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ECO-FRIENDLY REMEDIATION OF LAMPENFLORA ON SPELEOTHEMS IN TROPICAL KARST CAVES

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Abstract

This paper presents an experiment on lampenflora removal in show caves located in a tropical monsoon climate in southeast Asia. Lampenflora thrive in wet conditions on surfaces directly illuminated by white light. They colonize different levels in show caves, from the cave ceiling, with a biota characterized of mainly cyanobacteria (Oscillatoria, Spirulina), algae (*Chlorella, Oedoclarium*), and mosses (*Cyathodium, Thuidium*), to near the cave floor, with a more complex biota including higher plants like ferns (*Asplenium*) and flowering plants (*Centella*). Mature lampenflora mats also harbor non-phototrophic fungi and bacteria. With the use of environmental scanning electron microscopy, speleothem surfaces were found severely damaged by lampenflora and their associates. In this study, we used H_2O_2 as an environmentally friendly chemical to exterminate lampenflora. The applied solution should be at least 15% H_2O_2 to efficiently destroy microbiota such as green algae, diatoms, and bacteria. For a complex community including mosses, fungi, and vascular plants, repeated spraying of chemical and, if possible, water jet washing at carefully selected places are required to recover the aesthetic characteristics of speleothems. Only a combination of such cleaning practices, and then some modification of the illumination regime, can minimize lampenflora development in show caves.

Introduction

Lampenflora is a community of phototrophic organisms and their associates developing at sites where light is provided from artificial sources rather than natural circumstances (Mulec and Kosi, 2009). It is a widespread problem in many show caves, as it modifies the appearance of the cave's interior, and more importantly, bio-deteriorates the various types of substrate onto which it is attached (Roldán and Hernández-Mariné, 2009). Thus, removal of lampenflora is of particular interest to cave conservation and management (Piano et al., 2015).

In general, removal of lampenflora should be complete, practicable, and done harmlessly to the cave environment. Since caves are confined spaces, the use of herbicides, a usual method applied in agriculture, should not be considered. Mechanical cleansing with the use of brush or water jet is not effectual, as mature and complex lampenflora may entangle or intertwine with abiotic substrates that are not easily detached. The use of strong oxidating agents to remove lampenflora has been reported, and biota have been removed by a sodium hypochlorite solution (Mulec and Kosi, 2009), which effectively eradicates any presence of cyanobacteria, algae, and mosses. During the cleansing operation, however, measurable amounts of chlorine as well as other compounds are released into the atmosphere and pollute the cave environment. The disappearance of some insect species in some show caves could be connected with these toxic substances (Faimon et al., 2003).

Alternatively, hydrogen peroxide (H_2O_2) has recently been recently introduced and is thought environmentally friendly (Kubesova, 2000). Oxidation of organic matter, simplified as $(CH_2O)_n$, can be expressed as $(CH_2O)_n + 2nH_2O_2 \rightarrow nCO_2 + 3nH_2O$. Here, H_2O_2 appears to be a more eco-friendly agent than hypochlorites or herbicides, as fewer oxidation byproducts are released to the environment.

Our review concludes that previous studies of treatment by H_2O_2 were applied to caves in temperate region (e.g., Mulec and Kosi, 2009), and as H_2O_2 is a strong corrosive and low-pH solvent that could dissolve substrate, the studies established a threshold of H_2O_2 application compromising between halting lampenflora growth and avoiding damaging the substrate (Faimon et al., 2003). There is a possibility that such protocols and thresholds may not be optimal for every region and climate, since lampenflora should be representative of the local biological community and substrate in caves may varying depending on the regional hydrogeology and cave microclimate. The thresholds obtained from these studies need to be validated for tropical caves where biota, cave microclimate, and illumination systems are very different from temperate ones. For instances, autotrophs in tropical climate grow well throughout the year, while in temperate region, they grow mostly in spring and summer. Hydrology and biogeochemistry in tropics can result in caves of gigantic size with large entrances and usually water constantly flowing inside (Limbert et al., 2016). In these caves, connection between the outside atmosphere and cave interior is strong. Surrounded by tropical jungle of highly diverse biology,

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caves in this region have maintained some exquisite cave organisms (Luong and Holinska, 2015). It is also expected that anthropogenic impacts to the show caves in tropical countries like Vietnam are different from the ones in temperate regions of Europe or the US, where advanced technologies and infrastructures are usually employed to reduce undesired impacts. In tropical countries, illumination systems installed into the show caves are patchy (Ngoc et al., 2014), construction to facilitate sightseeing does not help cave preservation (Trinh and Guinea, 2014), or there is no control of number of visitors every day (Dukhach, 2016). Thus, the objectives of this study are characterization of the show-cave microclimate that supports lampenflora development in a tropical coastal region, central of Vietnam, assessment of the lampenflora impact on speleothems inside humid-tropical show caves, and re-evaluation and adjustment of an environmentally friendly lampenflora cleansing practice that was previously tested in case studies in temperate regions (Faimon et al., 2003; Mulec and Kosi, 2009).

Materials and Methods

Study Site Description

The Phong Nha-Ke Bang National Park covers an area of 857.54 km^2 and is a UNESCO World Heritage Site, reflecting its global importance. The park came under UNESCO protection in 2003 because of its extraordinary stratigraphical diversity from the Precambrian to the present day, the long development of its topography from the Oligocene to the present day, and the intensively developed tropical karst formations (Fig. 1). Over three hundred karst caves have been recorded in the park (Limbert et al., 2012). Along with geological and geomorphological diversity, the park has considerable biodiversity in fauna and flora and extraordinarily well conserved tropical karst forests.

The central limestone area is bordered by impermeable strata that collect water on the surface and in the southern part of the park discharge it towards the Chay River lying farther north. This inflow of allogenous water is the main factor for development of the underground caves explored to date. Excellent examples of caves of this type are the Phong Nha show cave and the Hang Vom system. With the entrenchment of the Chay River, the underground flows shift lower and lower and leave fossil caves at the higher levels. Examples of such caves are Tien Son Cave, rich in calcite deposits and open to tourists as a show cave, and Thien Duong Cave, part of the Hang Vom system. The caves follow the bedding planes into the thickly stratified Devonian–Carboniferous–Permian limestone and numerous faults tied to the predominantly north-south faults in the Alpine Orogeny (World Heritage List Nomination Form 2000) throughout a long history of karstification in limestone strata over 1,000 meters thick. This study targets three show caves, Phong Nha, Tien Son, and Thien Duong (Fig. 1; Table 1). The first two caves have been open for frequent visits since 1995, and the last one was opened in 2006. They receive thousands of visitors every day (Phongnhakebang, 2014; Dukhach, 2016) with highest numbers during summer and national holidays, and that number has increased over the years (Vietnamtourism, 2016).

Surveys and Sampling

Since 2014, five surveys of cave microclimate have been conducted in spring (March–May) and autumn (August–September). In each cave, the monitoring took approximately one working day from 10 a.m. to 4 p.m. from the cave entrance to the end of passages open to visitors. Variables of temperature (°C, accuracy: ±0.3 °C), relative humidity (RH, accuracy: ±0.1 %), wind speed (accuracy: ±2% rdg, range: 0–30 m s^{-1}), and CO_2 (accuracy: ±1 ppm) were monitored with the use of a GreyWolf Toxic Gas TG 501 (USA). Since all stairs are fenced to prevent visitors from crossing out of the paths, air measurements were taken a few meters away from the stairs to avoid direct interference from visitors. The Gas TG 501 was left to stabilize for a few minutes and then programmed to record five results at 2 minute intervals. For the whole monitoring period, only one person in charge of microclimate monitoring was within 1 m of the sensor to limit possible interference. The sensor was placed approximately 1 m above the cave floor. All microclimate data were recorded between 10 and 11:30 a.m. and 2:30 and 4 p.m. when visitor number was peaking during a day.

Sampling of speleothems covered with lampenflora was conducted in the 2014 autumn and 2015 spring surveys. At each cave, three representative experimental sites were selected near the cave entrance, around the middle of the show section, and near the end of the show section (Fig. 1 g,h,i). Depending on the caves' morphologies and their entrance sizes, the sites near entrances were chosen between 50 and 100 m from them to guarantee no natural light interference at the sites. Sites near the end of exhibited passages were about 50 m to the end of show path. It should be stated that in show caves in Phong Nha–Ke Bang, visiting pathways consist of wooden, plastic, and steel stairs or steps standing on top of the cave floor, and lamps were consistently positioned along the passages with the light beam directed away from the pathways into the speleothems. Around massive and exquisite speleothems along the visiting passages, additional lamps were placed to give visitors a better view. In fact, the representative experimental sites are where attractive speleothems are concentrated in the proximity of visiting passages. Therefore, the speleothems were well illuminated and accessible.

Samples were taken between 0.5 and 1.5 m above the ground at the most trophic and prolific surfaces that were

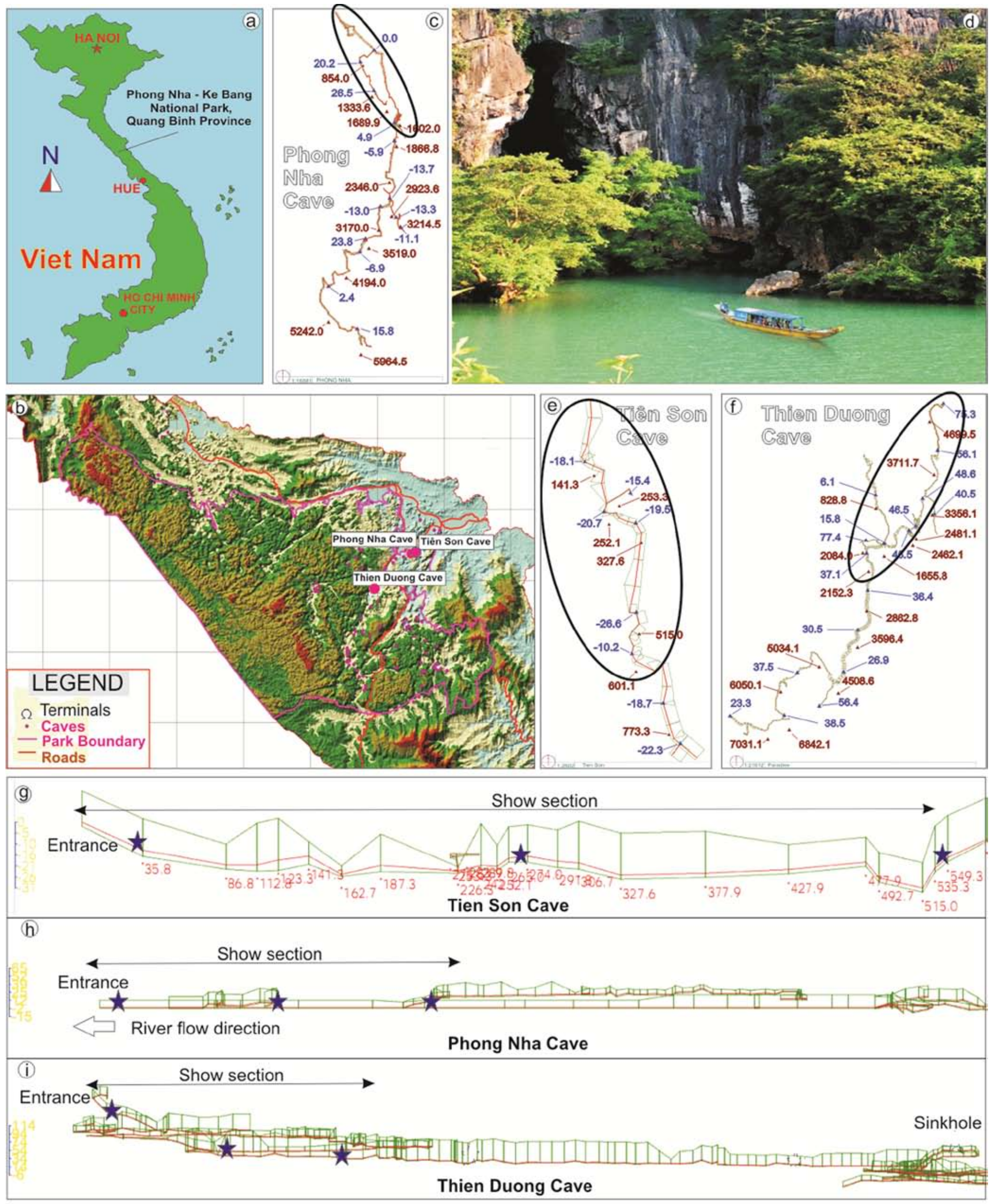

Figure 1. Map of the Phong Nha–Ke Bang National park and cave locations. (a) Vietnam Map and location of the Phong Nha–Ke Bang National Park, (b) Map of the Phong Nha–Ke Bang National Park and relative positions of the show caves studied in this research (Thien Duong, Tien Son, and Phong Nha), (c) Phong Nha Cave, (d) a photo taken in front of the Phong Nha Cave showing its enormous entrance with underground flow, (e) Tien Son Cave, (f) Thien Duong Cave, (g) cross-section of Tien Son Cave, (h) cross-section of Phong Nha Cave, (i) cross-section of Thien Duong Cave. Note: Oval shapes in (c), (e), and (f), indicate the artificially illuminated show-cave sections; Blue numbers represents elevation (m) as compared to the entrance; red numbers are relative distance (m) from entrance. Stars in (g), (h), and (i) indicate the locations of monitoring and sampling inside the caves.

constantly wet and directly illuminated with white lamps. All samplings were from hard substrates with comparable mineral composition of secondary microcrystalline calcite. With approval from the Phong Nha–Ke Bang National Park Management Board, hammers and knifes were used to detach the samples from the host speleothems. Each sample is about 1 cm thick and 5 cm across and fully covered with lampenflora. Samples were separately put in plastic bags, numbered, and kept in the dark and insulated cool box for transport to the laboratory. In the laboratory, they were stored in a refrigerator prior to analysis and experiments. It should be stated that microclimate conditions were monitored around the sampling speleothems andadjacent points where no lampenflora was observed for references. A Hioki 3423 Lux HiTester (Japan) was placed on the speleothem surface, and light intensity was averaged from five readings at 1 minute intervals.

Vascular plants (flowering plants and ferns) are identified by photographs taken at sites. Microbiota were identified according to morphology using light-microscope observation (Olympus BX51 at ×200/400 magnification). Identification of cyanobacteria was achieved by the use of the taxonomic literature of Komárek and Anagnostidis (1989, 1999, 2005). Diatom identification was based on the classification of Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b).

Environmentally Friendly Removal of Lamplenflora

In this study, industrial H_2O_2, 30% and medical H_2O_2, 3% (CPC1, Vietnam) were used for this treatment experiment. From the industrial H_2O_2, 30%, two solutions of H_2O_2, 10% and 15% were prepared, making up a total of four testing solutions; 3%, 10%, 15%, and 30%. Concentrated NaOH solution was used to adjust pH of the H_2O_2 solutions to 7–7.5 immediately before the treatment. pH paper was used to ensure the neutral pH of the solution.

Four sub-samples weighing within 0.5 and 1.5 g and size of less than 15 mm wide and 3 mm thick were placed in petri dishes and separately sprayed with the prepared H_2O_2 solutions. Two hours after spraying, the sub-samples were dried at 95 °C in atmospheric pressure for 24 hours and then subject to microscopic and mass-loss analyses. The procedure was then repeated with the same sub-samples after 2 weeks. The repeated treatments reflected a possible future practice when the remediation would be carried out twice per year.

Before and after each treatment step, all sub-samples were subjected to electron microscopic analysis to examine effect of H_2O_2 on lampenflora removal and substrate dissolution. The environmental scanning electron microscope (ESEM) XL30 microscope from FEI (Field Emission and Ion Company) is a low-vacuum ESEM (model Quanta) that enables high-resolution inspection and chemical analysis of non-conductive specimens.

In parallel, the sub-sample mass before and after each treatment step was weighed by micro balance (R200D Sartorius) to quantitatively evaluate the lampenflora removal efficiency by H_2O_2. Mass loss comparison was also made between untreated and treated sub-samples to evaluate the hydrogen peroxide dissolution effect on $CaCO_3$ substrate. In total, mass balances of sub-samples were checked before treatment (1), after first treatment (2), after second treatment (3), and after incineration at 550 °C (4). The reference sub-sample without treatment was also weighed, incinerated at 550 °C, and weighed again (5). Thus by comparing (1) and (2), (2) and (3), (3) and (4), and (4) and (5), we respectively have the removal fraction after the first spraying of H_2O_2, the removal fraction after the second spraying of H_2O_2, the lampenflora biomass remained in substrate (speleothem sub-sample) after treatments with H_2O_2, and the substrate fraction removed by H_2O_2. It is necessary to note that the removed substrate makes up a part of the removal fraction and is basically inorganic $CaCO_3$, not organic matter. The removed lampenflora organic matter is thus equal the removal fraction after treatment the second spraying minus the removed substrate. To avoid systematic errors during the heating procedures, substrate may gain or lose weight during drying or incinerating due to other causes unrelated to the H_2O_2 treatment. Pieces from inner sections of the samples not covered with lampenflora were sent to thermal-gravity analysis with the furnace set in atmospheric air environment with no purge gas. The mass change obtained by TGA at 100 °C and 550 °C was included in the calculation to achieve the exact mass loss after each treatment and incineration.

Results

Microclimates

During our surveys, temperatures inside the caves were generally lower than outside atmospheric temperature (Table 1). Inside the caves, temperatures measured near entrances and at deeper parts of the caves were similar during the sampling period, attesting the thermic stability of the caves and the consequent general atmosphere stability inside the caves. As shown in Figure 1, the cave entrances are typically high above the cave floor. Due to this morphology, a faster exchange of air between cave exterior and interior occurs in winter, when cave air is hotter and has a lower density than outside air. During that period, hot cave air blows out of the cave and cold exterior air descends into the cave. The cave's air temperature reduces quickly. When outside temperature starts to increase after winter, cave air is heavier and trapped inside the cave. A lower-than-outside temperature in cave air continues until the next winter (Mattey et al., 2016; Faimon and Lang, 2013). This explains why during our surveys in spring and autumn the cave air was found to be colder than the outside atmosphere. The Phong Nha Cave, characterized by an enormous entrance at a similar elevation with its interior, has a temperature more equilibrated with the outside temperature than other caves (Fig. 1).

The high humidity, a typical characteristic of tropical monsoon, has contributed to the caves' microclimates. Temperatures colder than outside increased relative humidity inside the cave. Depending on the entrance size, relative humidity would slightly fluctuate near the entrance, but it was always stable and close to the saturation level deep inside the caves. It should be noted that the water flow running inside the wet caves is another stabilizing factor for humidity in cave air. This factor could also be used to explain why in the dry cave of Tien Son humidity was somewhat lower than in the wet caves.

Monitored air circulation was fairly steady all over the show sections thanks to the large and multiple entrances and

Table 1. Atmospheric conditions in the surveyed caves in Phong Nha – Ke Bang. (ND = not detected)

Atmospheric Parameter	Thien Duong	Phong Nha	Tien Son	Outside
Temperature, °C	21.3 – 24.3	26.2 – 29.5	22.2 – 25.6	30.9 – 32.1
Relative humidity, %	89.5 – 99.5	85.8 – 98.5	78.4 – 94.5	75 – 81
Wind speed, m s^{-1}	0.0 – 0.2	0.0 – 0.1	0.0 – 0.1	0.3 – 0.4
Light, Lx	ND – 60	ND – 80	ND – 270	16800 – 42800
CO_2 concentration, ppmv	420 – 640	430 – 960	820 – 2500	240 – 590

the underground river. In addition, since most sampling-monitoring sites are in the vicinity of visitor pathways, cave air was also disturbed by thousands of visitors per day. There is an exception in term of air circulation in the Tien Son. This cave has only one entrance and has no underground water flow. Its deepest gallery is now closed to visits, thus yielding virtually non-detectable air flow there (Fig. 1c).

The illumination system inside Phong Nha–Ke Bang is generally equipped with two different color lamps; white (5500–6000K) and yellow (3700–4000K) (Ngoc et al., 2014). The white lamp spectrum in the visible range superimposed on the typical absorption peak of chlorophyll-a (660 nm; Piano et al., 2015) favors photosynthesis. The typical arrangement of illumination in the caves is to concentrate lamps around attractive speleothems, which has enhanced light intensity up to hundreds of lux on directly illuminated surface (Table 1).

Like other caves in karst regions (Fernandez-Cortes et al., 2015), the cave air CO_2 concentrations are higher than background atmospheric value (Table 1). In caves, CO_2 emitted from soil and epikarst, dripping water and visitors tends to be trapped due to a higher density of cave air compared with exterior, reaching value as high as 2500 ppm. Our surveys of the caves show that while temperature and humidity are stable and similar, CO_2 concentrations varied among galleries and peculiarly different among caves. In Tien Son, a dry and single-entrance cave, CO_2 reached 3 times higher in the deepest gallery than near the cave entrance section and 10 times higher than outside atmosphere. Carbon dioxide content was also highest among the surveyed caves. Since CO_2 was highly concentrated in the single-entrance cave and relatively low concentrated in the multiple-entrance ones, it is wise to conclude that CO_2 reflects exchange of cave air with the outside environment.

Lampenflora on Speleothems

Usually, light is switched on in the show caves every day from early morning (7 a.m.) till the afternoon (6 p.m.). In all show sections, lampenflora was observed from ceiling to floor where light and other microclimate conditions were favorable. In some parts, it covers an area of hundreds of square meters (Fig. 2).

Compared to the notion that lampenflora organisms are ubiquitous, fast-reproducing, and adaptable soil algae (Piano et al., 2015), lampenflora in the show caves of Phong Nha–Ke Bang appear to be more complex. In some parts, it was a lightly green, thin layer (Fig. 3a) or a dark green, thick layer (Fig. 3b). In other parts, it was a greenish yellow (Fig. 3c) or dark brown mat (Fig. 3d). Near the cave floor, where speleothems are usually covered by dirt or soil brought from outside by flows or visitors, lampenflora were more complex, with flowering plants (Fig. 3e) and ferns (Fig. 3f) in germination stage.

We observed a light-color preference of lampenflora growth. Between white and yellow, it was noticed that lampenflora grew well on surfaces directly illuminated with white light. The growth usually spread within 5 m from the light sources where light intensity was detected as between 50 and 200 lx. In several places where illumination was amplified by multiple light sources, lampenflora could be as far as 20 m from the lamps. Apart from light, water appears to be another essential factor for lampenflora growth. All lampenflora speleothems were soaked with drip water. Altogether, speleothems that are constantly wet, directly illuminated with white light, and within 5 m from the light source are likely covered with lampenflora.

Optical microscopic analysis reveals that, apart from green algae and cyanobacteria, diatoms, a class that are usually found on silicate based substrates, were abundant in the collected samples (Table 2, Supplement Fig. A). Filaments of fungi were also observed in colonies detached from white, yellow, and dark-brown mats.

The environmental scanning electron microscope images clearly show lampenflora as a complex community

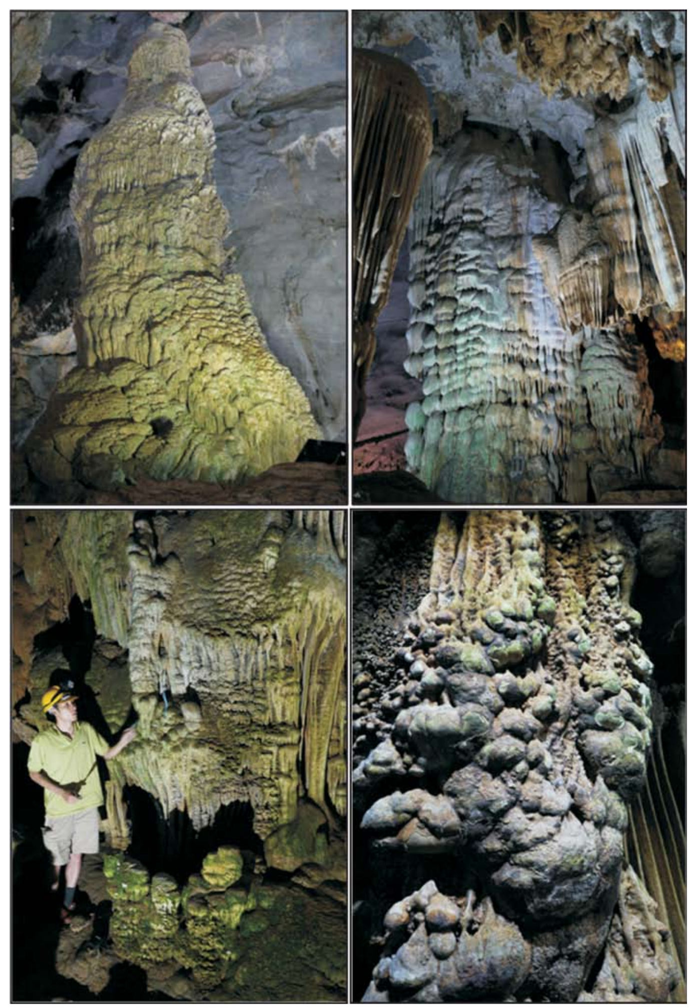

Figure 2. Lampenflora thrive on wet and white-light directly illuminated surfaces from stalactites near ceiling to flowstones and stalagmites on the ground of caves.

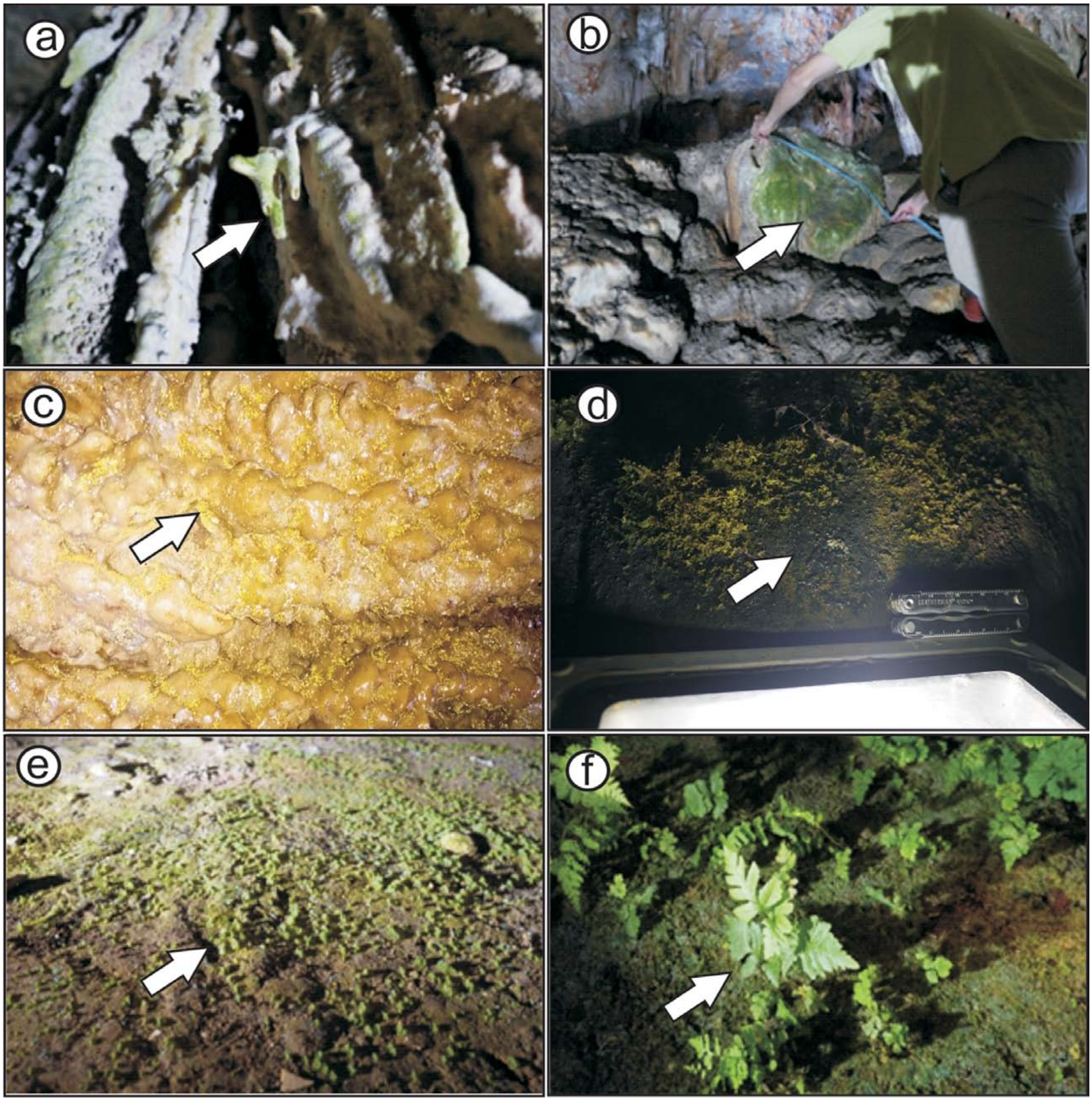

Figure 3. Images showing complex lampenflora biota: (a) light green thin layer, (b) dark green thick layer, (c) greenish yellow, (d) black and sticky mat, (e) germinating flowering plants, (f) germinating ferns

Table 2. Typical genus of lampenflora found in the caves in Phong Nha – Ke Bang.

Vascular Plants		**Non-Vascular Plants**				
Angiospermae (Phanerogams)	**Chryptogams (Ferns)**	**Bryophytes (Mosses)**	**Algae**		**Bacteria**	**Fungi**
...	...	...	Chlorophyta (Green algae)	Bacillariophyte (Diatoms)	Cyanobacteria	...
Centella	*Asplenium*	*Cyathodium*	*Chlorella*	*Nitzschia*	*Oscillatoria*	*Filamentous*
...	...	*Thuidium*	*Oedoclatium*	*Pinnularia*	*Spirulina*	...
...	...	...	*Microthamnion*	*Navicula spp.*	*Synechocystis*	...
...	...	...	...	*Achnanthes*	...	...
...	...	...	...	*Diploneis*	...	...

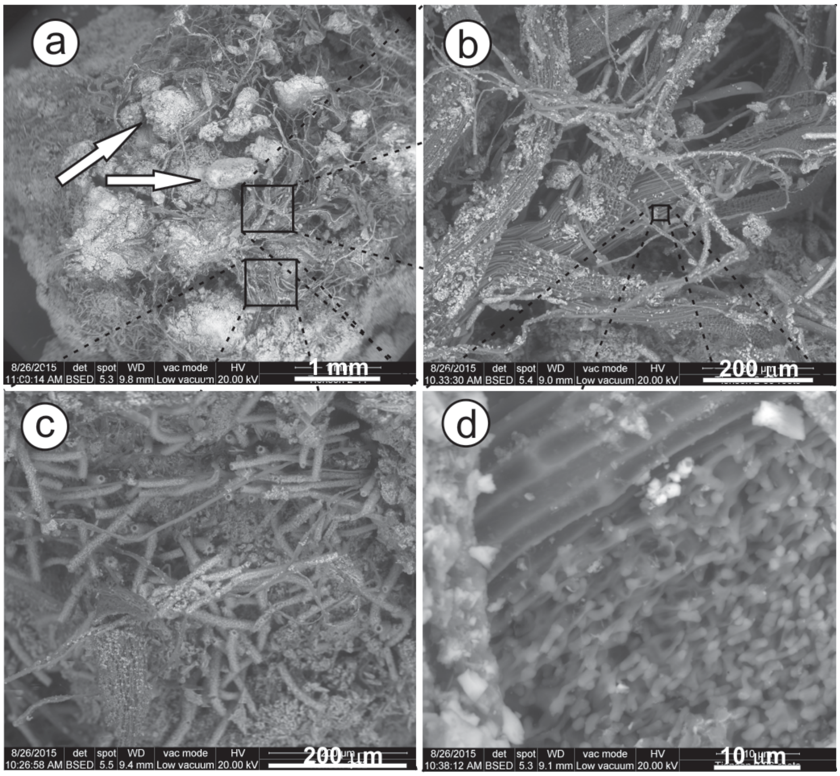

Figure 4. Microscopic images: (a) overview of speleothem surface destroyed by lampenflora (arrows point to the large $CaCO_3$ fragments detached from substrate), (b) large segment of fresh vascular roots, (c) calcite encrusted filaments (tubiform), and (d) fresh roots associated with calcite crystal fragments

that has a profound impact on speleothems (Fig. 4). There existed microbial assemblages directly or indirectly able to mediate a variety of constructive and destructive processes that resulted in the formation of distinctive fabrics. Destructive processes include dissolution of $CaCO_3$ due to respiration in rhizoids, microbes, moss, fungi, and lichens (Groth et al., 1999). It led to broken limestone pieces intertwined with root hairs and rhizoids as shown in Figure 4 a,b,c. Constructive processes include calcified microbes and bio-induced crystalline precipitates (Sanchez-Moral et al., 2003) as shown in Figure 4 d. Both processes, though opposing each other, impact the speleothem surfaces in undesirable ways, amplifying the porosity and crumbliness of the substrate. The smooth and scintillating surface of attractive speleothems is obliterated.

It is interesting to note that biogenic calcite crystals, which are not usually found in a highly oligotrophic environment, were abundant in collected samples here. The most abundant form of biogenic calcites results from the filamentous cyanobacterial calcification (Fig. 4d), with other forms of biogenic crystals (e.g., spherical) being less common (Supplement Fig. B). The calcification mechanism is probably sheath impregnation (Couradeau et a, 2013). The mechanism of cyanobacterial calcification by sheath impregnation in several occurrences, including the oldest calcified microfossils, remains controversial. Based on petrographic evidence, some authors have proposed that the calcification took place post-mortem (Pratt, 2001); others suggest it is a result of cell metabolic activity (Arp et al., 2002). Microscopic images here prove this calcification is a result of cell metabolic activity in which the filamentous crystals are in various types such as dendrite, needle, rhombohedra (Supplement Fig. B) and, in particular, always consists of an organic inner layer and a freshly formed $CaCO_3$ outer layer (Supplement Fig. C).

Hydrogen Peroxide Treatment

Organic Matter Removal

It was expected that concentrated H_2O_2 would largely remove lampenflora, but the results here did not confirm it. Microscopic images (Fig. 5) show that, at all H_2O_2 concentrations, lampenflora are dead and broken down but not massively oxidized. With H_2O_2 at 3% and 10 %, the filamentous lampenflora were still visible after repeated sprayings (Supplement Fig. D). With H_2O_2 at 15% and 30%, microbiota groups like algae and cyanobacteria were largely removed, but other groups like mosses, lichens, or ferns were only partly removed. Their remnant roots and stems were still seen on substrates.

The mass loss analysis confirms the conclusion derived from the microscopic analysis that all tested H_2O_2 solutions could not completely cleanse lampenflora (Table 3). With 3% H_2O_2 there is very little effect on lampenflora, with a mass loss of few percent. At 10%, for some types of lampenflora such as biofilms, the removal efficiency could be as high as 25.2% after the first spraying, but removal efficiency of vascular plants, mosses, or fens was low. Removal efficiency improved with the use of 15% and 30% H_2O_2, and a first spraying could remove up to 61.5% of total lampenflora mass.

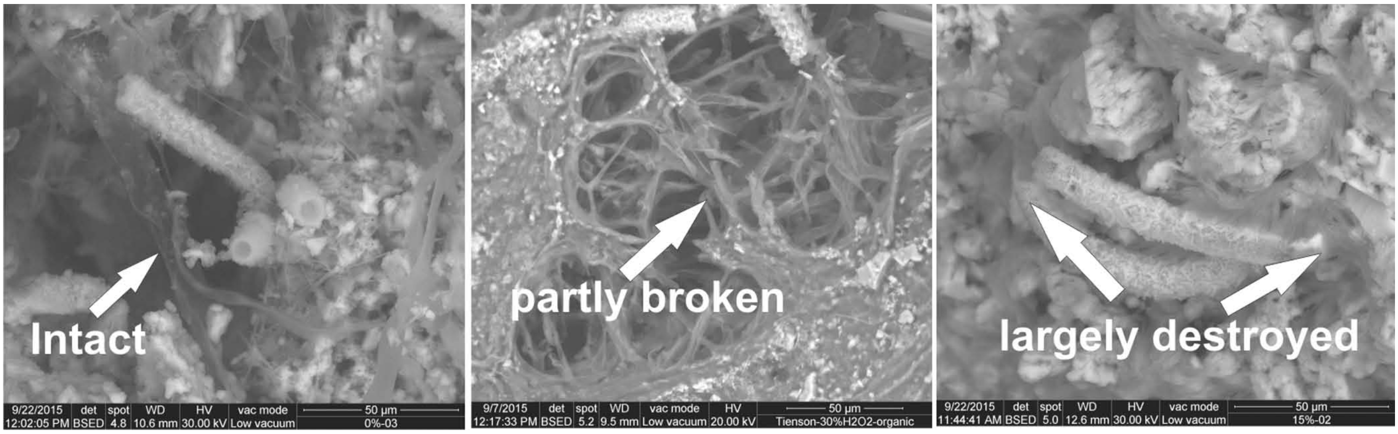

Figure 5. BSED images: Microbial mats at different stages of treatment, (a) before treatment, (b) after first treatment with H_2O_2, 30%, and (c) after second treatment with H_2O_2, 30%. Arrows point to the organic mat (a) or its remnants after treatments (b and c)

However, even after two sprayings about 30% of lampenflora mass remain in place.

This result is in agreement with previous studies stating that H_2O_2, 15% could efficiently destroy microbiota. For instance, Faimon et al. (2003) revealed that repeated applications of 15% peroxide solution with 2 or 3 weeks rest periods in between led to a total destruction of the algae, cyanobacteria, and slightly developed mosses. The highly developed growths of mosses yellowed and dried after these applications. They concluded the threshold hydrogen peroxide concentration for lampenflora destruction was 15%.

Another observation is that mass-loss analysis indicated a lower removal efficiency following the second spraying than the first (Table 3). This could be explained that the first spraying has removed the most labile and easily oxidized fraction of lampenflora. In the second spraying, the less oxidizeable one needed more H_2O_2 to be removed, leading to lower removal efficiency.

Dissolution of $CaCO_3$

One concern with the application of H_2O_2 for lampenflora removal is the potential risk of speleothem damage by chemical dissolution of $CaCO_3$ or bio-physical detachment of abiogenic and biogenic fragments associated with lampenflora mats (Fig. 4). We observed the effects of H_2O_2 on selected calcified filaments that were relatively in the same size and form and abundant in lampenflora substrates. We find that concentrated H_2O_2 (15% and 30%) has slightly reduced the thickness of calcified filaments (Fig. 6), indicating a possible $CaCO_3$ dissolution. Microscopic analysis also leads to a belief that calcite fragments (Figs. 4 a-c) as well as calcified microorganisms (Fig. 4 d) mixed inside the lampenflora mat were detached from the substrate when organic matters were cleansed by H_2O_2. Microscopic images also indicated that repeated sprayings increased the substrate dissolution. The mass-loss analysis taken between the treated and the non-treated sub-samples gives an averaged ratio of removed lampenflora biomass over removed $CaCO_3$ as 0.813. This number indicates that a substantial fraction (more than half) of removed lampenflora was actually inorganic $CaCO_3$. Probably this removed $CaCO_3$ consists mainly of the easily detachable biogenic $CaCO_3$, rather than the solid abiotic speleothems.

Discussion

The Show-Cave Environment and Lampenflora Growth

Usually cave fauna and flora are rare and perhaps adapt to extremely oligotrophic conditions (Holsinger, 1988). Our surveys, indicate that trophic condition in the show caves in Phong Nha–Ke Bang was not extremely oligotrophic. Irradiation, nutrients, and water, three essential conditions for autotroph growth, inside show sections were enough to sustain many groups of autotrophs. The condition is derived from both natural and anthropogenic factors. Indeed, natural factors such as large and multiple entrances, underground flow, and complicated seeping or dripping water patterns, leave the cave air and water well exposed to atmosphere. Our surveys, as well as another study on cave fauna (Luong and Holinska, 2015), found plankton and fishes in cave pools, lizards and spiders on cave walls, and bats flying near cave entrances. Anthropogenic impacts such as cave illumination, stairway construction, and visitors also accelerate caves' interior-exterior exchange. For instance, large or multiple entrances, together with a high frequency of visitors, facilitate air circulation. Typically, underground flows annually inundate some part of the caves. The flows have enough nutrients, such as 5 mg NO_3 and 0.03 mg L^{-1} PO_4, to sustain autotroph growth in inundated speleothems. Visiting activities increase the cave-air CO_2 content, as well as bring more material and organisms from outside into the caves. The caves' illumination systems have no motion sensors to restrict illumination to only when there are visitors. Lamps are

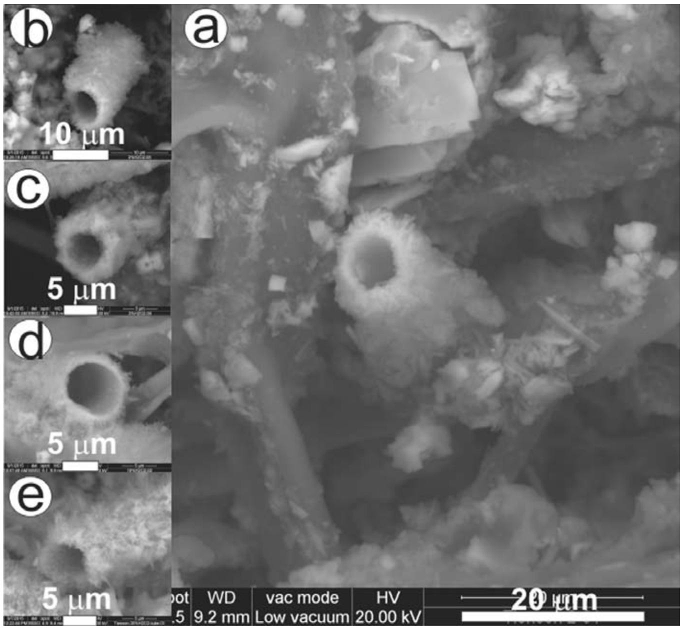

Figure 6: Fossilized tubes at the original and after spraying treatments with H_2O_2; from bottom to top are respectively, H_2O_2 3%, 10%, 15%, and 30%, showing relatively the effect of H_2O_2 on calcite dissolution.

Table 3. Lampenflora removal efficiency (% dw) after treatments with the use of H_2O_2.

H_2O_2	3%	10%	15%	30%
First treatment	1 – 4.4	2.8 – 25.2	30.5 – 61.5	48.1– 49.5
Second treatment	0.6 – 2.7	1.0 – 20.2	15.1 – 20.5	16.0 – 26.5

usually concentrated around beautiful speleothems, which intensifies photosynthesis (Table 1, Fig. 2).

Light intensity in show caves in Phong Nha–Ke Bang detected at sampling sites was more or less in the same range detected in show caves elsewhere (Piano et al., 2015). Here we found that light color was relatively decisive to lampenflora development. As stated in the result section, among the two main light colors used in the caves, white light is apparently more supportive to lampenflora development than yellow light. Basically, white light includes blue (about 450 nm wavelength) and red light (650 nm wavelength). They are the most favored wavelengths of photosynthesis. In particular, blue light is important during the germination stage of plant growth (Arnim and Deng, 1996). Yellow (wavelength around 550 nm) is less favorable to plants than blue and red. From the management point of view, our study suggests that particular attention should be devoted to implementing illumination system that would be less favorable for the growth of photosynthetic organisms. White lights should be replaced with colored lights with a spectrum out of the absorption peak of chlorophyll a, like green and yellow lights (Ngoc et al., 2014), and their distance from the speleothems should be increased to reduce direct illumination. The provision of motion sensors, at least along the tourist pathway, could be used to reduce the time of light exposure (Grobbelaar, 2000). A simple solution is to use appropriate LED lights, since LED light has a potential of tuning the desirable emission spectrum (D'Agostino et al., 2015).

Our macro- and microscopic analyses both reveal the importance of water. For instance, the abundance of cyanobacteria and its calcified structures (Fig. 4) is an indicator for the relationship between water and lampenflora in the show caves in Phong Nha–Ke Bang because the colonization of cyanobacteria (*Oscillatoria and Spirulina*) has been known to be dependent on water (Dayner and Johansen, 1991). Algae and flowering plants in their germination stage also require a lot of water (Fig. 3). Another effect of the presence of seeping water could be its role in transporting microorganisms inside the cave (Mulec et al., 2008). From this point of view, microorganisms, together with flora seeds, can enter the caves with the water seeping through the rock fractures, reaching the illuminated substrates. For management purpose, as seepage is a natural process, illumination should be limited in sections that are constantly wet.

As shown in Figures 2 and 3, mineral composition of substrates is another factor defining the lampenflora biodiversity. Generally, there is a stratification of the substrate mineral composition from purely limestone near the ceiling to a substrate contaminated with weathered or man-made materials near the bottom. Substrate that consists of a purely dense calcareous matrix allows mainly microbiota colonization. At mature stages, there will be lichens and fungal association. Near the ground, speleothems covered with significant amounts of weathering minerals sustains a more diverse biota (Warscheid et al., 1993). In addition, man-made materials, such as brick, mortar, or concrete brought to build the pathways, and sometimes artificial speleothems, are also susceptible to different forms of autotrophs and heterotrophs that are usually not found in karst caves. The degree of contamination will depend on the pore size distribution, as well as on the alkalinity of the artificial stones (Gu et al., 1998). For this mineral-composition issue, from a management point of view the advice is to keep clean the cave interior by daily washing, cleaning, and removing garbage, and avoid construction and new installations.

An Environmentally Friendly Treatment Protocol for Lampenflora Removal

The proliferation of lampenflora is a major threat for the conservation of show caves, since phototrophic organisms and their associates cause physical, chemical, and aesthetic damage to speleothems. To recover the aesthetic characteristics of speleothems, one apparently needs to eliminate completely lampenflora and its associates and in the meantime restore the physical appearance of speleothem's surface. Both microscopic and mass-loss analyses taken immediately after the second treatment reveals that the H_2O_2 chemical treatment alone has not met such expectation because organic matter are not completely removed and there are still $CaCO_3$-encrusted filaments and spores. The treated samples look yellow, dark, and even more crumbly than before treatment. Thus, it is impossible that sprayings with H_2O_2 will restore the aesthetic characteristics of speleothems.

In detail, our experimental results show clearly that treatment with diluted H_2O_2 (<15 %) has not much impact on lampenflora and their associates. The H_2O_2 treatment took effect only with more concentrated H_2O_2. The concentrated H_2O_2 treatments were able to remove microorganisms and destroy macroflora such as mosses and ferns. Together with that, many biogenic calcites were damaged because the dead organic-matter skeletons were burned by H_2O_2. Nevertheless, large fragments of roots and plant debris were still found after the concentrated H_2O_2 treatments (Fig. 5). Our results are, indeed, in agreement with a conclusion from Faimon et al. (2003) that lampenflora, mosses, and fungi died out and lost their structural integrity upon being sprayed with concentrated H_2O_2. This assumption leads to a suggestion to use water jets to clean the dead lampenflora and biogenic $CaCO_3$ on treated speleothems at carefully selected places. We recommend applying water jets to places near the cave floor where lampenflora are found more mature and complex and more difficult to chemically remove than the ones in higher places, and thus badly need further treatment after being chemically treated. Speleothems like flowstones and stalagmites are usually sturdy under a water jet, and due to natural and anthropogenic factors, speleothems near the floor are contaminated or covered with dirt and alluvium; an application of water to such surfaces would help washing as well. We believe that water jet application would not drastically enhance spreading of lampenflora inoculum in caves because the exchange of biota between the cave inner and exterior is already naturally strong. Their long history of karstification has made the caves spacious and highly accessible and, particularly, all studied caves are parts of active or inactive underground streams/rivers; Phong Nha and Thien Duong have large active rivers. Several studies have found invasive organisms to be abundant deep inside the caves (Luong and Holinska, 2015). In other pristine caves in Phong Nha–Thien Duong, such as Son Dong, life is thriving wherever the light reaches (CNN, 2016).

To conclude, the complete procedure for environmentally friendly removal of lampenflora in show caves in Phong Nha–Ke Bang consists of spraying at least two times with H_2O_2, 15% (no need to use a more concentrated solution), water-jet washing after chemical spraying at selected places, and illuminating caves with LED lamps of a different color such as yellow, installing motion sensors to illuminate only when necessary, and avoiding near and direct illumination onto constantly wet surfaces.

Conclusions

Caves in Phong Nha–Ke Bang National Park represent geomorphologic, geologic, biologic, historical, and paleoclimatic laboratories locating in a tropical monsoon coastal setting. This is, to our knowledge, the first time for tropical caves that lampenflora were studied and a remediation practice was proposed.

Our first conclusion is that with the addition of artificial light, white color in particular, lampenflora can easily develop in karst caves in tropical monsoon climate owing to a strong exchange between cave interior and exterior. A mature lampenflora community also harbors heterotrophs such as fungi and bacteria. Such complex biota is similar to the one in subsurface soil, where light is fairly limited but nutrients and water are available.

Second, remediation of lampenflora and its associates is not as simple as removal of soil-algae formed biofilm. In addition, the surfaces of speleothems colonized by mature lampenflora mat suffered from both destructive processes that break or dissolve carbonate and biogenic calcification that formed a porous and crumbly crystal layer.

Third, only a combination of chemical treatment, mechanical cleansing, and illumination modification can effectively eliminate lampenflora and recover the aesthetic properties of speleothems in show caves. This proposed protocol is cost effective and within the capability of local authorities.

It should be stated that as a strong oxidizer, H_2O_2 could be harmful to the indigenous cave fauna and flora. In Phong Nha–Ke Bang National Park, several new fauna species have recently been discovered in the caves (Lourenco and Pham, 2010, 2012; Luong and Holinska, 2015). All newly discovered species are highly vulnerable and rare cave dwellers. A direct hit of this chemical agent when sprayed could dispatch small invertebrates like scorpions, spiders, and millipedes or chase away vertebrates like lizards, which are found colonizing near the light sources for a better hunting. Thus it is compulsory that before applying H_2O_2 a thorough investigation of environment and biodiversity should be conducted at the sites. Preventive measures such as evacuation and dispersion of rare organisms should be taken. Spraying practice should be conducted carefully and avoid over-spraying. Water-jet should only be performed on solid flowstone, rimstone, and stalagmites near the cave floor.

Acknowledgements

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A. Faille and J.C. Lecoq. *Oedichirus spelaeus* n. sp., the first cave dwelling beetle from Madagascar (Coleoptera: Staphylinidae: Paederinae). *Journal of Cave and Karst Studies*, v. 80, no. 1, p. 13-18. DOI: 10.4311/2017LSC0112R2

OEDICHIRUS SPELAEUS N. SP., THE FIRST CAVE DWELLING BEETLE FROM MADAGASCAR (COLEOPTERA: STAPHYLINIDAE: PAEDERINAE)

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Abstract

During an expedition of the French National Museum of Natural History in the Tsingy de Namoroka National Park, we systematically collected the cave fauna. Various mainly troglophilic species were found, but one of the most surprising discoveries was a Staphylinidae belonging to a new species and remarkable for its morphologic adaptations: reduced eyes, long antennae and legs, and depigmentation. This species is the first cavernicolous beetle described from Madagascar. A description of the new species is provided, as well as molecular data and details of the ecology of the species.

Introduction

In spite of a high level of endemism of biodiversity in Madagascar, the cave fauna of this island is surprisingly poor. In particular, beetles, which are the most diversified group of insects in hypogean environments, are virtually lacking. The only species previously known from Madagascan caves are not considered as true troglobites (Rémillet, 1973). A single species of Scarabaeidae, *Cambefortantus myops* (Lebis), was considered as potentially troglobitic, but its microphthalmy is not a troglomorphic character, as it is also observed in epigean species of the same genus (Montreuil 2008).

Two other beetle species are known from Madagascan caves, a water beetle of the family Elmidae, *Elmidolia binervosa lamarcquei* Paulian, 1959, which is considered troglophilic but does not show any troglomorphic characters (Rémillet 1973), and a Staphylinidae Pselaphinae, *Centrophthalmus troglophilus* Jeannel, 1954 (= *Camaldus* Fairmaire, Jeannel, 1954). Although nothing is said regarding the collection of the latter species in Andranoboka Cave, Jeannel (1954) indicated that the species is winged and has eyes.

The genus *Oedichirus* belongs to the subfamily Paederinae, tribe Pinophilini (Schomann and Solodovnikov, 2017). Paederinae is the subfamily of Staphylinidae sensu stricto (i.e., excluding Pselaphinae) the most common in subterranean ecosystems (25 species quoted in Hlavàč et al., 2006), but only a single species of Pinophilini was described from a cave in the Galapagos islands (Campbell and Peck, 1989). The genus *Oedichirus* is characterized morphologically by the spiniform pencil of antennomere 11, the abdominal segments IV to VI having windows in the intersegmental membrane adjacent to the tergum and sternum, an abdomen with a strongly punctate surface, tergum and sternum VII fused except for an apical incision, and elytra without a long seta on the lateroapical angle (Herman, 2010, 2013). It is speciose and widespread, with 330 species described from all continents except for Antarctica (Herman, 2013; Assing, 2013, 2014; Irmler, 2015; Li et al., 2015). The genus *Oedichirus* has undergone a major evolutionary radiation in Madagascar, with 107 species described so far (Lecoq, 1986, 1991; Janák, 1995, 1996, 1998, 2003). Little is known regarding the ecology of these species, which were mainly collected by beating vegetation or sifting leaf litter (Janák, 2003; Assing, 2014; Li et al, 2015).

The tsingys of Madagascar are remarkable and distinctive geological formations, jagged, sharp-edged pinnacles whose dissolution led to the creation of a complex underground cave network (Salomon, 2006, Raharimahefa, 2012, Veress et al, 2008). The Tsingy de Namoroka is one of the four tsingy areas of Madagascar, together with Bemaraha, Ankarana, and Bemarivo (Rossi, 1977; Fig. 1); it is located in the Boeny region of the northwestern part of the island. It is composed of about 180 km² of needle-shaped Jurassic limestone formations, under a dry tropical climate (Middleton, 2004) (Fig. 2). Access to the tsingy is difficult, and it cannot be reached by car during the five months of the rainy season. As a result, its biodiversity—and especially the underground biocoenosis—is poorly known. The cave network has been explored by speleologists during various expeditions, but only limited data have been published regarding biological results and the cave fauna (Paulian and Grjebine, 1953; Rémillet, 1973; Soulier-Perkins et al, 2015).

Materials and Methods

Sampling: Specimens were collected by hand in the field and killed in ethyl acetate for morphological studies or 95% ethanol for DNA extraction.

Morphological Preparation: After dissection or DNA extraction, specimens were mounted on cards and the genitalia glued behind the specimen or stored in water-soluble dimethylhydantoin formaldehyde resin (DMHF) on transparent

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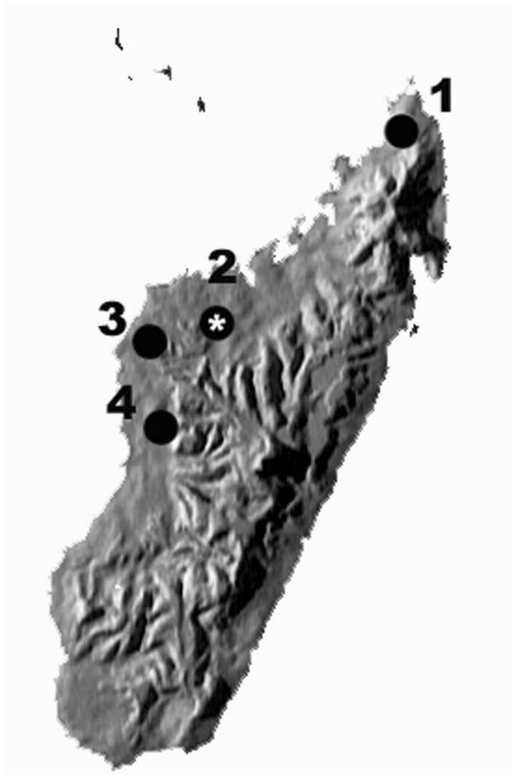

Figure 1. Location of the four main Tsingys areas in Madagascar. 1. Ankarana, 2. Namoroka, 3. Bemarivo, 4. Bemaraha. Asterisk: location of *Oedichirus spelaeus* n. sp.

Figure. 2. Tsingy de Namoroka, drone view of the canyons.

Figure 3. *Oedichirus spelaeus* n. sp. alive in the field.

cards pinned beneath the specimen. Pictures were taken with a Canon EOS 6D camera combined with a Cognisys Rail macro Stack Shot driven by the software Helicon Remote. Serial pictures were combined using the Helicon Focus 6 software, and finally processed using Adobe Photoshop CS.

Molecular Preparation: Non-destructive DNA extractions of single specimens were carried out using the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany). A partial fragment of the cox1 gene was PCR-amplified and sequenced. The primers used were Pat and Jerry, a couple frequently used in beetles (Simon et al, 1994).

Repository: Muséum national d'Histoire naturelle (MNHN), Paris.

Results

Description

Oedichirus spelaeus n. sp. (Figs. 3-5)

Type Material: Holotype ♂: Madagascar Namoroka 29.X.2016 / Grotte du Canyon S16°24.676′ E045°19.645′ alt. 161 m, A. Faille leg., extraction code: ZSM_L1464 (MNHN).

Paratypes: 1 ♂, 2 ♀, same data as holotype (MNHN); 1 ♂, Namoroka 23.X.2016 / Grotte des Chauves-souris, S16°24.501′ E045°18.662′ alt. 90 m, A. Faille leg., extraction code: ZSM_L1461 (MNHN).

Etymology: the specific epithet is derived from the Greek σπήλαιον, spḗlaion ("cave"), refering to the hypogean habitat of the new species.

Length: 8 to 10 mm (in extension). Forebody: 3.6 mm (from labrum to apex of elytra).

General appearance of body slender, with troglobiomorphic characteristics: legs slender, antennae long and slender, eyes strongly reduced. Apterous species, lateral border of pronotum complete.

Coloration: body uniformly reddish-brown; legs, antennae, and maxillary palpi pale-yellowish.

Head: eyes strongly reduced, not prominent, 0.5× as long as temples. Head nearly square, slightly longer (from labrum to neck constriction) than wide (R=1.14); temples subparallel, long, neck constriction well marked. Head much narrower than pronotum, strongly and densely punctate; punctation simple, strong and uniformly distributed. Distance between puncta greater than their diameter. Each punctum with fine lines radiating from its center. A small reticulate area at base of head. Some (ca. 50) long setae, uniformly distributed over surface of head. Antennae particularly long and thin, longer than the head and pronotum, measured from the labrum to the base of pronotum (R=1.3) (length 3.5 mm).

Maxillary palpi long, each of the three last segments longer than intermediary antennomere.

Pronotum: slightly longer than broad (R=1.14), much broader than the head (R=1.3), widest anteriorly in first third and tapering posteriorly; punctation strong and complex, each punctum surrounded by fine convergent lines. Sparsely pubescent; an irregular longitudinal sulcus on each side of the median line, each with ca. 15 punctae vaguely aligned. Lateral to the sulci, a succession of 5 or 6 weakly aligned punctae occurs in a shorter sulcus. Lateral border complete.

Elytra: short, as long as wide at apex, narrower than pronotum at the base and as wide as it at the apex. Humeral angles obsolete. Elytra progressively widening from base to apex. Surface covered with setiferous tubercles, the integument between the tubercles smooth, not reticulate. Lateral tubercles numerous. Setae long and thin. Hind wings absent. Scutellum wrinkled.

Abdomen: pubescent, with dense, uniform punctation. Abdominal segments wider than elytra, regularly covered with setigerous punctae slightly smaller than those of the forebody, regularly spaced, distance between adjacent punctae roughly equal to their diameter. Setae long and thin, similar to those on elytra. Integument nearly smooth, with some thin, fine lines around punctae. Tergite of genital segment (Tergum IX) with two long, parallel, sharp lateroapical processes (Fig. 4a, lower left). Sternite VIII of male with circular depression covered with short and recumbent setae, with some longer setae around this depression.

Aedeagus: asymmetrical, parameres slender, inserted near middle of median lobe, Lateral odd process enlarged in its median part (Fig. 5).

Diagnosis: *O. spelaeus* n. sp. shares apterism and a complete lateral border in pronotum with the species of *Oedichirus* of the first section of Fagel (1970), but it can be readily separated by the presence of troglobiomorphic characteristics: legs and antennae long and slender, eyes strongly reduced.

Molecular data: The cox1 partial sequence of the holo-

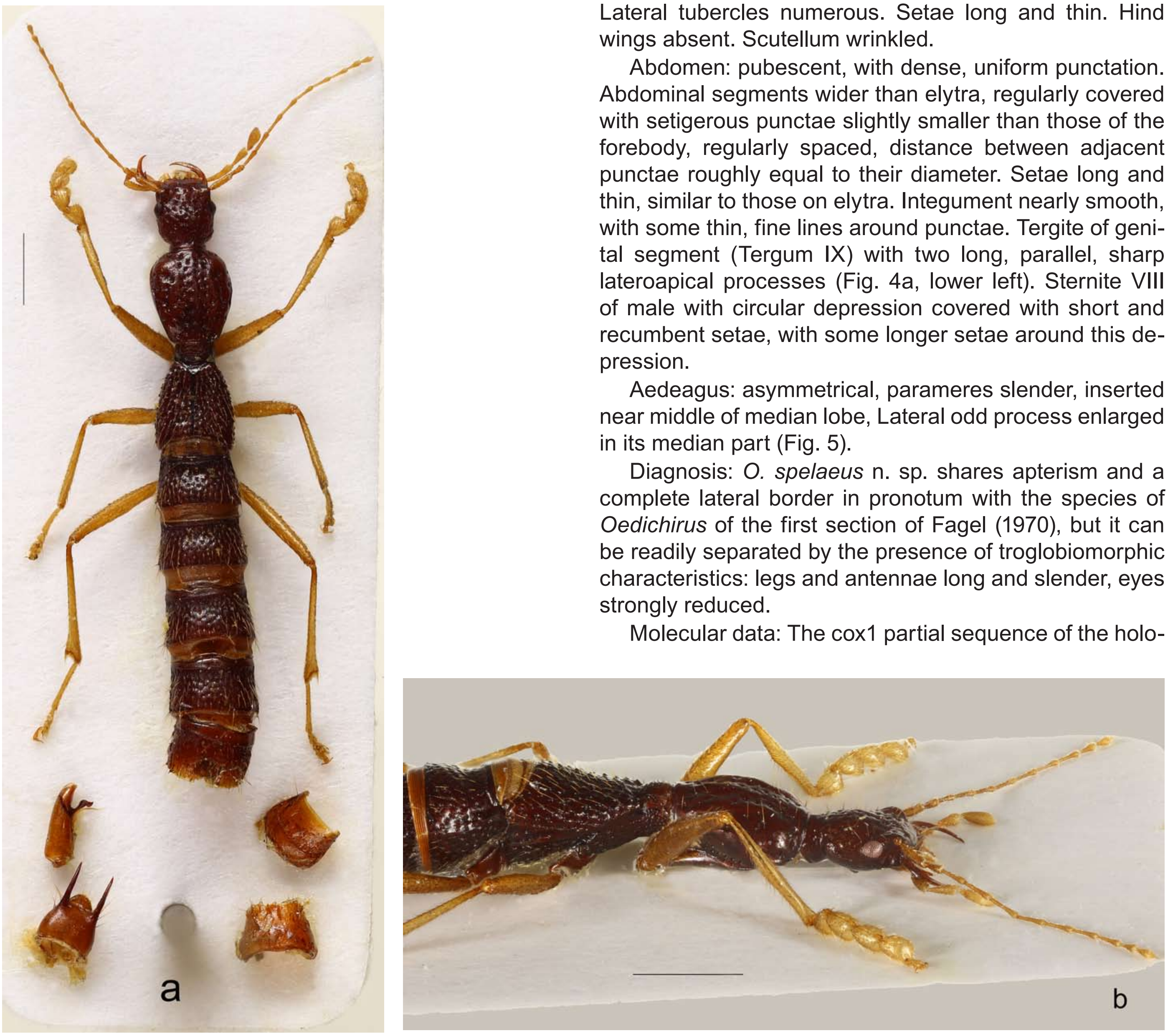

Figure 4.. *Oedichirus spelaeus* n. sp. habitus, in a. dorsal and b. lateral views. Scale = 1 mm.

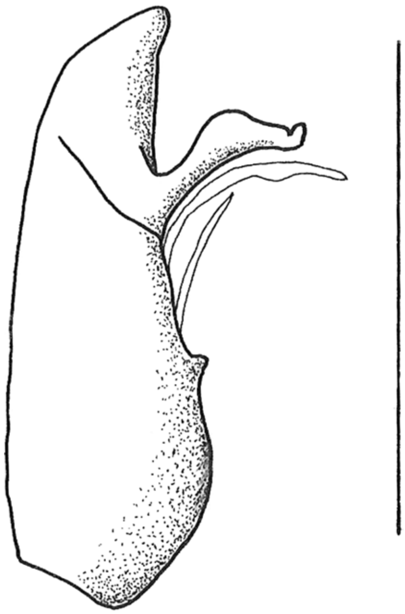

Figure. 5. *Oedichirus spelaeus* n. sp., holotype, median lobe in lateral view, scale = 1 mm.

Figure 6. Namoroka, entrance to Grotte du Canyon (Canyon Cave).

type is deposited in GenBank under the accession number: MF795058. These are the first data for *Oedichirus* available in Genbank.

Distribution and ecology: This species is known from four specimens found in the deepest parts of two caves of the northern area of the Namoroka Tsingy: Grotte du Canyon (Canyon Cave, type locality, three exemplars) (Fig. 6) and Grotte des Chauves-souris (=Bat Cave, one exemplar) (Fig. 7a).

Discussion

The new species belongs to the first section comprising apterous species with a complete lateral border in pronotum (Fagel, 1970, Lecoq, 1986). It is difficult to relate the species unambiguously to any species of this group, in particular, because of the numerous characters reflecting the hypogean lifestyle of *O. spelaeus*.

Oedichirus spelaeus n. sp. was collected in two caves located in the northern part of the Tsingy de Namoroka National Park, ca. 35 km southeast of Soalala. Like the other Madagascan tsingys, Namoroka hosts a remarkable biodiversity (Allorge and Haevermans, 2015). The new species was discovered during the 2016 expedition organized by MNHN in Namoroka, which concentrated on the exploration of the northern part of the tsingy. The Grotte des Chauves-souris (=Bat Cave) is a long cave, an important part of which is formed by a canyon, ending in a large chamber with various lateral galleries and small chambers, these generally being dry in the season of the expedition (Fig. 7a, b). The network is developed on two levels, the new species having been found in the second, lower level (Fig. 7b) in company with a rich fauna of invertebrates, including Araneae, Amblypygi, Diplopoda, Polydesmida, Chilopoda, Isopoda, Zygentoma Nicoletiidae, Blattodea Nocticolidae (blind), Hemiptera Reduviidae Emesinae, Homoptera Cixiidae T*yphlobrixia namorokensis* Synave, 1953, Coleoptera Carabidae *Abacetus (Astigis)* sp., Collembola. Canyon Cave, the second locality where the new species was collected, is located in a remote area of the massif, on the side of a deep canyon crossing the tsingy (Fig. 6). It is a large cave with various underground passages, ending by a narrow joint. This is the deepest part of this cave, and it is here that *Oedichirus spelaeus* n. sp. was collected, walking on the ground, together with various troglobites, including Opiliones, Araneae, Hemiptera Reduviidae, the Cixiidae *Typhlobrixia namorokensis*, Blattodea Nocticolidae, and a new species of Staphylinidae Scaphidiinae (Löbl and Faille 2017).

Acknowledgements

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Figure 7. Namoroka, Grotte des Chauve-souris (=Bat Cave), a. entrance, b. chamber in which the new species was collected.

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W. Hasegawa, Y. Watanabe, H. Matsuoka, S. Ohsawa, B. Brahmantyo, K.A. Maryunani, and T. Tagami. Environmental parameters controlling stalagmite growth in tropical areas: New insights from cave monitoring at Petruk Cave, central Java, Indonesia. *Journal of Cave and Karst Studies*, v. 80, no. 1, p. 19-27. DOI: 10.4311/2015LSC0118R3

ENVIRONMENTAL PARAMETERS CONTROLLING STALAGMITE GROWTH IN TROPICAL AREAS: NEW INSIGHTS FROM CAVE MONITORING AT PETRUK CAVE, CENTRAL JAVA, INDONESIA

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Abstract

To elucidate environmental parameters controlling stalagmite growth in tropical areas, we conducted cave monitoring throughout a year in Petruk Cave, central Java, Indonesia. We compared stalagmite growth rate with the cave's environmental parameters, air temperature, drip rate, calcium concentration of the drip waters, and pCO$_2$-air. We found a relationship where stalagmite growth rate is fast (slow) when pCO$_2$-air is low (high) during dry (rainy) season, suggesting that pCO$_2$-air controls stalagmite growth. Note that this is a first study that reports that dramatic pCO$_2$-air reduction occurring during dry season in a tropical cave controls stalagmite growth. Additionally, we discuss the mechanism of pCO$_2$-air fluctuation. Monitoring results show that pCO$_2$-air fluctuation is divided into two phenomena: seasonal fluctuations and daily fluctuations. Dramatic pCO$_2$-air reduction during the dry season is likely to result from a decline of plant activity due to little rainfall. On the daily scale, pCO$_2$-air reached to the minimum around 6 a.m. and maximum around 2–4 p.m., although it is not obvious whether this is due to plant activity or cave ventilation. Also, dynamic pCO$_2$-air reduction was observed following cave ventilation driven by the sudden drop of outside air temperature due to a downburst during severe rain. This suggests that heavy rainfall in short duration is also one factor that controls cave ventilation and pCO$_2$-air.

Introduction

Stalagmite geochemistry has been widely recognized as a useful proxy to reconstruct paleoclimate or paleoenvironment of terrestrial areas (e.g., Wang et al., 2001; Fairchild et al., 2006; Wang et al., 2008). If stalagmite growth rate has a seasonal variation, the geochemical signatures might be influenced as well by the seasonal variation in growth rate. Meanwhile, some previous studies have utilized stalagmite growth-rate itself as a paleoclimate or environment proxy (e.g., Proctor et al., 2000; regional precipitation reflecting the strength of the winter North Atlantic Oscillation; Polyak and Asmerom, 2001, wet/dry condition). It is, therefore, important to elucidate the mechanism of stalagmite growth for reconstructing paleoclimate or environment using stalagmites.

Recently, cave monitoring studies were conducted to understand the relationships between surface climate and stalagmite characteristics (e.g., Spötl et al., 2005; Banner et al, 2007; Baldini et al., 2008; Mattey et al., 2010; Boch et al., 2011; Tremaine et al., 2011). Previous studies from the mid-latitudes revealed that stalagmite growth rate is affected by several factors, such as air temperature, drip rate, calcium ion concentration, which is given as [Ca^{2+}] in this study (e.g., Baker et al., 1998; Genty et al., 2001), or partial pressure of cave air CO$_2$, which is given as pCO$_2$-air in this study (e.g., Spötl et al., 2005; Banner et al, 2007; Baldini et al., 2008). Especially, Banner and his colleagues conducted cave monitoring in central Texas, USA, and discussed seasonal variations of stalagmite growth. Banner et al. (2007) reported that the stalagmite growth rate inversely correlated to seasonal changes in outside air temperature, with nearly no growth rates during the warmest summer months and high growth rates from fall to spring, suggesting that the seasonal variations of stalagmite growth rates were primarily controlled by regional air temperature effects on ventilation of cave-air CO$_2$ concentrations or drip water CO$_2$ contents. At the study site of Banner et al. (2007), outside air temperatures have a large seasonal variation, ranging from 0 to 30 °C. In contrast, outside air temperature at our study site, central Java, Indonesia, is almost constant through the year, and precipitation has a distinctive seasonal cycle of wet and dry seasons.

Cave monitoring studies are very limited in the tropics, except small numbers of studies in such as Belize (Ridley et al., 2015) and Brazil (Sondag et al., 2003). Sondag et al. (2003) presents monitoring data for temperature, atmospheric pressure, and drip rate in two caves of Brazil. Also, Ridley et al. (2015) presented the results of cave monitoring in Yok Balum Cave, Belize, where outside temperature has a small seasonal variation, ranging from 21 to 24 °C. Their evidence shows that clear seasonal ventilation regimes exist, driven by thermally induced inside-outside air density differences (Ridley et al., 2015). The winter (summer) regime is dominated by air inflow (outflow), low (high) pCO$_2$-air, and lower (higher) epikarstic drawdown (Ridley et al., 2015). However, on previous studies of tropical cave monitoring,

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Ridley and his colleagues had no robust evidence of the relationship between stalagmite growth and surface climate. In this study, to elucidate environmental parameters controlling stalagmite growth rate in tropical area, cave monitoring was conducted throughout a year in Petruk Cave, central Java, Indonesia. We monitored stalagmite growth rate and environmental parameters of relative humidity, air temperature, drip rate, [Ca^{2+}], and $p$$CO_2$-air. Results of this study are a significant first step to reconstruct paleoclimate or environment precisely using stalagmites in a tropical area, especially in regions characterized by constant outside air temperature through the year and a seasonal rainfall pattern.

Study Area

Cave monitoring was conducted in Petruk Cave, central Java, Indonesia. The cave is located on the western side of Karangbolong karst area, and the total length of the cave is about 350 m (Fig. 1). The elevation of the main entrance is about 80 m a.s.l., and a river flows along the main corridor of the cave. Limestone bedrock overburden of the cave is approximately 70 m above the entrance and 120 m above the center of the cave (Fig. 1c).

The climate diagram of Jogjakarta city, which is located about 100 km east from Petruk Cave, is shown in Figure. 2. At the study site, the monthly mean temperature is almost constant throughout the year, and precipitation has a seasonal cycle, with the dry season from May to October and the wet season from November to April (Fig. 2), in conjunction with movement of the Intertropical Convergence Zone.

Monitoring Method

Monitoring was conducted at seven stations in the cave (P1-2, P1-river, P2, P3-1, P3-2, P3-river, and P4) and a station outside the cave (Fig. 1). The surface station is located 200 m away from the main entrance and at an elevation of 0 m a.s.l.. P1-2 is located on the cave river terrace that is about 50 m away from the main entrance and about 4 m above the cave floor. P1-river is located at the stream bank of the cave river near P1-2. P2 is located at the narrow corridor with a climb up the stairs from the large hall (approximately 30 m width, 70 m length, and 30 m height), which is about 150 m away from the entrance, and the relative elevation to the cave river is about 30 m. P3-1 and P3-2 are adjacent stations (approximately 3 m distance), and both stations are located on the cave river terrace on the east side of the large hall. P3-river is located at the stream bank of the river near P3-1 and P3-2. P4 is located 30 m away from the large hall and about 4 m above the river. An open corridor runs from the entrance to P1-2, P1-river, P3-1, P3-2, and P4, whereas P2 is located in a relatively closed part of the cave (Fig. 1b and c).

In this study, stalagmite growth rate and environmental parameters were monitored in Petruk Cave from October 2011 to December 2012. Environmental parameters that were monitored are relative humidity, air temperature in the cave and outside, cave airflow direction, wind speed, $p$$CO_2$-air, drip rate, precipitation, and chemical compositions of drip water (i.e., Ca^{2+},HCO_3^- , Na^+, Mg^{2+}, Cl^-, NO_3^- and SO_4^{2-}).

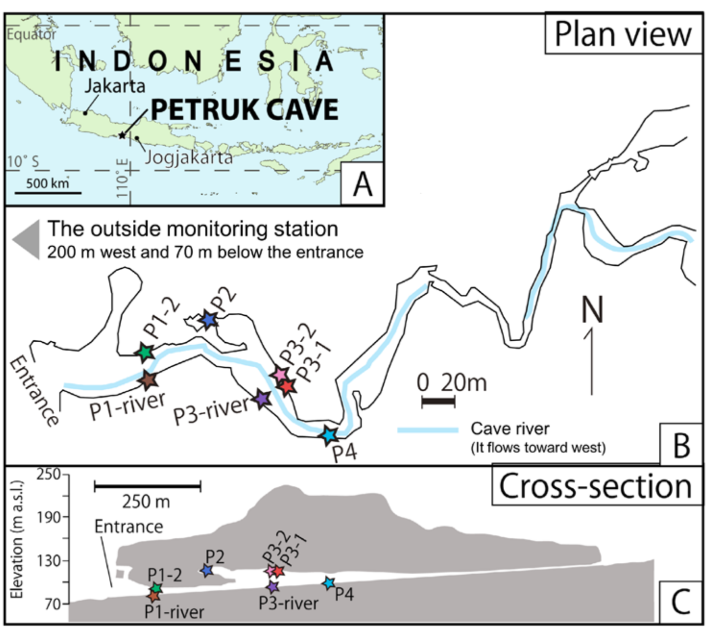

Figure 1. Location and monitoring station map of Petruk Cave. It is modified from cave map of Brahamantyo et al. (2006). (A) The location of the cave. (B) Plan view of the cave. The stars present monitoring stations inside the cave, and the outside monitoring station is 200 m west and 70 m below the entrance. (C) Cross-section of the cave.

Monitoring parameters and stations are summarized in Table 1 and Figure 1. Air temperature, airflow, and pCO2-air were measured at all cave stations. Relative humidity was measured at P1-2, P1-river, P3, and outside. Drip water sampling and the measurement of stalagmite growth rate were conducted at four stations (P1-2, P2, P3-1, and P3-2) and three stations (P1-2, P3-1, and P3-2), respectively.

Precipitation, drip rate, air temperature, and relative humidity were logged with Onset HOBO micro Station Logger (H21-002) in 15 to 20 minute intervals. Precipitation and drip rate were measured with tipping-bucket rain gauges (Onset S-RGB-M002). Air temperature and relative humidity were measured with air temperature and relative humidity smart sensors (Onset U-DTW-1).

$p$$CO_2$-air was measured and logged continuously with Sense Air A/N074001 CO_2 gauges in 20 minute intervals. Airflow direction was measured with incense sticks, and wind speed measured with Testo 425 hot-wire anemometer. Water temperature and pH were measured in situ with a HORIBA D-54 pH gauge.

Drip water was collected into 10.4 ml or 4.9 ml tubes

Table 1. Cave monitoring parameters and stations.

		Air				Drip Water						
Station Name	**Precip.**	**Temp.**	**Relative Humidity**	***p*CO$_2$-air**	**Air-Flow Direction and Speed**	**Drip Rate**	**Chemical Composition[a]**	**pH**	**Water Temperature**	***p*CO$_2$-drip**	**SI$_{cc}$**	**Speleothem Growth Rate**
Outside	L	L	L	···	···	···	···	···	···	···	···	···
Entrance	···	···	···	···	M	···	···	···	···	···	···	···
P1-2	···	L	L	Ls	M	L	S	M	M	C	C	S
P1-River	···	L	L	Ls	M	···	···	···	···	···	···	···
P2	···	Ls	···	Ls	M	···	S	M	M	C	C	···
P3-1	···	L (P3)	L (P3)	Ls (P3)	M (P3)	L	S	M	M	C	C	S
P3-2	···	L (P3)	L (P3)	Ls (P3)	M (P3)	L	S	M	M	C	C	S
P3-River	···	Ls	···	Ls	M	···	···	···	···	···	···	···
P4	···	Ls	···	Ls	M	···	···	···	···	···	···	···

[a] Measured drip water chemical composition = (i.e., Ca^{2+}, HCO_3^-, Na^+, Mg^{2+}, Cl^-, NO_3^-, and SO_4^{2-}).

Notes:
C = Calculated by other data.
L = Continuous logging.
Ls = A few days continuous logging in each season.
M = Manual measurement in each season.
S = Sampling and laboratory measurement.

and sealed with silicon plugs. Major ion compositions of drip water (i.e., Ca^{2+}, Na^+, Mg^{2+}, Cl^-, NO_3^- and SO_4^{2-}) were determined by ion chromatography (DIONEX ICS-1100). Bicarbonate ion [HCO_3^-] was determined by the spectrophotometric method according to Mishima et al. (2009). The saturation index of calcite (SI_{cc}) is defined as follows:

$$SI_{CC} = \frac{\log[Ca^{2+}] \times [CO_3^{2-}]}{K_C} = \log \frac{K_2 \times [Ca^{2+}] \times [HCO_3^-]}{K_C \times [H^+]}$$

K_c and K_2 are respectively the equilibrium constants for the reactions $Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3$ and $HCO_3^- \leftrightarrow H^+ + CO_3^{2-}$. K_c and K_2 are determined by drip-water temperature.

Stalagmite growth rate was measured by a stalagmite-farming experiment at three stations (i.e., P1-2, P3-1, and P3-2; Fig. 1; Table 1). A sandblasted glass plate was installed horizontally, and then the glass plate was weighted before and after the stalagmite farming to estimate stalagmite growth rate.

Results

Relative Humidity, Air Temperature, Drip Rate, and Precipitation

Monitoring results of relative humidity, air temperature, drip rate, and precipitation are shown in Figure 3. Outside relative humidity varied from 43 to 100 %, and the daily average was 75 to 95% (Fig. 3a). Outside relative humidity of the dry season was about 10% less than that of the rainy season. At P1-2, relative humidity was from 90 to 100% throughout the year and the daily range was little (1 to 3%). Relative humidity during the early dry season (May to July) was 3 to 5% less than the other season. At P3-1, relative humidity was almost 100%, and there were no variations on both daily and seasonal scale.

Although outside air temperature fluctuated from 21.8 to 34.5 °C, the daily average was 25 to 29 °C throughout the year and there was no seasonal variation (Fig. 3a). At P1-2 and P1-river, air temperature varied from 23 to 27 °C and daily range was less than 2 °C. At P3-1, air temperature varied from 26.7 to 27.3 °C and there was no fluctuation on both daily and seasonal scale.

The drip-rate varied from 0.042 to 0.061 ml s^{-1} at P1-2, from 0.058 to 0.068 ml s^{-1} at P3-1, and from 0.007 to 0.018 ml s^{-1} at P3-2, respectively (Fig. 3b). On each station, drip-rate was almost constant throughout a year and had no response to heavy rainfall (Fig. 3b).

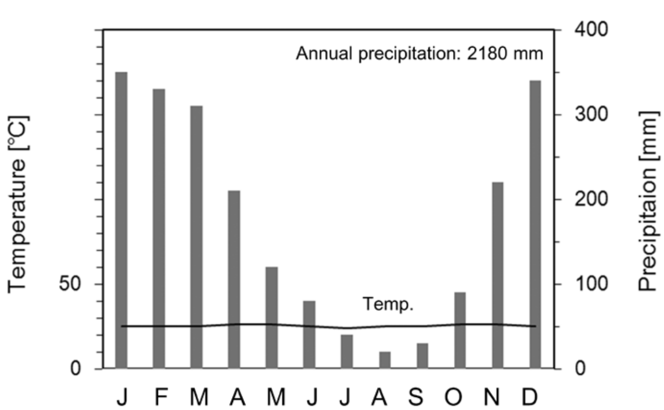

Figure 2. The climate of Jogjakarta city, which is located about 100 km east from Petruk Cave. The climate data are from http://www.weatherbase.com. The monthly means of air temperature are almost constant throughout a year. Annual precipitation is about 2180 mm, and there is a seasonal cycle with a dry season from May to October and a wet season from November to April.

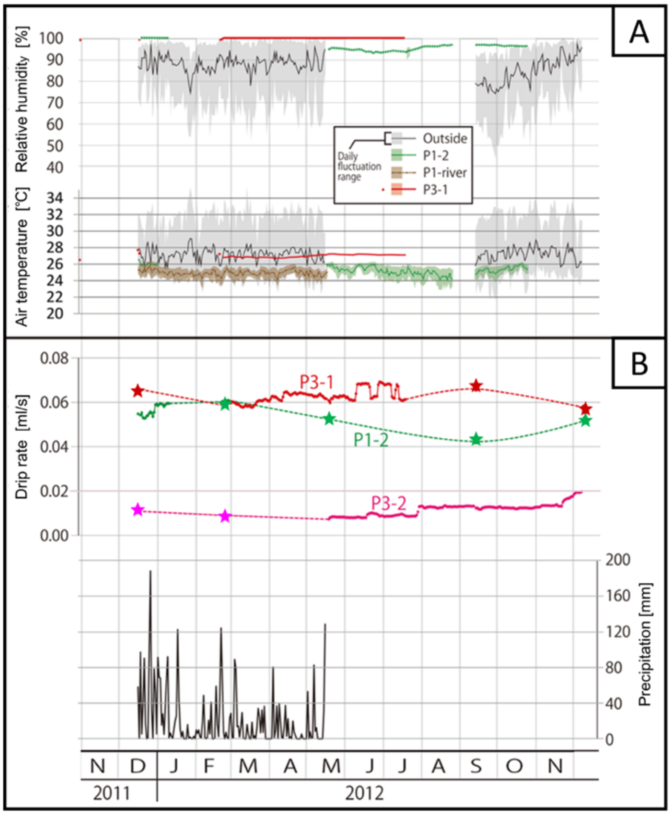

Figure 3. (Top) Temporal variations of relative humidity and air temperature. Shaded bands indicate daily fluctuation range. Relative humidity inside the cave (i.e., P3-1) is almost 100 % and there was no variation on both daily and seasonal scale. Air temperature inside the cave (i.e., P3-1) is also constant. (Bottom) Temporal variations of drip rate and precipitation. Solid lines indicate the results of continuous monitoring and the dashed lines represent interpolated trend of "snap-shot" data measured manually.

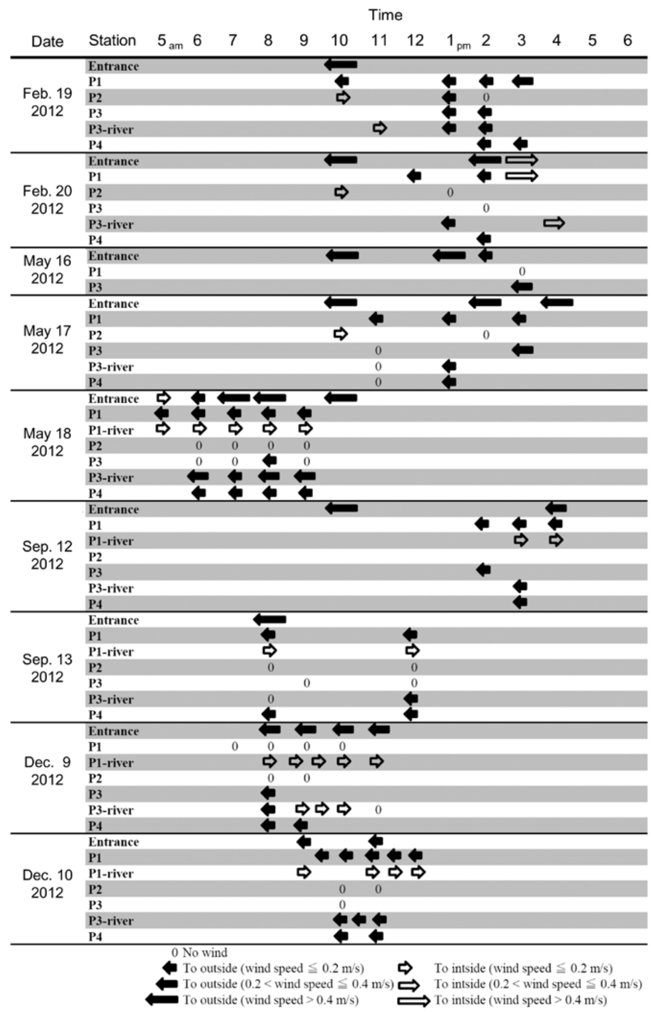

Figure 4. Results of airflow monitoring. The right arrows represent airflow toward the inside of the cave, whereas the left are toward the outside. The length of the arrows shows strength of airflow.

Cave Ventilation and pCO$_2$-air

Cave ventilation

The monitoring results for cave airflow are shown in Figure 4. Airflow speed at the entrance is higher than that of cave inside. No airflow was sometimes observed at P2 and P3, which are located at the upper part of the cave. Throughout a year, cave air almost always flows toward the entrance, except at P1-river, where it usually flows inward.

Seasonal fluctuation of pCO$_2$-air

The seasonal fluctuation of pCO$_2$-air is shown in Figure 5. In May 2012, September 2012, and December 2012, continuous monitoring was conducted during a few days each month. In February 2012, long continuous monitoring was not conducted, but snapshot pCO$_2$-air measurements were conducted by manual measurements. Results of the snapshot measurement were approximately 400 to 1700 ppm at P1-2, approximately 1400 to 2100 ppm at P2, approximately 1600 to 2400 ppm at P3-1, and approximately 2100 to 2500 ppm at P4, respectively. It is thought that the snapshot data are biased for daytime because the measurements were conducted in-situ only from 10 a.m. to 4 p.m.

In September 2012, pCO$_2$-air was dramatically lower than other seasons, and daily range was also low at all stations inside cave (Fig. 5). In contrast, in May 2012 and December 2012, pCO$_2$-air was higher and daily fluctuating range was also higher (Fig. 5).

Daily fluctuation of pCO$_2$-air

Daily pCO$_2$-air variations are shown in Figures 6 and 7. Figure 6 is continuous-monitoring data during 3 days or 4 days in May 2012, September 2012, and December 2012. In Figure 7 are the data of February 2012, when we conducted continuous monitoring during only 6 hours or 10 hours. Throughout the year, outside air temperature reached to the minimum around 6 a.m. and a maximum around 1 p.m. (Fig. 6; Fig. 7), whereas air temperature inside the cave stays constant (approximately 27 °C). pCO$_2$-air reached a minimum around 6 a.m. and a maximum around 2 to 4 p.m. (Fig. 6; Fig. 7).

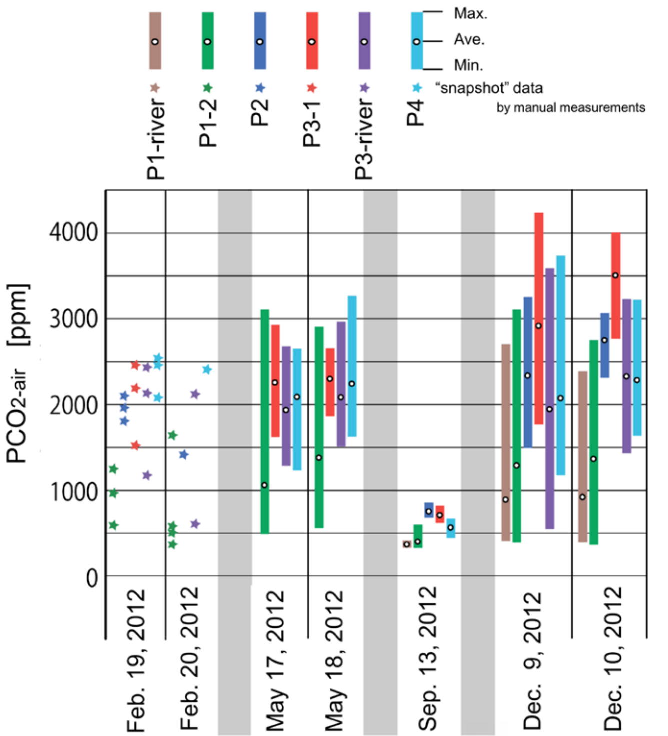

Figure 5. Seasonal fluctuation of pCO_2-air. The circles present daily average and bars present daily fluctuation based on the results of continuous monitoring. Stars indicate the snap-shot data measured by manual observation. Note that pCO_2-air in the dry season (i.e., September- 2012) was very low and the daily fluctuations small.

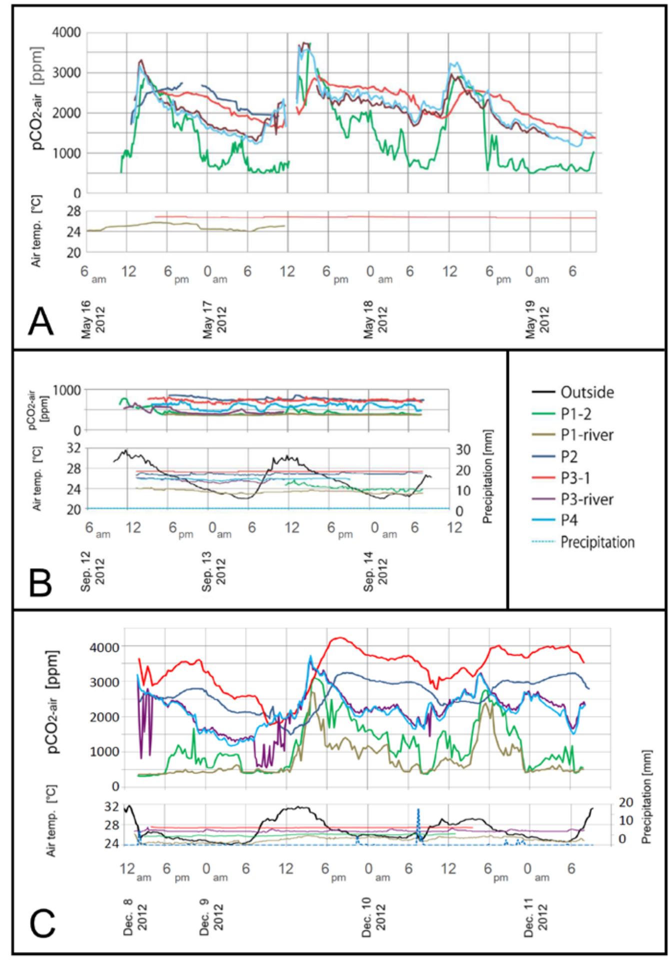

Figure 6. Daily fluctuation of pCO_2-air and air temperature. (A) May 16−19, 2012. (B) September 12−14, 2012. (C) December 8−11, 2012. pCO_2-air reached a minimum around 6 a.m. and a maximum around 2 to 4 p.m.

Chemical Composition of Drip Water

Chemical data of drip water are summarized in Table 2, and some data are graphed in Figure 8. Obvious seasonal variations on all data of drip water were regarded as unlikely (Table 2; Fig. 8).

Because there was no daily fluctuation data of pCO_2-air in December 2011 and February 2012, we chiefly describe seasonal variation of drip water based on the data of May 2012, September 2012, and December 2012. [Ca^{2+}] and [HCO_3^-] of September 2012 appear to be lower than those of other seasons (i.e., May 2012 and December 2012), and [Mg/Ca] ratio of September 2012 is slightly higher than that of other seasons (Fig. 8). The evidence that the [Mg/Ca] ratio becomes higher during lower pCO_2-air resembles results of previous researches (e.g., Mattey et al. 2010; Wong et al., 2011), showing that the amount of prior calcite precipitation (Fairchild et al., 2000) increases during dry season due to low pCO_2-air (Fig. 5). However, there is no definitive seasonal variations of pH, saturation index (SI_{cc}) and pCO_2-drip (Fig. 8), as shown in previous studies (e.g., Spötl et al., 2005; Boch et al., 2011), implying that prior calcite precipitation has a small influence on drip water geochemistry in this cave.

Stalagmite Growth Rate

During the stalagmite farming experiment, donut-shaped stalagmite was formed on the glass plate. Figure 9 presents stalagmite growth rates on the glass plate. The growth rates during the dry season (i.e., May 2012 to September 2012) were 2 to 5 mg day^{-1} and those in the rainy season were less than 1.8 mg day^{-1}. The growth rates of dry season were higher than those of rainy season.

Discussion

Environmental Parameters Controlling Stalagmite Growth

According to previous cave-monitoring studies, stalagmite growth was affected by air temperature, drip rate, and [Ca^{2+}] (e.g., Baker et al., 1998; Genty et al., 2001) or pCO_2-air (e.g., Spötl et al., 2005; Banner et al, 2007; Baldini et al., 2008). Herein we discuss environmental parameters controlling stalagmite growth rate based on the monitoring results of Petruk Cave.

Table 2. Chemical compositions of drip water samples.

Sample Location	Date (mmddyy)	Time	Temp. (°C)	pH	Drip Rate (mL/s)	pCO$_2$-air (ppm)	Na$^+$ (mmol/L)	Mg^{2+} (mmol/L)	Ca^{2+} (mmol/L)	Cl$^-$ (mmol/L)	NO$_3^-$ (mmol/L)	SO$_4^{2-}$ (mmol/L)	HCO$_3^-$ (mmol/L)	pCO$_2$-drip (ppm)	SI$_{cc}$
P1-2	121611	···	26.5	7.9	0.055	870	0.113	0.029	1.669	0.062	0.849	0.012	2.496	1833	0.565
P1-2	21912	11:23	26.1	7.76	0.0583	633	0.111	0.028	1.426	0.071	0.553	0.014	2.342	2378	0.331
P1-2	51612	12:11	26.3	7.8	0.0533	1061	0.129	0.031	1.645	0.07	1.293	0.014	2.08	1925	0.38
P1-2	51812	6:26	26	7.7	0.0557	751	0.11	0.03	1.63	0.08	1.22	0.014	2.231	2585	0.302
P1-2	51812	8:32	26.1	7.73	···	613	0.108	0.03	1.639	0.068	1.133	0.014	2.268	2454	0.342
P1-2	91312	8:28	25	7.98	0.0426	433	0.172	0.027	1.282	0.082	0.523	0.013	2.123	1286	0.452
P1-2	91312	12:18	25.3	7.87	0.043	599	0.108	0.026	1.33	0.057	0.514	0.012	2.198	1718	0.375
P1-2	91312	13:45	25.3	7.9	0.0471	423	0.11	0.026	1.328	0.058	0.513	0.012	2.212	1614	0.407
P1-2	120912	8:16	26.4	7.83	0.0516	436	0.12	0.029	1.65	0.066	0.997	0.012	2.133	1843	0.423
P1-2	121012	12:37	26.1	7.91	0.0533	924	0.114	0.029	1.624	0.07	1.075	0.011	2.177	1558	0.501
P2	121611	···	27	7.9	0.0153	2550	0.178	0.033	1.324	0.098	0.215	0.012	2.542	1899	0.488
P2	22012	10:42	27	8.01	0.0234	1592	0.124	0.037	1.043	0.07	0.124	0.014	2.022	1188	0.407
P2	51712	11:17	27.1	8.02	0.0126	1875	0.176	0.038	1.236	0.111	0.074	0.012	2.494	1420	0.574
P2	91312	13:14	28.3	7.87	0.0094	856	0.114	0.031	0.922	0.068	0.111	0.012	1.829	1523	0.194
P2	120912	13:42	27.3	8.02	···	1719	0.121	0.034	1.321	0.059	0.413	0.011	2.14	1223	0.54
P3-1	121511	···	27.7	7.6	0.0661	2618	0.137	0.036	1.595	0.062	0.528	0.014	2.681	4006	0.294
P3-1	121611	···	27.3	7.9	0.0598	1963	0.138	0.036	1.592	0.06	0.493	0.014	2.628	1957	0.58
P3-1	21912	14:06	27.2	7.69	0.0616	1948	0.13	0.034	1.351	0.072	0.293	0.015	2.546	3092	0.29
P3-1	51612	14:46	27.1	7.67	0.0544	2528	0.129	0.035	1.438	0.07	0.512	0.016	2.494	3160	0.285
P3-1	51812	7:17	27	7.54	···	2221	0.124	0.035	1.4	0.07	0.594	0.015	2.373	4058	0.122
P3-1	51812	9:19	27	7.67	···	1991	0.124	0.035	1.468	0.073	0.524	0.015	2.549	3220	0.3
P3-1	51712	14:54	27.1	7.6	0.0576	2343	0.137	0.036	1.442	0.077	0.677	0.016	2.31	3443	0.184
P3-1	91312	10:11	27.1	7.75	0.0662	745	0.13	0.033	1.152	0.062	0.112	0.014	2.416	2570	0.263
P3-1	91312	14:45	26.8	7.79	0.0721	816	0.132	0.033	1.18	0.062	0.118	0.014	2.41	2325	0.307
P3-1	120912	9:23	27.4	7.85	0.0576	1784	0.139	0.034	1.472	0.071	0.541	0.013	2.445	2055	0.47
P3-1	121012	14:14	27.1	7.9	0.0563	3384	0.131	0.035	1.457	0.06	0.524	0.014	2.525	1882	0.525
P3-2	121511	···	27.3	7.7	0.0113	2618	0.14	0.035	1.435	0.065	0.37	0.015	2.489	2953	0.316
P3-2	22012	14:23	27.1	7.76	0.0094	1277	0.132	0.033	1.201	0.074	0.344	0.016	2.072	2156	0.225
P3-2	51712	15:27	27.3	7.76	···	2665	0.13	0.034	1.354	0.071	0.471	0.015	2.332	2418	0.326
P3-2	91312	9:36	27.2	7.81	0.0122	705	0.136	0.034	1.134	0.177	···	0.013	2.358	2190	0.308
P3-2	121012	11:24	27	7.8	···	3051	0.135	0.034	1.557	0.063	0.21	0.013	2.887	2691	0.506

Notes:
Analytical errors are ±0.1 °C for temperature; ±0.01 for pH; ± 4% for drip rate; and ±3 % for pCO$_2$-air.
Analytical errors of concentrations are less than 1% for Ca^{2+}, NO$_3^-$, SO$_4^{2-}$; 3% for Na$^+$, Mg^{2+}; 12% for Cl$^-$; and 1% for HCO$_3^-$

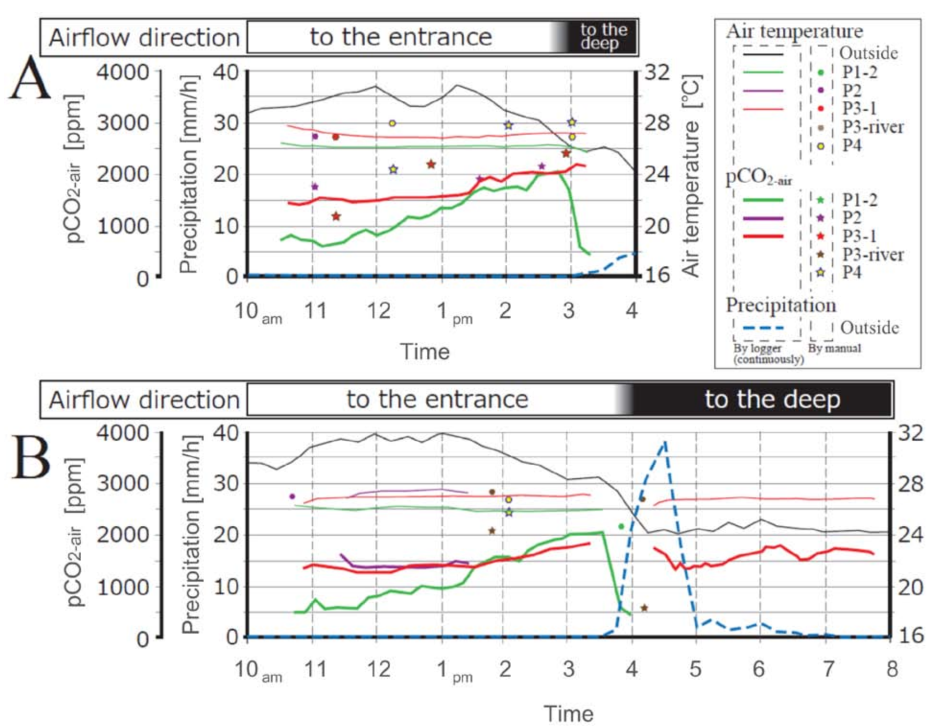

Figure 7. Relationships between air temperature, precipitation, and pCO$_2$-air on February 19, 2012 (A) and February 20, 2012 (B). After heavy rainfall, outside air temperature suddenly decreased and cave-airflow direction was reversed near the entrance. Then, outside air with low pCO$_2$-air was flowed into the cave and pCO$_2$-air dramatically decreased.

Air temperature: According to Baker et al. (1998) and Genty et al. (2001), stalagmite growth rate correlates positively with cave air temperature. In this cave, however, air temperature is almost constant throughout the year (Fig. 3), suggesting that cave air temperature has no influence on stalagmite growth.

Drip rate: According to Fairchild and Baker (2012), Dreybrodt and Franke (1987) show that stalagmite growth rate correlates positively with drip rate. On every sampling site of Petruk Cave, drip rates are almost constant throughout the year (Fig. 3), and there are changes on stalagmite growth rate throughout the year (Fig. 9), suggesting that drip rate is not the main influencing factor.

[Ca^{2+}]: Baker et al. (1998) reports that [Ca^{2+}] of drip water has a positive correlation with stalagmite growth rate. Assuming it to be true, [Ca^{2+}] of May and September 2012 should be higher than other seasons, because the growth rate is higher during this time (Fig. 9). However, this was not observed (Fig. 8), suggesting that [Ca^{2+}] is not the main factor of stalagmite growth in this cave.

***p*CO_2-air:** After the cave monitoring of Spötl et al. (2005), *p*CO_2-air has attracted attention as a key factor to control stalagmite growth (e.g., Spötl et al., 2005; Banner et al., 2007; Baldini et al., 2008). Comparing *p*CO_2-air of this cave with stalagmite growth (Fig. 5 and Fig. 9), there is the relationship that stalagmite growth rate is high (low) when *p*CO_2-air is low (high) during dry (rainy) season. This evidence suggests that *p*CO_2-air controls stalagmite growth in this cave, a result similar to previous studies in mid latitudes (central Texas, USA; Banner et al., 2007). Note that this is a first report that dramatic *p*CO_2-air reduction occurs during the dry season in a tropical cave and controls stalagmite growth. According to Banner et al. (2007), outside air temperature of central Texas has a large seasonal variation, and *p*CO_2-air reduction occurs during winter months due to active cave ventilation. On the other hand, outside air temperature of our study area is almost constant through the year, and precipitation has a distinctive seasonal cycle (Fig. 2). Even on such region of the tropics, it is worth noting that the dramatic *p*CO_2-air reduction occurs during the dry season.

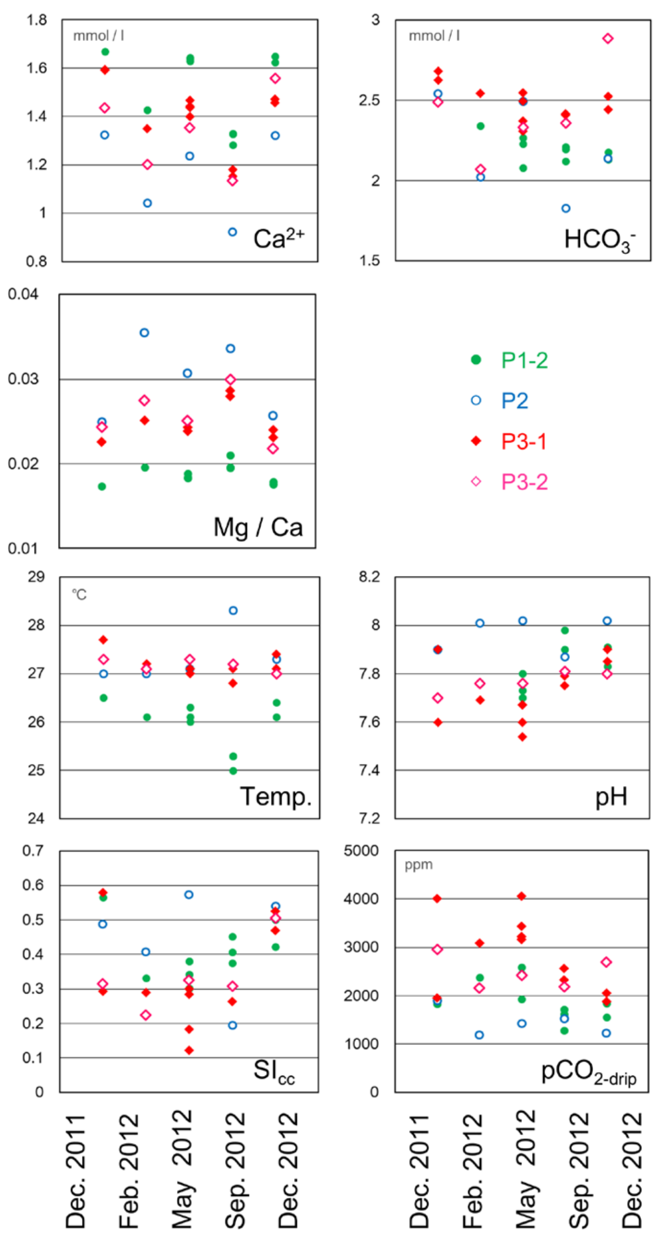

Figure 8. Chemical compositions of drip-water samples.

Possible causes of *p*CO_2-air fluctuation

Because *p*CO_2-air is the main factor controlling stalagmite growth in Petruk Cave as described above, we discuss the mechanism of *p*CO_2-air fluctuation. The monitoring results show that *p*CO_2-air fluctuations are divided into seasonal fluctuations (Fig. 5) and daily fluctuations (Fig. 6; Fig. 7).

Seasonal fluctuation of *p*CO_2-air: Daily averages of *p*CO_2-air are 400 to 800 ppm for September 2012, but 900 to 3500 ppm during the rainy seasons in May 2012 and December 2012 (Fig. 5). Seasonal fluctuation of *p*CO_2-

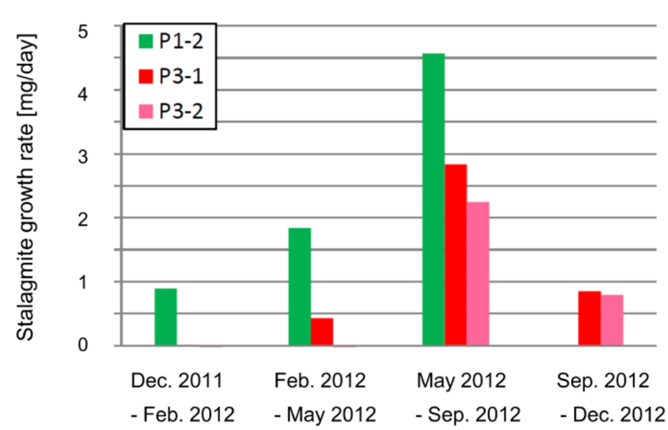

Figure 9. Stalagmite growth rates calculated by the weight of stalagmite farming on glass plates. Growth rates were higher during the dry season from May 2012 to September 2012 at all stations.

air observed in this study is typical, as in previous studies (e.g., 400 to 1400 ppm in Austria, Spötl et al., 2005; 500 to 8000 (30000 in extreme case) ppm in central Texas, Banner et al, 2007; 500 to 8000 ppm in Gibraltar, Mattey et al., 2010). Moreover, our seasonal fluctuation of pCO_2-air is similar to that of Ridley et al. (2015) although the values they observed in Belize are lower than those of this study. The summer is characterized by higher mean pCO_2-air (~500 ppm) and high temporal variability, whereas winter has lower pCO_2-air (420 ppm) and displays lower temporal variability. Consequently, summer and winter in Belize correspond to wet and dry season in this study, respectively. According to Spötl et al. (2005), pCO_2-air variation is driven by cave ventilation or CO_2 production in the soil zone. In the case of Belize, the change of airflow direction from inflow to outflow appear mainly due to thermally induced inside-outside air density differences (Ridley et al., 2015). However, in Petruk Cave, airflow direction is almost constant throughout the year, as shown in Figure 4, because seasonal variation of outside air temperature is much lower (Fig. 2). On the other hand, CO_2 productivity in the soil zone is assumed to have a seasonal variation due to rainy and dry season, similarly to the case of Thailand (Hashimoto et al., 2004). Accordingly, seasonal pCO_2-air fluctuation and stalagmite growth are because in the rainy season, the rainfall amount enhances plant activity that leads to an increase of CO_2 productivity in the soil zone, and therefore, higher pCO_2-air that reduces stalagmite growth. During the dry season, little rainfall results in declines in the plant activity that lead to a reduction of CO_2 productivity in the soil zone, and therefore, lower pCO_2-air, resulting in increased stalagmite growth.

Daily fluctuation of pCO_2-air: pCO_2-air reaches a minimum around 6 a.m. and a maximum around 2 to 4 p.m. (Fig. 6; Fig. 7). Air temperature inside the cave remains constant throughout the year, and outside air temperature reaches a minimum around 6 a.m. and a maximum around 1 p.m. This daily fluctuation of pCO_2-air is similar to that observed in Belize during summer (Ridley et al., 2015). As Ridley and his colleagues pointed out, daily pCO_2-air fluctuation observed in this study is also likely to be driven by the cave ventilation arising from the difference of air temperature between the outside and inside the cave or CO_2 productivity of soil zone. In order to understand exactly the detailed mechanism of daily pCO2-air fluctuation, it is important for future studies to examine the cave ventilation at night and CO_2 productivity of soil zone. It is also noteworthy that dynamic pCO_2-air reduction was observed after heavy rain in February 2012 (Fig. 7). This is likely to have been caused by cave ventilation change driven by the sudden drop of outside air temperature due to a downburst during severe rain (Fig. 7).

Conclusions

In this study, stalagmite growth rate and environmental parameters were monitored in Petruk Cave from October 2011 to December 2012. We compared stalagmite growth rate with the environmental parameters, air temperature, drip rate, [Ca^{2+}], and pCO_2-air. As a result, there is the relationship that stalagmite growth rate is fast (slow) when pCO_2-air is low (high) during dry (rainy) season, suggesting that pCO_2-air controls stalagmite growth.

The monitoring results show that pCO_2-air fluctuation is divided into seasonal fluctuation and daily fluctuation. For the seasonal type, dramatic pCO_2-air reduction during the dry season is likely result from a decline of plant activity due to little rainfall. Daily pCO_2-air reached a minimum around 6 a.m. and a maximum around 2 to 4 p.m., although it is not obvious whether plant activity or cave ventilation arising from the difference of air temperature between the outside and the inside cave. Dynamic pCO_2-air reduction was also observed after a heavy, intense rain, suggesting that heavy rainfall of short duration is also one factor that controls cave ventilation and pCO_2-air.

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SINKHOLE CLUSTERS AFTER HEAVY RAINSTORMS

Mario Parise [1,2,C], Luca Pisano[2], and Carmela Vennari[2,3]

Abstract

Sinkholes are the most common geological hazard in karst terrains. Generally triggered by rainstorms or by changes in the hydrological/hydrogeological regime, they display a wide range of morphologies and sizes. Typically, the main difficulty in evaluating the sinkhole hazard is represented by the collection of reliable data about time of occurrence of the events, which is a mandatory requirement for the estimation of the hazard. In this paper, we document a dozen sinkholes triggered by a heavy rainstorm that occurred during the first week of September 2014 in the Gargano Promontory of Apulia (southeastern Italy). Following a description of the rainstorm, two clusters of sinkholes are described, starting with the identification of the sinkhole type and of the main morphometric characteristics. Even though it is very likely that the documented sinkholes are only a fraction of those caused by the September 2014 rainstorm, this documentation provides insights for the collection of important sinkhole data produced by a specific, triggering storm.

Introduction

Sinkholes (or dolines) are closed depressions with internal drainage, widely regarded as one of the main diagnostic landforms of epigenic karst (Ford and Williams, 2007; Palmer, 2007; Gutiérrez, 2010; De Waele et al., 2011). Sinkholes display a wide range of morphologies (cylindrical, conical, bowl- or pan-shaped), varying in size up to hundreds of meters across and typically from a few to tens of meters deep (Gutiérrez et al., 2008, 2014). The origin of a sinkhole can be natural, if related to the presence of soluble rocks, or anthropogenic. In this latter case, anthropogenic sinkholes indicate a connection to a man-made underground cavity (De Bruyn and Bell, 2001; Parise and Gunn, 2007; Waltham and Lu, 2007; Parise, 2012, 2015).

Natural sinkholes are typically triggered by rainfall events and by the consequent effect these events might have on groundwater circulation (White and White, 1984; Kovačič and Ravbar, 2010; Lei et al., 2016). Another significant trigger is seismic shocks, which may work in creating new sinkholes, or in enlarging those already existing (Kawashima et al., 2010; Parise et al., 2010; Borgatti et al., 2013).

Sinkholes are typically described by their spatial distribution and density (Day, 1983; Hung et al., 2002; Angel et al. 2004; Gao et al., 2005; Kemmerly, 2006; Lyew-Ayee et al., 2006; Bautista et al., 2011), in relation to the built-up environment (He et al., 2003; Scheidt et al., 2005; Brinkmann et al., 2008; Cooper, 2008), or to geological and morphological settings (Panno et al., 1994; Denizman, 2003; Florea, 2005; Del Prete et al., 2010; Basso et al., 2013; Fragoso-Servón et al., 2014), often without entering into specific details about date of occurrence. Other studies focus on the integration of different approaches, for the identification of the sinkhole-prone areas, from stratigraphy to geophysical techniques, to the use of digital elevation models (Ezersky et al., 2009; Frumkin et al., 2011; Margiotta et al., 2012, 2016; Miao et al., 2013; Wu et al., 2016).

Detailed documentation about sinkholes, aimed at ascertaining their direct relationships with the triggering factor (rainfall, earthquake, etc.), is not always easily accessible in the scientific literature. With the exception of some states in the United States (namely, Florida, Kentucky and Illinois, where there is high awareness about sinkhole problems; see in this regard, White et al., 1986; Tihansky, 1999; Brinkmann et al., 2007, 2008; Brinkmann, 2013, and references therein; Polk et al., 2015), in the rest of the world, attention toward sinkholes is typically not so high. It is definitely lower than that paid to other geological hazards such as landslides, floods, or tsunamis. Post-event sinkhole surveys rarely have the amount of information necessary to fully link each event to its precise time of occurrence. This often represents the main drawback in the process of sinkhole hazard evaluation, as knowledge of the temporal occurrence is mandatory at this goal (Gutierrez-Santolalla et al., 2005; Farrant and Cooper, 2008; Galve et al., 2011; Heidari et al., 2011).

Even if sinkhole occurrence in Italy is not frequent, when compared to other geological hazards such as landslides or floods, there are many regions highly prone to these events due to the widespread presence of carbonate or evaporite rocks. These rocks are highly susceptible to dissolution processes, which may be locally enhanced or favored by a number of human activities (Iovine et al., 2010, 2016; Vigna et al., 2010; Parise, 2012; Zini et al., 2015; De Waele et al., 2017). Within the framework of a project by the Institute of Research for Geo-Hydrological Protection of the National Research Council of Italy (CNR IRPI), dedicated to evaluation of natural and anthropogenic hazards in karst, Parise and Vennari (2013) built a chronological database on sinkhole occurrence in Italy that contains information about sinkholes. These include morphometric data, damage, and triggering factors. In this database, sinkholes are divided on the

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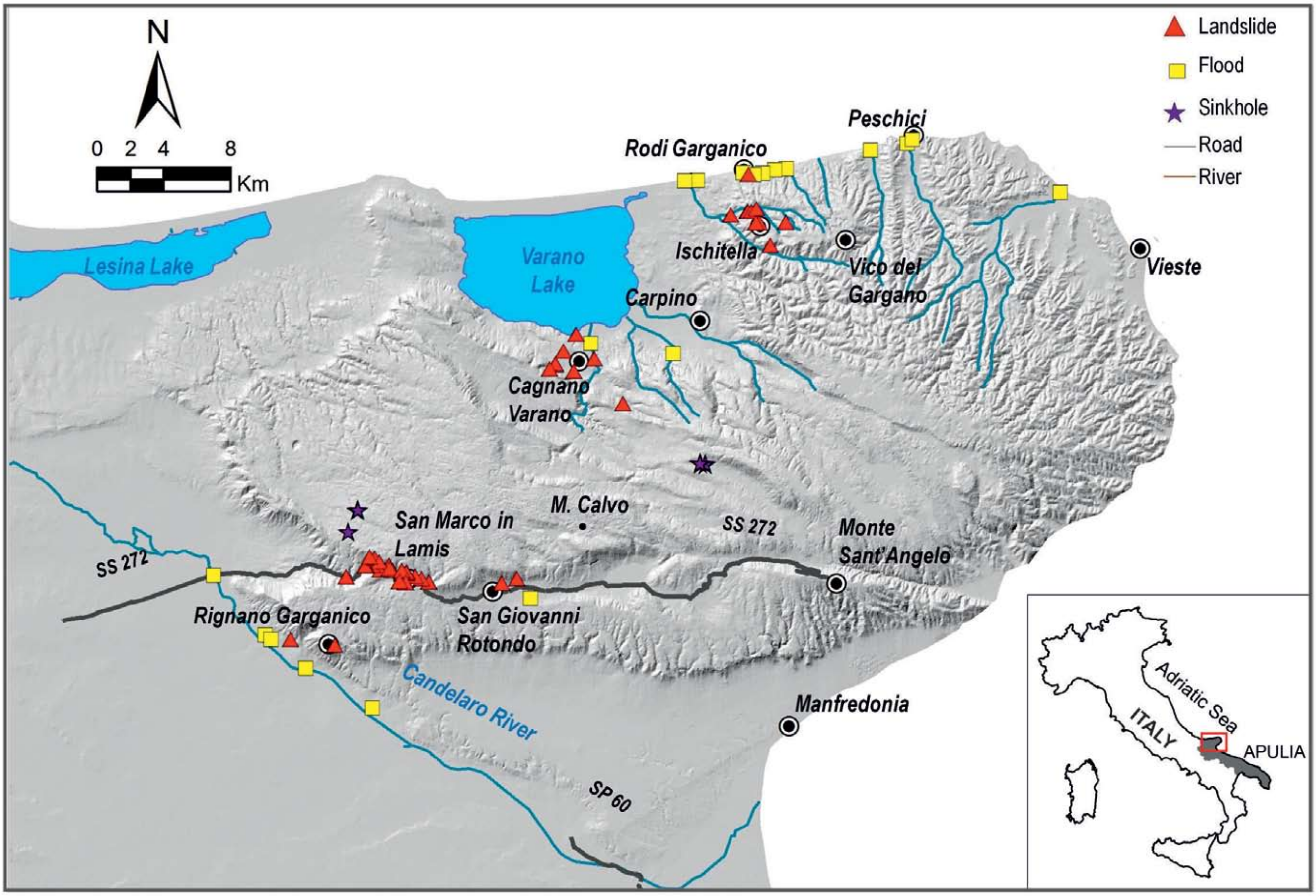


Figure 1. Map showing location of the study area and the events triggered by the September 1-6, 2014, intense rainfall event in the Gargano Promontory.

basis of cave origin: natural or anthropogenic. Time of occurrence and location of sinkholes are known for every event, with different levels of accuracy and certainty, based upon a careful examination of the information source from each event.

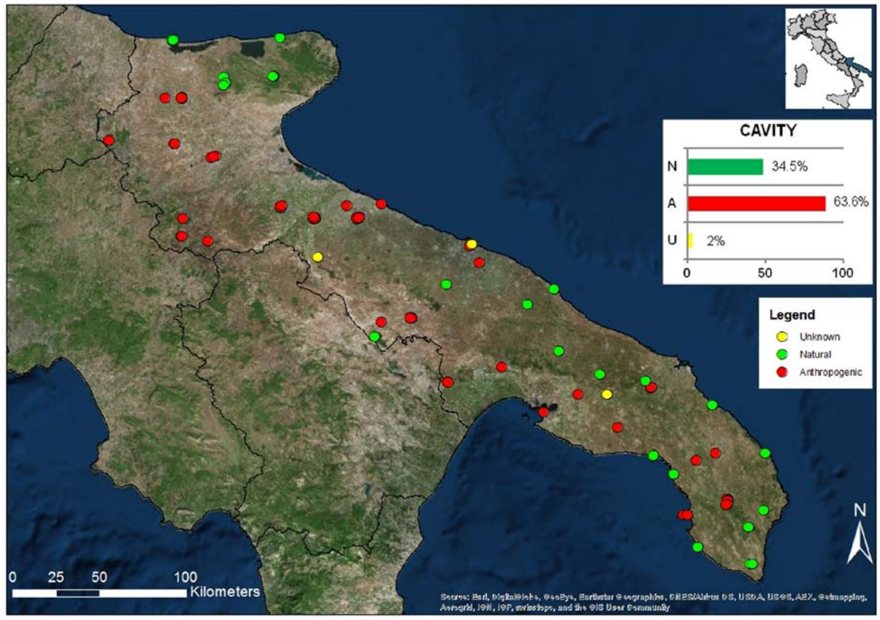
In this paper, aimed at contributing to increase the documentation between sinkholes and triggering factors, we present data about a number of sinkholes that occurred, together with other geological hazards such as landslides and floods (Fig. 1), in consequence of the rainstorm that hit the Gargano Promontory in northern Apulia, during the first week of September 2014. We are strongly convinced that availability of a large amount of reliable information about sinkhole occurrence, and their main morphometric features at the time of the formation, is a fundamental piece of evidence contributing to properly move toward assessment of the sinkhole hazard. With such a goal, we document and describe the observed sinkholes to improve the available sinkhole data from the karst of Apulia.

Sinkholes in Apulia

In the CNR IRPI database (Parise and Vennari, 2013), the Apulia region (southeastern Italy) is one region with the highest sinkhole occurrence (Delle Rose and Parise, 2002; Bruno et al., 2008; Fidelibus et al., 2011; Festa et al., 2012; Margiotta et al., 2012). Sinkholes have repeatedly caused serious damage to infrastructures and buildings, but above all, to human life (Parise and Lollino, 2011), with more than 650 evacuees and injured, and one victim reported. For example, 140 events have been collected from 1925 to 2017 in Apulia, 85 that have an anthropogenic origin, while 50 are related to the presence of a natural cavity (Fiore and Parise, 2013; Parise and Vennari, 2013, 2017; Lollino et al., 2013). The remaining events are with "unknown origin," meaning that the presence of an underground cavity is clear, but no certain data about the sinkhole origin is available.

Sinkhole occurrences in Apulia represent a serious geological hazard (Fig. 2), for which it is essential to have a complete knowledge of the cause. To properly evaluate such a hazard, it is necessary to reach a good understanding of the development and type of the caves at the sinkhole origin, and their stability conditions (Parise, 2010). Most of the sinkholes that produced damage are related to man-made underground cavities. Anthropogenic cavities are widespread in Apulia. They have been excavated since ancient time, due to distinct but complementary needs: urban development demanding building materials; and the necessity to keep developing agricultural practices at the surface,

Figure 2. Spatial distribution of sinkholes in Apulia Region (data from CNR IRPI catalogue, updated to January 2017). The different origin of sinkholes is marked by different colors (see key). The inset horizontal histogram shows percentages of sinkholes for the different origins.



in a land where products such as olive oil and wine have always been important (Parise, 2010; Negri et al., 2015).

The temporal distribution of sinkholes in Apulia during the period analyzed shows an increase of events in recent decades. The number of sinkhole occurrences in the last five years is higher than the other periods (Fig. 3). This is due to a growing availability of sinkhole data in scientific documents. Also, increasing attention is given to these phenomena, as more sinkhole information is published in the media. The number of sinkhole occurrences has grown. In particular, the number of events linked to natural caves is rising over the last decade. Anthropogenic sinkhole occurrences have gone down since 2010.

The most affected province by sinkhole is Foggia, in northern Apulia. Sinkholes can be triggered by several causes, both of natural and man-induced origin. Maintenance works, or a pipeline rupture can activate the event. However, the main triggering factor is rainfall, due to dissolution processes that can produce a sinkhole.

Materials and Methods

Aimed at characterizing the sinkholes formed in Gargano during the September 2014 storm, the rainfall data were collected, taking into account records from the available rain gauges closest to the sinkhole occurrence sites. They were analyzed to identify the rainfall intensity during different phases of the storm, and to evaluate rainfall intensity values and duration with respect to the average area rainfall.

The two clusters of sinkholes produced by the rainstorm - due to small size of the features and to unavailability of post-storm aerial photos - were analyzed by collecting geological and morphometric data through field surveys. For each sinkhole, several morphometric parameters were measured, following the classical studies about sinkhole morphometry. These included parameters such as diameter, shape and depth, sinkhole location in geomorphological unit at larger scale, and observations on the geological materials exposed along the sinkhole walls. Data from caving surveys were further obtained from local cavers. The data were field-checked, and discussed with the surveyors, to integrate additional data from underground explorations.

The data collection phase was important to record these small-size sinkholes, which are easily filled up or covered by vegetation, probably with the exception of the largest ones. In the process of sinkhole hazard evaluation, the collection of detailed sinkhole data linked to a specific triggering event - in this case, the September 2014

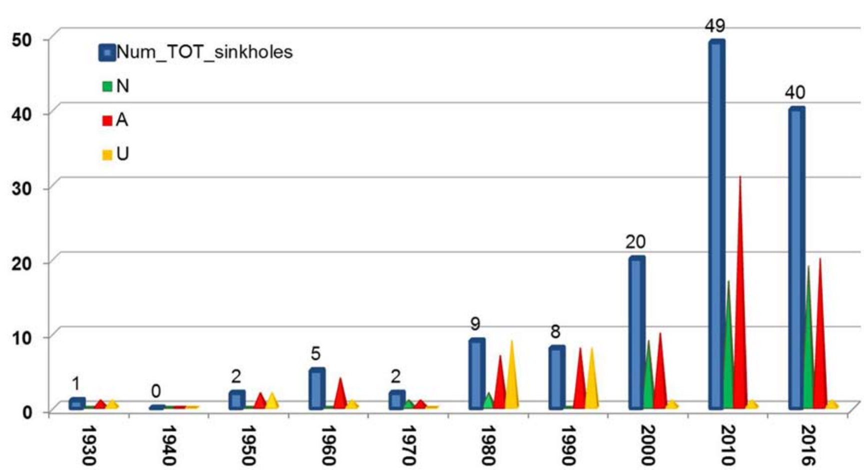


Figure 3. Time distribution of sinkholes in Apulia Region. Blue bars are the total number of sinkholes, green pyramids are natural sinkholes (N), red pyramids are anthropogenic sinkholes (A), yellow pyramids are sinkholes with unknown origin (U).

storm - represents a set of highly valuable information addressed toward a proper assessment of the return time of such events, and their relation with particular values of rainfall intensity or of cumulated rainfall.

Results

Morphology of the Gargano Promontory is mostly controlled by E-W and NW-SE-trending faults (Funiciello et al., 1991; Gambini and Tozzi, 1996; Brankman and Aydin, 2004). Karst is well-developed over the entire area, as well as in the rest of the region: the main karst features are represented by a multitude of sinkholes, reaching a maximum density of up to 100 per square kilometer in the Chiancate area (Castiglioni and Sauro, 2000; Simone and Fiore, 2014). Due to widespread outcroppings of soluble rocks (Bosellini et al., 1999), surface hydrography is limited to a few, short, ephemeral drainages along slopes that bound the elevated central plateau (reaching 1000 m a.s.l.) and to minor drainages in the alluvial and coastal plains surrounding the Gargano Promontory.

As concerns the September 1−6, 2014, rainstorm, 12 small sinkholes have been documented near the villages of Monte Sant'Angelo and San Marco in Lamis. This is certainly a conservative estimate, since the particularly dense forests covering large portions of Gargano make difficult the identification of such features, especially when small. Nevertheless, two sinkhole clusters have been identified and are described below. Due to the remote areas, where most sinkholes developed and the difficulty to detect them, no precise information is available on the time or period of sinkhole occurrence. We could only state that they were related to the September 1-6 rainstorm, based upon local witnesses. Morphology and shape of the documented features indicate an origin as collapse or cover-collapse sinkholes (Gutiérrez et al. 2008, 2014).

Storm Event Description

During the first week of September 2014, a complex, long-lasting rainstorm occurred in large sectors of the Apulia Region, with the most intense rainfall being registered in the Gargano Promontory. It was due to a vortex of low pressure that remained stationary for several days between the lower Adriatic Sea and the Balkans Area.

In particular, on September 1, 2014, a cold front coming from northern Europe moved to lower latitudes and caused scattered precipitation along the Apulia peninsula. In the following days, this cold front fueled the low-pressure vortex and, given the particular weather conditions, remained blocked until September 6, creating a prolonged instability, mainly on the Gargano area. This instability was powered by the thermal contrast between cooler air present in the vortex, from NE, and the lower warm and humid layers of the atmosphere in contact with the Adriatic Sea (Martinotti et al., 2015). Total rainfall measured during the event (September 1−6), in terms of cumulated rainfall, was especially high, with a peak of over 500 mm on some sectors of Gargano (Fig. 4).

The rain gauges located in Cagnano Varano, San Marco in Lamis and San Giovanni Rotondo (Fig. 5a) allowed to derive a cumulated rainfall chart that highlights how the heavy rainfall persisted for the entire week (Figs. 5b, c, d). It shows that the whole area was not hit at the same time: in San Giovanni Rotondo and San Marco in Lamis, the rainfall intensity was almost steady starting from September 3, while in Cagnano Varano two main, distinct rainfall events can be identified: namely, in the morning of September 4, and on September 6. Maps of cumulated daily rainfall (Fig. 6) highlight how the maximum values during September 3-4 are concentrated in the central area of the Gargano Promontory, while those during September 5−6 are focused in the NE sector. Severity of the storm event is particularly indicated by the fact that the majority of the rain gauges documented the maximum, cumulated rainfall ever registered in a period of five days (Table 1).

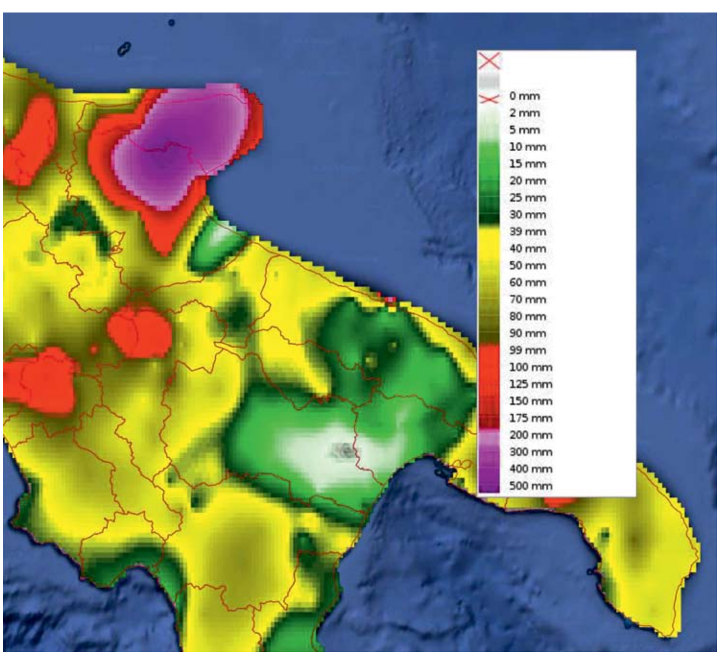


Figure 4. Cumulated rainfall for the whole event, from September 1-6, 2014 (source: Civil Protection Service of Apulia Region).

Observed Sinkholes

Sinkholes that occurred in Gargano are natural events quite common in karst areas (Parise, 2008; Gutierrez et al., 2014; Parise et al., 2015a), as a result of intense or prolonged rainfall events that increase the surface and underground outflow. The outflow speed of large water quantities may be able to remove unconsolidated soil, creating new paths for underground water circulation. As consequence, the surface material will collapse in the underlying cavities or karst conduits (Parise et al., 2015b).

During the heavy rainfall event in September 2014, several sinkholes occurred in the Gargano Promontory. Twelve sinkholes have been documented and became the object of surveys and measurements. They are likely only a small percentage of the events effectively triggered by the rainstorm, but the extremely dense forest covering large sectors of Gargano obscured sinkhole occurrences.

The documented sinkholes are grouped in two clusters, one in a rural area north of Monte Sant'Angelo, and the other in a small area near the village of San Marco in Lamis. Their main features are reported in Table 2, while in the following we provide a more detailed description.

Sinkholes at Monte Sant'Angelo were observed in a rural area north of the village: six sinkholes, linked to natural cavities, were documented in an area extending about 1500 m^2. Based upon the testimonies of local people, they were activated during the September 2014 storm and subsequently were slightly enlarged. In the Bosco Quarto area, terra rossa deposits, ranging in thickness from a few decimeters to several meters, cover the limestone, karstified bedrock - a situation very common in Gargano, as well as in the Murge sub-karst area of central Apulia.

Table 1. Maximum cumulated rainfall registered for five days by the rain gauges, compared with the maximum historical records.

Rain gauge	September 2014 event, mm	Maximum historical registered, mm
San Marco in Lamis	519.0[a]	430.6
San Giovanni Rotondo	585.8[a]	263.6
Vico del Gargano	309.8[a]	263.6
Cagnano Varano	342.2[a]	270.7
Sannicandro G.	75.8	311.4
Bosco Umbra	288.8	501
Monte S. Angelo	300.8[a]	296.8
Vieste	164.2	187

[a] Highest maximum cumulated rainfall events.

One sinkhole (No. 1, Table 2; Fig. 7c) is included within the limits of a larger solution doline, while most of the others are small openings in the ground (Nos. 2 and 3, Table 2; Fig. 7d), or slight depressions of limited size (the sinkhole pair No. 5 and 6, Table 2; Fig. 7e). The largest sinkholes (No. 4, Table 2; Figs. 7a, b) appeared to be deep (from the surface), 25.2 m, and large 12 m. Actually, speleological survey showed that after the first pit, visible from the ground, a passage allows access to other, deeper spaces, until reaching the maximum depth of 120 m (Figs. 8 and 9). The cave is vertically developed, and the bad conditions of the hosting rocks (characterized by several joint families and intense weathering, with the likely detachment of rocks) make the descent quite difficult. At the ground surface, the original circular shape of the sinkhole was later modified by secondary enlargements, due to minor failures involving the extremely steep to vertical walls. At least two more sinkholes have been recorded in the surroundings of Monte Sant'Angelo, but they were filled in by landowners before it was possible to observe, measure and document them.

Additional sinkholes were triggered in the village of San Marco in Lamis, again concentrated in a cluster (Figs. 10 and 11). This second cluster of sinkholes mostly affected the epikarst (Klimchouk, 2000; Williams, 2008), consisting of

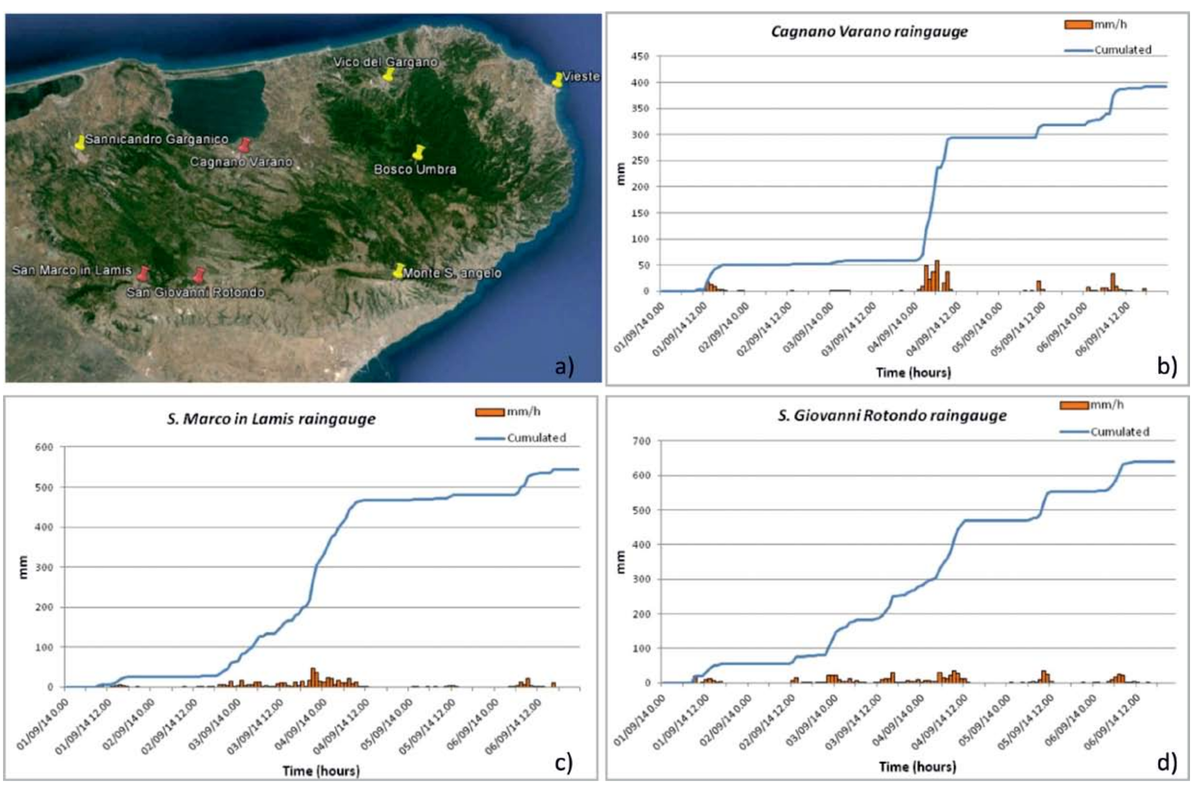


Figure 5a. Rain gauges in the Gargano Promontory. In the map (a), the red color marks the rain gauges used for reconstruction of the rainfall histograms, respectively shown in Figures 5b, c, d.

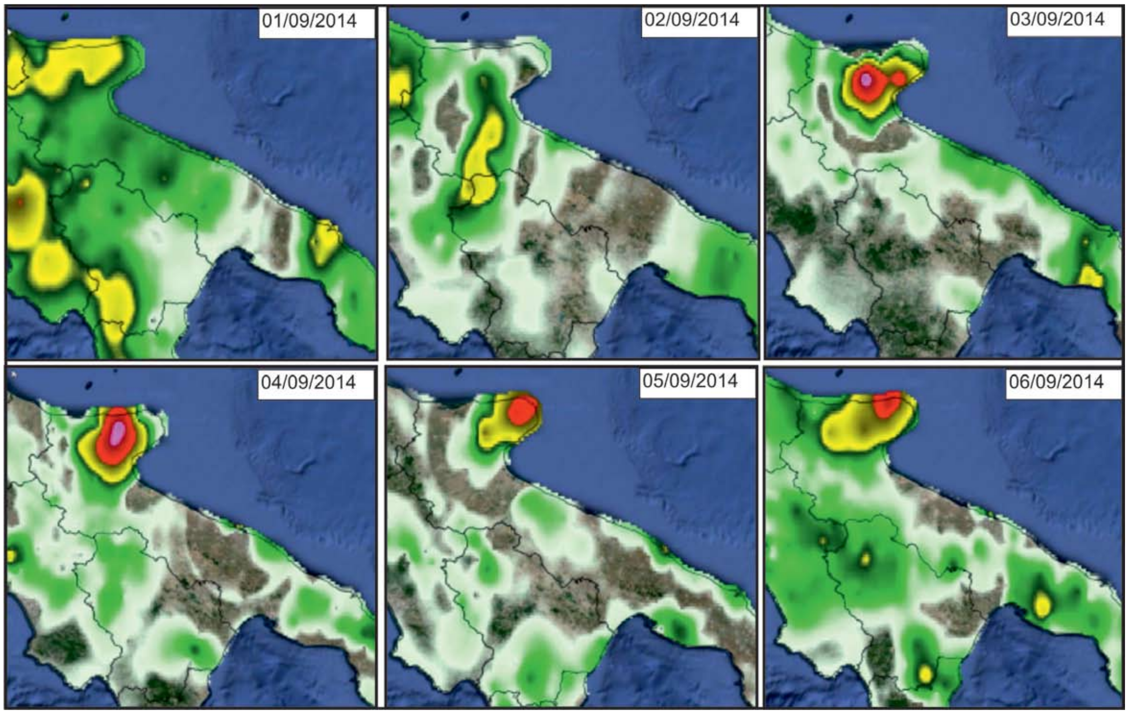


Figure 6. Cumulated daily rainfall registered during the first week of September, 2014 in the area (source: Civil Protection Service of Apulia Region). Key for colors as in Figure 4.

limestone rocks and pinnacles, immersed in terra rossa deposits. Five natural sinkholes were created (Fig. 11). In particular, four of them affect the lower sector of a large karst depression. The features' shapes are predominantly circular, but the dimensions are quite assorted. The smallest sinkhole has a diameter of 1.5 m and a depth of 1.5 m (No. 11, Table 2; Fig. 10d). It is placed along the boundary of a wider feature, outlined by circular cracks on the ground. This feature did not open as a true sinkhole. On the other hand, the biggest sinkhole (No. 8, Table 2; Figs. 10, 11a, c) has a diameter of 6 m and it is 5.4 m deep. At the base of this sinkhole, the presence of karst conduits (not accessible by man due to the small size), testify to the occurrence of subsurface flow, with likely removal of material, which left unsupported soil above, leading to sinkhole formation. Morphometry and shape of most of the observed sinkholes indicates that they belong to the typologies of collapse or cover-collapse sinkholes (Waltham et al., 2005; Gutierrez et al., 2014).

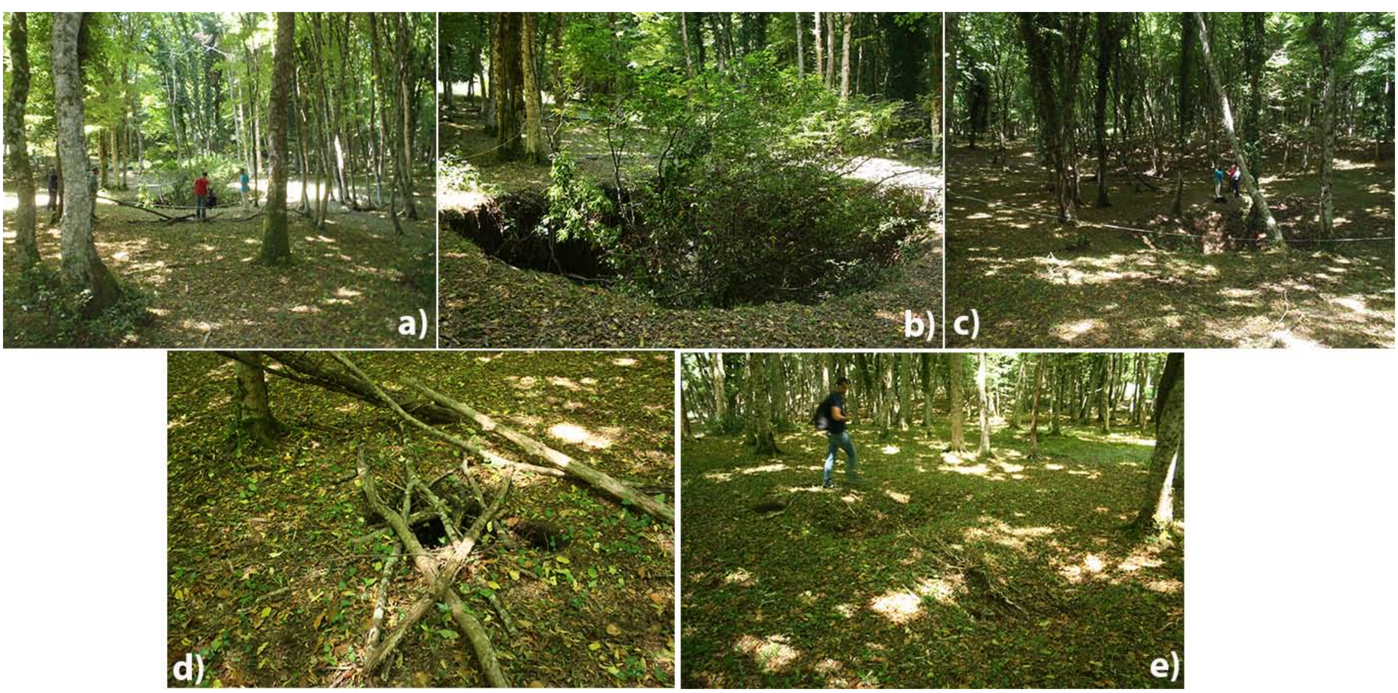


Figure 7. Sinkholes triggered by the September 1−6, 2014, intense rainfall event in the Gargano Promontory at Monte Sant'Angelo: a) approaching the Abisso San Matteo sinkhole (person for scale); b) close up of the entrance at Abisso San Matteo sinkhole (10.2 × 12 m wide); c) 2 m deep sinkhole; d) small sinkhole, covered by tree branches (1 × 1.5 m wide); e) couple of small-sized and slightly visible ground depressions, nearby the Abisso San Matteo sinkhole (person for scale).

Table 2. Morphometric data of documented sinkholes triggered by the September 1−6, 2014 intense rainfall event in the Gargano Promontory.

No.	Site	Depth, m	Width, m	Shape	Notes	Type
1	Bosco Quarto, Monte Sant'Angelo	1.5	3 × 1.5	circular		collapse sinkhole
2	Bosco Quarto, Monte Sant'Angelo	3.3	1 × 0.4	elongated		cover-collapse sinkhole
3	Bosco Quarto, Monte Sant'Angelo	0.5	1 × 1.5	circular		cover-collapse sinkhole
4	Bosco Quarto, Monte Sant'Angelo	120	10.2 × 12	circular		collapse sinkhole
5	Bosco Quarto, Monte Sant'Angelo	0.4	2 × 2	circular	close, likely connected, to # 6	cover-collapse sinkhole
6	Bosco Quarto, Monte Sant'Angelo	0.4	1.5 × 1.5	circular	close, likely connected, to # 5	cover-collapse sinkhole
7	Cime-Bosco Rosso, San Marco in Lamis	5	4 × 3	circular		cover-collapse sinkhole
8	road San Marco in Lamis – Sannicandro Garganico	5.4	5.7 × 6	circular		cover-collapse sinkhole
9	road San Marco in Lamis – Sannicandro Garganico	6.3	2.7 × 2	elliptical		cover-collapse sinkhole
10	road San Marco in Lamis – Sannicandro Garganico	1	5.5 × 5	circular		cover-collapse sinkhole
11	road San Marco in Lamis – Sannicandro Garganico	1.5	1.5 × 1.5	circular	within a larger doline	cover-collapse sinkhole
12	road San Marco in Lamis – Sannicandro Garganico	3.5	1.8 × 2	circular		cover-collapse sinkhole

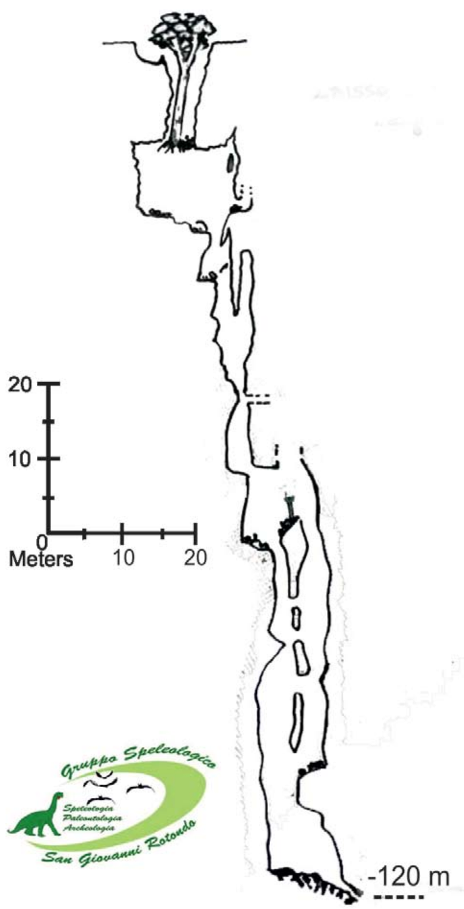


Figure 8: Profile of the Abisso San Matteo sinkhole (marked as PU 2656 in the regional register of natural caves, managed by Federazione Speleologica Pugliese, http://www.catasto.fspuglia.it), as obtained from the speleological survey (courtesy of Gruppo Speleologico San Giovanni Rotondo). The cave reaches a depth of −120 m from the ground surface.

Figure 9. Series of shafts within the Abisso San Matteo sinkhole (photos courtesy of Gruppo Speleologico San Giovanni Rotondo). The photograph at the bottom is an upward view of the first 25 m of the cave.

Discussion

In karst, it is not an easy matter to understand the main hydrological features at the surface and the groundwater circulation, due to peculiarity of karst terrains, and to their hydrogeological characteristics (Bonacci, 1995; Worthington, 1999; Worthington et al., 2001; White, 2002; Gunn, 2007; Palmer, 2010; Parise, 2016). This is even more complicated during heavy rainstorms and floods (White and White, 1984; Parise, 2003; Delrieu et al., 2005; Bonacci et al., 2006; Jourde et al., 2007; Delle Rose and Parise, 2010), with response to the storm that may heavily change with rainfall intensity or duration, as well as the cumulated amount of rainfall. For these reasons, it is extremely important to document any karst landscape changes occurring after significant rainstorms, both to contribute to future evaluation of the likely sinkhole hazard, and for collecting data aimed at the full comprehension of the water-flow circulation at the surface and subsurface (Parise and Pascali, 2003).

The Gargano Promontory is one of the main karst subsectors of Apulia. However, not many studies are available to provide data about origin and evolution of the karst features in the area. The information presented in this article, even though not numerous, and definitely not exhaustive, is, nevertheless, a worthy amount of data to characterize the effects of the heavy rainstorms that hit the area. Further, we were able to identify the typology of the produced sinkholes, and to link the timing of sinkhole formation to the September 2014 storm. Knowledge of the time of sinkhole occurrence is typically the most difficult information to obtain. This often hinders any possibility to link the sinkholes to triggering factors, and particularly to rainfalls (Brinkmann and Parise, 2010).

Conclusions

Data presented here, added to those already available about sinkhole chronology in Apulia (see Parise and Vennari, 2013, 2017), may help to build a sufficient amount of information to develop further studies. These can be aimed at evaluating the sinkhole hazard in the Gargano Promontory and in the entire region. Hazard evaluation is a complex task that is rarely actually reached, mostly due to lack of data about the connection between a triggering event and sinkhole occurrence.

Figure 10. The main sinkhole in the San Marco in Lamis group.

Figure 11. Sinkholes triggered by the September 1−6, 2014, intense rainfall event in the Gargano Promontory at San Marco in Lamis: a) and c) the main sinkhole of the group, 5.4 m deep; b) small-sized sinkhole opened at the boundary of a closed depression (person for scale); d) small-sized sinkhole within a greater depressed area, marked by lowering of the ground over an area about 25 m wide (person for scale).

Acknowledgements

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C.D.R. Stephen and M.S. Harvey. William B. Muchmore (1920-2017): His taxonomic contributions and complete bibliography. *Journal of Cave and Karst Studies*, v. 80, no. 1, p. 39-48. DOI: 10.4311/1700036R1

WILLIAM B. MUCHMORE (1920–2017): HIS TAXONOMIC CONTRIBUTIONS AND A COMPLETE BIBLIOGRAPHY

Charles D.R. Stephen[1,C] and Mark S. Harvey[2]

Abstract
William B. Muchmore (1920–2017) was the most influential worker on cave pseudoscorpions in North America and a globally-recognized expert on this arachnid order. Aside from brief stints in herpetological embryology and isopod taxonomy, he dedicated his 62-year career to the study of pseudoscorpions, focusing on their taxonomy. He described 278 new species, of which 167 were from caves. The majority of North American cave pseudoscorpions were either initially described or revised by him. He also wrote on the phoretic habits of pseudoscorpions, and speculated that cave pseudoscorpion distributions may be influenced by troglophilic rodents and bats. A complete bibliography of his scientific papers is provided.

William ("Bill") Breuleux Muchmore (Fig. 1) was born in Cincinnati, Ohio, on May 20, 1920. He was the first child of Oliver Charles Muchmore (1892−1968) and Ruby Breuleux (1895−1958), both of Ohio. His family has a long history in the United States (U.S.), with ancestry on his mother's side tracing to Philip Frederick Breuleux (born 1830, Busserel, Haute Saône, France; immigrated in 1860 to Sycamore, Ohio), and on his father's side to John Muchmore (born 1692, Windsor, Connecticut Colony; now the State of Connecticut). He had two children with his wife Marjorie Murrin: Susan Muchmore (born 1947) and Patricia Muchmore (born 1950). Marjorie predeceased Muchmore in 2007 at the age of 85. He died May 11, 2017 at the age of 96. At the time of his death he was an author of 145 publications on pseudoscorpions and was internationally recognized as a global expert on this arachnid order.

In his youth, Muchmore achieved the status of Eagle Scout, a path that likely influenced his interest in biology and fostered his mind into becoming a broadly-trained naturalist. After completing a B.A. at Oberlin College, Oberlin, Ohio, in 1942, from 1943−1946 he served with the U.S. Army Medical Corps in the southwest Pacific theatre of World War II (NARA, 2005). After the war ended he returned to the U.S. and enrolled at Washington University, Saint Louis, Mo., where he received his Ph.D. in 1950. He then moved to New York, where he spent his entire academic career at the University of Rochester, going up the ranks from Instructor (1950−1952), to Assistant Professor (1952–1958), to Associate Professor (1958−1970), to Professor (1970−1988), and finally "retiring" as Professor Emeritus (1985). Muchmore ceased working independently on pseudoscorpions around 2009. Continuing collaborations produced three additional papers, with his last published at age 93 in 2013. In his later years, he became a local historian for the Rochester area, participated in local cultural centers, and was involved in wildlife conservation.

Muchmore's career began as a herpetologist, not an arachnologist. His Ph.D. dissertation was on embryological development of the mesoderm germ layer in *Ambystoma maculatum* (Spotted Salamander). From 1947–1950, he collected this species and *Lithobates pipiens* (Northern Leopard Frog) egg masses from Missouri, New Hampshire, New York, and Tennessee. In his lab, he reared the eggs of these species into embryos and conducted his experiments with these developing embryos. His work followed two lines: (1) cell survival and differentiation of *A. maculatum* embryo mesoderm tissues, following experimental explantation into *L. pipiens* trunk mesoderm; and (2) cell survival and differentiation of *A. maculatum* embryo mesoderm tissues, following experimental removal of different ectodermal, mesodermal, and endodermal tis-

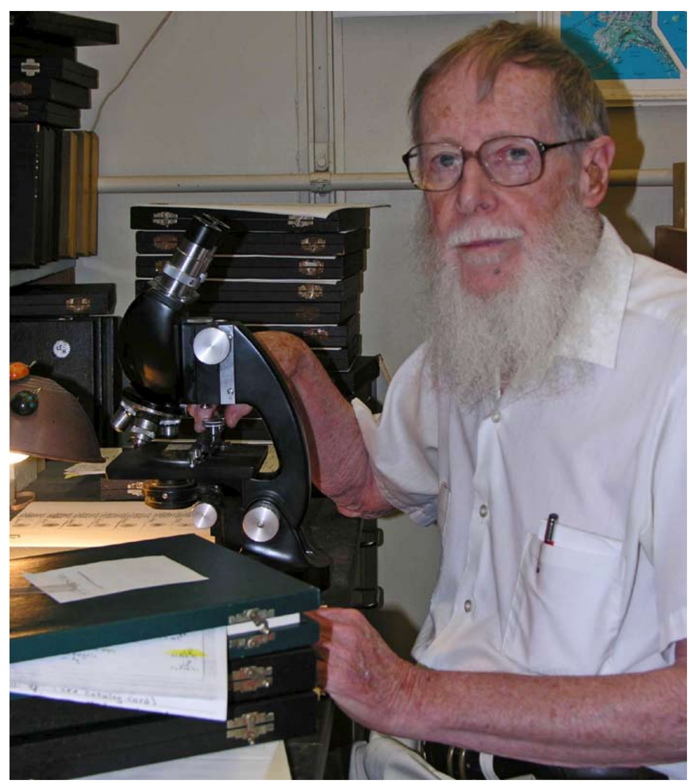

Figure 1. William B. Muchmore in his laboratory, June 11, 2006.

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sues from the developing embryo.

The central thesis of his dissertation was to test hypotheses developed by the Japanese herpetologist Tuneo Yamada that described how the mesoderm differentiates in the amphibian embryo. The meticulous nature of his dissertation engendered a familiarity with anatomy and esoteric nomenclature, taught him scientific illustration, and trained him in histological and embryological staining techniques. These skills were transferable to his later work with pseudoscorpions.

From 1951–1968, Muchmore authored seven papers that derived from his dissertation (Muchmore, 1951, 1957a, 1957b, 1958a, 1958b, 1964b, 1968). He never again published on embryology. Instead, he changed paths to become a highly productive taxonomist. In 1955, he authored his first taxonomic paper of any kind, in which he synonymized *Plethodon huldae* with northwestern New York populations of the nominate subspecies of the Red-backed Salamander (*Plethodon cinereus* (Green, 1818)) (Muchmore, 1955a). Also in this year, he published a taxonomic note motivated by a report of the Cave Salamander (*Eurycea lucifuga*) occurring in a hollow among bluffs of southern Ohio (Muchmore, 1955a, 1955b). He determined that the report of this species was, in fact, misidentified Longtail Salamander (*E. longicauda*), a troglophilic species that is presently known to occur in caves of the Interior Plateau and Appalachian karst regions (Buhlmann, 2001; Garton et al., 1993; Niemiller et al., 2016).

In 1955, he was awarded a Summer Research Fellowship at Edmund Niles Huyck Preserve, Rensselaerville, N.Y. His interests branched into arthropods here. He spent a summer looking for exotic arthropods in greenhouses and turning over logs and rocks across northern New York. Under the mentorship of Kenneth Cooper at the University of Rochester, this resulted in new reports of populations of exotic isopod, harvestman, and pseudoscorpion species in New York (Muchmore, 1957, 1963, 1969b). It was also from these early collections that he later described the epigean isopod *Miktoniscus ohioensis* and the epigean pseudoscorpion *Serianus enhuycki* (Muchmore, 1964a, 1968f).

These first forays into arthropods began to establish a reputation that would serve to induce worldwide collectors to send him specimens. The first example of this is the hypogean isopod *Caucasonethes paynei*, which he described from specimens collected in Tennessee by J.A. Payne from Offutt's Cave, Anderson County (now Offutt Cave (TAN12)) and Melton Hill Cave #1, Roane County (now Melton Hill Spring Cave (TLN4) and actually located in Loudon County) (Muchmore, 1970). On dedicating his career to arachnids, Muchmore later remarked that he was greatly encouraged by the eminent pseudoscorpionologists, Joseph C. Chamberlin, Max Beier, and C. Clayton Hoff, in the study of "those fascinating little critters, the pseudoscorpions" (Muchmore, 1998a).

Muchmore (1963b, 2000b) credited Hermann A. Hagen with initiating the study of North American cave pseudoscorpions in 1879 with his description of *Blothrus packardi* (now *Kleptochthonius packardi*). However, Muchmore has overwhelmingly made the most significant contribution to the study of cave pseudoscorpions on this continent. He remains one of the major authors of North American biospeleological taxonomy. His first paper on pseudoscorpions (Muchmore, 1962) described the hypogean *Microcreagris grandis*. This initial paper was based on specimens that were collected at Lehman Caves National Monument, Nevada, and that were borrowed from the Yale Peabody Museum of Natural History. Following Mahnert's (1979) redefinition of *Microcreagris*, most North American species were transferred to newly erected genera (Ćurčić, 1981, 1984, 1989), but *M. grandis* was not included in these or later treatments.

Defining North America as including the Caribbean archipelago and Central America, from this continent, Muchmore described 157 new species, co-authored two species, transferred 12 species to different genera, described one subspecies, *Ideobisium puertoricense cavicolum* (Muchmore, 1982e), and revised 19 taxa. These include 107 new species from the U.S.: in the Edwards Plateau and Balcones, Ozarks, Interior Low Plateau, and Appalachians karstic regions, as defined by Culver et al. (2003). Of the 151 pseudoscorpion species currently known from caves in the U.S. and Canada, 76 % have been either described or revised by Muchmore. South of the U.S., he increased the known cave pseudoscorpion fauna of Mexico from three species to 39, added three species to Belize, and two to Guatemala. From the Caribbean archipelago, he added eight cave-adapted species, including one new species from Antigua and Barbuda, one from the Bahamas, one from the Dominican Republic, and five from Jamaica. As of 2011, only five species that he authored have been synonymized with another species, and 15 have been transferred to a different genus (Harvey, 2013). Not only do these feats speak to his influence and the quality of his work, but also to the lack of any major pseudoscorpion taxonomist simultaneously based on the continent. Contemporaries of Muchmore, who were involved in taxonomy of North American cave pseudoscorpions, include James Cokendolpher, Bozidar Ćurčić, Ellen Benedict, Mark Harvey, and David Malcolm.

Muchmore contributed on a more minor level to biospeleology on other continents. He described five new cave-adapted species from Oceania (one from Australia and four from lava tubes on the Hawaiian archipelago), one new species from Africa, and two new species from South America. Additionally, he authored the first known troglobitic species from Hawaii, *Tyrannochthonius howarthi* (Muchmore, 1979d), and contributed five papers to the series "Cavernicolous fauna of Hawaiian lava tubes," that was initiated by Francis Howarth (1973), expanding the series' scope to include pseudoscorpions. In 2000, he published what was envisioned to be the first in a series of papers summarizing and describing all known Hawaiian pseudoscorpions, both epigean and subterranean. In this paper, which focused on the superfamily Chthonioidea, he listed nine species, provided a regional key, described one new genus, referred a species to this new genus, and described two new species. New species included *Vulcanochthonius howarthi*, known exclusively from volcanoes, and *V. aa*, known only from milieu souterrain superficial (MSS) of ‘a’a volcanic rock near a cave entrance. This paper also included the first plausible record from the North Pacific Rim of the synanthropic, globally distributed species *Chthonius tetrachelatus* (Preyssler, 1790). Unfortunately, subsequent papers in this series, meant to cover the remaining superfamilies, never made it to publication.

Muchmore’s ideas on adaptation to cave life in pseudoscorpions included several morphological features, which are seen in other troglomorphic arthropods and expanded upon by other authors (e.g., Barr, 1961). These include absence or reduction of eyes, appendage attenuation, and reduction or absence of pigment. However, in several instances he noted that some of these features were found in species that had only been collected from epigean habitats. Examples include elongate appendages in bark-dwelling species, such as *Bituberochernes mumae* (Muchmore, 1974d); eye loss in leaf litter-dwelling *Tyrannochthonius hypogeus*, a species known only from a sinkhole in Mammoth Cave National Park, Kentucky (Muchmore, 1996g); and depigmentation and eye reduction in *Apochthonius hypogeus*, a species known only from under rocks in the Appalachian Mountains of Virginia (Muchmore, 1976d). He struggled with explaining how troglobiontic species came to have disjunct distributions, as seen in *A. colecampi* (Muchmore, 1976d). In the first thorough review paper on phoresy in pseudoscorpions, he speculated that hypogean species of Chernetidae may be carried between caves via bats or rodents (Muchmore, 1971d, 1996b). However, molecular tools currently available for testing these ideas were either not yet invented or were, presumably, unavailable to him.

Despite much of his career having been dedicated to biospeleology (167 of 278 species he described or co-authored are hypogean), Muchmore rarely collected in caves himself. It is doubtful that he would have considered himself a caver. The only specimen from a cave that he both collected and used as material in a species description was *Apochthonius indianensis* from Donaldson-Bronson Cave, Indiana, which he collected in 1958 (Muchmore, 1967b). On one of the few subsequent occasions when he ventured into a cave with a fellow biologist, it was noted by his companion that he was both remarkably, and somewhat amusingly, unprepared for exploring the subterranean environment (Julian J. Lewis, pers. comm.). Instead, he relied on highly productive relationships with a wide assortment of cavers and biospeleologists. The most prolific collector from whom he received specimens was Stewart Peck, who contributed to 36 new species discoveries in caves of Belize, Jamaica, and the United States. In recognition of these survey efforts, he named six species in Peck’s honor.

Although Muchmore collected extensively from epigean localities, for type specimens he usually relied on material sent to him by other collectors. His preferred collection method for epigean fauna was sampling leaf litter in bulk and then extracting animals using Berlese-Tullgren funnels. He did not throw away by-catch from this sampling; instead, he deposited non-pseudoscorpion specimens that he collected into museums or gave them directly to specialists. Muchmore’s contributions to epigean pseudoscorpion taxonomy is on par with those to subterranean taxa: he described 92 new species and co-authored 19 new species. As with cave-adapted fauna, his focus was mostly North America, although he also described new species from Africa, Asia, Australia, South America, and remote island archipelagos. Two notable epigean species described by him include *Solinellus simberloffi* from Daniel S. Simberloff and Edward O. Wilson’s famous defaunation and recolonization studies of Florida mangrove islands, that led to the formation of their theory of island biogeography (Simberloff and Wilson, 1969); and *Wyochernes asiaticus*, which is known from a higher latitude than any other pseudoscorpion species, at 69° N, in Yukon, Canada (Muchmore, 1979a, 1990b, 1996f).

In addition to describing new species and subspecies, Muchmore named several higher taxa that cannot be easily divided amongst epigean or subterranean habitat. These include two subfamilies, 27 genera (three of which were co-authored), and one subgenus. Despite the common use of the subgenus rank in European pseudoscorpion taxonomy, as a rule Muchmore did not use this taxonomic rank. The only exception was the genus *Kleptochthonius*. In this genus, species only known from caves and with troglomorphic characters have been placed into the subgenus *Chamberlinochthonius*, while those collected exclusively from epigean environments and lacking troglomorphic characters have been placed into the nominative subgenus. In one of his last papers, Muchmore (2000a) described two new species of *Kleptochthonius* without assigning them to either subgenus. In this paper he also mentioned that he was working on a revision of the genus, but he never subsequently published on *Kleptochthonius*. Consequently, there is now some ambiguity in *Kleptochthonius*, with all but two of the 39 species in the genus assigned to either *K. (Chamberlinochthonius) or K. (Kleptochthonius)*.

Muchmore was known for his kindness in assisting new students of pseudoscorpions, regardless of their country of origin or the seriousness of their interest in publishing on specimens they had collected (René Barba Díaz, pers. comm.; Christopher M. Buddle, pers. comm.; William A. Shear, pers. comm.). Despite serving on the Board of Directors of the American Arachnological Society, he was not known among his peers to attend many conferences. As such, most of his colleagues came to know him through correspondence. He also influenced many pseudoscorpion scholars through his key to North American species, which remains the most comprehensive key for the continent (Muchmore, 1990c). Eighteen taxa have been named for Muchmore. These patronyms were given in respect of his being an international authority on pseudoscorpions, for his friendship, and in thanks for his personal collection efforts. One genus and six species of pseudoscorpions were named for him: *Muchmoreus* (Harvey, 2013), *Americhernes muchmorei* (Harvey, 1990), *Antillochernes muchmorei* (Dumitresco and Orghidan, 1977), *Austrochthonius muchmorei* (Harvey and Mould, 2006), *Ideoblothrus muchmorei* (Heurtault, 1983), *Pseudalbiorix muchmorei* (Harvey et al., 2006) and *Spelaeobochica muchmorei* (de Andrade and Mahnert, 2003). One pseudoscorpion species was named for Muchmore and his wife: *Tyrannochthonius muchmoreorum* (Cokendolpher, 2009). Patronyms in taxa other than pseudoscorpions include the edaphic minute brown scavenger beetle *Metophthalmus muchmorei* (Andrews, 1988); the eyeless weevil *Decuanellus muchmorei* (Howden, 1992); the edaphic unique-headed bug *Alienates muchmorei* (Wygodzinsky and Schmidt, 1991); the edaphic scolopendromorph centipede *Cryptops neocaledonicus muchmorei* (Lewis, 1989); the edaphic pauropod *Diplopauropus muchmorei* (Scheller and Muchmore, 1989); the epigean scorpion *Heteronebo muchmorei* (Francke and Sissom, 1980); three epigean spiders *Zimiromus muchmorei* (Platnick and Shadab, 1976), *Monoblemma muchmorei* (Shear, 1978), and *Khamisoides muchmorei* (Platnick and Berniker, 2015); and the epigean whip spider *Charinus muchmorei* (Armas and Teruel Ochoa, 1997).

Pseudoscorpion anatomical nomenclature has been interpreted differently over the years and in different regions (e.g., Chamberlin, 1931; Harvey, 1992). Muchmore used the schema developed by Chamberlin (1931) and studied specimens through permanent slide mounts made with Canada Balsam. He used slide-mounting methods described by Joseph C. Chamberlin and C. Clayton Hoff (Chamberlin, 1923; Hoff, 1944). This meticulous technique involves dissecting the chelicerae, a pedipalp, and two legs from the body, clearing the body in potassium hydroxide or beechwood creosote, and then mounting all parts onto a slide in a medium of Canada Balsam sap, liquefied with xylene. This method allows specimens to persist essentially unchanged for centuries. The slide-mounting technique was a passion of Muchmore. He confided to the junior author that he enjoyed the process to such an extent that he made his own thin glass tubes, used to prop coverslips over the thousands of specimens that he mounted over the course of his career. These slide mounts allowed the detailed examination of the specimens, which is required for species description. Some of Muchmore's material is stored in alcohol, but the vast majority is mounted on slides. Each is labeled with his personal catalog number.

Over five decades Muchmore accumulated a large collection of specimens and built a personal catalog of all specimens that he examined. In a paper on the arachnid order Schizomida, Reddell and Cokendolpher (1995) referred to Muchmore's private collection as "WBMC--William B. Muchmore collection, Rochester." However, in his own papers Muchmore never referred to his private collection by any acronym. Following the cessation of his work on pseudoscorpions around 2009, this collection, which included several important pseudoscorpion paratypes, was moved to the Florida State Collection of Arthropods (FSCA), Gainesville, Fla. Muchmore was a Research Associate at FSCA and this institute holds the majority of his type specimens. Other type material, including holotypes, paratypes, and topotypic specimens, are stored at Instituto de Ecología y Sistemática, Havana, Cuba; American Museum of Natural History, New York, N.Y.; Auburn University Museum of Natural History, Auburn, Ala.; Bohart Museum of Entomology, University of California, Davis, Calif.; Bernice P. Bishop Museum, Honolulu, Hawaii; California Academy of Sciences, San Francisco, Calif.; Canadian National Collection of Insects, Arachnids, and Nematodes, Ottawa, Ontario, Canada; Laboratorio de Acarología, Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico City, Mexico; Museum of Comparative Zoology, Cambridge, Mass.; Smithsonian Institution National Museum of Natural History (previously known as United States National Museum), Washington, D.C.; and Western Australian Museum, Perth, Western Australia, Australia.

Muchmore has left to future researchers a rich body of taxonomic literature on this rather poorly studied group of arachnids. This accumulation includes both the volume of publications he produced and the quality and quantity of specimens he has deposited into institutions situated worldwide. These treasures continue to be prized by pseudoscorpion researchers. The many insights he has provided into this arachnid order, through his meticulous scrutiny of the specimens coming under his microscope, will continue to influence pseudoscorpion researchers for many years to come.

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