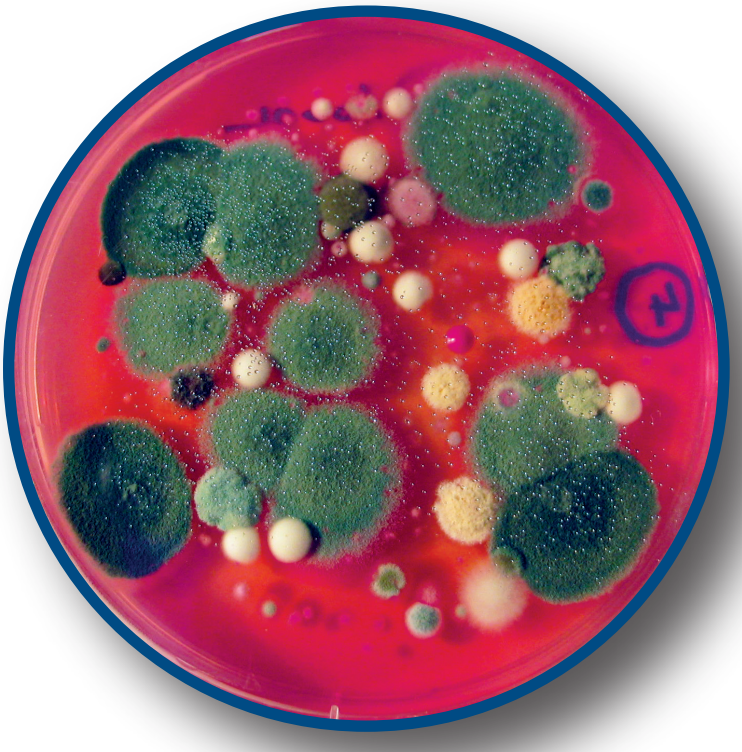
KARST RESEARCH INSTITUTE ZRC SAZU
Speleological Association of Slovenia
Slovenian National Commission for UNESCO
International Union of Speleology UIS
IGU Karst Commission

22nd INTERNATIONAL KARSTOLOGICAL SCHOOL
»Classical Karst«

KARST & MICROORGANISMS



Postojna, 2014

22nd INTERNATIONAL KARSTOLOGICAL SCHOOL
"Classical Karst"

KARST AND MICROORGANISMS

PROGRAM, GUIDE BOOK AND ABSTRACTS

Postojna, 2014

Editor
Janez Mulec

Published by
Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Titov trg 2, 6230 Postojna, Slovenia

Organizing committee
Franci Gabrovšek, Petra Gostinčar, Martin Knez, Janja Kogovšek, Peter Kozel, Andrej Mihevc, Janez Mulec, Bojan Otoničar, Metka Petrič, Tanja Pipan, Mitja Prelovšek, Tadej Slabe, Stanka Šebela, Nadja Zupan Hajna, Nataša Viršek Ravbar

Program committee
Alica Chroňáková, Marjutka Hafner, Václav Krištůfek, Alenka Mauko, Janez Mulec, Colin Murrell, Lejla Pašić, Blaž Stres, Annette Summers Engel

Organizer
Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Titov trg 2, 6230 Postojna, Slovenia

Supported and sposored by
Slovenian National Commission for UNESCO
Research Centre of the Slovenian Academy of Sciences and Arts
Municipality of Postojna
Postojnska jama, d.d.
Park Škocjanske jame, Slovenija
Notranjski muzej Postojna
SIBIOS International Society for Subterranean Biology

Front page
Airborne microorganisms on Dichloran Rose Bengal Chloramphenicol agar from Postojnska jama/Male jame, 1 October 2009. Photograph taken by Janez Mulec.

CIP - Kataložni zapis o publikaciji
Narodna in univerzitetna knjižnica, Ljubljana

551.44:579.8(082)
579.8(24)(082)
INTERNATIONAL Karstological School Classical Karst (22 ; 2014 ; Postojna)
Karst and microorganisms : program, guide book and abstracts / 22nd International Karstological School Classical Karst, Postojna 2014 ; [organizer Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts ; editor Janez Mulec]. - Postojna : Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, 2014

ISBN 978-961-254-705-9
1. Gl. stv. nasl. 2. Mulec, Janez 3. Slovenska akademija znanosti in umetnosti. Znanstvenoraziskovalni center. Inštitut za raziskovanje Krasa (Postojna)
274143744

CONTENT

GENERAL INFORMATION

Lectures and poster presentations
Lectures will be held in the lecture room at the Cultural centre Postojna (Kulturni dom Postojna, Prešernova ulica 1, Postojna). Presentation of posters will be held in the hall of the Karst Research Institute ZRC SAZU (Titov trg 2, Postojna). See schedule for details.

Poster presentation
Leave posters at the registration desk on Monday, June 16th, before the lunch break. Please be available during the poster session and stand by your poster during the session.

Unresolved mysteries of karst
This is a forum to challenge current paradigms in karst science. Participants are invited to prepare questions, comments and ideas to discuss during this session.

Lunches
Lunches are not organized on excursions and during session days. Lunch breaks are planned in the program during the session days and whole-day excursion. Organizer will provide information on recommended places to eat.

Excursions
Do not forget register your participation on excursions at the registration desk as soon as possible. Head lights are recommended, walking shoes and field clothes are necessary. The meeting point for the excursions will be announced during the conference.

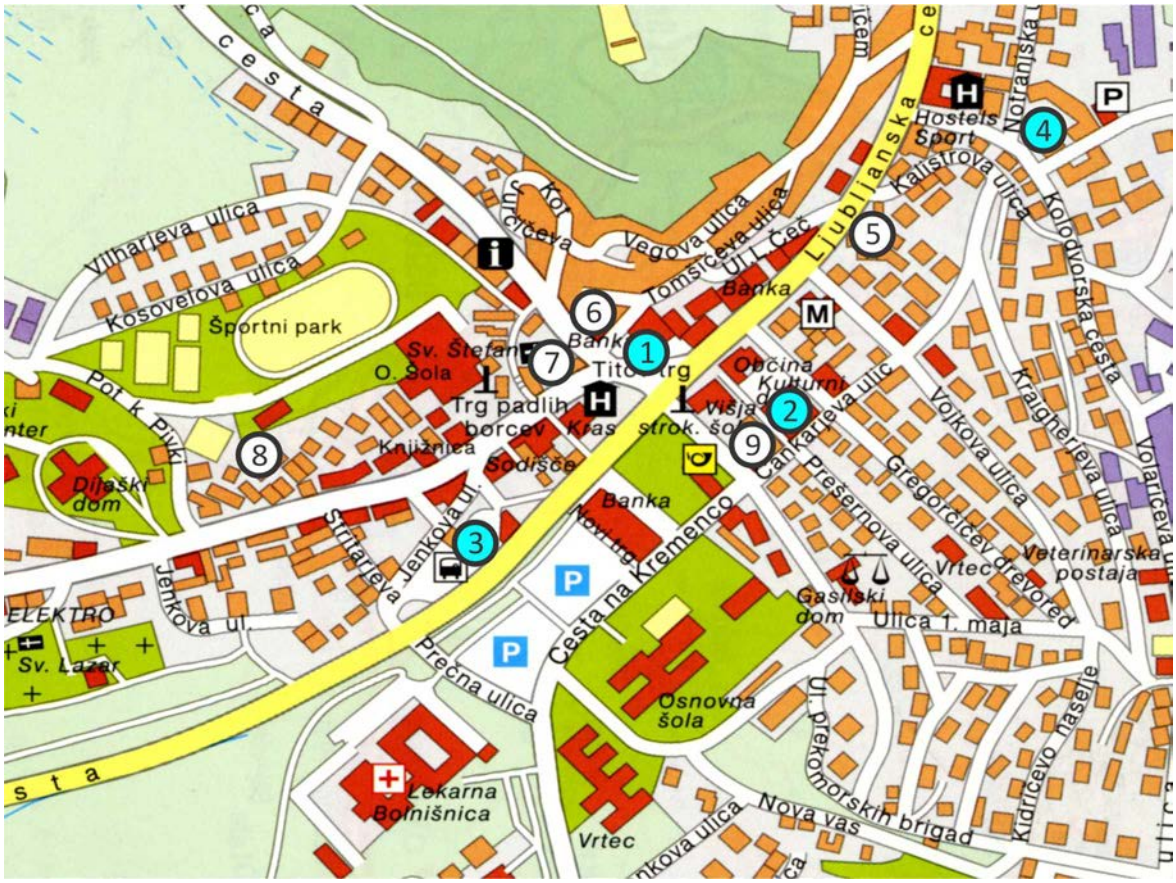
Organizers will supply some beverages for the whole-day field trip, take some additional ones if you need more.

For the whole-day excursion, use of insect repellents is highly recommended as we will be walking in the areas populated with ticks (*Ixodes ricinus*) which sometimes transmit Lyme boreliosis and tick-borne encephalitis.

Participation on the excursions is at your own risk.

Please follow annouces during the conference for eventual changes.

MAP OF THE TOWN CENTRE WITH IMPORTANT PLACES



1 – Karst Research Institute ZRC SAZU (Inštitut za raziskovanje krasa ZRC SAZU), Titov trg 2
2 – Kulturni dom (Cultural Centre of Postojna), Prešernova ulica 1
3 – Avtobusna postaja (Bus station), Titova cesta 2
4 – Notranjski muzej Postojna (Notranjska Museum, Postojna), Kolodvorska cesta 3

Places to eat:
5 – Pizzerija Minutka: restaurant with pizza, pasta, Balkan food and daily menu
6 – Restavracija Proteus: restaurant with local and "global" food and daily menu
7 – Bar Bor: restaurant, simple but good local food, also serves daily meals
8 – Pizzerija Čuk: restaurant at the sport park, pizzeria, good pasta, local and "global" food
9 – Gostilna Špajza: currently closed

PROGRAM

Monday, June 16th, 2014

8:00-12:00	**REGISTRATION**	**Cultural Centre Postojna**
9:00-9:20	**Opening Ceremony**	**Cultural Centre Postojna**
9:20-12:30	**SESSION 1** Chair: Alica Chroňáková	**Cultural Centre Postojna**
9:20-10:20	**Invited lecture** Microbial biodiversity of the subsurface, karst and rocky environments – critical comments and future perspectives *Natuschka M. Lee*	
10:20-10:40	Characterization of prokaryotic and eukaryotic diversity in microbial mats from the gouffre de la Sambuy Seythenex, Savoie, France *Didier Cailhol, Isabelle Domaizon, Émilie Chalmin, Fabien Hobléa, Yann Tual*	
10:40-11:00	Active and cryptic biodiversity of protozoa in underground habitats *Najla Kajtezović, Renata Matoničkin Kepčija*	
11:00-11:30	**Coffee break**	
11:30-11:50	The role of environmental factors on the cave biodiversity in Dinaric karst: an example of Vjetrenica cave *Dalibor Paar, Darko Bakšić, Nenad Buzjak, Ivo Lučić*	
11:50-12:10	Biogenic helictites in Asperge Cave, France *Nicola Tisato, Stefano Torriani, Sylvain Monteux, Francesco Sauro, Jo De Waele, Maria Luisa Tavagna, Ilenia M. D'Angeli, Daniel Chailloux, Michel Renda, Timothy I. Eglinton, Tomaso R. R. Bontognali*	
12:10-12:30	The cave biota of Slovakia - introduction of a new monograph *L'ubomir Kováč, Dana Elhottová, Andrej Mock, Alena Nováková, Václav Krištůfek, Alica Chroňáková, Alena Lukešová, Janez Mulec, Vladimír Košel, Vladimír Papáč, Peter L'uptáčik, Marcel Uhrin, Zuzana Višňovská, Igor Hudec, L'udovít Gaál, Pavel Bella*	
12:30-15:00	**Lunch break**	
15:00-17:00	**POSTER SESSION**	**Karst Research Institute ZRC SAZU**
18:00-20:00	Ice breaker and Unresolved mysteries of karst *Philipp Häuselmann*	

Tuesday, June 17th, 2014

9:00-12:50	**SESSION 2** Chair: Václav Krištůfek	**Cultural Centre Postojna**
9:00-10:00	**Invited lecture** Secondary mineral deposits and associated microbe-mineral interactions in subsurface rock environments *Ana Zélia Miller, Amélia Dionísio, Cesareo Saiz-Jimenez*	
10:00-10:20	Biogeochemistry of subsurface microbe/mineral associations *Aaron Alexander Jones, Philip C. Bennett*	
10:20-10:40	Bat guano as a driver of microbial metabolic activities in a cave *Alica Chroňáková, Václav Krištůfek, Petr Baldrian, Janez Mulec*	

10:40-11:00	Fibrous microcrystalline calcite in moonmilk: biogenicity, biosignatures and bioconfusion *Andrea Martín-Pérez, Adrijan Košir, Bojan Otoničar*	
11:00-11:30	**Coffee break**	
11:30-11:50	Exploring gut microbiota composition of the two caves coleopteran species *Neobathyscia pasai* and *Neobathyscia mancinii* (Coleoptera, Leiodidae, Cholevinae) *Leonardo Latella, Anna Castioni, Laura Bignotto, Elisa Salvetti, Sandra Torriani, Giovanna E. Felis*	
11:50-12:10	From soil microbial ecology to cave ecosystems – a better insight into microbial community dynamics by using high throughput sequencing techniques *Irena Maček, Nataša Šibanc, Thorunn Helgason, Alex J. Dumbrell*	
12:10-12:30	Moonmilk in Postojna cave, Slovenia *Magda Mandić, Andrej Mihevc, Tea Zubin Ferri, Helena Ćetković, Bruna Pleše, Ines Krajcar Bronić*	
12:30-12:50	Microbiological indicators for monitoring tourist use of Postojnska jama, Slovenia *Janez Mulec, Alenka Koželj, Erika Batagelj, Janja Vaupotič, Julia Walochnik*	
12:50-16:45	**Lunch break**	
16:45-19:30	**AFTERNOON FIELD TRIP** *Stanka Šebela, Janez Mulec*	**Postojnska jama, Črna jama, Pivka jama**

Wednesday, June 18th, 2014

9:00-12:30	**SESSION 3** Chair: Lejla Pašić	**Cultural Centre Postojna**
9:00-10:00	**Invited lecture** Microbial activities at environmental gradients in cave and karst ecosystems *Annette Summers Engel*	
10:00-10:20	Dense, shallow air-filled maze passage in coastal Quintana Roo, Mexico: mapping, investigation and resource preservation *Barbara Luke, Peter Sprouse*	
10:20-10:40	Reticulated Filaments found in a Subsurface Thermal Spring in Bad Gastein, Austria. *Marion Dornmayr-Pfaffenhuemer, Helga Stan-Lotter*	
10:40-11:00	High biodissolution rates in saturated karst freshwaters of NE Dinaric (Classical) Karst *Mitja Prelovšek, Janez Mulec*	
11:00-11:30	**Coffee break**	
11:30-11:50	Bacteria, bonds between classical karsts and karsts in silicated and non carbonated rocks? *Luc Willems*	
11:50-12:10	Diverse microniches in Škocjanske jame, Slovenia, offer an immense potential for future research *Janez Mulec, Gorazd Kosi, Elizabeth Covington, Alenka Mauko, Julia Walochnik*	
12:10-12:30	Poster award Closing remarks	
12:30-15:00	**Lunch break**	
15:00-18:30	**AFTERNOON FIELD TRIP** *Samo Šturm*	**Škocjanske jame**

Thursday, June 19th, 2014

8:30-19:00	**WHOLE-DAY EXCURSION** *Andrej Mihevc*	**Classical Karst**
20:30-23:00	**RECEPTION**	**Karst Research Institute ZRC SAZU**

Friday, June 20th, 2014

9:30-11:00	**MUSEUM COLLECTIONS** Speleology and speleobiology *Slavko Polak*	**Notranjska Museum, Postojna**
12:00-13:00	**CAVE BIOTA** Vivarium Proteus	**Vivarium Proteus, Postojnska jama**

LIST OF POSTERS

Screening the cultivable cave silver microbiome for the production of antimicrobial compounds
Jerneja Avguštin, Patricija Petrič, Rok Tkavc, Blagajana Herzog Velikonja, Lejla Pašić

Cave in Panorc
Fadil Bajraktari, Sami Behrami

Proliferation of green algae in the Moidons Cave: factors driving colonization and use of UV-C treatments to control their growth
Fabien Borderie, Nicolas Tête, Didier Cailhol, Laurence Alaoui-Sehmer, Faisl Bousta, Dominique Rieffel, Lotfi Aleya, Badr Alaoui-Sossé

Fossil attemsiid millipedes from the Hermannshöhle, Austria: *Polyphematia moniliformis* (Latzel, 1884)?
Pavel Bosák, Andrej Mock, Lukas Plan, Petr Pruner, Andrea Schober

Cave microclimate in the entrance part of the ice cave Ledena jama in Lomska duliba (Velebit Mt., Croatia)
Vinka Dubovečak, Nenad Buzjak, Dalibor Paar

The pilot research of allocation of gamma radionuclides in caves
Igor Kachalin, Oleksandr Liashchuk, Stanka Šebela, Janja Vaupotič

A simple mathematical model of cave eutrophication
Sergey Khizhnyak

Cold-adapted microbial communities in the limestone caves of Siberia, Caucasus and Far East and their biotechnological potential
Sergey Khizhnyak, Elena Nesterenko, Elena Lankina, Ilmira Ilients, Sofya Ovsyankina, Lyaysyan Kharlamova, Ekaterina Balmochnykh

Sediments of Béke Cave, Hungary - Preliminary Results I: stratigraphy and sedimentology
Klaudia Kiss, Balazs Bradak, Jozsef Szeberenyi, Gyorgy Varga, Gyorgy Czuppon, Gabor Vid

Olm conservation project (2012-2015)
Petra Kovač Konrad, Bojan Karaica, Marko Budić

First results of microbial inventory in caves: Dimnice and Sveta jama, Slovenia
Franc Malečkar, Violeta Lovko, Janez Mulec

Anthropogenic inscription of aerosol particles in the recent speleothems and in the cave air
Magda Mandić, Luka Mandić, Ivo Orlić

Bacteriological pollution of karst springs in Zlatar, Western Serbia
Dejan Milenic, Djuro Milankovic, Nevena Savic, Nenad Doroslovac

Bacteriological pollution of the groundwater of karst springs in Zlatibor
Dejan Milenic, Ana Vranjes, Bojan Doncev, Marina Jovanovic

Lampenflora in Postojnska jama, Slovenia: problems and solutions
Janez Mulec

Cultivation microbiological techniques for rapid determination of biological pollution during expeditions in water caves
Janez Mulec, Andreea Oarga

Detection of *Histoplasma* antigens in bat guano from caves in Slovenia using commercial ELISA for human samples was not supported by the molecular analyses
Janez Mulec, Sanja Stopinšek, Romina Kofol, Tadeja Kotar, Saša Simčič

Karst soil properties and men's impact: the case study of Pliskovica
Nina Peca

Chemical and isotope analysis of water samples from wells and springs of Rovte region, W Slovenia: an assessment of ongoing dedolomitization
Sven Philipp, Stephan Kempe, Bojan Otoničar

Chemical and microbiological analysis of water from Emilia Romagna gypsum caves (Parco dei gessi Bolognesi, Life + 08NAT/IT/000369)
Diana I. Serrazanetti, Chiara Montanari, Ilenia Maria D'Angeli, Jo De Waele, Lucia Vannini, Fausto Gardini

A novel survey of the distribution of *Proteus anguinus* by environmental DNA sampling
David Stanković, Gregor Aljančič, Špela Gorički, Magdalena Năpăruş, Miloš Pavićević, Ivan Skaramuca, Jasminko Mulaomerović, Matjaž Kuntner, Aleš Snoj, Zlatko Grizelj, William R. Jeffery

Microbiology of cave environments: specific or not?
Blaž Stres, Nadja Robič, Tina Zakotnik, Valentina Turk, Janez Mulec

Characterization of the cave environments for the palaeoclimate studies – from Dugi otok Island to Velebit Mt (Croatia)
Maša Surić, Nenad Buzjak, Robert Lončarić, Nina Lončar

Resistance of karst cave microbial strains to extreme factors
Olga Suslova, Oleksandr Tashyrev

Geomicrobiology of Irazu volcanic caves: preliminary results
Andrés Ulloa, Mariangela Vargas, Lorena Uribe, Walter Hernández, Marielos Mora

The biodiversity profile of cave drip water in Velika Pasica Cave, Central Slovenia
Allen Wei Liu, Anton Brancelj

FIELD TRIPS

AFTERNOON FIELD TRIP, TUESDAY, JUNE 17TH 2014

Postojnska jama, Črna jama, Pivka jama

Postojna cave system - geology, climate and sustainable use

Stanka Šebela

Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia

The Postojna Cave System is the second longest (20,570 m long; 115 m deep) known cave in Slovenia. The cave is connected to the Planina Cave (6.656 km long; 65 m deep) by unknown passages. There remains about 2,000 m unexplored separation, although an underground water connection has been known since the explorations of Gruber in 1781.

The Pivka River flows on impermeable Eocene flysch and sinks into the underground system of Postojna Cave System at an elevation of 511 m on the contact with Cretaceous limestones. Dye-tracing experiments in 1988 provided evidence for the bifurcation of the Pivka, for the surficial river also drains toward the sources of the Vipava River and thus forms part of both the Adriatic and the Black Sea drainage basins. The portion of the Pivka that enters the Postojna-Planina Cave System belongs to the Black Sea drainage basin.

Geologic setting

Slovenia is situated at the border between Adria microplate and Eurasia plate and characterized by complex and neotectonically active geological conditions.

Since the late Miocene to Pliocene paleomagnetic data had indicated about 30° counterclockwise rotation of Adria microplate. During Miocene to recent the thrust belts along the Adria margin include Dinaric thrust system, South-Alpine thrust system and Dinaric faults. Dinaric faults cut and displace both Dinaric and South-Alpine fold-and-thrust structures. Most of them are characterized by moderate historic and recent seismicity.

The area is part of the Javorniki-Snežnik thrust unit, which has been overthrust over the Eocene flysch. The Hrušica thrust unit, which is upper Triassic dolomite, overthrusts the Javorniki-Snežnik thrust unit. Overthrusting took place after the deposition of the Eocene flysch. During the Miocene and Pliocene, the overthrusting was accompanied by folding. The principal folding deformation in Postojna Cave is the Postojna Anticline (Fig. 1). Between Postojna and Planina Caves is the Studeno Syncline.

It is important to distinguish older overthrusting and folding deformations from younger faulting deformations. The Postojna Cave System is situated between two regionally important faults with the NW-SE Dinaric orientation. These are the Idrija Fault on the north and the Predjama Fault on the south. The tectonic structure of the area between those two faults has all the characteristics of the intermediate zone between two dextral strike-slip faults. The cave passages of Postojna Cave System follow strike and dip of the bedding planes, especially those with interbedded slips. They are developed in both flanks of the Postojna Anticline. They also follow Dinaric and cross-Dinaric (NE-SW) oriented fault zones and mostly north-south oriented fissured zones (Šebela, 1998; Šebela, 2012).

During the development stages of Postojna Cave System some of the geological structures were reactivated and appear to be connected with the formation of collapse chambers. The geological

structural elements were used as pathways for cave development. Along the same fault zone in the cave up to four different reactivations can be detected. The fault zone that runs from Pisani Rov and through Velika Gora shows sinistral horizontal movement in Pisani Rov, reverse fault movement at Velika Gora and dextral horizontal movement and normal fault movement in Lepe Jame (Fig. 1). The same Dinaric oriented fault zone is monitored at two places with TM 71 extensometers. It is also cut by the cross-Dinaric oriented fault zone in Lepe Jame. This neotectonic fault zone was active post cave development because it cuts older cave sediments which are at least 0.78-0.99 Ma in age.

On the southwestern flank of Postojna Anticline Dinaric oriented fault zones prevail, while on the northeastern flank the cross-Dinaric oriented fault zones prevail. Underground River Pivka passage follows the strike direction of bedding planes in the southern part and bedding strike dip direction in the northwestern part.

The Postojna Cave System is developed in Cretaceous carbonate rocks. The cave passages are developed in upper Cretaceous (Cenomanian, Turonian and Senonian) mostly bedded and thick bedded limestones (Fig. 2). The Cenomanian and Turonian limestones are more thin-bedded and can include chert lenses. The Senonian limestones are thick bedded to massive. The cave passages are developed in about an 800 m thick lithological column.

Recent micro-tectonic deformations have been monitored continuously in 3D in Postojna Cave with TM 71 extensometers since 2004. Two instruments, 260 m apart, were installed on the Dinaric oriented (NW-SE) fault zone that is situated about 1,000 m north of the inner zone of the regionally important Predjama Fault.

The locations for installation of the exstensometers in Postojna Cave were selected to evaluate if the monitored fault is tectonically active and to determine whether the tectonic activity has any influence on the speleogensis. The two devices were installed in the same fault zone but on different fault planes. Monitoring on both instruments has shown small tectonic movements *i.e.* a general dextral horizontal movement of 0.05 mm in 4 years (Velika Gora) and extension of 0.03 mm in 4 years (Lepe Jame). Between the longer or shorter calm periods, eleven extremes have been recorded regarding characteristic changes in displacement. The largest short-term movement was a compression of 0.04 mm in 7 days, detected in March 2005, which coincided with the 25 km distant Ilirska Bistrica earthquake (M_L=3.9). In the sense of speleogenesis the monitored fault zone represents a stable cave environment (Šebela et al., 2010).

Since 1932 in Postojna Cave in Tartarus passage the underground station with horizontal pendulums was in operations to measure Earth tides. The study that stopped before 1945 was important for karst hydrology and seismology as well. In the same place as 1932 station the accelerometer instrument was installed within the common project with Italian collegues (Univeristy of Trieste) and Slovene seismologists (Seismology and Geology Office, Ministry of Agriculture and Environment) in the begining of 2010. Our aim is to establish the first underground seismological station in Slovenia with real-time registration.

Since July 2010 the tilt data of vertical 20 m long pendulum in Magdalena Jama are collected with imminent work of Czech collegues. The two dimensional optical measurement of tilt of the rock mass and continous digital evaluation of the results is important to understand the behaviour of the stress changes within the rock mass (Kalenda et al. 2013).

Cave climate

First studies of Postojna Cave underground climate were published by Crestani and Anelli in 1939. Air temperature in different parts of the cave, underground River Pivka temperature and also temperature of the rock were accomplished. The ventilation and humidity in the cave were studied. Connection between blowholes on the surface and cave passages was described.

The recent systematical studies of underground meteorological conditions in Postojna Cave with the purpose to determin human impact on natural worth started in 2009 (Gregorič et al., 2013; Gregorič et al., 2014; Šebela & Turk 2011; Šebela & Turk 2014; Šebela et al., 2013; Muri et al. 2013, Mulec 2014). Beside four meteorological stations (air temperature, water temperature, humidity,

wind direction, wind speed, CO_2) other places are selected in the Postojna Cave as monitoring sites where air temperature and air pressure are continuously measured. The cave is well ventilated deep inside. The average air temperature (2009-2010) on the top of Velika Gora is 11.10 °C and 10.66 °C for Lepe Jame. For the same period the average air temperature on the surface was 9.20 °C.

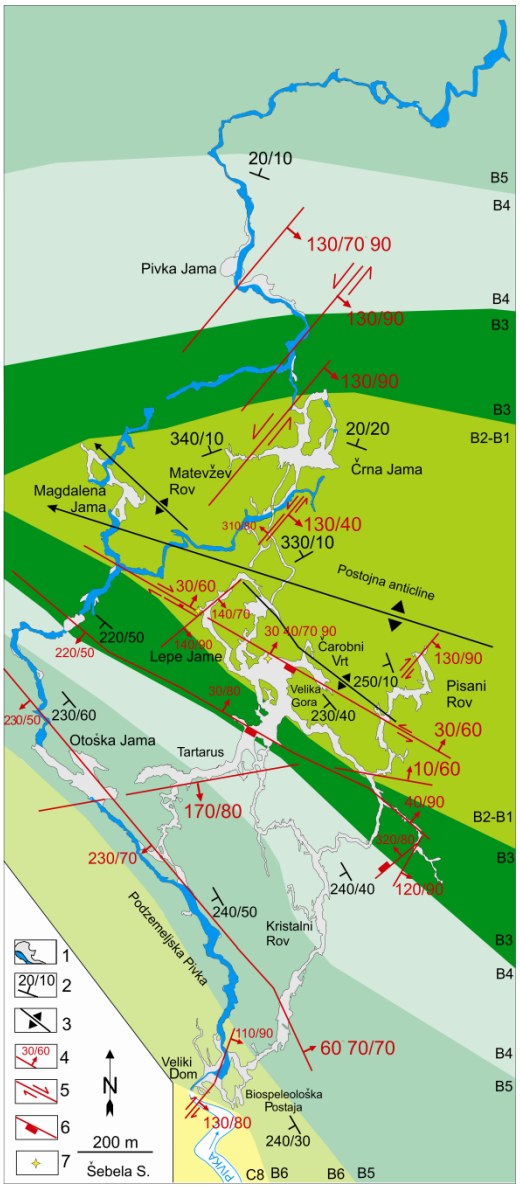


Fig 1. Geology of Postojna Cave System. 1-cave passage with underground River Pivka, 2-strike and dip direction of bedding plane, 3-anticline, 4-strike and dip direction of fault, 5-dextral horizontal movement, 6-vertical movement, subsidence of SW block.
B1 – thin-bedded Cretaceous limestone (165 m), B2 – thick-bedded Cretaceous limestone (50 m), B3 - very thick-bedded Cretaceous limestone with *Chondrodonta* horizon (up to 110 m), B4 - thick-bedded Cretaceous limestone with rudists (290 m), B5 - very thick-bedded Cretaceous limestone with rudists (420 m), B6 – thick-bedded Cretaceous limestone (130 m), C8 – Eocene flysch.

Almost 200 years of sustainable use of the cave

The Postojna Cave System has a lengthy history of tourism. Recently the cave reached a milestone of having welcomed 36 million visitors since 1818. In recent years about 500,000 tourists per year have visited the cave.

Modern tourism in Postojna Cave began in 1818, when local cave lamplighter and guide Luka Čeč discovered extensive new sections of the cave system. It was on the occasion of the visit of the Austrian emperor, when Luka Čeč climbed across the underground river Pivka in the Veliki Dom chamber.

Valvasor in 1689 described the cave as one of the most remarkable caves in the world. Since 1872 the railway was in operation for tourists in Postojna Cave. A small man-powered railway was

introduced for all who could afford it. In 1924 manpower was replaced by first locomotive power. Permanent installation of electric lighting is since 1884, what means that Postojna Cave was the third in the World.

All karst caves in Slovenia are natural worths that belong to the state property. Expert control and recommendations for sustainable management of natural worths, formulation of suitable directives for sustainable use of natural worths, and climatic and biologic monitoring of cave system needs to be fulfilled. Beside this the cave guardian collects data on old and actual scientific and popular researches of the cave system.

Bibliography

Gregorič, A., Vaupotič, J., Gabrovšek, F., 2013. Reasons for large fluctuation of radon and CO_2 levels in a dead–end passage of karst cave (Postojna Cave, Slovenia). Natural Hazards and Earth System Sciences 13, 287–297. DOI: 10.5194/nhess-13-287-2013.

Gregorič, A., Vaupotič, J., Šebela, S., 2014. The role of cave ventilation in governing cave air temperature and radon levels (Postojna Cave, Slovenia). International Journal of Climatology 34 (5), 1488–1500. DOI: 10.1002/joc.3778.

Kalenda P., Neumann L., Šebela S., 2013. Early results of micro-deformation measurements in Magdalena Jama (Slovenia) by a vertical static pendulum. Acta Carsologica 42(1): 143-154.

Mulec, J., 2014. Human impact on undergorund cultural and natural heritage sites, biological parameters of monitoring and remediation actions for insensitive surfaces: Case of Slovenian show caves. Journal for Nature Conservation 22 (2), 132–141. DOI: 10.1016/j.jnc.2013.10.001

Muri, G., Jovičić, A., Mihevc, A. 2013. Source assessment of deposited particles in a Slovenian show cave (Postojnska jama): evidence of long-lasting anthropogenic impact. International Journal of Speleology 42 (3), 225–233. DOI: 10.5038/1827-806X.42.3.6

Šebela, S. 1998. Tectonic Structure of Postojnska jama cave system. ZRC Publishing, vol. 18, 112 pp. Ljubljana.

Šebela, S. 2012. Postojna Planina Cave System, Slovenia. In W.B. White, & D. C. Culver (Eds.), *Encyclopedia of Caves* (pp. 618–624). Amsterdam, Boston, Heidelberg, London, New York, Oxford, Paris, San Diego, San Francisco, Sydney, Tokyo: Academic Press, Elsevier.

Šebela, S., Vaupotič, J., Košťák, B. and Stemberk, J. 2010. Recent measurement of present-day tectonic movement and associated radon flux in Postojna cave, Slovenia. *Journal of Cave and Karst Studies* 72/1, 21-34.

Šebela, S., Prelovšek, M., Turk, J., 2013. Impact of peak period visits on the Postojna cave (Slovenia) microclimate. Theoretical and Applied Climatology 111, 51–64. DOI: 10.1007/s00704-012-0644-8.

Šebela, S., Turk J., 2011. Local characteristics of Postojna Cave climate, air temperature, and pressure monitoring. Theoretical and Applied Climatology 105 (3-4), 371–386. DOI: 10.1007/s00704-011-0397-9

Šebela, S., Turk, J., 2014. Natural and anthropogenic influences on the year-round temperature dynamics of air and water in Postojna show cave, Slovenia. Tourism Management 40, 233–243. DOI: 10.1016/j.tourman.2013.06.011

AFTERNOON FIELD TRIP, WEDNESDAY, JUNE 18TH 2014

Škocjanske jame

Škocjanske jame

Peter Kozel

Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia

Škocjanske jame is the name given to a complex of caves and deep vertical-sided collapse dolines formed by the Reka river. Reka with mean annual discharge of 8,26 m^3 enters the cave at an altitude of 317 m; in the Martelova dvorana room, it is 214 m above sea level, the lowest point reaches at 190 m a.s.l. In dry periods of the year Reka sinks before it enters the cave. Floods usually reach up to 30 m. The largest known flood in the previous century raised the water table level for 132 m (Mihevc & Gabrovšek 2012). Škocjanske jame are located in the Škocjan karst, a unique extensive flat landscape that lies at an altitude between 420 and 450 m in the SE of the Karst (Kras) (Debevec et al. 2008). This area is formed on the impermeable flysch rocks of the Brkini region. Škocjanske jame are 6200 m long and 250 deep complex of four caves, and one of the most important cave systems in the world. Peculiarities that make them so fascinating, and distinguish them from few others, otherwise also outstanding caves on Classical Karst, are their large galleries and chambers, underground canyon of the Reka river and large collapse dolines (Mihevc 2004). The largest chambers are Martelova dvorana, with a volume of 2,100,000 m^3, and Šumeča jama with 870,000 m^3 (Knez et al. 2003, Gabrovšek & Mihevc 2009). Some big collapsed chambers forming the big collapse dolines like Velika and Mala dolina. Škocjanske jame for quite long time represent the biggest natural curiosity to speleologists, and likewise to general public. Because of their immense dimensions and historically important explorations taking place in the caves, they are almost 30 years included in the UNESCO's list of natural and cultural world heritage. In 1999 they were, together with the underground Reka river, included in the list of Ramsar wetlands of international importance as one of the longest karst underground wetlands in Europe. Since 2004 onwards Škocjan Caves Park is also included in the world network of biosphere reserves named MAB - "Man and the Biosphere".

Historical overview

People have always been very interested to the gorge where the river Reka disappears underground, and even more to the following underground phenomenon, Škocjanske jame. They have been given name after the Škocjan village, situated at the top of the rock wall above Reka sink. Residuals are demonstrating early man presence and dwelling within the cave and in near vicinity from the Mesolithic period through the Iron Age, classical antiquity and the Middle Ages up to the present days. Some finding are suggesting that cave in prehistoric times had not only local but at least regional importance. First written record about Škocjanske jame is that of Posidonius of Apamea in 135–30 BC, which dates back in antiquity. Caves were later also presented on the oldest printed maps (Lazius 1573, Mercator's Novus Atlas 1637). Even the world famous Slovenian polyhistor, J. V. Valvasor, did not overlook that remarkable phenomenon. In his encyclopedia "The Glory of the Duchy of Carniola" (1689) he discusses the underground flow of the Reka, adding an illustration of its swallow-hole. Few illustrations of French painter F. Cassas proves that Škocjanske jame were treated as one of the most brilliant natural attractions worth seeing in 18th century. At that time many of people already visited the bottom of Velika dolina collapse doline. 1st January 1819 is, however, nowadays considered as the beginning of modern tourism in Škocjanske jame (Debevec et al. 2008).

First serious speleological explorations of Škocjanske jame commenced in early 19th century. In 1823 Tominčeva jama (named after Matej Tominc) was discovered, and first paths within the cave were made. In that period some foreign researchers also investigated Škocjanske jame. Adolf Schmidl discovered "Schmidlova dvorana" and passage to "Rudolfova dvorana". More detailed and systematic work started after 1880. Mullerjeva dvorana, Dvorana ponvic, final siphon and Tiha jama were discovered between 1884 and 1904. Simultaneously the local cave workers constructed the footpaths and bridges (Mihevc 2004, Debevec et al. 2008). One of the greatest acquirements in 20th century was the electrification of the caves in 1959 and construction of elevator in Velika dolina in 1986. Besides, some other investigations took part in late 19th century. Overall, the most significant explorer of the caves was Anton Hanke. Another important milestone was reached on 15th September 1991 when two Slovene cave divers, Janko Brajnik and Samo Morel, discovered the continuation of Škocjanske jame downstream Reka river. This led to next discoveries to penetrate down the underground Reka and reach Kačna jama (Debevec et al. 2008).

Geology of Škocjanske jame

Škocjanske jame are developed in Cretaceous and Paleocene limestone, near the contact with the impermeable Eocene flysch rock. The contact is located only a few kilometers to the south from the caves and significantly influenced the cave development and formation. Flysch rock consists of quartz sandstone, conglomerates and marl (Mihevc 2002, Debevec et al. 2008). In Škocjanske jame we find the monocline of the Upper Cretaceous and Palaeocene beds. The monocline is composed of Turonian and Senonian rudistid limestone and Danian, Thaneatian and Eocene foraminiferal limestone. The monocline reaches under the Upper Eocene flysch.
Geological investigation indicated that accessible passages of Škocjanske jame system developed in Turonian and Senonian, mostly thick-bedded limestones with exception of Tiha jama, built in thin layered Cretaceous and Paleocene limestones (Fig. 1) (Mihevc 2002, Knez et al. 2003).

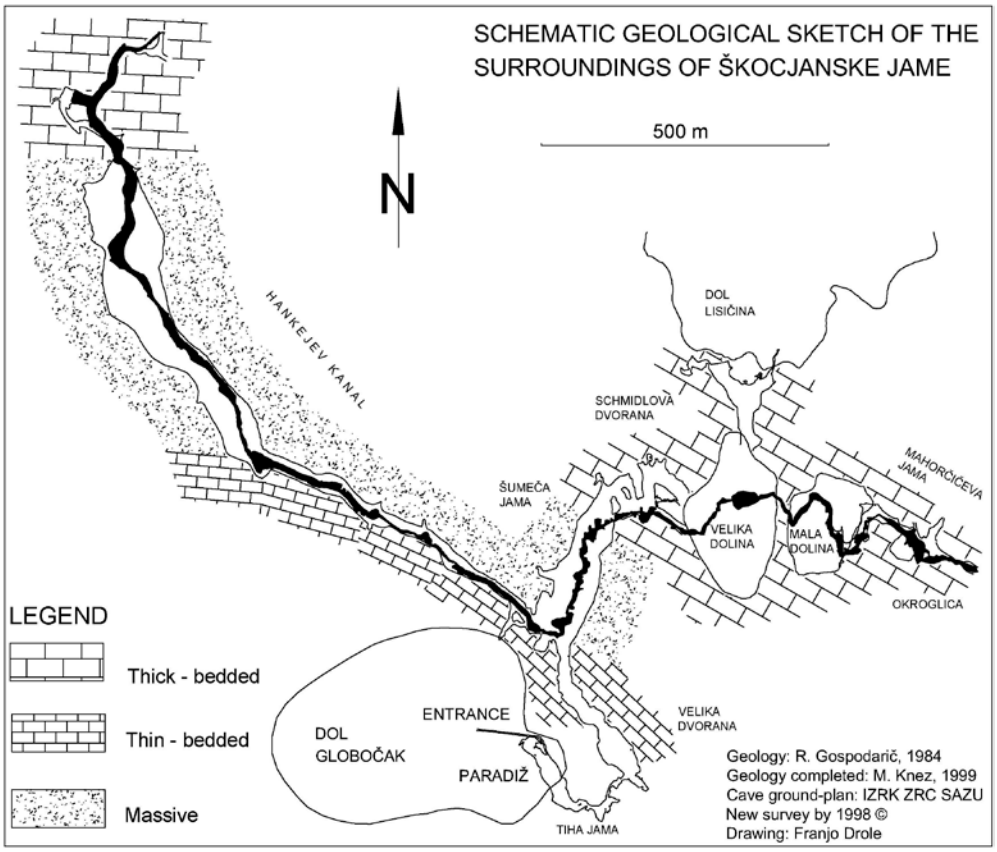


Figure 1. The geological map of the Škocjanske jame (Mihevc & Gabrovšek 2012).

The canyon passages between the swallow-hole of the Reka and the beginning of "Hankejev kanal" run primarily near singular bedding-planes and some fractures and faults in predominantly thick-bedded Senonian limestone. Velika dolina and Mala dolina collapse dolines are also formed in thick-bedded Senonian limestone. Speleogenetic processes in this area mainly occurred along bedding-planes. Šumeča jama and Hankejev kanal, with the exception of Zaliti kanal, are formed in massive limestone. Tiha jama is formed in characteristic thin-bedded, rather bituminous Maastrichtian limestone (Mihevc 2002, Knez et al. 2003).

The largest chambers of Škocjanske jame are formed in Cretaceous beds. In contrast to this, flat and thin-bedded Maastrichtian limestone strata are finely cracked in all directions, which causes them to crumble into small particles.

The dependence of passage morphology on lithostratigraphic rock composition, structural condition in the rock and on microtectonic characteristic of limestone is particularly noticeable in the Škocjanske jame. In massive limestone and thick-bedded limestone, with characteristic cracks and faults, recent canyon passages have been formed. In bedded and thin-bedded limestone, passages are wide, low and collapsed. Karstic relief over the cave and near it differs in this respect. There is no question that passages adapted to this geologic structure and to the lithological differences in limestone. Lithological differences undoubtedly preconditioned subterranean morphogenetic and speleogenetic processes (Knez et al. 2003).

Hydrology

Škocjanske jame were formed by the sinking Reka river which is the widest known disappearing stream of a Classical Karst (Brilly et al. 2002). Reka collects water from more than 350 km^2 of the surface. Its average measured flow before sinking in Škocjanske jame is 8.95 m^3/s, during heavy flooding can reach up to 387 m^3/s (Debevec et al. 2008). After sinking at Škocjanske jame, Reka flows through karst underground and reappears on surface about 35 km away in the Timavo spring in Italy. Even today a large part of its underground flow is still unknown and mysterious, moreover it is accessible only in few deep shafts up to 320 m deep.

It is assumed that previous to Pleistocene the Reka river flow was entirely superficial flowing from the flysch ground across the Karst towards the sea. Later, beacuse of the karstification, it was progressively sinking underground and began to incise a gorge in the limestone bedrock, which can be recognized by river terraces. This gradual lowering of the channels through which Reka river used to flow is also evidenced and visible within the caves.

Flora and fauna

In the literature Škocjanske jame are mostly recognized as one of the most important underground phenomena in the world. Additional, one aspect that should not be neglected, and makes these incredible caves so unique is their biotic diversity. Škocjanske jame were intensively explored by numerous botanists and zoologists due to their heterogeneous landscape which encompasses remarkably diverse flora and fauna. Special microclimatic conditions and habitats are providing adequate habitat for few glacial relicts, shelter to rare and endangered bird and several bat species. Collapsed dolines and underground world is situated in the sub-Mediterranean phytogeographic area. In addition to diverse surface fauna, underground habitats are as well inhabited by rich aquatic and terrestrial fauna (Debevec et al. 2002).

First botanical examination of Škocjanske jame was carried out by Carlo Marchesetti in 1887. Deep collapse dolines are inhabited by numerous kinds of frigophilous species and a variety of thermophilic species. In the Škocjanske jame Regional Park also nine plant species thrive that are included in the Red List of ferns and seed plants in Slovenia which are endemic, rare or threatened: *Orobanche hederae*, *Orobanche mutelii*, *Lamium wettsteinii*, *Campanula justiniana* (Fig. 2), *Aconitum anthora*, *Hyssopus officinalis*, *Juniperus oxycedrus*, *Ranunculus pospichalii* and *Salvia officinalis*.

Figure 2. Škocjanske jame are the locus classicus of *Campanula justiniana* (http://dinaricarcparks.blogspot.com/2012/12/justinova-zvoncica-campanula-justiniana.html).

Termophilous plant species characteristic of sub-Mediterranean grasslands and shrubs can be as well found in regional park: *Carpinus orientalis*, *Centaurea rupestris*, *Chrysopogon gryllus*, *Cleistogenes serotina*, *Digitalis laevigata* etc. Two glacial relicts like alpine auricular (*Primula auricular*, Fig. 3) and encrusted saxifrage (*Saxifraga crustata*, Fig. 4) are present due to relative low temperatures in certain parts of collapse dolines.

Figure 3. *Primula auricular* (http://www.park-skocjanske-jame.si/slo/index.shtml)

Figure 4. *Saxifraga crustata* (http://www.plant-world-seeds.com/store/view_seed_item/2380?itemname=SAXIFRAGA+CRUSTATA)

Most common and first noticeable birds in the Velika and Mala dolina are rock doves (*Coluumba livia*). Several bird species, for example alpine swift (*Tachymarptis melba*), eagle owl (*Bubo bubo,* Fig. 5), peregrine (*Falco peregrinus*) and raven (*Corvus corax*), find cave entrance sections and various parts of the collapse dolines as a suitable nest place.
Some other interesting species can be encountered within the territory of the Škocjanske jame Regional Park, like dormouse (*Glis glis*), red fox (*Vulpes vulpes*) and Eurasian badger (*Meles meles*), especially in the slopes of Reka gorge.

Figure 5. Eagle owl (*Bubo bubo*) sometimes nests in collapse dolines (http://en.wikipedia.org/wiki/Eurasian_eagle-owl).

Škocjanske jame are noted for their exceptionally rich and diverse subterranean fauna. Biospeleologists have quite intensively investigated aquatic and terrestrial fauna. At first they were mainly interested in aquatic fauna (Kiefer 1930); most of the research at that time, and continuously till 1980's, were devoted to Copepod studies. In recent years, special attention has been put on epikarst fauna from percolation water. From water drips and pools Pipan (2005) identified a wide range of animal groups: Turbellaria, Nematoda, Gastropoda, Oligochaeta, Acarina, Ostracoda, Copepoda, Isopoda, Amphipoda, Collembola and Diptera larvae. Copepods are species-richest group of animals in percolation water in Škocjanske jame. 32 species have been identified so far, five of them, members of the genera *Bryocamptus*, *Moraria*, *Parastenocais* in cf. *Stygepactophanes,* were also new for science (Pipan 2005). It is worth mentioning that *Elaphoidella karstica* is endemic species of Škocjanske jame.

Furthermore, investigations of the Reka river fauna have been carried out (Pipan 2000). Animals from different groups are present, among these are insects larvae (mainly Ephemeroptera and Diptera), Cnidaria, Turbellaria, Gastropoda (*Zospeum spelaeum spelaeum*), Bivalvia, Oligochaeta (*Haber monfalconensis*), Hirundinea, Acarina, Isopoda (*Asellus aqaticus cavernicolous*, *Trichoniscus stammeri*), Amphipoda (*Niphargus timavi*, *N.* cf. *stygius*) (Culver & Sket 2002) and Decapoda (*Troglocaris* sp.). Few rare stygobionts which can be found in the River are *Marifugia cavatica* (Polychaeta) and *Dendrocoelum spelaeum* (Turbellaria). *Proteus anguinus* (Amphibia) ranges from Italy to Croatia and in the river Reka reaches the most northwestern distribution.

Terrestrial subterranean fauna in Škocjanske jame is very diverse as well; in the last monitoring of tourist part of the caves 39 taxa have been listed. Fauna includes individuals from all three ecological groups, with troglophiles predominating (20 species), and troglobionts represented by only 8 species (Polak 2012). Common trogloxene taxa found in Škocjanske jame are *Scoliopteryx libatrix* (Fig. 6), *Triphosa dubitata*, *Troglophilus neglectus, Mitostoma chrysomelas* and several Phoridae species.

Among troglophiles *Oxychilus cellarius*, *Lithobius validus*, *Meta menardi*, *Metellina merianae*, *Androniscus roseus* and *Laemostenus cavicola* (Fig. 7). Surprisingly, number of troglobiont species is very low regarding the great dimension of Škocjanske jame and comparing to the faunisticaly richest caves like Postojnska jama, Jama pod Predjamskim gradom, Logarček, and many others (Culver & Sket 2000). Up to now, following troglobiont species have been found in Škocjanske jame: *Zospeum spelaeum spelaeum* (Fig. 8), *Eukoenenia* sp., *Titanethes dahli*, *Moserius percoi*, *Onychiurus canzianus*, *O. variotuberculatus, Oncopodura cavernarum, Troglopedetes* cf. *pallidus*, *Anophthalmus schmidti*, *Bathysciotes khevenhuelleri terghestinus*, *Brachydesmus subterraneus*, *Alpioniscus strasseri*, *Androniscus stygius tschameri* and *Typhloiulus illyricus* (Culver & Sket 2002, Polak 2012). List of subterranean species, particularly troglobiont species, is surely incomplete since the caves have not been thoroughly investigated so far.

Figure 6. Trogloxene moth *Scoliopterix libatryx* (Polak 2012)

Figure 7. Troglophile beetle *Laemostenus cavicola* (Polak 2012)

Figure 8. Cave snail *Zospeum spelaeum spelaeum* (Polak 2012).

References

Brilly, M., Mikoš, M., Petkovšek, G., Šraj, M., Kogovšek, J., Drobne, D., Štravs, L. (2002) The experimental monitoring of the water regime in the Reka river. *Acta Carsologica*, 31 (1): 65–74.

Culver, D. C., Sket, B. (2000) Hotspots of subterranean biodiversity in caves and wells. *Journal of Cave and Karst Studies*, 62 (1): 11–17.

Culver, D. C., Sket, B. (2002) Biological monitoring in caves. Acta carsologica, 31 (1): 55–64.

Debevec, A., Peric, B., Kranjc, A., Mihevc, A., Slapnik, R. (2008) *The Škocjan caves in the bosom of the classical karst*. Park Škocjanske jame, Škocjan, 44 pp.

Gabrovšek, F. Mihevc, A. (2009) *Cave climate. 17th International karstological school "Classical Karst"*. Karst Research Institute, Scientific Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, 92 pp.

Grgić, I., Iskra, I., Podkrajšek, B., Gerjevič Debevec, V. (2014) Measurements of aerosol particles in the Škocjan Caves, Slovenia. *Environmental Science and Pollution Research*, 21: 1915–1923.

Kiefer, F. (1930) Neue Höhlenbewohnende Ruderfusskrebse. *Zoolohishe Anzeiger*, 87: 222–228.

Knez, M., Kogovšek, J., Mihevc, A. (2003) Karst I: *Škocjanske jame*. In: Juvan, G., Črne, A.E., Pavlič, M.U. (Eds), *Mednarodni geološki tabor EUGEN 2003*. Zveza za tehnično kulturo Slovenije, Gibanje znanost mladini, Ljubljana, 13–45.

Kranjc, A. (2002*) Historical overview and description of the caves*. In: Peric, B. (Ed.), *The Škocjan caves regional park*. Park Škocjanske jame, Škocjan, 42–57.

Mihevc, A. (2002) *Climate, Geology and Geomorphology*. In: Peric, B. (Ed.), *The Škocjan caves regional park*. Park Škocjanske jame, Škocjan, 58–65.

Mihevc, A. (2004) *Development of the tourist pathways in Škocjanske jame*. In: Zupan Hajna, N. (Ed.), *Use of modern technologies in the development of caves for tourism*. Postojna: Postojnska jama, turizem, 117–120.

Mihevc, A., Gabrovšek, F. (2012) *Karst forms and processes. 20th International karstological school "Classical Karst"*. Karst Research Institute, Scientific Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, 80 pp.

Pipan, T. (2000) Biological assessment of stream water quality – the example of the Reka river (Slovenia). *Acta Carsologica*, 29 (1): 201–222.

Pipan, T. (2005) *Epikarst – a promising habitat. Copepod fauna, its diversity and ecology: a case study from Slovenia (Europe)*. ZRC Publishing, Karst Research Institute at ZRC SAZU, Ljubljana, 101 pp.

Polak, S. (2012) *Monitoring terestrične troglobiontske favne v turističnem delu Škocjanskih jam*. Final report, 46 pp.

WHOLE-DAY EXCURSION, THURSDAY, JUNE 19TH 2014

Classical Karst

Classical Karst

Andrej Mihevc

Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia

Classical Karst is a part of Dinaric Karst in Slovenia and Italy. It spreads between the coast of Adriatic Sea at Bay of Trieste and the springs of Ljubljanica River in the edge of Ljubljana tectonic basin. It belongs to Reka and to Ljubljanica river basins.

This karst area has abundance of karst phenomena like large sinking rivers and springs, intermittent lakes, large caves and relief features like dolines, collapsed dolines, uvalas, poljes, levelled surfaces and high plateaus. And the karst surface is mostly rocky and dry.

But it was the exploration of the people that were driven by curiosity or science and by land use or water management issues and tourism that make this karst famous. Since 17th century karst phenomena were studied by local and foreign naturalists and scientists. Well researched, mapped and described caves and other karst phenomena in 19th century made this karst as a reference karst. Together with that the name Kras was used to denominate the natural phenomena itself and a new scientific discipline – karst studies.

Postojna played important role in karst research and is situated in the middle of this area.

Ljubljanica River

The calculated size of the Ljubljanica drainage basin is 1779 km^2, of which about 1100 km^2 are composed of karstic rocks. The location of the water divide is approximate, but bifurcations have been proved at several boundaries by water tracing. According to studies during the complex water tracing experiments of the nineteen-seventies, the catchment area of the Vrhnika springs, where the main river definitively leaves karst terrain, covers 1108.78 km^2. The mean discharge is 38.60 $m^3\ sec^{-1}$, with a specific run-off of 34.8 $l\ sec^{-1}\ km^{-2}$.

After the river the capital of Slovenia, Ljubljana is named.

The karstic rocks are mostly limestones and dominantly late-diagenetic dolomites of Mesozoic age. They were formed on the Dinaric platform under conditions of continuous sedimentation which enabled high rock purity, generally with less than 5%, but locally as little as 0.1%, insoluble residue. The total thickness of the carbonate sequence is about 6850 m.

The highest parts of the basin are high karst plateaus Hrušica, Javorniki, Snežnik. On the poljes among them surface rivers appear only, but they have different names: Trbuhovica, Obrh, Stržen, Rak, Pivka, Unica and finally after the springs at Vrhnika the name Ljubljanica. Besides these rivers there are some sinking rivers that are collecting waters from dolomite or flysch. These are Nanoščica, Cerkniščica, Logaščica, Hotenka and Rovtarica and many smaller.

The highest lying is the karst polje near Prezid (770 m), followed by Babno polje (750 m), Loško polje (580 m), Cerkniško polje (550 m), Rakov Škocjan (520 m), Planinsko polje (450 m), Logaško polje (470 m) and finally by Ljubljansko Barje (300 m) where the Ljubljanica springs are at 300 m a.s.l. There are several large springs are dispersed along the edge of the Barje, part of Ljubljana tectonic basin, which is connected with gradual tectonic subsidence of the area. Mean annual discharge of the Ljubljanica at springs is 38.6 m^3.

There are 1540 caves, accessible fragments of underground drainage system known in the catchments area of the Ljubljanica. The average length of the cave is 48 m and the depth 18 m.

However, the largest caves are the ponor or spring caves; in them we can follow the 71 km of passages of the main rivers, tributaries of Ljubljanica.

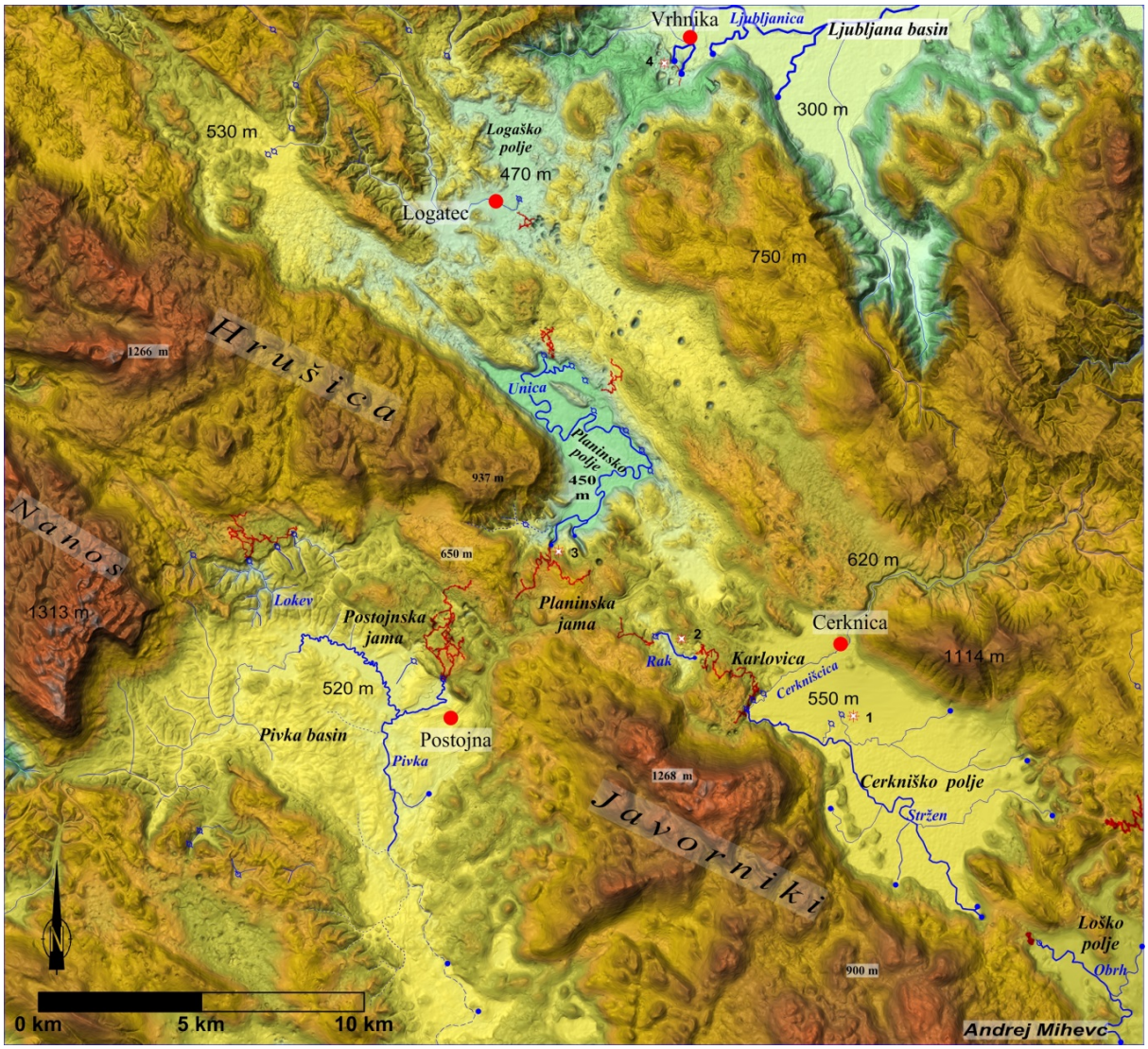


Fig. 1: Central part of the Ljubljanica basin with high karst plateaus, lower surfaces with karst poljes and surface rivers. Passages of larger caves are marked with red lines. Main field trips stops are marked with red star: 1 Cerkniško polje, 2 Rakov Škocjan, 3 Planinsko polje, 4 Springs of Ljubljanica river.

Cerkniško polje

Cerkniško polje is the largest karst polje in Slovenia and has regular floods, which last about half year. The intermittent lake covers 26 km^2 when is full; it is 10.5 km long and almost 5 km wide. Its hydrological properties caused that already in the beginning of New Age scholars from all round Europe were attracted to it. The lake becomes still more known through the Valvasor's description in 1689.

It is a karst polje developed in the important regional fault zone – Idrija fault. Idrija fault has "Dinaric" direction (NW-SE); in the same fault zone are developed: Planinsko polje, Loško polje and Babno polje. Bottom of Cerkniško polje covers 38 km^2 in elevation of about 550 m. Bottom is formed on Upper Triassic dolomite and Jurassic dolomite and on W and NW the Cretaceous limestone. Karst

springs are mostly are on E, S and partly on W sides of the polje. The only surface tributary to polje is Cerkniščica draining the dolomite catchments area.

Flat bottom of Cerkniško polje is regularly flooded for several months in autumn winter and spring time, during floods it alters to spacious karst lake. Lower waters of the main river Stržen on polje are sinking mostly in marginal swallow holes and in numerous grounds swallow holes and estavellas, which are disposed in bottom of the polje. Principal ponor caves and swallow holes are disposed at NW polje's border from where the water flows to Rakov Škocjan through Karlovica cave system.

Rakov Škocjan

Rakov Škocjan is a karst depression about 1.5 km long and 200 m wide. It is situated below the N side of Javorniki Mountain at elevation about 520 m between Planinsko and Cerkniško polje. Through the depression flows the permanent river Rak.

The river flows from Zelške jame cave, about 5 km long cave separated by collapse doline Velika Šujca, from Karlovica cave system, outlet of Cerkniško polje. Downstream the Rak valley widens and several springs bring additional water to the river. The valley is narrowed at the Great Natural Bridge, a remnant of Tkalca jama cave from where the water flows towards Planinska jama cave at Planinsko polje. The connections of the Rak with water from Cerkniško polje and with the Unica springs at Planinsko polje were proved by water tracing.

Planinsko polje

Planinsko polje developed in a levelled karst surface that follows the Idria fault zone. Its wider surrounding is built of Jurassic and Cretaceous limestone and Upper Triassic dolomite, dolomite also form a part of the polje bottom. Polje is of rectangular shape, 6 km long, 2 km wide. There are two narrow pocket valleys on SW part formed above the springs of Unica River and one on its NW side above temporal springs. Polje is about 50 m deeper then lowest surrounding surface and consists of 16 km^2 flat surface at height about 450 m.

Planinsko polje presents the most important water confluence in the river basin of Ljubljanica. The principal Unica spring is situated in the southern polje's part in Cretaceous limestone, where the confluence of waters from Cerknica, Javorniki Mt. and Pivka is located. Main spring is 6656 m long Planinska jama cave.

Planinsko polje is flooded several times in a year. The minimum inflow to the polje amounts to 1, 5 m^3/s; mean 23 m^3/s, maximal was estimated to 100-120 m^3/s, the total ponor capacity being about 60 m^3/s. At floods, lasting 1-2 months, the water increases up to 10 m and up to 40 million of m^3 of water inundate the polje.

The principal Unica swallow-holes are disposed at northern edge, where mostly medium and high waters are sinking. At low waters the whole Unica is disappearing in swallow-holes at eastern polje's border. The water is sinking directly from Unica bed through the polje's bottom across more than 150 swallow-holes and impassable fissure. Only few short ponor caves are known, but there are several horizontal caves in vicinity of the polje, where water oscillations to 20 m can be observed. Larger caves behind the ponors are over Najdena jama cave (5110 m), Logarček (4334 m) and Vetrovna jama (700 m).

Planinska jama

Planinska jama is the best known for the easy accessible confluence of relatively large rivers, the Pivka (arriving from Postojnska jama) and the Rak (arriving from Rakov Škocjan), about half a kilometre from the entrance. The former enters the cave through a sump, which has been explored about half a kilometre long and 60 m deep, without penetrating on the other side. When Cerkniško polje dries out, the eastern (Rak) branch of the cave receives no inflow and the water body begins to flow towards the Malni spring. The inflow/outflow sump has been explored some hundred meters

long, to the very location of bifurcation, which turned out to be extremely dangerous for the divers. Some 30 metres below the water table, the main passage is "crossed" by direct outflow from Javorniki Mountain, draining directly to Malni Springs. The total of all the explored passages in the cave is about 10 km.

Plateau Kras

The Kras is a low, 40 km long and up to 13 km wide, NW–SE trending limestone plateau lying between Trieste Bay, the northernmost part of the Adriatic Sea, Vipava valley in north-east, and Friuli lowlands and river Soča in north-west.

The name itself has a pre-Indo-European origin from word karra, which means rock – stone. The ancient word for "stone" gave the origin to the ancient name for the region (Carusadus, Carsus) and this word changed according to different languages into Kras (Slovene), Karst (German) and Carso (Italian). From this toponym the international term – karst – for such type of landscape is derived. The name and some other terms from the area like dolina, polje, and ponor have entered to international scientific terminology from here.

Climate is sub-Mediterranean with warm dry summers and most of the precipitation in autumn and spring. Cold winters, with NE wind "burja« (bora = borealis) show strong influence of the continent. Average yearly precipitation on Kras varies from 1400 to 1650 mm, and average yearly evapotranspiration from 700 to 750 mm. Because of different land use, pasturing, in past centuries, the Karst was bare, with rocky and grassy surface. Last decades the bushes and trees are overgrow the landscape.

The Kras belongs to Adriatic–Dinaric Carbonate Platform of the External Dinarides composed of shallow marine fossil-bearing Cretaceous and Palaeogene carbonates. Eocene flysch rocks encircle the carbonate plateau. Kras and Matarsko podolje tectonically belong to Komen thrust sheet, which is thrust over Eocene flysch and Palaeocene/Eocene limestone of the Podgorski kras.

The main part of the plateau is essentially levelled, inclined slightly towards the north-west, with numerous dolines, caves and other karst features. About 3490 caves are known on the Kras plateau. In seven of them we can reach 21 km of passages of the underground Reka which flows between 200 and 300 m below the surface. There is a belt of slightly higher relief in the central part of the plateau, formed by conical hills like Grmada (324 m a.s.l.), and dissected by large depressions. The higher relief divides the Kras into two separated levelled surfaces. In the north-western part, the plateau descends to below 50 m a.s.l. on the edge of the Friuli Plain; on its south-eastern edge altitudes are about 500 m a.s.l. There is about 300 m of accessible vadose zone with caves formed at all altitudes from the surface to the sea level and below it.

No superficial streams occur on the Kras surface, because all rainwater immediately infiltrates to carbonate rocks. There are two dry valleys crossing the plateau and some NW–SE-trending belts of lower relief which are result of young tectonics.

The age of the karst of Kras plateau can be defined as the time when the karst rocks were uplifted out of the sea after the Eocene, but there are no remnants of karst features from that time. Most likely denudation, (at present between 20 mMa^{-1} and 50 mMa^{-1}) has already destroyed them.

The oldest features in the karst relief are unroofed caves. On the surface, they are expressed as narrow and often meandering shallow trenches, shallow oblong depressions, and doline-like forms in rows.

The appearance of old unroofed caves and their fills resulted from denudation, erosion and chemical dissolution of limestone above the cavities. Fills exposed on the present surface include speleothems and cave fluvial deposits. The ancient directions of flow, different catchment areas of sinking rivers and different organisation of the ancient underground drainage were reconstructed from several unroofed caves. The thickness of rock overburden removed above cavities was established to have been 50–100 m. The age of cave fills was calculated from denudation rates and the expected thickness of missing overburden to 0.7–5 Ma. This large time range resulted from the expected minimum calculated or measured in the area.

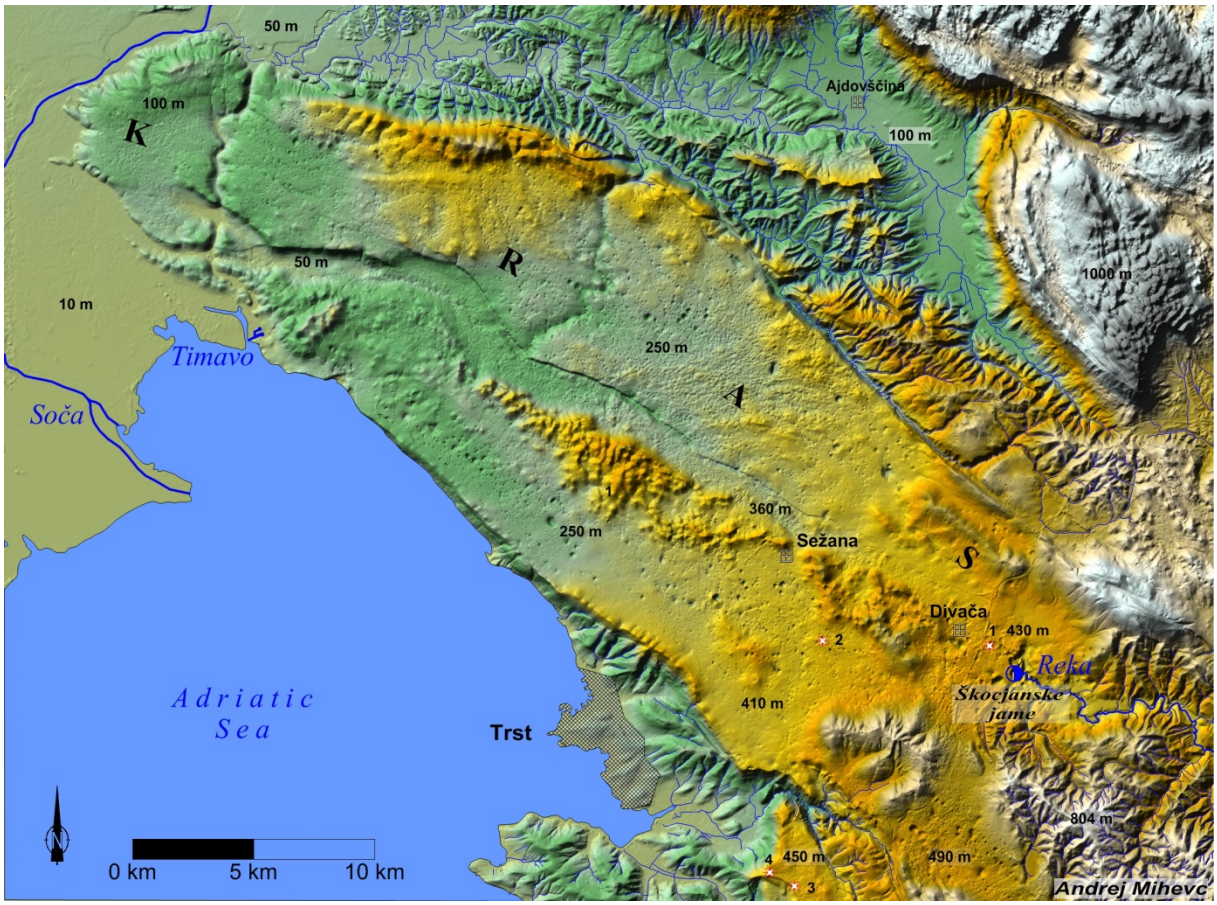


Fig. 2: Plateau Kras. Levelled surface without surface rivers is dissected by numerous dolines; large ones are of collapse origin. River Reka sinks on SE part of the plateau and appear on the coast as river Timavo after more than 30 km of the underground course. Main field trips stops are marked with red star: 1 unroofed cave at Lipove doline, 2 Lipica quarry, 3 Črnotiče quarry, 4 Socerb.

Divaški kras

Karst surface above Škocjanske jame, Divaški kras is a SE part of the Kras plateau between the sinks of Reka River and the village Divača. It is built mostly by Cretaceous and Palaeogene limestone. The surface is levelled in elevations between 420 and 450 m a.s.l, inclined slightly towards NW. The karst features here are exceptional; there are sinks of Reka River, 15 large collapse dolines and hundreds of dolines.

In the Divaški kras there are known 64 caves with the total passages length of 18,500 m. The largest caves of the area are Škocjanske jame, 5800 m long and 250 m deep cave. They were formed by the sinking river Reka that after sinking flows towards Kačna jama, Labodnica and then to springs of Timavo.

The largest collapse doline in the area is the Radvanj double collapse doline (volume 9 million m^3). It is followed by the 122 m Sekelak, the volume of which is 8.5 million m^3 and Lisični dol (6.2 million m^3). Then there are: Globočak (4.6 million m^3), Bukovnik (1.7 million m^3), Risnik (1.5 million m^3) and others. As rooms as big are not known in the Kras, we must assume that collapse dolines this large could develop only with simultaneous rock removal. If this were not the case, the room would fill up with caved-in rocks and only collapse dolines much smaller than the primary cave would appear on the surface (Mihevc 2001).

Kačna jama is the longest cave system of Reka River in the continuation of Škocjanske jame. The entrance lies west from Divača 435 m a.s.l. The total length amounts to 12,500 m. In the lower level the actual underground flow of Reka is met at 195 m respectively.

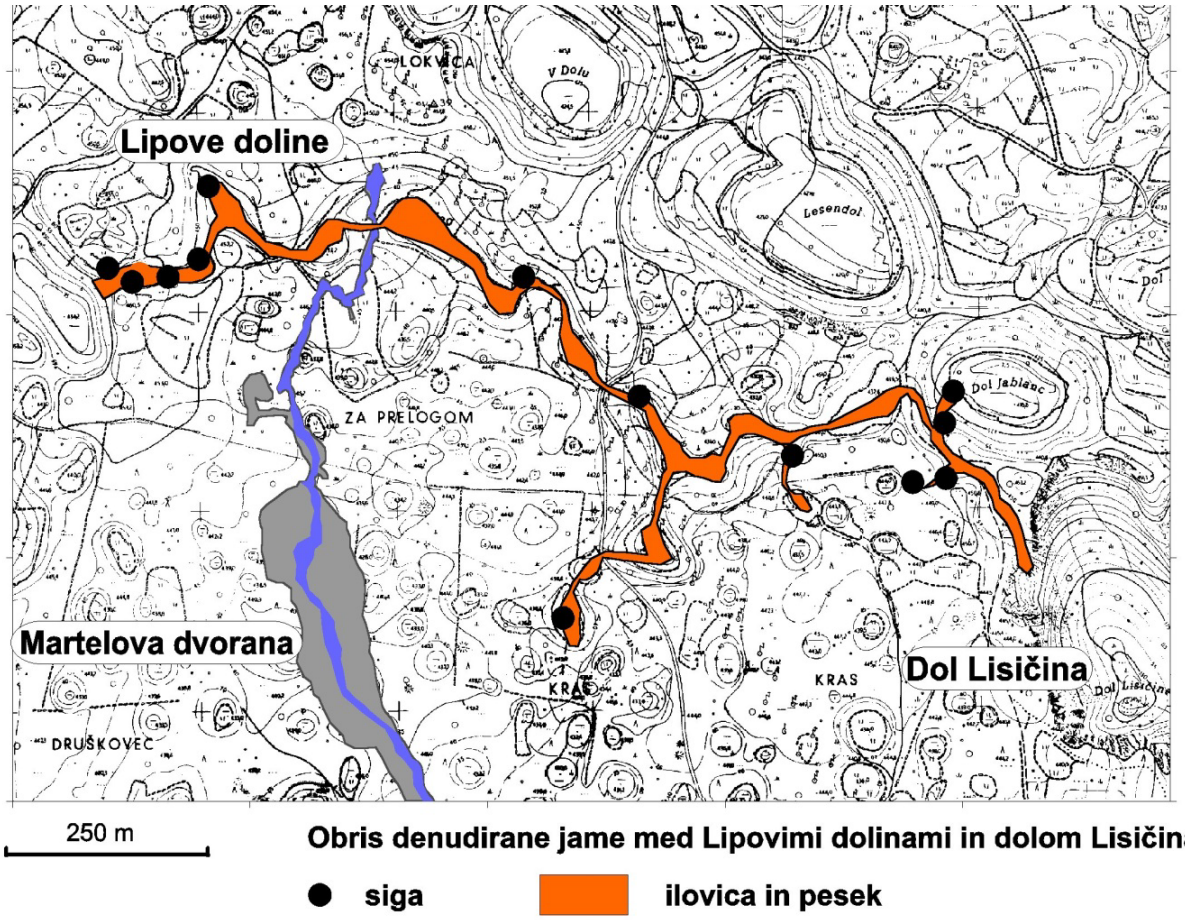


Fig. 3: Unroofed cave Lipove doline (Mihevc, 2001). The surface is between 420 and 440 m a.s.l. Black dots: outcrops of massive flowstone; orange colour: outline of the cave sediments; grey outline: the extreme N part of Škocjanske jame; blue outline: river Reka between 220 m and terminal sump at 190 m.a.s.l.

Unroofed cave at Lipove doline

Unroofed caves are an important part of the surface morphology of Divaški kras where 2,900 m of the unroofed caves was mapped (Mihevc, 2001). They are caves exposed to the surface due to the surface denudation lowering which re-shapes them into the surface relief forms. In such features flowstone, allochtonous sediments and morphology are testifying their cave origin. Several unroofed caves were studied and sediments were analysed; clastic sediments are dated to 1.6 - 1. 8 Ma or/and 3.8 to 5 Ma.

Lipica Quarry

Lipica Quarry is situated in levelled karst surface near stud farm Lipica. Quarry has a long tradition, it started in middle ages, but most of the pits were dug out in past decades.

The stone quarried is mostly thick bedded to massive Upper Cretaceous light grey grainy limestone with micritic matrix.

They use wire to cut the stone, and they also cut stone in galleries. Quarry cuts through many karst voids from small corrosionaly enlarged fissures, vadose channels pits and also some caves.

Podgorski kras and Črnotiče Quarry

The Podgorski kras is about 5 km wide and up to 15 km long karst plateau between Slavnik mountain (1025 m a.s.l.) on the north-east and littoral flysch hills of the Koprska brda on the south-west. The plateau represents the continuation of the Kras towards the south-east, but is separated from it by an important tectonic line with a drop of about 50 m.

Several flysch and limestone thrust slices are elongated in NW–SE, with a dip of about 20–30^{o} towards the north-east. The sub-thrusting is well expressed in the relief as an escarpment at the south-western edge of Podgorski kras, where carbonate rocks are thrust over marl and flysch.

Numerous shallow dolines with flat bottoms in which some sediments are preserved and represent the principal surface karst forms. There are also several unroofed caves, remnants of larger cave systems indicating that the karst is ancient. Old caves both, empty and filled, are dissected by younger vadose invasion shafts in places.

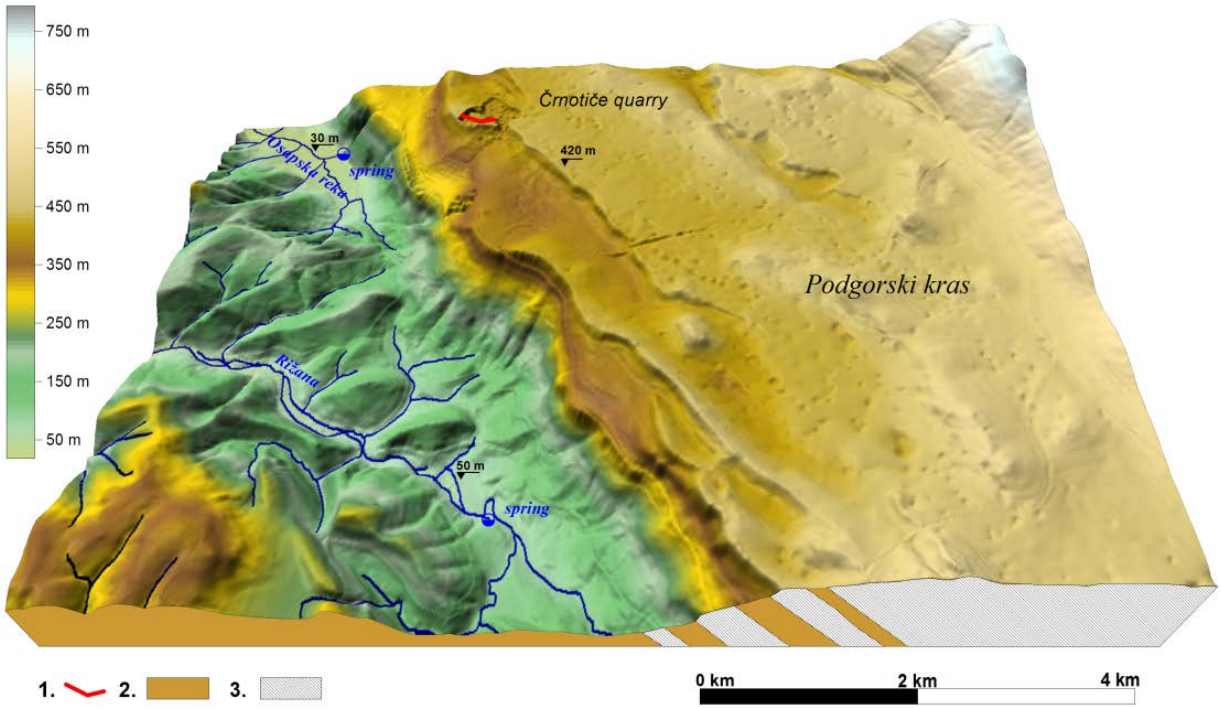


Fig. 4: Podgorski kras and position of Črnotiče Quarry. Red line presents the exposed cave where remnants of *Marifugia cavatica* were found attached to the wall. The animal now lives in caves of the springs of Rižana and Osapska reka which are about 400 m below the cave exposed in the quarry.

The Črnotiče quarry is situated on the western margin of the Podgorski kras, levelled surface at an elevation of 440 m a.s.l. (45°33' 57''N, 13°52' 48''E). A total of 92 caves are known on it, with maximum depths up to 150 m. The plateau is formed on Eocene bedded alveolinid and nummulitid limestone and narrow zones of flysch in imbricated structure with dips of about 20–30° towards the north-east.

Numerous caves have been opened during the Črnotiče Quarry operations. A cave inclined from the north-west to south-east and about 200 m long was opened progressively by quarry operations in 1990–1998. In successive years it was quarried away.

Only remains are left today in the faces on the edge of the quarry. The cave represented a relic of a huge passage with the diameter of about. The passage, about 7 m wide and more than 17 m high was filled by allogenic fluvial cave sediments overlain by several meters thick flowstone and some

collapse material. The flowstones extended up to the present surface where they were exposed by karst denudation as an unroofed cave. The cave was formed by a sinking river flowing from the Eocene flysch.

Calcareous tubes resembling to recent serpulid tubes of *Marifugia cavatica* were found attached to the wall in the lower part of the passage between 426 and 427 m a.s.l. The sessile serpulid tubes were buried when the passage was filled with fine sediment. These were eroded and replaced with new sediments. Teeth of small mammals were found in these new sediments; animals were probably washed in a cave by flood waters. The combination of paleontological and palaeomagnetic data indicates that the fauna and sediment are about 3.6 Ma old.

Marifugia cavatica is the only recent fresh-water species of the Serpulidae family (Annelida: Polychaeta). It is a filter feeder on free-swimming larvae. It has become widely, although not continuously, distributed within the Dinaric Karst. It is supposed recently that it has colonised cave waters from a habitat in fresh-water lakes since the Pliocene. Tubes attached to the cave wall are the oldest remnant of this cave animal and also the oldest known remnant of any cave animal.

References

Gams, I., 2004: Kras v Sloveniji v prostoru in času. Založba ZRC, 516, Ljubljana.

Kranjc, A., 1997: Kras : Slovene classical karst. Ljubljana: Znanstvenoraziskovalni center SAZU, Založba ZRC: Inštitut za raziskovanje krasa ZRC SAZU.

Mihevc A, 2001. Speleogeneza Divaškega krasa, (Zbirka ZRC, 27). Ljubljana: Založba ZRC, ZRC SAZU.

Mihevc, A. 2000: Fosilne cevke iz brezstrope jame – verjetno najstarejši ostanki jamskega cevkarja Marifugia (Annelida: Polychaeta). Acta Carsologica 29/2. Ljubljana.

Mihevc, A., B. Sket, P. Pruner, P. Bosák 2001. Fossil remains of a cave tube worm (Polychaeta: Serpulidae) in an ancient cave in Slovenia. V: *Speleology in the third millenium: sustainable development of karst environments : proceedings*. Campinas: Sociedade Brasileira de Espeleologia.

Zupan Hajna N, Mihevc A, Pruner P, Bosák P, 2008. Palaeomagnetism and Magnetostratigraphy of Karst Sediments in Slovenia. Carsologica 8, Založba ZRC, Ljubljana.

ORAL PRESENTATIONS

Characterization of prokaryotic and eukaryotic diversity in microbial mats from the gouffre de la Sambuy Seythenex, Savoie, France

Didier Cailhol [1], Isabelle Domaizon [2], Émilie Chalmin [2], Fabien Hobléa [3], Yann Tual [4]

[1]- Laboratoire EDYTEM, Centre Interdisciplinaire Scientifique de la Montagne Université de Savoie, Le Bourget du Lac Cedex, France
[2]- UMR CARRTEL Alpine Centre for Research on Lake Ecosystems and Food Webs, Equipe BioFEEL Biodiversity Functioning Evolution of Lacustrine Ecosystems, Thonon-les-Bains Cedex, France
[3]- Laboratoire EDYTEM, Université de Savoie / CNRS Campus Scientifique, Le Bourget du Lac Cedex, France
[4]- Comité départemental de spéléologie de Savoie, Chambéry, France

Gouffre de la Sambuy is an alpine cave, located in the French northern Western Alps, in the Bauges massif. This subalpine massif is included in the "delphinohelvetic" domain of the external Alps, drifted over the foreland (jurassian and molassic domains). Bauges massif presents with these folded structures a medium altitude (< 2 500 m asl) and mountainous relief built with only sedimentary rocks. To the east of the massif, the Sambuy summit is 2198 m asl, the main entrance of the cave (SB9) is open at 1 820 m asl. The average of temperature in this area is 1.6°C. The precipitations are over 2 100 mm/year.

The cave is approximately 900 m development and 120 m depth. The final part of the cave is on the top of the Hauterivian marls.

During speleogenesis studies on 2012, speleologists have founded bacterial mats and gypsum in the marly parts of the cave. Upper, in the middle part, the Barremian limestone wall of a 25 m shaft was covered by unusual fine and white deposits looking like lichen.

To understand these ecosystems, climate aerologic measurements like air temperature, pressure, and wind speed were realized during each visit in winter, spring and summer. Due to the difficulties to access to the cave with no possibilities to get electrical power, no monitoring was realized for the climate parameters.

In spring, the wall deposits have been sampled with swab and store in sterile tube for laboratory molecular analysis. DNA was extracted, PCR amplifications and cloning-sequencing were performed targeting bacterial 16SrRNA and eukaryotic 18S rRNA genes.

For the bacterial community, we identified 29 different OTUs and the richness estimator (Schao) showed a global richness of 65 OTUs. The bacterial community was mainly composed by Proteobacteria, Acidobacteria, Actinobacteria, Nitrospirae and an important part of uncultured bacteria. Some of these bacterial taxa are known to be involved in nitrogen cycling.

For the eukaryotic community, 32 OTUs were identified from our clones library, and the Schao index showed a global richness of 86 OTUs. Rhizaria (with mostly Cercozoa, genus *Cermononas*) was a dominant group, followed by Fungi (mostly Dikaria). Some Alveolata belonging to Ciliophora were also detected, as well as an important part of uncultured fungi and others.

Additional sequencing analyses should be performed to depict in depth the taxonomic composition in these microbial mats. Our preliminary results showed that these microbial mats comprised a rather high diversity of prokaryotic and eukaryotic taxa, which physiological/biochemical characteristics and biotic interactions have still to be described.

Keywords: Alpin cave, bacteria, eukaryotic, DNA extraction.

Bat guano as a driver of microbial metabolic activities in a cave

Alica Chroňáková*[1], Václav Krištůfek [1], Petr Baldrian [2], Janez Mulec [3]

[1]- Institute of Soil Biology, Biology Centre AS CR, České Budějovice, Czech Republic
[2]- Institute of Microbiology, v. v. i., Praha, Czech Republic
[3]- Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia

Nutrient cycling in oligotrophic karst cave is significantly influenced by organic matter inputs from aboveground, which can be extremely high, when cave hosts large roosting bat colonies. Animals produce considerable amount of guano, which at certain conditions stays concentrated in one place as a heap and persists for a long time. Bat guano is rich in chitin, proteins, and mineral nitrogen. In Domica Cave (Slovakia), guano heap remained preserved from the Middle Ages, reaches the height of 1 m. During maturation pH decreased and concentration of heavy metals (HM), particularly Cd, Cr, Cu, Hg, Pb, and Zn, increased. Previous studies showed the presence of non-degraded structures of insect tissues, bat hairs and pollens in the oldest deposit (~1000 y.) as well as surprisingly high microbial colonization and presence of cultivable bacteria and fungi along the vertical transect of the heap. Degradation of chitin into the soluble N-acetyl D-glucosamine is dependent on synergistic action of endo- and exo- chitinases of bacterial or fungal origin. The process is energetically demanding and usually inhibited by low oxygen availability and increased HMs. Here we aimed to evaluate the potential enzymatic activities, bacterial and fungal molecular profiles (454-pyrosequencing and PCR-DGGE, respectively) in five sections of the heap and to compare them with samples of the guano deposits from temperate karst caves from Slovenia: Huda luknja, Predjama, Škocjanske jame, and Turjeva Jama. We hypothesized, that overall microbial activity, mainly bacterial, would be inhibited by increased HMs and acidic pH. Therefore, we established chitin bag experiment to compare enzymatic activities in the bag and the surrounding guano. The results showed that potential enzymatic activities of different heap sections are dominated by phosphatase and chitinases, with 5-fold decrease of chininases in deeper sections. Bacterial and fungal community profiling showed significant differences among studied sections, showing the enrichment of chitin bags by colonizing species. Pyrosequencing revealed that dominant chitin-attracted bacteria were affiliated to *Herminnimonas*, *Rhodanobacter*, *Stenotrophomonas*, and *Tetrathiobacter* (β- and γ-Proteobacteria), while those of guano associated were related to *Dyella*, *Fulvimonas*, *Mycobacterium*, and *Acidobacterium* (γ- Proteobacteria, Actinobacteria, and Acidobacteria). In conclusion, increased HM content and low pH are the most likely factors affecting chitinolytic microbial community and its activity in bat guano in caves.

Keywords: bat guano, cave, nutrient cycling, bacteria, fungi, chitin.

Reticulated filaments found in a subsurface thermal spring in Bad Gastein, Austria.

Marion Dornmayr-Pfaffenhuemer, Helga Stan-Lotter

Molecular Biology, University of Salzburg, Salzburg, Austria

Isolated environments such as caves and other subsurface locations are of interest for geomicrobiological studies. The examination of natural biofilms grown on glass slides using several electron microscopical techniques suggests that the subterranean thermal springs in the Central Alps near Bad Gastein represent a novel and unique habitat for microbial life.

Filamentous microstructures have been found in moist subsurface environments such as limestone caves, basalt lava tubes, granitic tunnels and also in biofilms surrounding the thermal mineral springs of Bad Gastein. Initially termed microcholla, these structures are now called

reticulated filaments. They are tubes of 0.3 to 1µm in diameter. They appear commonly hollow and consist of an open web reminiscent of a fish net or honeycombs. The debate if they are of microbial origin or represent minerals is still not resolved. So far they cannot be associated with any known microorganisms or part of any organism, although recent results suggested biological features.

We describe the experimental retrieval of reticulated filaments from waters of the spring „Franz-Josef-Quelle" in Bad Gastein, using glass slides or pebbles immersed in the water (1). Scanning electron micrographs (SEM) of these microstructures are shown and revealed similarity to previous SEMs from naturally grown biofilms, obtained by the late Dr. Heinen. Results obtained during these studies revealed reproducibility of his micrographs.

Reticulated filaments have been reported from subsurface regions in New Mexico (USA), Cape Verde Islands, Mexico, Italy, Poland, Portugal and Austria. Given this wide range of locations they are probably a significant component of microbial communities in caves, albeit with unknown living habits.

(1) Dornmayr-Pfaffenhuemer, M., Weidler, G.W., Gerbl, F.W., Stan-Lotter, H. (2011) Prokaryotic morphological and phylogenetic diversity in biofilms from an alpine subsurface thermal spring. In: Aquifers: Formation, transport processes and pollution. Laughton, R.H. (Ed.), Nova Science Publishers, Hauppauge N.Y., USA, pp. 387-401.

Keywords: alpine subsurface thermal spring, reticulated filaments, biofilms, SEM.

Biogeochemistry of subsurface microbe/mineral associations

Aaron Alexander Jones, Philip C. Bennett

The University of Texas at Austin, Austin, Texas, USA

In the subsurface microorganisms live in contact with rock/mineral surfaces. Traditionally, those surfaces are assumed to be a homogeneous and only slowly reactive substratum. However, these surfaces can also act as electron sources/sinks, often contain trace elements and valuable nutrients that may be beneficial to microbial growth, and could allow highly localized geochemical conditioning such as pH buffering. I hypothesize that in a complex microbial community, these factors influence surface colonization and microbial diversity, as not every organism will be capable of taking advantage of every surface, giving rise to unique associations between specific microorganisms and specific rocks/minerals. We performed laboratory and field based experiments (within Lower Kane Cave (LKC), WY, USA) to investigate this selective relationship and examined the controls on rates of sulfuric-acid speleogenesis (SAS).

I utilized biofilm reactors containing a variety of rock/mineral surfaces, while using thiosulfate media that were nutrient-limited, non-nutrient limited, or favoring heterotrophic growth (10mM acetate), and inoculated with either a pure culture of sulfur-oxidizing bacteria (SOB) *Thiothrix unzii* or a mixed environmental (LKC) sulfur-metabolizing community. I also designed a flow-through bioreactor to quantify the rate of carbonate corrosion as a function of community membership within this dynamic microbial ecosystem. I continuously measured concentrations of $^{12}CO_2$ and $^{13}CO_2$ to determine if the community was dominated by heterotrophs or autotrophs while monitoring dissolved Ca^{2+} to determine limestone dissolution rate. We found that the dominance of autotrophic SOB in acetate-free media leads to enhanced carbonate corrosion (up to 13X faster) while the dominance of heterotrophic SRB in acetate amended media leads to inhibition of mineral dissolution.

I found that the combination of pH buffering-capacity, cell wall charge, nutrient content of the rock and media, and competitive exclusion of some populations control biomass density, rate of biofilm formation, α-diversity (local diversity), and β-diversity (global diversity) of the attached communities on mineral and rock surfaces. In nutrient limited media, the carbonates had nearly

identical communities that were significantly different from communities on non-carbonates. Also, acidophiles exclusively colonized non-buffering quartz while neutrophilic SOB preferentially colonized highly-buffering carbonate rocks, aluminum tolerant organisms colonized feldspars. Gram-positive bacteria were excluded from carbonates probably due to surface charge, while *Thiothrix* (a mid-ocean ridge organism) shows an affinity for basalt and outcompetes other sulfur oxidizing bacteria in aerobic conditions. In acetate-amended media, heterotrophic sulfur-reducers (SRB) dominate on most surfaces leading to an overall decrease in both α and β-diversity. These results suggest that adaptations to specific rocks are retained even when the organism is displaced in time and space from an ancestral rock habitat.

Keywords: sulfur, sequencing, diversity.

Active and cryptic biodiversity of protozoa in underground habitats

Najla Kajtezović [1,2,3], Renata Matoničkin Kepčija [4]

[1]- Croatian Microscopy Society, Zagreb, Croatia
[2]- SO HPD Željezničar, Zagreb, Croatia
[3]- Croatian Biospeleological Society, Zagreb, Croatia
[4]- Department of Zoology, Faculty of Science, University of Zagreb, Zagreb, Croatia

During the three years research of protozoa in karst underground habitats of Croatia and Bosnia and Herzegovina tens of species were detected. Protozoa, like other groups of microorganisms, show some specific characteristics about their biodiversity. In this research an emphasis was placed on the active biodiversity of protozoa that is composed of active stages of organisms in which they move, feed, and reproduce. Beside active, cryptic biodiversity of protozoa was also researched. It is composed of organisms that are in the stadium of cysts and also of those active protozoa that are so rare in the sample that they were very hard to detect. Cryptic biodiversity was researched using liquid growth media that allowed development of the protozoa that were in stadium of cysts and sufficient multiplication of the rare species. Using this growth method up to 40% of the species was detected.

Karst underground habitats are ideal environments for studying active and cryptic aspect of protozoa biodiversity. Understanding their dynamic is the key element that could help us to clarify the conceptual model that explains penetration of protozoa in the underground habitats, transformation of their communities and potential existence of stabile communities of protozoa that are not under significant impact of the surface communities. It also helps us to understand the presence of pathogenic protozoan taxa and general trophic interrelationships in underground habitats.

During this research in Croatia and Bosnia and Herzegovina the most studied group of protozoa were testate amoebas. It is phylogenetically diverse group of protozoa whose common characteristic is test built of organic or inorganic material. Research results showed large number of species from eleven genera of testate amoebas: *Trinema*, *Euglypha*, *Cyphoderia*, *Tracheleuglypha*, *Cryptodifflugia*, *Centropyxis*, *Cyclopyxis*, *Plagiopyxis*, *Difflugia*, *Microquadrula* and *Arcella*.

Keywords: Protozoa, underground habitats, cryptic biodiversity.

The cave biota of Slovakia - introduction of a new monograph

L'ubomir Kováč [1], Dana Elhottová [2], Andrej Mock [1], Alena Nováková [2], Václav Krištůfek [2], Alica Chroňáková [2], Alena Lukešová [2], Janez Mulec [3], Vladimír Košel [4], Vladimír Papáč [5], Peter L'uptáčik [1], Marcel Uhrin [1], Zuzana Višňovská [6], Igor Hudec [1], L'udovít Gaál [5], Pavel Bella [6]

[1-] Department of Zoology, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University, Košice, Slovakia
[2-] Institute of Soil Biology, Biology Centre AS CR, v. v. i., České Budějovice, Czech Republic
[3-] Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia
[4-] Department of Zoology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia
[5-] State Nature Conservancy of the Slovak Republic, Slovak Cave Administration, Rimavská Sobota, Slovakia
[6-] State Nature Conservancy of the Slovak Republic, Slovak Cave Administration, Liptovský Mikuláš, Slovakia

Slovakia may be considered as a karstologic pearl in the heart of Europe. On the small territory there are numerous karst areas, outstanding cave systems and chasms. More than 6 200 caves and chasms have been documented in the country, many are inhabited by cospicious organisms. The first findings of cave organisms in the Western Carpathians extended to the 18th century, since that time our knowledge in cave biology has increased considerably. Chapters "Cave habitats", "Cave organisms" and "Monitoring of cave biota" (including microorganisms) are the key parts of the book. The publication is devoted especially to naturalists but also to wide public that is interested in natural sciences, nature and caves.

Keywords: cave habitat, cave organisms, conservation, geology, history, invertebrates, microorganisms, monitoring, vertebrates, zoogeography.

Exploring gut microbiota composition of the two caves coleopteran species Neobathyscia pasai and Neobathyscia mancinii (Coleoptera, Leiodidae, Cholevinae)

Leonardo Latella [1], Anna Castioni [2], Laura Bignotto [1], Elisa Salvetti [2], Sandra Torriani [2], Giovanna E. Felis [2]

[1]- Museo Civico di Storia Naturale of Verona, Verona, Italy
[2]- Department of Biotechnology, University of Verona, Verona, Italy

Neobathyscia mancinii (Jeannel, 1924) and *Neobathyscia pasai* (Ruffo, 1950) are two cave-dwelling Coleoptera (Leiodidae, Cholevinae,), which inhabit two caves located in the Prealps of the Veneto Region (Damati Cave and Tana delle Sponde Cave, respectively). In detail, *N. mancinii* is confined in the internal part of Damati Cave characterized by a constant temperature during the year, while *N. pasai* mostly colonizes the cave entrance where the temperature is more variable and the energy sources might be more abundant (Latella et al. 2009; Bernabò et al., 2011).

Little is known about the behaviour and the physiological adaptation of these troglobite insects to cave environments, which are characterized by specific conditions as perpetual darkness, high humidity and sporadic food sources.

It has been observed that the complex microbial community associated to insects plays substantial biological roles for the host organisms and contributes to host fitness and adaptation (Colman et al. 2012; Shi et al. 2013).

The present project aimed at characterizing the gut microbiota of *N. pasai* and *N. mancinii* as a unique opportunity to gain insights about the behaviour of these insects and their eating habits in the cave environment.

A total of 90 insects (60 N. pasai and 30 N. mancinii) were collected from December 2013 to April 2014 both in Damati and Tana delle Sponde caves and they were pooled in samples of ten insects each. Total DNA was extracted from each pool and subjected to PCR amplification using universal primer designed on ribosomal gene sequences. Denaturing gradient gel electrophoresis (DGGE) was applied in order to investigate bacterial and fungal communities present in the gut of these insects. Selected DGGE bands were subjected to sequencing to identify the most relevant members of these complex communities at genus and species level. Results provide novel information on adaptation to cave environment of *N. pasai* and *N. mancinii*. Moreover, insects could be seen as natural reservoir of bacteria, therefore contributing to the microbial community involved in natural processes in the underground.

Bernabò P., Latella L., Jousson O., Lencioni V. Cold stenothermal cave-dwelling beetles do have an HSP70 heat shock response. Journal of Themal Biology. (2011) Vol 36: 206-208.

Colman D. R., Toolson E. C. and Takacs-Vesbach C.D. Do diet and taxonomy influence insect gut bacterial communities? Molecular Ecology (2012) Vol 21: 5124–5137.

Latella L., Bernabo' P., Lencioni V., 2008. Distribution pattern and thermal tolerance in two cave dwelling Leptoditrinae (Coleoptera, Cholevidae). Subterranean Biology, 6: 81-86.

Shi W., Xie S., Chen X., Sun S., Zhou X., Liu L., Gao P., Kyrpides N.C., No E-G., Yuan J.S. Comparative genomic analysis of the endosymbionts of herbivorous insects reveals eco-environmental adaptations: biotechnology applications. PLOS Genetics (2013) Vol 9(1): e1003131.

Keywords: gut microbiota, DGGE, leptodirins, Lessini Mountains, Northern Italy.

Microbial biodiversity of the subsurface, karst and rocky environments – critical comments and future perspectives

Natuschka M. Lee

Lab Microbial Systems Ecology, Department of Microbiology, Technische Universität München TUM, Freising, Germany

Since the early 1990´s a tremendous amount of different types of gene and genome sequences have been retrieved from various environments, but how well have we managed to map the actual biodiversity – in particular of rocky and karst environments and the vast hidden subsurface? Although the improvement of a number of molecular tools has facilitated the screening of the molecular biodiversity, the results that some of them produce are still too superficial to allow the recognition of the actual biodiversity. This presentation will give a contemporary survey of the general biodiversity of all domains on our planet, and compare that with the biodiversity described so far for rocky and karst environments and the subsurface, including the aquifers. Furthermore, an updated survey will be presented on the validity of biomarkers employed so far (e.g. for PCR, sequencing, and FISH) and on some novel technologies for improved mapping of the biodiversity.

Keywords: cave, karst, subsurface, molecular tools, biomarkers, biodiversity.

Dense, shallow air-filled maze passage in coastal Quintana Roo, Mexico: mapping, investigation and resource preservation

Barbara Luke [1], Peter Sprouse [2]

[1]- University of Nevada Las Vegas, Las Vegas, Nevada, USA
[2]- Zara Environmental LLC, Manchaca, Texas, USA

Some of the world's longest mapped cave systems lie beneath the coastal platform of the northeastern Yucatan Peninsula in Quintana Roo, Mexico. Most of the mapped passage is underwater, where exploration and mapping continues today. In recent years, the "dry" zone (no scuba required) has received renewed attention. About 140 km of dry passage has been mapped, all within ~8 meters of the ground surface. Much more is known and remains to be mapped. The passage character is most frequently broad rooms with flat floors, peppered with columns. Calcite decorations are common. There are many entrances and skylights. Roofs can be a meter or less below the ground surface. The caves are developed in Pleistocene limestone, to maximum depths of approximately 100 m. They have a dominant trend northwest-southeast, terminating at the coast, with a secondary set of passages parallel to the coast along old dune structures.

The caves house a rich diversity of terrestrial and aquatic macroinvertebrates as well as mammals. New species are being discovered, including an undescribed troglobitic schizomid. The underwater portions of the caves contain numerous crustacean species. Footprints and scat from jaguar are found in the soft sand near pools, and prey remains show that they retire to caves to eat after hunting. Thick and dense tree roots penetrate the cave passages. Rich evidence of historic use includes extensive systems of low stacked stone walls, carvings in stone, altars, and pottery.

Different groups of volunteer cavers are exploring and mapping dry caves in the area. Preceded by 30 years of underwater mapping, the new dry cave surveys are being tied to the existing underwater ones. Cave mapping software allows maps to be merged as they grow and connect. As survey base lines change with new data, drawn elements such as passage walls and floor detail can move with the survey baseline via "roundtripping". Frequent expeditions are mapping dry caves all along the coast between Tulum and Playa del Carmen. In one project in 2012, 92 survey teams fielded over the course of fifteen days mapped more than 27 km of passage. Such success is enabled by excellent cooperation between local landowners, local Mexican and expat cavers, and visiting cavers from around the world. Prominent caving groups involved in the mapping include the Paamul Grotto of the National Speleological Society and the Circulo Espeleológico del Mayab, both based in Playa del Carmen.

Caves are a tourist draw here in the wildly popular "Riviera Maya." Cave maps are in demand by land owners looking to develop their cave resources for tourism. The drive to welcome visitors can have a beneficial impact on the caves, which might otherwise be filled in or trashed. Land development for resorts is booming, and with it comes strain on infrastructure. Cave passages have been collapsed to support highways; recently, a truck carrying copious cargo wrecked, dumping oil and other waste into a roadside cenote. In the near absence of soil cover over the limestone, waste water is in some cases released untreated back into the shallow karst, to be diluted by the ocean but not without first impacting coastal sea life. The region receives over 1.5 meters of rainfall annually, virtually all of which flows through underwater conduits before reaching the sea. Water flows quickly, perhaps kilometers in a day, giving scant opportunity for filtration or natural attenuation. Drinking water supplies are collected up-gradient from wastewater release points, but the freshwater conduits are directly connected and tidal cycling can transmit contaminants back up-gradient. It is anticipated that mapping of the caves will raise awareness about these important and sensitive resources.

Key word: shallow caves, coastal caves, Quintana Roo, Mexico, cave mapping.

From soil microbial ecology to cave ecosystems – a better insight into microbial community dynamics by using high throughput sequencing techniques

Irena Maček [1,2], Nataša Šibanc [1,2], Thorunn Helgason [3], Alex J. Dumbrell [4]

[1]- Department of Agronomy, Biotechnical Faculty, University v Ljubljana, Ljubljana, Slovenia
[2]- Faculty of Mathematics, Natural Sciences and Information Technologies, University of Primorska, Koper, Slovenia
[3]- Department of Biology, University of York, York, UK
[4]- School of Biological Sciences, University of Essex, Colchester, UK

Fast development of new molecular tools enabled more detailed investigations of indigenous microbial communities in diverse ecosystems. Various molecular approaches, ranging from fingerprinting techniques, clone libraries and Sanger sequencing, to different platforms of high throughput sequencing, can support different depth of description of the present microbial communities. The latter usually correlates also with the number of samples that can be processed for a certain ecosystem, both in spatial and temporal scale. We used a range of different techniques, from t-RFLPs, clone libraries and Sanger sequencing, to amplified marker-based 454 sequencing, to study a complex and diverse community of plant root endosymbiotic arbuscular mycorrhizal fungi in a grassland ecosystem. An overview of our recent research with the focus on studies describing the impact of variable soil abiotic factors on arbuscular mycorrhizal fungal communities will be presented, the results of different approaches used will be compared, and their compatibility discussed. Our data indicate that sufficient spatial and temporal sampling effort in combination with new molecular approaches enable us to rank the relative importance of different regulators of soil microbial communities (e.g. environmental niche, yearly stochasticity and monthly fluctuations) in natural ecosystems. This is a relatively new approach to soil microbial ecology studies, supported by the fast development of the next-generation sequencing platforms that could also be applied to research of different microbial groups in other interesting and complex habitats, including highly underexplored cave ecosystems.

Keywords: microbial communities, community ecology, 454 sequencing, next-generation sequencing, fungi, arbuscular mycorrhizal fungi.

Moonmilk in Postojna cave, Slovenia

Magda Mandić [1], Andrej Mihevc [2], Tea Zubin Ferri [3], Helena Ćetković [4], Bruna Pleše [4], Ines Krajcar Bronić [4]

[1]- Department of Physics, University of Rijeka, Rijeka, Croatia
[2]- Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia
[3]- Research Centre METRIS, Istrian Development Agency (IDA) Ltd., Croatia
[4]- Ruđer Bošković Institute, Zagreb, Croatia

A comprehensive multidisciplinary study of Postojna Cave, the largest known cave in Europe and most visited cave in Slovenia, related to modern speleothem formation conditions that included one year monitoring of environmental conditions in the Cave and stable isotope analyses resulted in finding locations and samples which are promising candidates for paleoclimate investigations (Mandić, 2013). One of such samples is moonmilk from location Zgornji Tartar. Monitored environmental parameters of location microclimate and requirements for carbonate deposition under isotopic equilibrium on this location are fulfilled.

Mean air temperature at location Zgornji Tartar is 10.7 ± 0.9 °C and mean water temperature is 10.9 ± 1.0 °C. Mean values of conductivity, pH, and pCO_2 are 313 ± 123 µS/cm, 7.8 ± 0.3, and 937 ± 325 ppmv, respectively. Mean values of magnesium, calcium and bicarbonate concentrations are 0.8 ± 0.2 mg/L, 76 ± 4 mg/L, and 215 ± 24 mg/L, respectively.

Because this is the first time that moonmilk is described in Postojna Cave, additional investigations helped its better characterization. Petrographicaly, the moonmilk is composed of calcite needles approximately 100 µm long, and 10 µm wide. SEM micrographs of moonmilk show evidence of microbial biomediation or bioprecipitation Also used Fourier transform infrared (FTIR) analysis indicate presence of organic compounds.

Since Postojna Cave is touristic cave the question arose whether the organic compounds found in moonmilk sample are results of native cave microorganisms or if they are allochthonous species. Therefore, bacterial isolate DNA sequences were analyzed using BLAST, which identified sequence homologies with previously identified isolates. Sequences showing greater than 97% identity across the amplified region were considered to belong to the same operational taxonomic unit (OTU). Most of the sequences (68.4 %) were affiliated with the phylum Actinobacteria (species Streptomycetes). Uncultured bacteria from environmental samples were presented with 11.6% followed by Proteobacteria (1%) showing the highest identity with *Klebsiella* sp. thus indicating that microorganisms in moonmilk at the studied location are native.

Mandić M., 2013. Determination of equilibrium conditions of carbonate precipitation in Postojna Cave with possible application to paleoclimatology. Ph.D. Thesis, Zagreb, University of Zagreb, 205 p.

Keywords: moonmilk, DNA sequencing, microbial biomediation, cave microorganisms.

Fibrous microcrystalline calcite in moonmilk: biogenicity, biosignatures and bioconfusion

Andrea Martín-Pérez [1], Adrijan Košir [1], Bojan Otoničar

[1-] Institute of Palaeontology, Research Centre of the Slovenian Academy of Sciences and Arts, Ljubljana, Slovenia
[2-] Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia

Fibrous microcrystalline calcite (FMC) is one of the most common and widespread forms of secondary calcium carbonate in vadose terrestrial environments (caves, soils, surficial sediments, etc.). FMC is characterized by two basic habits: *needle fiber calcite* (NFC) and *nanofibers*, both varieties composed of low-Mg calcite. The origin of FMC crystals has generally been attributed either to purely physicochemical processes or to microbial biomineralization. The current biogenic hypotheses are largely based on common co-occurrence of fibrous calcite and microbial features, and the similarity in dimensions and morphology of calcite fibers and fungal hyphae and filamentous bacteria. Morphological arguments for biogenicity have been supported by geochemical and petrographic data, considered as biosignatures preserved in certain forms of FMC.

There is a considerable evidence that deposits of FMC make specific microbial ecosystems in the vadose zone, i.e., many studies have shown positive association of diverse microbial communities with fibrous calcite, what is particularly evident in soft, hydrated moonmilk. However, the controversy remains regarding the role of microorganisms in carbonate mineralization.

An aspect, mostly ignored in the previous studies, is a possible passive role of organic matter in FMC precipitation. Growth of unusual, fibrous morphologies of calcite in the presence of organic polymers has recently been observed in materials chemistry experiments. Precipitation of calcite in these experiments can be compared to natural processes of organomineralization (= microbially-influenced mineralization) where the organic matrix (mostly microbial exopolymeric substances - EPS) influences crystal morphology and composition - with no living organism and no metabolic processes required.

The purpose of our ongoing project is to test the hypothesis of biogenic precipitation of FMC using *in-situ* and laboratory investigation of calcite moonmilk from actively precipitating sites in four Slovenian karst caves. It is primarily aimed at evaluating the mechanisms of biologically-induced vs. biologically-influenced vs. inorganic precipitation.

Keywords: calcite moonmilk, needle fiber calcite, biogenicity, exopolymeric substances – EPS, microbial origin.

Diverse microniches in Škocjanske jame, Slovenia, offer an immense potential for future research

Janez Mulec [1], Gorazd Kosi [2], Elizabeth Covington [1], Alenka Mauko [3], Julia Walochnik [4]

[1]- Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia
[2]- National Institute of Biology, Ljubljana, Slovenia
[3]- Slovenian National Building and Civil Engineering Institute, Ljubljana, Slovenia
[4]- Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Vienna, Austria

Škocjanske jame as a show cave and a natural phenomenon listed on the UNESCO World Heritage List deserve special attention. The most distinctive underground attribute for tourists is a canyon along the Reka River, but from a microbiological point of view, the cave system offers very diverse microbial habitats. In Schmidlova dvorana, which is occasionally fully illuminated by sunlight, are developed stromatolitic stalagmites and tufaceous stalactites, speleothems linked to the

biolithogenic activity of cyanobacteria. In a complex microbial community of a stromatolitic stalagmite, a new vahlkampfiid amoeba, *Allovahlkampfia spelaea*, was identified in 2009. Škocjanske jame hosts 12 different species of bats, making this an important site for preservation of these endangered mammals. In a guano heap with recent bat droppings we retrieved a DNA sequence which was closely related to several unidentified *Geomyces* clones and to pathogenic *G*. *destructans*, a causative agent of White-nose syndrome (WNS) at bats. However, to date no reports exist on WNS at bats in Škocjanske jame. Tourist use and illumination brings light eutrophication in the underground. Tourist part of the cave was equipped with electric lighting in 1959 and since then only some parts have been treated for lampenflora removal, which makes this an almost ideal site to study succession in a phototrophic/lampenflora community. For example, the percentage of Cyanobacteria in the lampenflora community increases with time. Tourists bring into the cave system a lot of cave allochtonous material, including microorganisms. Elevated concentration of cave airborne bacteria in the area of Tiha jama named Šotor was clearly related to the presence of tourists and persisted for a few hours even after tourists had left the sampling area. In a period when this part of the cave was closed for tourist visit, the concentration of total culturable bacteria was very low, 0-6 CFU/m^3, which can be attributed a natural background of airborne bacteria. In huge underground voids such as those in Škocjanske jame, one has to consider also external weather conditions which affect the dynamics and concentration of airborne microbiota. In recent years, water quality of the Reka River at entry into the cave system improved. Physical and chemical parameters of the Reka River are generally comparable with other surface rivers, but further actions are needed to reduce a relative high input of organic C and accompanied high concentrations of bacteria, especially *Escherichia coli* - an indicator of fresh faecal contamination.

Keywords: biogenic speleothems, white-nose syndrome, lampenflora, cave management.

Microbiological indicators for monitoring tourist use of Postojnska jama, Slovenia

Janez Mulec [1], Alenka Koželj [2], Erika Batagelj [2], Janja Vaupotič [3], Julia Walochnik [4]

[1]- Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia
[2]- Center za kemijske analize živil vod in drugih vzorcev okolja, Nacionalni laboratorij za zdravje, okolje in hrano, Koper, Slovenia
[3]- Radon Center, Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia
[4]- Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Vienna, Austria

The tourist part of Postojnska jama has a long record of mass visitation (1819) which leaves its imprint on the cave ecosystem. The tourist visit typically consists of a 1.6 km of train transport from the cave entrance to Velika gora where tourists continue walking 1.8 km on a footpath to the train station in Kongresna dvorana where they take a return train ride to the cave exit. Train motion causes movement of air masses which disperse a lot of microbes, especially fungi, >700 CFU/m^3. During visits, tourists bring and disperse high a quantity of microorganisms, being responsible for the high concentration of culturable airborne bacteria deep underground (51-137 CFU/m^3, in the period 2009-2010). In 2011 a disinfection barrier was introduced at the cave entrance for the tourists to reduce the introduction of organic material on tourists' soles. Postojnska jama was the third cave in the world equipped with electric lighting (1884) that is why lampenflora - proliferation of mainly phototrophic organisms near artificial light sources - early become an urgent issue for the cave management to fix. Manual removal of moss thalli and an application of 15 and 20% v/v solution of hydrogen peroxide for microscopic phototrophs have been used in Postojnska jama since 2011 to eliminate and restrict lampenflora. A growth experiment with *Chlorella vulgaris*, a lampenflora

representative, was carried out in the cave where different LED lamps were used as a light source, emitting light resembling natural light (cool- and warm white LEDs). The results revealed that the selection of emission spectra is less important in the long-term (> 6 months) from the perspective of lampenflora growth, and more attention should be paid to the lighting regime and preventing re-colonization of lampenflora. The cave environment of Postojna Cave is largely affected by the quality of the Pivka River within it. Levels of physical and chemical parameters of the Pivka River at the ponor are generally comparable with other surface rivers, but relative high values of organic C accompanied with high concentrations of bacteria (specifically coliforms and *Escherichia coli*) affects the cave ecosystem downstream and air quality in the cave. Elimination and/or proper management of sources of organic pollution are necessary steps towards a natural state of the river underground.

Keywords: cave management, lampenflora, aerobiology, water quality.

The role of environmental factors on the cave biodiversity in Dinaric karst: an example of Vjetrenica cave

Dalibor Paar [1,2,3], Darko Bakšić [4], Nenad Buzjak [5,6], Ivo Lučić [7,8]

[1]- Department of Physics, Faculty of Science, University of Zagreb, Zagreb, Croatia
[2]- Speleological Society Velebit & Zagreb Speleological Union, Zagreb, Croatia
[3]- Speleological Committee of the Croatian Mountaineering Association, Zagreb, Croatia
[4]- Faculty of Forestry, University of Zagreb, Zagreb, Croatia
[5]- Department of Geography, Faculty of Science, University of Zagreb, Zagreb, Croatia
[6]- Caving club Samobor, Samobor, Croatia
[7]- Speleološka udruga Vjetrenica – Popovo polje, Ravno, Bosnia and Herzegovina
[8]- Croatian Biospeleological Society, Zagreb, Croatia

The Vjetrenica cave is one of the most important caves of Dinaric karst with very high cave biodiversity, from diverse and specialized cave fauna to sprout-like microbial aggregates (Kostanjšek et al, 2013; Ozimec&Lučić, 2009; Lučić, 2003).

Why Dinaric karst comparing to other European regions has so high cave biodiversity? One possible explanation is that the in South-eastern Europe lower glaciation level in combination with much moderate climate, diversified geomorphology, and hydrology during the Pleistocene have resulted in a remarkable range of different underground habitats. If this is true, the important question about Vjetrenica cave remains: why this cave has so extraordinary biodiversity comparing to thousands of other caves of Dinaric karst with similar geological, hydrological and outside climate settings? The aim of this study is to argue that special microclimate and hydrological conditions define environment that slightly, but essential for biodiversity, differ from the most of the caves in this region.

Vjetrenica cave has specific climate and hydrological conditions that vary trough the cave, but also have time component connected to hydrological regime, The interest in climatic features of Vjetrenica cave dates from 77 BC (Historia naturalis by Pliny the Elder) and lasts until today, with special focus to strong wind at the entrance of the cave. Systematic and quantitative measurements of cave climate in entrance and deeper parts started ten years ago with new speleological exploration and making a new cave map. Strong wind at the entrance (up to 9.8 m/s in summer) is one of the key signals that suggest strong exchange of energy and materials with surface environment. Average annual air temperature is between 11.0 and 11.35 °C, depending on location in the cave. The highest temperature corresponds to Donja Vjetrenica passage (300 m from the entrance, -23 m deep) that has separate air temperature regime. Air temperature amplitude drops with distance from the entrance, but not so fast; it is 3.45 °C at 520 m and 1.59 °C at Veliko jezero (1275 m). In Donja Vjetrenica it is only 0.87 °C. One of the factors that have big influence to climate

conditions is periodical siphon at 1007 m. The closing of the siphon with water during wet season stops strong air circulation along the passage and at the entrance.

Unlike the many caves in Dinaric karst, these conditions are not so stable, even over 1 km away from the entrance. These oscillations may be small but important switches that affect biodiversity.

Bakšić D., Paar D., Buzjak N., Lučić I. (2011): Mikroklimatska opažanja u Vjetrenici. Međunarodni znanstveno-stručni skup "Čovjek i krš". Knjiga sažetaka. Lučić I., Mulaomerović, J. (eds.). Sarajevo-Međugorje, 13-14.

Kostanjšek R., Pašić L., Daims H., Sket B. (2013): Structure and community composition of sprout-like bacterial aggregates in a Dinaric Karst subterranean stream. Microb Ecol. Jul 66 (1):5-18.

Lučić I. (2003): Vjetrenica, insight into the Earth's soul. ArTresor, Zagreb

Lučić I., Bakšić D., Mulaomerović J., Ozimec R. (2005): Recent research into Vjetrenica cave (Bosnia-Herzegovina) and the current view of the cave regarding its candidature for the World Heritage List. Proceedings of the 14th International Congress of Speleology. Petreas C. (ed.). Athens: Hellenic Speleological Society, 2005, 144-147.

Ozimec R., Lučić I. (2009): The Vjetrenica cave (Bosnia and Herzegovina)—one of the world's most prominent biodiversity hotspots for cave-dwelling fauna. Subterranean Biology 7:17–23

Keywords: cave climate, hydrology, cave fauna, microbial aggregates, biodiversity, Vjetrenica, Dinaric karst.

High biodissolution rates in saturated karst freshwaters of northern Dinaric (Classical) Karst

Mitja Prelovšek, Janez Mulec

Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia

Biofilm develops on practically all rock surfaces (epilithic), and can penetrate several mm deep into the rock (endolithic). Its biogeochemical role and influence on weathering and denudation is a result of attaching, nutrition uptake from the substratum, respiration and/or metabolism (acid exudation).

Although biodissolution is widely observed, measurements of biodissolution rates in surface karst waters are almost completely absent. To define biodissolution rates, 56 pre-weighted limestone tablets were exposed to 7 different light conditions for one year at Malenščica and Unica spring (in front of Planina Cave entrance). After exposure, limestone tablets were dried and weighted again. Weighing after ignition enabled calculation of biomass dry weigh and overall dissolution rates.

Diatoms were the predominant group of phototrophs identified on the tablets. Contribution of microbial biofilms in nutritient-poorer water to overall dissolution in underground of Unica River seemed to be of minor importance and since chemical dissolution was almost absent due to the saturated waters, overall denudation rate was extremely small (about -1 µm/a). Much higher dissolution rates were recorded few tens of meters downstream after the resurgence in the illuminated riverbed (up to -143 µm/a), what is attributed to the biological activity. Similar impact of biofilm was reported also from other lighted terrestrial aquatic systems with high total hardness of water in northern Dinaric karst. Results showed that complex biofilms, especially phototrophs are important biogeomorphological agents for landform evolution in freshwater streams of temperate karst areas. The study showed weak correlation with physical and geochemical parameters of waters that are usually taken into account to calculate dissolution rates and landform evolution.

Key word: biodissolution rates, biological dissolution, limestone, role of microorganisms, Classical Karst.

Microbial activities at environmental gradients in cave and karst ecosystems

Annette Summers Engel

Department of Earth and Planetary Sciences, University of Tennessee–Knoxville, Knoxville, Tennessee, USA

Microorganisms colonize environmental gradients at air-water, water-rock, and air-rock interfaces in cave and karst ecosystems. Colonization of, and active microbial metabolism at, these interfaces control geochemical processes, such as mineral equilibria, organic matter transformations, and metal and contaminant speciation and transport. Current technological advances that utilize isotopic and genomic approaches allow for the study of microbial taxonomic and functional diversity at key environmental gradients in karst systems. To understand how interface and gradient processes regulate metabolic function, more experimental and analytical studies are needed. This abstract summarizes what is currently known from each interface and that is attributed to distinct types of aquatic and terrestrial cave ecosystems, including what potential processes contribute to the geochemical and ecological evolution of cave and karst ecosystems. Based on particle density and cell hydrophobicity, microbes colonizing air-water interfaces overcome surface tension and can develop into biofilms and floating microbial mats, which are of interest from chemolithoautotrophic cave ecosystems. The biofilms can serve as food for higher trophic levels, but the microlayers also affect aerosol formation and the exchange of organic and inorganic gases. In particular, oxygen consumption by microbes in biofilms decreases oxygen diffusion into the water column, which can lead to anaerobic conditions. In turn, anaerobic production of hydrogen sulfide and methane gases in the lower water column can be oxidized by microbes at the air-water interface. Cycling of these gases, along with other nutrients, affect the trophic structure of the foodweb in these aquifer habitats. But, regulation of gases by microbes at the air-water interface impacts potential reactions between gases and the cave walls at the air-rock interface in air-filled passages. Visibly different pigmented colonies, subaerial cave-wall corrosion, sediment formation, and deterioration of cave-wall surfaces are evident at the air-rock interface, whereby microbial colonization is influenced by gas exchange, water availability, and air circulation. The nutrient content of the underlying rock, mineral solubility, and surface charge also affects colonization, and microbial cell hydrophobicity and charge can lead to extensive air-rock interface biofilms. Terrestrial cave fauna can be dependent on these biofilms for food, particularly near moist, hydrated surfaces that result from epikarstic input of nutrients and organic matter. Microbial colonization of water-rock interfaces in aquatic habitats is also influenced by the nutrient content of the rock, as well as mineral solubility. But, biofilm development in subaqueous environments depends on how dynamic the hydrological conditions are over time. Solute transport and feedback processes associate with fluid geochemistry, ion exchange, and metabolic preferences of colonizing microbes also impact processes at the water-rock interface. At both the air-rock and water-rock interfaces, pH and ion concentration gradients may form between the biofilms and rock surface. The biofilms become hydrophobic barriers to solute and gas exchange between the underlying rock and the surrounding bulk fluids, which may lead to chemical disequilibrium and the dissolution of certain mineral phases and the precipitation of others. Subaqueous biofilms in deep aquifers can be important food sources for higher trophic levels, and many of the studied biofilms consist of chemolithoautotrophic microbial groups that are also important for ecosystem energy as primary producers.

Keywords: ecosystem,geochemistry, gradient, equilibria, genomics, function, diversity.

Biogenic helictites in Asperge Cave, France

Nicola Tisato [1], Stefano Torriani [2], Sylvain Monteux [3], Francesco Sauro [4], Jo De Waele [4], Maria Luisa Tavagna [5], Ilenia M. D'Angeli [4], Daniel Chailloux [6], Michel Renda [6], Timothy I. Eglinton [5], Tomaso R. R. Bontognali [5]

[1]- ETH Zurich, ERDW, Zurich, Switzerland; now at: University of Toronto, Toronto, Canada
[2]- ETH Zurich, Institute of Integrative Biology, Zurich, Switzerland; now at: Syngenta Crop Protection, Münchwilen AG, Werk Stein, Stein AG, Switzerland
[3]- Université Montpellier 2, Montpellier Cedex, France; now at Climate Impacts Research Centre, Universitet Umeå, Abisko, Sweden
[4]- Department of Biological, Geological and Environmental Sciences, Bologna University / Italian Institute of Speleology, Bologna, Italy
[5]- ETH Zurich, ERDW, Zurich, Switzerland
[6]- FFS –Fédération Française de Spéléologie, Lyon, France

Asperge Cave is 7 km long, 126 m deep and is located in the region of the "Montagne Noire" - Hérault (Fr). The cave opens in Cambrian terrains at the contact between marbles and phyllites. Certain zones of the cave present bouquets of acicular-, coralloid- and bulb- shaped helictites. Thanks to their spectacular morphology and colors, Asperge has been proposed as UNESCO World-Nature-Heritage. Speleothems with shapes as those found in Asperge are rare but not unique and have been described elsewhere in the world. In fact, some have been suggested to be the result of biological processes.

Here we present the results of a series of field observations, mineralogical-chemical and microbiological analyses of some of these unusual Asperge concretions.

The speleothems are composed of large tubular branches, growing in an upward curved way, and they locally merge in a non-coincidental manner. These features are difficult to explain by purely abiotic processes, and invoke biological mediation. Our investigations show that a biofilm is, indeed, an integral component of the speleothems. We infer that its presence actively determines the macroscopic morphology of the mineral concretion. Our results may lead to a revised, partly biogenic interpretation of similar speleothems present in other caves worldwide.

Keywords: excentric speleothems, biogenic speleothems, geomorphology, microbiology, terrestrial carbonates.

Bacteria, bonds between classical karsts and karsts in silicated and non carbonated rocks?

Luc Willems

HECh & University of Liège, Amay, Belgium

For several years, similar karsts to those found in the limestone are observed in various silicated and non-carbonated rocks. Several morphological arguments show that a complete dissolution of rocks should be considered to explain the formation of some caves and other related forms independently of hydrotermalisme phenomena. However, the physico-chemical processes generally considered not provide a satisfactory explanation to this dissolution. Bacterial activity found deep in many lithologies provides a solution to this apparent contradiction.

Keywords: Classical karsts, karsts in silicated and non carbonated rocks, bacteria.

Secondary mineral deposits and associated microbe-mineral interactions in subsurface rock environments

Ana Zélia Miller [1], Amélia Dionísio [2], Cesareo Saiz-Jimenez [1]

[1]- Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Sevilla, Spain
[2]- CEPGIST/CERENA, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

Subsurface rock environments are one of the least explored habitats of microbial life. These environments contain a wide variety of redox interfaces and stable physicochemical conditions which enhance microbial growth and secondary minerals precipitation. In particular, caves host an especially interesting variety of mineral-utilizing microorganisms, which may contribute to the formation of secondary mineral deposits, such as moonmilk, pool fingers, coralloids and ferromanganese deposits. These secondary minerals were usually considered to be abiotic in composition and genesis, but researchers have demonstrated they are indeed biologic in origin arising from important geomicrobiological interactions. Such interactions have been evidenced by the presence of microorganisms and microbial mats concealed within mineral deposits. Electron microscopy combined with ancillary chemical analyses have been essential tools to assess microbe-mineral interactions, to evaluate biogenicity, as well as to describe unusual mineral formations and microbial features. Despite numerous studies performed to determine biogenicity and biomineralization mechanisms predominantly of calcium-containing minerals, the formation mechanisms of biominerals containing other cations are poorly investigated. Moreover, while there are several reports on biominerals from surface and aqueous environments, examples from subsurface rock environments, especially caves, are comparatively few. Hence, the interest in the geomicrobiology of caves is derived from (i) the evaluation of biogenicity of secondary mineral deposits, (ii) the acquaintance of subsurface microbial diversity, (iii) the finding of new species of bacteria, (iv) the role of microorganisms in biogeochemical and biomineralization processes, and (v) the recognition of biosignatures, as fossil bacteria, microbial fabrics and unusual biominerals, used in the understanding of life on early Earth and on other planets. In light of the outstanding examples of ongoing geological, ecological and biological processes, the geomicrobiology of subsurface environments has an added-value related to the conservation of caves that house exceptional testimonies of human history. Here, the findings on the association of microorganisms with secondary mineral deposits from a number of case studies conducted by our research group are presented.

A.Z. Miller acknowledges the Marie Curie Intra-European Fellowship (PIEF-GA-2012-328689- DECAVE) within the 7th European Community Framework Programme.

Keywords: caves, secondary mineral deposits, geomicrobiology, biogenicity, biospeleothems, biomineralization.

POSTER PRESENTATIONS

Screening the cultivable cave silver microbiome for the production of antimicrobial compounds

Jerneja Avguštin, Patricija Petrič, Rok Tkavc, Blagajana Herzog Velikonja, Lejla Pašić

Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

The development and/or discovery of novel and effective antimicrobials has become a necessity in the age of increasing antimicrobial resistance. One way to approach this issue is to screen culture collections originating from different environments. Accordingly, we used various sample pretreatments and culture media in order to isolate indigenous bacterial microbiota that forms 'cave silver' in Pajsarjeva jama, Slovenia. This way, we recovered 80 unique isolates and affiliated them with *Streptomyces* (25%), *Micrococcus* (16%), *Rhodococcus* (10%), *Pseudomonas* (9%), *Agrobacterium* (8%), *Lysobacter* (6%), and *Paenibacillus* (5%), while *Microbacterium*, *Agrococcus*, *Arthrobacter*, *Bacillus*, *Kocuria*, *Oerskovia*, *Sphingomonas*, *Aerococcus*, and *Bosea* represented minor portion of cultivable diversity.

In our preliminary screens we tested the cultivable cave silver microbiome for the production of antimicrobial compounds by growing it on five different media and overlaying it with a number of laboratory and clinically important strains. Out of 78 isolates tested, 15.3% showed activity against *Escherichia coli* DH5, 15.3% against extended-spectrum β-lactamase producing *Escherichia coli*, 3.8% against *Salmonella enterica* serovar Typhimurium TL747, 9% against *Klebsiella pneumoniae* ATCC BAA-1706, 9% against carbapenem-resistant *Klebsiella pneumoniae* ATCC BAA-1705, 7.7% against *Bacillus cereus*, 20.5% against *Bacillus subtilis*, 9% against *Listeria monocytogenes*, 19.2% against methicilin-resistant *Staphylococcus aureus,* and 20.5% against methicilin-resistant *Staphylococcus pseudointermedius*. These results indicate that caves are a useful source of microorganisms that produce antimicrobial agents.

Keywords: caves, actinomycetes, antimicrobial activity, multidrug resistant bacteria.

Cave in Panorc

Fadil Bajraktari, Sami Behrami

Kosovo Institute for Nature Protection, PristinaKosovo

The cave is located about 3 km, south of the Panorc village in northern hillside of Okovan Mountain (977 m). Cave's entrance has northern exposition at altitude of about 581 m. It is usually known by the name of the Panorc location, but also it is known by other names as "Shadow Cave" and "Breezy Cave".

Near cave's entrance are two water sources which are used for drinking purpose and most likely these sources have hydrological relation with cave's water flows. The cave is developed in rudist limestone of upper cretasic featured with lithologic and tectonic elements.

So far, this is the largest and the most interesting cave of Mirusha River basin. Cave's entrance has small dimensions, on the transom about 1 m, width and 0.5 m height. The overall length of explored corridors and galleries is about 925 m. Cave Panorc has also scientific, geomorphological, educational, biological, hydric and tourist values.

Keywords: Ponorc, Cave, Mirusha.

Proliferation of green algae in the Moidons Cave: factors driving colonization and use of UV-C treatments to control their growth

Fabien Borderie [1], Nicolas Tête [1], Didier Cailhol [2], Laurence Alaoui-Sehmer [1], Faisl Bousta [3], Dominique Rieffel [1], Lotfi Aleya [1], Badr Alaoui-Sossé [1]

[1]- Laboratoire Chrono-Environnement, UMR CNRS 6249, Université de Franche-Comté, Besançon, France

[2]- Laboratoire Environnement, Dynamique et Territoires de Montagne, UMR CNRS 5204, Université de Savoie, Le Bourget du Lac Cedex, France
[3]- Laboratoire de Recherche des Monuments Historiques, USR 3224, Champs-sur-Marne, France

The proliferation of epilithic algae that form biofilms in subterranean environments, such as show caves, is a major problem for conservators. In an effort to reduce the use of chemical cleansers when addressing this problem, we proposed investigating the effects of UV-C on combating algal biofilm expansion in a cave located in northeastern France (Moidons Cave). First, the biofilms and cavity were studied in terms of their algal growth-influencing factors to understand the dynamics of colonization in these very harsh environments. Next, colorimetric measurements were used both to diagnose the initial colonization state and monitor the UV-C-treated biofilms for several months after irradiation. The results indicated that passive dispersal vectors of the viable spores and cells were the primary factors involved in the cave's algae repartition. The illumination time during visits appeared to be responsible for greater colonization in some parts of the cave. We also showed that colorimetric measurements could be used for the detection of both thin and thick biofilms, regardless of the type of colonized surface. Finally, our results showed that UV-C treatment led to bleaching of the treated biofilm due to chlorophyll degradation even one year after UV-C treatment. However, a re-colonization phenomenon was colorimetrically and visually detected 16 months later, suggesting that the colonization dynamics had not been fully halted.

Keywords: epilithic algae, biofilms, cave, growth-influencing factors, colorimetric measurements, UV-C treatment.

Fossil attemsiid millipedes from the Hermannshöhle, Austria: Polyphematia moniliformis (Latzel, 1884)?

Pavel Bosák [1], Andrej Mock [2], Lukas Plan [3], Petr Pruner [1], Andrea Schober [3]

[1]- Institute of Geology ASCR, v. v. i., Praha, Czech Republic; Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia
[2]- Department of Zoology, Institute of Biological and Ecological Sciences, Faculty of Science, University of Pavel Josef Šafařík, Košice, Slovakia
[3]- Karst and Cave Department, Museum of Natural History, Vienna, Austria

The Hermannshöhle (Kirchberg am Wechsel, Lower Austria), is developed in an isolated block of weakly metamorphic Middle Triassic banded calcite marble. Within only 140 × 160 m ground area and 73 m of elevation difference a total of nearly 5 km of corridors formed in a 3-D maze together with nearby Mäanderhöhle, Antonshöhle and Rauchspalten caves (map; Schober et al. 2013).

The fragment of white ovate cuticle, partly decomposed in situ to white powder, was found in profile of sediments filling highly corroded fissure up to narrow passage known as the Teichkluft. Cave walls were weathered up to sandy residuum, in places. Profile consisted from several parts

separated by disturbed sections by digging, built by complicated sequence of brown and violet clays with yellow micaceous clayey sands. Abundant Mn-rich schlieren pinched out from cave walls into the sediment. Small erosion surfaces were often stained/coated by Fe-Mn compounds. Erosion channels filled with cubes of brown clays with yellowish-sandy matrix occur in the upper part of the profile. The profile was covered by flowstone crust dated to 162.7 ± 3.9 ka.

Specimen was found in a crack in brown clays, close to cave wall. Unfortunately, the rest decomposed completely during the microscopic analysis. The fragments of cuticle of ring-like shape clearly are parts of trunks (segments) of millipedes (Diplopoda). Organic parts of cuticle were lost or were substituted by anorganic material. Microstructure of prosomites and metasomites were also lost (like grooves, fine nodes, setae etc.). Sole bigger fragment of body shows noticeable nodes on metasomites. The shape of metasomite with node indicates it could be some representant of the millipede order Chordeumatida. In this locality, there is well-known occurrence of recent species from this order (Diplopoda: Chordeumatida: Attemsiidae), *Polyphematia moniliformis* (Latzel, 1884), published by several authors, reviewed by Strouhal and Vornatscher (1975, pp. 472–475). Character of external morphology (size, shape) of this species (as same as other attemsiids) is similar to controlled fragments (other species recently dwelling this cave, *Haasea flavescens*, has somites without clear nodes). The probability that it is the same species is high.

The ecology of the attemsiid millipedes is specific: they are cave dwellers and they occupy also surface and subsurface habitats closely connected with underground spaces with stable microclimate. Some genera and species: (1) are distributed mainly in Alps, especially in the east part (Austria, Slovenia); (2) most of them are endemitic with small occurrence areas; (3) they survived in situ probably for a long period (e. g., 2–3 species distributed in isolated areas of the Western Carpathians are expected as Tertiary relicts, Mock and Tajovský 2008); (4) they utilize underground as refuges during periods with unfavorable climate, and (5) they are only partly adapted to underground (juvenile stages are almost without pigment) with possibility to live also on/at surface.

Fragile fragments of millipedes are found rarely in fossil records (Donovan and Veltkamp 1994; Duncan et al. 1998; Shear and Edgecombe 2010). Tubes of attemsiid millipedes encrusted in speleothem were recently found also at Ölberg site in the cave.

The age of profile was studied by the U-series dating (Schober et al. 2013) and by the high-resolution paleomagnetic analysis. The standard paleomagnetic analysis was adopted (e.g., Zupan Hajna et al. 2008) in the Department of Paleomagnetism, IG ASCR, v. v. i. in Praha–Průhonice. Profile showed prevailing normal polarized magnetization and two very short reverse polarity excursions at 0,41 and 2.19–2.26 m (graph). The paleomagnetic directions (D, I) are very close to the present magnetic field. We assume deposition of sediments within the Brunhes chron (<780 ka) and the excursion may be correlated with Jamaica-Pringle Falls (205–215 ka) and/or Calabrian Ridge 1 (315–325 ka; Langereis et al. 1997) excursions of the magnetic field owing to the age of cover flowstone (162.7 ± 3.9 ka).

Acknowledgement. We are grateful to Hermannshöhle – Kirchberg am Wechsel for permission of field work and arrangement of lodging. We acknowledge the help of doc. RNDr. Ľubomír Kováč, PhD (University of Pavel Josef Šafárik, Košice, Slovakia). The paleomagnetic analysis (Kristýna Čížková, Jiří Petráček), evaluation and preparation of the report were carried out within the Plan of the Institutional Financing of the Institute of Geology ASCR, v. v. i. No. RVO67985831.

Donovan S.K., Veltkamp C.J. (1994): Unusual preservation of late Quaternary millipedes from Jamaica. – Lethaia, 27: 355–362.

Duncan I.J., Briggs D.E.G., Archer M. (1998): Three-dimensionally mineralized insects and millipedes from the Tertiary of Riversleigh, Queensland, Australia. – Paleontology, 41: 835–851.

Langereis C.G., Dekkers M.J., Lange G.J. de, Paterne M., Santvoort P.J.M. van (1997): Magnetostratigraphy and astronomical calibration of the last 1.1 Myr from an eastern Mediterranean piston core and dating of short events in the Brunhes. – Geophysical Journal International, 129: 75–94.

Mock A., Tajovský K. (2008): Mecogonopodium carpathicum n. sp. (Diplopoda: Chordeumatida: Attemsiidae), a new troglophilic millipede from Slovakia. – Zootaxa, 1778: 26-36

Schober A., Plan L., Scholz D., Spötl Ch. (2013): Speleogenesis of a 3D-Maze cave (Hermannshöhle, Lower Austria). – in: Filippi M., Bosák P. (Eds.): Proceedings of the 16th International Congress of Speleology, July 21–28, Brno, Vol. 3: 408–411. Czech Speleological Society. Praha.

Shear W., Edgecombe G.D. (2010): The geological record and phylogeny of the Myriapoda. – Arthropod Structure & Development, 39: 174–190.

Strouhal H., Vornatscher, J. (1975): Katalog der rezenten Höhlentiere Österreichs. – Annalen des Naturhistorischen Museums in Wien, 79: 401–542. (http: //www.landesmuseum.at/ pdf_frei_remote/ANNA_79_0401-0542.pdf)

Zupan Hajna N., Mihevc A., Pruner P., Bosák P. (2008): Palaeomagnetism and Magnetostratigraphy of Karst Sediments in Slovenia. – Carsologica 8: 1–266. Založba ZRC. Ljubljana.

Keywords: attemsiid millipedes, Diplopora, U-sedies dating, magnetopstratigraphy, Hermannshöhle, Austria.

Cave microclimate in the entrance part of the ice cave Ledena jama in Lomska duliba (Velebit Mt., Croatia)

Vinka Dubovečak [1,2], Nenad Buzjak [3,4], Dalibor Paar [5,6,7]

[1]- Speleological Society Velebit, Zagreb, Croatia
[2]- Speleological Committee of the Croatian Mountaineering Association, Zagreb, Croatia
[3]- Department of Geography, Faculty of Science, University of Zagreb, Zagreb, Croatia
[4]- Caving club Samobor, Samobor, Croatia
[5]- Department of Physics, Faculty of Science, University of Zagreb, Zagreb, Croatia
[6]- Speleological Society Velebit, Zagreb, Croatia
[7]- Speleological Committee of the Croatian Mountaineering Association, Zagreb, Croatia

The study is focused on specific microclimate conditions in the ice cave Ledena jama in Lomska duliba and their connection with geomorphological, hydrogeological, general climate and physical properties, with special emphasis to perennial ice and snow and the influence of climate change on ice and snow deposits. This 536 m deep cave is situated on mountainous region of Northern Velebit (1200 – 1700 m a. s. l.) that is located between the Adriatic Sea and Lika in Croatia. This area is in the transition zone between the temperate humid climate with warm summer (Köppen climate type Cfb) and humid boreal climate (Df) which result is low mean annual air temperature and high amount of precipitation. Ledena jama entrance (perimeter 50 x 60 m) is situated at the bottom of the Lomska duliba valley (1235 m a. s. l.). This large karst depression of glacial origin is acting as a cold air trap and known for frequent temperature inversion. Due to above mentioned micro-location factors in the upper part of the Ledena jama (-50 m) that was included in this study notably deposits of ice and snow are accumulated with significant dynamics in the last 20 years. This large cold body (diameter 20 x 30 m) spreads from 50 to 90 m depth in the cave and the estimated ice deposit age is about 140-450 years. The Lomska duliba valley with low air temperatures due to temperature inversion is the factor influencing snow accumulation and percolating water freezing. One year monitoring of microclimate parameters showed correlation between microclimate of Lomska duliba valley and Ledena jama. In the winter period dominant process is turbulent inflow of external cold air in the upper part of the cave. Absolute minimum air temperature at the bottom of Lomska duliba valley (temperature inversion) was -29.9°C and in the 50 m depth in Ledena jama was -19.3°C that same day, with 1 hour delay. In the summer period external air is warmer (maximum air temperature 29.7°C), while air in the cave is cold and trapped (maximum air temperature 4.6°C), so the air exchange does not exist. Air temperature also changes due to fissure air circulation. This annual

variation of air temperature effect on ice mass balance (ice accumulation and melting) in the upper part of the cave.

Keywords: cave microclimate, ice and snow deposits, Ledena jama ice cave, Lomska duliba valley.

The pilot research of allocation of gamma radionuclides in caves

Igor Kachalin [1,2], Oleksandr Liashchuk [1], Stanka Šebela [3], Janja Vaupotič [4]

[1]- National Antarctic Scientific Center, Kyiv, Ukraine
[2]- AtomKomplexPribor, Kyiv, Ukraine
[3]- Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia
[4]- Jožef Stefan Institute, Ljubljana, Slovenia

Within the BlackSeaHazNet Project (FP7 MCA PIRSES-GA-2009-246874), the pilot research of allocation of radon and gamma radionuclides was realized in three karst caves in Slovenia (Postojna Cave System, Županova Jama and Škocjan Caves) and at one cave in Northern Italy (Grotta Gigante). In all four caves, radon and gamma ray monitoring was performed to understand the relation between radon and its daughter products. In situ monitoring of natural level of gamma radiation and spectrometry of gamma radionuclides in karst caves was for the first time accomplished in this study. Measurements were performed by radiometric and spectrometric instrument PRS–01 (produced by AtomKomplexPribor, Ukraine), which is designed for determination of qualitative and quantitative composition of gamma-emission radionuclides in field and in laboratory, for investigation of radioactive sources and anomalies, and for gamma-survey of surface. In the Pisani Rov corridor in the Postojna Cave System, maximum radon concentration was 20,000 Bq/m3 and highest gamma doze rate, 526 nSv/h. In the Škocjan Caves, maximum radon concentration was 7,500 Bq/m3 and highest gamma doze rate, 550 nSv/h. In the Županova Jama cave maximum radon concentration was 4,930 Bq/m3 and highest gamma doze rate, 290 nSv/h. In the Grotta Gigante, maximum radon concentration was 2,140 Bq/m3 and highest gamma doze rate, 180 nSv/h. We did not find Cs-137 contamination, but we detected daughter isotopes of Ra-226 and Th-232, such as Rn-222, Rn-220, Bi-214, Pb-214. Detected radioactive elements have natural origin and are probably connected with natural uranium deposits in north-central Slovenia.

Keywords: radon concentration, gamma radiation, karst caves, Slovenia, Italy.

A simple mathematical model of cave eutrophication

Sergey Khizhnyak

Krasnoyarsk State Agrarian University, Krasnoyarsk, Russia

In spite of obvious oligotrophy of the Middle Siberian caves as ecosystems, the ratio of oligotrophs to total heterotrophs in sediments and water ranges from 7% to 76% with the average ratio 55%. Such a low ratios of oligotrophs in the oligotrophic ecosystems as soon as a great variation in oligotrophs proportion among cave sites can be explained using a simple model based on the Monod- Haldane's equations of substrate inhibition applied with irregular income of nutrient-rich exogenous substrate into cave ecosystem.

Keywords: cave eutrophication, mathematical model, Siberia.

Cold-adapted microbial communities in the limestone caves of Siberia, Caucasus and Far East and their biotechnological potential

Sergey Khizhnyak [1], Elena Nesterenko [1], Elena Lankina [1], Ilmira Ilients [1], Sofya Ovsyankina [1], Lyaysyan Kharlamova [1], Ekaterina Balmochnykh [2]

[1]- Krasnoyarsk State Agrarian University, Krasnoyarsk, Russia
[2]- Siberian Federal University, Krasnoyarsk, Russia

Researchers performed in nineteen Siberian, two Far East and two Caucasian cold (temperature of air, water and cave sediments varies from 0 to +6°C depending on the cave type and cave site) limestone caves (depth 7 m to 1830 m, length 40 m to 43470 m) revealed abundant and diverse autochthonous cave microbial communities presented with bacteria and fungi feeding on the exogenous organic matter. Both bacteria and fungi are cold-adapted (psychrophilic) with the optimal growth temperature in the range of 14–20°C and the maximal growth temperature in the range of 26–29°C depending on strain. Total number of fungi varies from less then 10^3 colony forming units (CFU) per gram of cave sediments in low impact caves and cave sites to $3.6x10^6$ CFU per g in high impact areas such as underground camp sites or other areas with organic income such as bat's colonies sites or near-entrance sites. Among predominant fungal isolates members of *Penicillium*, *Chrysosporium*, *Mucor*, *Mortierella*, *Absidia*, *Thamnidium*, *Doratomyces*, *Echinobotryum*, *Cryptococcus* and *Rhodotorula* genera were found. Total number of cultivated bacteria varies from 10^4 to 10^7 CFU per g of cave sediments and from 10^2 to 10^5 CFU per mL of water. The most abundant and diverse bacterial biota is present in high human impact areas where number of bacteria (up to $5.75x10^7$ CFU per g) is comparable with those in the soils of region. In contrast with the fungi, high numbers of cultivated bacteria (up to 10^5-10^6 CFU per g) are found in low human impact areas including previously non-visited caves and cave sites. Cave bacteria are metabolic active with minimal observed in situ generation time 48-72 hours.

Both oligotrophs and eutrophs were found in the cave sediments and in the underground water bodies, with the percentage of obligate oligotrophs ranged from 6.8 to 94.5% to total number of cultivated bacteria. Simple math model based on Monod-Haldane equations and stochastic income of substrate explains this phenomena. Among predominant cave bacteria the members of genera *Flavobacterium*, *Janthinobacterium*, *Pseudomonas*, *Pedobacter*, *Arthrobacter*, *Pseudochrobactrum*, *Paenibacillus*, *Sporosarcina*, *Bacillus*, *Sphingopyxis*, *Rhodococcus*, *Acinetobacter* and *Planococcus* were found using 16S rRNA Gene sequence analysis. Besides psychrophilic bacteria and fungi, both mesophiles typical for land ecosystems of region and atypical for lend ecosystems psychrotolerants are found in the cave microbial communities with significant variations of psychrophiles: psychrotolerants: mesophiles proportions within and among the caves. Computer simulation based on the idea that caves are colonized by mesophiles penetrating from the land ecosystems with their subsequent evolutional step-by-step "psychrophilization" showed that all the researched microbial communities are immature and their formation is still in progress.

A lot of strains of cave bacteria and fungi can suppress growth of the soil-borne phytopathogenic fungi such as *Bipolaris*, *Alternaria* and *Fusarium* species. Laboratory and field assessments showed that cold-adapted cave strains are good alternative to the traditional biopreparations based on mesophilic microorganisms for biological control of plant pathogens because of their ability to grow at the temperature range typical for agriculture in cold and temperate climatic zones and inability to infect warm-blooded organisms.

Key word: cave bacteria, cave fungi, Siberia, Far East, Caucasus, psychrophiles, psychrotolerants, agricultural biotechnology.

Sediments of Béke Cave, Hungary - Preliminary Results I: stratigraphy and sedimentology

Klaudia Kiss, Balazs Bradak, Jozsef Szeberenyi, Gyorgy Varga, Gyorgy Czuppon, Gabor Vid

Geographical Institute, Research Centre for Astronomy and Earth Sciences, Hungarian Academy of Sciences, Budapest, Hungary

In this study stratigraphical and sedimentological analysis of some sediment sections of Béke Cave (Aggtelek Karst Region, NE Hungary) are presented. The sediment sections were preserved in safe geomorphological situations.

Different kind of sand layers were identified at the 16th point of Main Passage ("Főág") of the cave. The basal layer was a poorly compacted and sorted, fine-medium gravel grained, clast-supported conglomerate (BCC - Béke Cave Conglomerate). The matrix of the conglomerate is a fine grained sand, partly compacted by carbonate concretion. The clasts of BCC were poorly-rounded and some sand lentils were identified in the upper part of the layer. The overlaying sediments of BCC was a fine laminated sandy material. A possible hiatus due to fluvial activity (cave stream) was identified between the gravel and sand stratas. Graded/inverse graded bedding, cross bedding sand and palaeochannels were identified in the different part of the overlaying sand layers. The complex fluvial activity of a cave stream could be derived by the paleogeomorphological development of the cave and the climate and environment change on the surface (cave terraces, migration and infill of palaeochannels).

More than 1 meter thick, laminated, various coloured and grain sized clay dominant section was identified in a narrower and lower cave passage („Felfedező-ág"). The development of this section started with a strong corrosional and erosional phase characterized by paleogeomorphological phenomenom (cave terrace) and basal strata of this profile (coarse grained gravel). The coarse grained, poorly sorted gravel layer were overlaid by a thick clayey strata. Thin (0.5 cm) carbonate bed was intercalated in the clayey sediment. The decreasing transport energy of the stream is indicated by the decreasing grain size of the accumulated sediments. The carbonate bed possibly developed during the period when the fluvial activity stop. The cave environment of this phase possibly characterized by cave ponds. The final event of the sedimentation was the accumulation period of the overlying red clay.

Two important components of the development of sediment sequence were characterized based on the study of two different profiles in Béke Cave (Hungary):

1. 'In situ' cave sediments: characteristic sediments and paleogeomorphological phenomenom indicated 'cave facies' (e.g. 'gravels of cave terrace'). Surface paleoenvironment could be characterized by these phenomenom indirectly.

2. The 'surface' sediments: originated from surface, accumulation was controlled by the system of the cave environment but store direct information about the paleoenvironment of the surface (redeposited materials and surface processes, facies, paleoclimate etc.)

In the future the complex paleoenvironment reconstruction may be revealed by the simultaneous study of surface and cave facies and the interpretation of the interaction between these two factors above.

Keywords: cave sediments, palaeoenvironment, stratigraphy, Béke Cave.

Olm conservation project (2012-2015)

Petra Kovač Konrad [1,2,3], Bojan Karaica [1], Marko Budić [1,3]

[1]- Croatian herpetological society-HYLA, Zagreb, Croatia
[2]- Society for karst research-Freatik, Zagreb, Croatia
[3]- Croatian mountaineering society Željezničar- Caving section, Zagreb, Croatia

Proteus anguinus is one of the most evolutionary distinct amphibian species in the World and the only European cave adopted vertebrate. The *Proteus anguinus* as a biological indicator of status of underground karst waters (important ecosystem) are of fundamental importance to environmental function and sustainability, and they provide many goods and services to individuals and societies. Its skin is totally white and it has lost its eyes. It lives in total darkness so it uses minute vibrations and electric signals to find its food. Amazing is that it can live up to 100 years due to its cold water environment (average 6°C) and very slow metabolism. Its populations are in decline due to pollution, climate change and habitat destruction but still very little is known about these animals and how they behave in their natural environment. Research team from Croatian Institute for biodiversity, Croatian Herpetological Society and Society for karst research-Freatik follows these animals deep into the cave systems. In this project using cave-diving techniques, a new method has been developed to study population of *Proteus anguinus* in 6 cave systems. Cave divers count number and distance of the species along transect line as well as number of adult and juvenile members of population. The data is then written in specially developed protocols as part of the project and later statistically processed. The sizes of the population in certain cave systems can be estimated then, as well as a raise or decline in size of the population. As well as biological factors associated with the species the project includes study in hydrogeological, geological and geomorphological elements. There is a need to define optimal protection zone, or preferably zones, within NATURA 2000 for *Proteus anguinus* habitat. This is not possible if hydrological, geological and hydrogeological studies are not conducted for a specific habitat site (cave system). Collecting data such as water quality and composition, underground water level, fault and fracture orientation (structural geology), lithology, cave orientation and geographical setting (2D and 3D), karst geomorphology for a specific area, doline (sinkhole) geographic distribution (defining density with GIS technology) and defining watersheds. This data will be compared with biological research of *Proteus anguinus* populations. As a consequence it will be possible to better understand the connections between populations and/or habitats. Information gathered in the project will be given to government institutions which will result in raising awareness of lack of integration with those involved in decision making and consultation activities; promoting a more interdisciplinary approach to policy making and the provision of guidelines reinforced with training programs on how to undertake integration sensitive to different contexts; and the expansion and improvement of research on the environment, social and economic factors in development processes and improvement of data for integrating appraisal and decision making.

Keywords: *Proteus anguinus* protection, cave diving, project, new methodology, optimal protection zone.

First results of microbial inventory in caves: Dimnice and Sveta Jama, Slovenia

Franc Malečkar [1], Violeta Lovko [1], Janez Mulec [2]

[1]- CŠOD, Ljubljana, Slovenia
[2]- Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia

Dimnice and Sveta jama are show caves with intense air streaming in some of the galleries. Sveta jama serves also as a church. In Dimnice, the outside air descents through the main shaft in the colder part of the year, it mixes with the warmer cave air, what consequently causes intense air circulation. Water condensation on the walls is formed at the junction between warm and cold air where microbial mats of different colours are observed. The preliminary results will be presented on the poster as a basis for further research and presentation to visitors.

Keywords: show caves, air streaming, microbiology.

Anthropogenic inscription of aerosol particles in the recent speleothems and in the cave air

Magda Mandić, Luka Mandić, Ivo Orlić

Department of Physics, University of Rijeka, Rijeka, Croatia

New investigation of cave environment and aerosol particles has been initiated in abandoned war shelters in city center of Rijeka, Croatia. These shelters are natural caves up to 300 m long, and are specific because of the soda straw speleothems samples of impressive length (2 – 3 m) growth inside them.

Because the investigated sites are not isolated from air circulation, migration of aerosol particles is constant process, and therefore the particles can be incorporated in speleothems and used as trace elements. The aerosol particles that this investigation is focused on are of anthropogenic origin. Path of particles are going to be monitored and modeled through elongated profile of location i.e. from the location entrance, walls and flour, through the end where the chamber with speleothems. Drip water geochemical and stable isotope parameters are going to be monitored.

Keywords: cave environment, aerosol particles, shelters, stable isotopes.

Bacteriological pollution of karst springs in Zlatar, Western Serbia

Dejan Milenic, Djuro Milankovic, Nevena Savic, Nenad Doroslovac

Centre for renewable water energy resources, Department of Hydrogeology, Faculty of Mining and Geology, University of Belgrade, Belgrade, Serbia

Zlatar mountain is situated in the western part of The Republic of Serbia and thanks to its authentic nature and specific climate, it represents a significant Serbian tourist resort. With the activity of endogenous and exogenous forces in a wider area of Zlatar mountain, a typical hilly-mountainous landscape was formed, with the altitudes that go above 1000 m. As opposed to mountain tops, the lowest terrain points are gorges of the Uvac, Zlosnica and Tisovica rivers, which were formed by means of erosive activities of the aforementioned water flows.

Geotectonically speaking, the area of Zlatar mountain belongs to the region of the Inner Dinarides.The geological structure of the area is characterized by a dominant distribution of the

mesozoic formations, that is limestone of triassic age and limestone, marl, sandstone and cherts of jurassic age. The presence of carbonate formations on Zlatar mountain caused the formation of karstic aquifer type, which is discharged via numerous karst springs. The most significant springs of the area are : Curcica vrelo spring (Qav= 50 l/s), Gacevo vrelo spring (Qav= 35 l/s), Crno vrelo spring (Qav= 20 l/s), Oman spring (Qav= 20 l/s), Vrelo spring (Qav= 20 l/s), wellspring Rekaca (Qav= 20 l/s), Stitkovo vrelo spring (Qav= 17 l/s), Saponjica vrelo spring (Qav= 10 l/s), Lakomica spring (Qav= 10 l/s), Bursac spring (Qav= 5 l/s) and Kusica vrelo spring with Qav= 4,5 l/s. The groundwater of these springs belongs to the hydrocarbonate class-calcium group.

For the sake of water supply of the Nova Varos settlement, people use karstic water from the following springs: Lakomica, Gacevo vrelo, Crno vrelo, Saponjica vrelo, Sopot vrelo and spring Muhica jama (pothole). Executing bacteriological analyses (during 2013) of the groundwater from these springs for the sake of water supply, bacteriological pollution was identified. Namely, all the analysed groundwater samples contained the bacteria *E. coli* and *Enterobacter* sp., and the groundwater sample from Gacevo vrelo spring also contained the bacterium *Citrobacter*. Likewise, all the groundwater samples from the springs for the water supply of Nova Varos contained bacterial flora of iron and manganese of the Leptotrix type.

The bacteriological irregularity of the groundwater from karst springs in the area of Zlatar mountain is the consequence of great many pollutants, such as dairy farms, mushroom farms, numerous sawmills which dispose of their byproducts directly in the open karstic surfaces or they deposit them at the banks of surface flows. In the process of recharging karstic aquifers with the infiltration of atmospheric sediments and water of surface flows, the bacteria which are formed in the raw material processing and the decomposition of deposited byproducts (sawdust), get into the groundwater and are manifested in karstic springs as microbilogical pollution.

Keywords: karst springs, bacteriological pollution, Zlatibor mountain, Western Serbia.

Bacteriological pollution of the groundwater of karst springs in Zlatibor

Dejan Milenic, Ana Vranjes, Bojan Doncev, Marina Jovanovic

Centre for renewable water energy resources, Department of Hydrogeology, Faculty of Mining and Geology, University of Belgrade, Belgrade, Serbia

Zlatibor mountain massif is situated in the southwestern part of the Republic of Serbia and, administratively speaking, it belongs to Zlatibor county. It spreads over the territory of the following municipalities: Cajetina, Nova Varos and Uzice. Its south and east boundaries are The Uvac and Veliki Rzav rivers, the north boundary is The Djetinja river, and in the west it borders with Bosnia (Mokra Gora, Semegnjevo and Jablanica).

Zlatibor mountain massif is predominantly built of the rocks of mesozoik age. Within the mesozoik complex of rocks, there are ultramaphites, diabase-chert formations and the carbonate complex of rocks of triassic age. The distribution of karstic aquifer type, which is the most significant from the stance of the groundwater reserves and the possibilities of their multi-purpose usage, is related to the triassic carbonate complex, whose thickness in the area of Zlatibor ranges from 150 m to 750 m. The karstic aquifer type in the area of Zlatibor mountain massif is drained by numerous springs which occur at the contact of triassic limestone and impermeable layer bottom, then at the tectonic contacts of triassic limestone and slightly water permeable rocks, and finally at the contact of triassic limestone and neogene sediments at the edges of the neogene basin. Karstic aquifers with the highest yield in the area of Zlatibor mountain massif are: Susicko vrelo spring, Dobroselicka vrela sprigs, Zmajevac spring, Ljubisko vrelo spring, Gostilje vrelo spring, Kotren spring, Golovsko vrelo spring.

Due to the unique hydrogeological characteristics of a karstic aquifer, karst springs in the area of Zlatibor are most often bacteriologically contaminated. The inadequacy of the karstic springs' groundwater is mainly caused by coliform bacteria which were observed when executing chemical analysis in the Susicko vrelo spring and Dobroselicka vrela springs. Owing to a continual monitoring of the bacteriological contents within one year, Susicko vrelo is a representative example with 4 chemical analyses. During dry periods, that is at the beginning of June and at the end of August, the number of coliform bacteria is on the increase and reaches 880 bacteria per 100 ml of water, and the maximum allowed value is 10. Dobroselicka vrela springs had "only" 24 bacteria out of the allowed 10 in March 2014. As a type of coliform bacteria, there are those of fecal origin and they are found in huge quantities (up to 500 bacteria per 100 ml of water). Streptococci of fecal origin, that is enterococci, also pollute the water in Susicko and Dobroselicko springs, and the water shouldn't contain them at all. Aerobic bacteria in the Susicko vrelo spring are also found in larger than allowed quantities.

Such a bacteriological structure of the groundwater in Susicko and Dobroselicko springs, is a consequence of a few factors and among them the leading part is played by the lack of the spring's ordered management (the spring is not captured), hydrogeological structure type, developed cattle breeding in the basin area.

Keywords: bacteriological pollution, fecal origin, karst springs, Zlatibor mountain.

Lampenflora in Postojnska jama, Slovenia: problems and solutions

Janez Mulec

Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia

Postojnska jama is one of the most famous tourist caves in Europe and attracts more than 500,000 visitors per year. Similary to other show caves that are open all year round, in the illuminated parts of the cave lampenflora develops and proliferates around lamps. Lampenflora is a community of mainly phototrophic organisms that is involved in biodeterioration of substrata, including those of cultural value, such as historical inscriptions. More than 30 different species of mosses and ferns have been identified as a part of lampenflora. As a sum of independent surveys since 1981, among microscopic phototrophs, cyanobacteria were the most diverse, followed by diatoms and green algae. In show caves it is crucial to restrict lampenflora growth at its early stage of development. Starting with 2011, manual removal of moss and fern thalli and an application of 15 and 20% v/v solution of hydrogen peroxide (pH 7.0-7.5) have been used in Postojnska jama to restrict lampenflora. A complete round of applications consists of three consecutive applications with one week between each application. This procedure is usually repeated twice a year, and is suitable only for treatment of insensitive carbonate surfaces without the presence of cave fauna. Light quality and quantity have an impact on the physiology and ecology of phototrophs; that is why the recent approaches to restrict lampenflora growth include manipulation of the emission spectra of lamps that are installed in show caves. In a six-month experiment in Postojnska jama with different LED lamps that emitted white light of different quality, the growth and biomass level of *Chlorella vulgaris*, a frequent microscopic algae in lampenflora, was almost undisturbed. This was attributed to the ability of this organism to synthesise different accessory photosynthetic pigments. Instead of just manipulation of the emission spectra of lamps without knowing the dominant phototrophs in the lampenflora, more attention should be dedicated to the lighting regime, positioning of lamps and frequent removal of re-emerging lampenflora.

Keywords: cave management, lampenflora, hydrogen peroxide, lamp spectra, photosynthetic pigments.

Cultivation microbiological techniques for rapid determination of biological pollution during expeditions in water caves

Janez Mulec [1], Andreea Oarga [2]

[1]-Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia
[2]-University of Nova Gorica, Nova Gorica, Slovenia

The development of techniques and media for immediate field use is useful for the assessment of environmental health of underground ecosystems, and especially for water quality at sites where it is used for human consumption. We present the use of microbial indicator groups based on RIDA®COUNT cultivation plates in two case studies: The Great Cavern of Santo Tomás, Cuba, where underground water is used for domestic needs, and Postojnska jama, Slovenia, to monitor the underground flow of the Pivka river. In The Great Cavern of Santo Tomás microbial indicators in water of different pools fed by constant percolation water or occasional underground floods (total cultivable bacteria, coliforms, *Escherichia coli*, Enterobacteriaceae) showed that the microbial load depended very likely on the sampling position in the vertical transection of the cave system and its closeness to the entrance. During the underground flow of the Santo Tomás stream through the cave system, the concentration of isolates typical for *E. coli* was reduced from 56 CFU/ml at the ponor to a concentration below the detection limit at the spring. In Postojnska jama sampling was performed during different hydrological conditions of the Pivka river between 2009 and 2013. In the underground downstream from the ponor, the concentration of the cultivable bacteria gradually reduces. The microbial parameters at the ponor reflected human interferences upstream in the catchment area; for example high total bacterial counts during flood in December 2010 (total bacteria >2000 CFU/ml) and high waters in October 2013 (total bacteria 2730 CFU/ml) were very likely connected to the overflow of untreated water from the municipal wastewater treatment plant of Postojna directly into the Pivka river. At the outflow from the wastewater treatment plant during normal operation the total number of culturable bacteria is reduced 90% and *E.coli* 97%. At the low discharge of the Pivka river in July 2011, the elevated microbial indicators, e.g. total bacterial counts 2020 CFU/ml, might be connected with intensive fertilization of fields in the catchment area. The microbial detection system RIDA®COUNT was proved useful for monitoring, evaluation of the organic load, alerting to the presence of potentially hazardous microbes that enter underground, and to determine the points of organic pollution.

Keywords: caves, microbial indicators, feacal contamination.

Detection of *Histoplasma* antigens in bat guano from caves in Slovenia using commercial ELISA for human samples was not supported by the molecular analyses

Janez Mulec [1], Sanja Stopinšek [2], Romina Kofol [2], Tadeja Kotar [3], Saša Simčič [2]

[1]- Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia
[2]- Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[3]- Clinic for Infectious Disease and Febrile Illnesses, University Medical Centre Ljubljana, Ljubljana, Slovenia
Address correspondence to S. Simčič, University of Ljubljana, Faculty of Medicine, Institute of Microbiology and Immunology, Zaloška 4, SI-1000 Ljubljana, Slovenia

Histoplasmosis is a disease frequently encountered during caving in tropical and subtropical regions after inhalation of spores of *Histoplasma capsulatum* which might originate from bat guano. In Europe, medical reports exist on the acquirement of histoplasmosis after visitation of confined spaces in Cyprus (1964) and Topolniţa Cave in Romania (1966). Infection with *H. capsulatum* is often asymptomatic but has considerable morbidity and mortality in the immunocompromised population. The key question for speleologists is the presence and eventual distribution of *H. capsulatum* in temperate caves related to bat guano. Isolation of *H. capsulatum* from environmental samples is difficult; the fungus is typically retrieved as a cultivable strain after inoculation of laboratory animals. Commercially available, a *Histoplasma* antigen detection Enzyme-Linked Immunosorbent Assay (Immy Alpha *Histoplasma* Antigen ELISA; Immuno-Mycologics, Inc., Norman, OK) was developed to rapidly and noninvasively diagnose and manage patients with disseminated histoplasmosis.

In the study we evaluate the utility of a *Histoplasma* Antigen ELISA as a rapid and cost effective alternative to hazardous culturing of bat guano samples for detection of the fungus in caves in Slovenia. All tested guano samples were positive for *Histoplasma* antigen with the highest antigen content in the sample of fresh guano collected under a nursery bat colony. Fungi specific PCR on isolated DNA from guanos using two sets of the universal fungal primers ITS1-ITS4 and ITS4-ITS5 confirmed the presence of fungal DNA. However, *H. capsulatum*-specific PCRs using additional two sets of primers H-anti3-H-anti4 and Msp2F-Msp2R (Muniz M, et al, Appl. Environ. Microbiol. 2010; 76: 4438-47. Guedes H, et al, J. Clin. Microbiol. 2003; 41: 535-9.) were negative for all the samples. Positive *Histoplasma* Antigen ELISA might be linked to cross-reactivity with other fungal organisms which are present in guano or to the long-term persistence of *H. capsulatum* antigen in the environment due to its stable polysaccharide nature. On the other hand, negative results of specific PCRs can point out that the conditions in guano might not be supportive for the preservation of *H. capsulatum* DNA, or the concentrations of *H. capsulatum* DNA in the samples were extremely low. More studies are needed to optimize the sensitivity, threshold and applicability of ELISA on the environmental samples.

Keywords: histoplasmosis, bat guano, *Histoplasma* antigen, *Histoplasma* DNA.

Karst soil properties and men's impact: the case study of Pliskovica

Nina Peca

Regionalni razvojni center Koper, Koper, Slovenia

Typical karstic soils Rhendzic Leptosols and Chromic Cambisols were studied near the village of Pliskovica on the Kras plateau. Kras plateau has been settled continuously from the Paleolithic age. Due to hard living and farming conditions on shallow and rocky soils farming land was improved by

agricultural meliorations and extended by deforestation, consenquently man became an important soil forming factor. With hand and machine agromeliorations typical karst landscape with »cleared« meadows, cultivated dolines and stone structures was formed. However soil properties were also affected, specially by removing the stones, relocating and adding allochthonous soil. The aim of the study was studying the effect of men through agricultural use on soil properties. Chemical and texture analyses of soil from 18 locations under different land use (forest, meadow, vineyard, garden) were carried out. The results show that agromeliorations' effect mainly results in higher rubble and calcium carbonate content in the soil.

Keywords: karst soil, terra rossa, land use, agriculture meliorations, Kras.

Chemical and isotope analysis of water samples from wells and springs of Rovte region, W Slovenia: an assessment of ongoing dedolomitization

Sven Philipp [1], Stephan Kempe [1], Bojan Otoničar [2]

[1]- Institute for Applied Geoscience, TU Darmstadt, Germany
[2]- Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia

Speleological and geological research in the area of Rovte (W Slovenia) revealed that some of the caves may be of hypogenic origin. Among other processes, dedolomitization could have played an important role in their speleogenesis. To assess if dedolomitization is still occurring, we sampled and analysed water from three deep wells and two springs. The wells penetrate gypsum strata at a depth of several hundred meters. Therefore samples from wells Z and R show high SO_4^{2-}-concentrations and low Mg^{2+}/Ca^{2+}-ratios. Water samples from surface springs show very low sulfate but high nitrate and chloride concentrations in contrast. These are clearly impacted anthropogenically. The isotopes ($\delta^{18}O$ and $\delta^{2}H$) of the wells Z and R are in the same range suggesting that the waters from Z and R derive from the same aquifer system. Calculation of saturation indexes with PHREEQC shows that most samples from the wells were supersaturated with respect to calcite and mostly undersaturated with respect to dolomite. Furthermore, the plot of pH vs. SO_4^{2-} from Z and R show a negative correlation. This and the preferential removal of Mg^{2+} as suggested by the molar Mg^{2+}/Ca^{2+}-ratio of < 1 indicates that dedolomitization may still be occurring.

Keywords: dedolomitization, PHREEQC, ground water chemistry, deep wells.

Chemical and microbiological analysis of water from Emilia Romagna gypsum caves (Parco dei gessi Bolognesi, Life + 08NAT/IT/000369)

Diana I. Serrazanetti [1], Chiara Montanari [1], Ilenia Maria D'Angeli [2]*, Jo De Waele [2], Lucia Vannini [1,3], Fausto Gardini [1,3]

[1]- Centro Interdipartimentale di Ricerca Industriale Agroalimentare, Università degli Studi di Bologna, Cesena, Italy
[2]- Dipartimento di Scienze Biologiche, Geologiche ed Ambientali, Università degli Studi di Bologna, Bologna, Italy
[3]- Dipartimento di Scienze e Tecnologie Agro-alimentari, Università degli Studi di Bologna, Cesena, Italy
* Corresponding author

The Project Life + 08NAT/IT/000369 "Gypsum", co-financed by the European Union, has started in the spring of 2010. This project aims to protect and manage the main karstic caves and sites of Emilia-Romagna region. The A3 action provides a periodic monitoring of the main karstic aquifers in terms of chemistry and microbiology.

During the first two years, karst waters of 50 control points were analysed (sinking streams, rivers and streams in caves, and resurgences). The objective of the first two years of study was to evaluate the impact, in the waters of the gypsum karst systems, of agricultural substances or other forms of pollution or settlements related to human activities or natural factors. Waters were analysed for major chemistry (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, SO_4^{2-}, HCO_3^{-}, Cl^{-}, F^{-}, Br^{-}, NO_3^{-}, and NH_4^{+}), measuiring pH, electrical conductivity and temparature in situ. The same samples were analysed with traditional microbiology techniques (plate counting) and molecular biology techniques (sequencing of 16S rRNA segment and PCR-DGGE), focused on the characterisation of microbial populations in the different sampling sites and determination of their variations and/or changes. During the third and fourth years the dynamics of contamination have been analysed using traditional microbiology techniques focused on total microbial count and on faecal microbiota as index of human and animal contamination. As expected, waters were increasingly mineralised from sinking streams to resurgences, with only local and temporaly high contents in nitrates and ammonium, often related to the presence of bat colonies. The results of the first and second years outlined the presence of numerous bacterial species (*Agrobacterium tumefaciens*, *Pseudomonas* spp., *Rahnella aquatilis*, *Stenotrophomonas maltophilia*, *Pedobacter swuonensis*, *Enterobacter* spp., *Aeromonas hydrophila*, *Citrobacter*, *Klebsiella* and *Raoultella*). The organisms identified have different origins: some come from the soil, others are common water contaminants and others derive from human activities (faecal bacteria). Up to now, PCR-DGGE revealed the ecological changes, in terms of microbial populations present in the samples, and different sampling sites within the same cave. Although the impact of faecal microorganisms never exceeded 2 logCFU/ml, the results of the third and fourth years evidenced fluctuations of these microorganisms strictly correlated to the season and to the biological activity of bats.

Keywords: geochemistry, microbiology, habitat, gypsum karst.

A novel survey of the distribution of *Proteus anguinus* by environmental DNA sampling

David Stanković [1,2,3], Gregor Aljančič [1], Špela Gorički [1,3], Magdalena Năpăruş [1], Miloš Pavićević [4], Ivan Skaramuca [5], Jasminko Mulaomerović [6], Matjaž Kuntner [7], Aleš Snoj [2], Zlatko Grizelj [8] and William R. Jeffery [9]

[1]- Society for Cave Biology, Tular Cave Laboratory, Kranj, Slovenia
[2]- Department of Animal Science, Biotechnical faculty, University of Ljubljana, Domžale, Slovenia
[3]- Herpetological Society of Slovenia – Societas Herpetologica Slovenica, Ljubljana, Slovenia
[4]- Biospeleolološko društvo Crne Gore, Podgorica Montenegro
[5]- Hercegovačka gorska služba spašavanja, Mostar, Bosnia and Herzegovina
[6]- Centar za krš i speleologiju, Sarajevo, Bosnia and Herzegovina
[7]- Institute of Biology, Scientific Research Centre, Slovenian Academy of Sciences and Arts, Ljubljana, Slovenia
[8]- Laboratorija za vode, Javno komunalno poduzeće Ljubuški, Ljubuški, Bosnia and Herzegovina
[9]- Jeffery Lab, Department of Biology, University of Maryland, USA

Proteus anguinus is one of the most famous cave animals worldwide, the flagship species of the Dinaric karst groundwater and its endangered cave fauna. As such, *Proteus* represents our joint aspiration to raise scientific and public attention to preserve it in the future. An active alliance of organizations committed to nature conservation of the Dinaric karst was built in order to embark on the ambitious task to promote, share and implement an advanced action plan to save *Proteus*, Europe's only cave vertebrate, along its fragmented range in the Western Balkans.

Since the beginning of the research on the olm, the basic but particularly important question of its exact distribution has been difficult to address. Because only small fragments of its subterranean habitat can be accessed by man, this task seems to be impossible to adequately resolve even today. The current knowledge on the species distribution is incomplete, and the extent of its decline cannot be estimated without deeper knowledge of its distribution.

P. anguinus presence in inaccessible habitats cannot be ascertained by classical survey methods such as trapping and visual encounters. For this reason, the Tular Cave Laboratory is developing an alternative indirect research tool to overcome the critical problem when determining *Proteus* occurrence – inaccessibility of its subterranean habitat. This highly efficient, non-invasive and innovative forensic method helps to detect traces of *Proteus* DNA by filtering water samples from springs and caves. Namely, during the process of skin growth and regeneration, fragments of epidermal cells, along with the DNA they contain, are constantly shed from the skin of aquatic vertebrates and carried away by water. Such DNA is called environmental DNA (eDNA). The environmental DNA collection from water habitats in combination with real-time PCR approach has already been shown as suitable tool for monitoring surface dwelling vertebrates. Real-time PCR approach allows detection of lower quantities of DNA in the water samples when compared to the classical PCR approach. In its most basic form, the methodology aims at detecting traces of *P. anguinus'* DNA in cave/spring water samples; however as new genetic markers are found, it can also be expanded to for fast and routine genotipization of water samples.

We developed a SYBR chemistry-based assay with two sets of specific primers to amplify short mitochondrial DNA sequences in the 16S rDNA gene (153 bp) and in the control region (106 bp). The specificity of the primers was tested on trout, crested newt and human DNA. In the majority of cases there was no DNA amplification; if amplicons were present, however, they could easily be separated from amplified DNA of *P. anguinus* by the shape of the melting curve. The eDNA filtration coupled with real-time PCR amplification was tested in controlled conditions of *Proteus* husbandry in the Tular Cave Laboratory (Slovenia), followed by testing in nature.

With real-time PCR-based method of eDNA detection we significantly improved the efficiency of detection of a cave-dwelling organism and reduced the possibility of contamination as post-PCR analysis can be cancelled out. Furthermore, the DNA-based method to detect the olm from water samples coupled with local spatial data will provide a vulnerability map, which will help visualize

zones most threatened by human impacts along. Our real-time PCR approach is the most time-efficient method currently known for detection of the cave-dwelling *Proteus anguinus*. Furthermore, when integrated in an accurate Geographic Information System (GIS) distribution model, the patterns of genetic variability of *Proteus* within the karst landscape will emerge and it will help to prepare urgently needed models for assessing potential impacts of hydrotechnical and water extractions *P. anguinus* populations.

Here we present the results of this method that was tested in Slovenia (Tular Cave Laboratory, Vir pri Stični, Mahniči and Kompoljska jama), Bosnia and Herzegovina (Trebižat river tributary and Hutovo blato) and Montenegro (Grahovo, Nikšič, Boka Kotorska and Skadar Lake area), within a research project funded from the Critical Ecosystem Partnership Fund - Mediterranean Basin Biodiversity Hotspot, Jeffery Lab at the University of Maryland, Department of Animal Science at the Biotechnical Faculty and EZ Lab at the Research Centre of the Slovenian Academy of Sciences and Arts.

Keywords: *Proteus*, environmental DNA, monitoring, real-time PCR.

Microbiology of cave environments: specific or not?

Blaž Stres [1], Nadja Robič [1], Tina Zakotnik [1], Valentina Turk [2], Janez Mulec [3]

[1]- Department of Animal Science, University of Ljubljana, Domzale, Slovenia
[2]- Marine biological Station Piran, National Institute of Biology, Piran, Slovenia
[3]- Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia

Cave microbiology, recently acknowledged as an integral part of speleology, has yet to determine the structure of microbial communities and its relation to the subsurface environment. Due to 43 % of surface as karst and 10419 caves registered to this day, Slovenia has ideal settings as well as responsibility to invest in research of these delicate environments. The purpose of this study was to asses the difference in microbiological and environmental properties of clastic sediments between four sampled caves in comparison to the surface soil. In addition, incubation experiments were used to model the spring-autumn inflow of nutrients and organisms into cave sediments. Finally, a sediment transect spanning significant differences in cave conditions encompassing also sediment age up to sediments from Middle Quaternary (<0.78 Ma) was analyzed. Within-cave sediment characteristics variability was higher than between cave variability, suggesting high spatial heterogeneity of environmental conditions within each particular cave. The variability in bacterial microbial communities followed that of the sediments, but was significantly different from surface soil community. Incubation experiments showed that despite the influx of cell and nutrients into cave sediments, the microbial community structure remained characteristics of cave environment. In addition, characteristic communities resembling cave microbial community structure developed on sterilized glass beads incubated under the same conditions. These findings were corroborated by the results of Middle Quaternary transect. In order to determine the robustness of these observations, global comparison of 16S rRNA sequences of microbial communities from deep subsurface, mine, cave sediments, polluted soils, cold, polar, alpine and extreme soil, rock endoliths and temperate soils was conducted. With increasing breadth of environments from extremely oligo- to nearly eu-trophic with primary production it became evident that cave bacterial communties did not represent a specific subterranean cluster but were largely similar to other sediment-soil communities with limited substrate supply and low temperatures.

Keywords: community structure, incubation experiment, activity, environemntal parameters.

Characterization of the cave environments for the palaeoclimate studies – from Dugi otok Island to Velebit Mt (Croatia)

Maša Surić [1], Nenad Buzjak [2], Robert Lončarić [1], Nina Lončar [1]

[1]- Department of Geography, Center for Karst and Coastal Researches, University of Zadar, Croatia
[2]- Department of Geography, Faculty of Science, University of Zagreb, Zagreb, Croatia

Speleothems have been recognized as excellent archives of palaeoenvironmental changes since they form from the solution that carries information from the atmosphere in its temperature dependent isotopical composition. In order to assess eligibility of the particular cave and cave sediments for reconstruction of the palaeoenvironments at the different altitudes, we analysed cave microclimate settings and isotopic composition of precipitation and cave dripwater along the transection from Dalmatian islands to Velebit Mt peaks (Croatia). We selected Strašna peć Cave on Dugi otok Island (70 m a.s.l.), Manita peć Cave in Velika Paklenica canyon (Velebit Mt, 570 m a.s.l.) and Spilja u Zubu Buljme Cave (Velebit Mt, 1305 m a.s.l.). The aim was to determine cave microclimate conditions and to characterize the hydrological behavior of drip sites which fed the stalagmites already taken for analyses from Manita peć Cave, and of those predicted for future analyses from Strašna peć Cave and Spilja u Zubu Buljme Cave.

Due to the topographic locations of the caves in epikarst and shallow vadose zone near the summits, recharge areas of all three caves are limited, and source of the cave drip water is restricted to local precipitation without any influence of mixing with the groundwater aquifer. Because of the relatively shallow overburden, infiltration elevation i.e. altitude of the catchment, is just slightly above the elevation of the cave. Modification of the isotopic composition can occur during transition through the soil zone and epikarst, but in our case of bare karst, soil (sparse *terra rossa*) is negligible, and overlaying bedrock is 2-80 m thick.

The lowermost Strašna peć Cave (SP), although relatively small, shows quite stable microclimatic conditions in its farthermost part, having T_{av}= 10.8 °C (ampl. 1.8 °C), and RH = 100%. However, it stands out with significant aberration from the mean annual surface air temperature (T= 16.4 °C). Although we have only one year data set, this difference could be attributed to the general cave morphology (descending passage suitable for colder air trap, long enough passage that separates entrance part from the back chamber where measurements have been performed and fissure air flux during colder winter winds). Dripwater isotopic values cluster along the local meteoric water line (LMWL) $\delta^2H = 6.82 \times \delta^{18}O + 6.86$ which has lower slope than the global meteoric water line (GMWL $\delta^2H = 8 \times \delta^{18}O + 10$) due to the enhanced evaporation during the warm season. Drip intensity at the site chosen for future study showed stable discharge with no significant response to rain events.

Mid-altitude Manita peć Cave (MP) has T_{av}= 8.8 °C (ampl. 1.7 °C), and RH = 100% in the distant part of the cave where two stalagmites (MP2 and MP3) have been collected. Rainwater isotopic composition, defined by LMWL $\delta^2H = 6.61 \times \delta^{18}O + 4.92$ resembles the Strašna peć Cave record, both by the slope and intercept, in spite of the altitude difference of 500 m. In epikarst zone dripwater isotopic signal is attenuated (rainwater $\delta^{18}O_{MPR}$ amplitude of 6‰ is reduced to dripwater $\delta^{18}O_{MPW}$ amplitudes of 0.9-1.6‰ for different sites), but the average $\delta^{18}O_{MPW}$ (-7.1‰) value is controlled by altitude effect when compared to average $\delta^{18}O_{SPW}$ (-6.4‰). Recorded drip intensities showed different hydrological regimes: stalagmite MP2 had relatively low and quite stable discharge rates with only weak response to rain events, while stalagmite MP3 site shows a fracture flow response whit drip rate highly correlated with rain events.

The highest Spilja u Zubu Buljme Cave (ZB) significantly differs from previous caves by lower temperature T_{av}= 4.0 °C, but also by quite large amplitude of 5.3 °C, and relative humidity which occasionally decreases to 95%. The reasons for those microclimate settings are the highest altitude, high tertiary porosity that sustains ventilation and ascending passage suitable for seasonal warm air accumulation. Nevertheless, dripwater $\delta^{18}O_{ZBW}$ signal does not vary a lot ($\delta^{18}O_{ZBW}$ = -8.2 – -7.4‰) unlike the rainwater composition with $\delta^{18}O_{ZBR}$ = -13.4 – -5.9‰. The LMWL $\delta^2H = 7.83 \times \delta^{18}O + 14.45$

has the slope of the GMWL, but the interception deviates to the higher value that are characteristic for the water vapour deriving from Mediterranean area.

In accordance with the expected altitude effect, isotopic composition of rainwater becomes lighter with increased altitude being: $\delta^{18}O_{SPR}$ = -6.4‰, $\delta^{18}O_{MPR}$ = -6.9‰ and $\delta^{18}O_{ZBR}$ = -9.2‰. Average gradient is $\Delta\delta^{18}O/100m$ = -0.2‰, but with different lower ($\Delta\delta^{18}O/100m$ = -0.1‰) and higher parts ($\Delta\delta^{18}O/100m$ = -0.32‰). As for the groundwater, values of $\delta^{18}O_{SPW}$ = -6.4‰, $\delta^{18}O_{MPW}$ = -7.1‰ and $\delta^{18}O_{ZBW}$ = -7.8‰, give the gradient of $\Delta\delta^{18}O/100m$ = -0.1‰.

Deuterium excess ($d = \delta^{2}H - 8 \times \delta^{18}O$) controlled by the kinetic fractionation associated with evaporation helps to define the source and trajectories of the atmospheric moisture. Obtained values of d_{ZB} = 16.0‰, d_{MP} = 14.6‰ and d_{SP} = 14.5‰ fit between Atlantic (10‰) and Mediterranean (22‰) values, indicating both continental and maritime influences.

Cave air temperatures do not follow the external trend since, due to the morphology, the remote parts of the caves with stable cave conditions act as the "pockets" for the cold (in descending chamber) or warm air (in ascending chamber) throughout the year. Cave air temperature variations are governed by conduction during the warm season and by advection during the colder part of the year when ventilation occurs. Unlike two lower caves (Strašna peć and Manita peć), the highest one (Spilja u Zubu Buljme) does not fulfil requirement of low-amplitude temperature variations. Moreover, in spite of well attenuated dripwater signal, investigation on equilibrium condition and palaeoclimate records will not continue in this cave due to the lack of recent carbonate precipitation and no adequate flowstone formations. Strašna peć and Manita peć caves appeared to be suitable study sites regarding cave climate and drip hydrological settings, and it is likely that speleothem calcite has been precipitated in isotopic equilibrium with dripwater, providing the reliable records of past events.

Keywords: cave climate, speleothems, dripwater, isotopic composition, altitude gradient, palaeoclimate, Croatia.

Resistance of karst cave microbial strains to extreme factors

Olga Suslova, Oleksandr Tashyrev

Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, Kyiv, Ukraine

Background. Karst caves can be characterized as extreme ecosystems with complete absence of light, stable physical and chemical conditions (temperature, humidity, concentration of organic compounds) for hundreds of years. There could be find rare and extreme microorganisms, which are relics of the past geological periods. Usually microorganisms from extreme ecosystems are resistant to extreme factors (e.g. toxic metals or organic xenobiotics) and are able to interact with them. Thus, these ecosystems can serve as a testing ground for bioprospecting - screening industrially-promissing microorganisms. We suggest a hypothesis of cave microorganisms' high resistance to model toxic metal (Cu^{2+}) and organic xenobiotic (p-nitrochlorobenzene) because of high adaptive potential of cave microorganisms.

Objectives. We have chosen 25 chemoorganotrophic strains isolated from Mushkarova Yama (Ukraine) and Krubera-Voronya (Abkhazia) cave clays.

Aim. Our work was aimed to determining the quantitative indices of cave strains resistance to the three types of extreme factors: electromagnetic radiation (UV), inorganic xenobiotics (toxic metal Cu^{2+}) and organic xenobiotics (p-nitrochlorobenzene - NCB).

Methods. Microbial strains were tested by microbiological, chemical and analytical methods.

Results. Cave strains have low resistance to UV-radiation. Most strains characterized by LD99,99 40-100 J/m^2. But 20% of strains were resistant to 150-200 J/m^2. Approximetely 30% of tested strains

were highly resistant to toxic metals (maximum permissible concentration is 200-1000 mg Cu^{2+}/l) which is in 3-4 orders higher than those in cave clays. These strains can also interact with Cu^{2+} by cation accumulation and reduction it to Cu(I). More then 90% of cave strains were highly resistant to NCB in the concentration range 200-300 mg/l, which is 10 times higher than bactericidal concentrations. Cave strains are not olny resusistant to NCB but also interact with it within transformation to chloroaniline and further destroying of aromatic ring.

Conclusion. The studies carried out suggest the validity of our hypothesis about high resistance of cave strains to extreme factors. Isolated microbial strains could be used as a base of new environmentaly friendly bioremediation biotechnologies (for example, wastewater treatment).

Keywords: cave strains, extreme factors, resistance, biotechnologies.

Geomicrobiology of Irazu volcanic caves: preliminary results

Andrés Ulloa [1,2,5], Mariangela Vargas [4,5], Lorena Uribe [3], Walter Hernández [3], Marielos Mora [3]

[1]- Centro de Investigaciones en Ciencias Geológicas, Universidad de Costa Rica, San José, Costa Rica
[2]- Karst Research Institute, Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia
[3]- Centro de Investigación en Biología Celular y Molecular, Universidad de Costa Rica, San José, Costa Rica
[4]- Instituto Clodomiro Picado, Universidad de Costa Rica, San José, Costa Rica
[5]- Grupo Espeleologico Anthros, Costa Rica

Irazú Volcano is the highest volcano in Costa Rica (altitude 3432 masl), part of an andesitic shield located in the southeast of the Central Volcanic Range. The main crater (active between 1962 and 1965) has a nearly circular shape and presently hosts an intermittent lake. Three volcanic caves have been found at northwest of the main crater, in a geological unstable area affected by intense hydrothermal alteration: Cueva de los Minerales, Cueva de los Mucolitos and Cueva del Pizote Espantado. These caves present a particular environment, at an altitude near to 3000 masl, with volcanic vents with high temperature and H_2S concentrations, low pH, and high diversity of minerals, mainly sulfates (Ulloa et al., 2013) Acidophiles organisms similar to "snottites" have been found. Some of these organisms are growing in the volcanic pyroclastic rocks, on a mineral substrate (presumably gypsum) and others are associated with mineral precipitations. Most of them are living in low pH (< 2) and in different ranges of luminosity. Preliminary microscope observations suggest the presence of: bacteria including cocci, bacilli and spirilli; flagellates; a variety of diatoms and in areas of the cave with natural illumination algae have been observed growing in association with crust minerals in the walls and Geysermites. Due to the extreme environmental conditions described, possible thermophiles and chemolithothrophs might also be growing inside the caves in the active volcanic vents and in the sulfur and iron-enriched minerals, respectively.

Ulloa, A., Campos-Fernández, C. S., Rojas, L., 2013. Cueva Los Minerales, volcán Irazú, Costa Rica: descripción, mineralogía y origen. Revista Geológica de América Central: 169-187.

Keywords: volcanic caves, snottites, minerals, acidophiles, thermophiles, chemolithotropic.

The biodiversity profile of cave drip water in Velika Pasica Cave, Central Slovenia

Allen Wei Liu, Anton Brancelj

Department of Freshwater and Terrestrial Ecosystem Research, National Institute of Biology, Ljubljana, Slovenia

Epikarst harbours a rich fauna of obligate subterranean-dwelling aquatic species, i.e., stygobionts, especially among the Copepoda (Crustacea). From May 2006 until February 2013, 80 sample sets were collected and twelve species (predominantly stygobionts) were identified from four permanent drip sites in the Velika Pasica Cave, Central Slovenia. The abundance of stygobionts differed from site to site, and VP2 was the site with majority of specimens. Based on the abundance and distribution of collected specimens, these stygobiotic species could be cluster into four different and distinct groups. Additionally, long-term composition of stygobiotic species varied from site to site, especially at the drip site VP4. Further research will focus on the impacts of the hydrological and hydrochemical characteristics of the epikarst water on the composition and variation of stygobiotic species present there.

Keywords: cave drip water, stygobionts species, Velika Pasica Cave.

LIST OF AUTHORS


ZNANSTVENORAZISKOVALNI CENTER
SLOVENSKE AKADEMIJE
ZNANOSTI IN UMETNOSTI