for the formation of the joints is less clear. They could have formed during any of the earlier glacial stages. Given the size of the passages and declining base level implied by the phreatic/vadose transition expressed in passage morphology, it is likely that the structural template upon which the cave formed was in place by the end of the Illinoian Stage (MIS 6).

It would be useful in the future to compare the results of this study of Ohio Caverns to caves in similar topographic settings in other glaciated regions with similar rock types, and to caves in non-glaciated regions. This would allow a more definitive linkage between the observed features and the glacial history. The viability of the fracturing mechanism could be further tested by finite element modeling of the rock response to ice-generated stresses, though definition of robust boundary conditions is not simple.

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REFERENCES CITED


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A decade of modern cave surveying with terrestrial laser scanning: A review of sensors, method and application development

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Abstract: During the last decade, the need to survey and model caves or caverns in their correct three-dimensional geometry has increased due to two major competing motivations. One is the emergence of medium and long range terrestrial laser scanning (TLS) technology that can collect high point density with unprecedented accuracy and speed, and two, the expanding sphere of multidisciplinary research in understanding the origin and development of cave, called speleogenesis. Accurate surveying of caves has always been fundamental to understanding their origin and processes that lead to their current state and as well provide tools and information to predict future. Several laser scanning surveys have been carried out in many sophisticated cave sites around the world over the last decade for diverse applications; however, no comprehensive assessment of this development has been published to date. This paper reviews the state-of-the-art three-dimensional (3D) scanning in caves during the last decade. It examines a bibliography of almost fifty high quality works published in various international journals related to mapping caves in their true 3D geometry with focus on sensor design, methodology and data processing, and application development. The study shows that a universal standard method for 3D scanning has been established. The method provides flexible procedures that make it adaptable to suit different geometric conditions in caves. Significant progress has also been recorded in terms of physical design and technical capabilities. Over time, TLS devices have seen a reduction in size, and become more compact and lighter, with almost full panoramic coverage. Again, the speed, resolution, and measurement accuracy of scanners have improved tremendously, providing a wealth of information for the expanding sphere of emerging applications. Comparatively, point cloud processing packages are not left out of the development. They are more efficient in terms of handling large data volume and reduced processing time with advanced and more powerful functionalities to visualize and generate different products.

Keywords: 3D scanning, LiDAR, cave modeling, speleology, geomatics, geomorphology

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INTRODUCTION

Caves are underground space formed by the process of natural weathering in carbonate rocks. The mystery of the origin of cave formations has a long history with different theories postulated during the early 19th century. Practical exploration to document this subterranean environment, in order to advance knowledge, took its modern form by the turn of 20th century. This was the time when Edouard-Alfred Martel (1859–1938) and his contemporary speleologists believed that a combination of both vadose solution and erosion provided the complete explanation for speleogenesis (Shaw, 2004a). Martel, referred to as the father of speleology (Shaw, 2004b), was active in advancing a method of cave surveying that eventually metamorphosed to a distinct field of science called speleology.

The use of caves has been a behavioral trait of human and animals alike, whose activities span several hundred thousand years until present. Prehistoric residents lived in caves on a short or long term basis as necessitated by the circumstances of the activities carried out. Even after Industrial Revolution...
hydrogeologists have realized that 3D information (see section 5). In another development, modern applications have benefited from this methodology resolution imaging camera to create a photorealistic combines 3D scanning and images taken with high heritage documentation technique in caves, which of a spatial context. Caprioli et al. (2003), El-Hakim 3D digital documentation, visualization and analysis was initiated by the realization of its potentials for improvement in underground surveying in terms of be determined. The use of total station was a major drawbacks of these early studies are, one, the TLS technology has revolutionized cave surveying, norther Spain, using a triangulation-based Minolta VI-700 scanner was carried out between 1988 and 2004). Almost 10 years after the Altamira project, a particular example is the well-known Batu Cave in Kuala Lumpur.

Ever since the foundation of speleology as a scientific discipline was established, various methods to map caves have evolved in line with advances in survey instrumentation. Early speleologists used freehand drawings as a simple way to depict and document their experience (Fryer et al., 2005). Succeeding the freehand drawing is the use of simple traditional surveying instruments like compasses, tapes and clinometers (Tsakiri et al., 2007), that was later rendered obsolete with the advent of total stations, which allows single 3D coordinate points to be determined. The use of total station was a major improvement in underground surveying in terms of methodology and accuracy, but these methods are not efficient for capturing irregular geometry such as caves (Haddad, 2011). Photogrammetry is another proven technique with potential similar to terrestrial laser scanning (TLS). The perpetual darkness in many caves renders the technique a rather arduous task (Fryer et al., 2005). In recent years, high resolution TLS technology has revolutionized cave surveying, resulting to significant shifts in the prospect for 3D cave research.

The idea of using TLS survey in cave environments was initiated by the realization of its potentials for 3D digital documentation, visualization and analysis of a spatial context. Caprioli et al. (2003), El-Hakim et al. (2004) and others pioneered today's cultural heritage documentation technique in caves, which combines 3D scanning and images taken with high resolution imaging camera to create a photorealistic models. Since then, a number of significant scientific applications have benefited from this methodology (see section 5). In another development, modern hydrogeologists have realized that 3D information from TLS is a fundamental base data to the study of karst and its geomorphological structure. According to Buchroithner and Gaisecker (2009), calcareous mountains are known for hosting large amount of ground water, so connection between hydrology and climate over time can be analyzed from TLS data (Silvestre et al., 2015). Moreover, having data depicting the shape and volume cavity of a cave will allow projecting water storage capacity. Also, a combination of elusive surface geological structure with precise observation in the cave will enable modeling scenarios such as seepage/percolation (Yumin et al., 2013) and water run-off (Silvestre et al., 2013).

From the foregoing, it is evident that there has been growing interest in three-dimensional cave mapping for different applications long before laser scanning was invented. The development and incorporation of laser technology in surveying instruments have witnessed successive improvement over the past few decades with expanding applications beyond industrial and engineering surveying. The emergence of laser scanning systems do not come as a surprise to the geomatics community, but rather an evidence of consistent stride for making available state-of-the-art surveying hardware (and software) for the geospatial professionals. TLS offers unparalleled possibilities in accuracy, speed and point density for small and medium scale topographical mapping of open and closed environments (El-Hakim et al, 2004). These factors therefore made it an acceptable and mature tool for accurate 3D cave surveying and mapping. This study intends to present a review of scanning in world’s great caves for different purposes through the exploration of high quality works published in various international journals. The paper examines cave surveying with TLS, specifically for 3D cave modeling and as base data to reference other cave information. The study attempts to clarify progress made in standardizing the method for 3D scanning particularly in cave, hardware/software design, and applications development during the last decade.

**EVOLUTION OF 3D CAVE SURVEYING WITH TLS**

Literature search reveals that protracted efforts to map cave in true 3D geometry has a long history, however, the introduction of short and medium range terrestrial laser scanners is a phenomenon. The first attempt to replicate Altamira cave, located in northern Spain, using a triangulation-based Minolta VI-700 scanner was carried out between 1988 and 2001. The project took a very long time to complete due to scanner range limitation (0.7-1.1m), excessive modeling/CAD packages and manual handling (Blais, 2004). Almost 10 years after the Altamira project, a team of researchers experimented with 3D mapping in the Upper Palaeolithic cave of Cap Blanc, southwest France in March 1999 (Robson et al., 2001). They used Surveyor Autoscanning Laser System (Surveyor ALS) to produce accurate 3D model of the cave. Two major drawbacks of these early studies are, one, the method adopted is not empirically rigorous and, two,
the instrument has limited storage capacity. Despite that, both projects, indeed, signposts the evolution of a new era in modern cave surveying.

Cyrax 2400 and Riegl LMS-Z210 are the first set of products that came to the market in 1998. Almost immediately, Cyrax 2400 was put to test in May, 1999 through a pilot project jointly initiated by Cyrax Technology and National Park Service to comprehensively explore the documentation of the complex tourist Chapel’s cave in the southwestern Oregon, USA, and also to assess its viability for 3D cave mapping (Perperidoy et al., 2010). Accurate 3D model of the cave was built from which precise horizontal and vertical sections were obtained. Cyrax 2400 was equally tested by Kanaya et al. (2001) to scan and reconstruct a prominent Japanese prehistoric Shofukuji Tomb. Another pilot study was conducted in Castellane Grotte cave, Bari, Italy by Caprioli et al. (2003). The team tested two 3D techniques; scanning with Mensi-GS100 scanner and independent photogrammetric method. A limited number of scans were taken and merged. The surface model was not reconstructed but the mesh produced depicts the structure of the stalagmite with the horizontal section. These pilot studies, obviously, proved TLS as viable and reasonable alternative method to execute 3D cave mapping. Nonetheless, the success recorded ignites renewed perspective in cave research around the world’s famous caves as several other projects follows with a chain of improvement in all ramifications.

**Previous case studies**

In this section we discuss previous caves around the world where TLS had been used for 3D cave surveying and mapping. For the purpose of comprehension, the topics are treated on regional basis.

**Australia** - El-Hakim et al. (2004) pioneered research effort in developing methodology to record the shape and appearance in the cave. In Baiame rock art cave, New South Wales, Australia, the researchers combined data obtained with Riegl LMS-Z210i and images acquired with cheap digital camera using bundle adjustment to create photorealistic 3D model. A significant achievement was made to automatically register image and 3D geometry without the need for corresponding points. The authors concluded that the geometry of cave cannot be satisfactorily represented using image-based method alone. Fryer et al. (2005) revisited the cave using different approach. They generated DEM and orthophotographs using automated image correlation software from survey control points and digital photogrammetry images. The product and accuracy was compared with the work of El-Hakim et al. (2004) and the author claimed both techniques exhibit similar capability.

**Austria** – The first sketch of Dachstein South Face Cave was produced in 1913, since then consistent visits have been made to the cave in order to accurately describe the intricate nature on map. Buchroithner and Gaisecker (2009) successfully scanned with Riegl LMS-Z420i and created a 3D model of the cave chamber from where approximate height and volume were obtained. The authors established that laser scanning has the potential to elicit richer information that can be useful for geologists to explore the structural and hydrogeological characteristics of rocks in the cave. Marchenhohele cave, Northern calcareous Alps, Styria, is another site where the efficiency of laser scanning for 3D modeling for geomorphological applications was established with Z+F Imager 5006i (Roncat et al., 2011). High precision data collected using scanner permitted the researchers to identify, analyze and describe the internal formation of the cave surface.

Eisriesenwelt, Werfen, is the world’s largest ice-filled rock cave that has attracted intense investigations on different research issues because of its prominent touristic and commercial values. With FARO Photon 20/120, Buchroithner et al. (2011) built 3D model of the cave and accurately computed the ice surface. Similarly, Petters et al. (2011) analyzed the cave morphology and identified areas prone to hazard within the cave system using the 3D model. Milius and Petters (2012) focused on providing methodical solutions to generating realistic 3D model. According to the researchers, the data will serve as the baseline for change detection and monitoring.

**The Americas** – The United States of America is one of the early places where the idea of 3D cave scanning was conceived with Chapel’s cave as pilot project (Perperidoy et al., 2010). The successful scanning of a section of the historic world longest cave, the mammoth cave in central Kentucky, running to about 4km in length, further strengthened the conviction that TLS is, undeniably, a mature technique for cave surveying (Addison, 2011). The project produced about 18 million points, which was decimated to 500,000 points in order to generate 3D model and digital animation. Again similar expedition was carried out in Coronado cave with Leica ScanStation C10 to produce 3D model and analyze passage stability for risk and hazard management (Lyons-Baral, 2012).

Another cave on record is the Preacher’s Cave, Eleuthera, Bahamas, where high definition scanning was carried out by a group of researchers from the University of South Florida’s Alliance for Integrated Spatial Technologies, GeoArch Division, in February 2006 with Leica HDS 3000 for 3D modeling and archaeological documentation (Doering et al., 2006). In Mexico, the endangered Naica Cave, Chihuahua, equally played a host to researchers of different speleological orientations to provide comprehensive understanding and knowledge of the origin and development of the famous massive gypsum crystals (Canevese et al., 2009). The geomatic research group from Canesve Surveying Company undertook the scanning task with FARO laser scanner for digital documentation of 3D data for research and educational purposes.

In a similar development, a number of European world-class caves have delivered impressive applications that rely on high resolution 3D data. In Italy, a foundational test to promote 3D scanning was inaugurated in the Italian Grotta dei Cervi, Porto Badisco in 2004 (Beraldin et al., 2006) following similar pilot project in Castellane Grotte in southern Italy (Capiroli et al., 2003). The cave was scanned...
with a prototyped laser scanning system ‘Big scan’ capable of mapping at different resolutions to produce 3D model. The Santa Barbara karst system (Sardinia), and the surrounding topography was scanned with Leica HDS6100 and Riegl LMS-Z210i to advance understanding of the morphological relationship of the two environments (Canevese et al., 2011). Furthermore, Canevese and Tedeschi (2013) used Leica HDS6100 to document Re Tiberio Cave in Mount Tondo. The cave is rich in historical and archaeological relics but faces the threat of extinction. Arma Pollera Cave (Cosso et al., 2014) and Santa Croce Cave (Marsico et al., 2015) are also among the Italian cave that have been accurately surveyed with TLS for archaeological and geomorphological applications.

France – The yearnings of paleontologist to enrich analysis of their investigations in Tautavel Cave, southern Corbieres, called for 3D scanning with Trimble GS200 equipment. The work produced a database that facilitates visualization and examination of cave finds in relation to the geometry interactively in a single system (Chandelier & Roche, 2009). The archaeological Bronze Age cave “Les Fraux”, Dordogne, France, is probably one of the most studied caves in Europe with the largest number of researchers with different background (Grussenmeyer et al., 2012). The cave consists of a network of constricted horizontal corridor adorned with a range of historical artifacts such as ceramic, metal deposits, fireplace and parietal engravings meticulously emplaced across a length of more than 1000 m. The documentation work kicked off in 2007 and lasted for six years (Burens et al., 2013). Three novel 3D technologies; FARO photon 80, FARO Focus3D and Trimble Spatial Station, and close range photogrammetry were used to produce the geometric and virtual 3D model of the cave (Grussenmeyer et al., 2010; Grussenmeyer & Guillemin, 2011). As part of the objectives to deliver a system that will be handy for the use of the multidisciplinary team, Grussenmeyer and his team (2012) proposed an adaptable recording processing workflow to integrate information collected at different scales with the 3D model of the entire cave. In a recent paper, Burens et al. (2013) further demonstrated the possibility to combine data by merging topographic, archaeological and magnetic information in the same depiction system.

Spain – is one of the European nations that have actively engaged in 3D scanning in caves for various applications. González-Aguilera et al. (2009) scanned “Las Caldas” and “Pena de Candamo” with Trimble GS200 to create 3D model of the caves at global point resolution of 20 mm. From the high-resolution model metric measurements, sections and plan were derived. In order to produce photo-realistic model of the caves, the author proposed an automatic co-registration technique to merge high resolution images acquired with digital camera with the model in a two-steps processing chain (González-Aguilera et al., 2009). In another move to consolidate strategy to facilitate recording rock arts, advance processing procedure for 3D reconstruction allowed to capture different information related to a cave to be combined in a common spatial information system. This was the case with Paleolithic rock art caves, la Loja and Buxu, which were scanned with Trimble GX scanner to provide data for efficient management and cartographic shrewdness (González-Aguilera et al., 2011). Unfortunately, the efficiency of the method is hampered with technical limitation of handing large volume of data.

Olerdola Cave is another test site in Spain where laser scanning data was combined with GIS data of the environment to allow archaeologists to reconstruct and interactively view the cave system in 3D environment. Pucci and Mambro (2009) used Riegl LMS-Z420 to generate the 3D model, which was introduced to a mobile visualization environment, ALICE, together with other GIS data for position tracking and stereo viewing. La Cova del Parpallo Cave located in Iberian Peninsula was also scanned with FARO LS 880HE to produce 3D model for geomorphological analysis. Lerma et al. (2010) combined the model with close range photogrammetric images to create the 3D model and virtual navigation in 3D. The subject of research in Pena Castil Ice Cave is to quantify temperature differences within the cave surface in relation to the geometry. Hence, thermographies and 3D model built from data collected using Leica ScanStation C10 were merged to quantitatively analyze interrelated phenomena such as ice morphologies, climate evolution, air and heat flow dynamic and other measurements (Berenguer-Sempere et al., 2014). Last but not the least, the Ardales Cave and its environment was scanned with TLS to generate accurate 3D model (Hoffmeister et al., 2014).

Other isolated cases in Europe are recorded in Greece, Portugal, and Croatia. Kefala Cave in the Greek Island of Kalymnos was surveyed with iQsun 880HE80 for rendering of 3D model of the cave structure. The researcher discovered that the accompanied processing software package could not define the center of the targets, which affects the registration process and as a result caused holes in the model (Tsakiri et al., 2007). Perpideroy et al. (2010) later used Leica Cyclone processing package to register point clouds and produced a better 3D model. Additionally, the accuracy of the model was evaluated. Another Greek site, Skoteino Cave located in Crete was scanned with Riegl LMS-Z420i laser scanner to generate 3D model as basis to understand what function the prehistory generation used the cave for (Tyree et al., 2014). In Portugal, 3D model of the karst cave, Algar do Penico (Algarve) was produced from point clouds collected with Leica ScanStation C10 to describe the morphological characteristics of the cave on the web for visualization (Silvestre et al., 2013). The researchers further their work focusing on 3D analysis by developing algorithm to extract speleothem and delivering the model on web (Silvestre et al., 2015). Similar to the French Les Fraux, the Croatian fortified cave of Kuca, is another location in Europe with abundant remnant of human presence that enlists it one of the European “Karst Underground Protection” project (Kordic et al., 2012). The cave was scanned with FARO Photon 120 to create the 3D model for digital documentation and visualization as basis
upon which targeted research findings in the fields of geodetic, geology, archaeology and anthropology can be built.

Malaysia – is among the countries in South-eastern Asia with the most revered historic caves (McFarlane et al., 2013). The presence of dozens of species of swiftlet birds and bats in large quantities vis-à-vis the symbiotic relationship with their environment, and the fallout of uncontrollable bird’s nest harvesting causing sharp reduction in species population has been a subject of investigations of late. The Niah Great Cave, Borneo (Buchroithner et al., 2012), Gua Kelawar Cave, Langkawi (Azmy et al., 2012), and Gomantong Cave, Sabah (McFarlane et al., 2015) have been scanned with FARO scanner products to produce 3D model, estimate species population, describe roosting pattern, and analyze cave morphology. Also in China, Yumin et al. (2013) documented 3D models of Grottoes and carving produced from TLS data.

Two African countries, South Africa and Egypt, are the only countries on the continent where TLS have been used in cave. Wonderwerk Cave in South Africa was fully scanned with Leica HD3000 to build 3D model as part of the African Cultural Heritage Sites documentation project (Rüther et al., 2009). The output provides realistic model for visualization and permits deriving other metric measurements. In the North African country of Egypt, the 3D model of Sodmein Cave derived from point clouds collected with Riegl LMS-Z210i was used to evaluate morphological features (Hoffmeister et al., 2014).

A general overview of these case studies shows that the rest of the world is not as active in cave research as the European nations. Does it mean that Europe have more caves than others? It is doubtful. This probably may be related to factors such as perceived importance of cultural heritage, impact of educational institutions, economy, and even access to instrument as it can be observed that most of the producers of TLS are based in Europe. It is hoped that this trend will change with the level of awareness scientific publications are rolling out.

TERRESTRIAL LASER SCANNING TECHNOLOGY

Light detection and ranging (LiDAR) systems are contact-free ranging instruments that measure and record geometric (and at times texture) information of surface targets using pulse of laser lights to create three-dimensional representations. Since late 1990s, TLS has been seen as a promising and reliable alternative to land-based survey and close-range photogrammetry for different applications (Tamás, 2010). According to findings, Riegl (Austria) and Cyrax (USA) are the first producers to break a new ground in 1998 with Riegl LMS-Z210 and Cyrax 2400 scanners respectively (see Table 1). Since then, TLS technological development has witnessed unimaginable scale of production that floods the market with different types and model of scanners available to the user community (Lerma et al., 2010). To avail users the opportunity to identify suitable scanner for specific application, scanners are generally categorized on the basis of measuring principles (pulse-based i.e. time-of-flight and phase measurement, and triangulation); scan angle (panoramic, hybrid, and camera scanners); and distances at which ranging can be achieved – (short, medium, and long range) (Caniveze et al., 2011).

Classification of TLS according to range is based on some defined maximum distance limits at which laser light can collect data. For scanners that can acquire data below 150m, they are classified as short-range scanners, while scanners with maximum range between 150m and 350m are said to be in the medium-range. TLS with effective range of up to 1000m and beyond belong to the long-range class (Petrie and Toth, 2008). Before the recent introduction of FARO Focus3D x330, the short range TLSs comprises of instruments that employ phase measurements. TOF (Time-of-flight) scanners have range measurement advantage over phase-based instruments, however, the gain in range is accompanied by reduction in the accuracy of measured distances. On the other hand, limitation in range measurement with phase instruments is offset by very high distance accuracy and faster data rate. The last category, the triangulation-based scanners, is designed to measure distances less than 5 m. They are portable and sometimes handheld devices restricted to applications such as industrial inspection and high-resolution 3D detailed documentation of archaeological artifacts such as petroglyphs and pictographs (Fryer et al., 2005; Beraldin et al., 2006; Grussenmeyer et al., 2010). Short and medium range scanners are commonly used for indoor or enclosed space like cave, tunnel, industrial plants, whereas, long-range scanners are most suitable for topographic applications. Detailed information on classification of scanners can be found in the books edited by Shan and Toth (2008) and Vosselman and Maas (2010).

In principle, scanners operate by emitting pulse of laser light to the target and receive the inbound signal which carries along with it range, elevation differences, angle and horizontal directions (González-Aguilera et al., 2009). These observables are translated into scanner Cartesian coordinates and internally processed as a set of 3D xyz points, called point cloud. In addition to the xyz coordinates, the reflectance of the surface at scanned points is simultaneously recorded as gray scale or RGB intensity image. And most recently, full-waveform laser data are increasingly available. These products and their derivatives have been widely used by geomorphologists in their research (discussed in section 4). Fig. 1 shows the gray scale intensity image of the first cave chamber in Simud Hitam, Gomantong cave.

TLS in caves

The market today is inundated with varieties of TLS with varying design, operational principles, range, accuracy and resolution. Market evaluation reveals that the number of producers and the types/models of scanners currently available are enormous, beyond what can be discussed in application-specific paper like this. We therefore limit the discussion to scanner producers and model of scanners used exclusively for 3D cave surveying.
**FARO** - based in North America, had been in business since 1981 developing varieties of advanced hand-held computer-based technologies such as laser trackers, gauge and measuring arms for 3D medical diagnosis and industrial plants metrology (www.faro.com). In 2005, FARO acquired IQvolution, a German based company that specializes in manufacturing terrestrial laser scanner and its IQsun phase shift product. FARO rebranded the IQsun 880 introduced to the market in 2004 to LS 880 after it took possession (Shan & Toth, 2008). Since then other products such as LS420, LS840, FARO photon 80 and 120, FARO Focus3D have been introduced. The introduction of light-weight FARO Focus3D in 2010 is an exceptional technological advancement that makes it easy to scan complex environments like cave.

The latest series of FARO products, Focus3D x330 and x130 launched in October 2013 and March 2014 respectively are lightest and smallest high-speed scanners designed for outdoor and indoor applications available in the market. Small weight and size, touch-screen, SD-card, and a battery life of 4.5 hours make the Focus3D X 330 supreme and easy to use. Furthermore, the capability to scan objects up to 330m away up to 330m takes the normally considered middle range Phase-shift scanning technology into the realm of the long-range. In addition to this, both scanners are multi-sensor with integrated GPS receiver, compass, height sensor and Dual Axis Compensator capabilities permit correlating individual scans. This has significantly reduced the efforts required during data collection and in post-processing.

The native software, FARO SCENE, is now optimized to automatically recognize objects that provides tool for automated target-less scan positioning with high-quality colorized scans. SCENE is efficient for 3D visualization, meshing, and exporting into various point cloud and CAD formats of third-party software for different applications. Another achievement is the ability to publish scan project on web server using the SCENE WebShare Cloud. In short, scanning is increasingly going mobile with remote scanning and virtually unlimited scan data sharing through this platform.

**Leica Geosystems** – founded in 1997 in Heerbrugg, Switzerland is a company with a mark of distinction in the manufacturing of state-of-the-art surveying instruments. Leica herald its interest in terrestrial laser scanner by first investing in Cyra Technology in 2000 and took over ownership of the company a year later. Cyra Technology, an American company incorporated in 1993, produced its first terrestrial laser scanner Cyrax 2400 in 1998 and a later model Cyrax 2500. The company initially kept its identity as a unit of Leica Geosystem, but was officially renamed Leica Geosystems HDS Inc. in 2004 (Shan & Toth, 2008). To this effect, Cyrax 2500 was changed to HDS 2500. Subsequent model with enhanced functionality called HDS3000 have emerged in 2004. Ever since, several other models such as the ultra-high speed HDS 6100 scanner and the later long range HDS8810, HDS8400 and Leica ScanStation P and C series have been put into market (www.hds.leica-geosystems.com).

In April 2, 2015, Leica Geosystems rolled out the eight generation of its high performance laser scanners, ScanStation P40, P30, and P16. This products advance into entirely new dimension in terms of range, very high speed and high-quality 3D data and HDR digital imaging that offers complete scanning solutions for varieties of applications, even under severe environmental conditions. P40 and P30 improve survey capabilities with longer range (120 m for P30 and up to 270 m for P40) while P16 operates at short-range.

Beside the advances in hardware, Leica Geosystems have improved the proprietary Cyclone software to support automatic target finding, fitting and matching to complement automated registration capability. Furthermore, a suit of other specialized packages, such as Leica CloudWorx, TruView, and JetStream, has been developed. CloudWorx is a plug-in for CAD and virtual reality applications whereas TruView provides access to view and measure scans via internet browser on any mobile devices and computer with no additional plug-in or App installed. Like the FARO Cloudshare, Leica JetStream facilitates sharing Cyclone data over data streaming server for third party users. Overall, Leica range of software packages offer the essential tools to manage, process, and distribute point cloud and other scanning products efficiently.

**Riegl Laser Measurement Systems (LMS)** – founded by Dr. Johannes Riegl in 1978, Riegl is the pioneer company that advances the development of 3D laser
technology into airborne, land-based and industrial measurement tools. Riegl put its first medium-range terrestrial laser scanner, LMS-Z210, into market in 1998, and subsequently long-range scanners LMS-Z420i and LMS-Z2110i in 2003 and 2004, respectively. In 2008, LMS-Z620 was added to the Z-series (Cheves, 2013). The Z-series have similar design features but vary in range. Concurrently in 2008, the VZ family was introduced starting with VZ-400. Subsequent very long range (a classification common to scanners with range distance above 1000 m) models VZ-1000, VZ-4000, and VZ-6000 followed, where numbers that suffix model indicate measurement range (www.riegl.com). All Riegl terrestrial scanners employ the time of flight measurement principle and have GPS-Sync option available for time stamp. RiSCAN Pro and Ri Profile are the two data manipulation software dedicated to Riegl scanners. The software equally supports automated registration, point cloud filtering using hue, saturation and brightness.

**Trimble** – established in 1978 as Trimble Navigation Limited, has placed itself as a company with outstanding reputation in providing high-tech navigation and positioning solutions. Although Trimble specializes in GPS and allied software packages to improve positioning solutions, it has successfully combined it with inertial and laser technologies to expend its market and operational sphere (www.trimble.com). Trimble entered into the terrestrial laser scanner production front in 2003 through the purchase of the Mensi Company. Mensi is a French-based company that has been established since 1986 producing short-range laser scanners utilizing triangulation measurement mode for industrial purpose. In 2001, Mensi made their first terrestrial laser scanner, GS100, that use TOF. This was followed with GS200 in 2003 with longer range and higher precision (Shan & Toth, 2008). Trimble introduced its GX 3D model in 2005, and Trimble VX Spatial station, which integrates precision total station, imaging and 3D scanning in 2007.

The latest series include advanced models Trimble TX8 with maximum range of 340m and Trimble TX5 (a repackaged FARO Focus3D model with Trimble branding and software). This was possible after FARO and Trimble signed original equipment manufacturer (OEM) and distribution agreement for 3D laser scanners in August 2012 to expand market network (Trimble, 2012). Trimble RealWorks is the proprietary point cloud processing and analysis software to efficiently register, analyze, model and create deliverables. It supports automated scan registration using both target-based and target-less workflow and can handle large volume of data. Other advance features of the latest version of RealWorks include powerful fitting tools and SketchUp Pro interoperability far accurate modeling to the point cloud.

**Zoller + Frohlich GmbH** – founded in 1963 by Hans Zoller and Hans Frohlich, the company stepped into laser scanning market in 2002 with it first compact standalone scanner IMAGER 5003 and later IMAGER 5006 in 2006. Advanced models 5006i and 5006h with superior precision, longer range and point density of the order of million points per seconds came out in 2008 and in 2010 respectively (Zoller+Frohlich, 2013). Similarly in 2010, IMAGER 5010 with integrated control panel and high-resolution color display was added (www.zf-laser.com) followed by IMAGER5010C in November, 2012 designed with touchscreen interface that make it easy to operate.

The most recent 3D scanner in the line of succession, Z+F IMAGER 5010X released in April 1 during the SPAR International 2015, is a revolution in 3D scanning technology. The device is the first-industry maneuvering for indoor navigation and movements tracking between scan positions. The navigation system will estimate the scanner position and orientation that enable automatic registration of scans immediately in the field, without the use of external targets. In fact, the integration of GPS, compass, bar, IMU, and HDR i-Cam camera brings IMAGER 5010X to the multi-sensor category, providing complete survey solution with remote scanner control. Likewise, improved WiFi speeds permits easy communication and fast scan data streaming to other portable devices for other users. Z+F scanners come along with in-house developed data processing software Z+F LaserControl Professional PLUS. The new Z+F LaserControl Scout is designed to automatically register scans on site and as well verify data quality in the field.

The list above is not exhaustive; other producers of terrestrial laser scanners are Callidus, Topcon, Optech, I-SiTE, etc. whose products are equally up to the task for the same purpose. Table 1 specifically discusses TLS products and models reported in referenced academic publications for three dimensional cave surveying.

### 3D CAVE SCANNING METHODOLOGICAL DEVELOPMENT

**Laser scanning workflow**

Surveying with TLS in the cave environment has a workflow that is somewhat comparable with that of above ground. Generally, caves are characterized with complex and naturally constrained shape and size, harsh environmental conditions, and darkness (Addison, 2011). Total darkness in caves and issue of targeting in such confined spaces are the most significant factors that differentiate the two environments, which are more of logistical concerns than scanning workflow. Although absence of light is not a barrier to collection of point data, it limits the ability to capture color data and photograph. This may not be an issue for applications such as 3D modeling and geomorphological analysis, but for applications such as photo realistic modeling and documentation of cave arts (pictographs and petroglyph), external lighting is required. In view of this, it is fundamental to apply systematic and efficient strategy that guarantees data collection and processing steps to adequately capture cave cavities in their correct three dimensional orientations and geometry, and where necessary, make provision for adequate lighting for photo capture.
Today we dare to say that in theory and practice, the methodological approach to cave surveying and 3D modeling using terrestrial laser scanners has attained full maturity with undisputable acceptance within and outside the geomatics discipline. Discussion on the standard method adopted for field procedure and data processing exercise involved in cave surveying and 3D model are classified into two stages: fieldwork and data processing (Lerma et al., 2010; Perperidoy et al., 2010; Diego Gonzalez-Aguilera et al., 2011).

Fieldwork: Planning and data collection

A standard practice in surveying that comes first before data collection is reconnaissance. In cave surveying however, this exercise is even much more critical because the cave environment is confronted with several challenges such as high temperature and humidity, movement restriction and darkness (Canevese et al., 2013; Berenguer-Sempere et al., 2014). So cave surveying needs proper planning to ensure that appropriate methods are determined and efficient processing scheme is designed ahead of fieldwork. This will reduce the length of time spent in the cave, allowing maneuvering geometric complexity and environmental unfriendliness on one hand. On the other hand, proper planning will secure the quality of data collected and invariably the 3D model (González-Aguilera et al., 2009).

The first assignment at the planning stage is to define the project objectives and to ensure that the purpose of the task and user needs is clearly understood. Next is a visit to site to identify technical and environmental constraints that need to be resolved prior to data collection. According to Lerma et al. (2010) and González-Aguilera et al. (2011), the planning stage should take into account the intricacies of the cave shape, coverage, and other physical characteristic such as illumination, temperature and mobility of the equipment and crew members before mobilizing to the cave site. In most cases, the physiological arrangement of the cave may render single point clouds insufficient to cover the entire area of interest. So, scanner positions must be painstakingly selected in a way that successive scans have sufficient overlap that will make it easy to combine point clouds from different scans. With that, issues related to instrument, number of scans, their positions and resolution, and reference coordinate system are clarified (González-Aguilera et al., 2009; Milius & Petters, 2012). It must be noted that the decision to use a particular scanner has to be

### Table 1. TLS systems discussed and their basic properties with respect to cave surveys.

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Product</th>
<th>Year</th>
<th>FOV</th>
<th>Range (m)</th>
<th>Points/Sec</th>
<th>Accuracy</th>
<th>Weight (kg)</th>
<th>Wave-length (nm)</th>
<th>Meas. mode</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FARO</strong></td>
<td>iQsun 880HE80</td>
<td>2004</td>
<td>360H</td>
<td>30</td>
<td>120,000</td>
<td>±3 mm</td>
<td>14.5</td>
<td>-</td>
<td>TOF</td>
</tr>
<tr>
<td></td>
<td>FARO LS 880HE</td>
<td>2004</td>
<td>360H</td>
<td>76</td>
<td>120,000</td>
<td>±3 mm</td>
<td>14.5</td>
<td>785</td>
<td>PS</td>
</tr>
<tr>
<td></td>
<td>FARO Photon 80</td>
<td>2008</td>
<td>360H</td>
<td>76</td>
<td>120,000</td>
<td>±2 mm</td>
<td>14.5</td>
<td>785</td>
<td>PS</td>
</tr>
<tr>
<td></td>
<td>FARO Photon 120</td>
<td>2009</td>
<td>360H</td>
<td>120</td>
<td>120,000 - 976,000</td>
<td>±2 mm</td>
<td>14.5</td>
<td>785</td>
<td>PS</td>
</tr>
<tr>
<td></td>
<td>FARO Focus 3D 120</td>
<td>2010</td>
<td>360H</td>
<td>120</td>
<td>12,200 - 976,000</td>
<td>±2 mm</td>
<td>5.0</td>
<td>905</td>
<td>PS</td>
</tr>
<tr>
<td><strong>Leica Geosystems</strong></td>
<td>Cynax 2400</td>
<td>1998</td>
<td>40H</td>
<td>50</td>
<td>800</td>
<td>±6 mm</td>
<td>20.5</td>
<td>532</td>
<td>TOF</td>
</tr>
<tr>
<td></td>
<td>Leica HDS 3000</td>
<td>2004</td>
<td>360H</td>
<td>100</td>
<td>1,800 - 4,000</td>
<td>±4 mm</td>
<td>17</td>
<td>532</td>
<td>TOF</td>
</tr>
<tr>
<td></td>
<td>Leica HDS6100</td>
<td>2009</td>
<td>360H</td>
<td>79</td>
<td>500,000</td>
<td>±1 mm</td>
<td>14</td>
<td>650 - 690</td>
<td>PS</td>
</tr>
<tr>
<td></td>
<td>Leica ScanStation C10</td>
<td>2011</td>
<td>360H</td>
<td>300</td>
<td>50,000</td>
<td>±4 mm</td>
<td>13</td>
<td>532</td>
<td>TOF</td>
</tr>
<tr>
<td><strong>RiegI</strong></td>
<td>RiegL LMS-2210</td>
<td>1998</td>
<td>330H</td>
<td>150</td>
<td>12,000</td>
<td>±25 mm</td>
<td>13</td>
<td>900</td>
<td>TOF</td>
</tr>
<tr>
<td></td>
<td>RiegL LMS-2210</td>
<td>2004</td>
<td>360H</td>
<td>400</td>
<td>12,000</td>
<td>5 mm</td>
<td>13</td>
<td>900</td>
<td>TOF</td>
</tr>
<tr>
<td></td>
<td>RiegL LMS-Z2420</td>
<td>2003</td>
<td>360H</td>
<td>350 - 1,000</td>
<td>8,000</td>
<td>±10 mm</td>
<td>16</td>
<td>NIR 905-1,550</td>
<td>TOF</td>
</tr>
<tr>
<td><strong>Trimble</strong></td>
<td>MenSi GS100</td>
<td>2001</td>
<td>360H</td>
<td>100</td>
<td>5,000</td>
<td>±5 mm</td>
<td>13.6</td>
<td>532</td>
<td>TOF</td>
</tr>
<tr>
<td></td>
<td>Trimble GS2000</td>
<td>2003</td>
<td>360H</td>
<td>200</td>
<td>5,000</td>
<td>±2 mm</td>
<td>12.8</td>
<td>532</td>
<td>TOF</td>
</tr>
<tr>
<td></td>
<td>Trimble GX 3D</td>
<td>2005</td>
<td>360H</td>
<td>200 - 350</td>
<td>5,000</td>
<td>±3 mm</td>
<td>12.2</td>
<td>532</td>
<td>TOF</td>
</tr>
<tr>
<td><strong>Zoller + Frohlich GmbH</strong></td>
<td>Z+F Imager 5006i</td>
<td>2008</td>
<td>360H</td>
<td>79</td>
<td>500,000</td>
<td>±6.5 mm</td>
<td>14</td>
<td>Visible</td>
<td>PS</td>
</tr>
<tr>
<td></td>
<td>Z+F Imager 5010</td>
<td>2010</td>
<td>360H</td>
<td>187</td>
<td>1,016,027</td>
<td>±1 mm</td>
<td>9.8</td>
<td>1,500</td>
<td>PS</td>
</tr>
</tbody>
</table>

TOF, PS are time-of-flight and phase shift
weighted relative to the technical specifications and its adaptability relative to the conditions in the cave (Canevese et al., 2011).

The underlining factor in 3D cave scanning is to provide accurate three dimensional data that can provide detail and high-quality morphometric data layer as a bedrock upon which other cave information can be integrated and analyzed spatially. This, however, requires some level of expertise and technical know-how on the part of the operator. Buchroithner and Gaisecker (2009) described two possible ways to set up terrestrial laser scanners for data collection. The first procedure is analogous to using total station where the instrument is leveled over ground control point whose coordinates are known and ‘backsight’ to another visible control point to compute the correct bearing and angle. Nevertheless, structural irregularity and perpetual darkness inside cave make this orientation-based setup impracticable. Because of this, an alternative instrument setup particularly was adopted for cave surveying. The approach allows scanner to be mounted at any location that guarantees optimal scan coverage. Fig. 2 demonstrates the later setup option with artificial targets placed around the scanner’s field of view. The targets are made of reflective surface materials for easy identification; nevertheless the accuracy of automatic registration improves with increase in scan resolution (Roncat et al., 2011). A common practice is usually to strategically place targets inside the cave before the actual survey such that at least three (preferably more) targets are at any time visible on two adjacent scans and their global positions measured accurately using theodolite (González-Aguillera et al., 2009; Rüther et al., 2009), total station (Perperidoy et al., 2010; Canevese et al., 2011), or combination of GPS and total station (Tsakiri et al., 2007; Chandelier & Roche, 2009). The availability of alternative scan registration procedures such as cloud-to-cloud and automated registration in target-less mode is gradually phasing out the use of artificial targets.

As mentioned earlier, the output of laser shots is a dense xyz point clouds computed from the signal that bounces off from the surface and stored in scanners’ local coordinate system (see Fig. 3) for 3D point cloud of the entrance of Simud Hitam. The time taken on a station to complete a scan differs from one scanner to another, depending on the scanner’s coverage (field of view) and operating scanning resolution. Scan density defines the interval between adjacent point in the horizontal and vertical plane usually in millimeters. An unavoidable choice in selecting point spacing is that compromise has to be made between scan time, coverage and scan density (Rüther et al., 2009). Meanwhile, most of the authors advocate high point density scanning which can be decimated in the course of data processing to meet specific applications.

Scanning cave in most cases will require multiple setups to cover area of interest, so overlap must be well planned for before data acquisition. A general rule of thumb is that about 25 percent overlap between two contiguous scans will yield high quality registration. Points scanned from different stations are in different local coordinate system. Therefore, registration is needed to align these individual point clouds into a single Cartessian reference frame. Moreover, in order to accurately georeference cave data and aggregate cave information, it must be tied to global coordinate system. This is usually accomplished by using surveying techniques (GPS and Total Station or Theodolite). Since GPS is ineffective inside the cave, a usual practice is to drop control points on the ground at the cave entrance and use total station to translate the coordinates underground through a network of survey points. Point clouds processing packages are designed to automatically extract target point coordinates used during registration to stitch point clouds acquired from different scans together and again to transform scanner’s Cartesian coordinate system to local or global reference framework. The data is further processed to generate 3D model and other derivatives subject to the application requirement.

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Data processing

The huge volume of data acquired with laser scanner need to be processed before it can be of value to users. Point clouds processing is a time demanding task that has to be endured (Rüther et al., 2009; Milius & Petters, 2012). To stress how time intensive it could be, González-Aguilera et al. (2011) approximated as much as three times the duration taken to complete the fieldwork. All scanners come along with proprietary software packages developed by the manufacturers to efficiently manage scans and process point clouds. Data processing stage goes through these three steps in their order of sequence: filtering and registration, 3D meshing and post-processing.

Filtering and Registration – comprises of all corrections made to point clouds to eliminate erroneous points before generating mesh surface. Editing functions usually carried out at this level are scan registration, filtering, and noise reduction (Fabio et al., 2003). The process of combining scans obtained from several positions using artificial target points is called scan registration. As mentioned earlier, scanners come with software packages that facilitate this. Iterative closest points (ICP) developed by Besl and McKay (1992) remains so far the most effective and widely used algorithm for scan alignment (Kanaya et al., 2001; Tsakiri et al., 2007; Buchroithner & Gaisecker, 2009). The algorithm employs a minimum of three points common to successive adjacent scans to automatically compute accurate transformation parameters (translation, rotation and scale factors) to glue set of scans and bring them to single point clouds (González-Aguilera et al., 2009, 2011). Occasionally the cave structure may hinder the likelihood to have adequate overlap or to identify sufficient target points, in that case homologous points appearing on adjacent scans are visually identified and interactively selected as data input into the registration process (González-Aguilera et al., 2009; Cosso et al., 2014). All point clouds processing software provide the flexibility of using the automatic and manual point correspondence to create high quality registered point clouds.

Once scans are aligned the next step is to reduce noise in the data and get rid of unnecessary points. Cleaning point clouds of unwanted data eliminates invalid data caused by instruments, lessen data redundancy, and maximizes data processing speed and efficiency (Pucci & Marambio, 2009; Cosso et al., 2014). Filtering and allocation of point clouds to feature class usually involve automatic and manual procedure. points outside the cave walls are discarded statistically using predetermined distance range or surface curvature threshold (González-Aguilera et al., 2009; Rusu & Cousins, 2011), while those not required or belonging to object groups not related to the surface are deleted manually (Petters et al., 2011). The success of this process is highly dependent on the technical skill and familiarity of the operator with the environment (cave). No matter how carefully executed, it is very rare to attain absolute point clouds coverage without gaps. The possible ways to fill the holes is either to manually add points or fill the void automatically using cluster of points in the immediate surroundings (Tsakiri et al., 2007).

3D Meshing – triangulated irregular network (TIN), created from a set of x, y, and z coordinates values, is the most widely used topological data structure to depict 3D surfaces. A Triangulation first divide input surface into regular polygonal model that encloses the sampled points. Then the polygons are further partitioned into triangles by connecting the each point to boundary points with straight lines that do not intersect. Two basic rules are critical in defining best triangles that accurately model the surface; one that the triangles are as equilateral as possible, and two that the circumference passing through the three vertexes of a triangle does not contain any other point. According to Pucci and Marambio (2009), triangulation performs three key functions: transform point clouds to a more visually perceptive facsimile, reduce data size, and permit interactivity within and across platforms.

Converting point clouds to polygonal model is more complicated in true three dimensional space than digital terrain model because it involves correct modeling of closed or freeform shapes like caves and overhang that contain multiple elevation values at the same x, y positions (Besl & McKay, 1992; Fabio et al., 2003). Two classes of surface reconstruction algorithms are particularly used for closed surfaces; they are volume oriented Delaunay triangulation (DT) and the parametric function-based B-spline curve (Fabio et al., 2003; Temizer et al., 2013). Delaunay triangulation is an optimal triangulation algorithm that satisfies all the rules for proximal surface reconstruction. The method partitions point clouds into assemblage of adjoining tetrahedrons that meet only at shared edges, summits and sides (Temizer et al., 2013). DT is efficient for storing surface representation while the model thus generated offers an advantage for quantitative analysis of volume of cavity, slope, aspect, elevation, and other geometric elements. The parametric Non Uniform Rational B-Spline (NURBS) curves produce a close-to-surface reconstruction using a set of parameters. The method describe continuous surface using polynomial equation that model as patches of curves (Buchroithner & Gaisecker, 2009). Fig. 4 is a 3D model of a section of Gomantong cave in North Borneo island.

Post-Processing – this is the final processing stage where a number of actions are taken to manually correct surface defects. Filling of holes, correcting edge defects and modification of polygons are the key-editing task done to refine three-dimensional model (Fabio et al., 2003). Triangles can be split up into two; moved to other location, completely removed or even new triangle added to fill void or fix edge problem while still respecting the integrity of the surface. Similar modification can be done to polygons by adding points, adjusting edge or vertexes in order to repair, perfect and preserve shapes. For the purpose of dissemination, visualization or manual interaction and analysis, mesh may be also compressed to reduce data to manageable size and yet preserving the geometric quality.
Point clouds processing software packages

Point clouds are basically the primary output of laser scanning which are by themselves not useful without software packages to process them. Though not at equal pace with hardware development, point cloud processing packages have received tremendous attention during the last years on three different fronts. Table 2 presents the different modeling and visualization software for 3D reconstruction of the cave geometry.

The first source is from scanner manufacturers who usually accompany their products with dedicated point clouds processing packages. This category includes FARO Scene, Leica Cyclone, RiSCAN Pro and Ri Profile, Trimble RealWorks, and Z+F LaserControl. This group of software is dedicated to TLS hardware and designed to manage scan projects and to process point clouds. Current versions have improved data storage capability and can perform both target-based and automatic scan registration. Also, they are equipped with powerful tools for point cloud editing, visualization and measurement, efficient 3D meshing, and sharing scan projects almost instantaneously via internet streaming thereby enriching point cloud mobility. Applications of these software has expended beyond the traditional point cloud processing and 3D model to other specialized purposes such as infrastructure management, forensic, multimedia, and modeling complex scenarios.

The second front, commonly referred to as third party software, comes from independent point clouds processing software developers with similar capabilities with the scanner companion packages. This group of packages accepts point clouds acquired with any scanner in the standard ASCII 3D coordinate xyz file format as input for processing and analysis. Third party software provides standalone workflow for efficient point cloud processing, editing, manipulation, animation, visualization, and analysis. In this class are PolyWorks, Geomagic, MeshLab, CloudCompare, 3DReshaper, and Bentley 3D imaging and point cloud tools (Pointools, Descartes, Map Enterprise) offering industry standard applications in infrastructure, GIS and mapping, engineering design, arts and entertainment, and manufacturing industries for prototyping, product design and inspection.

The third source is point clouds processing plug-in packages that run on AutoCAD platform. Usually professional CAD software does not offer tools to import point clouds, edit, visualize and manipulate them. So, one common way to allow editing and 3D polygonal modeling is the development of auxiliary CAD/CAM add-ons like Cloudworx (Intergraph), RapidForm (Donelan, 2002), CloudCUBE (Canese et al., 2013), PointSensese Heritage and pointCloud for AutoCAD (Milius & Petters, 2012), and Autodesk 3D Studio Max (Petters et al., 2011; Milius & Petters, 2012). CAD-enabled point clouds processing software are reverse engineering packages oriented towards providing engineering and industrial solution for automatic solid shape reconstruction. They offer powerful edge detection capability to define boundaries of surfaces and extract geometric standard shapes like pipe, steel structural elements, bridge, etc. (Rüther et al., 2009; Lundberg & McFarlane, 2012; Milius & Petters, 2012) from point clouds, and also to compute volume based on user define lines or planes.

High-resolution laser scanners with powerful point cloud processing software are resourceful tools for many applications. Powerful all-in-one survey production packages such as PointSense Heritage that combines laser scanning and photogrammetry within AutoCAD environment is gradually bringing together different surveying and engineering production workflows under a single platform.

Most of the software packages have capability for basic editing functions like point cloud cleaning and visualization, registration, meshing, quantitative measurement and support for different 3D export file formats like LiDAR Exchange Format (LAS), Virtual reality Modeling Language (VRML), XYZ, X3D, etc. (Boehler et al., 2002; Silvestre et al., 2013).
Table 2. Point clouds processing software packages.

<table>
<thead>
<tr>
<th>Package</th>
<th>Developer</th>
<th>Availability</th>
<th>Basic Point Cloud Management Capability</th>
<th>3D Modeling</th>
<th>Visualization</th>
<th>Other products</th>
</tr>
</thead>
<tbody>
<tr>
<td>FARO Scene</td>
<td>FARO Technologies</td>
<td>Commercial</td>
<td>Automated (target &amp; target-less) registration, powerful processing &amp; editing capability, colour coded 3D point cloud view. Map external photo to point cloud, data sharing via internet with SCENE WebShare</td>
<td>3D meshing &amp; editing</td>
<td>3D visualization, Texturing, fly-through, and orbits of point clouds</td>
<td>Horizontal and vertical section, volume calculations</td>
</tr>
<tr>
<td>Leica Cyclone</td>
<td>Leica Geosystems</td>
<td>Commercial</td>
<td>Automated (target &amp; target-less) registration. Efficient processing &amp; editing. Full 3D point clouds visualization, map external photo to point cloud. Web-based data sharing with Cyclone-PUBLISHER. Direct point cloud importing from other products. Supports distributed parallel computing with Cyclone-SERVER.</td>
<td>3D meshing and 3D CAD geometry/meshes from point clouds as for engineering applications</td>
<td>Fly-through animations of point cloud and model</td>
<td>Cross-sections, profiles, contours, Volumes &amp; areas,</td>
</tr>
<tr>
<td>RISCAN PRO &amp; Ri Profile</td>
<td>Regl LMS</td>
<td>Commercial</td>
<td>Automated (target &amp; target-less) registration. Efficient processing &amp; editing. Full 3D point clouds visualization, map external photo to point cloud. Web-based data sharing with Cyclone-PUBLISHER. Direct point cloud importing from other products. Supports distributed parallel computing with Cyclone-SERVER.</td>
<td>3D meshing &amp; editing, 3D CAD geometry model of pipe from point cloud</td>
<td>3D navigation, Animation with camera focus, texture mapping</td>
<td>Mesh texturing, orthophoto, create geometric objects (Point, line, cylinder, section &amp; plane)</td>
</tr>
<tr>
<td>RealWorks</td>
<td>Trimble</td>
<td>Commercial</td>
<td>Automated (target &amp; target-less) registration, 3D point cloud visualization, Advanced-Tank edition. Data sharing with publisher.</td>
<td>3D meshing &amp; editing, 3D CAD geometry model of pipe from point cloud</td>
<td>Mesh coloured with inspection colours. Photo-realistic model</td>
<td>Profile/cross-sections, volume &amp; Surface calculation, contour, video output.</td>
</tr>
<tr>
<td>Z+F LaserControl</td>
<td>Zoller + Frohlich GmbH</td>
<td>Commercial</td>
<td>Automated (target &amp; target-less) registration. Map external photo to point cloud for visualization in 3D. Data sharing via internet streaming.</td>
<td>3D meshing &amp; editing</td>
<td>Automatic texture mapping &amp; 3D movie/fly-through</td>
<td>Geometric measurements, field of view simulation</td>
</tr>
<tr>
<td>PolyWorks</td>
<td>InnovMetric Software inc</td>
<td>Commercial</td>
<td>Target-based registration, powerful point cloud editing. View point shaded by intensity or colour.</td>
<td>Real-time quality 3D meshing &amp; editing</td>
<td>Rendering &amp; 3D view, walkthrough and animation</td>
<td>Volume calculation, profile/cross-section, pipe and planar steelworks modeling and measurement in 3D</td>
</tr>
<tr>
<td>Geomagic</td>
<td>Geomagic Inc. (3D Systems)</td>
<td>Commercial</td>
<td>Scan registration and editing. Point cloud visualization</td>
<td>3D meshing &amp; polygonal editing</td>
<td>Animation</td>
<td>3D virtual and actual realities</td>
</tr>
<tr>
<td>MeshLab</td>
<td>ISTI-CNR, Italy</td>
<td>Open Source</td>
<td>Scan registration and editing</td>
<td>3D meshing &amp; polygonal editing</td>
<td>Interactive mesh painting &amp; colour</td>
<td>Supports linear measurement</td>
</tr>
<tr>
<td>CloudCompare</td>
<td>Cloud Compare Project</td>
<td>Open Source</td>
<td>Scan registration and edit point cloud. 3D colour view</td>
<td>Meshing &amp; polygon editing</td>
<td>Orthophoto, orthographic and perspective 3D view</td>
<td>Cross-section, volume &amp; surface measurement, local statistics</td>
</tr>
<tr>
<td>3DReshaper</td>
<td>Hexagon Metrology</td>
<td>Commercial</td>
<td>Scan registration and edit, extract geometric shapes, Planar &amp; cross-sections. Light model of building from point cloud.</td>
<td>3D meshing &amp; editing,</td>
<td>3D presentation, animation, &amp; photo-realistic rendering</td>
<td>Volume calculation, inspection &amp; 3D comparison, reverse engineering</td>
</tr>
<tr>
<td>*Cloudworx</td>
<td>Leica Geosystems</td>
<td>Commercial</td>
<td>Runs on Cyclone and CAD packages; allows interactive point cloud visualization</td>
<td>3D CAD geometry model of Pipe &amp; other planar surface</td>
<td>Intensity &amp; photo-quality true color mapping, TruSpace panoramic viewing</td>
<td>3D coordinate extraction Engineering design &amp; reverse engineering</td>
</tr>
<tr>
<td>RapidForm</td>
<td>3D Systems</td>
<td>Commercial</td>
<td>Manages scan</td>
<td>3D mesh &amp; polygonal editing</td>
<td></td>
<td>Rapid prototyping, product design</td>
</tr>
<tr>
<td>*CloudCUBE</td>
<td>VirtualGeo</td>
<td>Commercial</td>
<td>Manage, edit, and visualize point cloud and 3D model on AutoCAD. Direct modeling from point cloud</td>
<td>3D model</td>
<td></td>
<td>Vertical and horizontal sections, linear, angular, slope</td>
</tr>
<tr>
<td>PointCloud for AutoCAD</td>
<td>Kubit International</td>
<td>Commercial</td>
<td>Add-on to AutoCAD tool to view and work directly with Point cloud. Speed up 2D drawing, detail image mosaicing.</td>
<td>As-built piping model and other 3D construction</td>
<td></td>
<td>Create geometric model from point cloud semi-automatically. Geometric measurements</td>
</tr>
<tr>
<td>PointSense Heritage</td>
<td>FARO</td>
<td>Commercial</td>
<td>Combine laser scanning and photogrammetry within AutoCAD. Visualization of point cloud</td>
<td>3D model</td>
<td>Photo-realistic rendering</td>
<td>2D and 3D Plans, detail and sectional drawings</td>
</tr>
<tr>
<td>3D Studio Max</td>
<td>Autodesk</td>
<td>Commercial</td>
<td>3D animation, and rendering</td>
<td>3D modeling</td>
<td>3D graphic animation with camera focus in flight &amp; rendering</td>
<td></td>
</tr>
<tr>
<td>Pointools</td>
<td>Bentley</td>
<td>Commercial</td>
<td>Point cloud visualization, manipulation, editing, and animation in standalone workflow</td>
<td>As-built piping model and other 3D construction</td>
<td>Virtual fly-through with camera path and videos</td>
<td>Scaled true ortho-images, plans, facades and sections</td>
</tr>
</tbody>
</table>
Whereas others have extended capabilities such as cloud-to-cloud registration, fusion with other data, rendering, animation, fly-through, and other sophisticated visualization resources (see Fig. 5 for an advance visualization using RiScan Pro and Pointools View).

Fig. 5. Color point cloud image of Skoteino Cave looking from the back toward the entrance; advance visualization allows stratigraphy within the limestone bedrock to be clearly denoted in the cave walls (Source: Tyree et al., 2014, p. 187).

APPLICATIONS THAT HAVE BENEFITED FROM 3D CAVE SCANNING

Archaeology - Preserving cave environment through the process of recovery, documentation, analysis and archiving of things left behind is one of the key applications that promote the use of TLS in caves (El-Hakim et al., 2004; Beraldin et al., 2006; González-Aguilera et al., 2011; Grussenmeyer et al., 2012). Due to human presence for a long time in the past, caves has become a symbolic community identity venerated by generations as remain of their lineage and cultural attraction. Prehistoric human populations used cave environments for different purposes: shelter, protection, cultural, and spiritual functions. Archaeologists exploit information obtained from such materials, artifacts, and inscriptions of classical antiquities using various devices to understand the evolution of human history and culture. Documentation of cave arts requires a number of integrated hardware such as camera to capture photograph, handheld scanners like FARO Arms for high resolution scan. Integrating these information with cave scan is a complicated and time consuming task that older hard/software cannot process. This is expected to change in the near future with the evolution of more powerful hardware and efficient and high performance software.

Geomorphology - The study of geomorphological processes and characterization of the structure of cave is another scientific application that proves the relevance of 3D scanning. TLS point cloud has been efficiently utilized for 3D visualization of caves with animated fly-through and 3D colored point cloud. 3D mesh derived from point clouds accurately represents the geometry of cave topography which is very useful for geomorphometry, geomorphological mapping, landform process modeling, and, also, 3D visualization (Höfle & Rutzinger, 2011). TLS provides very detail DEM as important resources for quantitative analysis and visualization. Morphometric information such as volume of cavity, area, plan, sections, slope, elevation, etc. (Lerma et al., 2010; Canevese et al., 2011) have been accurately computed from 3D scanning. In addition to this, third level derivatives such as shaded relief maps can assist in both manual and semi-automatic feature detection and interpretation. The high vertical accuracy of TLS data makes it possible to detect geomorphological features that are difficult to access or rarely perceptible in the field. Laser intensity is another product that has been used to complement other data for geomorphological analysis in cave. Intensity image enhances geometric description, surface classification (Milius & Petters, 2012), and object detection (Azmy et al., 2012; McFarlane et al., 2015). With repetitive scanning, deformation measurement and change in cave structure and form can be detected. High-resolution 3D data is a promising offer for future comparison and analysis of changes at millimeter level that will advance understanding of the dynamic transformation within cave system.

Ice surface morphology change - Closely related to geomorphology is the scientific problem of measuring ice surface morphology and changes. 3D data is a potential basis for change detection and monitoring in the near future. Practical application of TLS has been demonstrated in Buchroithner et al. (2011) and Berenguer-Sempere et al. (2014). 3D model provides opportunities to accurately measure ice surface area and volume. Future scanning will improve understanding of ice surface morphology and dynamics and will equally benefit from glacier mass/area balance estimation.

Ecology - Caves have been a natural habitat for prehistoric human generation; and till this present time a home to varieties of animals and organisms. Ecologists have recognized the importance of 3D data in the study and analysis of inter and intra-species interaction with cave environment as they compete for food and space. For example, Burens et al. (2013) studies the interaction of Bronze Age residents of...
Les Fraux Cave through integration of magnetic field measurement with 3D scanning. In his study, he could locate position of fireplaces by analyzing thermal impact on sediment causes local distortion of magnetic field relative to the cave geometry. Biospeleology dominates studies in Malaysian caves where species population counting, roosting pattern and biogenetic modifications are analyzed from TLS intensity data (Azmy et al., 2012; McFarlane et al., 2013). Palaeoclimatology and Palaeontology - The scientific study of past climate and fossil is another field of research with interest in 3D cave scanning. Scientists exploit physical characteristics of data previously preserved within earth such as rocks, sediments, ice sheet, et cetera to reconstruct past earth climatic condition. Chandelier and Roche (2009) provide extensive analysis of paleontological information relative to Tautavel cave geometry. Ice surface morphology plays a key role in ecosystem and climate change. According to Berenguer-Sempre et al. (2014), ice melting contributes to changes in wind temperature condition in and around the cave, the process, which can be better comprehended when viewed and represented in 3D. So, he merged thermal observations with cave model to analyze temperature variation within the cave surface in relation to the geometry. In addition to that, it was possible to extract climatic variables to model the evolution and the behavior of air and heat flow (Petters et al., 2011).

Passage stability/Hazard - As more and more caves are opened to tourists, proactive measures must be taken to ensure safety of their lives at all time. Geologist and geotechnical engineers have proven the capability of TLS data for soil/rock stability analysis (Beraldin et al., 2006). There had been growing concern to identify areas in the cave that are prone to the risk of collapse and rock fall. The works of Lyons-Baral (2012) and Petters et al. (2011) identified vulnerable locations within cave system through the measurement of surface displacement from high-resolution 3D model. Beside passage stability assessment, engineers use 3D data for facility development plan (Rüther et al., 2009; Addison, 2011).

Visualization and education applications – High-level 3D models provide the basic means of viewing the structure of cave. Meshed surface on its own brings home the feel of the shape and form of caves, in addition to being able to make accurate and precise measurements. Another engaging channel of visualization combines photographic imagery with 3D scanning to create virtual cave model (Pucci & Marambio, 2009; Tyree et al., 2014). Currently digital animation and virtual fly-through are the means to convey adequately the picture of the shape of caves (Buchroithner & Gaisecker, 2009). At advanced level however, researchers are exploiting building immersive virtual reality that will give tourists a taste of physical visit to caves - virtual tourism (Cosso et al., 2014), and as well provide special opportunity to engage students, scientists, and marketers in education and outreach while still preserving history for the future generations. The GIS community has also inject geovisualization into cave research for data exploration, transmission and decision making process by combining cave information with other geospatial data for knowledge construction using different medium such as 3D color coded point cloud, 3D PDF, and 3D web-based visualization (Pucci & Marambio, 2009; Canevese et al., 2011) and web visualization (Silvestre et al., 2013).

**DISCUSSION**

TLS has become a pivotal tool that brings people of different research orientation together to open new lines of thoughts and inquiries. In general an overview of the duration under consideration can be described in three stages: proof of concept, method standardization and application development. 3D scanning in cave, which began as a “prove of concept” has been accepted today as a scientific tool of enquiry in cave. Starting from 1999 to 2003, scanners were deployed to caves just to assess its capability and thus developed new method of data collection. Then, by establishing universal procedure for scanning in cave was not given a priority, but rather how to generate models that can accurately depict the form and shape of cave and as well facilitates visualization in 3D. When it became certain that TLS provide unparalleled potential, research focus gradually shifted to “method standardization”.

Starting with the work of El-Hakim and his team in the Aboriginal rock arts cave in Baiame, Australia (El-Hakim et al., 2004; Fryer et al., 2005), the method discussed in section 4 have emerged following series of refinement between 2004 and 2012; that was the period when validation of 3D scanning in cave went through the process of maturation as a technique. Then there were concerns about the best possible way to define the level of accuracy needed to achieve optimum visual and geometric fidelity, how to maneuver with different 3D modeling packages with minimum loss in quality of data and products, in addition to coping with large volume of data and excessive processing time (El-Hakim et al., 2004; Grussenmeyer et al., 2010; Lyons-Baral, 2012). Testing with applications outside 3D reconstruction, geomorphology and archaeological documentation, particularly between 2009 and 2012, provides opportunity to scale these issues. The current state of 3D scanning sees application specific researches taking over, a situation that is influenced by technological advances in sensor (Terrestrial scanners for data collection) and improved efficiency of computer system and software solutions.

Advances in sensor technology have been evolving with such rapidity that is well ahead of software solutions. Ever since the first generations of scanners appeared, the design of TLS devices has become more and more reduced in size and weight. For example, it was observed that FARO Focus3D weighs 65 percent less than the first generation FARO LS 880HE model. In the same way, Leica HDSS6100 is about 32 percent less than the weight of Cyrax 2400 (see Table 1). This makes the new generation scanners much more mobile and suitable to survey constricted and longer
caves (Lyons-Baral, 2012). The reduction in size of these scanners has made it possible to get into confined spaces that were not possible to scan before. Beside reduction in weight, design of compact standalone scanners like Leica HDS6100, Focus3D, Imager5006i, Imager5010, etc. with soft touch screen to manage and view scanning in the field is becoming common. In fact the use of external laptop and other peripherals attached to scanners is seemingly an outdated technology. Also, an overall market trend in the newer scanners unveils an evolution of multi-sensor scanning hardware. The integration of other hardware sensors (i.e., GPS receivers, inclinometers, IMU, compass, powerful imaging cameras, and height compensator) with scanners like Focus3D x330, x130 and Z+F Imager 5010X, which have increased the capabilities, brings in sight a new era of total survey solution in the possible near future. Other physical improvement includes enhanced field of view to obtain full panoramic system i.e. 360º by 360º. This can be noticed in Riegl LMS-Z420i, which has a default 80º vertical coverage but now customized to achieve 360º via stepwise tuning at an angle of 5º (Caneseve et al., 2011).

Aside the physical characteristics discussed above, technical specifications have also steadily improved during the last decade. Initially, absence of scanners that can provide the needed 3D data for high resolution visualization was a problem (Beraldin et al., 2006), in contrast today, dealing with large volume of points acquired with high resolution scanners turn out to be a subject of concern, though still an advantage. Scanners like Focus3D, Imager5010 and the later scanner models are capable of collecting million and above points per second at relatively short time and higher precision (Burens et al., 2013; Cosso et al., 2014).

Speed and measurement accuracy that use to distinguish CW from TOF is gradually becoming neutralized as both methods now compete favorably in both aspects. Compare FARO photon 120 with Focus3D and Trimble GX with Imager5006i in Table 1. Similarly, the assertion that TOF scanners have range superiority over phase-based scanners is shifting, depending on the application. This can be observed in FARO Focus3D x33 and Z+F Imager 5010X which are PS scanner stepping into the long-range category with exceptional performance in penetration, noise reduction and measurement accuracy. As much as the aforementioned advances in hardware progresses, it cannot, in isolation, deliver without software to complement it.

There has been rapid shifting from 32-bit to 64-bit processors to increased computational performance and the speed at which tasks can be completed in modern computing. This advances in processors, coupled with cores level grading (i.e., 64-bit Core i3, i7, etc.), allow for an increased number of calculations per second that can be performed. This development has increased the processing power and makes computers run faster and more efficiently for processing large volume of point cloud which require many calculations to run smoothly. Apart from performance, the amount of memory (RAM) supported by a computer system depends on the type of processor. 32-bit computers have maximum of 3-4 GB memory it can accommodate whereas 64-bit computers support over 4 GB memory space. Another progress made in computer processing is in graphic components, which enhance the way pictures, video, animation, and 3D data are displayed on computer screen. Advancements in graphic card technology have augmented the efficiency of translating binary data from the CPU and turn it into a picture on the screen. Also worth mentioning is the growing use of distributed computing using internet infrastructure as a link to share data. This often happens between scanning hardware and other remote devices (computer and mobile platforms) that allow remote user access and process the data.

A major advance in software development is handling data volume (Silvestre et al., 2015). Most of the regular point clouds programs have been optimized to counter excessive processing time and accommodate large volume of data at reasonable processing time. Developers provide enhanced super-highway interface capabilities that make interoperability between different software possible through different data formats that allow exchange from one platform to another with minimal loss of data quality as alternative (Zoller + Fröhlich, 2013). An important achievement in this direction is the growing adoption of E57 compliant data (ASTM E57 File Format for 3D Imaging Data Exchange), which have been accepted as industry standard 3D format by most scanner producers, including all those discussed in this paper. ASTM E57 File Format provides a single common format that reduces the need to convert from one file format to another. Other advantages of the format include efficient storage and data compression (Huber, 2011). This will allow easy transfer of 3D data and other products across different processing platforms (Fabio et al., 2003), which used to be difficult with the binary format of the older versions of point cloud processing programs (Petters et al., 2011; Kordic et al., 2012).

Another development is the increasing convergence of point cloud software developers and laser scanner manufacturers. In February 2013, Geomagic Inc. was taken over by 3D Systems to emphasize CAD applications within 3D scanning. There are numerous examples of smaller software and applications companies being purchased and repackaged through scanning companies like FARO, Leica, and Trimble. These market shifts, purchases and application streamlining will continue to have a major impact on the industry and will likely increase user options and accessible use of tools that work together, rather than separate and hard to work with packages for each specific deliverable. The outcome of this takeover is expected to bolster more powerful and robust platforms that can offer complete solution for reverse engineering, 3D imaging and inspection, and virtual rendering.

Future outlook

The future holds an excellent outlook for incredible innovations in sensor technology, software for
data processing and visualization, and amazing applications. In the near future, the likelihood that miniaturized terrestrial laser scanners capable of collecting data at micro and macro resolution is envisaged. The recent novel laser scanning device used by Schiller and Pfeiler (2015) in underwater cave hints on this. Furthermore, powerful multipurpose system capable of delivering simultaneous 3D scanning, optical positioning, high resolution imaging and video technology, and at the same time communicate with external remote platform for real/near-real time data processing – with appellation such as “RoboScan” (robotic scanner) may soon be a reality.

As hardware becomes more and more sophisticated, so also there will be a pressing need for software packages to handle those huge amounts of scanned data. The problems faced with processing large data and merging images with 3D scan still persists, although less critical (Silvestre et al., 2015). Current virtual reality rendering engines are yet to reach optimal satisfaction in handling mesh structure (Silvestre et al., 2013). Progress is expected in future in response to raising number of immersive intelligence applications such as gaming and immersive virtual realities. Developing operative geovisualization packages is expected to be on the rise due to increasing need to integrate data collected by different interest groups. This decade could see the growth of solution targeted applications and packages such as Split-x (Lyons-Baral, 2012) in the fields like hydrology, structural hydrogeology, volcanology, geodetic and geodynamic, change detection, and so on.

**CONCLUDING REMARKS**

In this study, the paper presents a comprehensive account of scanning in cave during the last ten years with emphasis on 3D modeling from different perspectives, sensor (hardware), software, and applications through exploration of case studies around the world. It is clear that a lot have been achieved in all the components examined and more is still expected looking through the periscope of current developments and future direction. On sensors, great progress have been made in terms of physical features like size, weight, compactness with new innovations like touch screen, increased memory capacity, battery life time, powerful imaging and video cameras that will counter the current limitations imposed by darkness in the cave. On the technical side too, scan resolution and accuracy, coverage and range have significantly improved. Point cloud processing packages have also advanced with more functionalities, enhanced efficiency and user friendliness. It is expected that future studies will explore further the potential applications of LiDAR intensity either as a product or in combination with 3D scan, in particular to further understand the relationship between the terrestrial topography and the hypogean environment and their contributions to micro and macro climate modification. In conclusion, the current state of cave research is interesting; however the future awaits more innovative products and scientific discoveries.

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**REFERENCES**

Besl P. & McKay N., 1992 - A method for registration of 3-D shapes. IEEE Transactions on Pattern Analysis and Machine Intelligence, 14 (2): 239-256. [http://dx.doi.org/10.1109/34.121791](http://dx.doi.org/10.1109/34.121791)
Blais F., 2004 - Review of 20 years of range sensor development. Journal of Electronic Imaging, 13 (1): 231-240. [http://dx.doi.org/10.1117/1.1631921](http://dx.doi.org/10.1117/1.1631921)
A review of modern cave surveying with TLS


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Diversity of cultured bacteria from the perennial ice block of Scărişoara Ice Cave, Romania

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Abstract: Cave ice ecosystems represent a poorly investigated glacial environment. Diversity of cave ice bacteria and their distribution in perennial ice deposits of this underground glacial habitat could constitute a proxy for microbial response to climatic and environmental changes. Scărişoara Ice Cave (Romania) hosts one of the oldest and largest cave ice blocks worldwide. Here we report on cultured microbial diversity of recent, 400, and 900 years-old perennial ice from this cave, representing the first characterization of a chronological distribution of cave-ice bacteria. Total cell density measured by SYBR Green I epifluorescence microscopy varied in the $2.4 \times 10^4$ – $2.9 \times 10^5$ cells mL$^{-1}$ range. The abundance of cultured bacteria ($5 \times 10^2$ – $8 \times 10^4$ CFU mL$^{-1}$) representing 0.3-52% of the total cell number decreased exponentially with the ice age, and was higher in organic rich ice sediments. Cultivation at 4˚C and 15˚C using BIOLOG EcoPlates revealed a higher functional diversity of cold-active bacteria, dependent on the age, sediment content and physicochemical properties of the ice. The composition dissimilarity of ice microbiota across the ice block was confirmed by growth parameter variations when cultivated in different liquid media at low and high temperatures. PCR-DGGE and sequencing of bacterial 16S rRNA gene fragments from the cultured ice samples led to the identification of 77 bacterial amplicons belonging to Gammaproteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria, showing variation in distribution across the ice layers. Several identified OTUs were homologous to those identified in other glacial and karst environments and showed partial conservation across the ice block. Moreover, our survey provided a glimpse on the cave-ice hosted bacteria as putative biomarkers for past climate and environmental changes.

Keywords: ice cave; bacteria; biodiversity; 16S rRNA gene; Scărişoara

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INTRODUCTION

The increased interest in microbial communities from various frozen environments has focused on understanding their mechanism of adaptation and role in these habitats. In spite of the in depth investigation of the structural and functional microbial diversity in frozen environments (Price, 2007; Priscu et al., 2007; Margesin & Miteva, 2011; Gunde-Cimerman et al., 2012) such as polar ice sheets and glaciers (Miteva et al., 2004, Lanoil et al., 2009; Rehakova et al., 2010; Anesio & Laybourn-Parry, 2012), permafrost (Rivkina et al., 2004), mountain glacier forefields (Lapanje et al., 2012; Zumsteg et al., 2012), frozen lakes (Felip et al., 1995), sea ice (Deming, 2002), Arctic (Varin et al., 2010; Adams et al., 2014) and Antarctic permanent lake ice (Priscu et al., 1998; Dieser et al., 2010, Murray et al., 2012), very little is known about microbial communities present in cave-hosted perennial ice accumulations. Such habitats are found in caves from mid-latitude, mid-altitude mountains, where the combination of cave morphology and local climatic conditions allow for the year-round preservation of ice and associated azonal glacial climatic conditions (Perșoiu & Onac, 2012). These limited reports include isolation of bacterial species (Margesin et al., 2004) and diatom flora (Lauriol et al., 2006) from this type of habitat. Recent investigations of the microbial communities from volcanic ice caves formed on Mount Erebus, Antarctica, revealed a low diversity of bacteria and fungi in this extreme subsurface habitat (Tebo et al., 2015). However, no data on the chronological
distribution of bacteria in ice caves has been reported so far.

Scărișoara Ice Cave, located in the Bihor Mountains, NW Romania, (46°29′23″N 22°48′35″E, 1165 m asl) is a limestone cave hosting one of the oldest (>3,500 years) and largest (>100,000 m³) perennial underground ice blocks in the world (Holmlund et al., 2005; Perşoiu & Pazdur, 2011). Over the last century its underground ice block was extensively studied in order to understand climatic and glaciological processes (Racoviță, 1927; Șerban et al., 1947; Racoviță, 1994, Racoviță & Onac, 2000). More recently, it became the subject of studies aiming to reconstruct climatic and environmental changes in the region (Onac et al., 2007; Feurdean et al., 2011; Perşoiu et al., 2011a; Perşoiu & Pazdur, 2011). Unlike surface glaciers and glacier caves, the perennial ice from this cave was formed by water freezing (Perşoiu et al., 2011a). Between spring and late autumn, water infiltrates in the Great Hall area (Fig. 1A,B), forming a shallow lake (up to 20 cm deep) on top of the existing ice block. During winter, the entire lake freezes resulting in a ~20-cm thick ice layer that traps at the bottom various sediments deposited during summer. Therefore, the resulted ice block consists of ice layers of variable thickness separated by organic- and inorganic-rich sediment layers (Perşoiu et al., 2011a; Perşoiu & Pazdur, 2011). Nitrifying bacteria were identified in the limestone area of Scărișoara Ice Cave more than six decades ago (Pop, 1949). Our recent investigation of the ice deposits from this cave reported the presence of cultured bacteria in one-year old ice stalagmites formed in the Little Reservation area (Fig. 1A, C), belonging to Pseudomonas, Bacillus and Paenibacillus genera (Hillebrand-Voiculescu et al., 2013). In addition, preliminary data indicated the presence of bacterial and eukaryotic SSU rRNA genes in samples collected from 1, 400 and 900 years old ice-block layers, and the occurrence of phototrophic microorganisms in sun- and light-exposed ice using epifluorescence microscopy (Hillebrand-Voiculescu et al., 2014).

Here, we investigated the structural and functional diversity of cultured bacteria throughout the perennial ice block of Scărișoara Cave in correlation with its physicochemical and chemical parameters, using BIOLOG EcoPlates, PCR-DGGE and phylogenetic analysis of bacterial 16S rRNA gene sequences from the culturable microbial fraction isolated from five different locations of the subterranean ice. Our findings highlighted the heterogeneous distribution of cave ice-bacteria in sequential ice layers up to 900 yr-old characterized by various light exposure regimes (dark, indirect and direct sunlight) and organic matter content (clear ice and sediment-rich ice). This data, contributing to the microbial characterization of Scărișoara Ice Cave ecosystem, represents the first report on bacterial chronosequence in ice deposits from a limestone cave.

MATERIALS AND METHODS

Ice sampling

Ice samples of different ages were collected from C-dated ice layers of the perennial ice block from Scărișoara Ice Cave (Perşoiu & Pazdur, 2011). Five different samples of 1 (AD 2012), 400 (385 cal BP) and 900 (943 cal BP) years old ice were extracted from the ice block. Recent (1 year old) ice samples originated from the Great Hall from a sun-exposed site (sample 1-S) in the immediate vicinity of the entrance, and from an indirect light exposed area (sample 1-L), in the center of the cave (Fig. 1B). These samples were collected from the top of the ice block by vertical drilling, after removing ~5 cm of the superficial layer. The 400 (400-O), and 900 (900-O and 900-I) yr-old ice samples were collected from the Little Reservation ice wall (Fig. 1C) by horizontal drilling, after removing ~20 cm of the ice wall surface. Both 400-O and 900-O samples correspond to organic-rich ice layers, while 900-I represent clear ice (Fig. 1D). Sampling was carried out in triplicate from each location, under aseptic conditions (Hillebrand-Voiculescu et al., 2014). The ice surface was flame for 5-10 s prior to
the drilling procedure, and both the outer and inner surfaces of the coring auger (5-cm diameter, 50-cm length) were decontaminated with 96% ethanol and flaming after each drilling step. Ice samples were collected in sterile 1-L flasks, in the presence of an open-flame laboratory torch.

Physicochemical and chemical analyses
Carbon and nitrogen contents of the ice samples (0.5 mL) melted at 4°C were determined using a Multi N/C 3100 elemental combustion analyzer (Analytik Jena, Jena, Germany). Total nitrogen content (TN) was measured using a furnace temperature of 850°C, and the carbon content, comprising total carbon (TC) and inorganic carbon (IC), was determined at 800°C. Total organic carbon content (TOC) was calculated from two experimental data sets. The average values of the carbon and nitrogen contents, expressed as mg mL⁻¹ melted ice, and the standard deviations were calculated from three replicates. The pH, electrical conductivity (EC), and total dissolved solids (TDS) values of the melted ice samples were measured at 22°C using a Multiparameter HI9828 water quality meter (Hanna Instruments, Woonsocket, RI, USA).

Cultivation and cell density of ice-contained bacteria
Ice-contained heterotrophic bacteria were cultivated on various growth media: T1 (5 g L⁻¹ peptone, 0.15 g L⁻¹ ferric ammonium citrate, 0.2 g L⁻¹ MgSO₄·7H₂O, 0.05 g L⁻¹ CaCl₂, 0.05 g L⁻¹ MnSO₄·4H₂O, 0.01 g L⁻¹ FeCl₃·6H₂O (Bidle et al., 2007)), T2 (1 g L⁻¹ glucose, 1 g L⁻¹ peptone, 0.5 g L⁻¹ yeast extract, 0.2 g L⁻¹ MgSO₄·7H₂O, 0.05 g L⁻¹ MnSO₄·4H₂O (Bidle et al., 2007)), Luria broth (LB), and LB containing 10 g L⁻¹ glucose (LBG). Ice samples (0.5 mL) were thawed at 4°C and used for liquid media inoculation (1:10 v:v). Bacterial cultures were incubated at 4°C and 15°C with shaking for 20 and 17 days, respectively. Cell growth was monitored by measuring the OD₅₀₀ using a FluoStar Omega plate reader (BGM Labtech, Ortenberg, Germany). The growth parameters, doubling time (DT), and lag time were calculated using the DoublingTime exponential least square fitting software (Roth, 2006), and the linear fit of the exponential phase slope, respectively. The average values and standard deviations were calculated from two experimental data sets.

The total cell density of ice bacteria was determined by enumeration of SYBR Green I labeled cells using epifluorescence microscopy (Noble & Fuhrman, 1998). Melted ice samples (2 mL) were sonicated for 30 minutes in the presence of 10% Tween 20 (Fluka Chemie GmbH, Buchs, Switzerland), and further incubated for 15 minutes with SYBR Green I Dye (Thermo Fisher Scientific, Waltham, MA, USA). After passing on 0.22 mm pore size filters (Merck Millipore, Billerica, MA, USA), the strain cells were counted using an AXIO Scope A1 epifluorescence microscope (Carl Zeiss, Oberkochen, Germany).

The cell content of cultured heterotrophs from ice samples was determined by cultivation on R2A medium, commonly used for cultivation of heterotrophs from cold habitats (Reasener & Goldreich, 1985; Miteva et al., 2004; Yu & Margesin, 2014), at both 4°C and 15°C. The plates were inoculated with 100 mL of melted ice diluted 1:1, 1:10, 1:100 and 1:1000 in sterile water, and incubated at 4°C and 15°C for 51 and 13 days, respectively. The average cell density and standard deviation values were calculated from triplicate data sets, and expressed as number of colony forming units (CFU) mL⁻¹ of melted ice.

BIOLOG EcoPlates
The functional diversity of culturable bacteria from recent and old ice layers was investigated using BioLog EcoPlates (Garland & Mills, 1991; Lehman et al., 1995). The 96-well microplates containing 31 different carbon sources in triplicate were inoculated with 300 mL of melted ice, and incubated at 4°C for 68 days and at 15°C for 46 days. The color development was monitored daily using a FluoStar Omega plate reader (BGM Labtech, Ortenberg, Germany).

The carbon-source utilization pattern was analyzed based on the calculated parameters average well-color development (AWCD), substrate richness (R), Shannon-Weaver diversity index (H), and Shannon substrate evenness (E), computed for each well (i) as
\[
AWCD_i = \frac{\sum p_i}{\sum \ln (R)}
\]
and using a 0.25 (OD₅₀₀) threshold value for positive growth response. The computed parameters corresponded to 42-days growth at 4°C, and 30-days growth at 15°C, representing the shortest incubation time for reaching maximum AWCD (plateau phase) for all the samples (Pessi et al., 2012).

DNA extraction and PCR amplification
Genomic DNA was extracted from the melted ice samples cultivated on T1, T2, LB and LBG media using DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) by a modified protocol including two initial cell lysis steps. The cell pellet from 4-mL culture was incubated for 1 hour at 37°C in the presence of 200 mL TE containing 20 units mutanolysin (Fermentas, Waltham, MA, USA) per gram of cell pellet, to disrupt the Gram-positive bacterial cell wall. The resulted extract was incubated for 30 min at 56°C with 12 units proteinase K per gram of cell pellet, in the presence of ZR bashing beads (Zymo Research Corporation, Irvine, CA, USA), using a Homogenization system SpeedMill PLUS (Analytic Jena), and further processed according to the manufacturer protocol.

PCR amplification of bacterial 16S rRNA gene fragments for DGGE analysis was performed using a Thermal Cycler C1000™ (Bio-Rad Laboratories, Hercules, CA, USA). The reaction consisted of an initial 2-min denaturation step at 95°C, 35 cycles of 30 s at 95°C, 1 min at 54°C, and 1 min at 72°C, and a final 5-min elongation step at 72°C (Muyzer et al., 1993). The amplification mixture contained 40–100 ng DNA template, 0.2 μM of forward (F357-
GC: 5'- CGC CCG CCG CGC GGC GGG GGC GGG GGC CGG CCT ACG GGA GGC AGC AG-3’ and reverse (R518: 5’-ATT ACC GCG GCT GCT GG-3’) primers, 1xDreamTaq buffer containing 2 mM MgCl₂, 0.2 mM dNTP mix (Thermo Fisher Scientific), and 5 U DreamTaq DNA polymerase (Thermo Fisher Scientific), in a final volume of 50 ml. The presence and size of the PCR products were visualized by 1% agarose (w/v) gel electrophoresis.

**DGGE**

To assess the diversity of cultured ice-contained bacteria, DGGE analysis of the 16S rRNA gene fragments was performed using a DGGE-4801-220 system (CBS Scientific). Amplicons (0.5-1 mg) were loaded onto 8% denaturing polyacrylamide gels containing 37.5:1 acrylamide/ bisacrylamide (Carl Roth, Karlsruhe, Germany) and a 30-55% urea-formamide linear gradient, where 100% corresponded to 7 M urea (Serva, Heidelberg, Germany) and 40% formamide (Carl Roth, Karlsruhe, Germany). Samples were migrated for 4 h in TAE buffer (Lonza Group, Basel, Switzerland) at 60°C and 220 V. After staining for 90 min at 20°C with 0.1 µg ml⁻¹ ethidium bromide (Sigma-Aldrich, St. Louis, MO, USA), the DNA fragments were excised from the gel, incubated in 20 ml sterile MilliQ water at 4°C for 48 h, and reamplified by PCR as described above. Amplicon sequencing was carried out using the R518 bacterial primer (Macrogen, Amsterdam, The Netherlands).

The sequences reported in this study (bacterial 16S rRNA DGGE fragments) were assigned the GenBank accession numbers KP85203-KP853221, KJ454416-KJ454425, and KP219085-KP219133.

**Sequence analysis**

The DNA sequences were edited using CodonCode Aligner (www.codoncode.com) and BioEdit (Hall, 2007) for eliminating the false gaps and sequencing errors.

**RESULTS**

**Ice physicochemical and chemical properties**

In order to correlate the cell density and diversity of cultured bacteria with the physicochemical characteristics of the ice substrate, the carbon and nitrogen content, along with the pH, EC, and TDS parameters of recent and old ice samples were determined. The results (Table 1) indicated mildly alkaline pH values (7.5-7.6) for the recent (1-S, 1-L) and 400-O ice samples, with a slight increase for the older 900-O (pH 7.9) and 900-I (pH 8.0) samples. The electrical conductivity of melted ice samples varied with both the age and organic content of the sediment. Thus, the highest EC value (124 mS cm⁻¹) was measured for 1-S, showing a 2-fold decrease for both 1-L and 400-O samples. The lowest conductivity (15 - 17 mS cm⁻¹) was recorded for the oldest ice samples 900-O and 900-I. In accordance, TDS concentrations decreased with the ice age, ranging from 7 mg L⁻¹ to 62 mg L⁻¹, with the exception of 1-L and 400-O that showed similar TDS values (Table 1).

### Table 1. Physicochemical and chemical properties of ice samples. The pH, electrical conductivity (EC), and total dissolved solids (TDS) values of melted ice samples 1-S, 1-L, 400-O, 900-O and 900-I were measured at 22°C. Samples differ by their age (1, 400 and 900 years old), light regime (sun (S) and indirect light (L) exposure), and sediment content (organic sediment (O) and clear ice (I) content). The average values and standard deviation of total carbon (TC), inorganic carbon (IC), total organic carbon (TOC) and total nitrogen (TN) contents of ice samples were calculated from three different measurements. TC/TN represents the ratio of TC and TN values.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>EC (µS cm⁻¹)</th>
<th>TDS (mg L⁻¹)</th>
<th>TC (mg L⁻¹)</th>
<th>IC (mg L⁻¹)</th>
<th>TOC (mg L⁻¹)</th>
<th>TN (mg L⁻¹)</th>
<th>TC/TN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-S</td>
<td>7.48</td>
<td>124.2</td>
<td>61.9</td>
<td>41.97 ± 0.47</td>
<td>8.60 ± 0.07</td>
<td>33.38 ± 0.48</td>
<td>21.5 ± 0.03</td>
<td>19.52 ± 0.47</td>
</tr>
<tr>
<td>1-L</td>
<td>7.57</td>
<td>65.1</td>
<td>32.0</td>
<td>20.52 ± 0.44</td>
<td>9.25 ± 0.04</td>
<td>11.28 ± 0.45</td>
<td>0.53 ± 0.01</td>
<td>38.72 ± 0.44</td>
</tr>
<tr>
<td>400-O</td>
<td>7.45</td>
<td>61.6</td>
<td>30.3</td>
<td>43.72 ± 0.95</td>
<td>13.76 ± 0.07</td>
<td>29.96 ± 0.95</td>
<td>2.23 ± 0.03</td>
<td>19.60 ± 0.95</td>
</tr>
<tr>
<td>900-O</td>
<td>7.87</td>
<td>17.3</td>
<td>9.0</td>
<td>12.32 ± 0.30</td>
<td>5.84 ± 0.04</td>
<td>6.48 ± 0.30</td>
<td>0.62 ± 0.01</td>
<td>19.87 ± 0.30</td>
</tr>
<tr>
<td>900-I</td>
<td>8.03</td>
<td>15.0</td>
<td>7.0</td>
<td>8.46 ± 0.12</td>
<td>5.42 ± 0.01</td>
<td>3.03 ± 0.12</td>
<td>0.64 ± 0.09</td>
<td>13.22 ± 0.15</td>
</tr>
</tbody>
</table>

Chemical analysis of melted ice samples showed variations of the total carbon (TC), organic carbon (TOC) and total nitrogen (TN) contents (Table 1). The highest TC, TOC and TN content (~40 mg L⁻¹) were measured for 1-S and 400-O ice samples, while 1-L and 900-O/I samples had 4-fold lower values. The two organic-rich samples 1-S and 400-O also had the highest TOC content (~30 mg L⁻¹), representing 80% and 68% of the TC content, respectively. Moreover, a 3-fold lower TOC value was measured for the recent ice sample 1-L, while the oldest ice samples 900-O/I showed 5 to 8-fold lower values as compared to that of 1-S. The ice inorganic carbon IC content was generally low (5-14 mg L⁻¹), representing 20-31% of the TC content in 1-S and 400-O, and 45-64% in 1-L, and 900-O/I samples. The highest TN content was found in 1-S and 400-O, which decreased by about 4-fold in 1-L and 900-O/I. The organic rich sediments 1-S, 400-O and 900-O presented similar TC/TN values of approximately 20 mg L⁻¹, while the clear recent ice sample 1-L had a 2-fold higher TC/TN ratio. The lowest TC/TN score (13 mg L⁻¹) was found for the 900-I ice deposit.
Growth and enumeration of cultured heterotrophic bacteria

Heterotrophic bacteria from Scărișoara ice samples were cultivated on both solid (R2A) and liquid (T1, T2, LB, and LBG) media at 4°C and 15°C, in order to calculate the cell density and growing parameters of the culturable bacterial communities present in the ice block.

Bacterial abundance

The total cell density of ice bacteria stained with SYBR green 1 and measured by epifluorescence microscopy varied in the 2.4 - 22.3 x 10^4 cells mL^-1 range (Table 2). The highest bacterial content was found in 1-S and 400-O ice samples, while both 900 yr-old ice samples (900-O/I) showed a ~10-fold decrease in microbial cell density. 1-L recent ice exhibited 2-fold lower cell content relative to that of the same aged ice sample 1-S.

When grown on R2A medium at 4°C and 15°C, the culturable cell number ranged from 0.7 x 10^2 CFU mL^-1 to 7.8 x 10^2 CFU mL^-1 (Table 2). At 4°C, 1-S, 1-L and 400-O samples contained 5 - 8 x 10^4 CFU mL^-1, while in the oldest ice 900-O and 900-I the cell density was 50-100-fold lower. The bacterial communities from the recent ice samples 1-L and 1-S also exhibited the highest cell density (1-4.3 x 10^4 CFU mL^-1) when cultivated at 15°C (Table 2), whereas the old ice layers showed a 10-fold (400-O), 100-fold (900-O), and 600-fold (900-I) lower culturable population at this temperature. The density of bacteria cultivated at 4°C was higher than that growing at 15°C for all analyzed ice sediments (Table 2), suggesting a higher viability of cold-active microorganisms, particularly in the old ice layers. Moreover, on R2A, the cell density of bacteria from organic-rich ice layers 1-S, 400-O and 900-O cultivated at both 4°C and 15°C showed an exponential decrease with the age of the ice (Fig. 2). The viability of the analyzed bacterial communities showed a significant decrease with the ice age (Table 2); when cultivated at 4°C, the highest viability was found in recent ice layers (35% for 1-S and 52% for 1-L), with a significant drop to 13% in 400 yr-old ice, and as low as 2-5% in 900 yr-old ice. We found less viability of culturable bacteria when grown at 15°C; varying from 10-19% in recent ice samples to 0.3-1.5% in both 400 and 900 yr-old ice, with the lowest cultured fraction found in 900-I sample (Table 2).

Table 2. Cell density of ice-contained microorganisms. The cell content of melted ice samples 1-S, 1-L, 400-O, 900-O and 900-I was determined by SYBR Green I staining using epifluorescence microscopy. The culturable cell density was measured by plating melted ice samples on R2B medium at 4 and 15°C, respectively. The average values and standard deviations were calculated from 15 visual fields for ice-contained cells, and from triplicate data sets for cultured cells. Cell viability was expressed as percentage of cultured cells density relative to the total cell density.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ice-contained cell density (cells mL^-1)</th>
<th>Cultured cell density 4°C (CFU mL^-1)</th>
<th>Cell viability 4°C (%)</th>
<th>Cultured cell density 15°C (CFU mL^-1)</th>
<th>Cell viability 15°C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-S</td>
<td>(2.2 ± 0.3) x 10^5</td>
<td>(7.8 ± 1.4) x 10^4</td>
<td>35.0</td>
<td>(4.3 ± 0.5) x 10^4</td>
<td>19.3</td>
</tr>
<tr>
<td>1-L</td>
<td>(1.0 ± 0.3) x 10^3</td>
<td>(5.2 ± 0.9) x 10^4</td>
<td>52.0</td>
<td>(1.0 ± 0.2) x 10^4</td>
<td>10.0</td>
</tr>
<tr>
<td>400-O</td>
<td>(2.9 ± 0.9) x 10^3</td>
<td>(3.7 ± 0.4) x 10^4</td>
<td>12.8</td>
<td>(4.4 ± 0.8) x 10^4</td>
<td>1.5</td>
</tr>
<tr>
<td>900-O</td>
<td>(2.9 ± 0.3) x 10^4</td>
<td>(1.0 ± 0.5) x 10^4</td>
<td>53.0</td>
<td>(4.0 ± 0.9) x 10^4</td>
<td>1.4</td>
</tr>
<tr>
<td>900-I</td>
<td>(2.4 ± 1.1) x 10^4</td>
<td>(0.5 ± 0.3) x 10^4</td>
<td>2.1</td>
<td>(0.7 ± 0.6) x 10^4</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Fig. 2. Ice age dependence of cultured cell density. Cell density from each melted-ice sample cultivated on R2B-agar at 4°C (grey) and 15°C (black) was determined by serial dilution inoculations, as described in Methods. The cultured cell density (CFU mL^-1) of 1-year and 900-years old ice-contained microbota represent the average values (Table 2) for ice samples of same age, 1-S/1-L, and 900-O/I, respectively. Curve plot at 4°C (R = 0.956); 15°C (R = 0.999). Two sample test provided a variance value of 0.55.

Liquid media growth of ice microbiota

When the cave ice was cultivated for 20 days at 4°C and 15°C, heterotrophic bacteria from Scărișoara ice samples were cultivated on both solid (R2A) and liquid (T1, T2, LB, and LBG) media at 4° and 15°C, in order to calculate the cell density and growing parameters of the culturable bacterial communities present in the ice block.

Bacterial abundance

The total cell density of ice bacteria stained with SYBR green 1 and measured by epifluorescence microscopy varied in the 2.4 - 22.3 x 10^4 cells mL^-1 range (Table 2). The highest bacterial content was found in 1-S and 400-O ice samples, while both 900 yr-old ice samples (900-O/I) showed a ~10-fold decrease in microbial cell density. 1-L recent ice exhibited 2-fold lower cell content relative to that of the same aged ice sample 1-S.

When grown on R2A medium at 4°C and 15°C, the culturable cell number ranged from 0.7 x 10^2 CFU mL^-1 to 7.8 x 10^2 CFU mL^-1 (Table 2). At 4°C, 1-S, 1-L and 400-O samples contained 5 - 8 x 10^4 CFU mL^-1, while in the oldest ice 900-O and 900-I the cell density was 50-100-fold lower. The bacterial communities from the recent ice samples 1-L and 1-S also exhibited the highest cell density (1-4.3 x 10^4 CFU mL^-1) when cultivated at 15°C (Table 2), whereas the old ice layers showed a 10-fold (400-O), 100-fold (900-O), and 600-fold (900-I) lower culturable population at this temperature. The density of bacteria cultivated at 4°C was higher than that growing at 15°C for all analyzed ice sediments (Table 2), suggesting a higher viability of cold-active microorganisms, particularly in the old ice layers. Moreover, on R2A, the cell density of bacteria from organic-rich ice layers 1-S, 400-O and 900-O cultivated at both 4°C and 15°C showed an exponential decrease with the age of the ice (Fig. 2). The viability of the analyzed bacterial communities showed a significant decrease with the ice age (Table 2); when cultivated at 4°C, the highest viability was found in recent ice layers (35% for 1-S and 52% for 1-L), with a significant drop to 13% in 400 yr-old ice, and as low as 2-5% in 900 yr-old ice. We found less viability of culturable bacteria when grown at 15°C; varying from 10-19% in recent ice samples to 0.3-1.5% in both 400 and 900 yr-old ice, with the lowest cultured fraction found in 900-I sample (Table 2).

Community-level physiological profile (CLPP) of cave-ice microorganisms

The calculated parameters AWCD, R, and H for the melted ice incubated at 15°C and 4°C using BIOLOG EcoPlates clearly indicated a higher functional diversity in 1-S and 400-O ice layers, corresponding to AWCD of 0.7 ± 0.02 and 0.94 ± 0.09, substrate richness R of 18.38 ± 3.23 and 24.08 ± 1.44, and substrate selectivity H of 38.9 ± 4.2 and 33.5 ± 3.8, respectively.
Table 3. Growth parameters of cave ice microbiota cultivated at 4°C and 15°C. (The doubling time (DT) and Lag time values were calculated from the corresponding growth curves, as indicated in Materials and Methods).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Temperature (°C)</th>
<th>DT1 (day)</th>
<th>Lag time1 (day)</th>
<th>DT2 (day)</th>
<th>Lag time2 (day)</th>
<th>LB (day)</th>
<th>Lag time LB (day)</th>
<th>LBG (day)</th>
<th>Lag time LBG (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-S</td>
<td>4</td>
<td>3.06 ± 0.01</td>
<td>13.40 ± 0.25</td>
<td>0.91 ± 0.14</td>
<td>4.45 ± 0.18</td>
<td>1.22 ± 0.01</td>
<td>5.55 ± 0.10</td>
<td>1.26 ± 0.05</td>
<td>5.37 ± 0.16</td>
</tr>
<tr>
<td>1-L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.19 ± 0.10</td>
<td>8.97 ± 0.10</td>
<td>1.49 ± 0.03</td>
<td>7.75 ± 0.02</td>
<td>1.96 ± 0.14</td>
<td>8.39 ± 0.29</td>
</tr>
<tr>
<td>400-O</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>2.00 ± 0.05</td>
<td>9.04 ± 0.16</td>
<td>2.77 ± 0.01</td>
<td>7.84 ± 0.20</td>
<td>2.16 ± 0.02</td>
<td>10.20 ± 0.11</td>
</tr>
<tr>
<td>900-O</td>
<td>-</td>
<td>-</td>
<td>1.81 ± 0.06</td>
<td>9.84 ± 0.39</td>
<td>1.99 ± 0.10</td>
<td>10.92 ± 0.21</td>
<td>2.00 ± 0.03</td>
<td>9.41 ± 0.35</td>
<td></td>
</tr>
<tr>
<td>900-I</td>
<td>-</td>
<td>-</td>
<td>2.24 ± 0.09</td>
<td>7.95 ± 0.15</td>
<td>1.89 ± 0.05</td>
<td>7.24 ± 0.24</td>
<td>2.11 ± 0.09</td>
<td>7.31 ± 0.30</td>
<td></td>
</tr>
</tbody>
</table>

Shannon-Weaver diversity index H of 2.55 ± 0.02 and 2.7 ± 0.09, respectively (Fig. 3). In the case of the 1-L and 900-O/I samples, the AWCD, R and H values showed a significant decrease (up to 20-fold). The lowest values were obtained for 900-I (AWCD of 0.04 ± 0.01 and 0.08 ± 0.01, R of 1.04 ± 0.62 and 1.22 ± 0.51, and H of 0.18 ± 0.01 and 0.35 ± 0.11) at 15°C and 4°C temperature, respectively. All calculated parameters showed higher values for the microbial communities cultivated at 4°C as compared to 15°C. Evenness (E) values varied in the 0.88 ± 0.05 - 0.97 ± 0.12 interval, with lower average and higher standard deviation values for 900-O (0.74 ± 0.27) and 900-I (0.84 ± 0.12) samples cultivated at 4°C.

The carbon substrate utilization profile of the Scărişoara cave ice bacterial communities (Supplemental Table 4) indicated the use of Tween 40 by all samples, regardless their growth temperature. Interestingly, the extensive use of this non-ionic detergent was also found in the cases of all analyzed strains belonging to Sphingomonas sp. isolated from lake sediments of southern Finland (Rapala et al., 2005). 1-S and 400-O microbiota could utilize most of the substrates (24 and 26, respectively), with some variations with the growth temperatures, while a more limited number of substrates was used by 1-L (10) and 900-O/I (3/1).

Principal component analysis (PCA) of the BIOLOG EcoPlates variation in bacterial growth (OD595) at 4°C and 15°C on 31 different C-sources (Fig. 4) explained 64.84 % of the total variance (PC1). The ordination plot showed that the substrate utilization profiles of ice bacterial communities were location-specific; cultured bacteria from the 1-S and 400-O samples grown at 4°C and 15°C were distributed close to each other along PC1 axis, and distant from the other ice samples. The substrate utilization profiles of 900-O and 900-I samples formed a distinct group, in close proximity to the 1-L group, and independent of their growth temperature. Recent ice samples 1-S
and 1-L were separated along PC2 axis from the older (400-O and 900-O/I) ice layers. PC1 correlated (r = correlation coefficient) with specific C-sources, showing significant (p < 0.05) positive correlation with the carbohydrates D-xylose (r = 0.32) and D-mannitol (r = 0.35), the carboxylic acid 4-hydroxy benzoic acid (r = 0.31), and aminoacids L-asparagine (r = 0.33) and L-serine (r = 0.39). In addition, PC2 correlated positively (p < 0.05) with the carbohydrate α-D-lactose (r = 0.32) and carboxylic acid 4-hydroxy benzoic acid (r = 0.34), and negatively with both carbohydrates i-erythritol (r = -0.44) and D-mannitol (r = -0.44).

The PCA plot of the Shannon diversity index (H) calculated for cultured bacteria at 4°C and 15°C, in relation with the physicochemical and chemical parameters of ice samples (Fig. 5), explained 93.4% of the data variation for the separation on the first two axes. For the PC1 axis, all chemical parameters (Table 1) had approximately equal contribution to sample partition except for TC/TN, which had a large contribution to the sample separation along the PC2 axis. Therefore, sample 1-L showed a high score on PC2, being well separated from all other samples based on the TC/TN contribution. Samples 1-S and 400-O formed a separate group based on similar physicochemical characteristics. The oldest ice samples, 900-O and 900-I, were also grouped due to the pH of the ice substrate, which was more alkaline (Table 1). The Shannon-Weaver diversity H index of microbial communities grown at high (H4) and low (H15) temperatures appeared to be explained by EC (p < 0.04), TOC (p < 0.01) and IC (p < 0.03) carbon contents, and showed significant (p < 0.01) negative correlation with the pH.

**PCR-DGGE and phylogenetic composition of ice bacterial community**

The DGGE profile of PCR-amplified bacterial 16S rRNA gene fragments of ice microbial communities cultivated in different media at low and high temperatures (Fig. 6) indicated the presence of different species in all five samples. The distinct DGGE patterns of bacterial amplicons from T1, T2, LB and LBG cultures at 4°C (Fig. 6A) and 15°C (Fig. 6B) confirmed the diversity of culturable bacterial communities from each ice sample and their distinctiveness across the cave ice block. A total of 77 DGGE amplicons (Fig. 6) of cultured bacteria at 4°C (37) and 15°C (40) were excised from the gels, reamplified and sequenced, corresponding to 68 distinct bacterial OTUs showing 86-100% identity with environmental sequences (Supplemental Table 5). The closest identity to 18 OTUs originated from cold environments (glaciers, snow pits, ice nuclei, permafrost, Arctic and Antarctic soil, lake sediments, mats, etc.), with 3 from cave-related habitats (lava cave, cave drip water, and karst water rivulet), while 40 others OTUs corresponded to sequences from soil, dust and sand, ground water, coastal and deep sea sediments, rivers and streams, sea and lakes water, biofilms and sediments, thermal springs, etc. (Supplemental Table 5).

Eight bacterial OTUs were conserved among ice layers of different age and/or sediment content and demonstrated homology to *Pseudomonas*, *Serratia*, and *Rahnella* species, and uncultured clones (Supplemental Table 5). A soil bacterium 5V-07 [EU839205] homolog was found in 1-S and 900-O/I ice samples (SM4.1-S.46, SM15.900-O.84 and SM15.900-I.99) was cultivated at 4°C and 15°C, respectively. Also, two ice cave OTUs homologous to *Pseudomonas* sp. [FM161544] (SM15.1-S.93 and SM15.900-I.102) and *Rahnella* sp. [FM161540] (SM15.400-O.96 and SM15.900-O.98), were common to different aged ice layers (Supplemental Table 5). A glacier isolated *Serratia* sp. [LN680099] homolog was found in both 1-S and 900-O samples (SM15.1-S.80 and SM15.900-O.74) cultivated at 15°C. Recent (SM4.1-L.43) and 900 yr-old (SM15.900-I.101) clear ice samples contained a homologue of an Arctic thermal spring uncultured clone [JX257866]. The deep sea sediment homologue *Pseudomonas* sp. [AM111029] was encountered in both SM4.1-S.1 and SM15.900-I.64. In addition to various aged strata, common OTUs were also found in organic rich and clear ice samples of same age (SM4.900-O.10 and SM4.900-I.13), and in the recent ice samples SM4.1-S.37/44 (Supplemental Table 5, Fig. 6).
The similar migration pattern of several DGGE amplicons (Fig. 6) suggested the occurrence of additional bacterial strains common to different cave ice layers, such as the soil bacterium 5V-07 [EU839205], which was present in four of the five analyzed samples (1-S, 400-O and 900-O/I), while Pedobacter steynnii [KF583713] (1-L/400-O), Bacillus sp. [KC160801] (1-L/400-O), bacterial clone VS16-38 [JX257866] homologue (1-S/900-I), Pseudomonas clone [AY881672] (400-O/900-I), and uncultured bacterium [FJ527575] (1-S/900-O) could be identified in two different aged ice samples.

The relative abundance of the identified OTUs in Scărișoara ice samples (Fig. 7) highlighted the composition heterogeneity of cultivable bacterial communities throughout the cave’s ice block, indicating the ubiquitous presence of Gammaproteobacteria that dominated 1-S and 900-O/I ice layers, and of Firmicutes that was less represented, but with a major presence in 400-O. Bacteroidetes phylum was encountered only in 1-L and 900-O/I samples. Moreover, 1-L sample appeared to contain a higher number of taxa, comprising Actinobacteria and Betaproteobacteria in addition to Gammaproteobacteria, Firmicutes, and Bacteroidetes.

**DISCUSSION**

Our investigation revealed the presence of cultivable bacteria in all analyzed samples of Scărișoara Cave ice block up to 900 yr-old, varying in the range of $5 \times 10^2 - 7.8 \times 10^4$ CFU mL$^{-1}$, similar to other glacial habitats (Skidmore et al., 2000; Lee at al., 2011; Bell, 2012). The viability of cultured bacteria, as compared to the total cell content ($2.2 \times 10^4 - 2.9 \times 10^5$ cell mL$^{-1}$), showed a remarkable drop in older ice strata, shifting from 35-52% culturability in recent ice to 0.3-5.3% in the 900 yr-old ice samples.

Cultivation in the presence of different substrates and at different temperatures revealed a various response in terms of growth lag time and doubling time of bacterial communities from all analyzed ice samples, demonstrating their compositional heterogeneity. The abundance of the cultured cave ice-contained bacteria appeared to be influenced by the age and physicochemical properties of the ice substrate, with a lesser impact of ice chemistry (carbon and nitrogen contents).

The cell density of culturable microbiota decreased exponentially with the age of the ice layer. The recent ice samples 1-S and 1-L exhibited the highest cell content when cultivated on R2A at low and high temperatures, while the lowest viable cell content was found in 900 yr-old ice. The viability of cells cultivated at 4°C was generally higher than that at 15°C in all the analyzed ice samples, suggesting a higher resilience of cold-adapted microorganisms in this habitat. A significant difference between bacterial communities cultured at 4°C and 15°C was observed in 400 yr-old ice, favoring low-temperature culturable microbiota.

Physicochemical parameters of the ice samples appear to have a strong effect on the cultivable bacterial cell content of this habitat. The high cell density of heterotrophs from the sediment-rich ice samples 1-S and 400-O suggested that the activity and survival of cells embedded in cave-ice was influenced by the high sediment content and TDS content of the ice layers. No clear dependence of the cell content and functional variability of the...
samples on the ice chemical characteristics was observed; the recent ice samples 1-S and 1-L showed a comparable culturability, despite of different TOC and TN concentrations and utilized carbon-sources (BIOLOG EcoPlates). Considering the similarities of 1-S and 400-O ice formation (Perşoiu & Pazdur, 2011), this quantitative characteristic of cave-ice bacterial communities could be strongly related to the physicochemical characteristics of the cave ice layers. In the case of the common-origin ice layers 900-O and 900-I, both the chemical properties and cultured microbial contents do not showed significant variations, confirming a strong correlation of the bacterial content mainly with the ice age.

The C/N composition of the ice sediments varied considerably with the distance from the cave entrance. The distinct ice formation pattern of 1-S and 1-L samples, corresponding to surface-enriched organic sediments, and cryogenic cave carbonate enriched ice, respectively, was reflected in the total and organic/inorganic carbon and nitrogen contents of the two recent ice samples. Thus, the higher TOC and TN contents of 1-S could originate from both the direct influx of surface-derived organic matter, due to the proximity of the site to cave entrance, and from the activity and decomposition of photrophs flourishing in the sunlit supraglacial lake formed during the warm period (data not shown). Surprisingly, the inorganic carbon (IC) content of the two surface samples 1-S and 1-L was similar, indicating that the variations of dissolved carbonate and cryogenic cave carbonates formed in the two locations (Zak et al., 2008; Perşoiu et al., 2011b) were removed in the supraglacial lake, with little or no contribution from bacteria to the calcification process.

As shown by Perşoiu & Pazdur (2011), the ice formed near the cave entrance (sample 1-S, Fig. 1A) was rich in surface-originating materials (Feurdean et al., 2011), while that in the central part of the Great Hall (sample 1-L, Fig. 1A) contains mainly autogenic material composed of cryogenic cave carbonates and carbonates derived from the weathering of the cave walls (Zak et al., 2008).

In addition, climate at the time of ice layers formation appeared to play a role in geochemical composition of ice strata, and influence the culturable fraction of embedded bacterial communities. Thus, the climate associated to sample 900-O formation (~1550 AD, the peak of the Medieval Warm Period - MWP) was slightly warmer and drier than during the genesis of 400-O sample (~1050 AD, the peak of the Little Ice Age - LIA). These climatic differences implied changes in the forest composition above the cave, with dominance of beech (Fagus sylvatica) during MWP, and of spruce (Picea abies) during the LIA, respectively (Feurdean et al., 2011). The high TOC and TN contents of the 400-O ice sample relative to 900-O could be related to the enrichments in carbon and nitrogen of the cave-surrounding soil formed during spruce-dominated forests period (LIA), as compared to the one formed during beech-dominated forests period (MWP) (Vesterdal et al., 2008).

The functional heterogeneity of cultured ice bacteria throughout the cave ice block was revealed by the various responses in terms of growth lag time and doubling time when cultivated at 4°C and 15°C on different substrates. The CLPP analysis using BIOLOG EcoPlates revealed an overall higher functional diversity of cold-active bacteria throughout the cave ice block, based on higher H values when cultivated at 4°C. Except for the general use of Tween 40 by all analyzed samples of the cave ice block (Rapala et al., 2005), the carbon-source utilization varied with the age and sediment content of the ice substrate. The functional heterogeneity estimated by BIOLOG EcoPlates cultivation showed a relatively grouped PCA distribution of ice bacterial communities based on the age of the ice, which was independent of their sediment content (900-O and 900-I samples) and light regime (1-S and 1-L samples). In the case of 1-S and 400-O samples, a correlation of cultured bacterial composition and functional heterogeneity with the physicochemical properties of the ice layers could be observed, in direct correlation with their relatively grouped PCA distribution.

In addition, the bacterial functional diversity of the cultured segment of ice microbiota appeared to be dependent also on the origins of water source that formed the different areas of Scărişoara ice block, as indicated by the large shift (2-5-fold reduction) of the H values of the 1-S vs.1-L and 400-O vs. 900-O samples, respectively, and their PCA distribution associated to the physicochemical parameters. Meanwhile, the ice samples of a high sediment-content (1-S, 400-O and 900-O) were characterized by the same TC/TN ratio, but had different cell numbers and culturable bacteria substantiating a moderate impact of the cave-ice chemistry on the microbial community structure from Scărişoara ice block. This culturable ice-embedded microbiota appears to be dependent in particular on the age and physicochemical properties of the ice substrate.

The 77 identified bacterial sequences in cultured ice samples up to 900 years old were assigned to various species of Pseudomonas, Carnobacterium, Bacillus, Lysinibacillus, Flavobacterium, Pedobacter, Arthrobacter, Serratia, Yersinia, and unspecified uncultured bacteria. These strains were affiliated to Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria phyla, dominated by Gammaproteobacteria. Common bacterial strains with those described in various cold habitats (Segawa et al., 2010; Margesin & Miteva, 2011; Wong et al., 2011; Anesio & Laybourn-Parry, 2012) were found in Scărişoara cave ice block, confirming a common distribution of specific strains in glacial environments. Among these, the Arthrobacter strain SM4.1-L.33 [KF853212] identified in recent ice deposits (1-L sample) of Scărişoara cave and grown at 4°C corresponded (97% identity) to a strain isolated from a 25,000 years old permafrost ice wedge (Katayama et al., 2007). Facultative psychrophilic species belonging to this genus (Arthrobacter psychrophilicus) have also been identified in an Austrian alpine ice cave (Margesin et al., 2004).
Interestingly, the presence in old ice strata (400-O and 900-O) of a karst water-specific *Rahnella* species, an endophytic bacteria from spruce (*Picea abies*) seeds (Cankar et al., 2006), provides a putative microbial biomarker candidate for distinguishing between the two periods based on the quantitative representation of this bacterium in ice layers from dominating (LIA) or scarcer (WMP) spruce forests occurring in the surroundings of the cave at the time of the ice deposition (Feurdean et al., 2011). However, quantitative analysis of both *Rahnella* representation as well as climate and vegetation dynamics outside the cave is required in order to confirm this hypothesis. Another cave-associated bacterium was the cultured strain SM15.400-O-0.62 [KJ454424] from 400-O ice sample, homologous (99% identity) to a lava-cave bacterium clone, indicating the presence of specific bacteria for cave environments. Conservation of bacterial species in increasing aged ice sediments, most of them as culturable organisms, was confirmed in the cases of different OTUs belonging to particular *Pseudomonas* and *Paenibacillus* genera, and uncultured bacteria from soil and Arctic thermal springs.

In addition to the identified cultured phytypes, the high ratio (28.2% average value) of unclassified bacterial OTUs found in Scărişoara Cave ice block, reaching 42.8% in the case of 400-O sample, suggested a higher bacterial diversity of the cave ice microbiota. Also, the low identity score (86-96%) of several cultured ice bacterial sequences suggest the ability to identify novel species in this glacial habitat. Further investigations of environmental samples should provide a more accurate overview of the cave ice-microbiota, overcoming the limitations induced by cultivation and PCR amplification, and revealing also the autotrophic bacterial communities that are expected to be prominent in this type of habitat.

This first report on the time and space-dependence of cultured bacterial communities from a perennial cave ice block and continues to contribute to the characterization of Scărișoara Ice Cave, which is already well-documented glacial habitat from geological and palaeoclimatic perspectives (Racoviță & Onac, 2000; Persoiu & Pazdur, 2011). By identifying bacterial species, and highlighting differences on the abundance, distribution, and diversity of the cave ice block-embedded bacterial communities for the last millennium, our data support the hypothesis of a close relationship between climatic-related source microbiota and the cave-ice bacterial community composition, allowing for identifying possible climate biomarkers in this underground glacial habitat.

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**REFERENCES**


Castresana J., 2000 - Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution, 17: 540-552. [http://dx.doi.org/10.1093/oxfordjournals.molbev.a026334](http://dx.doi.org/10.1093/oxfordjournals.molbev.a026334)


Deming J.W., 2002 - Psychrophiles and Polar regions. Current Opinion in Microbiology, 5: 301-309. [http://dx.doi.org/10.1016/S1369-5274(02)00329-6](http://dx.doi.org/10.1016/S1369-5274(02)00329-6)


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In Memoriam: Gheorghe Racoviţă (1940-2015)

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Dr. Gheorghe Racoviţă, an eminent Romanian biospeleologist, died on 1 December 2015, at the age of 75. His passing leaves many in the national and international speleological community (and beyond) reflecting on his significant contributions to the discipline and the various ways he impacted the careers of those who knew him.

Dr. Racoviţă was a Senior Scientist at the “Emil Racoviţă” Institute of Speleology (ERIS) in Cluj-Napoca, where he joined the research group in 1963. He also held an appointment as Associate Professor in the Department of Biology, Babeş-Bolyai University in Cluj-Napoca. Gheorghe earned his BS (1962) and PhD (1978) in Biology from Babeş-Bolyai University in Cluj and the Institute of Biological Research in Bucharest, respectively. As a grandson of the great Romanian scientist Emil Racoviţă - well-known for his role as chief biologist of the “Belgica” scientific expedition in Antarctica (1897-1899) and later as the founder of world’s first Speleological Institute (1920) in Cluj - Gheorghe Racoviţă was inspired by his grandfather’s passion for researching and understanding the subterranean fauna. Gheorghe’s legacy is evidenced by almost five decades of scientific contributions to the fields of quantitative taxonomy, origin and evolution of cave fauna, cave climatology, and karst protection. While serving in the ERIS, he authored and co-authored more than 140 papers, 12 books, 20 book chapters, and 20 articles, disseminating science to a broader audience. A selection of his publications are listed below.

Dr. Racoviţă helped establish quantitative taxonomy and statistical analysis of cave climate data as viable and important tools in biospeleological and physical karstology studies. Like his grandfather, he was a strong advocate for using science to promote and advance environmental protection of karst regions. He supervised over 25 Bachelors, Masters, and PhD students at the Babeş-Bolyai University and ERIS. Gheorghe was soft-spoken, a good listener, a tireless worker, a very conscious editor, and a patient supportive mentor.

Listed below are some of Racoviţă’s many scientific accomplishments:

• collaborated with other ERIS researchers to study cave topoclimate (e.g., statistical analysis of various climate parameters, modeling cave ventilation, etc.);
• pioneered cave glaciology by investigating ice dynamics and climate evolution studies based on the ice block from Scărişoara Ice Cave;
• studied the anthropic impact on cave climate and fauna;
• made definitive contributions to the success of the biospeleological research conducted during the 1969 Cuban-Romanian Expedition. The monograph that published the results of this expedition received the Romanian Academy Award for Excellence in Research (1973).

Dr. Racoviţă was truly extraordinary - he was revered by generations of scientists and colleagues worldwide, and also by the eclectic group of biologists, geologists, geographers, paleontologists, and others that make up the Speleological Institute.

The karst and cave community will deeply miss this awe-inspiring scientist, mentor, and friend.

Selected books, chapter, edited (co-edited) volumes, and papers by Gheorghe Racoviţă:


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