



**21st International Conference  
on Subterranean Biology**

**2 — 7 September 2012, Košice, Slovakia**

**ABSTRACT BOOK**



Pavol Jozef Šafárik University in Košice





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on Subterranean Biology  
2–7 September, 2012, Košice, Slovakia**

## **Abstract book**

Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University  
in Košice, Slovakia

State Nature Conservancy, Slovak Caves Administration, Liptovský Mikuláš,  
Slovakia

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Organizers:

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in Košice, Slovakia

State Nature Conservancy, Slovak Caves Administration, Liptovský Mikuláš,  
Slovakia

Organized under the auspices of the International Society for Subterranean Biology.

Published by Pavol Jozef Šafárik University in Košice, Slovakia.

Edited by Ľubomír Kováč, Marcel Uhrin, Andrej Mock & Peter Ľuptáčik

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permission from the publisher.

The authors are responsible for scientific and linguistic standard of the book.  
Manuscript did not pass through editorial or linguistic correction.

Typography: Marcel Uhrin

Cover design: Pavel Maria Smejkal

Printed by: Equilibria, s. r. o., Košice

ISBN 978-80-7097-959-4

**21st International Conference on Subterranean Biology  
2–7 September, 2012, Košice, Slovakia**

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## **History of conferences of subterranean biology ...**

- I<sup>st</sup> International Symposium of Biospeleology**  
10–14 September 1979, Moulis, France
- II<sup>nd</sup> International Symposium of Biospeleology**  
28–29 June 1980, La-Chapelle-En-Vercors, France
- III<sup>rd</sup> International Symposium of Biospeleology**  
7–11 September 1982, Beziers, France
- IV<sup>th</sup> International Symposium of Biospeleology**  
2–5 July 1984, Dijon, France
- V<sup>th</sup> International Symposium of Biospeleology**  
1986, Roma, Italy
- VI<sup>th</sup> International Symposium of Biospeleology**  
21–24 September 1987, Saint-Amand-Montrond, France
- VII<sup>th</sup> International Symposium of Biospeleology**  
3–7 October 1988, Hamburg, Germany
- VIII<sup>th</sup> International Symposium of Biospeleology**  
10–15 September 1990, Reims, France
- IX<sup>th</sup> International Symposium of Biospeleology**  
1991, Liège, Belgium
- X<sup>th</sup> International Symposium of Biospeleology**  
7–12 September 1992, Puerto de la Cruz, Spain
- XI<sup>th</sup> International Symposium of Biospeleology**  
28 August – 2 September 1994, Firenze, Italy
- XII<sup>th</sup> International Symposium of Biospeleology**  
28 August – 2 September 1995, Moulis, France
- XIII<sup>th</sup> International Symposium of Biospeleology**  
20–27 April 1997, Marrakech, Morocco
- XIV<sup>th</sup> International Symposium of Biospeleology**  
19–26 September 1999, Makarska, Croatia
- XV<sup>th</sup> International Symposium of Biospeleology**  
8–15 July 2001, Intervales, Brazil
- XVI<sup>th</sup> International Symposium of Biospeleology**  
8–15 September 2002, Verona, Italy
- XVII<sup>th</sup> International Symposium of Biospeleology**  
25–30 November 2004, Raipur, India
- XVIII<sup>th</sup> International Symposium of Biospeleology**  
10–15 July 2006, Cluj – Napoca, Romania
- XIX<sup>th</sup> International Symposium of Biospeleology**  
21–26 September 2008, Fremantle, Australia
- XX<sup>th</sup> International Conference on Subterranean Biology**  
29 August – 3 September, Postojna, Slovenia

# Foreword

Welcome to the 21st International Conference on Subterranean Biology! It is great honour for conference organizers to held this event in Košice, the second largest city of Slovakia. During previous conference in Postojna in 2010 General Assembly of the International Society of Subterranean Biology (ISSB-SIBIOS) approved candidature of Slovakia for 2012. These conferences are scheduled in two-year time span and are held under the auspices of ISSB-SIBIOS. Thus, Slovakia follows after Slovenia, both small European countries in the centre of interest of speleobiology. Program of the present conference adopted principal schedule established in Postojna.

There are more than 6200 caves in Slovakia, all belonging to the state property. The Slovak Caves Administration in Liptovský Mikuláš manages the exploitation, research and protection of caves and their safe and sustainable use. The conference logo was inspired by endemic troglobiotic pseudoscorpion *Neobisum (Blothrus) slovacum* discovered by Gulička in 1977 in a cave on the Plešivská Plateau, Slovak Karst. The discovery represents a landmark in biospeleology of our country. *N. slovacum* and troglobiotic millipede *Typhloiulus polypodus* (Loksa, 1960) are among the first documentations of obligate cave fauna on territory of the Western Carpathians in Central Europe.

The scientific program of the conference is divided into several symposia offered in advance, the tradition started in Postojna 2010. "Subterranean realm – fragile ecosystems and unique biodiversity: towards new concepts" is a central topic of this scientific event. It highlights the necessity of complex study of subterranean environments.

By far the highest number of oral and poster presentations is listed in symposium „Subterranean Biodiversity“. It simply reflects the fact that we are just at the beginning of explorations of subterranean biodiversity. Even in temperate regions of the world still new invertebrate species highly adapted to cave life are discovered every year. Along with drastic changes in the landscapes that are induced by human activities a question appears: „Will we manage to monitor all the underground diversity before the particular areas are destroyed?“ Every data on the distribution of endemic taxa, the most threatened creatures since their high sensitivity to disturbance, may be very valuable for conservation of particular subterranean habitat or karstic area. Symposium "Phylogeography, Phylogeny and Evolution" includes high number of contributions thus reflecting dynamic development of such studies supported by molecular biology. Symposia "Adaptation, Development, Physiology and Behaviour", "Population and Community Ecology of Subterranean Organisms" and "Subterranean Ecosystems" are logically ordered within the program with well established background of lectures and posters. Symposium "Microbiology, Geomicrobiology and Sedimentology" became

an organic part of the conferences since drawing gradually increasing attention. Symposium “Conservation of Subterranean Life” scheduled at the end of program represents an essential part of the conference underlining the growing importance of cave protection.

In total abstracts of 70 oral and 67 poster presentations, and two documentaries were submitted for the conference. Such number allowed us to arrange scientific program within four conference days avoiding parallel symposia and giving the space for all participants that were interested to give lectures and present posters. A bit busy program, however, we hope that it will draw your full attention in comfort. The conference is visited by altogether 114 registered participants from 23 countries.

Other events include welcome reception in Guest House Grand on Sunday evening and Košice downtown visit by walk with guide on Monday. The mid-conference excursion is planned for Wednesday and is described in detail in these proceedings. The program during the excursion is again rather busy since we would like to accompany you to two of the most outstanding show caves of the country. The conference will end with the social dinner on Friday evening at House of Art.

We hope that during the conference many new ideas and co-operations will arise between the participants of different countries. As conference organizers we are lucky to be a part of the community of enthusiastic people that not only want to discover secret life of caves but also want to protect for further generations this fascinating world hidden from man eye.

Have a pleasant stay in Košice – the European City of Culture 2013!

On behalf of organizing committee

**Lubo Kováč**



# General information

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## Conference venue

The conference will take place at:

### Conference Hall

Theological Faculty of Catholic University, Hlavná street 89, see the map on cover page 3

### Guest House Grand

Kováčska street 65–67, see the map

Welcome reception – Sunday evening

Lunches during conference days

### House of Arts

Moyzesova street 66, see the map

Conference Dinner – Friday evening

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## Registration

Registration is on Sunday, 2 September from 17:00 hours onward at the Guest House Grand (see the map), or every conference day, except Wednesday, from 8:00 onward at the registration desk in the main foyer in front of Conference Hall. Extra tickets for single events and single day registration fees can be paid directly at the registration desk. If you wish to purchase extra meals please let us know at least two days in advance.

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## Lunch

Lunches are organized during the conference and are included in the registration fee. Lunch tickets will be handed out at the registration.

Dinner will be organized on Wednesday, 5 September (at the excursion) and on Friday, 7 September (Conference Dinner).

During breaks, non-alcoholic beverages and refreshments will be served.

## Other places to eat

Conference Hall is located right near the main street (pedestrian zone) in Košice downtown. In the close distance you will find many restaurants and pizzerias.

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## Mid-conference excursion

Wednesday, 5 September

Please confirm your participation in the excursion at the time of registration. Later registrations will be accepted till Monday 3 September.

Buses will depart from the public parking place in front of Hotel Doubletree by Hilton (Hlavná street 1, see the map) **exactly at 7:00 hours**. This rather early time is important to manage all the schedule of mid-conference excursion.

Walking shoes are required, light hiking gear is recommended. Be prepared for rainy weather and do not forget that we will visit Dobšinská Ice Cave where the temperature moves around zero (Celsius). A flashlight or headlamp will be useful, too.

Water and snacks will be available on the busses.

All transportation and admittance costs, lunch, dinner, and basic insurance during the excursion are included in the registration fee.

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## **Instructions for speakers, chairpersons and poster presenters**

### **Oral presentations**

The time slot of each talk can be seen in the program. Please respect strictly the time limit of 15 minutes per presentation. For the key-lectures time limit of 25 minutes is offered. Five minutes are available for discussion after each presentation.

Standard MS PowerPoint presentations (saved also in 2003 version) on a USB memory stick (or CD, DVD) should be handed to the person in charge at the presentation computer at least 15 minutes before the start of your session. If you use other presentation formats or older versions of MS PowerPoint, please check that your presentation runs smoothly on the presentation computer.

If you need an overhead projector (transparencies) please let us know at least one day beforehand. A 35 mm slide projector will not be available.

Asterisk (\*) indicates presenting author.

### **Chairpersons**

Have the very important task of assuring that the program proceeds exactly according to the time plan. You are authorized to stop a speaker if he/she exceeds his/her time limit.

Please check the contents of your session and the speakers well before the time of the session.

Introduce each speaker and the title of his lecture briefly. There is no time to give a detailed account of the speaker and his topic.

After each talk questions are welcome if there is enough time available.

### **Posters**

Posters can be hung on Monday, 3 September at any time in lounge of the same building as oral presentation will be realized. Look for your name and title on the poster boards. Mounting tape and pins will be provided.

Please stand by your poster during session on Thursday 6 September at 18:20–19:30.

Please leave your posters mounted until Friday so that they can be seen throughout the conference.

# Scientific program

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## Sunday, 2 September: Guest House Grand

18:00 and ongoing

Registration

20:00

Welcome reception

Welcome to the conference participants will be given by Ass. Prof. Gabriel SEMANIŠIN, Dean of the Faculty of Science, P. J. Šafárik University in Košice

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## Monday, 3 September: Conference Hall

8:00 and ongoing

Registration, poster hanging

9:00–10:00

Opening ceremony

Dr. Zdenko TREBULA, President of the Košice Self-governing Region

Dr. Richard RAŠI, Mayor of Košice

Prof. Ladislav MIROSSAY, Rector of P. J. Šafárik University in Košice

Dr. Giuseppe MESSANA, President of the International Society of Subterranean Biology

10:00–10:30

Opening Lecture

KOŠEL V.: History of the speleozoological research in Slovakia

10:30–11:00

*Coffee break*

### Symposium: Subterranean Biodiversity and Biogeography

Chair: D. C. Culver

11:00–11:30

**Key-lecture** – TRAJANO E.: Describing biodiversity: implications for subterranean biology and conservation

11:30–11:50

HUDEC I.\* & MOCK A.: Genus *Niphargus* in Slovakia – a permanent surprise?

11:50–12:10

MAURICE L.\*, SORENSEN J., NEWBOLD L., READ D., ALLEN D., EDWARDS F. & BLOOMFIELD J.: Hydrogeological borehole investigations of groundwater ecology in the English Chalk

12:10–12:30

DUMNICKA E.: Is the troglobiotic and stygobiotic fauna rich in Kraków – Częstochowa Upland (karstic region in southern Poland)?

12:30–13:00

**Key-lecture** – STEIN H. & HAHN H. J.\*: Bioregions or stygoregions? Biogeographical patterns as references for groundwater assessment

13:00–14:00

*Lunch*

14:20–14:50

**Key-lecture** – HOLSINGER J. R.\*, HAZELTON E. A., HOBBS III H. H. & ANDERSON K. A.: Distribution of stygobiotic amphipod species in glaciated regions of carbonate and non-carbonate bedrock in Ohio, USA

- 14:50–15:10 ESMAELI-RINEH S.\* & SARI A.: Description of two new species of *Niphargus* from cave systems in Iran
- 15:10–15:30 OLIVEIRA M. P. A.\* & FERREIRA R. L.: The potentially troglomorphic fauna in Brazilian iron ore caves
- 15:30–16:00 **Key lecture** – ZAGMAJSTER M.\*, EME D., FIŠER C., GALASSI D., MARMONIER P., STOCH F. & MALARD F.: Groundwater crustacean biodiversity in Europe
- 16:00–16:30 *Coffee break*
- Chair: P. Trontelj**
- 16:30–16:50 **Key lecture** – HALSE S.\* & PEARSON G.: Why trap troglofauna: nets provide superior catches
- 16:50–17:10 PALACIOS-VARGAS J. G.: Biogeography of Mexican cave Collembola after Morrone provinces
- 17:10–17:30 TAJOVSKÝ K.: Diversity of millipedes, centipedes and terrestrial isopods in subterranean habitats of the Czech Republic
- 17:30–17:50 BICHUETTE M. E.\* & SIMÕES L. B.: Influence of substrate type in the fauna composition of the Angelica Cave, central Brazil – the test of visibility and relevance
- 17:50–18:10 BEDEK J.\*, LUKIĆ M., JALŽIĆ B., OZIMEC R., BILANDŽIJA H., DRAŽINA T., HAMIDOVIĆ D., PAVLEK M., PATARČIĆ I. & KOMERIČKI A.: Subterranean community from Lukina jama – Trojama cave system, the deepest cave in Dinaric Karst (Northern Velebit Mt., Croatia)
- 18:30–19:30 **Košice historical centre – sightseeing by walk**

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## Tuesday, 4 September: Conference Hall

### Symposium: Subterranean Biodiversity and Biogeography (continued)

**Chair: P. Trontelj**

- 8:30–8:50 LUKIĆ M.\*, ČUKOVIĆ T., PORCO D., BEDOS A. & DEHARVENG L.: Species diversity and distribution of cave Collembola in Dinaric karst
- 8:50–9:10 SPENGLER C. & HAHN H. J.: Effects of groundwater warming on fauna
- 9:10–9:30 OZIMEC R.\* & PAVIČEVIĆ D.: Dinaric troglotrophic Pselaphinae (Coleoptera, Staphylinidae) – biodiversity and potential
- 9:30–10:00 *Coffee break*

### Symposium: Adaptation, Development, Physiology and Behaviour

**Chair: E. Trajano**

- 10:00–10:20 BILANDŽIJA H.\*, ČETKOVIĆ H. & JEFFERY W. R.: Convergent evolution of albinism in diverse cave adapted animals
- 10:20–10:50 **Key-lecture** – JEFFERY W. R.: An evolutionary advantage of albinism in *Astyanax* cave fish

- 10:50–11:10 FONG D. W.: Metabolic rate and behavioral responses of a stygobiont to different temperatures
- 11:10–11:30 GOTTSSTEIN S.\*, OSTRIHON Ž. & ŠKALEC S.: Can head morphology reflect habitat preference in the crustacean *Synurella ambulans* (Amphipoda, Crangonyctidae)?
- 11:30–11:50 POLAK S.: Vertical migration of the subterranean beetles as a respond to annual temperature fluctuations
- 11:50–12:10 SMRŽ J.\*, KOVÁČ L. MIKEŠ J., LUKEŠOVÁ A., ŠUSTR V. & TAJOVSKÝ K.: Nutritional biology of animals in some caves
- 12:10–12:40 **Key-lecture** – CULVER D. C.\* & PIPAN T.: Convergence and divergence in the subterranean realm – a reassessment
- 13:00–14:00 *Lunch*

**Symposium: Phylogeography, Phylogeny and Evolution**  
**Chair: O. Moldovan**

- 14:00–14:30 **Key-lecture** – FIŠER C.\*, BLEJEC A. & TRONTELJ P.: Cave amphipods as Antillean lizards: *Niphargus* ecomorph classes expose a new aspect of morphological diversity in caves
- 14:30–14:50 DELIĆ T.\*, ZAKŠEK V., FIŠER C. & STOCH F.: Revealing the consequences of Pleistocene events on present day distribution of *Niphargus stygius* (Crustacea: Amphipoda: Niphargidae)
- 14:50–15:10 KING R. A. \*, FAGAN-JEFFRIES E., COOPER S. J. B., FINSTON T. & HUMPHREYS W. F.: Resolving the systematics of stygobiotic Amphipoda using morphology and genetic analyses – Melitidae of central Western Australia
- 15:10–15:30 KONEC M. \*, TRONTELJ P., VEROVNIK R., PREVORČNIK S. & SARBU S.: Same and yet different: cave populations of *Asellus aquaticus* across Europe
- 15:30–16:00 **Key-lecture** – ABRAMS K. M. \*, GUZIK M. T., COOPER S. J. B., HUMPHREYS W. F., KING R. A., CHO J.-L. & AUSTIN A. D.: What lies beneath: molecular phylogenetics, biogeography and ancestral state reconstruction of the ancient subterranean Australian Parabathynellidae (Syncarida, Crustacea)
- 16:00–16:30 *Coffee break*
- 16:30–17:00 **Key-lecture** – EME D. \*, MALARD F., KONECNY L. & DOUADY C.: Phylogeographic history of subterranean aquatic species reveals contrasted dispersal scenarios
- 17:00–17:20 LEIJS R. \* & KING R. A.: Comparative analyses of ancestral distributions of Yilgarn (Western Australia) diving beetles and chiltoniid amphipods inferred from biogeography and phylogenies
- 17:20–17:40 KETMAIER V. \*, DI RUSSO C., RAMPINI M. & COBOLLI M.: The cave cricket genus *Troglophilus* (Orthoptera; Rhaphidophoridae): a combination of vicariance and dispersal drove diversification in the East-Mediterranean area
- 17:40–18:10 **Key-lecture** – ZAKŠEK V. \* & TRONTELJ P.: The paradox of syntopy in cryptic stygobiont lineages from the Dinaric Karst

- 18:10–18:40 **Key-lecture** – WILKENS H.: Mexican cave catfish genus *Rhamdia*: a case of parallel speciation?
- 18:40–19:00 KRIŠTŮFEK V.\* & others: Eight wonder of the Domica Cave (Slovak Karst, Slovakia) (scientific documentary) – movie
- 19:00–19:20 LUKIĆ M.\*: Collembola in caves – movie

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## Wednesday, 5 September: Mid-conference excursion

For details on excursion see page 131.

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## Thursday, 6 September: Conference Hall

### Symposium: Phylogeography, Phylogeny and Evolution (continued)

**Chair: M. Zągmajster**

- 9:00–9:30 **Key-lecture** – TRONTELJ P.\* & POLAK S.: Replicated adaptive radiations in leptodirine cave beetles
- 9:30–9:50 WEIGAND A. M.\*, JOCHUM A., SLAPNIK R., PRIETO C. E., SCHNITZLER J., ZARZA E. & KLUSSMANN-KOLB A.: Evolution of subterranean microsnailes (Carychiidae, *Zospeum*) – phylogeny and cryptic diversification
- 9:50–10:10 TAITI S.\*, ARGANO R., MARCIA P., SCARPA F., SANNA D. & CASU M.: Morphology and phylogeny of the subterranean genera *Alpioniscus* and *Utopioniscus* from Sardinia, Italy (Crustacea, Oniscidea, Trichoniscidae)
- 10:10–10:30 BEDEK J.\*, RISTORI E., TAITI S. & BARATTI M.: Phylogeography of the subgenus *Alpioniscus* (*Illyrionethes*) (Isopoda: Oniscidea: Trichoniscidae) in the Dinaric Karst
- 10:30–10:50 RŮŽIČKA V.\*, ŠMILAUER P. & MLEJNEK R.: Colonization of subterranean habitats by spiders in the Czech Republic
- 10:50–11:20 *Coffee break*
- 11:20–11:50 **Key-lecture** – OROMÍ P.\*, REBOLEIRA A. S. P. S. & LÓPEZ H.: Hotspot within hotspot: subterranean fauna of the Canary Islands
- 11:50–12:10 MELEG I. N.\*, ZAKŠEK V., FIŠER C. & MOLDOVAN O. T.: Groundwater biodiversity in Western Romanian Carpathians: the case of *Niphargus* (Amphipoda, Crustacea)
- 12:10–12:30 PELLEGRINI T. G.\*, SOUZA SILVA M., MUNARO P. & FERREIRA R. L.: Patterns of biodiversity in natural and artificial iron ore caves in Brazil
- 12:30–12:50 REBOLEIRA A. S. P. S.\* & SENDRA A.: Is there a vertical limit for the distribution of subterranean arthropods?
- 13:00–14:00 *Lunch*

## **Symposium: Microbiology, Geomicrobiology and Sedimentology**

**Chair: H. Wilkens**

- 14:00–14:20 ELHOTTOVÁ D.\* , PETRÁSEK J., JIROUT J., CHRONÁKOVÁ A., KYSELKOVÁ M. & VOLNÁ L.: The antibiotic resistance in cave environments
- 14:20–14:40 CHRONÁKOVÁ A.\* , MULEC J., KRIŠTŮFEK V. & BALDRIAN P.: Bat guano heap – the hot spot of microbial activity in cave
- 14:40–15:00 NOVÁKOVÁ A.\* , HUBKA V. & HILLEBRAND-VOICULESCU A. M.: Preliminary results on microfungal community of Movile Cave, Romania
- 15:00–15:20 TAYLOR E. L. S.\* , FERREIRA R. L. & DE RESENDE-STOIANOFF M. A.: Inventory of filamentous fungi and the relations between edaphic factors and the soil fungal community in a dolomitic cave in Brazil
- 15:20–15:40 PLEŠE B., OZIMEC R., POJSKIĆ N., ČETKOVIĆ H. & LUKIĆ-BILELA L.\*: Aquatic bacterial mats in Dinaric range caves: molecular and ecological approach
- 15:40–16:10 *Coffee break*

## **Symposium: Population and Community Ecology of Subterranean Organisms**

**Chair: H.-J. Hahn**

- 16:10–16:40 *Key-lecture* – MALARD F.\* , COLSON-PROCH C., EME D., CALVIGNAC S., JEAN P., KONECNY L., HERVANT F. & DOUADY C. J.: The role of history of place, dispersal and selective factors in shaping the distribution of a subterranean isopod
- 16:40–17:00 PELLEGRINI T. G.\* & FERREIRA R. L.: Structure and interactions in a cave guano-soil continuum community
- 17:00–17:20 CORDEIRO-BORGHEZAN L. M.\* , TRAJANO E. & BORGHEZAN R.: Distribution and population data on troglotrophic catfishes, genus *Trichomycterus*, from Serra da Bodoquena karst area, southwestern Brazil (Siluriformes: Trichomycteridae)
- 17:20–17:40 BORGHEZAN R.\* , CORDEIRO-BORGHEZAN L. M. & TRAJANO E.: Population data on two syntopic troglotrophic catfishes from Fadas cave system, Bodoquena karst area, southwestern Brazil
- 17:40–18:00 RUSDEA E.: Population dynamics of *Laemostenus schreibersi* (Coleoptera, Carabidae) in a cave in Austria – adult longevity stabilizing population size: results of a long-term research over 20 years
- 18:00–18:20 MIKOVÁ E.\* , ANDREAS M., BOLDOGH S., DOBRÝ M., JEHLIČKOVÁ V., LOBBOVÁ D., NAĐO L., ŠEVČÍK M. & UHRIN M.: *Rhinolophus euryale* (Chiroptera: Rhinolophidae): the summary results of ecological research of cave dwelling bat
- 18:20–19:30 **Poster session**

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## Friday, 7 September: Conference Hall

### Symposium: Subterranean Ecosystems

Chair: F. Malard

- 8:30–9:00 *Key-lecture* – PIPAN T.\* & CULVER D. C.: Organic carbon in aquatic shallow subterranean habitats (SSHs)
- 9:00–9:20 MOLDOVAN O. T.: Do we use the correct terms for subterranean habitats?
- 9:20–9:40 FERREIRA R. L.\*, BUENO SILVA A. P. & SOUZA SILVA M.: Trophic enrichment in underground environments and its applications in the biodiversity conservation of aquatic invertebrates
- 9:40–10:00 HAHN H. J.\* & GUTJAHR S.: Testing groundwater fauna habitats in landscape
- 10:00–10:20 ORTUÑO V. M., SENDRA A., GILGADO J. D.\*, PÉREZ-SUÁREZ G., JIMÉNEZ-VALVERDE A. & HERRERO-BORGOÑÓN J. J.: Early advances in the knowledge of the Mesovoid Shallow Substratum in calcareous massifs of the eastern Iberian Peninsula
- 10:20–10:40 SOUZA SILVA M.\*, DE MEDEIROS BENTO D., VASCONCELLOS A. & FERREIRA R. L.: Changes in the invertebrate community of caves between dry and rainy seasons in the Brazilian Savannah and Caatinga biomes
- 10:40–11:10 *Coffee break*

### Symposium: Conservation of Subterranean Life

Chair: R. Leijts

- 11:10–11:40 *Key-lecture* – MOLDOVAN O. T.\*, MELEG I. N. & EPURE L.: Fauna is in the cave mud too – is there an interest to protect cave sediments?
- 11:40–12:00 ALJANČIĆ G.\* & NĀPĀRUŞ M.: Stygobionts washed out to surface: a case of *Proteus anguinus*
- 12:00–12:20 MELEG I. N.\*, NĀPĀRUŞ M., FIERS F., MELEG I. H., VLAICU M. & MOLDOVAN O. T.: Predictive GIS modelling and conservation of copepods in groundwater habitats of the Romanian Carpathians
- 12:20–12:40 CORBETT J.\* & NOBLE B.: Conserving natural caves in the American West: unique challenges in a multiuse landscape
- 13:00–14:00 *Lunch*
- 14:00–14:20 FERREIRA R. L.\* & PELLEGRINI T. G.: Species-area model predicting diversity loss in an artificially flooded cave in Brazil
- 14:20–14:40 LUKIĆ\* M., BILANDŽIJA H., BEDEK J., JALŽIĆ B., KLJAKOVIĆ GAŠPIĆ F., OZIMEC R. & PAVLEK M.: Dinaric type localities of cave dwelling fauna: research and conservation
- 14:40–15:00 NUÑEZA O. M.\*, ENGUIITO R., LABAJO Y. & PONCE A.: Distribution and conservation of cave bats in Mindanao, Philippines
- 15:00–15:20 REBOLEIRA A. S. P. S.\*, OROMÍ P., GONÇALVES F. & CULVER D. C.: Biodiversity patterns and conservation of subterranean fauna from Portugal



**15:20–15:50**     *Coffee break*

**16:00–16:30**     **Conference close**  
**Obituary of Lazare Botoseanu**  
**Presentation of next conference venue**

**16:30–19:00**     **General Assembly of the International Society for Subterranean  
Biology**  
**Meeting of the new Council**

**20:00**             **Conference dinner – House of Art**

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OZIMEC R.\*: Juliana Pichler Stiegler (1827–1901), one of the first world female biospeleologists

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- ALJANČIĆ G.: The 250th anniversary of the first scientific description of *Proteus anguinus*
- BARJADZE S.\*, BURKHARDT U., SCHULZ H.-J., XYLANDER W. & DJANASHVILI R.: Revision of the genus *Argonychiurus* Bagnall, 1949 (Collembola: Onychiuridae)
- BICHUETTE M. E.\*, FARIA L., FERREIRA T. F., MOREIRA M. A., SANTOS P. V. S., ROCHA A. & GALLÃO J.: Cave fauna from iron caves of southeastern Brazil – first data
- CADEDU B.\*, MELIS P., LEDDA F. D. & MANCONI R.: Biodiversity assessment of Mediterranean marine caves: the taxon Porifera
- CAMPOS-FILHO, I. S., DE ARAUJO, P. B. & TAITI S.: Terrestrial isopods (Crustacea: Isopoda: Oniscidea) from Brazilian caves
- CORDEIRO-BORGHEZAN L. M.\* & SAMPAIO-COSTA C.: A high diversity spot for Onychophora in Serra da Bodoquena karst area, southeastern Brazil
- ČUKOVIĆ T.\* & LUKIĆ M.: Diversity and distribution of cave dwelling Collembola of the Velebit Mts., Croatia
- ČUKUŠIĆ A.\*, PAVLEK M. & OZIMEC R.: Diversity and distribution of cave dwelling spiders of the families Nesticidae and Agelenidae (Araneae) in Croatia
- FENĐA P.\*, LUPTÁČIK P. & MOCK A.: The family Coprozerconidae (Acari, Mesostigmata) in the caves of Palaearctic region
- GEOFFROY J.-J.\* & GOLOVATCH S. I.: The cave-dwelling millipede highly rich fauna from China and south-east Asia: additional results and new perspectives (Diplopoda)
- JALŽIĆ B., BILANDŽIJA H.\* & CUKROV M.: Distribution of the Dinaric cave-dwelling tube worm *Marifugia cavatica* Absolon & Hrabě, 1930 in Croatia
- KOMERIČKI A.\* & WEIGAND A. M.: DNA barcoding of genus *Eupolybothrus* (Chilopoda, Lithobiidae) from Dinaric Karst reveals unexpected cryptic diversity
- KOVÁČ L.\*, MOUREK J. & RENDOŠ M.: Troglotrophic collembolan *Neelus koseli* Kováč & Papáč, 2010 (Collembola, Neelidae) – contribution to its morphology, ecology and distribution
- KUČINIĆ M.\*, OZIMEC R. & JALŽIĆ B.: Diversity and distribution of troglophilic fauna of caddisflies and moths (Insecta: Trichoptera, Lepidoptera) of Croatia
- LUPTÁČIK P.: Morphology and distribution of a cave dwelling oribatid mite *Belba clavigera* (Acari, Oribatida)
- MELIS P.\*, CADEDU B., MANCA B., LEDDA F. D. & MANCONI R.: Porifera inventory from anchialine caves
- MESCOLOTTI M. B.\*, BERNARDI L. F. O. & FERREIRA R. L.: Fauna associated to artificial cavities in the state of Minas Gerais, Brazil
- MOCK A.\*, TAJOVSKÝ K. & PAPÁČ V.: Study on the genus *Hylebainosoma* sensu lato (Diplopoda, Chordeumatida, Haaseidae) – the millipedes with affinity to subterranean habitats
- MULAOMEROVIĆ J. & HODŽIĆ M.\*: *Proteus anguinus* in Bosnia and Herzegovina: from the middle ages to today

- NJUNIĆ I.\* & PAVIĆEVIĆ D.: *Parapropus pfeiferi* Apfelbeck (Leiodidae, Cholevinae, Leptodirini), a poorly known leptodirine from the north-west Bosnia
- PALACIOS-VARGAS J. G.\* & VARO DE LA ROSA G.: Cave fauna from Chimalacatlán, Morelos State, México
- PAPÁČ V.\* & KOVÁČ L.: Unexpected diversity of the genus *Megalothorax* Willem, 1900 (Collembola: Neelidae) in caves of the Carpathians (Slovakia, Romania)
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- SIMÕES L. B.\*, FERREIRA T. S. & BICHUETTE M. E.: Diversity and distribution of stygobiont fauna in karstic systems from Goiás, central Brazil – the test of visibility and relevance of habitats
- SAIT TAYLAN M., RAMPINI M.\*, DI RUSSO C. & COBOLLI M.: Cave crickets (Orthoptera, Rhaphidophoridae) from Turkish subterranean habitats: an update of taxonomy and geographical distribution
- SVATOŇ J. \* & GAJDOŠ P.: Spider fauna (Araneae) of Slovak caves
- SUSAC R.\* & ZAKRZEWSKA B.: Filling the gaps; using biodiversity surveys of cavernicolous invertebrate fauna to provide a spatial analysis of diversity patterns from Gunung Mulu National Park, Sarawak
- UCHOA M. P.\* & BICHUETTE M. E.: Malacofauna diversity recorded in Brazilian caves and their ecological importance
- ZAGMAJSTER\* M., TURJAK M. & SKET B.: Database on subterranean biodiversity of the Dinarides and neighboring regions – SubBioDatabase

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- AVRAMOV M.\*, ROCK T., RIEB J., PFISTER G., SCHMIDT S. I., SCHRAMM K.-W. & GRIEBLER C.: Do catecholamines reflect temperature stress in groundwater and surface water amphipods?
- GIURGINCA A., TAJOVSKÝ K. & ŠUSTR V.\*: Morphological structures on the integument of *Mesoniscus graniger*
- MIKOVÁ E.\*, UHRIN M., VARCHOLOVÁ K. & BOLDOGH S.: Trophic basis of winter activity in *Rhinolophus euryale* (Chiroptera)
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- KING R. A. \*, LEIJS R. & MURPHY N. P.: Evidence for numerous subterranean colonisations: using molecular and morphological approaches to define the Australian chiltoniid amphipods
- MURIEL-CUNHA J.\* & TRAJANO E.: The phylogenetic mapping and evolution of subterranean habit in catfishes (Teleostei: Siluriformes) based on molecular data
- NĀSTASE-BUCUR R., BORDA D. & KETMAIER V.\*: Comparative phylogeography in two

obligate cave beetle species from Romania (genus *Pholeuon*; tribe Leptodirini) based on mitochondrial DNA

TULIĆ U., KARAMAN I. M. & LUKIĆ-BILELA L.\*: Preliminary results of molecular genetics characterization and phylogenetic analysis of selected taxa within family Nemastomatidae (Simon, 1872) in the Western Balkans

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### **Population and Community Ecology of Subterranean Organisms**

BALOGOVÁ M.\* & UHRIN M.: Wintering of *Salamandra salamandra* (Lissamphibia) in subterranean roosts

MATOČEC N., OZIMEC R.\* & KUŠAN I.: *Polycephalomyces ramosus* (Hypocreales, Ascomycota) an interesting troglophilic entomogenous fungus, new for Croatia

PAPI F.\* & PIPAN T.: Ecological studies of epikarst communities: results from caves in Slovenian Alps

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### **Subterranean Ecosystems**

HAJKOVÁ B. & MOCK A.\*: Tufa cellars in the Tokaj wine region (Zemplínske vrchy Mts., SE Slovakia) as artificial subterranean habitats

- MESSANA G.\*, BARATTI M., DI LORENZO T., FILIPPELLI M.T., MASCALCHI C., PARIBELLI S., RISTORI E., VENTURA S. & SAMBUGAR B.: The RIPARI project: a vegetated buffer zone and its effect upon a groundwater biocoenosis
- REBOLEIRA A. S. P. S.\* , GONÇALVES A. M. M., BESSA M., PEREIRA R., DE FIGUEIREDO D., GONÇALVES F. & ABRANTES N.: Quality assessment of groundwater and sediments of the Iberian Peninsula largest karst aquifer
- SENDRA A., ORTUÑO V. M., REBOLEIRA A. S. P. S., GILGADO J. D.\* & TERUEL S.: Colonization of hypogenic caves by terrestrial arthropods: consequences in the biodiversity patterns of subterranean species
- SOUZA SILVA M.\*, REZENDE R. K. S. & FERREIRA R. L.: Detritus processing in a shallow groundwater without invertebrate shredders action

### **Conservation of Subterranean Life**

- BEDEK J., HORVATOVIĆ M. & KARAMAN I.: A new isopod genus (Crustacea: Isopoda: Oniscidea) from the cave system Vilina špilja – izvor Omble (Dubrovnik, Croatia): extinction before description?
- GALLÃO J. E.\* & BICHUETTE M. E.: A new hotspot for Brazilian subterranean fauna – the remarkable Igatu, a pseudokarstic region from Chapada Diamantina, northeastern Brazil, with a test of data repeatability
- ŠTURM S.\*, BELTRAM G., ZORMAN T.\*, PERIC B., MULEC J., PIPAN T. & POLAK S.: Tourism and conservation: monitorings in show caves (Škocjan Caves)
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# Abstracts

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Phylogeography, Phylogeny and Evolution: oral presentation

## **What lies beneath: molecular phylogenetics, biogeography and ancestral state reconstruction of the ancient subterranean Australian Parabathynellidae (Syncarida, Crustacea)**

Kym M. ABRAMS<sup>1\*</sup>, Michelle T. GUZIK<sup>1</sup>, Steve J. B COOPER<sup>1,2</sup>, William F. HUMPHREYS<sup>3</sup>, Rachael A. KING<sup>1,2</sup>, J.-L. CHO<sup>4</sup> & Andrew D. AUSTIN<sup>1</sup>

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The putatively ancient subterranean crustacean family Parabathynellidae has been poorly studied, in part because of the problem of obtaining material from difficult to access subterranean habitats in which they live. Further, the systematics of the group has been complicated by their generally simplified morphology and isolated descriptions of new taxa in the absence of any phylogenetic framework. Here, we use a comprehensive molecular systematics framework for Australian Parabathynellidae based on COI and 18S sequence data, to explore phylogenetic relationships amongst parabathynellids, their diversity, some aspects of character evolution and their biogeographic history within Australia. Our results suggest that genera are largely monophyletic and revealed numerous unknown taxa. They also provide evidence for high levels of endemism in Australia, in addition to uncovering ancient connections amongst clearly disparate geographic locations. The tendency towards short-range endemism has rendered parabathynellids vulnerable to perturbations of groundwater, which has significant implications for their management. The conservation value of these parabathynellids is a high priority not only because of their uniqueness, but also because of their role in biofiltration and as bioindicators of groundwater quality. These results also emphasize the conservation importance of groundwater habitats.

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Adaptation, Development, Physiology and Behaviour: poster presentation / student

## **Larval stages of Leptodirini from Cantabrian region of Iberian Peninsula (Coleoptera, Cholevinae)**

Beata ADAMCZYK

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Tribe Leptodirini belongs to Cholevinae (Coleoptera, Leiodidae) and is the second largest group of beetles living in the subterranean environment (Fresneda et al. 2007). It is distributed in the

Mediterranean basin, southern half of the continent as well as in non-Mediterranean countries (Romania, Slovakia, the Czech Republic, Austria and Great Britain) (Moldovan 2008). The tribe includes about 1800 species and almost 95% cave-dwelling representatives are troglobiotic (Peck 1998). As result of living in such restrictive environment there are many morphological modifications, progressive (elongation of the legs and antennae) or regressive (absence of wings, pigmentation and eyes). Their life cycle is also modified. Part of Leptodirini have two or one larval stages, some larvae do not feed and the size of eggs grows, when number of larval stages is reduced. The study focuses on larval stages of sections *Quaestus* (genus: *Quaestus*, *Breuilia*, *Breuilites*, *Espanoliella*, *Leonesiella*) and *Speonomidius* (genus *Notidocharis*) from caves of Cantabria region of Iberian Peninsula (Spain). Chaetotaxy, measurements, morphology and DNA sequencing of larval stages of 21 species are studied to their type of development and phylogeny.

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Subterranean Biodiversity and Biogeography: poster presentation

## **Canae Gabru cave: a chiropteran dominated ecosystem in south of Iran**

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The Canae Gabru Cave (the name means “house of the fire worshippers”) is locating in the village of Tadovan, about 65 km north of Jahrom in the Fars province. Just north of the village a tributary passes through a deep cleft in a ridge and forms a canyon. This cave is located high on the west-facing cliff. Portions of the cave entrance and many other clefts in the cliff wall adjacent to and across from the cave are walled up with rocks. These are too high on the cliff face to have been used as livestock pens and are probably remnants of human habitation. Subterranean habitats are always unusual and fascinating. These ecosystems are characterized by darkness, constant temperature and high humidity with limited air currents which some organisms are attracted to live in such ecosystems at least for part of their life cycle due to low predation pressure. The Canae Gabru Cave is a large and complex cave inhabited by many species of bats. The cave is a home to at least nine species of bats including *Rhinopoma microphyllum*, *R. muscatellum*, *Myotis blythii*, *M. capaccinii*, *Rinolophus ferrumequinum*, *R. blasii*, *R. euryale*, *R. hipposideros*, and *Miniopterus schreibersi*) in four seasons. In the first chamber, we found approximately 300 *Rhinopoma* individuals of both species. In other parts of the cave, *R. euryale*, *R. blasii*, *R. hipposideros*, *Myotis blythii*, *M. capaccinii*, and *M. schreibersi* hung from the cave ceiling. Several bat specimens were surveyed for ectoparasites. The ectoparasites included the genera *Spinturnix*, *Eyndhovenia*, *Ixodes*, *Pencilidia*, and the family Sterblidae. Moreover, we found several species of cave-dwelling animals. In small chamber we found the *Calomyscus bailwardi* and several geckos (*Hemidactylus persicus*) and large spider.

## **The 250th anniversary of the first scientific description of *Proteus anguinus***

Gregor ALJANČIČ

Tular Cave Laboratory, Oldhamska cesta 8A, 4000 Kranj, Slovenia

*Proteus anguinus* is most probably the first cave animal ever scientifically described. The name with a brief description of this species was published by Joseph N. Laurenti in 1768, and is still valid today. However, the very first description had been presented even earlier, by the famous naturalist Joanes Antonius Scopoli (1723–1788), in his letter to Carl Linnaeus, dated on May 3, 1762. Scopoli was an Italian physician, appointed at the mercury mine in Idrija, today Slovenia (1754–1769). He became one of the important naturalists of his time, with special dedication to fungi, plant and animal taxonomy of Carniola (Central Slovenia). Among higher taxa, Scopoli introduced Caudata. Many of his descriptions of new species from Carniola were included in the Linnaeus' Systema Naturae, and Linnaeus himself named him as one of the "auctores reformatoris". In this letter, a blind amphibian, discovered in a karst spring near Stična, Slovenia, was offered to be included in the Systema Naturae, under the name *Lacerta caeca*. The letter is preserved in the collections of the Linnean Society, but the two drawings and the specimen are lost. While Linnaeus' reply is also lost, it is clear that the apparent larval characteristics of a neotenic *Proteus* postponed the final decision of Linnaeus – until further observations would have confirmed that the metamorphoses could not occur. In 1772, four years after Laurenti, Scopoli finally published his description, opposing the Laurenti's erroneous note on the habitat and locus classicus of *Proteus*. Scopoli concludes with a comment, illustrating a puzzle of the early biospeleology; in Latin: "The famous Linnaeus, to whom I have sent the picture, considers it to be the larva of *Lacerta*. In my opinion it is a discrete genus."

## **Stygobionts washed out to surface: a case of *Proteus anguinus***

Gregor ALJANČIČ\* & Magdalena NĀPĀRUŠ

Tular Cave Laboratory, Oldhamska cesta 8A, 4000 Kranj, Slovenia

As all stygobionts, the olm, *Proteus anguinus* (Amphibia: Urodela: Proteidae), is restricted to its subterranean aquatic habitat. Occasionally, during the night, it may appear in springs close to cave entrances. However, during seasonal flooding, some individuals get washed out onto the surface, far from their subterranean environment. The earliest description of this, apparently a not so rare phenomenon, was presented already by J. V. Valvasor in 1689. Until the early 19th century, this was the only way to obtain fresh specimens, and has revealed many new localities of *Proteus*. The Tular Cave Laboratory has extensively studied this phenomenon since 2008, documented 16 cases in Slovenia, and successfully returned 6 of these animals to their source population. In nature, the fate of these individuals is diverse, but is often predictable – the chance to reenter the underground and to survive is minimal. Animals are often deposited



on temporarily flooded fields and may survive for up to several weeks, until the high water retreats. Some are carried further into superficial streams where they are preyed upon by fish or birds. Seasonal flooding has probably been an important selective force in the evolution of *Proteus* behaviour. We presume that *Proteus* has developed several responses to reduce the danger of being washed out of its subterranean habitat, and has tuned its feeding and reproduction strategy. On the other hand, floods may also be considered as a highly hazardous way for *Proteus* to disperse into new habitats. While these adaptations remain unclear, the study of this phenomenon is opening a conservational discussion – whether to salvage these animals or let them be. The conservation efforts should also involve other stygobiont vertebrates. A rare case of good practice is the Texas blind salamander, *Eurycea rathbuni*. Because of the extreme lifespan of *Proteus* (close to 100 years in captivity) and long reproduction cycles (approx. every 8 years in captivity), this case needs an urgent conservation action plan. We suggest using the washed-out individuals for scientific investigation, education and *ex situ* breeding program, rather than collecting new animals from nature. We also propose veterinary care and a strict protocol if individuals are to be returned to their source population.

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Subterranean Biodiversity and Biogeography: oral presentation / student

## **New data to the distribution of the aquatic and terrestrial troglobiont macroinvertebrate endemisms in eight caves of the Mecsek Mountains (SW Hungary)**

Dorottya ANGYAL

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However biospeleology used to be a prosperous discipline in Hungary, at the last decades the number of the related researches has significantly decreased. In the Mecsek Mountains (SW Hungary) only two, the Abaliget Cave and the Mánfai-kölyuk Cave were previously explored in biospeleological aspect. Between 1889 and 1927 six troglobiont, endemic macroinvertebrate species were described from this two caves. The aim of the repeated sample collection carried out between May 2010 and October 2011 was to find out the presence or absence of these rare species. Six other caves which were not researched previously were also included. The blind aquatic Isopod, *Protelsonia hungarica* Mehely, 1924 occurred in 4 caves, while *Niphargus* specimens (*molnari* or *gebhardti*) were collected from 6 caves. From the Mánfai-kölyuk Cave the taxa has disappeared, as well as the blind planarian species, *Dendrocoelum pannonicum* (Mehely, 1927) which could be related to the cave's utilization by the waterworks. The Hungarian Blind Snail, *Bythiospeum hungaricum* (Soos, 1927) was collected only from the two previously explored caves. Molecular taxonomic assay conducted on the individuals of each cave has shown that the genetical divergence between the two populations is high; they should be treated as two different conservation biological units. A population of the only terrestrial troglobiont macroinvertebrate from the region, the diplopod *Brachydesmus troglobius* (Daday, 1889) was considered to be an endemic species of the Abaliget Cave. During the present study the species was found in a sinkhole as well. Sample collection should be extended to other unknown caves from the region. Phylogenetic studies on each taxon would be also desirable.

## Do catecholamines reflect temperature stress in groundwater and surface water amphipods?

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Catecholamines (CAs) are well known neurotransmitters in humans as well as in many other organisms. Under stress conditions, these substances are released into the blood stream where they regulate the physiological stress response. The function of CAs in invertebrates is far less understood, but changes in catecholamine levels under stress have been recently observed in surface water crustaceans. Concerning the function of CAs in groundwater invertebrates, no information is available so far. Groundwater fauna and surface water organisms differ considerably in terms of physiology due to specific adaptations to their native environments. Therefore, we hypothesize that the stress responses of both groups differ. Such differences might manifest particularly strongly with temperature stress: surface water animals are frequently exposed to temperature fluctuations, whereas groundwater fauna live at quite constant water temperatures throughout the whole year. With increasing usage of shallow geothermal energy, temperature stress in groundwater has become an important issue. In this study we compare the changes in CA-levels in response to sudden, short-term temperature elevations for two closely related species of amphipods: *Gammarus pulex* (surface water) and *Niphargus inopinatus* (groundwater).

## Wintering of *Salamandra salamandra* (Lissamphibia) in subterranean roosts

Monika BALOGOVÁ\* & Marcel UHRIN

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The fire salamander (*Salamandra salamandra*; family Salamandridae) occurs across large parts of Central, Western and Southern Europe. In Slovakia it inhabits deciduous and mixed forests in altitude 200–600 m, where belongs to the most common amphibians. In winter salamanders often overcome adverse conditions in subterranean roosts and despite of the importance of these environments, there is still a lack of information on the conditions occurred in such type of roosts. We monitored their wintering during November 2011 – April 2012 in three different underground sites in the eastern part of Slovakia. During 24 inspections we studied sex and age structure, body mass and location within roost of all recorded wintering individuals. Males

dominated in each of the monitored sites. Mobility in the underground shelter was evaluated only in one roost (old mine gallery). The most of individuals displayed the significant movement during wintering. They preferred freely position and half-hidden position in cavities inside the gallery. There were found larvae of the fire salamander inside hypogeous pools in two roosts. The mobility and weight changes of pregnant females during time of the laying of larvae were also observed.

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Subterranean Biodiversity and Biogeography: poster presentation

## **Revision of the genus *Argonychiurus* Bagnall, 1949 (Collembola: Onychiuridae)**

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The genus *Argonychiurus* was revised based on investigations of primary types of all species included. Before our investigation the following six *Argonychiurus* species were listed: *A. bertrandi* (Denis, 1936), *A. bogheani* (Gruia, 1989), *A. fistulosus* (Gisin, 1956), *A. lenticularius* (Gisin, 1962), *A. papulosus* (Gisin, 1964), and *A. perforatus* (Handschin, 1920). Based on our investigation the cave-dwelling *A. bogheani* (Gruia, 1989) was separated from the genus *Argonychiurus* because its morphology does not match the diagnosis of the genus. Furthermore, the cave-dwelling *A. lenticularius* (Gisin, 1962) was separated from the above-mentioned genus and a new genus *Pomorskonychiurus* was erected based on differences of the sensory clubs between *A. lenticularius* (smooth clubs with ribs) and all remaining *Argonychiurus* species (granulated clubs). One new species – *A. multiocellatus* n. sp. was described from the Tvishi Cave of Georgia and differentiated diagnosis with its morphologically closely related species – *A. fistulosus* (Gisin, 1956) was given. A key to the species in this genus was provided.

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Conservation of Subterranean Life: poster presentation / student

## **A new isopod genus (Crustacea: Isopoda: Oniscidea) from the cave system Vilina špilja – izvor Omble (Dubrovnik, Croatia): extinction before description?**

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Cave system Vilina špilja – izvor Omble near the town of Dubrovnik is the second longest cave in Dalmatia (3.036 long, 192 meters deep), containing numerous different habitats, both terrestrial and aquatic. With more than 30 troglobiotic species recorded up to date (majority

stenoendemic), it is one of the richest caves in Croatia. Seven bat species inhabit the cave and four of them have their nursery colonies in the top most level of the cave. Additionally to its high biological value it is also an important archeological site. The majority of habitats in the cave system are threatened by the construction of hydro power plant Ombla. The dam, power plant and its entire facility is planned to be built inside the cave and hill causing the water level raising up to 130 meters high and consequently flooding two entire lower levels of the cave. The top most level, the only non flooded part, should be drilled to compensate occasional high air pressure from raising water, and therefore will be destroyed too. In the entrance chamber of the topmost level, very unusual terrestrial isopod was found during biospeleological research of Croatian Biospeleological Society. It has extremely elongated body and same width of cephalon, pereon and pleon, which is very rare within Oniscidea and could be compared to the subterranean genus *Cylindroniscus* Arcangeli, 1929 (Trichoniscidae) from Mexico and Cuba. The new genus belongs to the family Styloniscidae, which has mainly Gondwanian distribution. This family is represented in Europe only by the predominately troglobite genus *Cordioniscus*, and two still undescribed new troglobite dinaric genera. The new genus shows slight affinity to the undescribed genus present in some caves in Montenegro. The new isopod has capability to bend the pereon laterally in the shape of letter „S“, and even spiral along the longitudinal axis of the body. This ability is unique within Oniscidea, and has not been reported to date. This could be adaptation to terrestrial interstitial sediment habitat, where the majority of specimens have been found, which is in concordance with extremely elongated body.

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Subterranean Biodiversity and Biogeography: oral presentation / student

## **Subterranean community from Lukina jama – Trojama cave system, the deepest cave in Dinaric Karst (Northern Velebit Mt., Croatia)**

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First biological research of deep pits in the Northern Velebit area started in 1992 with discovery of the cave system Lukina jama – Trojama. It is the deepest pit of the Dinaric Karst (-1421 m), situated in the Northern Velebit National Park. From its entrance zone to the 320 m of depth it is filled with ice and snow and therefore not suitable for the majority of cave-dwelling fauna. The temperature range (measured in 2010) gradually increases from below 0°C (entrance) to 4.9 °C (depth of 1381 m). Deepest part of the pit reaches phreatic zone which is explored up to 40 m in depth. During high water stands water can rise more than 100 m. Altogether 29 taxa are recorded in Lukina jama – Trojama cave system, 20 troglobionts (Gastropoda 1; Palpigrada 1; Acari 2; Opiliones 2; Pseudoscorpiones 1; Isopoda 2; Chilopoda 1; Diplopoda 1; Collembola 5; Diplura 1; Coleoptera 3); 7 stygobionts (Porifera 1; Bivalvia 1; Hirudinea 1; Polychaeta 1; Amphipoda

2; Decapoda 1); 1 tentative troglophile (Diptera) and 1 troglaxene (Chiroptera). Almost total absence of trogliphiles can be explained with low temperatures in the entrance parts of the pit. The number of collected specimens of most taxa is extremely low considering the collection effort. In total 16 troglobionts and 2 stygobionts found in the cave system are endemic for Velebit Mt. and Lika region. However, several troglobionts recorded in deep pits only few kilometers away from Lukina jama – Trojama have not been found (Hirudinea 1; Araneae 2; Pseudoscorpiones 1; Isopoda 1; Collembola 1; Coleoptera 1). Along with a species of Coleoptera adapted to hygropetric habitat, extremely troglomorphic representatives of Collembola and Isopoda terrestria were found. In comparison to the world's deepest subterranean community from Krubera – Voronja Cave (Western Caucasus), Lukina jama – Trojama has higher species richness (29 vs. 16 taxa) probably as a result of more frequent collection effort but also biogeographical position. The vertical distribution of species richness is different where the highest number of collected taxa in Voronja-Krubera is in the entrance zone (60 m deep) and in Lukina jama – Trojama in a chamber at 1000 m depth. About half of the taxa collected in Lukina jama – Trojama are still undescribed, including several new genera, but more specimens are needed for the taxonomical studies.

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Phylogeography, Phylogeny and Evolution: oral presentation / student

## Phylogeography of the subgenus *Alpioniscus* (*Illyrionethes*) (Isopoda: Oniscidea: Trichoniscidae) in the Dinaric Karst

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At present the genus *Alpioniscus* includes two subgenera: *Alpioniscus* s. str. and *Illyrionethes*. The latter includes 13 species in the Dinaric Karst, two species from Sardinia (*A. fragilis* (Budde-Lund, 1885) and *A. thanit* Taiti & Argano, 2009) and one from Spain (*A. escolai* Cruz & Dalens, 1989). All known species of the genus *Alpioniscus* are troglotrophic except for the Sardinian endogean species *A. thanit*. A total of 3763 specimens of *Illyrionethes* were found in 308 caves from the Dinaric Karst. Molecular analyses were conducted on 35 different populations from 52 caves (1–4 specimens for each population), using two mtDNA genes (16S rRNA and COXI) and a nuclear one (H3). On the basis of morphological analyses two different groups were identified, while by molecular analyses some distinct groups are recognized, each with a merodinaric distribution. The *strasseri* group consists of six subgroups, and has a northwestern Dinaric distribution. Four groups have a southeastern distribution pattern: two monotypic groups (*absoloni* and *verhoeffi*) and two groups (*heroldi* and *herzegowinensis*) each consisting of two subgroups. Two groups (*magnus* and *haasi*) include four subgroups and have a paralittoral distribution. The *strasseri*, *absoloni*, *verhoeffi*, and *heroldi* groups are well characterized both morphologically and molecularly, the *haasi* and *herzegowinensis* groups are morphologically the most diversified each other, and *magnus* group is morphologically not well supported. Some groups are geographically overlapping: on the Biokovo Mt. three different groups (*strasseri*, *heroldi* and *verhoeffi*) are present. Syntopic species are relatively rare, more than two species together were never reported. The distribution pattern of *Illyrionethes* phylogroups seems to be different from that of phylogroups of other cave dwelling fauna in the Dinaric Karst.

## **Cave fauna from iron caves of southeastern Brazil – first data**

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Studies in iron caves are rare in Brazil and we have no precise data about the number of caves in ferruginous lithology. Most faunistic inventories in this lithology are developed in huge mining areas (mainly in southeastern and north Brazil) and, unfortunately, the data about biotic environment are not available in publications. We present herein preliminary results about subterranean fauna in iron caves from southeastern Brazil (Minas Gerais state). We studied the fauna in three iron caves from Caeté County, located in a region called “Quadrilátero Ferrífero” because its ferruginous landscape is highly developed. These caves are formed by “Canga”, defined as superficial fragmented deposits with high concentration of hematite minerals cemented by limonite minerals. Caves developed in this lithology present many crevices and micro tunnels and consequent potential passageways for distribution and dispersion of fauna, which is, nowadays extremely threatened due iron extraction in large scale. Indeed, the dynamic of these communities is still unknown. We recorded a richness of 72 species and abundance of 382 individuals, distributed in 44 families. These data show a high diversity in a high hierarchy (Family), suggesting a tendency to endemism. The number of species per cave shows that the size of the accessible habitat is not correlated to the richness: 43 species at Piedade Cave (400 m of mapped passageways), 25 species at Triângulo Cave (103 m of mapped passageways), 26 species at Chuveirinho Cave (27 m of mapped passageways). The most representative group were the Araneae (15 species), Collembola (five species), Hemiptera (five species), Acari (four species) and gastropods – Pulmonata (four species). In relation to the troglomorphic taxa, we recorded 15 species showing regression of eyes (ocelli) and/or pigmentation (Diplopoda – Spirostreptida, Palpigradi, Opiliones, Pseudoscorpiones – Chthoniidae and Chernetidae, Araneomorphae – Prodidomidae, Orsolobidae and Ochyroceratidae, Coleoptera – Carabidae, Collembola). In the same occasion we conducted collections in the epigeal environment, close to the caves. In this case, 24 species recorded. No one troglomorphic species was recorded in the surface, suggesting also a troglotrophic status for those found in the iron caves. However, a collection effort must be higher to corroborate this idea.

## **Influence of substrate type in the fauna composition of the Angelica cave, central Brazil – the test of visibility and relevance**

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Available substrates for cave fauna can vary in composition, physical conditions and distribution and influence the spatial distribution of the cave fauna. Part of terrestrial habitats of a limestone cave (Angélica Cave) was sampled by Quadrat method to understand the influence of different substrates on the local fauna composition. We sampled the fauna on three fieldtrips in the years of 2011 and 2012, covering a terrestrial area of 64.3 m<sup>2</sup>. We established five stations over a length of 250 m from the entrance zone to aphotic conduits. We sampled systematically the substrates, including eight categories of substrates: bedrock (7.2 m<sup>2</sup>), mixed 1 (sand + gravel, 25.1 m<sup>2</sup>), mixed 2 (clay + gravel, 8 m<sup>2</sup>), wet sand (5.2 m<sup>2</sup>) dry sand (1.3 m<sup>2</sup>), mixed 3 (rock + sand + gravel, 9.4 m<sup>2</sup>), litter (4 m<sup>2</sup>) and dry unconsolidated (4 m<sup>2</sup>). We verified similarities between the substrates using cluster analysis (Single linkage, Euclidian distance). The substrate with higher richness was litter (36), followed by mixed 1 (34), while the poorer was bedrock (2). We did not record any taxa on unconsolidated dry substrate, probably due the impacts of touristic visitations. Some recorded taxa showed preference for specific substrates, while others were widespread (e.g., *Zelurus* sp.). There was a predominance of Carabidae, Staphylinidae (Coleoptera) and Blattellidae (Blattaria) in litter substrate. Wet sand showed the highest richness of Araneomorphae. Litter substrate, close to the cave entrance, showed the highest richness, suggesting an influence of the epigeal environment, considering the food support. However, the proximity of the surface is not the only factor influencing the diversity, since other substrates showed a high richness too (e.g., mixed 1). The substrate complexity offers micro-habitats and food resources for cave fauna. Indeed, sampling replication is essential to effectively access the richness of a habitat and a single visit occasion, even with sampling direct methods (such as *Quadrat*), is not enough to infer the minimal richness of the subterranean fauna.

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Adaptation, Development, Physiology and Behaviour: oral presentation

## Convergent evolution of albinism in diverse cave adapted animals

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Albinism, the regression or loss of melanin pigmentation, is an omnipresent feature that has evolved by convergence in all phyla with cave dwelling representatives. We are interested in the molecular mechanisms that are underlying the evolution of albinism in diverse cave animals. The molecular basis of albinism is currently known in only one cave adapted animal: *Astyanax mexicanus* cavefish. In this species, different loss-of-function mutations in the *oca2* gene cause albinism in at least three independently evolved cavefish lineages. The function of OCA2 is not completely understood, but it is known to act during the first step of melanin biosynthesis, the conversion of L-tyrosine to L-DOPA. Since the melanin synthesis pathway is generally conserved among animals, we have used a melanogenic substrate assay to survey for defects in this pathway in albino cave animals belonging to many different phyla. The assay involves supplying exogenous substrates, such as L-tyrosine or L-DOPA, to lightly fixed specimens, and subsequently detecting the presence of melanin as deposits of black pigment. The addition of L-DOPA, but not L-tyrosine, produced black pigment in diverse albino

cave animals, including a sponge, a planarian, annelids, mollusks, arthropods, and several vertebrates other than *Astyanax*, indicating that the initial step of the pathway is defective in all these animals. In some of the cases, L-DOPA treatment restored melanin pigmentation in patterns resembling those of closely related surface-dwelling relatives. Therefore, albinism has evolved by a defect at the first step of melanin biosynthesis in all cave-adapted animals tested thus far, and L-DOPA can restore pigmentation, indicating that all downstream steps of the pathway are present and potentially functional in these animals. Our results show that albinism has evolved by a defect in the first step of melanin biosynthesis in cave animals belonging to many different phyla, suggesting that there is an adaptive advantage for a block at the beginning of the pathway.

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Population and Community Ecology of Subterranean Organisms: oral presentation / student

## **Population data on two syntopic troglobiotic catfishes from Fadas cave system, Bodoquena karst area, southwestern Brazil**

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We present the results of population ecological studies on two syntopic undescribed species of siluriform fishes, the catfish *Rhamdia* sp. (Heptapteridae) and the armored catfish *Ancistrus* sp. (Loricariidae), endemic to four caves from the same cave system in Salobra River Basin, SE Brazil. These species present a mosaic of morphological variations concerning pigmentation and eye development. The population study was conducted in April, July and October of 2010 and 2011 (dry seasons), based on visual censuses and capture-recapture methods with permanent marking. Individuals of *Rhamdia* sp. were marked and recaptured in two localities (Fadas and Cinco de Ouros caves) whilst the *Ancistrus* sp. population was studied only in Fadas Cave. A total of 253 individuals of *Rhamdia* sp. was marked (41 in Cinco de Ouros Cave and 212 in Fadas Cave), of which 68% had no externally visible eyeless, 19% had eyes as developed as in the epigean sister species, and 13% had asymmetric eyes. The *Ancistrus* sp. population presented individuals with reduced or asymmetric eyes, but none was completely eyeless. This mosaic of morphological variations suggests a relatively short time of isolation in the subterranean environment. According to the Comark-Jolly-Seber analysis using the MARK software, the survival rate of *Rhamdia* sp. is related to the troglomorphic degree, with eyeless individuals presenting a higher survival rate than those with normal or asymmetric eyes. During the studied dry seasons, the density of *Rhamdia* sp. in Fadas Cave was relatively constant, around 0.106 ind/m<sup>2</sup> (SD = 0.029) and tended to concentrate in slow-moving water pools (0.12 – 0.17 ind/m<sup>2</sup>). The observed density was lower at the end of the rainy season (0.06 ind/m<sup>2</sup> in April), when the fish could be dispersed throughout their habitat. The *Ancistrus* sp. population showed much lower, with constant densities around 0.019 ind/m<sup>2</sup>. Both species showed sedentary behavior, being often captured at the same site of their previous capture. One specimen of *Rhamdia* sp. displaced between the studied caves, confirming the hypothesis that they are part of the same karst system. The overuse of the surface cave system, such as housing, land use for livestock and mining, poses a considerable risk of extinction for both species due their high endemism.



## Is natural attenuation of pollution occurring through karst?

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Natural attenuation is the least expensive way of remediation of polluted environments. Besides physical means of reduction of pollution loads, such as dilution, sorption, precipitation or decantation, microbial biodegradation is the only mean really decreasing the mass and concentration of pollution. The aim of our study is to show whether and how natural attenuation of pollution occurs through karst systems. We examine whether the amount of organic pollution that enters the system decreases while water passes through karst. The study focuses in particular on the contribution of microbial communities to pollutant biodegradation. The research is carried out in areas in Romania where pollution generated by the upstream anthropogenic activity enters the karst systems, passes underground and emerges downstream in karst streams that are used as drinking water sources by the local human communities. A reduction of pollutants concentration is expected through the underground passage, with lower concentrations downstream the karst system. To test this hypothesis, we determine the concentration of selected contaminants and the presence of degrading microorganisms using culture independent molecular techniques. The capacity of certain microorganisms to degrade pollution is being assessed and quantified using functional genes known to be involved in biochemical pathways of pollutant biodegradation. To further demonstrate the involvement of selected species in the degradation of certain contaminants, *ex situ* exposure experiments are being performed to assess the capacity of degradation for dominant species.

## Biodiversity assessment of Mediterranean marine caves: the taxon Porifera

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The aim of this paper is to assess the taxonomic richness of sponge fauna in the Mediterranean marine caves. Systematic researches started in the fifties by snorkelling in submerged caves (Sarà 1958) and were strongly increased by the development of SCUBA techniques after the pioneer cave diving activity of the Austrian team (Riedl 1966). Our up to date knowledge on cave-dwelling sponges of the Mediterranean subregion focus on records included in 76 papers ranging from alpha taxonomy to faunistics and ecology. Additional recent data from karstic caves of three Marine Protected Areas scattered in the western-central Mediterranean were also considered (Ledda et al. 2010, Manconi et al. 2010, Manconi et al. 2011, Cadeddu 2012). In total less

than 95 caves were bioprospeted until now in the entire Mediterranean. As far as taxonomic richness a total of 303 species belonging to 138 genera, 69 families, 19 orders and 4 classes were recorded. The analysis of the chorological categories shows that most of the biogeographic patterns of Mediterranean sponges occurring in caves are Atlanto-Mediterranean (n=127, 42%) followed by the Mediterranean endemics (n=122, 40%), cosmopolitan (n=40, 13%) and a few ampho-Atlantic (n=9, 3%) and Indo-Mediterranean (n=5, 2%) species. Only 31 species (10%) of sponges are endemic to Mediterranean caves, with 18 species (6%) exclusively recorded each from a single cave. Data on taxonomic richness of the caves in the Mediterranean geographical areas were tested by statistical analysis. The data clearly show that in the Mediterranean caves i) knowledge of the sponge fauna is far from uniform, ii) taxonomic richness is directly proportional to the number of studies performed in each cave/subMediterranean Basin, iii) the rich and diversified biota harbours a highly structured sponge taxocenosis, iv) sciophilous sponge species are dominant on other benthic sessile taxa (Cnidaria, Bryozoa).

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Subterranean Biodiversity and Biogeography: poster presentation

## Terrestrial isopods (Crustacea: Isopoda: Oniscidea) from Brazilian caves

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Up to date only six species of terrestrial isopods (*Benthana iporangensis* Lima & Serejo, 1993; *Amazoniscus eleonora* Souza, Bezerra & Araujo, 2006; *Trichorhina guanophila* Souza-Kury, 1993; *Circoniscus buckupi* Campos-Filho & Araujo, 2011; *Circoniscus carajasensis* Campos-Filho & Araujo, 2011; *Gabunillo aridicola* Souza, Senna & Kury, 2010) were known from Brazilian caves but only four could be considered as troglobionts. We have lately examined a large collection of Oniscidea from many Brazilian karst caves in the states of Pará, Bahia, Minas Gerais and São Paulo. The specimens are deposited in the collections of the Museu de Zoologia, Universidade de São Paulo, and the Coleção de Carcinologia, Universidade Federal do Rio Grande do Sul. The Pará caves (10) are located along the Xingu River in the Amazonian forest; Bahia caves (5, some of which are tourist caves) in the Atlantic forest area, Minas Gerais caves (2) in the Cerrado, and the São Paulo Cave in a karst system near the Betari River. Twenty species of Oniscidea have been identified, of which 12 in the families Styloniscidae, Philosciidae, Scleropactidae, Platyarthridae, Dubioniscidae, and Armadillidae are new to science. Three new genera (two in the family Styloniscidae and one in Scleropactidae) are also recognized. Four new species in the families Styloniscidae and Scleropactidae, are highly troglomorphic and can be considered as troglobionts. All the remaining species are troglaxene, including the already known species *Miktoniscus medcofi* Van Name, 1940, *Circoniscus intermedius* Souza & Lemos de Castro, 1991, *Dubioniscus goeldii* (Lemos de Castro, 1967) and *Dubioniscus marmoratus* Lemos de Castro, 1970 from Pará caves, *Porcellionides pruinosus* (Brandt, 1833) and *Cubaris murina* Brandt, 1833 from Bahia caves, and *Benthana taeniata* Araujo & Backup, 1994 and *Armadillidium vulgare* (Latreille, 1804) from Minas Gerais caves.

## Bat guano heap – the hot spot of microbial activity in cave

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Migratory animals with their activities provide as a significant source of nutrients in oligotrophic karst cave and thus may enhance nutrient cycling. Roosting bat colonies 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 and is characteristic by acidic pH and elevated concentrations of heavy metals (HM), particularly Cd, Cr, Cu, Hg, Pb, and Zn. Scanning electron microscopy of this guano showed that non-degraded structures of insect tissues, bat hairs and pollens are still present in the deepest and oldest deposits (~1000 y.), what raised the question about inhibition of microbial degradation processes through time. 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. This process is energetically demanding, being inhibited by oxygen limitation and increased HMs. Previous studies showed microbial colonization of different parts of the heap, including archaea, bacteria, and fungi. In this study, we aimed to evaluate the potential enzymatic activities, bacterial and fungal molecular profiles (PCR-DGGE), and diversity of bacterial chitinase genes (F18, F19) 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 chitinases in deeper sections. Bacterial and fungal community profiling showed significant differences among studied sections, showing the enrichment of chitin bags by colonizing species. 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.

## Microbiome of diverse sediments in Slovakian wild caves

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Temperate karst caves are dominated by autochthonous microbiota due to prevalence of oligotrophic conditions and long-term isolation. Different inputs of organic matter associated with penetrating water, river, roots, air circulation, animals, caving, and tourism, may cause imbalance in nutrients and shifts in microbial community composition. Human associated pollution of karst ecosystem influences the quality of karst water and cause eutrophication. The increase of allochthonous microorganisms, associated with higher availability of organic carbon and enhanced microbial pollution may indicate changes in geochemical processes. It was evidenced, that nutrient rich deposits of animal feces, e.g. guano heaps, serves as hot spot of microbial activity in oligotrophic cave as well as source of invading species and/or genes. In this work, the bacterial heterotrophic composition was determined in eight wild (Ar dovská C., Bobačka C., Drienovská C., Demänovská C. of Peace, Modrovská C., Pružinská Dúpna C., Stará brzotínska C., and Suchá C.) and one tourist (Harmanecká C.) karst caves in Slovakia. The diversity of cultivable aerobic heterotrophic bacteria in diverse cave sediments was determined. Till now, we obtained 109 bacterial species in cave sediments including 30 species in bat guano deposits, from those 39 and 24 species, respectively were resistant to tetracycline. In cave sediments, common inhabitants of colder terrestrial and aquatic habitats were detected, including g. *Arthrobacter*, *Bacillus*, *Carnobacterium*, *Janithobacterium*, *Oerskovia*, *Paenibacillus*, *Pseudomonas*, and *Psychrobacter*. In moonmilk samples, g. *Arthrobacter*, *Bacillus*, *Carnobacterium*, *Delftia*, *Kocuria*, *Microbacterium*, *Pseudomonas*, *Psychrobacter*, and *Variovorax* were observed. The obtained data showed that bat guano deposits and sediments of caves inhabiting bats, were enriched by bacteria indicating eutrophication (g. *Enterococcus*, *Ewingella*, *Myroides*, *Nocardia*, *Oerskovia*, and *Sphingobacterium*), the potential reservoirs of antibiotic resistance genes (g. *Chryseobacterium*, *Ochrobactrum*, *Sphingopyxis*, *Staphylococcus*, and *Streptomyces*), and potential risk for human or animal health (g. *Curtobacterium*, *Hafia*, *Chryseobacterium*, *Mycobacterium*, *Neisseria*, *Nocardia*, *Ochrobactrum*, and *Rhodococcus*).

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Conservation of Subterranean Life: oral presentation

## Conserving natural caves in the American West: unique challenges in a multiuse landscape

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A significant number of natural caves are clumped and scattered across the American West's vast landscape. The majority of these caves are on and under publicly owned lands administered by US Federal resource management agencies such as the US Forest Service (USFS). Caves located on these lands are technically protected by the Federal Cave Resources Protection Act of 1988 but the reality of their protection is much different. Local politics and disagreements between user groups such as organized cavers and the USFS have created and fostered relationships that can be strained at best. As a result, management agencies often are not aware of the caves on their lands and thus are unable to protect them during management activities

such as forest fuels reduction, timber sales, or fire fighting. A recent landscape scale plan to remove fuels from four National Forests in Arizona, totalling 2.4 million acres, encompasses over 200 natural caves, many of which are/were unknown to the USFS. Over a period of two years three extremely dedicated individuals from 3 separate organizations met, crafted, and hammered out a unique conservation strategy for the 200 “nonexistent” caves and the life within them thus ensuring their survival for generations to come. The considerations and techniques used were not standard and we look forward to sharing and discussing this example of cave conservation with you!

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Subterranean Biodiversity and Biogeography: poster presentation / student

## **A high diversity spot for Onychophora in Serra da Bodoquena karst area, southeastern Brazil**

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The presence of syntopic species of velvet worms (Onychophora) is rarely recorded in the literature. Currently, 16 species of Peripatidae are recognized in Brazil, in general one species per area. Onychophorans are usually found in microhabitats with high relative humidity and a high concentration of organic matter. These invertebrates are occasionally recorded in caves, with two troglomorphic species so far known: *Peripatopsis alba* (Peripatopsidae), from Table Mountains, in South Africa, and *Speleoperipatus spelaesus* (Peripatidae), from Jamaica. In Brazil, velvet worms are widely distributed, except in Southeastern and South regions. Herein we extend the distribution Brazilian onychophorans to the southwestern region, in Mato Grosso do Sul State, and discuss their richness in the Bodoquena Plateau. Since the first specimen was discovered, in 2006, several expeditions were carried out in epigeal and hypogean localities of this karst area. Four new species were found in caves, all Peripatidae, including a troglomorphic one and three non-troglomorphic. A new genus was elected to include the troglomorphic species and one of the non-troglomorphic ones (for which no epigeal specimens have been found). The other two non-troglomorphic species, with both epigeal and cave (troglophilic) populations, were preliminary assigned to the genus *Epiperipatus*, pending on further confirmation, since no adult males were found till now. The new genus is identified by the large size (more than 80 mm in length), the body color pattern, long legs, the unique tegument arrangement and a single anal gland pore in the male. The troglomorphic species differs from the non-troglomorphic one by the paler, pinkish coloration, higher number of antennal rings and feet papillae and the larger number of legs (35-36 pairs versus 34 pairs of legs in the females). This is the first record of a troglomorphic Onychophora in South America and the second for the Neotropical region. The Bodoquena records represent the second case of sympatric onychophorans in Brazil and the unique case of three syntopic Neotropical species. Three species (one *Epiperipatus* and the new genus) are distributed in the highlands (about 500 m of altitude) on the left margin of Salobra River canyon, which seems to be a biogeographic barrier for subterranean terrestrial communities.

## **Distribution and population data on troglotrophic catfishes, genus *Trichomycterus*, from Serra da Bodoquena karst area, southwestern Brazil (Siluriformes: Trichomycteridae)**

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Trichomycteridae catfishes have successfully colonized subterranean habitats in the Neotropical region, with 11 troglotrophic species in Brazil, in addition to several troglomorphic populations so far recorded. Recently, the high troglomorphic catfish *Trichomycterus dali* was described based on specimens from three localities within a distance of 100 km along the Miranda River basin. Between 2010 and 2012, potential areas of occurrence for these catfishes were prospected in the Bodoquena Plateau, resulting in nine new records in the Salobra microbasin, and one record in the Formoso River Basin (Taquaral spring), representing an extension of the distribution to an area of 60 km of radius. However, morphological studies pointed to the presence of a differentiated species occupying the headwaters of the Salobra basin. *Trichomycterus* cf. *dali* from Urubu Rei Cave was investigated in detail, using visual censuses and mark-recapture, in April, July and October of 2010 and 2011, and May 2012. Seasonal variations in the population densities and frequency of adults were noted. The highest and most variable densities were observed at the entrance plus twilight zone (till 50 m from the entrance – stream resurgence), with a peak in April 2010 (0.6 ind./m<sup>2</sup>) and declining sharply afterwards: the lowest values were obtained in 2011, with a slight recover in May 2012. The same pattern was observed in the aphotic zone, but with lower (maximum = 0.1 ind. /m<sup>2</sup> in April 2010) and less variable values. These catfish move for longer distances inside the cave than other studied Brazilian fishes, for instance, a mature female (SL = 5 cm) displaced 350 m upstream the Urubu Rei Cave in 15 days, indicating larger foraging areas. The observed fluctuations in population densities may be due to natural infra-annual cycles, as a response to exceptional flash floods during the rainy season of 2010/2011, and/or due to uncontrolled touristic activities. The ability to produce melanine was retained in nine out of the 10 known populations of *T. cf dali*. “Black” individuals, yet eyeless as all specimens, were commonly found in illuminated zones, for example, 90% of the specimens in the entrance zone of Urubu Rei Cave were pigmented, the opposite being observed in the aphotic zone. In contrast with *T. dali*, these catfish react intensively to the exposition to light by producing melanine, which is slowly lost (months to years) after returning to aphotic conditions.

## **Diversity and distribution of cave dwelling Collembola of the Velebit Mts., Croatia**

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The Velebit Mt. is one of the largest mountains of the Dinarides with the length of 145 kilometers and the highest peak at 1.757 m above the sea level. Due to its geological, geomorphological and hidrogeological characteristics various karst forms were developed, including remarkable caves. Both longest and deepest cave of Dinarides are situated on the Velebit (cave system Lukina jama - Trojama, 1421 meters deep, 3731 meters long and cave system Kita Gaćešina - Draženova puhaljka, 665 meters deep, 22767 meters long). Numerous caves and pits have been explored by speleologists, two more of them being deeper than 1000 meters and 28 pits deeper than 250 meters. Samples from Velebit Mt. collected from 1999 till 2011 from the Croatian Biospeleological Society Collection were analyzed and 27 different taxa from 114 caves were identified. Eleven families have been recorded: Entomobryidae, Isotomidae, Oncopoduridae, Paronellidae, Tomoceridae, Neelidae, Neanuridae, Hypogastruridae, Onychiuridae, Arrhopalitidae and Sminthuridae. The most diverse are families Tomoceridae with 8 and Entomobryidae with 5 recorded taxa. Out of total number of 27 there are 11 troglotrophic, 3 troglophilic and 13 troglotenic taxa. Five out of 11 troglotrophic taxa are new to science and were collected in the deep pits up to depth of 950 meters. The most dominant troglotrophic species is *Pseudosinella heteromurina* (Stach, 1929) followed by troglotrophic species from family Onychiuridae. *Heteromurus nitidus* Templeton, 1836 is the most common troglophilic species recorded in the caves. There are no literature data for the cave dwelling Collembola of the Velebit Mt. and this study gives the first insight into their distribution and diversity and reveals a great number of new taxa from the deep pits, a cave habitats usually inaccessible to researchers.

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Subterranean Biodiversity and Biogeography: poster presentation

## Diversity and distribution of cave dwelling spiders of the families Nesticidae and Agelenidae (Araneae) in Croatia

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Croatian caves are characterized by a remarkable species richness of cave dwelling invertebrates, and spiders are one of the most abundant groups. So far known, 10 families with cave dwelling species are recorded from Croatian caves with Agelenidae and Nesticidae making up a significant part, especially at cave entrances. Agelenidae are represented by six genera (number of species in brackets): *Hadites* (1), *Histopona* (7), *Inermocoelotes* (2), *Malthonica* (3), *Tegenaria* (2) and *Textrix* (1), altogether with 16 species showing different levels of adaptation to the cave environment. The most interesting is the monotypic genus *Hadites* with only one species, *Hadites tegearoides* Keyserling, 1862, which is the most troglomorphic species of the family and found only in caves on Island of Hvar. Nine species are endemic to the Dinarids and three to Croatia. Some specimens of the genus *Tegenaria* could not be determined to the species level, suggesting that the species number could even be greater. The family Nesticidae is represented by the genus *Nesticus*, which is widespread and abundant in most Croatian caves. Both species, *Nesticus cellulanus* (Clerck, 1757) and *N. eremita* Simon, 1879, are troglophiles with Holarctic and European distributions, respectively. They inhabit

cave entrances but also can be found deeper in caves. A very interesting finding is a potential new species of the same genus, so far found only on Biokovo Mountain and with more troglomorphic features than two known species. Field work and analyses of these families have been performed using all available references, lists and systematic taxonomical survey in the Croatian Biospeleological Society collection. Spiders of the two families have been collected from around 400 speleological sites throughout of the Croatian Karst. This study gives the first comprehensive insight into the distribution and diversity of cave dwelling spiders of families Agelenidae and Nesticidae in cave habitats of Croatia.

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Adaptation, Development, Physiology and Behaviour: oral presentation

## **Convergence and divergence in the subterranean realm – a reassessment**

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The dominant neo-Darwinian paradigm of the evolution of cave animals is that the severe aphotic, low food environment with little environmental cyclicity imposes strong selective pressures leading to convergent (troglomorphic) morphology of reduced pigment and eyes, and elaborated extra-optic sensory structures. Challenges to the paradigm come from two fronts. First, troglomorphic animals occur in many aphotic habitats with relatively abundant food and environmental cyclicity. Second, many permanent reproducing populations in caves are not troglomorphic. A review of data on patterns of troglomorphy confirms both of these points. This suggests that absence of light, rather than resource level and environmental cyclicity, is the important selective factor, and that other forces are at work, including competition and differences in age of lineages in subterranean environments.

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Phylogeography, Phylogeny and Evolution: oral presentation / student

## **Revealing the consequences of Pleistocene events on present day distribution of *Niphargus stygius* (Crustacea: Amphipoda: Niphargidae)**

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The Pleistocene climate has had a strong impact on the distribution of subterranean fauna. The Last Glacial Maximum (21,000 years ago) presumably caused mass extinctions of subterranean fauna in Northern Europe and Alps, leaving fewer species which mainly survived in ice-free refugia. Some recent publications, however, provide the evidence that at least four



stygobiotic amphipod species survived Upper Pleistocene events under the glacial mass. We explored the feasibility of this scenario for the Alpine subterranean amphipod *Niphargus stygius* (Schiödte, 1847). *N. stygius* is distributed in western Slovenia and in the easternmost karstic areas of Italy, from the sea level and up to 1,600 m above sea level and inhabits both, formerly glaciated and ice free areas. We tested two evolutionary hypotheses: (I) populations covered by glacier suffered extirpation, present-day populations in this area originate from post-glacial colonization; (II) populations survived under the glacier. We analyzed 35 specimens from 26 different localities (caves, springs) using two mitochondrial (COI and 16S rRNA) and one nuclear (ITS) markers. We reconstructed phylogeny for each marker separately and altogether using two different Bayesian methods. Analysis of combined data sets identified three well supported lineages: Southern, Central and Northern (Alpine) lineage, while mtDNA and nDNA gene datasets gave incongruent population trees with some of the groups being paraphyletic. Genotypes present in area covered by an ice sheet during the maximum extent of Quaternary glaciations are absent elsewhere within the species range, suggesting that at least some populations of Northern (Alpine) lineage survived underneath the glacier cover.

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Subterranean Biodiversity and Biogeography: oral presentation

## **Is the trogllobiotic and stygobiotic fauna rich in Kraków-Częstochowa Upland (karstic region in southern Poland)?**

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The studies on invertebrate fauna in various terrestrial and aquatic subterranean habitats of this region started some 150 years ago, but they have never been intense systematic. Moreover information concerning some taxonomical groups such as Amphipoda, Ostracoda or Araneae dates back to 40 years ago. Among stygobionts two *Niphargus* species, four (or five) oligochaete species (including genus *Trichodrilus* and *Ganius aquaedulcis*) and prosobranch gastropod *Falniowskia neglectissima* (closely related to genus *Bythiospeum*) were found. Ostracoda and recently studied Copepoda were represented by stygoxenic and stygophilic species only. Each stygobiotic species occurred in a single or a few localities, situated mostly outside the caves (in wells or springs). The patchy distribution of old stygobionts such as *Niphargus leopoliensis*, *Trichodrilus* spp., *Ganius aquaedulcis* and *Falniowskia neglectissima* suggests continuous presence of these taxa in subterranean waters (at least in the glacial refugia), but re-colonization along river courses (by hyporheos) should be taken into consideration for *Niphargus tatrensis* and *Trichodrilus* spp. Trogllobiotic, endemic subspecies of the beetles *Catops tristis infernus* and *Choleva septentrionis gracilentia* live in two static, cold caves. Remaining trogllobionts such as spider *Porhomma egeria*, 2–3 springtail species and 2 acari species are widely distributed in the Upland. It seems that local subspecies of cave beetles and *P. egeria* are subtrogllobionts (or neotrogllobionts). Their isolation from surface forms took place after one of the cold periods. Springtail *Arrhopalites pygmaeus* could be treated as a local trogllobiont, because in northern Europe it lives in the litter. Other collembolan and acari species, known from deep soil layers or mammal burrows also, could colonize subter-

ranean habitats of the Upland even after the last glacial period. In addition, troglotrophic fauna was enriched by the beetle *Speonomus normandi hydrophilus*, introduced by dr A. Skalski to one cave about 20 years ago. In Kraków-Częstochowa Upland the number of troglo- and stygobiotic species is small, but further studies could enlarge this list and allow determining its specificity by the evaluation of genetic distance of particular species from their populations living elsewhere.

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Microbiology, Geomicrobiology and Sedimentology: poster presentation

## **Inventory of soil filamentous fungi in an iron ore cave inserted in the Quadrilátero Ferrífero Region (Brazil)**

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Iron ore caves have been recently registered and studied in Brazil (past decade). The “Quadrilátero Ferrífero” region presents interesting geomorphologic and environmental features due to its unique lithological characteristics and is also an ecotone of two important biodiversity hotspots (Atlantic Forest and Cerrado). However, the caves inserted in this region are also endangered by mining activities and urban development. Although there are a few studies concerning the subterranean fauna in this type of caves, there is a lack of more detailed microbiologic inventories in iron ore caves in the country. The aim of this study was to perform an inventory of soil fungal community in an iron ore cave located in an environmentally protected area of the Quadrilátero Ferrífero region. It is important to highlight that this area is surrounded by mining companies and developing municipalities. A total of five sampling stations were selected. Soil samples were removed with sterilized equipment and placed in sterilized plastic bags (Whirl-pack) for laboratory analysis. The isolates were obtained through the serial dilution technique ( $10^{-1}$  –  $10^{-6}$ ), in triplicates and incubated on DRBC media at room temperature (25°C). Purification was performed on MEA and the identification was performed by morphologic characterization (macro and microscopic) in specific media. A total of 2718 isolates (CFU) were counted. These isolates were distributed among 27 species of 10 genera (*Aspergillus*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Mucor*, *Paecilomyces*, *Penicillium*, *Rhizopus* and *Trichoderma*). The most representative genus was *Aspergillus* (33.3%), followed by *Penicillium* (29.9%). The isolates not growing reproductive structures were clustered in the *Mycelia Sterilia* group. It was possible to observe at least three possibly different species in this group. These isolates were identified as morphotypes and included in the mycological collection for further molecular analysis. This is the first more detailed inventory of soil filamentous fungi performed in an iron ore cave inserted in the “Quadrilátero Ferrífero” region in Brazil. The vicinity with mining and urban areas highlights the importance and urgency of performing more studies on iron ore cave microbiota.

## **The selective effect of earthworm activity on bacterial community: comparison of cave and soil population of *Aporrectodea rosea***

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Earthworms affect microbial populations mainly by passing huge amounts of soil substrate through their digestive tracts. In this study, we compared the effects of cave and soil populations of an endogeic earthworm *Aporrectodea rosea* on passaged bacterial communities. The earthworms and cave sediment were repeatedly collected in the Amateur Cave (Moravian Karst, Czech Republic), the only cave in the Central Europe inhabited by viable population of *A. rosea* isolated from above-ground environment for many decades. Corresponding soil samples and earthworms were collected on the banks of a stream flowing into the cave. Phospholipid fatty acid analysis (PLFA<sub>bact</sub>) was used to assess bacterial biomass in the gut and fresh faeces of earthworms and in the substrates. Plate cultivation of bacteria was used to determine the number of culturable bacteria, their growth strategy and species representation (MIS Sherlock System; MIDI Inc., USA). The antibiotic resistance (ATB-r) of the isolates was determined by growth on selective media enriched with antibiotics and by antibiotic diffusion disc test (Bio-Rad, France). In total, 15 antibiotics were tested. Cave earthworms increased both the culturable bacterial counts and the PLFA bacterial biomass and stimulated the fast-growing bacterial r-strategists in the consumed cave sediment. The species screening indicated a shift from Firmicutes endosporing bacteria dominated community to that enriched with Proteobacteria. Soil earthworms did not affect the number or the growth pattern of the culturable bacteria and the increase of total bacterial PLFA biomass in consumed soil was lower than that in cave sediment. Species richness of bacteria was higher in soil and faeces of soil earthworms than those in cave sediment and faeces of cave earthworms. The antibiograms of ATB-r isolates were described for all substrate types. The pattern of bacterial resistance to antibiotics differed between earthworm populations. In general, the effects of *A. rosea* activity on the quantitatively and qualitatively poor bacterial communities of cave sediment were much more pronounced than those on rich communities of soil bacteria.

## **The antibiotic resistance in cave environments**

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Antibiotic resistance (ATB-r) in bacterial pathogens is a global hot topic that has been solved intensively. With respect to considerable heterogeneity of ATB-r determinants in different ecosystem and lack of information about factors affecting its evolution, dissemination and persistence, the ATB-r should be studied as an ecological problem. Data from different ecosystems are highly desirable. This contribution informs about recent knowledge on occurrence of ATB-r bacteria in subterranean environment and provides original results concerning occurrence of bacteria resistant to tetracycline (TET-r) based on our three-year monitoring of caves in Slovakia. In total, eleven karst caves monitored in years 2010–2012 were located in protected areas, majority of them (8) were inaccessible to the public. The culturable microbiome of subterranean environment (STE) was characterized based on samples of cave sediments and speleo-aerosols collected from 42 different monitoring microsites. In parallel, culturable microbiome of surface environment (SFE) was represented by soils and air sampled outside of the 11 caves. Plate cultivation of bacteria (Tryptic soya agar, BD and Tryptic soya agar + 0.003 % chlortetracycline) was used to determine the number of total and TET-r culturable bacteria and TET-r prevalence in the both microbiomes. Following single colony isolation, these isolates were identified based on whole cellular fatty acid profile (MIS Sherlock System; MIDI Inc., USA) and on DNA sequencing of 16S rRNA PCR products and comparison in the BLAST Database. The antibiotic multiresistance of the isolates was determined by antibiotic diffusion disc test (Bio-Rad, France). In total, 15 antibiotics were tested. The TET-r bacterial abundance of cave sediments and soil laid within the range  $10^4 - 10^8$  CFU  $g^{-1}$  dw, and  $10^3 - 10^6$  CFU  $g^{-1}$  dw. The both speleo-aerosol and air showed low abundance  $0-10^1$  CFU  $m^{-3}$ . The Proteobacteria and Firmicutes dominated in TET-r of STE, in contrast to SFE enriched by TET-r Firmicutes and Actinobacteria. The pattern of ATB multiresistance of SBE and SFE was identified. This contribution is important to our understanding of the occurrence of ATB-r, and shows that even wild microbiomes and environments isolated from practice of antibiotic application are reservoirs of ATB-r.

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Phylogeography, Phylogeny and Evolution: oral presentation

## **Phylogeographic history of subterranean aquatic species reveals contrasted dispersal scenarios**

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Biodiversity patterns are shaped by interactions among a limited set of processes, the relative strength of which is influenced by environmental factors. Pleistocene climatic oscillations in Europe and North America might have repeatedly changed the relative strength of dispersal, selection and drift. Dispersal heavily influenced species distribution at the onset of colonization, but species-environment relationships progressively became important as colonization proceeded. Earlier phylogeographic studies showed that biodiversity patterns at northern latitude supported a scenario of recent dispersal over large areas from distant Mediterranean or continental refugia. However, subsequent studies also documented recent

but local dispersal from *in situ* refugia. The shift from small to large geographic ranges among subterranean animals with increasing latitude has often been considered as an argument for the recent and large-scale dispersal model. However, the discovery from molecular studies of cryptic species (i.e. highly divergent evolutionary units) within widely-distributed morphospecies severely questioned this argument. Even so the wide distribution range of a cryptic species is evidence for dispersal; it does not necessarily indicate that dispersal is recent. In this study, we used species delineation methods and phylogeographic methods to explore the likelihood of the recent and large-scale dispersal model for five aquatic subterranean isopods, the large distribution range of which extends in regions severely affected by cold Pleistocene climate. First, the occurrence of cryptic species within the 5 morphospecies was tested using a threshold method and the general mixed Yule and coalescence model. Second, we searched for evidence of recent dispersal by reconstructing the phylogeographic history of species using Bayesian relaxed random walked models. This research was funded by the Agence Nationale de la Recherche (ANR-08-JCJC-0120-01, "DEEP"), the Institut Universitaire de France and the European Commission (7th EU Framework Programme, Contract No. 226874, BioFresh).

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Microbiology, Geomicrobiology and Sedimentology: poster presentation / student

## **Differences in microbial assemblages of sediment deposits from two Romanian caves**

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Microbial diversity of sediments in two caves (Urșilor Cave and Cave cu Apă din Valea Leșului) from north-western Romania was investigated by analyzing 27 samples. The sediments are represented by alluvial deposits of the subterranean rivers and the sampling was done at different depths. ITS and 16S rRNA gene diversity was assessed by PCR, DGGE and cloning techniques. The results revealed the presence of members belonging to Chloroflexi, Nitrospirae, Proteobacteria, Firmicutes, Gemmatimonadetes and Acidobacteria. The bacterial species richness was higher in the Cave cu Apă din Valea Leșului, comparing to the fungal species richness, which was higher in the Urșilor Cave. Half of the Bacteria sequences belonging to the Cave cu Apă din Valea Leșului had up to 99% similarity with those retrieved from deep subsurface sediment at the Hanford Site 300 Area, Washington state (USA). The majority of the bacterial sequences from Urșilor Cave had no close relatives in GenBank, being grouped in two separate clades of the phylogenetic tree. Most of the fungus clones had high similarity with *Gymnascella* members isolated from Lascaux Cave (France). The obtained sequences have high similarity with bacterial species found in extreme environments, like deep sea, geothermal springs and hypersaline lakes. Besides *Bacillus* sp., all the sequences represent uncultured organisms, making difficult a precise interpretation of the results. The present study represents the first attempt to identify microbiota in old cave sediments, of Pliocene-Pleistocene age.

## Description of two new species of *Niphargus* from cave systems in Iran

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Caves are nearly stable environments with characteristics favouring the organisms such as arthropods. There are recent progresses in the knowledge of Iranian cave biodiversity in Iran. In this study, we described two new subterranean species of the genus *Niphargus* Schiödte, 1849 from two cave systems from Iran. These are named *Niphargus alisadri*, n. sp and *N. danieli*, n. sp after the cave names. The material are described and their taxonomic status within the genus *Niphargus* is discussed with several known species from Iran, Turkey and Iraq based on the characters such as epimeral plates, uropod I, uropod III, maxiliped and pereopods.

## The family Coprozerconidae (Acari, Mesostigmata) in the caves of Palearctic region

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Family Coprozerconidae was established by Moraza & Lindquist (1998) on the basis of mites from Mammoth Cave in Kentucky, U.S.A. *Coprozercon scopaeus* Moraza & Lindquist, 1998 is known from feces of the wood rat, *Neotoma floridana magister*. *Neocoprozercon europaeus* Fend'a & Mašán, 2012 was collected from soil detritus and animal excrement in Hoľa Cave in the Čierna hora Mts., Western Carpathians, Slovakia. The first specimen was collected on feces of the European badger, *Meles meles*. Seven years later, 70 specimens were found in powdery soil in the same collection site (both dysphotic and aphotic zone). Most of these specimens (55 individuals) were found in twilight zone of the cave, where the soil was markedly rebored and dissected, probably after hibernating of the raccoon dog *Nyctereutes procyonoides*. There are only these two findings of the family Coprozerconidae so far. Both findings of the family Coprozerconidae are known from caves and substrates contaminated by mammal excrements so their life cycle seems to be restricted to the cavernicolous and/or nidicolous environment. The mites of the family Coprozerconidae seem to be troglophilous and saprophilous animals (or even troglobite).



## **Species-area model predicting diversity loss in an artificially flooded cave in Brazil**

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Caves are good models for answering ecological questions. Furthermore, those ecosystems are considered by some authors as “islands”, although it is clear that their degree of isolation is not comparable, in most cases, to real oceanic islands. Unfortunately, the effects of area loss under a cave invertebrate community were never tested, and such effects are thus, unknown. We sampled the invertebrate community of a Brazilian limestone cave before the filling of a reservoir (built for hydroelectric powering) that submerged 76% of the cave floor. During the filling of the reservoir, we conducted an experimental management that included direct transport of invertebrates to upper areas inside the cave (not directly affected by submersion) and indirect transport of fauna through the installing of artificial “bridges” from the irregular topmost areas in the floor to those upper galleries in the cave. After such management, we conducted a three-monthly monitoring of the cave fauna. We calculated the  $z$  value from species-area relationship equation after the area loss. Our results support the species-area relationship hypothesis, since the cave community showed drastic reduction in richness after the area loss. Moreover, the richness reached certain “stability” over time. However, although the dissimilarities presented by the community become less evident on time, this community apparently did not stabilize. As expected, the  $z$  value from the species-area relationship was high, indicating an isolation of the cave after the filling of the reservoir, and the reduced mobility of the cave dwelling species. In conclusion, studies considering the cave community before and their responses after disturbances are important, since they help researchers in proposing strategies for cave fauna conservation.

## **Trophic enrichment in underground environments and its applications in the biodiversity conservation of aquatic invertebrates**

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Up to date there is only few evidence of the effect of artificial increase in food resources over cave communities. Furthermore, the effects of reduction on food and light availability over

epigean communities are also unknown. The present study aimed to experimentally evaluate the influence of food resource availability on invertebrate communities present in the epigean and hypogean areas of a stream that enters in a quartzite cave in Brazil. Enrichment plots were used in different areas of the hypogean stream in order to increase the quantity of vegetable matter (food resource) in the cave. The methods used in the enrichment were based on the amount of vegetable food resource imported to the upstream area outside the cave. It was possible to verify a potential leveling between number of species present in the hypogean and the epigean community. The scarcity of food resource is, therefore, an important selective pressure that influences the aquatic community in the studied cave, although other factors, such as absence of light, may also restrict the establishment of organisms. A structure simulating an underground environment, with low penetration of light and trophic resources, was installed in the epigean environment and caused structural changes on the epigean community. There were no changes on the species richness, but there was a decrease on absolute abundance. The study could provide information for the conservation of aquatic invertebrate fauna in quartzite caves similar to the studied cave.

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Microbiology, Geomicrobiology and Sedimentology: poster presentation

## **Molecular approaches to study the underground bacterial assemblages – a case study from a Portuguese karst aquifer**

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In spite of the known relevance of groundwater as the main freshwater reservoir, it has been increasingly evident that it is also a vulnerable ecosystem. Therefore, it is of major importance to assess potential changes in biodiversity and the main factors driving these changes. However, scarce background information is available on its microbial diversity, particularly for the Portuguese karst aquifers. The present work takes advantage of molecular methodologies such as PCR (Polymerase Chain Reaction), DGGE (Denaturing Gradient Gel Electrophoresis), cloning and sequencing in order to study the seasonality in bacterial community from underground karst water samples in central Portugal. These data will be integrated with information from physical and chemical parameters to assess potential modulation factors for bacterial assemblage variation. Preliminary data suggest that seasonality in bacterial community (depicted from DGGE profiles) from groundwater samples may be primarily driven by precipitation levels rather than typical seasonal parameters such as temperature. This highlights the vulnerability of aquifers to long and severe drought periods, as predicted for future decades.



## **Cave amphipods as Antillean lizards: *Niphargus* ecomorph classes expose a new aspect of morphological diversity in caves**

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Extreme selective environments are commonly believed to funnel evolution towards few predictive outcomes. Caves are well known extreme environments with characteristically adapted faunas that are similar in appearance, physiology and behavior all over the world even if not closely related. Morphological diversity between closely related cave species has been explained by difference in time since colonization and different ecological influence from the surface. Here we tested a more classical hypothesis: morphological diversity is niche-based, and different morphologies reflect properties of microhabitats within caves. We explored seven communities of the amphipod genus *Niphargus*, each community counting three or more species. In the first step, we found that *Niphargus* communities consist of species that are morphologically more diverse than expected by chance alone. This is an indication that competition between co-occurring species is driving them to evolve different functional morphologies to exploit different ecological niches. In the second step we explored the relationship between morphology of species and the type of cave-microhabitats they occupy. We used two physical dimensions to describe aquatic microhabitats within a cave: (1) size of available spaces, and (2) velocity of water currents. Morphometric analyses grouped the species into four distinct ecomorph classes – a concept first used to explain the recurring pattern of morphological diversity of *Anolis* lizards on different Antillean islands. *Niphargus* ecomorphs were consistently associated with a certain type of microhabitat: small pore habitats (cave interstitial and epikarst), cave streams, and cave lakes (with two differently-sized ecomorphs). Each ecomorph class has multiple independent phylogenetic origins. Individual traits are associated with microhabitat properties and thus might represent adaptations to a particular dimension of internal cave microhabitats, rather than to the subterranean environment in general. For example, long antennae and legs are typical only of low water currents. These results shed new light on the morphological diversity of subterranean animals. What appear to be different degrees of troglomorphy could in fact be adaptations reflecting fine-level niche partitioning within caves.

## **Metabolic rate and behavioral responses of a stygobiont to different temperatures**

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The individual activity patterns and metabolic rates of the stygobiotic amphipod crustacean, *Stygobromus tenuis potamacus*, from a hypotelminorheic habitat were examined from 6–18 C,

the natural range as measured at the seepage spring. Measures of both activity and MR increased with increasing temperature. The increase in activity was linear, and showed large individual variation in slope. The increase in MR was sigmoidal, with the inflection point at 14 C on average, and showed only slight individual variation in the shape of the response. Variation in rates of increase in activity was not correlated with variation in the increase in MR among individuals. This pattern suggests that this amphipod actively avoids warmer water when foraging at the seepage spring, probably to avoid the energetic cost of higher MR. This scenario also explains the pattern of variation in abundance of this population observed over a two-year span at the seepage spring. The lack of individual variation in MR response to temperature suggests that this population may be unable to evolve in response to increasing groundwater temperature resulting from global warming.

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Conservation of Subterranean Life: poster presentation / student

## **A new hotspot for Brazilian subterranean fauna – the remarkable Igatu, a pseudokarstic region from Chapada Diamantina, northeastern Brazil, with a test of data repeatability**

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Hotspots are areas with high biological diversity, high species richness, endemism and unique ecological and/or evolution events, besides the presence of rare taxa. Due the absence of photoperiod and consequently plants, the hypogean environment would not fix on the classical definition of hotspot. Works focusing biological diversity in Neotropical caves are relatively rare and recent, and concerning pseudokarstic areas, no one was conducted with repeated samples. Aiming to understand the faunistic composition and distribution of cave fauna in a pseudokarstic area from northeastern of Brazil (Igatu region), we formulated some questions: 1. According to the proposed typical hotspot cave areas in the World, Chapada Diamantina region could be considered a new hotspot for subterranean fauna in Brazil? 2. Are two samples in one single annual cycle, enough to access the minimal species richness, and hypothesized any faunistic patterns, as proposed in new environmental laws in Brazil? Does lithology influence the faunistic distribution in caves from Chapada Diamantina? We made six collections along three years in a quartzitic area from northeastern Brazil, establishing the same collection effort (time and number of collectors) and methods (visual inspection). We recorded 127 taxa: 18 troglolobiotic ones distributed in small area of 24 km<sup>2</sup> and, until the moment, endemic. Indeed, we recorded at least 10 new species (among the troglolobiotic and troglolophilic ones), which will put the area as type-locality of many cave organisms. Igatu represents a new *hotspot* of subterranean fauna in Brazil, since, besides the cave fauna richness, endemism and relict taxa were recorded (such as the troglolobite scorpion *Troglopholurus translucidus* Lourenço, Baptista & Giupponi, 2004 and the basal cave catfish *Glaphyropoma spinosum* Bichuette, de Pinna and Trajano, 2008). Indeed, herein we demonstrated that two samples are not enough to access the minimal species richness and, more than the lithology, many other factors must influence the faunistic composition, such as latitudinal and altitudinal gradients and substrate type.

## The cave-dwelling millipede highly rich fauna from China and south-east Asia: additional results and new perspectives (Diplopoda)

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China is one of the last great *terra incognita* for diplopods now and hotspots of biodiversity are clearly identified in southwestern China, Laos, Vietnam, Cambodia, Thailand and Indonesia. As collections of Diplopoda from China have only recently become available for study, a relatively small number of cave-dwelling millipede species have hitherto been recorded there. Although a brief review of the cave millipede fauna exists, it fails to emphasize that the limited number of Chinese diplopod cavernicoles known include several unusual higher taxa. These are especially remarkable among the orders Glomerida, Callipodida, Polydesmida, Chordeumatida and Spirostreptida (family Cambalopsidae). The work treats collections by biological-speleological expeditions made in 1997–2012 to southern China, Laos, Vietnam, and Indonesia (Kalimantan), and additional material from Museum collections (MNHN Paris France, VMNH Martinsville USA). It emphasizes the descriptions of taxa recently proposed by an international group of myriapodologists. 31 species have been described in the order Polydesmida, belonging to the genera *Desmoxytes* (Paradoxosomatidae), *Trichopeltis* (Cryptodesmidae), *Epanerchodus*, *Glenniea*, and *Pacidesmus* (Polydesmidae) and *Eutrichodesmus* (Haplodesmidae). The latter family has been recently revised and synonymised with Doratodesmidae. New Callipodida have been described in the genera *Paracortina* and *Sinocallipus* and the family Paracortinidae have been reviewed. In Chordeumatida, one family, two genera and six species were described (genera *Lipseuma*, *Nepallela* and *Guizhousoma*). In Glomerida, the genus *Hyleoglomeris* was reviewed, in addition of 21 new species and proposals for species groups. The order Stemmiulida was enriched by one new genus and two species of *Stemmiulus* and *Eostemmiulus*. Two new species of Julida are described in the genus *Nepalmatoiulus* from China. In the order Spirostreptida, 61 new species of Cambalopsidae have been described in the genera *Glyphiulus*, *Plusioglyphiulus*, *Hypocambala* and *Trachyjulus* in relation to a revision of the genera in the tribe Glyphiulini. The family Cambalopsidae is obviously the most rich and diverse millipede taxa in the area, particularly abundant in deep subterranean ecosystems and appears as the most interesting model for barcoding investigations. In the light of this knowledge on rich millipede taxonomy, hotspots of biodiversity are confirmed in south-east Asia among karst landscapes in Vietnam and China, Mulun and Maolan karsts ranking as the richest Chinese karsts for subterranean biodiversity. The clustered distribution of the surveyed caves, strong difference in faunal composition between the studied karsts, evidence of micro-endemism inside the Mulun karst suggest that more species are expected in the region.

## **Morphological structures on the integument of *Mesoniscus graniger***

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The integument surface of the cave dwelling terrestrial isopod *Mesoniscus graniger* collected in Magura and Zidita caves, Romania was investigated using scanning electron (SEM) and fluorescence microscopy. Surface structures were observed on the surface of the isopods under SEM. Almost their entire surface is covered by honeycomb-like polygonal scales arranged like a net. Only two fields on both sides of the cephalon seem to be smoother with less pronounced and somewhat morphologically altered scale structures. Tricorns, characteristic for isopod body surfaces, were not observed. The surface granulae looking as a ring or chalice of partly elevated scales with a brush or spine-like structures or setae in the centre were documented as almost regularly arranged structure around the body. Possible functions of these structures are discussed. *Mesoniscus* body surface showed blue auto-fluorescence under UV light. Some morphological structures shine much more brightly than the remaining parts of the body. Chemical principle of this phenomenon and its possible adaptive significance are discussed.

## **Can head morphology reflect habitat preference in the crustacean *Synurella ambulans* (Amphipoda, Crangonyctidae)?**

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In the absence of light body morphology reflect features of the life style of crustaceans. Various populations of the species *Synurella ambulans* is adapted to the epigeal and subterranean habitat types in Croatia. These populations show great similarities in size and body shape and are thus difficult to discriminate in the level of adaptation to the subterranean life. We tested the hypothesis that differences in the habitat demands in alluvial and karstic Croatian environment are reflected by differences in the morphology, especially of their compound eyes. Five populations of the species *S. ambulans* from five different habitat types (limnocrene and rheocrene spring, seepage, karstic river, and alluvial river) were compared for morphological measurements of the head and eyes. The populations not only differ in the head capsule length but also in the relative length of their antenna, and the number and density of their ommatidia. We suggest that the population with the lowest number of ommatidia inhabited intermittent spring and thus it is adapted to the partial subterranean life. These differences can be interpreted functionally and correlate well with features of the various habitats in which the species are found.

## Testing groundwater fauna habitats in landscape

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On the 20th International Symposium on Biospeleology in Postojna, we started a discussion about what is groundwater and what this means to fauna. There, we mentioned that groundwater communities reflect the strength of the hydrological exchange and the origin of the water either from the saturated (surface water bodies) or from the unsaturated zone (soils). With this background, we tested faunistic data from 30 wells, situated in three adjacent landscapes (Naturraums, major physiographic units/MPU). All sites were sampled monthly over the period of 15 months. Multivariate analyses of the faunal data identified five ecological groups of habitats. In general, the results support our hypotheses from Postojna and imply that the strength of the hydrological exchange and the origin of the water strongly influence community structure and species composition. Additionally, fauna was affected by different stressors, forming a distinct habitat group: stressed habitats. However, the correlations between these factors, fauna and classifications were not always clear, since there were also biogeographical or MPU-effects visible.

## Tufa cellars in the Tokaj wine region (Zemplínske vrchy Mts, SE Slovakia) as artificial subterranean habitats

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Spacious complex of wine cellars was excavated in neogene tufa material – a volcanic pyroclastic – in the famous Tokaj/Tokai viticultural region, the Zemplín/Zemplén district on the border between Hungary and Slovakia since the 16<sup>th</sup> century. Long corridors were initially created as a hiding place during the invasions of the Ottoman Empire, later they were used for long maturation of specific Tokaj wines. Due to suitable microclimate (10–12 °C, high humidity) and organic material (oak barrels and evaporated alcohol), diversified communities of moulds were found previously. The walls of the tunnels are completely covered with grey and black velvety moulds (*Penicillium* spp., *Aspergillus* spp. and *Cladosporium* spp. predominating). Fauna of these cellars has not been studied so far. The aim of our research was to test potential occurrence of obligate subterranean fauna and to describe structure of permanent, seasonal and episodic fauna, especially arthropods in such peculiar artificial underground. The study was carried out during winter and spring 2011/12 in two cellars with different history and climate: a) cellar at the village Viničky, about 600 m long, excavated around 1960, with stable “wine” part in tufa (aeration through narrow vertical tubes) and one part with dynamic climate (aeration through great shafts with openings of 2×2 m) within paleozoic sediments or with cement concrete walls; b) tufa cellar under the

village Malá Třňa, exceeding 2 km in length, with medieval history and stable climate. We used direct observations and collecting, pitfall traps with ethylene-glycol/wine solution and heat extraction of standard samples of various organic materials. According to preliminary results both cellars have permanent fauna of invertebrates, with dominance of springtails, millipedes and mites, followed by beetles and flies, locally also spiders, woodlice and earthworms. The fauna is accumulated at the sites with higher humidity and abundant organic material, but it colonises all underground spaces, even those close to the places, where some ricks of sulphur are frequently burned for disinfection of the cellars. Common synanthropic species were identified at the moment. Temporal fauna – various insects, woodlice and bats – enters the underground during winter season through ventiducts and stay near them pro tempore. It avoids the corridors with stored wine processed with sulphurous gas. Vertical shafts give access to accidental fauna, various invertebrates but also rodents, hedgehogs and especially frogs. The study is continued, supported by the Slovak Grant Agency in the project VEGA 1/0139/09.

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Subterranean Biodiversity and Biogeography: oral presentation

## **Why trap troglofauna: nets provide superior catches**

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One of the problems when documenting subterranean biodiversity is low capture rates of animals. The problem is particularly pronounced when trying to document the fauna of the deeper underground matrix. In the Pilbara region of Western Australia, where intensive mining occurs, there has been much sampling of small holes (c. 150 mm diameter) drilled for geological characterization of the landscape. Historically, the most common method of collecting troglofauna from these holes has been setting a trap deep in the hole and leaving it in place for up to 6 weeks before retrieval. The need to visit a site to collect its fauna twice is a significant sampling constraint in remote areas. This talk examines the efficiency of a new technique that we refer to as scraping. Efficiency was measured by comparing capture success of scraping and trapping. Essentially, the scraping technique consists of dropping a net down the hole and dragging it back up against the edges of the hole. It is an extension of the net-hauling technique used for stygofauna and the sample is available immediately. In 9475 comparisons of scraping and trapping in the Pilbara, 4468 troglofauna were caught by scraping and 2029 by trapping. Hemipterans, spiders, pseudoscorpions, beetles and several other groups were much better represented in scrapes than traps, while isopods and polyxenid millipedes were caught in similar numbers by both techniques. Of the 20 Orders recorded, only dipterans (sciarid flies) and two Orders of millipedes (Spirobolida, Spirostreptida) were better represented in traps. In summary, it appears that scraping is a cost-effective method of sampling that collects the same faunal groups as trapping, albeit it in much greater numbers for most groups. Further details will be provided of comparative capture rates and designs of traps and nets for scraping.



## Molecular taxonomy of the cave sponge *Eunapius subterraneus* Sket & Velikonja, 1984

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The only known stygobiotic sponge *Eunapius subterraneus*, Sket & Velikonja, 1984 (Porifera, Spongillidae) lives in a small region near the town of Ogulin, Croatia. Two subspecies were described: *E. s. subterraneus* and *E. s. mollisparspanis*. The latter is known from a single cave (Rudnica VI), situated within the range of the more widely distributed *E. s. subterraneus*. In 2010 a new cave sponge population was found in a sump 1420 meters below ground in the Lukina jama – Trojama Cave system on Mt. Velebit, Croatia. To infer the overall phylogeographic structure of these cave sponges, we amplified an intron region of the ATP Synthetase Subunit  $\beta$  (ATPS  $\beta$ ) gene in 93 specimens sampled throughout the entire range. The results show no population structure within the main range but clearly separate the Lukina jama – Trojama population from the others. To further corroborate these results we analyzed 18S rRNA and ITS2 molecular markers, which also did not support an independent taxonomical position of *E. s. mollisparspanis* but confirmed the divergence of the population from the Lukina jama - Trojama Cave system. The results of molecular analyses point to the need for a revision of taxonomy of cave sponge *Eunapius subterraneus*.

Subterranean Biodiversity and Biogeography: oral presentation

## Distribution of stygobiotic amphipod species in glaciated regions of carbonate and non-carbonate bedrock in Ohio, USA

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Historically, stygobiotic and non-obligate amphipods have been documented in Ohio's surface streams and groundwater (nine non-obligate species of *Crangonyx*, *Synurella*, and *Gammarus*; four obligate species representing *Bactrurus* and *Crangonyx*). Recent discoveries have yielded specimens of three previously unknown populations of the exclusively stygobiotic amphipod genus, *Stygobromus*, collected from ground water via wells (Greene, Montgomery, and Morrow counties) and a rock shelter/natural spring (Licking County). The Morrow and Licking county collections were made in Devonian Berea and Mississippian Blackhand sandstones, respectively, while the Greene and Montgomery county populations occur in Silurian dolomite, all of which are located in or immediately adjacent to formerly glaciated regions of the state. Three

of these populations represent new, distinct species although additional specimens are required for formal descriptions. Like *S. canadensis*, endemic to Castleguard Cave in Banff National Park, southwestern Alberta, Canada and *S. borealis* in a springhouse in Rensselaer County, New York and in Morris Cave in Rutland County, Vermont, USA these newly discovered species of *Stygobromus* in Ohio may have survived the Pleistocene glaciers in groundwater refugia beneath the ice. This kind of isolation north of the southern limits of Pleistocene glaciation clearly would have played an important role in their evolution. The discovery of the new Ohio populations from wells and springs in carbonate and non-carbonate bedrock provide further evidence that stygobiotic amphipod species commonly occupy interstices within the highly fractured bedrock and epikarstic groundwater in addition to the more typical aquatic habitats in caves.

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Subterranean Biodiversity and Biogeography: oral presentation

## Genus *Niphargus* in Slovakia – a permanent surprise?

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Nine species of the genus *Niphargus* are known from Slovakia at present time. Four of them are relative common. *N. tatrensis* Wrześniowski, 1888, is distributed widely in northern part of Carpathians (the area overruns length of 200 km from the Slovak/Ukraine border to Bohemia) in diverse habitats: caves, wells, permanent and temporal springs, even lakes of glacial origin in the Tatra Mts. (as Anitino očko Tarn) in different bedrock and altitudes (150–1830 m a. s. l.). *N. aggtelekiensis* Dudich, 1932, has limited distribution in karstic areas of Central and South Slovakia (Slovak/Aggtelek Karst and Muráň Plateau) with length of area below 100 km. Only these two species were sampled in the Slovak caves. Both species are characteristic with extreme variability in numerous characters, not completely evaluated up till now (ontogeny, heterochrony, individual variability, regeneration ability etc.). Other two “common” species, *N. hrabei* (Karaman, 1932) and *N. valachicus* (Dobreaun & Manolache, 1933) are dwellers of surface water habitats in separate lowlands of Slovakia. *N. hrabei* in the Podunajská nížina Lowland on the SW Slovakia and Pannonian Lowland in Hungary and *N. valachicus* on SE Slovakia (East Slovakian Lowland), what is the northernmost border of this Ponto-Caspic species. All other species inhabit groundwater habitats recorded sporadically in the springs and manholes. Couple of these species has affinity to areas at tectonic dislocations manifested by numerous of mineral springs: *N. bajuvaricus* Schellenberg, 1932 was at Rajec and Turčianska Valley and *N. inopinatus* Schellenberg, 1932, at Rajec and Bojnice. Three other species were recently recorded at foothill of in neogene volcanic hills: *N. baloghi* Dudich, 1940, at foothill of the Zemplínske vrchy Mts.) and two unidentified taxa in the Slanské vrchy Mts. and Poľana Mts. The study of their morphology and taxonomy is just in process. Last group of species includes enigmatic *N. carsicus* Straškraba, 1956 (sole type sample from the Zádiel Gorge in Slovak Karst) and *N. dudichi* Hankó, 1924, known from groundwater of SW Slovakia. Detailed description of morphology, ecology and distributional pattern will be commented. The study was supported by the grant Vega 1/0139/09.



## **Distribution of the Dinaric cave-dwelling tube worm *Marifugia cavatica* Absolon & Hrabe, 1930 in Croatia**

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The tube worm *Marifugia cavatica* Absolon & Hrabe, 1930 is an endemic species of the Dinaric Karst and the only known stygobiotic serpulid. Recently, due to the development of cave diving techniques and the intensification of cave biology research, many new localities of this species were discovered in Croatia. *M. cavatica* is recorded in 71 localities in Croatia. Seven localities are known only from the literature and have not been confirmed by field research, and 11 sites contain only fragments of tubes flushed out of subterranean habitats. Except for the K2 cavern on the Island of Brač, all of these localities are on the mainland. Most of the localities (57.7%) belong to the Adriatic Sea Basin. Systematic research of the biology and ecology of *M. cavatica* in Croatia have never been carried out. It is known that *Marifugia* tolerates a range of water temperatures varying between 4 °C (Lukina jama -Trojama Cave System) and 19 °C (Jama u Predolcu, Pukotina u tunelu polje Jezero – Peračko blato). *Marifugia* can withstand prolonged periods outside the water but was never recorded in brackish conditions, although research of anchialine and other caves close to the Adriatic coast has been quite intense in recent years. The largest aggregations of *M. cavatica* were recorded in Pukotina u tunelu polje Jezero – Peračko Blato and Crni vir caves. Both of these caves are situated in the Jezero Karst Polje near Vrgorac, South Dalmatia, suggesting that the conditions there are very favorable for *Marifugia*. *M. cavatica* is the most widespread sessile species in groundwater of the Dinaric Karst. It is interesting that it inhabits all localities where other subterranean sessile species, the bivalve *Congeria kusceri* Bole, 1962 and sponge *Eunapius subterraneus* Sket & Velikonja, 1984, have also been recorded. *M. cavatica* is strictly protected by Croatian law, and its aggregations are enlisted as a unique habitat in National Habitat Classification (H 1.2.1.2.). Due to the insufficiency of information, *M. cavatica* was assessed as Data Deficient (DD) according to IUCN criteria in the Red book of Croatian cave dwelling fauna. As a sessile species it is directly threatened by changes in water regimes due to different hydrotechnical interventions as well as by different types of pollution of subterranean waters.

## **An evolutionary advantage of albinism in *Astyanax* cavefish**

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Albinism has evolved independently at least three times in the Mexican cavefish *Astyanax mexicanus*, as evidenced by different loss-of-function mutations in the *oculocutaneous albinism 2* (*oca2*) genes. The OCA2 protein functions at the first step of melanin biosynthesis, facilitating

the conversion of L-tyrosine to L-DOPA by the multifunctional enzyme tyrosinase. L-DOPA is subsequently converted into black melanin pigment by several additional downstream reactions catalyzed by tyrosinase and other enzymes. The initial step of this pathway is also affected in the American cavefish *Amblyopsis spelaea* and *Typhlichthys subterraneus*, which are distantly related to *Astyanax*, and in the European cave salamander *Proteus anguinus*, suggesting a broad convergent evolution of albinism in cave-adapted vertebrates. In surface dwelling vertebrates, however, genetic defects can occur at many different steps in melanin synthesis, leading to at least four types of albinism. Accordingly, what is the advantage, if any, of evolving a type of albinism in cave adapted vertebrates in which melanin synthesis is blocked at its first step? The initial step of the melanin synthesis pathway involves L-tyrosine transport into the melanosome, probably mediated by OCA2, where it is used as a substrate to produce melanosomal L-DOPA and eventually melanin, and effectively removing it as a precursor of alternative reactions in the cytoplasm. Therefore, blocking the pathway at its first step could prevent transport and accumulation of L-tyrosine into melanosomes and instead make it available for use in the catecholamine synthesis pathway (L-tyrosine > cytoplasmic L-DOPA > dopamine > norepinephrine > epinephrine), which occurs in the cytoplasm. To test this possibility, we quantified the levels of L-tyrosine, L-DOPA, dopamine, and norepinephrine by high performance liquid chromatography (HPLC) during embryonic and larval development in *Astyanax* surface fish and Pachón cavefish. The results showed a four-fold increase in L-tyrosine, along with the expected decrease in L-DOPA due to blocked melanin synthesis, in cavefish relative to surface fish larvae. The increased L-tyrosine levels were detected at developmental stages when pigment cells differentiate and produce melanin in surface fish but not in cavefish. We also detected significant increases in dopamine and norepinephrine in cavefish relative to surface fish larvae. Therefore, we conclude that an evolutionary advantage of blocking melanin synthesis at its first step in albino cave vertebrates may be to increase the available pool of L-tyrosine substrate for the production of higher levels of dopamine, norepinephrine, and perhaps its derivative epinephrine. The increased synthesis of catecholamines, which serve as important neurotransmitters and paracrine factors, could have key modulatory effects on neural circuitry and behavior that are beneficial for living in a cave environment.

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Phylogeography, Phylogeny and Evolution: oral presentation

## **The cave cricket genus *Troglophilus* (Orthoptera; Rhaphidophoridae): a combination of vicariance and dispersal drove diversification in the East-Mediterranean area**

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In this study, by using a selection of mitochondrial and nuclear genes and by adopting a geographically and taxonomically exhaustive sampling, we reconstructed the evolutionary history of the cave cricket genus *Troglophilus*. The genus is discontinuously distributed throughout the

East-Mediterranean area from Turkey to Northern Italy, including some Aegean islands along the Hellenic arc (Crete, Rhodos and Santorini). This distribution largely overlaps with that of the only other representative of the family Raphidophoridae in the area, the genus *Dolichopoda*. These insects, being apterous and bound to the subterranean environment, have a very low potential for long-distance dispersal and thus offer a unique opportunity to test whether their evolution parallels the complex geological events that shaped the circum-Mediterranean geography. Phylogenetic analyses and molecular-based time estimates suggest a complex scenario. Contrary to what expected in terms of pure vicariance, patterns of relationships are best explained by invoking a combination of vicariance and dispersal events. The species distributed on the Aegean islands form a strongly supported monophyletic clade. Their basal placement in the genus' phylogeny hence agrees with previous hypotheses on the Aegean origin of the group. For the Balkan and Turkish species, active dispersal must be invoked to reconcile molecular data with their current geographical distribution. Here, the most likely scenario is based on a combination of active epigeal dispersal during temperate and humid interglacial periods and subsequent fragmentation of populations during the cold and dry glacial phases. Biogeographic relationships are discussed in light of the data available for the genus *Dolichopoda* to search for shared evolutionary trajectories.

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Phylogeography, Phylogeny and Evolution: oral presentation

## **Resolving the systematics of stygobiotic Amphipoda using morphology and genetic analyses – Melitidae of central Western Australia**

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The Pilbara region of Western Australia contains one of the world's most diverse assemblages of stygofauna. Many of these invertebrates have been shown to be endemic to the region, with restricted short range distributions – many limited to single aquifers. The stygofauna of the Pilbara is dominated by crustaceans, in particular the Amphipoda. Members of one amphipod family, the Melitidae, have been extensively collected and documented in the Pilbara and the adjacent offshore Barrow Island, however many of the species are inadequately and in some cases contentiously defined (many genera are monotypic, and most species are known from single localities). A multi-year project is underway and attempts to resolve the evolutionary history and systematics of this group. Species boundaries will be defined using a comprehensive approach and include morphological, molecular, and biogeographic and hydrogeographic data. Here, we present the latest results. Analyses of *COI* molecular data shows significant lineage diversity at the broader catchment scale, suggesting the presence of a great diversity of potential genera and species isolated within each of the five Pilbara catchments.

## **Evidence for numerous subterranean colonisations: using molecular and morphological approaches to define the Australian chiltoniid amphipods**

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Groundwater associated with springs of the Great Artesian Basin in South Australia and calcrete aquifers in Western Australia have in recent years been shown to contain a significant diversity of invertebrate species with highly restricted distributions. A dominant feature of this faunal diversity is amphipods of the family Chiltoniidae, yet the family has remained poorly known with only four contentiously defined species described from Australia. In this project we used genetic data as a framework for morphological analyses of the Australian Chiltoniidae and attempted to provide a systematic framework to the group. Molecular analyses identified considerable genetic (28S, *COI* mtDNA) diversity among discrete mound spring and calcrete chiltoniid amphipod populations, suggesting numerous subterranean colonisation events and unique species being present within individual calcretes and spring groups. Morphological analyses revealed considerable phenotypic plasticity within chiltoniid species. The outcomes of the project are a robust analysis of chiltoniid species diversity in these groundwater ecosystems, information that has important conservation and management implications for Australian groundwater systems.

## **DNA barcoding of genus *Eupolybothrus* (Chilopoda, Lithobiidae) from Dinaric Karst reveals unexpected cryptic diversity**

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In total, 54 specimens of the genus *Eupolybothrus* collected in a period from 2005–2010 from caves in Croatia, Bosnia and Herzegovina and Montenegro were DNA barcoded. Additionally, DNA barcodes of *E. transylvanicus*, *E. kahfi*, *E. fasciatus*, *E. litoralis* and *E. nudicornis* were included in our analysis. The morphology of our specimens match the original descriptions of *E. leostygis*, *E. gloriastygis*, *E. obrovensis*, *E. tridentinus*, and *E. grossipes*; but our material comprised several indeterminate *Eupolybothrus* sp. as well. The genetic results confirm our morphological diagnosis by forming monophyletic morphospecies: *E. leostygis* (Verhoeff, 1899) cluster includes eight specimens found in three caves in the wider area of the city of Dubrovnik (Croatia); *E. gloriastygis* (Absolon, 1916) cluster includes ten specimens collected in two caves (Bosnia and Herzegovina and Montenegro), one of which is type locality for *E.*

*magnificus* (Hoffer, 1935) – a junior synonym of *E. gloriastygis*; *E. obrovensis* (Verhoeff, 1930) cluster consists of three specimens collected in two caves in Mt. Učka (Croatia); *E. tridentinus* (Fanzago, 1874) cluster comprising of 13 specimens shows the most heterogeneity, since specimens were collected from a larger distribution area: six different caves in wider area of Krka National Park (Croatia) and one cave near Trebinje (Bosnia and Hercegovina). The *Eupolybothrus sp.* does not cluster with the other ten DNA barcoded morphospecies, but form 14 distinct genetic lineages. Two clusters, each comprising four specimens, are particularly interesting. The “Miljacka cluster” includes specimens collected in two caves in Krka NP (Croatia). Specimens from the “Zrmanja cluster” were collected in four caves in a wider area of Zrmanja river canyon (Croatia). Both clusters are morphologically well supported and differ from all species of the genus *Eupolybothrus* so far recorded for Croatia. Several individuals form single evolutionary lineages, which are morphologically supported and differ from all other analyzed specimens. Conclusively, the genus *Eupolybothrus* demonstrates a high degree of cryptic diversity and undescribed species are expected to inhabit the Dinaric Karst.

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Phylogeography, Phylogeny and Evolution: oral presentation / student

## Same and yet different: cave populations of *Asellus aquaticus* across Europe

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Parallel and convergent evolution provide strong evidence for the role of natural selection: similar phenotypes evolve in populations that colonized similar environments independently. However, populations in similar environments always also show some differences. Non-convergent traits can reflect adaptation in some cases or neutrality in others, and are a valuable source of information about evolutionary processes. One such example is the isopod crustacean *Asellus aquaticus*. Despite its ubiquitous ecology and Europe-wide distribution there are only two areas where troglomorphic populations can be found: in the northern Dinaric Karst, Balkan Peninsula, and in Dobrogea, Romania. The two areas are 1000 kilometers apart, separated by largely non-karstic terrain. Applying phylogenetic analysis of mitochondrial and nuclear DNA sequences, we demonstrated that the Romanian troglomorphic population (*A. a. infernus*) does not share the most recent common ancestor with northern Dinaric ones. In both areas the cave populations are genetic subgroups of the surface populations from the local area and are younger than the splits among the main European phylogeographical groups, suggesting their recent colonization of the subterranean realm. In both areas, however, there are signs of multiple colonization events and in many places they can be found parapatrically. There are several similarities among independently evolved cave populations, like loss of pigment and eyes, longer antenna II, less articles in the flagellum of antenna I and less widened propod of the pereopod I. Yet the cave systems of both areas are strikingly different. In the northern Dinaric Karst the *Asellus* populations inhabit hydrologically complex cave systems with sinking rivers and poljes, forming sequential sections of subterranean and surface habitats. In Romania, they are part of a complex chemoautotrophic ecosystem in thermal sulfidic groundwater. Why no other groundwater system was colonized, remains a mystery.

## History of the speleozoological research in Slovakia

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Within the speleozoological research in Slovakia, three periods can be distinguished according to the territorially-political situation (1) period of historical Hungary from 1841 to 1918, (2) period of the independent Czechoslovakia from 1918 to 1945, (3) period of Czechoslovakia from 1945. In the first period of the research, four explorers can be mentioned – J. S. Petényi (Petian), I. Frivaldszky, J. Frivaldszky and A. Schmidl, who between 1841 and 1856 visited and explored the Baradla Cave, lying today at the Slovak-Hungarian border. Later, in the second half of the 19th century, the research of cave fauna was only sporadic and occasional. New and intensive research of cave fauna started since 1903, when a new species of *Duvalius* has been discovered in Baradla cave. In this respect, the brothers E. Bokor and entomologist E. Csiki were the main researchers till the year 1928. In Czechoslovakia after 1918, E. Bokor was still active in searching of cavernicolous beetles. After the Domica Cave (an upper part of the Baradla cave system) had been discovered in 1926, the geologist Kettner initiated the foundation of the Commission for Karst Research in 1933. The field research lasted up to 1939 (V. J. Staněk, J. Štorkán, Z. Frankenberger, S. Hrabě, F. Miller and J. Kratochvíl). During this period, E. Dudich was the first to study crustaceans in phreatic waters in Slovakia. After 1945, research in caves and underground waters was soon restored. The main research was focused to Chiroptera and their parasites (Diptera, Siphonaptera, Acari, helminths) (J. Vachold, J. Gaisler, V. Hanák, K. Hůrka, F. Dusbábek, J. Weiser, J. Hůrková). In phreatic and hyporheic waters, crustaceans – mainly copepods had been studied very intensively (O. Štěrba, V. Kulhavý, J. Brtek, M. Straškraba). On the other hand, dry land arthropods had been studied only sporadically (J. Paclt, J. Gulička, J. Nosek, J. Rusek, I. Zajonc). In 1975, J. Gulička published the first summarizing paper on underground fauna in Slovakia. Since 1972, V. Košel has started his research of cave water and dry land fauna.

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Subterranean Biodiversity and Biogeography: poster presentation

### Troglobiotic collembolan *Neelus koseli* Kováč & Papáč, 2010 (Collembola, Neelidae) – contribution to its morphology, ecology and distribution

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Springtail species *Neelus koseli* Kováč & Papáč, 2010 (Collembola, Neelidae) has been described recently from several caves of the Čierna hora Mts. and the Slovak Karst in Western Carpathians, Slovakia. In contrary to edaphic congeners, it exhibits clear troglomorphic features, i.e. larger



body, elongated unguis on legs and elongated sensilla on antennal segments. The cuticular surfaces of *N. koseli* were studied in detail using scanning electron microscopy. Structures only weakly visible in light microscope are described and documented, including sensilla on antennal segments and modified setae of sensorial fields on dorsal side of the body (head, thorax, abdomen) and coxae of legs. Recent extensive study of endogean arthropods in “milieu souterrain superficiel – MSS” on limestone substrate (Čierna Hora Mts.) was conducted by Rendoš et al. (unpubl.) using subterranean traps installed to a maximum depth of 95 cm. The traps revealed presence of *N. koseli* in depth range 45 – 95 cm, number of specimens caught gradually increased up to the maximum depth. Thus this troglobiotic representative is also an inhabitant of MSS on limestone. Rather disjunctive distribution of *N. koseli* restricted to small and isolated karst units within the Western Carpathians may be explained by their common historical origin within the Silicikum carbonate platform of the Late Permian – Late Jurassic origin. It may be considered as a descendant of older fauna of Kenozoic origin that inhabited subterranean environments of the Silicikum platform before its later tectonic fragmentation and desintegration into smaller units. However, the species has been recently discovered in the Pieniny Mts. in northern Slovakia belonging to different geological unit (Pieniny Klippen Belt). Up till present the karst of the Pieniny Mts. has been assumed as generally poor in cave fauna with absence of obligate cave representatives.

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Phylogeography, Phylogeny and Evolution: Scientific documentary

## **Eight wonder of the Domica Cave (Slovak Karst, Slovakia)**

Václav KRIŠTŮFEK & others

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The Domica Cave in the Slovak Karst is peculiar with its seven wonders, such as sinter shields, spherical stalactites, sinter neolithic earthen vessels, prehistoric drawings, colonies of bats, sinter dams and underground sailing. On two sites in the cave bat droppings accumulate in heaps more than one meter high and 3–4 m wide produced by great colonies of Mediterranean horseshoe bat (*Rhinolophus euryale*) and Schreiber's bat (*Miniopterus schreibersii*). What could the bat guano reveal? Thousand-years old guano heap tell us about the life of bats, insects and plants occurring in the proximity of the cave centuries ago. Moreover, it tells us also about the human activities in the surroundings of the cave. The movie introduces the famous cave, interconnection of man with above-ground and underground ecosystems, and the work performed by scientists who reveal the hidden secrets of the eighth wonder of the cave.

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Subterranean Biodiversity and Biogeography: poster presentation

## **Diversity and distribution of troglophilic fauna of caddisflies and moths (Insecta: Trichoptera, Lepidoptera) of Croatia**

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The Republic of Croatia is placed in the south-east part of Europe. Its territory covers 56,000 km<sup>2</sup> of land and 32,000 km<sup>2</sup> of sea. According to the climate and geomorphological particularities three zones can be distinguished: a continental region between the rivers Sava and Drava with continental climate (hot summers and frosty winters), a central highland region with typical mountain climate (short summers, long winters with plenty of snow) and a Mediterranean region with Mediterranean climate (warm and long summers and- mild and- rainy winters respectively). A geomorphological important part of Croatia is the karst- region (calciferous area) which covers the central-highland and the Mediterranean part and its rich in different speleological objects. The fauna of caddisflies (Trichoptera) and moths (Lepidoptera) is represented by a small number of species of troglobiotic fauna. The first data on the Trichoptera and Lepidoptera cave fauna of Croatia are known from the first half of the 20th century (e.g. Wettstein, 1912; Girometta, 1914, Langhoffer 1912, 1915; Pax, 1937, 1938). Systematic investigation of cave Trichoptera and Lepidoptera started 20 years ago with intensive fieldwork in the karst region of Croatia conducted by the Croatian Natural History Museum, Faculty of Science in Zagreb and Croatian Biospeleological Society. The aim of these surveys was to define ecology, diversity and distribution of trogliphilic caddisflies and moths in Croatia. About twenty species of trogliphilic moths and caddisflies are recorded in Croatia so far.

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Phylogeography, Phylogeny and Evolution: oral presentation

## **Comparative analyses of ancestral distributions of Yilgarn (Western Australia) diving beetles and chiltoniid amphipods inferred from biogeography and phylogenies**

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The Yilgarn region of central Western Australia contains hundreds of isolated calcrete aquifers lined up in a number of palaeo drainage valleys. These aquifers are inhabited by a diverse range of stygofauna taxa. The majority of the stygofauna species are endemic to individual calcrete aquifers. Here we compare the evolution of two groups that have very different life histories: insects (diving beetles, Dytiscidae) and crustaceans (Amphipoda, Chiltoniidae), which are both common taxa in these aquifers, often with multiple species per aquifer. By combining phylogenetic and biogeographic data we test whether the colonization of these aquifers by ancestral surface species is structured according to the palaeo drainage patterns, as is hypothesized for amphipods that are confined to permanent water, or does not follow the palaeo drainage pattern, as is hypothesized for diving beetles, because surface species are known to disperse over large distances. We find that the diving beetles had ancestral species that were widespread and colonized the aquifers in a random pattern. Although the historical biogeographic pattern of the chiltoniid amphipods was more or less structured according to palaeo drainage pattern, the pattern was not as clear as expected. Phylogenetic analysis showed that this was caused by the apparent existence of multiple ancestral amphipod lineages that must have been present in the drainages before the colonization of the aquifers. Ultimate species composition in individual aquifers is explained by random colonization processes, competition and local extinction.



## Collembola in caves

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Collembola play an important role in the subterranean communities forming large populations and being predated by numerous predators. Due to their minute size, ethology, biology and adaptations to cave life is rarely studied in situ. Collembola are filmed in numerous situations as feeding, cleaning, jumping, fighting over food and being hunted. The act of predation is rarely seen and documented and in this short movie predators from several taxonomic groups (Aranea, Acari, Palpigrada, Pseudoscorpionida) are shown predated and feeding on Collembola. As many other cave dwelling taxa they have also developed numerous adaptive features to cave life e. g. elongation of the claw as adaptation to wet conditions. Another adaptation, jumping over small water puddles, is recorded by new species of *Tritomurus* (Tomoceridae) from a Croatian cave. Reduced jumping power is used to cross the water puddles compared to normal jumping in the case of predation escape. This unique ability has never been reported for Collembola and is for now observed only for the new species known from a single cave. All of the scenes are filmed in their natural environment in situ using Sony camcorder DCR-HC1000 with some additional photos included in the movie.

## Dinaric type localities of cave dwelling fauna: research and conservation

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The Dinaric Karst is the world's hotspot of cave fauna biodiversity with a large number of cave dwelling species. So far, around 1200 taxa have been described from numerous caves scattered all over Dinaric Karst. In order to prepare a platform for conservation of this heritage in Croatia, the Croatian Biospeleological Society (CBSS) launched a long term project in 2000. First aim was to collect the data on Croatian type localities of cave fauna. Several reasons pointed to the need for urgent action regarding type localities. Some of the type localities are becoming impossible to find due to the rapid decrease in number of local inhabitants who know cave names and their exact positions. Furthermore, a certain number of type localities faced complete devastation or are in danger due to the high pressure of economic development and ignorance and negligence of relevant governmental bodies. Also, the number of type localities is increasing as new taxa are being frequently discovered. As a result of the CBSS project the "Catalogue of cave type localities of Croatian fauna" was published in 2006. It initiated the

cave type localities protection on the state level. In 2010 “The cave type localities atlas of Croatian fauna, Volume 1” was published which presents 102 type localities and 133 taxa in a popular and illustrative way suitable for education of wider community about the values of underground world. During 2011 the entire Dinaric area became included in the project by launching the open access database Biospeologica Dinarica ([www.biospeologica-dinarica.org](http://www.biospeologica-dinarica.org)) and establishment of Scientific and Expert Network consisting of members from all countries of Dinaric Karst. The database will serve as a portal for all Dinaric Karst cave species and their type localities. It will include species and cave images, references, regional and global species conservation status, cave maps, endangerment assessments etc. In 2012, data for 300 caves and 650 taxa will be included in the database. General long term goal is to generate a complete checklist of the species and type localities of Dinaric cave fauna. Another important aim of Scientific and Expert Network establishment is to initiate and intensify cooperation and collaboration on cave fauna research in the region and facilitate taxonomic research of many invertebrate groups.

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Subterranean Biodiversity and Biogeography: oral presentation

## Species diversity and distribution of cave Collembola in Dinaric karst

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Although cave dwelling fauna of the Dinarides is relatively well known, one of the most diversified group of cave terrestrial invertebrates, Collembola, remain heavily understudied and undersampled. Altogether 26 species from 14 genera (8 families) have been described to date from caves of Italy (1), Slovenia (13), Croatia (4), Bosnia and Herzegovina (5) and Montenegro (2) with scarce distributional data. Extensive Collembola sampling during last 5 years and analysis of part of Croatian Biospeological Society Collection resulted in data set for 650 caves (95% from Croatia) presented in this study. A total number of 13 000 specimens were examined and 260 specimens were DNA-barcoded using 658 bp 5' region of COI. Some genera were not morphologically studied in details and for them the MOTUs were used as surrogates to species. Two new genera and 45 new troglomorphic species and MOTUs from 10 families were identified. First troglomorphic taxa of family Isotomidae (*Parisotoma* sp. nov. and two new genera) and Neelidae (*Neelus* sp. nov.) were recorded for Dinarides. For some genera holodinaric (*Pseudosinella*, *Tritomurus*, *Oncopodura*, *Troglopedetes*, *Disparhopalites*, *Onychiuroides*), north-western merodinaric (*Absolonia*, *Neelus*) and south-eastern merodinaric (*Verhoeffiella*, *Typhlogastrura*, *Galeriella*, *Ongulonychiurus*) distribution patterns were recognized. Few new taxa (*Tritomurus* sp. nov., *Ongulonychiurus* sp. nov., Isotomidae gen. nov.) found in deep pits are outstanding by their degree of troglomorphy, their phyletic isolation and their unique adaptive features. They have no known epigeal relatives in their respective distribution areas suggesting a relictual status, but share some non-adaptive characters with

microendemic cave species of the Pyreneo-cantabric range (*Tritomurus veles* Lukic, Houssin & Deharveng 2010 and *Tritomurus* sp. nov. with *Tritomurus falcifer* Cassagnau, 1958; *Ongulonychiurus* sp. nov. with *Ongulonychiurus colpus* Thibaud & Massoud, 1986). The puzzling disjunct distributions of these genera remain unexplained, and is not supported by distribution patterns in any other terrestrial cave dwelling fauna. This study shows that Collembola is one of the most diversified groups of terrestrial cave fauna in Dinarides together with Coleoptera, Araneae, Pseudoscorpiones and Oniscidea.

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Subterranean Biodiversity and Biogeography: poster presentation

## **Morphology and distribution of a cave dwelling oribatid mite *Belba clavigera* (Acari, Oribatida)**

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The most of oribatid mites recorded in caves of temperate zone belong to troglonexes. However, several species have close relation to cave environment. First and foremost is *Belba clavigera*, described from the Balcarka Cave in Moravian Karst (Czech Republic) by Willmann in 1954. This mite belongs to a group of epigeic species with prolonged legs. Pale body color and extremely long legs we consider as troglomorhic feature of the species. Simplified and insufficient original description of the species and recent records of *B. clavigera* in Slovak caves were the stimuli for detailed redescription of morphology and completion of data on its distribution and ecology.

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Population and Community Ecology of Subterranean Organisms: oral presentation

## **The role of history of place, dispersal and selective factors in shaping the distribution of a subterranean isopod**

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Disentangling the importance of interactive processes shaping biodiversity patterns at scales ranging from local to global is a key issue in ecology. The mess stands from the fact that there are numerous ways to get from process to pattern. In this study, we combined genetic, ecological and physiological data to examine how dispersal and selective processes might have interacted over time to shape the present day distribution of the subterranean isopod *Proasellus valdensis*. This species exhibits striking distributional features. First, its geographic range almost entirely fits within the limits of the last glacial maximum. Second, *P.*

*valdensis* occurs in isolated limestone massifs. Third, the species has a large altitudinal range, but occurrence data suggest that it is more frequent at high altitude. These distributional features lead to contrasted scenarios about the role of dispersal and selective factors. The range limits suggest a post-glacial dispersal whereas the disjunct distribution may indicate the occurrence of cryptic and allopatric units within *P. valdensis*. Although correlation does not imply causality, the putative altitudinal distribution of *P. valdensis* also suggests a role of temperature. This species could maximize its physiological performance at low temperature, a trait that is equally advantageous for surviving *in situ* or dispersing in vacant habitats during ice melting periods. Alternatively, the altitudinal distribution of *P. valdensis* may also reflect a thermally dependent biotic interaction. In this study, we attempted to select the best supported scenario using a multifaceted approach. First, we used phylogeographic methods to determine which of the dispersal and cryptic speciation scenario was more plausible. Second, a logistic regression model was performed to quantify variation in the probability of occurrence with groundwater temperature. At last, we measured variation in survival and respiration over a range of temperatures within 4 populations to test for a causal relationship between temperature and the distribution of *P. valdensis*. This research was funded by the Agence Nationale de la Recherche (ANR08JCJC012001, “DEEP”), the Institut Universitaire de France and the European Commission (7th EU Framework Programme, Contract No. 226874, BioFresh).

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Population and Community Ecology of Subterranean Organisms: poster presentation

## ***Polycephalomyces ramosus* (Hypocreales, Ascomycota) an interesting troglophilic entomogenous fungus, new for Croatia**

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During the regular monitoring on Veternica Cave (Mt. Medvednica, Croatia) performed in cooperation of Croatian Biospeleological Society experts and Natural Park Medvednica, particular synnematosus entomogenous fungus parasitizing on imago of subtroglophilic fly *Heteromyza* sp. (Heleomyzidae, Brachycera, Diptera) has been found. Specimens, found in the dark zone of the cave were sampled, microclimate data recorded and photo documentation performed. The fungus is identified as *Polycephalomyces ramosus* (Peck) Mains (Hypocreales, Ascomycota) according to the Seifert's monograph on stilbellaceous fungi. This anamorphic ascomycetous fungus is often found in caves, living as a hyperparasite on entomogenous fungi viz. *Hirsutella guignardii*, *Cordyceps barnesii* and *C. entomorrhiza*. Relying on data compiled in form of preliminary checklist of Croatian cave fungi, this species is considered as first record for the Croatian mycobiota. This first finding of *Polycephalomyces ramosus* parasitizing on *Heteromyza* fly, give us opportunity to perform *in situ* morphological and ecological assessment and to work on its taxonomical status. Similarly to *Cordyceps riverae* treated in previous detailed ecological research, *Polycephalomyces ramosus* too is always

found to produce fruitbodies (in this case synnemata) at extremely high air humidity (100%) and condensed water regularly appears on the surface of both synnemata and host bodies. Air temperature (10 °C) is also very constant ecological factor, and occurring in such large cave as Vjeternica, without daily fluctuations and negligible seasonal fluctuations. Both *Polycephalomyces ramosus*, as well as and *Cordyceps riverae*, are members of the same order and may well be rather closely related, especially due to their common subterranean and cavernicolous habitat where they parasitize or hyperparasitize on adult stages of arthropods and both do not require sunlight for completion of their whole life cycle. They are therefore able to live constantly inside cave habitats. However, final conclusions on both taxonomical and ecological issues will be possible only after the planned molecular research is done focused on these troglophilic fungal species.

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Subterranean Biodiversity and Biogeography: oral presentation

## Hydrogeological borehole investigations of groundwater ecology in the English Chalk

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Estimating the numbers of stygobiotic invertebrates in aquifers, and understanding their distribution within boreholes and how this relates to their distribution within aquifers is important for understanding their environmental and habitat requirements, as well as the ecosystem services they might provide (e.g. their role in biogeochemical cycling and their potential as water quality indicators). Single borehole dilution tracer tests and borehole imaging were used to identify the location of major flowing fissures intercepted in two boreholes of approximately 100 m depth in the English Chalk. To investigate variability in invertebrate faunal communities and numbers with depth, 3 flowing fissures were selected in each borehole – one near the water table, one near the bottom of the borehole and one in the middle. A double packer system was used to isolate each of these fissures, enabling them to be directly sampled. At each interval, invertebrates were collected from 5000 litres of pumped water. Previous net hauling from above the bottom of boreholes and borehole CCTV images indicated that stygobiotic invertebrates live in the water column (particularly on the borehole walls) at substantial depths above the bottom. Fauna from the isolated packer intervals therefore comprise both animals living in the borehole water column within the isolated interval and those living within the water pumped from the aquifer. The 5000 litres of pumped water was divided into 13 samples in which fauna were collected separately to investigate how numbers and types of invertebrates relate to the amount of water that has already been pumped from the interval. In each interval, water chemistry and microbiological samples were collected at the beginning, middle, and end of pumping. Results indicate that different intervals contain different numbers and types of fauna, and have different microbial populations, and provide new information on the ecology of Chalk groundwaters.

## **Predictive GIS modelling and conservation of copepods in groundwater habitats of the Romanian Carpathians**

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The distribution of subterranean copepods as related to environmental parameters may reflect the integrity of the groundwater ecosystem. Our aim was to assess copepod diversity and to depict suitable areas for copepod population persistence in groundwater habitats of the vadose zone using ArcGIS. We then identified the environmental parameters influencing subsurface copepod distribution – a proxy for groundwater quality. Data for copepod distribution in groundwater habitats of the vadose zone in karst areas of the Romanian Carpathians were compiled from different sources (i.e. personal surveys, published data). Six environmental features were selected from the available data to serve as model parameters. We used habitat-based modelling to predict suitable areas for seven subsurface copepod taxa. Ordinary least squares regression and geographically weighted regression were used to identify the significant predictors for explaining copepod habitat suitability. The most constant predictor was land cover, a measure of human impact, followed by precipitation and elevation. Hypogean taxa were the main taxa correlated with land cover. The model performed well for the majority of analyzed taxa and the predicted suitable areas for narrowly distributed taxa overlapped with the observed distribution. ArcGIS can be useful in predicting habitat suitability, and also allows us to test spatial autocorrelation. The sustainable management of the surface areas of karst landscape should be a priority in protecting and conserving the groundwater resources.

## **Groundwater biodiversity in Western Romanian Carpathians: the case of *Niphargus* (Amphipoda, Crustacea)**

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In the past decades, several studies have shown that subterranean aquatic habitats importantly contribute to overall diversity of freshwater aquatic fauna. Subterranean taxa show narrow distribution ranges and as such, significantly increase species richness and endemism at regional



scale. Until now, taxonomic and ecological research on *Niphargus* in Romania has been based exclusively on study of morphological characters. Several recent studies on *Niphargus* from other countries, however, imply that morphologically cryptic species within this genus might be common and that accurate species identification and delimitation requires molecular tools. We studied the area of the Apuseni Mountains (NW Romania) that includes the protected areas of the Pădurea Craiului Natura 2000 Site and the Apuseni Natural Park. This small (500 km<sup>2</sup>) region presumably harbors ten niphargid species. Our aim was to revise the taxonomic status of these species and to assess their phylogenetic diversity, i.e. to identify the number of phyletic lineages living in the region. We analyzed eleven cave populations using two independent molecular markers (COI and 28S). Phylogenetic analyses showed that studied populations belong to seven different species that derive from two independent phylogenetic lineages. Taxonomic results are in contrast with our expectations: several species reported from individual caves have not been re-collected, suggesting either species' rarity, past misidentifications or both. Two species endemic to this region, however, appeared to be complexes of cryptic species. The newly discovered sister species imply that speciation in *Niphargus* can occur at scale of few tens of kilometers. These findings can be used in further taxonomic researches and conservation strategies.

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Subterranean Biodiversity and Biogeography: poster presentation / student

## Porifera inventory from anchialine caves

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To contribute to the global biodiversity assessment this paper aims to review available information on the cave-dwelling anchialine sponge fauna worldwide. For this purpose, investigations on anchialine sponges from the historical point of view are presently in progress (papers, grey literature, private collections etc.) In addition, we analysed new samples of sponges recently collected in two sites: Canary Islands, Corona lava tube, Tunnel of Atlantis at 722 m from the entrance, 18 m of depth (courtesy of A. Martinez); Sardinia, oriental karst, shallow water in estuarine caves. Results indicates from the historical point of view that sponge fauna, and in general sessile benthic taxa, from anchialine caves is sparsely investigated (Manconi, 2009; Martinez et al., 2009; Manconi et al., 2010; van Soest et al., 2012), and that the few records of sponges from anchialine caves refer to species endemic to a single cave or a single cave system. The latter condition is valid for *Higginsia ciccaresei* Pansini & Pesce endemic to the Mediterranean Zinzulusa Cave (Pansini & Pesce, 1998) and *Protosuberites geracei* (van Soest & Sass) and *Oceanapia penicilliformis* (van Soest & Sass) endemic to the Bahamian Dixon Hill Lighthouse Cave of San Salvador (van Soest & Sass, 1981). Another species, *Cinachira subterranea* van Soest & Sass, 1981 previously considered endemic to the latter cave is at present considered junior synonym of a widespread species *Cinachyrella alloclada* (Uliczka, 1929). Also, is remarkable the recent record of *Protosuberites* cf. *epiphytum* (Lamarck, 1815)

and a haplosclerid species (Manconi & Ledda, in prep.) in the Bue Marino Cave (Sardinian karst). The latter investigation focus on cryptobiosis by resting bodies as adaptive strategy of the cave-dwelling sponge fauna. As for present taxonomic investigations, analyses on sponges from the Corona lava tube and Sardinian karst are currently in progress. Our investigations highlights that very few published data exist. Although samples of sponges are registered in some collections by T. Iliffe (in litt.), no taxonomic investigations were performed on these collections (Martinez et al., 2009). Moreover new samples are under study from Sardinia and new sampling campaigns are planned for the next future in the Bahamas, Canary Islands and Sardinia.

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Subterranean Biodiversity and Biogeography: poster presentation

## **Fauna associated to artificial cavities in the state of Minas Gerais, Brazil**

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The goal of this study was to understand the ecological aspects related to artificial galleries systems, and compare communities found in these systems with those observed in caves. Furthermore, we measured abiotic factors related to the temperature in artificial cavities. We sampled a total of 110 artificial cavities located in 12 municipalities in the state of Minas Gerais. Over 90% of the cavities exhibited aphotic zones. Temperature ranged from 16 °C to 29.1 °C, and humidity ranged between 67% and 100% of saturation. The most abundant resource in hypogean environment was plant organic matter (91%), which were concentrated especially in the entry region. Other abundant resources in this environment were organic matter carried by human action (32%) and bat guano (28%). We found 594 invertebrate species by manual collection. The richer orders were Diptera (102 spp.), Coleoptera (83 spp.) and Araneae (62 spp.). Araneae represented 21% and Orthoptera 16.6% of the total number of individuals observed. We observed significant differences of Mean Value of Population Distribution (VMDP) ( $F(1, 213) = 63.657, p < 0.001$ ) and Ecological Complexity Index (ICE) ( $F(1, 213) = 15,778, p < 0.001$ ) between artificial cavities and caves. Using comparisons between pairs of artificial cavities, the similarity between them ranged from “0” (found for 1,238 combinations between pairs of cavities) to 61.5% (two artificial cavities 140 meters-apart). Similarity between communities presented in the same municipality ranged from 13% (Novo Oriente de Minas municipality) to 46.8% (Caraí municipality). The homogeneity of these artificial cavities, their lack of resources and the constant presence of anthropogenic activities, make that these environments present less total richness than caves. The distribution of invertebrates populations is affected by these factors, being these animals concentrated especially near the organic resources in the entry of artificial cavities. Despite artificial cavities present a smaller number of species than caves, these structures could be an important refuge facilitating the permanence of many species in areas where the external environment are intensely impacted.



## **The RIPARI project: a vegetated buffer zone and its effect upon groundwater biocoenosis**

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“Buffer zones” are widely utilised along rivers since several year in different parts of Europe and their effect upon the control of various polluting agents is recognised. The RIPARI project is a POR-FESR project of Tuscany Region (Italy), financed by EU. It’s main objective was the installation of a “Buffer zone” along a small channel in an agricultural area at risk of nitrate and pesticides pollution, to evaluate the fine-scale effects on groundwater biome. The scheme of the project is presented, together with the methods utilised and the first preliminary results upon physico-chemistry of runoff water and groundwater and it’s biological and microbiological status. To study the groundwater fauna, nine piezometers were installed each with three different levels of capture devices ranging from 1.5 to 10 m depth. The groundwater resulted in a suspended aquiclude. The inhabiting biocoenosis resulted strictly stygobiotic with a good biodiversity in accord with similar ecosystems, mainly composed of Copepods and Oligochaetes. The tree species of stygobiotic copepods present in the nine piezometer utilised for the study, and utilised as target species, resulted in fact, after molecular study, as belonging to four genetic lineages. The nine piezometers resulted homogeneous in their physico-chemical and microbiological parameters irrespective of position and depth range while the fauna was mainly concentrated on the top levels.

## ***Rhinolophus euryale* (Chiroptera: Rhinolophidae): the summary results of ecological research of cave dwelling bat**

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*Rhinolophus euryale* is originally cave dwelling bat species reaches the northern border of its area in central Europe (Slovakia and Hungary). Here it formes isolated subpopulation with

some ecological differences from the core population in the Mediterranean. We summarise current knowledge about the species based on the wide-oriented research in the Slovenský kras Mts. (southern Slovakia). The telemetry method was used to observe foraging habitat and home range of the species. There were marked altogether 29 adult females during the period 2010–2011. We have found out that the forest edges are the main foraging habitats and whole foraging area reached (expressed as a minimal convex polygon) 192 ha. The analysis of seasonal changes in the diet shows that medium-sized Lepidoptera is the main component of diet during the whole season, with increased portion of Tipulidae in the spring. Observations advert to considerable vagility of individuals in the hibernacula and the foraging and grooming activity during the hibernation. Altogether 4161 individuals of parasites from 259 bats were collected (families Ixodidae, Spinturnicidae and Macronyssinae) with dominance of *Eyndhovenia euryalis euryalis* (4087 inds, 98.2%). The data analysis of known roosts shows that species use for hibernation only subterranean shelters (27% mines, 73% caves; n=30 roosts). However there is a major change in roost preference in summer observed; the species starts to use lofts and roofs since the 70's of the 20th century with the result of the beginning of the synanthropisation process. Permanent temperature measurements in the roosts during the whole season refer to big differences (ca. 5 °C) between average temperatures in the cave roosts and lofts.

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Adaptation, Development, Physiology and Behaviour: poster presentation / student

## **Trophic basis of winter activity in *Rhinolophus euryale* (Chiroptera)**

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*Rhinolophus euryale* is originally cave dwelling bat species reaches the northern border of its area in central Europe (Slovakia and Hungary). Species shows the high vagility within and/or between its winter roosts during the hibernation period and species is often rousing from torpor. We hypothesized that the species should forage during the flights between the hibernacula and thus compensate energetic losses. We studied winter prey composition collecting the fresh guano samples under the winter aggregations of *Rhinolophus euryale* in six sites (Slovakia: Jasovská jaskyňa Cave, Drienovská jaskyňa Cave, Ardovská jaskyňa Cave, Domica Cave; Hungary: Baradla Cave; Romania: Galaşeni Cave) during November 2011 – April 2012. We found out that the analyzed pellets had two forms: (1) containing only Lepidoptera and (2) containing non-prey items. Male genitals (valve) were used to determine lepidopteran taxa in the foraged prey. Second pellet type includes unidentified organic material resembles tissue or gel, where hairs were often presented and in few samples were found mites. We suppose that gel-like guano is the result of intensive grooming, continuous digesting processes, changing of intestinal tissues and/or endobacteria activity. Data shows that Lepidoptera plays the crucial role in compensating energy losses during the winter activity of *Rhinolophus euryale*.

## **Study on the genus *Hylebainosoma* sensu lato (Diplopoda, Chordeumatida, Haaseidae) – the millipedes with affinity to subterranean habitats**

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Genus *Hylebainosoma* Verhoeff, 1899 was erected for the type species *Hylebainosoma tatranum* Verhoeff, 1899, originally recorded in soil of alpine habitats of the High Tatra Mts., Slovakia. The occurrence of this species was subsequently confirmed in other regions of the Western Carpathians in Slovakia, Hungary and the Czech Republic together with easternmost records in the Bieszczady Mts., Poland, all on different bedrocks and both on the surface and within the caves. The variability of particular populations led subsequently to the description of separate subspecies and supported the confusion within the given taxon. Later, Ceuca (1967, 1979) described four other species from the Northern Romanian Carpathians, all of them partly or fully adapted to cavernicolous life and he placed them into the new genus *Romanosoma*, evidently closely related to *Hylebainosoma*. Unfortunately the description of the new genus as well as diagnostic differentiation between both genera was not complete. Another species, preliminary assigned to the genus *Hylebainosoma*, was found completely outside the Carpathians in surface habitats of the French Alps and described as *H. nontronensis* by Mauriès and Kime (1999). Furthermore, several records of unidentified specimens of these genera group were reported from mountain localities of the Ukrainian Carpathians (Kosyanenko 2003, 2005). Evidently the whole history of the taxonomic complex *Hylebainosoma*-*Romanosoma* was followed by doubts. Recently, a new undescribed troglobite species from the genus *Hylebainosoma* was recorded in Central Slovakia. Based on the light and scanning electron microscopy of numerous materials its morphology and taxonomic status with notes to the ecology and biogeography will be presented. Outline of critical revision of the separate genus *Romanosoma* as well as known species and subspecies of the *Hylebainosoma* s. l. will be proposed. The study was supported by the grant Vega 1/0139/09.

## **Do we use the correct terms for subterranean habitats?**

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There is a general trend to use simple terms/syntagms for very different subterranean habitats. One of the often mentioned habitats is the epikarst. It was defined by geologists, hydrologists and biologists in different ways, though it must have the same meaning to

all specialists. The prefix *epi*, or *ep* is derived from the Greek preposition  $\pi\acute{\iota}$  meaning: above, on, over, nearby, upon. Originally described by Mangin, the epikarst is located at the top of the vadose zone. Most of the strictly adapted fauna collected from drips or pools in caves belongs to the vadose zone. The same misunderstanding appears in case of the milieu souterrain superficiel (mesocavernous shallow substratum, MSS), which was originally defined as “any cavity of any size if food resources reach the cavity and if it has the characteristics of the underground climate”. Juberthie has defined these characteristics, such as absence of light, moderate annual temperature range, short food chains, relative humidity near saturation. Both, the vadose zone and the MSS are typical subterranean habitats. Where seasonal or daily fluctuations are present we are dealing with a shallow, subsurface habitat. The presence of a majority of epigeal species and some scarce cave species is the most striking indication that we are not in a typical MSS and the presence of many true cave aquatic animals means we are not collecting only epikarst fauna. The correct use of the terms in subterranean biology will allow clear and logical interpretations and avoid speculations. The biological community and intra-community interactions do not depend on terms, but understanding the processes is related to the correct use of terms. We are not working with axioms and theorems, but we create paradigms that can alter the true significance of our results.

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Conservation of Subterranean Life: oral presentation

## **Fauna is in the cave mud too – is there an interest to protect cave sediments?**

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Fossil invertebrate fauna was found in the cave “mud” or the cave sediments deposited during Pliocene-Pleistocene periods. Almost all investigated caves from Slovenia and Romania (south-eastern Europe) provided fossil invertebrates belonging especially to Oribatida, Ostracoda, Collembola and Coleoptera. Most of the identified invertebrates belong to surface fauna but very few cave beetle elytrae and groundwater ostracod valves were also found. Many of the identified taxa are new. The importance of these findings is given by the age of the fossils. Relatively well preserved, the invertebrates from cave sediments are the oldest continental invertebrate remains from the continental settings, except for the Arctic sites. Although rare, represented by only a few individuals in some of the sediment layers, fossil invertebrates can yield useful information about the vegetation on the surface and the flow regime during the deposition of sediments. The good preservation of fossil invertebrates is due to several causes and among them there is also the small density of microorganisms in the fine structured sediments. In some of the sites, the presence of fossil invertebrates has been cross-correlated with other proxies such as magnetic susceptibility, stable isotopes derived from speleothems or fossil vertebrate remains. New taxa and the information that can be obtained from the fossil invertebrates are strongly suggesting that cave sediments are not just the mineral part of the caves but archives that need to be protected and studied.

## ***Proteus anguinus* in Bosnia and Herzegovina: from the Middle ages to today**

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The earliest reference to a speleological feature in Bosnia and Herzegovina in the vernacular appears on a mediaeval tombstone known as a stećak, where the deceased associates his origins with a place called Jama (pit, pot-hole). The same tombstone bears the figure of an animal that is strongly reminiscent in appearance of the man-fish (*Proteus anguinus*). The place called Jama may be the easternmost part of Hutovo blato, where man-fish were recently found in a shallow well not far from Babino oko spring. This suggests that mediaeval people might well have known about this species and, since it comes from underground, have associated it with images of the afterlife, which would explain its appearance on a tombstone. As far as is known, this would be the earliest pictorial representation of this species. There are no references to the man-fish between the middle ages and the late 19th century, not surprisingly given the social circumstances of Bosnia and Herzegovina. There can be no doubt that the residents of Trebinje in the Popovo plain were familiar with the creature, which can be found in many family wells dating back to the Ottoman period. It was only when Bosnia and Herzegovina became part of the Austro-Hungarian monarchy that serious research in general began, including that of the plant and animal kingdom, and in particular of subterranean fauna. The first record of *Proteus* dates from 1895, from the Studenci spring in the Trebižat valley. Since then, sixty sites of the man-fish have been found. Of particular importance in this regard are cave-diving investigations, which have been stepped up in recent years. The most recent site, a well near Babino oko spring, not far from the hamlet of Čore (Hutovo blato), was confirmed thanks to the clue left by a mediaeval stonemason on the stećak from Boljuni. All the habitats of the man-fish in Bosnia and Herzegovina have been marked on the map, revealing an interesting biogeographical distribution. Though it depends on a number of factors, it should encourage speleologists to continue the quest for new habitats of this endemic Dinaric species.

## **The phylogenetic mapping and evolution of subterranean habit in catfishes (Teleostei: Siluriformes) based on molecular data**

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We aim to discuss the evolution of catfishes specialized to the life in subterranean and similar habitats, based on troglomorphic optimization in a phylogenetic context, offering a chronistic

and time perspective for the descriptions done before. More than 52 species of troglomorphic siluriforms have been recorded, which exhibit the classical troglomorphisms in various degrees, when compared to their epigean congeners. Most are endemic to distinct karst areas or other patchy subterranean habitats, thus being the result of independent speciation events. In addition, several troglomorphic catfish species are known from epigean, poorly illuminated habitats, such as murky waters in the Amazon and Congo basins. The mosaic distribution of different troglomorphic character states in congeneric species from the same, continuous subterranean habitat can be tested for vicariant events. Preliminary we tested three character states: 1) subterranean habit; 2) red color in life (hyperemia); and 3) troglomorphy (absent or reduced eyes and/or dark pigmentation in the tegument). We used a strict consensus cladogram from most parsimonious trees (900bp – nuclear rag2 gene), for nine genera (seven families) including troglomorphic species. We combined our data with the remaining siluriforms families DNA sequences from literature. The subterranean habit in catfishes first arrived in the basal clade Loricarioidei super-family, more specifically in the Trichomycteridae family. All others Loricarioidei families, except for Nematogenyidae and Scoloplacidae, present at least one subterranean species diverged early in the phylogeny. Within the Siluroidei super-family, troglomorphy has evolved in five independent clades from 29 family groups (Clariidae, Siluridae, Ictaluridae, Heptapteridae and *Phreatobius*). The red color in life originated four times independently in the order (Trichomycteridae, Clariidae, Siluridae and all known *Phreatobius* species). All subterranean species mapped show some level of regression of eyes and melanic pigmentation, but some epigean species also shows such regressions (Trichomycteridae, Aspredinidae, Cetopsidae). Hypotheses of independent colonization by epigean, non-troglomorphic species versus direct derivation other from troglomorphic species through subterranean dispersion, followed by isolation, may be further tested by character mapping into phylogenetic trees.

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Phylogeography, Phylogeny and Evolution: poster presentation

## **Comparative phylogeography in two obligate cave beetle species from Romania (genus *Pholeuon*; tribe Leptodirini) based on mitochondrial DNA**

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Although remarkable advances have been achieved in the last decades in insect molecular systematics, resulting in the accumulation of large amounts of DNA sequence data, terrestrial cave organisms have been comparatively less considered than their aquatic counterparts in molecular studies. The tribe Leptodirini with about 1800 species represents one of the few cases of a subterranean adaptive radiation. A recent phylogenetic hypothesis for the 57 Leptodirini species from all major lineages present in Western Mediterranean brought back to the



Oligocene-Miocene transition the initial phases of diversification of the group in the area. Here we present a study aimed at producing a comparative phylogeographic scenario for the two geographically most widespread obligate cave Leptodirini from the Apuseni Mountains (Romania). One species, *Pholeuon* (s. str.) *leptoderum*, includes five subspecies, the description of which was based on morphological characters (external morphology or the anatomy of the male copulatory organ) or numerical taxonomy. The subspecies occur in about ten different caves in the Bihorului Mountains. The other species, *Pholeuon* (*Parapholeuon*) *gracile*, is divided into three subspecies, altogether being distributed in more than ten caves in the Padurea Craiului Mountains. For the present study we sampled 56 individuals of *Pholeuon* (s. str.) *leptoderum* from five caves and 95 individuals of *Pholeuon* (*P.*) *gracile* in eight caves. For each individual we sequenced an 800 base pair fragment of the mitochondrial Cytochrome Oxidase I gene in order to identify the degree of genetic divergence between and within species. Molecular data clearly separate the two species. At the intraspecific level we found relatively little variation with sharing of the same haplotypes among different caves – six haplotypes for the first species and nine haplotypes for the second species. At the microevolutionary scale, the relatively low intra-specific genetic divergence identified for the two species could be explained either by a recent, post-glacial origin of the studied populations and/or by an ongoing gene flow between caves, presumably through the system of crevices.

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Subterranean Biodiversity and Biogeography: poster presentation

## ***Parapropus pfeiferi* Apfelbeck (Leiodidae, Cholevinae, Leptodirini), a poorly known leptodirine from the north-west Bosnia**

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The representatives of the genus *Parapropus*, Ganglbauer 1899 (Leiodidae, Cholevinae, Leptodirini) are troglobiotic beetles widely distributed in the Dinaric range of the south-west Slovenia, west Croatia and west and north-west Bosnia. This genus belongs to “*Apholeuonus*” phyletic group and contains 21 taxons (Perreau, 2000). One of the less known and rare species of this genus is *Parapropus pfeiferi* Apfelbeck, described on the basis of specimens collected by lieutenant L. Pfeifer in an unnamed cave of the village Vrhpolje in north-west Bosnia (between Sanski Most and Ključ). In his book “Revision des Bathysciinae (Coleoptera, Silphidae)” from 1911, apart from type locality (cave near Vrhpolje), Jeannel mentions *P. pfeiferi* in the Hrustovacka Cave (village Hrustovo) and in a cave near Zavolje near the river Sanica. All the aforementioned caves are located between Sanski Most and Ključ. In his book “Monographie des Bathysciinae” from 1924, Jeannel described the subspecies *P. sericeus muelleri* from the cave Glibaja (Bujan Mt. south of Sanski Most, district Ključ, valley of the river Sana). Geologist from Zemaljski muzej in Sarajevo, A. Polić, in his article from 1939, “Pećina Hrustovača kod Sanskog Mosta”, states that Glibaja is a spring beneath the Hrustovacka Cave which flows into the river Sanica. Since Hrustovačka pećina is the only cave close to this spring, we came to the conclusion that the very same cave is a synonym



for pečina Glibaja. In his catalogue from 1968, “Catalogus Faunae Jugoslaviae (Coleoptera, Catopidae, Bathysciinae)”, E. Pretner wrongly mentions the Hrustovačka Cave as type locality for *P. pfeiferi*. In May and April 2012, one of the authors (I. NJ.) investigated Hrustovačka Cave and one small cave on the bank of the Sana River in the village Donji Kamičak (nearby Vrhpolje). The result of the investigation was a series of *P. sericeus muelleri* in the Hrustovačka cave, but no specimen of *P. pfeiferi* (yet mentioned by Jeannel (1911; 1924) and Pretner (1968)) has been found. In the cave in the village Donji Kamičak, a small series of *P. pfeiferi* specimens has been collected. We are representing the habitus of this rare species and the male and female genitalia for the first time.

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Microbiology, Geomicrobiology and Sedimentology: poster presentation

## Microscopic fungi associated with bats

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Microscopic fungi colonise all organic substrates in caves including cave inhabiting animals. Caves are suitable environment for bat hibernation or bat summer colonies, nevertheless hibernating bats or bat cadavers are colonised with various microfungi species. Some of them belong to saprotrophic microfungi which take part in decomposition processes and some are agents of bat diseases, e.g. *Geomyces destructans*, an agent of the white nose syndrome (WNS), a fatal bat disease in North America. Results from keratinolytic activity tests of several strains of *G. destructans* which have been isolated in the Czech Republic and Slovakia are presented. Microscopic fungi associated with dead bats in caves were studied in several caves in the Czech Republic and Slovakia. Microfungal colonization of *Mortierella* species (Mucoromycetida) was isolated from bat cadavers in the Domic Cave and the Dobšinská Ice Cave. *Mortierella humilis* was isolated from dead bat from Sloup-Šošůvka Caves as well as from the Cave of Dead Bats. This study was supported by the project GAČR „Bat adaptations to the fungal disease geomycosis“ and by the project „Monitoring and management of selected caves“.

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Microbiology, Geomicrobiology and Sedimentology: oral presentation

## Preliminary results on microfungi community of Movile Cave, Romania

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Movile Cave, a ground-water ecosystem in southern Romania, was discovered in 1986. This chemoautotrophic cave contains an abundant and diverse fauna with terrestrial and

aquatic invertebrate communities including 24 endemic species. In the past, several studies were focused on cave chemoautotrophic bacterial community, nevertheless microfungi community was so far neglected. Microscopic filamentous microfungi were studied in upper dry level of cave system from cave air, cave sediments, corroded limestone walls, and isopod faeces and cadavers. The gravity settling method for the estimation of air-borne microfungi and the dilution plate method were used as isolation methods. The cave microfungi community was also compared with above environment (outdoor air-borne and soil microfungi). Results of a pilot study show a broad microfungi spectrum in cave air and sediments including several species of the genus *Aspergillus* (e.g., *A. baeticus*, *A. ustus*, *A. versicolor*) and *Penicillium* (e.g., *P. manginii*, *P. chrysogenum*, *P. expansum*), *Paecilomyces lilacinus*, *Microsporium gypseum*, *Myriodontium keratinophilum*, *Oidiodendrum griseum*, *Spiniger meineckellus* etc.

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Microbiology, Geomicrobiology and Sedimentology: poster presentation

## Human effect on microfungi community in show caves

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This study has been focused on microfungi communities (species diversity, CFU counts) in several show caves in the Czech Republic, Slovakia, Romania, and Spain. Considering ubiquitous presence of microfungi spores, microscopic fungi are very good model organisms for human impact assessment in cave environment. Regular tourist visits, working activity and treatments during tourist season as well as during low-season periods such as washing and reparation of pavements, banisters, stairs, and lights etc. or total cave reconstruction affect mainly cave airborne microfungi community. Nevertheless obtained results are very variable – microfungi communities in some caves or cave sites were importantly affected with touristic or working activities (e.g., Balcarka Cave, Bozkov Dolomite Caves, Czech Republic, Nerja Cave, Spain), but in other ones no influence on microfungi community was found (e.g., Harmanecká Cave, Domicca Cave, Slovakia, and Gruta de las Maravillas, Spain). Differences in quantitative microfungi occurrence (CFU counts) during day and night exposition in 2009 were found in the Ursilor Cave, Romania, whereas in 2010, no differences were estimated. The human effect is also evident in microfungi occurrence, mainly presence of species of genera *Aspergillus*, *Trichophyton*, and *Microsporium* was observed in some show caves or in their touristic more exposed sites which are contaminated with organic material of human origin, such as hairs, skin residua as well as any waste material. Increased occurrence of *Aspergillus* species was found for example in the Nerja Cave and especially in the Cueva del Tesoro (Spain), in which a lot of *Aspergillus* species were found in cave air, but also as visible microfungi colonies of organic residua on pavements or cave sediments.

## **Distribution and conservation of cave bats in Mindanao, Philippines**

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The Philippine bat fauna is well documented and several species of bats are known to roost in caves. However, in Mindanao, the second largest island in the Philippines, cave bat fauna is poorly known. In this study, 37 caves in Mindanao were surveyed from April–July, 2010. Bats were captured by mist nets to document the bat species present, giving particular attention to endemic, threatened, and socioeconomically important species. Eighteen species were recorded, of which five (28%) are endemic. Only 72% are known cave dwellers. Bigger caves were observed to be more species-rich. Bats were totally absent in the Bluewaters cave, which is a tourist destination, implying that this cave is already severely disturbed. Two species were found to be socioeconomically important and locally threatened primarily due to bat hunting for food. Threats to the cave biodiversity include guano harvesting and treasure hunting which could greatly disturb the bat fauna. Bat nurseries were found in the inner zones in two caves indicating the conservation importance of these caves. Results indicate the need to protect the caves through strict policy implementation. The total number of bat species recorded could increase with the assessment of more caves in Mindanao.

## **The potentially troglomorphic fauna in Brazilian iron ore caves**

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Recent studies in iron ore caves in Brazil have revealed that these, when compared to other caves lithologies, have unique characteristics regarding biodiversity, trophic functionality, vulnerability and troglomorphic species richness. Due to the economic importance of the areas where they located, studies concerning cave fauna are being conducted by private companies in different regions of the country, mainly in the Amazon and Cerrado (the latter considered a biodiversity hotspot by Conservation International). Thus, this paper aims to demonstrate the richness of the fauna potentially troglomorphic found in Brazilian iron ore caves. This study presents the results of a research of specimens deposited in Subterranean Invertebrate Collection of the Universidade Federal de Lavras (ISLA – Brazil). These come from inventories cave fauna in 257 cavities between 2010 and 2012, covering two sampling events. In total, 160 species potentially troglomorphic were found, belonging to at least 41 families distributed in 16 orders. It is important to note the difficulty in confirming

many species such as trogllobiotes because some characters considered troglomorphic traits might occur in species not yet known to the epigeal systems. This situation occurs due to lack of information on the fauna of arthropods that occur in epigeal environment. Another consequence of the lack of studies of external areas is to determine the actual distribution of these species. Confirmation of troglomorphic traits and actual distribution of species will determine the conservation status of the cavities where they were found. These factors are considered important by the Brazilian legislation for determining the degree of impact which these cavities may be subject.

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Phylogeography, Phylogeny and Evolution: oral presentation

## Hotspot within hotspot: subterranean fauna of the Canary Islands

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The Canary archipelago is formed by seven main islands, roughly aligned from the oldest eastern to the youngest western ones. Its genesis is associated to a particular hotspot model for which, contrarily to other volcanic archipelagos, the islands do not subside under the sea along time. Thus, there are islands at very different stages of geological evolution which, combined to the east-west climatic diversity, condition the availability of subterranean habitats and trogllobion diversity. Most of their caves are lava tubes that have a short life span, and different kinds of inhabited MSS are widespread over an important part of the islands. After 30 years surveying the subterranean fauna we have a reasonable representation of the terrestrial trogllobion fauna, reaching for the whole archipelago as much as 160 species, 138 of which have been found in 104 caves from five islands (La Gomera has no caves, and those from Lanzarote are too dry for adapted cave-fauna). Each island has a local endemic fauna of trogllobions that has evolved independently to each other, so islands can be considered as independent units in terms of subterranean diversity. We have investigated richness patterns of terrestrial cave-dwelling species using islands as units. To assess total trogllobion diversity, including unknown species, we have used Mao-Tau accumulation curves, Chao 2 and ICE estimates on an insular scale. Tenerife is the richest island (61 spp.) with two caves recording more than 30 trogllobions. La Palma harbors more caves and has the higher ratio of observed vs. estimated cave-dwelling species. El Hierro is the smallest and youngest island, reaching less than 10 species per cave. Gran Canaria, in spite of having very few lava tubes, is the most promising island for finding new hypogean fauna, given the high difference between observed and expected trogllobions. Fuerteventura has very few caves and only 4 trogllobion species. The different results of diversity, either observed or estimated, are interpreted for each island according to their biogeographic parameters such as island age, geographic isolation, abundance and diversity of subterranean habitats, local climate, and surface ecological diversity, together with the relative sampling effort carried out in the underground.

## **Early advances in the knowledge of the Mesovoid Shallow Substratum in calcareous massifs of the eastern Iberian Peninsula**

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The Iberian hypogean fauna is mostly known from the exploration and study of the caves and abysses in limestone landscapes; however, there is little information on the fauna of the Mesovoid Shallow Substratum (MSS). Given this knowledge gap, we took initiated a research project to study the colluvial MSS in some of the most important calcareous massifs of eastern Iberian Peninsula, particularly in the northern half of the province of Alicante. This area was not only selected because of its special orography (massifs separated by narrow ravines) but also for: 1) Its climate, given its gradual desertification process; 2) Its faunistic interest; since there is a well known cave fauna in that region, which allows to establish a comparison with the MSS; 3) its biogeographic potential, since massifs may act like islands for the speciation of subterranean fauna, with the fluvial Miocene basins among them as barriers. We adopted the sampling methodology proposed by other researchers, but with some modifications for our purpose and study area. A series of perforated PVC cylinders or USI (Underground Sampling Installation) were buried in our sampling areas. In each USI a baited pitfall trap was set, using propylene glycol as conservator. A digital thermo-hygrometer was introduced in some USI, in order to register the changes in temperature and humidity of the MSS each hour, as well as another one in the epigeal surroundings of the USI. In the same way, we also set epigeal pitfall traps to compare the epigeal and hypogean fauna, and determine which one is exclusive of each environment and which one is shared by both. All obtained data are contributing to test our hypotheses: A) Are those calcareous massifs islands for subterranean fauna? B) Is there any connectivity among them? C) Is the MSS a refuge for stenohygrobic fauna without troglobiomorphic characters? Finally, some previous results are presented, as well as the further steps of this investigation.

poster presentation

## **Juliana Pichler Stiegler (1827–1901), one of the first world female biospeleologists**

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Juliana Pichler was born in year 1827 in Košice, Slovakia, by father Antun Pichler and mother Barbara Sabina, born as von Špišić de Jappra et Bukovica. Already in year 1842, at age 16, she was married with Austro-Hungarian officer Stiegler. In year 1852, at age 26, she move to Croatia, where she has bought an estate Donja Lomnica in Turopolje region, but after 25 years she sold estate and move to Zagreb city, where she died in year 1901, at age 74. During she's life in Croatia she make decision to establish a collection of Croatian coleopterans and she start to collect them in region of Turopolje, Zagreb city, Medvednica Mt, Karlovac city, Pokuplje, Banovina and Lika. She has write no one article, but have permanent correspondence with several eminent entomologist in that time, as Austrian Clemens Hampe, English Charles Owen Waterhouse and Croatian entomologist with Polish descent, Slavoljub Wormastiny. She has established excellent collection with many new coleopteran taxa for Croatian fauna. On 21. June 1867, she visited Ozaljska špilje Cave in Pokuplje region, where she have collected three taxa of cave coleopterans, two of them new for science. New taxa were described by Austrian entomologist Clemens Hampe (1802–1882) at year 1870 and 1871 as: *Leptoderus intermedius* and *Anopthalmus croaticus*, now redefined as: *Typhlotrechus bilimeki croaticus* (Hampe, 1871) and *Parapropus sericeus intermedius* (Hampe, 1870). There are no data available if she has researched the same cave after, or some other caves. At 1871 in Zagreb, she received honorary award by First universal Croatian teacher's assembly for her work and especially for her insect collection, which has been stored at the end of her life in High agricultural school in Križevci town. Julijana Stiegler born Pichler died at 17. January 1901 in Zagreb, where she was buried on Mirogoj cemetery. With her work she is one of the first female entomologists in SE Europe, but also one of the very first world female biospeleologists.

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Subterranean Biodiversity and Biogeography: oral presentation

## **Dinaric troglobiotic Pselaphinae (Coleoptera, Staphylinidae) – biodiversity and potential**

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Thanks to work of biospeleologists in the very first decade of 21. Century, in Dinarid range many new taxa of cave Pselaphinae have been explored. Some of them are already described, as new genera: *Nonveillera*, *Pauperobythus*, *Thaumastocephalus*, *Seracamaurops* (*Cordiamaurops*) and many new taxa of genera *Gasparobythus*, *Machaerites* and *Seracamaurops*. According to the Catalogue of the troglobiotic Pselaphinae of Balkan Peninsula (Hlaváč et al., 2008) in whole Dinarid range 12 genera with 45 species of cave Pselaphinae have been known so far, but several new species have been already described after this publication. In custody of Croatian Biospeleological Society (CBSS) collection, there are many new taxa for science collected in Dinaric range, as several new genera, some of them peculiar and relictual, same as many new troglobiotic taxa belong to genera: *Bryaxis*, *Machaerites*, *Nonveillera*, *Pauperobythus*, *Seracamaurops*, *Thaumastocephalus*, *Troglamaurops* and *Tychobythinus*. According to new acquisitions, some important biogeographical, but also taxonomical revisions for tribe Amauropini and genera *Machaerites* and *Nonveillera* should be done. Anyhow, descriptions of some new genera and species are just



in process of preparing or publishing, but for some taxa description additional material should be collected. The biggest reasons for unsatisfied level of knowledge for cave Pselaphinae was their very small size and fact that they are not attracted with attractant traps. In fact, there is almost no one cave in Dinaric range without cave Pselaphinae, and in some most frequently researched caves already two or more cave Pselaphinae taxa are recognised, with a peak with one cave on Biokovo Mt. in middle Dalmatia, where one troglophilous and four troglotibiotic taxa have been recorded. At the moment, biodiversity of Pselaphinae cave fauna is in Dinaric range on third place among Coleopterans, but far away after Trechini (Carabidae) and Leptodirini (Leiodidae). It seems that finally, after more careful biospeleological field research and specialist work, group of Pselaphinae will be almost of same taxonomical richness.

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Subterranean Biodiversity and Biogeography: oral presentation

## Bioecography of Mexican cave Collembola after Morrone provinces

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The purpose of this contribution was to detect which cave Collembola species characterize the provinces proposed by Morrone (2005). An overview of families and genera of springtails found in caves is undertaken. From 49 genera cited from caves, only 50% are present in Mexico. Hypogastruridae with a high number of cave and soil taxa (in parenthesis Mexican/world species) as *Acherontiella* (4/20), *Acherontides* (6/11), *Schaefferia* (5/34) and *Typhlogastrura* (2/18) is well represented. Among the Entomobryidae, only *Pseudosinella* are abundant in caves and soil, with 28 species cited, some of them exclusive of caves. *Trogolophysa* (9/37) that is highly diversified in Central and South Mexico, and *Oncopodura* (4/52) only in Northeastern Mexican caves. In the Neelidae (6/24) only *Megalothorax* has two endemic Mexican species in caves, but *Arrhopalites* (7/46) (Arrhopalitidae) seems to have more cave adapted forms and within the genus *Pararrhopalites* (4/11) (Sminthuridae) four cave endemic species have been described. Some cave Collembola characterizes the biotic provinces proposed by Morrone as follows. Mexican Plateau (MPL): *Typhlogastrura elsarzolae*. Sierra Madre Oriental (SME): *Typhlogastrura veracruzana*, *Trogolophysa marimutti*, *Oncopodura prietori*, *O. atoyacense*, *O. susanae* and *O. dura*, *Pararrhopalites anops*. Transmexican Volcanic Belt (VOL): *Paleonura colimana*, *Agraphorura acuitlapanensis*, *Pseudosinella huautla*, *P. palaciosi*, *Trogolophysa relicta* and *Megalothorax tonoius*. Balsas Basin (BAL): *Pseudosinella finca*. For Sierra Madre del Sur (SMS): *Acherontides juxtlahuacaensis*, *Acherontiella colotlipana*, *Trogolophysa nacionalica*, *T. yoshiia*. The distinctive elements from the Mexican Pacific Coast (MPA) are: *Pseudosinella petrustrinatii*, *P. rolfsi*, *P. vita* and *Pararrhopalites hennigi*. For the Mexican Gulf (MGU) they are: *Schaefferia oaxacana*, *Pseudosinella gisini*, *P. hirsuta*, *P. volca*, *P. voylesi* and *Trogolophysa variabilis*. Chiapas province (CHIS) is characterized only by *Trogolophysa toroi*. Yucatan Peninsula (YUC) is the richest with *Sinella avita*, *S. barri*, *Metasinella falciferea*, *M. nunezi*, *M. rapoportii*, *M. totopytica*, *Pseudosinella aerea*, *P. nata*, *P. spinosa*, *P. yuca*, *Trogolophysa maya*, *T. xtolokensis*, *Megalothorax spinotricosus* and *Pararrhopalites christianseni*.



## Cave fauna from Chimalacatlán, Morelos state, México

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Mexico is well known for its numerous caves and their fauna in different areas. At Morelos State there were 33 caves recorded in 1981 (Reddell, 1981), and most of them are of basaltic origin and some are very small and of limited interest. According to Reddell (1981) there were no troglobiotes cited from this state. Further studies (Hoffmann et al 1986) increased the number of species (mainly arthropods) recorded from Morelos caves and allowed to compare the fauna with adjacent states such as Guerrero. Recently important discoveries have been made of vertebrate fossils from Cueva Encantada (close to Chimalacatlán) where mammoth's bones were discovered. At least 40 fossil bones were found at 2 m under the soil, including femora longer than one meter, molars, teeth and many bone fragments. Nevertheless arthropods have remained largely unknown. Chimalacatlán is located in the Municipality of Tlaquiltenango and important archaeological sites have been found there; it is close to the ecological reserve of Sierra de Huautla, in Morelos; this area lacks water mainly from October to May and some of the caves were explored looking for water sources where bones of Pleistocene mammoths were found. The only reference about actual cave fauna from this area is the contribution of Palacios-Vargas et al (2005) who recorded some arthropods from Cueva del Toro and other small cavities with *Glossophaga* bats. Since then, a couple of expeditions have been done in Cueva Encantada, one in April of 2011 and another in April of 2012. Hand collecting and sampling soil and guano (from the bat *Artibeus jamaicensis*) were undertaken. The new records for this cave are the Collembolan families Brachystomellidae, Isotomidae and Entomobryidae, two families of Astigmata mites: Acaridae and Histiostomidae; two of Mesostigmata: Uropodidae and Ascidae and one of Cryptostigmata (Oppiidae), among other less abundant groups. In Cueva Salitrosa, close to Cueva Encantada, a big colony of the bat *Artibeus jamaicensis* was found (which had many parasitic Diptera: Streblidae) and many tenebrionid beetles in their guano and some blood sucking bugs (Hemiptera: Triatomidae: *Triatoma* sp.) which are known to transmit Chagas disease (*Trypanosoma cruzi*) in the cited State.

## Unexpected diversity of the genus *Megalothorax* Willem, 1900 (Collembola: Neelidae) in caves of the Carpathians (Slovakia, Romania)

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Genus *Megalothorax* involves small Collembola representatives with sphaeric body belonging to the family Neelidae. Because of minute body size (0.5–1.0 mm) they are often overlooked in caves. At present 19 species of the genus *Megalothorax* are described throughout the world. They inhabit various environments, such as arable soils, forest litter, moss, occasionally also habitats with specific conditions, e.g. saline soils or surface of the water. Five species may be classified as troglobiotic: *M. boneti* from Afganistan, *M. spinotricosus* and *M. tonoius* from Mexico and *M. massoudi* and *M. tuberculatus* from Western Europe. Of six European species only last two shows troglomorphic characters. During the systematic explorations in caves of Slovakia within last decade three troglomorphic species of the genus were discovered. Two species (*Megalothorax* sp. 1, sp.2) belong to *minimus*-group and were discovered in caves of central and eastern Slovakia. Both are similar to cosmopolitan *M. minimus* in the setal pattern of dorsal manubrial setae and antennal segments, while differing in pattern of setae on head, thorax and abdomen, and in structure of claws and mucro. Two other species (*Megalothorax* sp. 3, sp. 4) belong to *incertus*-group being strikingly different compared to widespread *M. incertus* in body setal pattern, and shape of mucro and claws. *M. sp. 3* is distributed in four caves of central Slovakia. *M. sp. 4* was discovered during an intensive exploration of the Dră-coaia Cave in western Romania. The four new *Megalothorax* species represent first known troglobiotic forms of the genus within the Carpathian Mountains with the distribution restricted to caves. They exhibit clear troglomorphic features: larger body, and elongated unguis, mucro and body setae. We observed that in the territory of the Western Carpathian caves the new species (*M. sp. 1–3*) are often co-occurring with *M. minimus* and/or *M. incertus* within the same subterranean locality.

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Population and Community Ecology of Subterranean Organisms: poster presentation / student

## Ecological studies of epikarst communities: results from caves in Slovenian Alps

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Epikarst fauna from four caves, located at different altitude (from 310 to 1556 m a. s. l.) in Slovenian Alps, was investigated. In each cave five sampling sites were monitored for fauna in percolation water in the period of one year. Temperature, conductivity, discharge, pH, dissolved organic carbon (DOC) total hardness and concentrations of various ions (calcium, chloride, nitrite, sulphate and phosphate) of water were measured. Epikarst biodiversity was related to environmental conditions. Results were compared with findings from Dinaric karst (where the epikarst aquatic fauna is by far the best known) and patches of isolated karst in Slovenia. Alpine epikarst fauna consists mostly of troglobiotic species, but is less diverse and less abundant than the epikarst fauna from the Dinaric karst. Many individuals of both aquatic and terrestrial invertebrate taxa (Nematoda, Clitellata, Arachnida, Amphipoda, Copepoda, Insecta and Gastropoda) were found, Copepoda being the most abundant. High proportion of immature copepods in drips shows that epikarst is their primary habitat. Different statistic analyses were used to relate copepod abundance and measured parameters. Investigations of

the alpine epikarst fauna can help to understand better the ecology of epikarst fauna and its roles within the large range of different shallow subterranean habitats.

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Population and Community Ecology of Subterranean Organisms: oral presentation / student

## **Structure and interactions in a cave guano-soil continuum community**

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Bat guano represents an important feeding resource for cave-dwelling organisms, hosting up to entire communities in a single deposit. A sampling conducted in guano and its interface with the adjacent soil revealed a wide variation in physicochemical parameters (nitrogen, phosphorus, organic matter, pH and moisture) along that continuum. The orders that showed the greatest abundance and species richness were: Mesostigmata, Sarcoptiformes and Trombidiformes, all belonging to subclass Acari. The greatest invertebrate species richness is positively related to local moisture and negatively related to phosphorus concentration. Diversity is directly related to moisture as well. Conversely, the abundance of individuals increases with the increase in nitrogen concentration in guano. Canonical correlation analysis showed that moisture is the factor that most influences distribution patterns and number of individuals of the most abundant species. Parameters phosphorus, nitrogen, organic matter and pH are all correlated, although they showed less influence in the patterns presented by the invertebrates associated with the guano deposit. Lastly, a trophic web was devised of the most abundant species in the guano, based on literature data, interspecies correlation, preferences for microhabitats and abundance rates of the populations.

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Phylogeography, Phylogeny and Evolution: oral presentation / student

## **Patterns of biodiversity in natural and artificial iron ore caves in Brazil**

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The aim of this work was to identify the differences presented by caves of different historical uses, besides verifying if relationships exist among the biological and abiotic variables of the

caves. 8 anthropized caves (used by slaves in the 19th century), 7 natural and 7 prospecting galleries (open for about 50 years) were sampled. The results support the hypothesis that the high porosity of the ironstone allows troglobiote traffic through canaliculi, since such species were found in galleries. Besides, significant differences were observed regarding the subterranean community structure in the different cave types sampled. Anthropized cavities present more similar communities between different seasons, indicating a higher ecological stability, followed by the natural cavities and finally the galleries, which presented the highest variations between the two seasons. Furthermore, the anthropized caves presented the highest values for species richness, diversity and troglobiote richness. Natural caves presented intermediate values for diversity and equitability, followed by the galleries. The highest dominances for a same substrate type were observed in galleries and natural cavities, there being higher substrata heterogeneity in anthropized caves. Among the environmental variables, those that best explained the differences in the composition and structure of the communities were the cavity type, the organic matter percentage in the substrate and also the percentage of substrate water presence. The anthropized cavities present in the area were shown more complex from an ecological point of view. Considered this scenario an explanation for the pattern found in the anthropized caves lies in the intermediary disturbance hypothesis. The disturbance caused by the slaves in the XIX. century, besides increasing the size of the caves, the excavation waste increased the heterogeneity of micro-habitats which enabled the colonization of such differentiated habitats by a larger number of species. It can be observed with the present study that, although anthropized or totally artificial cavities present important faunal composition, the structure of those communities can be differentiated.

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Subterranean Ecosystems: oral presentation

## Organic carbon in aquatic shallow subterranean habitats (SSHs)

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Organic carbon is likely to be a limiting factor in shallow subterranean habitats (SSHs). Data on dissolved organic carbon (DOC) for hypotelminorheic and hyporheic SSHs are reviewed, and compared to nearby streams and springs, all on Nanos Mountain in Slovenia. These results are compared to extensive estimates of organic carbon in epikarst sites in Postojna Planina Cave System (PPCS) and Škocjanske jame in Slovenia. The four hypotelminorheic sites showed both spatial and temporal heterogeneity. Median DOC values ranged between 1.1 and 7.0 mg L<sup>-1</sup>. The most diverse hypotelminorheic habitat had a DOC value of 2.7 mg L<sup>-1</sup>. Median DOC value for the one hyporheic site was 1.7 mg L<sup>-1</sup>, and varied from 1.2 to 10.4 mg L<sup>-1</sup> throughout the year. Two springs had median DOC values of 3.0 and 4.3 mg L<sup>-1</sup>, and a surface stream had a median DOC value of 4.0 mg L<sup>-1</sup>. SSH sites appear to be more variable but generally lower DOC values than surface habitats. A previous study of DOC in PPCS resulted in a mean DOC value of 0.70±0.04 mg L<sup>-1</sup>, and a preliminary set of measurements from Škocjanske jame indicated a mean value of 2.2 mg L<sup>-1</sup>. These results suggest that organic carbon in aquatic SSHs is lowest in epikarst.

## Environmental features influencing the harvestmen population dynamics in caves

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Population dynamics is influenced by a number of factors acting from local (i.e. habitat) to landscape scale. We have studied the dynamics of two cave populations of *Paranemastoma silli* (Opilionida) using mark-recapture data gathered monthly, during one year. The two caves differed in their both local (i.e. stable *versus* relatively variable climate) and surface (i.e. bare rock and dominating scrubs *versus* humid and dominating beech forest) conditions. *Paranemastoma silli* commonly inhabits beech forests and cave habitats, and it is the most frequent harvestmen species found in Romanian caves. We estimated monthly-different survival and recapture probabilities for the two caves. Both cave features and external factors influenced the dynamics of the studied harvestmen populations. Temperature and rainfall during the week prior to the captures were the main surface environmental variables influencing the harvestmen population size. Our study highlights the importance of considering cave and wider environmental-related features in understanding the population dynamics of cave inhabitants.

Microbiology, Geomicrobiology and Sedimentology: oral presentation

## Aquatic bacterial mats in Dinaric range caves: molecular and ecological approach

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Diversity of bacterial mats from three caves in Dinaric range was analyzed using a culture-independent molecular approach combining 16S rDNA and universally conserved genes/proteins. Bacterial diversity obtained with 16S gene-based analysis showed dominance of *Gamma*- and *Deltaproteobacteria*. To support an initial glimpse of genetic variation within these communities' five conserved protein coding genes were chosen. Heterogeneity among the protein clone libraries showed dominance by one phylotype (with exception of *pyrG* from Izvor Bistrac). Majority of analyzed phlotypes formed separate cluster within known bacterial classes thus suggesting phylogenetically novel sequence types. Conducted phylogenetic analysis generally supported 16S rDNA sequence phylogeny and further revealed the presence of phylum Nitrospirae thus giving us the opportunity to link genetic identity of yet uncultured microorganisms with ecosystem functionality. Fauna associated with bacterial biofilms was analyzed in order to

enable insights into ecological concepts and their potential connection to microbial ecosystems, as a nutrition resource in otherwise nutrient-poor cave habitat. Observed ecological concepts indicate that complex interspecies interactions should be considered among the cave-dwelling animal taxa and microbial communities in the mats.

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Adaptation, Development, Physiology and Behaviour: oral presentation

## **Vertical migration of the subterranean beetles as a response to annual temperature fluctuations**

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It is widely accepted that temperature in caves is stable and similar to the surface annual average temperature. What is generally called “cave” is just a small part of the entire subterranean environment. A cave is connected with the surface through shafts, cracks, fissures and micro fissures and therefore the influence of the annual surface meteorological fluctuations is to be expected in the subterranean environment too. While studying the phenology of the subterranean beetles in a particular ice cave in the Slovene high dinaric karst area of Snežnik we found significant fluctuations in cave beetle numbers and their space distribution. The studied cave Jama v Kovačiji is situated at the bottom of a steep karstic doline surrounded by dense mountainous forests at an altitude of 1000 m. At the bottom of the doline the snow usually persists until late spring. Strong air currents from the fissures at the bottom indicate a larger cave system underneath. In late spring and summer the leptodirine beetles *Parapropus sericeus* and *Prospelaebates brelihi* were commonly found in a cave chamber where ice accumulates during winter. *Bathyscimorphus (Drovenikia) serkoi*, *Bathysciotes khevenhuelleri* and *Anopthalmus schmidti* were common in the M.S.S. and deep soil environments of the doline. In late autumn the species composition significantly changed. The subterranean beetles in the cave chamber completely disappeared. Surprisingly enough, *P. brelihi* and *B. serkoi* appeared in the M.S.S. and even on the surface in great numbers at the end of November. Permanent temperature measurements carried out in different environments of the studied site throughout the year clearly show that the subterranean beetles' seasonal vertical migrations are affected by significant changes in temperature. Periodic extreme low temperatures in ice caves are forcing the beetles to retreat either to deeper parts of the cave underneath the studied site, or to seek refuge in M.S.S. or deeper soils where winter temperatures do not drop below 0 °C.

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Subterranean Ecosystems: poster presentation

## **Quality assessment of groundwater and sediments of the Iberian Peninsula largest karst aquifer**

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Karst aquifers are particularly exposed and impacted contaminants from point and diffuse sources of pollution. The protection of groundwater is an imperative need for nature conservation and for economic reasons, due to the strategic importance of water as a resource for future generations. The risk assessment of karst areas uses ecotoxicological information that in the case of subterranean ecosystems should be adjusted with the stygobiont responses to the most important contaminants threatening the area. The karst areas occupy a considerable area of the Portuguese territory where about 3000 caves and more than 115 obligate hypogean species are known. This contribution presents the preliminary results on the characterization of the sediments and groundwater quality of Estremenho karst massif, located in the centre of Portugal. Physical and chemical (PAHs, PCBs, pesticides and metals) were analysed. In general, PAHs, PCBs and pesticides were all below to the detection limit except naphthalene, fluorene, phenanthrene and acenaphthene. The metals analysed (Cr, Cd, Ni, Pb and Cu) were all below the detection limit excepted the Cr, what can be related to the tanning industry in the area (Alcanena). Sacrificing large number of stygobiont organisms for ecotoxicological tests can be problematic from ethical and technical reasons, so using toxicity tests with standard species can be an effective option to circumvent the prior problem. In the present work were selected two species: the bacteria *Vibrio fischeri* and the algae *Pseudokirchneriella subcapitata*. The results of the luminescence inhibition tests with *V. fischeri* showed low toxicity effects, whereas the growth rate inhibition tests using *P. subcapitata* showed significantly effects. Although this is a preliminary study, the results emphasize the need to increase knowledge about the impacts of anthropogenic activities on the groundwater communities, as a starting point to generate useful information for their protection.

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Adaptation, Development, Physiology and Behaviour: poster presentation

## **Acute toxicity with stygobiont *Proasellus*: can a wide environmental conditions tolerance be a key factor in groundwater colonization?**

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Subterranean aquifers host a unique biodiversity, composed mainly by stygobionts that play a role in water purification, providing important economic ecosystems service for human health and ensuring the equilibrium of groundwater dependent ecosystems. Understanding the impact of contaminants in stygobiotic taxa is an important for manage and set environmental quality of groundwater. The highly adapted features of the stygofauna towards life underground, may



lead them to lose the ability to face sudden changes on their ecosystems. Considering the geographic isolation provided by the groundwater aquifers, the risk of extinction is remarkably high for microendemic stygobionts. The knowledge on biodiversity of stygofauna increased significantly, but a little is known about their physiological responses, especially those linked to the contamination pressure in urbanized karst areas. Although it is wide known that several stygobiont species are sensible to changes in their environment, there seem to be no direct relation between the degree of troglomorphy and the loss of sensitivity to contaminants in stygobiont invertebrates compared with epigeal species. We have studied acute toxicity of copper and potassium on two endemic stygobiont species of the genus *Proasellus* from the Estremenho karst massif and Assafora karst, in Portugal. The first species is a highly specialized stygobiont and the second a less troglomorphic species. *Proasellus* is the most well represented genus among stygofauna in karst areas of Portugal, playing an important role on karst aquifers. The low metabolic rates of hypogean species may reduce the short-time uptake of toxicants in responses, eluding its long-term effect. Even so, our results suggest that both species of *Proasellus* are remarkably resistant to potassium and copper, being the less troglomorphic species the most resistant one. This observation, together with the previous data, leads us to think that a wide environmental conditions tolerance can be an advantage in groundwater colonization.

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Conservation of Subterranean Life: oral presentation / student

## **Biodiversity patterns and conservation of subterranean fauna from Portugal**

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The subterranean fauna from karst areas of Portugal has been considered moderately poor with some endemic relicts, but after five years of intense sampling, the species richness patterns of cave-dwelling species have changed. Several new species for science and a better knowledge of hypogean species distribution, revealed new biogeographical and species richness patterns for the cave fauna of the western Iberian Peninsula. To assess total diversity, including missing species, we used Mao-Tau accumulation curves, Chao 2 and ICE estimates on a regional scale. We estimate that the observed troglobiont biodiversity (20 species in the Algarve, 8 species in Estremenho and 14 species in Sicó) compared to total diversity in the major karst massifs of Portugal is 91% for Algarve, 85% for Estremenho and 48% for Sicó. We mapped the distributions of all hypogean species in karst areas of Portugal. Based on species similarity analysis, several factors were tested to explain diversity in individual caves. Evapotranspiration and the consequent high productivity on the surface may be an important determinant of species richness in the different karst units, but depth of the caves and the unique geological features of every massif seemed to play a more important role. Because subterranean animals are among

the most rare, threatened and worldwide under protected, we rank sites to prioritize conservation in karst areas of Portugal. Criteria for ranking are based on four quantitative measures: 1) species richness, 2) number of endemics, 3) weighted richness and 4) weighted observed and estimated richness. The richest caves of Portugal are in the south, but estimates of missing species indicate that the richest estimated area should be the center of the country.

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Subterranean Ecosystems: oral presentation / student

## **Is there a vertical limit for the distribution of subterranean arthropods?**

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It is wide known that subsurface biota extends over a wide variety of habitats that can be spatially interconnected. Information about communities of arthropods living below 500 meters is rare. The increase of the distance between surface and subterranean galleries decreases the amount of available food, affecting species richness and the composition of subterranean communities. During the summer of 2010, the CAVEX Team expedition to world's deepest cave Krubera-Voronja (Western Caucasus) performed the first effort to access its biodiversity, revealing an interesting subterranean community. The deep cave biota is composed of more than 12 species of arthropods, including several new species for science. Several obligate hypogean and epigean species were found living below 2000 meters. This contribution provides the biocoenosis and the vertical distribution of invertebrate fauna of Krubera-Voronja until the final siphon at -2140 meters, as well as a discussion about the vertical limit for the distribution of hypogean and epigean species.

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Subterranean Biodiversity and Biogeography: poster presentation / student

## **Collembola in superficial subterranean habitat of a karstic valley (Slovakia, Western Carpathians)**

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Up till present the existing data on communities of Collembola of superficial subterranean habitats are very scarce, although the group has dominant position within the arthropod communities dwelling this environment. We investigated the spatial distribution, seasonal dynamics and species richness of springtails at study site situated in a small karstic region in the Čierna Hora Mts. (Slovakia, Western Carpathians). The site represented *Tilio-Aceratum* forest association on the talus deposits covered by soil at altitude 530 m in a karstic valley.

The specimens were collected using series of subterranean traps consisted of plastic cups filled with 4% formaldehyde that were inserted into the depths 5–95 cm through a plastic tube. The traps were checked monthly from September 2008 to November 2009. Among 24 higher invertebrate taxa trapped, springtails were the most abundant with dominance of 67.6% and with year-round activity along the vertical gradient. Most of collembolan specimens (65.2%) were captured at depth of -5 cm. The number of individuals gradually decreased up to -35 cm with an erratic trend between 35–95 cm. Seasonal dynamics of springtails fluctuated during the year in each studied horizon. Their activity cumulated twice, during spring and autumn. Preliminary, material from sole sampling in May 2009 (5495 ex.) was analyzed to the species level with overall number of 31 species identified. The majority of the surface species were concentrated in upper horizons, however, *Desoria tigrina* and *Lepidocyrtus lignorum* showed activity along the whole profile studied. Several species with high affinity to underground habitats were detected: troglaphiles *Arrhopalites pygmaeus*, *Ceratophysella granulata*, *Plutomurus carpaticus*, *Protaphorura armata*, and troglobiont *Neelus koseli*. Some of these subterranean species were also present in upper horizons, however they occurred more regularly from depth of 45 cm down the profile. In deep zone *A. pygmaeus*, *N. koseli* and *P. armata* prevailed. Troglobiont *Arrhopalites aggtelekiensis*, inhabitant of the nearest cave, was not registered in subterranean traps. The study was supported by the grant VEGA 1/0139/09.

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Population and Community Ecology of Subterranean Organisms: oral presentation

## **Population dynamics of *Laemostenus schreibersi* (Coleoptera, Carabidae) in a cave in Austria – adult longevity stabilizing population size: results of a long-term research over 20 years**

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In this long-term investigation a population of the microphthalmic beetle *Laemostenus schreibersi* (Coleoptera, Carabidae) was studied over 20 years in a cave near Villach in Carinthia (Austria). During 27 periods of investigation (between 1986 and 2005) the beetles were trapped in pitfalls, individually marked and released in the cave near the trapping site. Using the mark-recapture method it was possible to estimate population size and to characterize the patterns of movement between the different cave-compartments and between the cave and the surrounding fissure-system. Other aspects of population structure and dynamics are concerned with: seasonal and spatial distribution of the beetles in the cave, seasonality of breeding and development, reproduction rate, mortality, age-structure, sex-ratio and population size. The size of the population was small, but rather constant (50–110 individuals). According to the several recaptures throughout the years, it was possible to estimate individual age and survival time. Some beetles of *Laemostenus schreibersi* attained the remarkable age of 7.5 and >8 years. Population size is stabilized by the longevity of the individuals (immigration from the surrounding fissure-system seems to be less important). The low mortality or rather the longevity of adults is discussed as a possibility of survival for small, local populations with low reproduction rates.

## **Colonization of subterranean habitats by spiders in the Czech Republic**

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We studied the distribution of spiders in soils, crevice systems, scree and caves, i.e. subterranean habitats at depths spanning from 10 cm to 100 m. In total, we collected 161 species. We found a great overlap in spider assemblages across the four habitat types. Seven species were recorded in all four habitats. Number of species declined with increasing depth, with a major drop in species richness at the depth below 10 meters. Thirteen species exhibited morphological adaptations to life in subterranean environment. They inhabited all four types of studied habitats. The highest number of these species (eight) was recorded in scree and caves. Species exhibiting only initial eyes reduction inhabited usually superficial subterranean spaces. At depths greater than ca 10 meters, spider assemblage was almost exclusively composed of troglomorphic spider species. However, no species were exclusively specialized to life in deep caves and we registered the occurrence of species with advanced troglomorphisms also in subsurface and surface habitats far from karst areas. The evolution of advanced troglomorphisms must have followed Quaternary climatic cycles. During warm (interglacial) periods, caves were accessible and could have been colonized by spiders, with a consequent evolution of troglomorphic characters. Cold (glacial) periods are characterized by formation of ice in caves during their maxima. Terrestrial animals cannot survive in caves filled with ice. Troglogenesis was blocked. We hypothesize, that during Quaternary glaciations, troglomorphic populations repeatedly migrated from caves to subsurface and even surface habitats, and troglomorphisms were introduced into populations inhabiting these habitats. Therefore, in the present time, advanced troglomorphic characters occur both in deep subterranean and in surface habitats and the broad variability of morphological characters complicates their taxonomic evaluation.

## **Cave crickets (Orthoptera, Rhaphidophoridae) from Turkish subterranean habitats: an update of taxonomy and geographical distribution**

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Five *Dolichopoda* (*D. aranea*, *D. pusilla*, *D. sbordonii*, *D. noctivaga*, *D. lycia*) and five *Troglophilus* species (*T. escalerai*, *T. gajaci*, *T. adamovici*, *T. bicakcii*, *T. tatyanae*) were so far known from Turkish subterranean habitats. Our investigations in different regions of Anatolia allowed us to identify at least one new taxon of *Dolichopoda* from the Aegean region and four new *Troglophilus* from the Marmara and Mediterranean regions. On the basis of this study, it can therefore be assumed that the distribution of Turkish Rhabdiphoridae is not limited to the North-East Black Sea region and South Anatolia, but it also includes caves from Western Anatolia, Middle Black Sea and Western Black Sea regions. According to the new data, we found that the distribution patterns of cave crickets can be strongly related to climatic and karst regions of Turkey. Although cave crickets can live in caves of the Mediterranean, Black Sea and Marmara regions, they are completely absent in the continental areas, where the climatic conditions (hard winter and dry summer) are not favourable to the Rhabdiphoridae life cycle. Furthermore, we found a maximum of species richness (12) in Southern and Western Turkey, whereas only two species live in Northern Turkey. This high diversity can be explained with the developed karst phenomena and the Mediterranean climatic conditions of this area that could have favoured the isolation of cave crickets populations and their consequent speciation during successive glacial and interglacial periods. On the contrary, the Black Sea region is poor of karst morphologies and its climatic conditions are characterized by a permanent humid-temperate climate, responsible for the occurrence of Colchic deciduous wet forest. In these conditions, cave crickets can exploit the epigeal habitats maintaining a high degree of gene flow in a wide geographical range. Finally, although important karst areas do exist in Southern-Eastern Turkey, we did not find any cave crickets populations there. This fact, observed also for many other invertebrate taxa, can be due to the presence of the Anatolian Diagonal, playing an important role as a barrier between the Western and Eastern Turkish fauna. Moreover, South-Eastern Turkey is characterized by a severe continental and dry climate that could have prevented the colonization of this area by Rhabdiphoridae species.

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Microbiology, Geomicrobiology and Sedimentology: poster presentation

## Evaluation of *in vitro* susceptibility of cave isolated opportunistic fungi to antifungal drugs

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Caves are the habitat of a large diversity of microorganisms. They are important part of the cave ecosystem. However, among these microorganisms there may be some species capable of causing opportunistic infections in humans. This could pose a medical risk to cave visitors, especially considering that wild fungal strains may be more resistant to antifungals. Among the opportunistic fungi, special attention has been given to the genera *Aspergillus*, *Cladosporium*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Mucor* and *Rhizopus*. The genus *Aspergillus* is highly

incident in systemic opportunistic infections. The main antifungal agents used to treat infections caused by filamentous fungi are Amphotericin B, Itraconazole, Terbinafine and Voriconazole. The aim of the present study was to evaluate the susceptibility of ten strains isolated from three important touristic caves and one seldom visited iron ore cave in Brazil. The drugs tested were Amphotericin B, Itraconazole, Terbinafine and Voriconazole. A clinical isolate (*A. fumigatus*) was also tested for comparison. The species selected were: *Aspergillus caespitosus*, *Aspergillus flavus*, *Aspergillus niveus*, *Aspergillus niger*, *Aspergillus ochraceus*, *Penicillium oxalicum*, *Paecilomyces lilacinus*, and *Mucor* sp. The selected strains were submitted to susceptibility testing (CLSI M38-A2). The strains presented different levels of tolerance to the different drugs. A wild strain (*A. niveus*) obtained from the iron ore cave was resistant to all drugs. This species has been reported to cause opportunistic lung infection. This was also the only species resistant to amphotericin B, which is the most largely used antifungal in the treatment of opportunistic infections. *P. lilacinus* presented the highest susceptibility to the same drug. Resistance to Voriconazole was observed in strains of *A. caespitosus*, *P. oxalicum*, and *Mucor* sp. Resistance to Itraconazole was observed in *Mucor* sp, and resistance to Terbinafine in *A. flavus* and *Mucor* sp. Amphotericin B presented the best results controlling the growth of the selected isolates. This study showed that some wild cave strains had a high resistance to antifungal drugs and that more studies should focus on the identification of opportunistic species in cave environments, especially in touristic caves.

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Subterranean Ecosystems: poster presentation

## **Colonization of hypogenic caves by terrestrial arthropods: consequences in the biodiversity patterns of subterranean species**

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Not all karst hypogean systems harbor troglobiotic fauna; there are some confined areas such as hypogenic caves where animals cannot reach through a subterranean path. Two hypogenic caves (“Autopista” and “Far” Cave), were biologically surveyed in the eastern Iberian Peninsula, in Prebetic Mountains. Both caves are characterized by the typical maze morphology of hypogenic caves. “Autopista” Cave extends over more than ten kilometers of subterranean galleries while “Far” Cave reaches one-kilometer. They are located within a region with caves inhabited by several troglobiont species. Both caves have proper environment conditions to harbor subterranean fauna (high humidity, thermic stability and reduced air flow). The connection of “Autopista” Cave to surface occurred twenty-five years ago during the construction of a highway, and “Far” Cave has a very small entrance opened several hundreds years ago. These hypogenic caves lack of troglobiotic fauna in both caves. “Autopista” Cave is scarcely



inhabited by epigean parietal fauna, while “Far” Cave harbors guano communities and few typical endogean species. The recent connection to surface of these two and their isolation during the hypogenic formation seems to have impeded the colonization by geographically close troglobiont species, allowing only very recent colonization by non specialized arthropods. The relation between the hypogenic origin of those caves and their lack of fauna may have relevant consequences in our knowledge on the biodiversity patterns of subterranean species. Some questions about the dispersion of the arthropod fauna through the subterranean environments, MSS included, and the extension of these habitats, are also discussed.

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Subterranean Biodiversity and Biogeography: poster presentation / student

## **Diversity and distribution of stygobiont fauna in karstic systems from Goiás, central Brazil – the test of visibility and relevance of habitats**

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Underground environments often feature large pathways of water infiltration, including the capture of epigean rivers or rainfall. The volume of water in each system varies depending on seasonality, oscillating its chemical composition and spatial distribution. The epikarst represents an unsaturated zone, with intense circulation of water and organic matter through micro fissures in rocks and varying according to their porosity. The water percolation produces an extensive network with potential micro-habitats, from small channels filled with air until permanent or temporary water pools. The speed and origin of water flow directly influence the diversity and abundance of stygobiont fauna on each system. The caves of Terra Ronca State Park, São Domingos, Goiás, central Brazil, have extensive drainage systems. To elucidate the regional diversity of stygobiont fauna, we sampled water from some wells using the indirect method of plankton nets, 20µm mesh. We have performed sampling on three visit occasions in the years of 2011 and 2012, covering a total volume of 6893 litres in five caves. We filtered water from different sources at each location, including points from the entrance zone to aphotic conduits. The samples were categorized according to its origin, which were – water drips (96 l), travertines (252 l), epikarst rivers (6528 l), karst resurgence (157.5 l) or epigean river (110 l). We found similarities between the samples using cluster analysis (*Euclidian, single linkages*). The most distinct, with greater richness and abundance of species was a karst resurgence ( $r = 13$ ). Moreover, no species were collected on cave Bezerra River, probably due to its high flow and type of substrate, mainly composed by rocks. There were unique records from single locations, such as karst resurgence (*Anuraeopsis* sp., *Euchlanis* sp.), epigean river (*Bosmina* sp.), underground rivers of São Bernardo Cave (*Brachionus* sp.) and Angelica (Chironomidae, Chaoboridae), and on a drip from the latter (*Brachionus falcatus*, *Conochilus* sp., *Filinia* sp, *Kellicotia bostoniensis*, *Keratella cochelaris*, *Trichocerca* sp.). Moreover, we recorded taxon Calanoida only in epigean sampling sites (Palmeiras River and karst resurgence). The data indicate the origin of water over a karst system is a relevant factor in the distribution of planktonic organisms.



## Nutritional biology of animals in some caves

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Several arthropod species were found in Slovak and Romanian caves. Their feeding habits were studied by microanatomical, especially histological methods including fluorescent light and confocal microscope. The system of microanatomical parameters was applied to be established the palatability of the grazed and digested food. The food offer seemed to be limited in caves. The saprophagous animals dominated, but our studies revealed the variability inside that nutritional group. *Mesoniscus graniger*, cave isopod, consumed cave substrate including some organismal thread-like remains, but never any fungal propagula. Such feeding habit was the same in all isopods in several as Slovakia as Romania caves. On the other hand, the diplopod *Trachysphaera costata* exhibited the fungal propagula in gut in all cases from Slovakia caves. Oribatid mite *Pantelozetes cavaticus* preferred the guano heaps. Its food bolus contained mainly bacteria. Microwhip scorpion, *Eukoenenia spelaea* (Palpigradida), however, was specialized, according its gut contents and gut walls activity to cyanobacteria. The latter appears to be peculiar due to the assumed photoautotrophy of those microorganisms. But it confirms their adaptability and phenoplasticity, hence, a variability of characteristics under pressure of extreme environmental factors.

## Changes in the invertebrate community of caves between dry and rainy seasons in the Brazilian Savannah and Caatinga biomes

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In Neotropical region variation in the amount of rain, temperature and humidity between the dry and rainy seasons are predictors of the seasonal pattern of epigeal insect communities. The objective of the present study was to evaluate, richness, diversity, equitability and turnover

variations, between the dry and rainy seasons, in invertebrate communities of 43 limestone caves, in Brazilian Savannah and Caatinga biomes. Furthermore, the influence of the cave sizes and number and extension of entrances on the variations of the communities was evaluated. In the general, significant differences were only observed in the richness (KW-H(1;78) = 12.52;  $p = 0.00$ ) and diversity values (KW-H(1;76) = 8.07;  $p = 0.00$ ) between rainy and dry periods of the year. These variations were also significant within each biome. The richness, and diversity averages were 56 spp. (sd = 29 spp.) and  $H' 2.41$ , (sd = 0.66) in the rainy season against 39 spp. (sd = 28 spp.) and  $H' 2.00$  (sd = 0.66) in the dry season. However, the collections which provided most species occurred at two caves, both with wide entrances, during the dry season of the year (111 and 159 spp.). In these same caves 100 and 141 spp. were found in the rainy season. The average richness in the Savannah was 80 spp. in the rainy season and 55 in the dry season. In the Caatinga the average richness was 41 spp in the rainy and 27.6 in the dry season. Significant differences were observed in the average temporal turnover of species between the caves of the Savannah (average 61%, sd = 14%) and Caatinga (average 50%, sd = 14%) (KW-H(1;45) = 7.0859;  $p = 0.00$ ). In the Caatinga biome positive and significant correlations were observed between the number ( $R_s = 0.56$ ;  $p < 0.05$ ) and extension ( $R_s = 0.46$ ;  $p < 0.05$ ) of the entrances with temporal turnover and between the number of entrances and the diversity in the dry season ( $R_s = 0.60$ ;  $p < 0.05$ ). However, in relation to the size of the caves, negative and significant correlation was observed ( $R_s = 0.48$ ;  $p < 0.05$ ) with the temporal turnover. Epigeal variations between the dry and rainy periods of the year influence invertebrate communities in Brazilian caves. However, more detailed studies are necessary to better to explain which of these environmental variations are the most notable.

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Subterranean Ecosystems: poster presentation

## **Detritus processing in a shallow groundwater without invertebrate shredders action**

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Groundwater systems are heterotrophic habitats where food and oxygen availability are determined by import from the surface. This hydrological exchange seems to be the key factor shaping most groundwater communities. Litter processing in groundwater environments has not been experimentally studied as much as it has in lotic subterranean systems. However, detritus are likely a critical resource for organisms inhabiting shallow groundwater habitats. The present study sought to evaluate the processing rates and the nitrogen and phosphorous dynamics in plant debris deposited in groundwater of two Neotropical limestone caves during 99 days. 84 – 10×10 cm<sup>2</sup> litterbags with mesh of 0.04 mm<sup>2</sup> and 9 mm<sup>2</sup> were used. In each litter bag, 50 green, intact plant leaf disks, previously weighed ( $\pm 2.0$  gr/bag), were conditioned. At the end of the experiment, the average weight loss was from 17.4%. Macroinvertebrates associated to the debris were not found but, significant differences in the processing rate

in relation to the site and mesh size were observed. The weight loss rate of the plant debris was considered slow (average 0.003 K<sup>-day</sup>). The amount of nitrogen and remaining phosphorous in the plant debris in the two caves showed variations over time but, with a tendency to increase probably due to the growth of microorganisms rich in nitrogen and phosphorus. The slow processing rate of the plant debris can be due mainly to the fact that groundwater ecosystems of the two caves is trophically restrictive to colonization by coarse particulate organic matter shredder invertebrates. Besides, the abrasive force of the water, which plays an important role in the processing and availability of fragmented debris for colonization by microorganisms, is absent.

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Conservation of Subterranean Life: oral presentation

## **Effects of groundwater warming on fauna**

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One of the central characteristics of groundwater habitats is stability of temperature. Compared to habitats on earth's surface, there are less fluctuations regarding groundwater temperature - both seasonally and daily. In the temperate zone, groundwater temperatures are generally low, with a cold stenothermous stygofauna adapted to these conditions. Shifts of groundwater temperature by increasing usage of groundwater for private air conditioning, industrial cooling and the predicted global warming, are thus very likely to affect stygofauna. For groundwater as a habitat no data about future trends are available so far. We consider artificially warmed groundwaters as models for the effects of climate change and increasing temperatures. For the study presented here, 70 wells situated in warmed and normally temperatured groundwaters in the southwest of Germany were sampled six times over a period of one year. Biodiversity and composition of communities in cold references and artificially warmed groundwater habitats will be compared. In the course of this year's conference first results of this study will be presented on a taxonomic main group level.

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Subterranean Biodiversity and Biogeography: oral presentation

## **Bioregions or stygoregions? Biogeographical patterns as references for groundwater assessment**

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As a result of biological processes, purification of water and provision of clean drinking water is the main service of groundwater ecosystems. Consequently, and other than in very most

countries, sustainable groundwater management should be expected not to refer to the good physicochemical and quantitative status only, but also to groundwater ecosystem health. It's ecologically sound integrative assessment schemes and protection measures, what is urgently required. Such assessment schemes need biogeographical references, so-called bioregions (Illies 1978) to be defined not only for surface waters, but also for the groundwater. Two years ago, at Postojna, Stein et al. (2010) presented a first approach to a bioregional classification of central European groundwater fauna, based on data from around 180 wells. Meanwhile, comparable data from 515 groundwater monitoring wells from many parts of Germany were analysed for the distribution patterns of groundwater dwelling invertebrates. Our results underline that the distribution patterns of invertebrates in groundwaters are not in accordance with any existing bioregional classification systems established for surface habitats. It seems that, in addition to the surface bioregions, more or less independent underground stygoregions exist. In consequence, we propose a new classification scheme for groundwater habitats based on stygoregions. The first four stygoregions delineated may form the base for re-discussing European groundwater faunal patterns and defining biogeographical references for the underground – the stygoregions.

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Conservation of Subterranean Life: poster presentation

## **Tourism and conservation: monitorings in show caves (Škocjan Caves)**

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Škocjan Caves ranks among the most important karst phenomena not only in Slovenia's Karst region, but worldwide. Škocjan Caves is on the UNESCO list of natural and cultural world heritage sites. It is also featured on the Ramsar list of wetlands of international importance as the first registered underground wetland in Europe. So far, twenty-seven stygobiotic and troglobiotic organisms have been described in Škocjan Caves. Epikarst fauna (i.e., Copepoda) is particularly abundant. Moreover, numerous troglophile organisms make their home in Škocjan Caves. Of particular importance are the greater horseshoe bat, the long-fingered bat, and the common bent-wing bat (Natura 2000 protected species). The Park's absolutely main priority is nature conservation which is a key task for the managing such a vulnerable area as caves are. We are executing a monitoring and set up the observations of percolation water in the underground (epikarst fauna). Furthermore, we observe the state of the underground troglobiont fauna in the tourist-accessible part of Škocjanske Jame. We also start project with replacing halogen lights in tourist part of the cave to decrease lampenflora (energy input in the cave system). We also monitor the quality of cave air with the microbiological parameters, which will help in ways to prevent the spread lampenflora the air flow (1), determining the quality of the cave air (2) and "load" the cave of the number of visitors, especially in the silent cave (3). On walking surfaces we monitor the impact of visitors (swabbing a variety of surfaces to determine the impact of tourist visits to the pit entry of alien organisms and their viability).

## **Filling the gaps; using biodiversity surveys of cavernicolous invertebrate fauna to provide a spatial analysis of diversity patterns from Gunung Mulu National Park, Sarawak**

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Gunung Mulu National Park, Sarawak is world renowned as a biodiversity hot spot; with its cavernicolous fauna being no exception. A recent (May 2012) speleological expedition to the area resurveyed some of the cave fauna sites visited previously by Chapman (1982) and included some new locations. These sites included caves considered as ‘wild caves’ and those used for adventure caving. This was to provide an insight into potential impacts and allow preliminary strategies to be formulated for protecting sensitive areas. The new information gathered was compiled with previous data to determine spatial patterns of biodiversity using GIS tools. The reported results will be used to inform park management when planning future caving activities within the park.

## **The feeding biology of cave invertebrates – methodological experiments**

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Feeding preferences of springtails originating from caves of Slovak Karst were studied in laboratory conditions. Several methods were combined and methodically tested. Springtails contained all tested digestive enzymes (trehalase, cellulase, chitinase). It indicates that they can digest storage sugars and cell walls of plants and fungi. The between-species differences among species in proportion of the three enzyme activities are showed. In feeding preference tests several parameters characterizing food preference such as presence of animals and their excrements on different food samples were recorded. Feeding preference tests of “cafeteria” type with 30 individuals and 8 microbial food types (algae and microscopic fungi species isolated from caves of Slovak Karst) in two variants confirmed preference of some of the microbial cultures (mainly algae *Chlamydomonas* sp. and the fungus *Cladosporium herbarum*) in the springtail *Heteromurus nitidus*. Individual paired preference tests (one individual and two food species) were methodically tested revealing impact of movability of springtails on usability of some parameters of feeding preference tests. Microscopic analyses of excrements under normal and fluorescence microscope can tell us clearly which food was really consumed and digested. The last evaluated method was based on the detection of food marked by fluorescent stain in the

gut of living animals under UV light. We verified successfully applicability of epifluorescence and fluorescent marking for food biology studies in speleobiology in combination with more traditional feeding preference tests and digestive enzymes analyses.

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Subterranean Biodiversity and Biogeography: poster presentation

## Spider fauna (Araneae) of Slovak caves

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Spiders (Araneae) of the Slovak cave ecosystems belong to one of the less knowing animal groups. Until now only one summarized article dealing with spider fauna of the Slovak caves were published (Svatoň 2000) where the author presented occurrence of 43 spider species recorded from the caves located in 13 geomorphological units of Slovakia. Several sporadic records about occurrence of other spider species in the Slovak caves were published by Majkus, Miller, Gajdoš, Gulička, Svatoň, Franc and co-authors. Later, spider fauna was studied in detail in small karst units of the Čierna hora Mts. and in the pseudokarst caves of the Cerová vrchovina Mts. In framework of a project „Monitoring of the state and trends of the invertebrate communities’ development in non open caves to the public” realized in 2010–2012, fauna of 26 model caves were investigated. In these caves authors found out the occurrence of 72 spider species belonging to 19 families. The linyphiid spiders (family Linyphiidae) were dominant and the genus *Porrhomma* with 9 documented species was the most numerous one. The findings of species *Porrhomma profundum* in 8 karst and pseudokarst caves of the southern and eastern Slovakia are very important from faunistical point of view because this species was considered as endemic species for the unit Slovak Karst. Other 8 species from the genus *Porrhomma* are *P. convexum*, *P. egeria*, *P. errans*, *P. microphthalmum*, *P. microps*, *P. myops*, *P. pallidum* and *P. pygmaeum*. Out of the identified species of all cave spider fauna, 15 species were rare and threatened ones: *Anguliphantes tripartitus*, *Centromerus brevivulvatus*, *Centromerus cavernarum* *Formiphantes lepthyphantiformis*, *Improphantes improbulus*, *Lepthyphantes notabilis*, *Liocranoeca striata*, *Micrargus apertus*, *Micrargus georgescuae*, *Palliduphantes insignis*, *Pardosa ferruginea*, *Saloca diceros*, *Scotina palliardii*, *Thyreosthenius parasiticus* and *Zora pardalis*. Only *Nesticus cellulanus affinis* we consider to be troglobiont. All other identified species from the Slovak caves are trogloniles – they occur in caves regularly, others are troglonile, with accidental occurrence in caves.

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Phylogeography, Phylogeny and Evolution: oral presentation

## Morphology and phylogeny of the subterranean genera *Alpioniscus* and *Utopioniscus* from Sardinia, Italy (Crustacea, Oniscidea, Trichoniscidae)

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At present the genus *Alpioniscus* Racovitza, 1908 includes two species in Sardinia: *A. fragilis* (Budde-Lund, 1909), distributed in many caves in the central- and south-eastern part of the island, and *A. thanit* Taiti & Argano, 2009, collected in endogean habitat and in a cave in the central-eastern part. According to the present taxonomic definition, both species are included in the subgenus *Illyrionethes* Verhoeff, 1927, which also includes several species from the Balkan area and a species from Spain. The genus *Utopioniscus* is presently known with one aquatic species (*U. kuehni* Schmalfuss, 2005) from two anchialine caves off the eastern coast of central Sardinia. For its morphology and aquatic life the species was considered to be the most primitive representative of the family Trichoniscidae. Recent investigations in many Sardinian caves revealed the presence of *U. kuehni* also in freshwater lakes in the Bue Marino Cave, and of a new aquatic species in Su Palu Cave, which shows intermediate morphological characters between *Alpioniscus* spp. and *Utopioniscus kuehni*. Moreover, different cave populations of *A. fragilis* showed some morphological differences (e.g. granulated vs. smooth dorsum) which might prove to be more than one species. Several populations from Sardinia belonging to *Alpioniscus*, the population of *U. kuehni* from Bue Marino Cave and the aquatic population from Su Palu Cave have been analysed morphologically. A molecular analysis was performed using mtDNA sequences (COI) to reconstruct their molecular phylogeny. In the molecular analysis also *Alpioniscus (Alpioniscus) feneriensis* (Parona, 1880) from two caves in Piedmont (north-western Italy) and *A. (Illyrionethes) strasseri* (Verhoeff, 1927) from a cave near Trieste have been included. Results show that: 1) both the aquatic species from Su Palu and the aquatic *Utopioniscus kuehni* are included in the same clade with *Alpioniscus* spp., suggesting that the latter species is not the most primitive Trichoniscidae but rather a species within *Alpioniscus* secondarily returned and morphologically adapted to an aquatic way of life; 2) high levels of genetic divergence among the Sardinian populations of *Alpioniscus* from caves and endogean habitats were found, suggesting the occurrence of more species than just *A. fragilis* and *A. thanit*. Combined analysis of mitochondrial and nuclear genes will shed more light on the phylogeny of this group.

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Subterranean Biodiversity and Biogeography: oral presentation

## **Diversity of millipedes, centipedes and terrestrial isopods in subterranean habitats of the Czech Republic**

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Regardless the almost complete absence of characteristic subterranean representatives of millipedes (Diplopoda), centipedes (Chilopoda) and terrestrial isopods (Isopoda: Oniscidea) in the Central Europe categorized as troglobiotic, the research undertaken in past decades in the Czech Republic brought together quite rich data about the occurrence of these arthropods



in diverse subterranean habitats. Presentation summarises recent knowledge of 46 species of millipedes, 17 species of centipedes and 27 species of terrestrial isopods recorded in karstic as well as non-karstic systems. Beside the mostly troglone representatives, several troglophilic species and species otherwise closely tied to underground habitats were found. Characteristic troglophilic species represent the millipedes *Brachydesmus superus*, *Brachychaeteuma bradeae*, the centipede *Lithobius lucifugus* and the isopods *Androniscus dentiger*, *Androniscus roseus* and *Cylisticus convexus*, all of them more or exclusively related to karstic cave systems than to non-karstic subterranean habitats. Nevertheless non-karstic systems were also rich in number of recorded species of all three groups, but troglone forms prevailed and species composition was strongly determined by the local above-ground fauna and migration abilities of individual forms. The millipedes *Brachychaeteuma bradeae*, *Geoglomeris subterranea* and *Macrosternodesmus palicola* and the isopod *Trichoniscus alemannicus* were historically recorded for the Czech Republic originally in caves. Some species known as rare inhabitants of uppermost organic and mineral soil layers showed to be more frequent in deeper structural strata including MSS and cave systems, which indicates much closer relations between soil and subterranean fauna. The real pattern of distribution remains unknown for difficulties to explore inaccessible parts of soils and bedrocks. The results suggest that the Central European subterranean habitats represent very important refuge for some quite rare faunistic element of soil fauna with less known or questionable distribution throughout the Europe.

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Microbiology, Geomicrobiology and Sedimentology: poster presentation

## **Inventory of filamentous fungi for the management plan of a touristic cave (Rei do Mato cave, Minas Gerais – Brazil)**

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Caves are environments with conditions favourable for fungal growth. The tourist use of caves is a common practice in Brazil. However, the risks of exposure to certain fungal species may cause serious problems to the visitors. *Histoplasma capsulatum* is a pathogenic fungus that causes a systemic and pulmonary infection known as histoplasmosis. This is a soil species that may grow on guano in caves. Furthermore, several pathogenic and opportunistic species have already been isolated in other caves in the world. There is a lack of studies concerning the microbiota in Brazilian touristic caves. “Rei do Mato” Cave is one of the main tourist caves in the country. The present study aimed to perform a more detailed microbiologic inventory as well as to search for possible pathogenic species (opportunistic) in “Rei do Mato” Cave (Minas Gerais state, Brazil). The specific objectives were: 1) to make an inventory of filamentous fungi, 2) to verify the presence of pathogenic or possible pathogenic

species, 3) to create a map of distribution of these fungi and plot areas of microbiological risk and 3) to build a management plan for the studied cave. The cave was divided in four sections where the samplings were performed. The fungi were isolated from the air through the settle plate method and from the soil through the serial dilution method. Both methods were performed using Mycosel and Sabouraud agar. Guano samples were collected and the fungi also isolated through the serial dilution method (brain-heart agar, Sabouraud and Mycosel). Samples were also taken from speleothems and touristic facilities with a swab. This material was incubated on Sabouraud at room temperature. A total of 1127 isolates belonging to the genera *Aspergillus*, *Cladosporium*, *Emericella*, *Fusarium*, *Mucor*, *Paecilomyces*, *Penicillium*, *Trichoderma* and *Torula* were isolated. Although *H. capsulatum* was not isolated, eight species related to opportunistic fungal infections and allergic reactions in humans were recorded in touristic and non-touristic areas. In addition, growth of *Fusarium solani* and *Aspergillus sydowii* was observed throughout the energy cables on the floor (touristic and non-touristic areas). The areas of microbiological risk and some management actions are suggested in this study.

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Microbiology, Geomicrobiology and Sedimentology: oral presentation / student

## **Inventory of filamentous fungi and the relations between edaphic factors and the soil fungal community in a dolomitic cave in Brazil**

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Caves are unique ecosystems that hold a diverse biota. Filamentous fungi are an important part of this subterranean biodiversity and they play key role in the maintenance of the hypogean ecosystem. Different species of fungi have their own niche and respond in a very particular manner to different environmental conditions. Abiotic factors may function as regulators of the fungal community. The objective of this study was to conduct an inventory of filamentous fungi in a dolomitic cave and verify the relations between the structure (composition, richness, abundance and distribution) of the soil fungal community and some edaphic variables (N, Fe, Al, Mn, Zn, pH, organic matter, and moisture). The inventory was performed by isolating filamentous fungi from the air, soil, guano and contaminated substrate. The air samples were obtained through the settle plate method using DRBC and Sabouraud media. The soil samples were collected *in situ* with sterilized material in the rainy and dry seasons. These samples were then processed through the serial dilution method ( $10^{-1}$ – $10^{-5}$ ) and incubated on DRBC (triplicated) at room temperature. Samples of contaminated substrates were placed (direct plating method) on Petri dishes containing DRBC and incubated at room temperature (25 °C). A total of 8,554 filamentous fungi were isolated (81 species/18 genera).

These isolates included species from the genera *Acremonium*, *Aspergillus*, *Calcarisporium*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Emericella*, *Eurotium*, *Eupenicillium*, *Fusarium*, *Geotrichum*, *Gliocladium*, *Humicola*, *Mucor*, *Paecilomyces*, *Penicillium*, *Rhizopus*, *Trichoderma*. Seven other unidentified morphotypes were registered. The diversity (Shannon-Wiener Index) of soil fungi varied from 0.025 (rainy season) to 2.71 (dry season). Zinc (Zn) had a significant positive relation with the fungi richness in the rainy season and iron (Fe) had a significant negative relation with the richness of soil filamentous fungi. It was also possible to observe a significant effect of organic matter, soil moisture, nitrogen and pH on some of the most frequent fungal species (CCA analysis). The present study highlights the importance of performing studies on the subterranean microbiota and their relations with the environmental variables.

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Conservation of Subterranean Life: poster presentation

## **Steps for specific vulnerability maps, management and conservation in Yucatan Peninsula subterranean habitats**

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Cenotes (sinkholes) are the most dynamic communications between the subterranean habitats and the surface. Intrinsic vulnerability refers to the properties of the karst system itself, while specific vulnerability is the degree of vulnerability caused by actual threats, mainly anthropogenic. Due to expanding anthropogenic activities management and conservation becomes critical for the conservation of subterranean biology & fresh water resource. Before this project geo-referenced cenotes data bases (DB) were scatter and not homogeneous. The aim 1 of this project was to get an insight of the current knowledge and information among stakeholders related to cenotes information and management. A consolidated data base (DB) of cenotes (sinkholes) and probable cenotes was generated and a geographical information system (GIS) was developed for the Yucatan Peninsula. Information was obtained through governmental and civil data bases, satellite imagery analysis and field work. Spatial analysis of geo-referenced cenotes and probable cenotes were conducted using 1:250 000 geographic information available: geology, land use and vegetation, soil type, protected areas or conservation priority areas, urban areas, population size of human settlements and mayor and minor roads. Danger and Risk models for ecological degradation of cenotes are proposed. Danger Models comprises fire susceptibility for vegetation, soil permeability and rock type & age. Risk Models comprise danger & urban areas, population size of human settlements and mayor and minor roads. Out comes of the project includes: >5000 records DB, GIS, thematic cartography & spatial analysis and Risk & Danger Models. Along with geomatics, a review of present natural and anthropogenic threats, recommendations for a regional cenotes census, as well as management and regulation actions were proposed to Mexican water federal agency.

## **Describing biodiversity: implications for subterranean biology and conservation**

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Conservation aims to preserve representative samples of geo- and biodiversity, their processes and patterns. Therefore, how to describe the hypogean biodiversity is a central issue in subterranean biology, both for scientific and practical reasons. So far, descriptors of alpha (inside habitat) diversity have been used to characterize subterranean habitats, locally and regionally, with emphasis in species richness and relative abundances. However, considering only alpha diversity is a major pitfall, especially when based only on few sampling occasions. Hypogean alpha diversity indices will always be within the range for epigeal habitats, from the lowest in extreme habitats such as deserts to the highest in tropical forests. Far more important than calculating absolute values of subterranean alpha diversity (including indices such as Shannon's, which have long been questioned) is to determine its relative importance to the gamma (regional) diversity, which requires estimating the beta diversity. Therefore, comparable epigeal surveys are basic not only to determine the troglomorphic status for troglomorphic taxa (the presence of troglobites increases alpha diversity), but also to understand the special feature of subterranean biodiversity. An important factor of singularity of hypogean habitats is dark diversity (taking into account species that belong to a particular species pool but that are not locally present), which tends to be high in subterranean and other extreme habitats. Moreover, phylogenetic and functional diversity may also differ, presenting special components in subterranean habitats. Troglomorphic populations may contribute to ecological diversity, because, as frequently observed, their dynamics is different from that of epigeal populations of the same species. Species that are rare in epigeal habitats, but with high potential for colonizing hypogean habitats, may become key populations in caves due to the lowered competition, and may even represent stocks of re-colonizers for epigeal habitats strongly affected by environmental fluctuations. Current problems for studies on subterranean diversity are taxonomic impediment, use of parataxonomy not validated by specialists, and inclusion of accidentals in faunal lists, which tends to artificially overestimate alpha biodiversity.

## **Replicated adaptive radiations in leptodirine cave beetles**

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Evolution of troglobionts and stygobionts has mostly been regarded as gradual transformation from non-troglomorphic surface ancestors to troglomorphic, highly adapted subterranean specialists. Only recently has it become clear that in caves, and the subterranean environ-

ment in general, a second type of evolutionary process is happening as well. This process is divergent evolution of lineages or species that already have become troglobionts in an earlier phase, and are diversifying and adapting to different ecological niches, all within the subterranean realm. It is the same process – known also as adaptive radiation – that takes place after a new island is colonized by the first representative of a taxon, or after a key innovation (like flight) evolves that enables the use of previously unexploited resources. Replicated adaptive radiations, in which the same pattern of diversification occurs independently in different, yet sufficiently closely related clades, are particularly instructive cases. They allow us to explore how predictable and repeatable evolution of complex systems actually is. So far, only few such cases are known, for example *Anolis* lizards from the Antillean Islands, cichlid fishes from East African lakes, or South American tropical butterflies. Recent molecular phylogenetic work showed that subterranean biology has to offer its own example of replicated adaptive radiations. The phylogeny of leptodirine cave beetles (Leiodidae, Cholevinae) shows four main clades within which a similar pattern of morphological divergence has evolved independently. However, only two clades from the Dinaric Karst have evolved the full array of morphological types: edaphic, or muscicole (which is probably the ancestral form in all four clades); bathyscioid; pholeuonoid; leptodirid; hygropetric. The first four types form a nearly perfect transitional series toward what has been traditionally explained as increased troglomorphy, i.e. longer legs and antennae, larger body and abdomen, longer prothorax. As such a gradient view is incompatible with the notion of adaptive radiation, alternative hypotheses explaining morphological diversity need to be developed and tested.

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Phylogeography, Phylogeny and Evolution: poster presentation

## **Preliminary results of molecular genetics characterization and phylogenetic analysis of selected taxa within family Nemastomatidae (Simon, 1872) in the Western Balkans**

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In Bosnia and Herzegovina have been identified 54 valid species of harvestmen and two subspecies. Many cavernicolous forms of harvestmen are endemic in the Dinarides. Due to the long adaptation period almost all cave species have typical troglomorphic traits, with total eye reduction, depigmentation and extraordinary long extremities. During biospeleological research of cave Pećina na Vrelu Mokranjske Miljacke in 2008. new genus and species of harvestmen with typical troglomorphic traits was found and currently under description by I. M. Karaman. Two nuclear markers were, 18S rRNA and 28S rRNA, used to infer the position of new genus and species within the family Nemastomatidae. In this research the results from molecular-genetics characterization of species *Hadzinia karamani* (Hadži, 1940) and *Paranemastoma bureschi* (Rower, 1926) were also included.

## **Malacofauna diversity recorded in Brazilian caves and their ecological importance**

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Subterranean ecosystems have a representative biodiversity for the Brazilian fauna nevertheless, studies on the diversity and ecology of terrestrial molluscs in these habitats are scarce in Brazil. In general, recorded molluscs have been considered accidental, with no ecological and evolutionary relation to the hypogeal environment, however, current researches has shown a high biological richness and has recorded some species with troglomorphic characters. The purpose of this paper is to analyze the diversity of molluscs found in several Brazilian caves, as well as the ecological importance of these species. The malacological material studied was composed mainly of empty shells, although they were well preserved. For species identification, a comparison test was conducted between the shells, using as reference the illustrations and descriptions found in the specialized scientific literature. Then, we elaborated distribution maps of species, emphasizing the potentially troglomorphic species. Some material was deposited in storage for the reference collection of the Laboratorio de Estudos Subterrâneos of UFSCar and part deposited in the Malacology Section of Museu de Zoologia da Universidade de São Paulo, under the responsibility of Dr. Luiz R. L. de Simone. A detailed identification of individuals cannot rely solely on the analysis of shells; therefore Dr. Luiz R. L. Simone helped to confirm the taxonomic identifications. Thus far, 946 specimens from the Phylum Mollusca were analyzed, belonging to 21 families of the Gastropoda and Bivalvia Class. Among the 49 species recorded, it is possible to confirm two new species, both highly troglomorphic and also troglomorphic, but this number can reach 40 new species, including troglomorphic and accidental ones. Therefore, this study showed that the cave environment has a high diversity of molluscs, confirming the need for further studies focusing its ecological relevance.

## **Antibiotic resistome of bacteria isolated from bat guano**

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Because of mass production of antibiotic and broad applications in medical care and agriculture, the antibiotic resistance became a global problem. Information about the occurrence of antibiotic resistance (ATB-r) genes in environment and factors affecting its dissemination and persistence are not fully understood with respect to heterogeneity of resistance genes distribution in different



ecosystems. Wild animal populations and their deposits are suggested as reservoirs of ATB-r in the environment. The aim of the study was to screen ATB-r of the culturable bacteria of bat guano in the inaccessible caves in protected areas in Slovakia. In total, bat guano samples from 5 caves (Ardovská c., Drienovská c., Demänovská jaskyňa mieru c., Bobačka and Pružinská Dupná c.) were collected. Plate cultivation on complex (TSBA) and selective (TSBA+0.003 % chlortetracycline) agar was used to determine the number of both total and tetracycline resistant (TET-r) culturable bacteria and species representation (MIS Sherlock System; MIDI Inc., USA; and/or 16S rRNA gene sequencing and BLAST search). The antibiotic multiresistance of the isolates was determined by an antibiotic disc diffusion test (Bio-Rad, France). In total, 15 antibiotics were tested. The TET-r bacterial abundance of bat guano was within the range  $10^3$ – $10^7$  CFU g<sup>-1</sup> dw, what corresponded to low prevalence TET-r in total culturable bacteria (0.01–3.6%). The species screening indicated that bat guano TET-r bacteria were dominated by Proteobacteria (64%), followed by Firmicutes (18%) and Actinobacteria (18%). The potential pathogens were isolated (*Salmonella enterica enterica*, *Enterococcus faecalis*, *Serratia* sp., *Staphylococcus epidermis*, *Stenotrophomonas maltophilia*, *Carnobacterium piscicola*). The multiresistance pattern of biohazardous species was determined. This study revealed bat guano as the significant source of ATB-r bacteria, including biohazardous ones. Bats should not be therefore neglected as vectors of ATB-r bacteria in the environment.

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Phylogeography, Phylogeny and Evolution: oral presentation

## Evolution of subterranean microsnails (Carychiidae, Zospeum) – phylogeny and cryptic diversification

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The taxon *Zospeum* (Ellobioidea, Carychiidae) comprises terrestrial microsnails with a maximum shell height of only a few millimetres. They inhabit humid subterranean microhabitats in the Alps, the Dinaric Alps, the Cantabrian Mountains and the Pyrenees. Recent records from Asian caves in China and South Korea considerably enlarge their distribution range. Until now, almost nothing is known about the true number of species, their diversification and evolutionary history. Here we present the first phylogenetic study for the European *Zospeum* spp. After integration of molecular data, we revealed an astonishing amount of 50% cryptic diversity. Several of the so far recognized morphospecies are formed by more than one distinct genetic lineage. In particular, *Z. isselianum*, *Z. spelaicum schmidti* and *Z. suarezi* are in the process of a larger revision. Two Cantabrian lineages are probably new to science and their taxonomic descriptions in progress. A wide geographical range and assumedly variable phenotype can be seen as valid predictors for cryptic lineages in *Zospeum*. In general, geographically close species are also revealed to be monophyletic and *in situ* ‘mountain-range radiations’ seem likely. Lineage



through time (LTT) plots fit best a model with constant rate of diversification (Yule-process). We discuss likely evolutionary scenarios and request for Asian material.

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Phylogeography, Phylogeny and Evolution: oral presentation

## **Mexican cave catfish genus *Rhamdia*: a case of parallel speciation?**

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Phenotypic convergence is a central characteristic of the evolution of cave living animals. Very often this phenomenon complicates the taxonomic separation of troglomorphic species, which derive from one single ancestor, but evolve in separate caves. They differ from their ancestral surface form only in features related to cave adaptation. In fish only minute morphological or highly variable meristic differences exist between them, which can be used and are applied for their taxonomic discrimination. The Mexican cave fish *Astyanax* (Characidae) shows evidence for parallel speciation, resulting in a large number of independently evolved troglomorphic populations being included in a single species. A different situation is found in the Mexican pimelodid catfish *Rhamdia*, where one surface and at least 6 troglomorphic species have been described. In contrast to *Astyanax*, they occur in caves that are separated from each other by long distances. Each cave population was described as a distinct species based mainly on the form of specific hooks along the first pectoral ray. By studies of mtDNA for one of the cave species, *R. reddelli*, and several river populations of the surface ancestor, *R. laticauda*, the species status of the troglomorphic form was questioned. Cave species were found to be more closely related to surface species of the same drainage than the latter were to surface species from different drainages. However, crossings between the troglomorphic species *R. reddelli* and another cave species, *R. zongolicensis*, as well as with the surface species *R. laticauda*, revealed that all three F1-generations exclusively contain females (Haldane's rule). No male hybrids developed indicating that the species status of all three species is justified. The different troglomorphic *Rhamdia* species are genetically coherent and represent independent evolutionary units. Conclusions about the phylogeographic history and taxonomic status should not be based on single genetic markers. I suggest that the rapidly evolving part of the male sex chromosome may be responsible for this incompatibility.

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Subterranean Biodiversity and Biogeography: oral presentation

## **Groundwater crustacean biodiversity in Europe**

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Groundwater presents exclusive habitats for many specialised animal species and there has been a growing interest in understanding their biodiversity patterns. Many studies focused on smaller geographic regions, while attempts to unravel large scale biodiversity patterns are rare. Studies of groundwater biodiversity patterns at the scale of whole Europe have been made using political or large biogeographical regions. The most ambitious and detailed approach in assembling and analysing the data was achieved in Pascalis project, but involved only six European countries. Data for other European regions remained scattered in different databases and various published sources. To overcome this impediment and make a leap toward understanding large scale groundwater patterns of biodiversity in whole Europe, an informal initiative of collaborators was formed. Crustaceans, representing over 60% of species in groundwater, were chosen as first group to collect the data for. Occurrence records were gathered from many existing datasets, literature sources and some museum collections and new localities were geo-referenced to the greatest possible accuracy. The European Groundwater Crustaceans Database (EGCD) was formed, including more than 25,000 records mainly for Europe but also some non-European areas. We performed the first set of analyses of biodiversity patterns in Europe, using data on freshwater subterranean crustaceans. More than 1550 species and sub-species have been identified in the continent, of which nearly 75% belong to only three orders: Amphipoda (28%), Harpacticoida (24%) and Isopoda (22%). Cyclopoida, Bathynellacea and Podocopida present about 24% of species, with remaining orders having less than 30 species. We mapped biodiversity patterns, identified hotspots of species richness, endemism as well as beta diversity patterns, while considering the effect of differences in sampling effort. We evaluated the latitudinal pattern of groundwater biodiversity. The next stage of studies will focus on identifying processes which shape the observed patterns. The assembly of EGCD was enabled by national research agencies supporting collaborators, and also via 7th EU Framework Programme (Contract no. 226874, BioFresh).

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Subterranean Biodiversity and Biogeography: poster presentation

## **Database on subterranean biodiversity of the Dinarides and neighboring regions – SubBioDatabase**

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Research of subterranean fauna in the Dinarides in the Western Balkans has a long history, reaching back to the first discoveries of cave animals. It is recognised as the world hotspot in subterranean biodiversity, with about 1000 obligate species described and new species still being discovered. The region has always attracted numerous scientists working on different aspects of the subterranean biology, therefore large amounts of data can be found in numerous literature sources. With the goal of connecting such data in one place, and making a powerful and useable tool for studies of the regional subterranean biodiversity as well as for planning its conservation, a common database of records was formed. Collection of data in spreadsheet form started in the 1980s at the Department of Biology of the Biotechnical Faculty of the University of Ljubljana. A few years ago, the database was rearranged into a relational form, in which a set of tables is joined together via ID fields, uniquely identifying each input. Repetitions due to different

names of localities and taxa are avoided and a huge amount of data is saved in a smaller space for fast access. The most important upgrade, however, was the addition of spatial coordinates for each locality. Subterranean animals in the region gained much attention in molecular studies, representing an interesting field for phylogeographical studies. The database therefore needed proper extension, to connect the distribution and locality data with the molecular data. The relational form of the database allowed for easy addition and crossconnection of tables holding such different information. The database was put on a central MySQL server, allowing simultaneous access for multiple users. Queries were designed to meet the demands of users, allowing data export for spatial analyses in GIS, as well as searches for DNA sequences from a particular taxon or locality. Online presentation of the database contains a tool for data contribution. Data arranged in a database are used in studies of regional biodiversity patterns and conservation, and are being extracted for inclusion in various cooperative projects and initiatives.

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Phylogeography, Phylogeny and Evolution: oral presentation

## **The paradox of syntopy in cryptic stygobiont lineages from the Dinaric Karst**

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In the last decade, phylogeographic analyses have uncovered a high level of cryptic diversity in subterranean taxa. In most cases, closely related cryptic species are geographically defined and allopatric. Recent extensive phylogeographic studies of the cave shrimp *Troglocaris* s. str. and the cave tube worm *Marifugia cavatica* in the Dinaric Karst using mitochondrial and nuclear genes, showed substantial level of cryptic diversity in both taxa. Most recovered cryptic lineages and phylogroups seem to be allopatric. By more detailed sampling and analyses (adding more specimens and populations), areas of sympatric occurrence of morphologically indistinguishable lineages were discovered in both taxa. We found them occurring syntopically in two areas: in the Italian Carso (cave shrimps and tube worms) and on Istra Peninsula (cave shrimps). Following the principle of competitive exclusion, two closely related species should not coexist in a stable environment; they must differ in their ecological niche, because without differentiation one species will exclude the other through competition. At least three possible hypotheses can be put forward to explain this apparent paradox. First, as there are only few sites of syntopic occurrence, we might not be observing a long-term stable coexistence but rather the transient result of accidental immigration of one species into the range of another. Second, if co-occurring cryptic species indeed form real ecological communities, they could avoid competition through differentiation of niches by traits other than morphology. The problem with these two hypotheses is that we have yet to explain the nature of the putative reproductive barrier that both of them imply. The third hypothesis circumvents this problem by conjecturing that syntopic lineages are not species, but clones that reproduce asexually. That way, no reproductive barrier is needed to account for the occurrence of unique and stable mitochondrial and nuclear allelic combinations. Asexual reproduction has been described for decapods as well as serpulid tube worms. This mode of reproduction could represent an alternative evolutionary strategy for subterranean species.

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# Excursion guide

## Mid-conference excursion Wednesday, 5 September 2012

**Departure at 7:00** from the parking place in front of DoubleTree by Hilton.

The rather early departure time is important to manage all the schedule of mid-conference excursion. Thank you for your kind understanding and for keeping in timetable (see below).

Walking shoes are required, light hiking gear is recommended. Be prepared for rainy weather and do not forget that we will visit **Dobšinská Ice Cave where the temperature moves around zero** (Celsius). A flashlight or headlamp will be useful, too.

The price for the excursion is included in the registration fee. It covers all transportation and admittance costs, lunch, dinner, and basic insurance.

### Program and timetable of excursion

7:00–9:30	Košice – Dobšinská Ice Cave
10:00–11:00	entry and display of the cave
12:00–13:30	Dobšinská Ice Cave – Pribylina
13:30–15:30	Museum of the Liptov Village, Pribylina – lunch and museum
15:30–16:30	Pribylina – Demänovská Liberty Cave
16:30–18:00	entry and display of the cave
18:30–20:00	Hotel Repiská – excursion dinner
20:00–22:30	Demänovská Valley – Košice

**Total ca 15.5 hours, 407 km**

### Legend

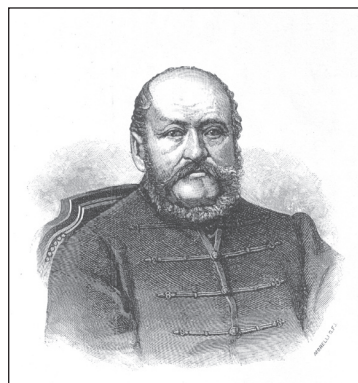
- will be seen from the bus      ● will be visited (in bracket = possibility to visit)

### Košice

- 240 000 inhabitants, city with long and agitated history and splendid medieval town, place of the 21st International Conference on Subterranean Biology, 2–7 September 2012.

### Slanské vrchy Mts.

- Mountain chain of Neogene volcanoes of ca 100 km length in N-S direction extending to Hungary (Zempléni Mts.). On the east foothill there is a birthplace of Imrich Frivaldský (Imre Frivaldszky), an important zoologist and biospeleologist (\*1799, Bačkov, †1870 Jobágyi).



**Imrich Frivaldský**



## Slovak Karst

- The largest karstic area in Slovakia (361.65 km<sup>2</sup>) divided into several plateaus with well developed surface and underground karst phenomena. It is a part of the Slovak-Aggtelek Karst orographic unit located between Slovakia and Hungary, the largest karst region in Central Europe. Several caves from the area belong to the UNESCO World Heritage List. National park and Biosphere reserve.



**Zádiel Valley**

## Turňa nad Bodvou

- Village with medieval church and ruins of the Turniansky hrad Castle from the 13th century on top of the conspicuous cone-shaped hill. Rare flora grows in its limestone slope including the local endemic *Onosma tornensis* (Boraginaceae).

## Zádiel Valley

- In the first part of the valley there is 3 km long and up to 400 m deep karstic canyon. The dominant rock is the slender formation with a matching name Cukrová homoľa (Sugar Cone).

## Krásna Hôrka Castle

- It stands on top of the conspicuous denuded mountain. The original Gothic castle was built around 1320. The castle acquired fortifications in time of the Turkish threat.



**Turňa nad Bodvou, castle**

Recently under reconstruction due to expansive fire in spring 2012.  
National cultural monument.



### **Rožňava**

- The largest town and centre of Gemer region. The settlement was first mentioned in the historical document from 1291. History of this medieval town was always connected with mining. The best time of its development were the 14th and 15th centuries when iron, copper and antimony, including precious metals like silver and gold were extracted from the ores in the town and its environs.

### **Slaná River (Salt River)**

- Small river, important for transport of salt from Baltic and Carpathian regions to Old Hungary in the Middle Ages.

### **Slovak Paradise**

### **Slovenské Rudohorie Mts. (Slovak Ore Mts.)**

- The ore-bearing mountain region, the largest orographic unit of Slovakia (ca 4,000 km<sup>2</sup>). On the south foot of the hill Radzim there is a village Kobeliarovo, a birthplace of Pavol Jozef Šafárik (\*1795, +1861 in Prague)

### **Betliar**

- Village with two-storied manor house surrounded by a wonderful English park. One of the most remarkable things about the manor house is its library that contains more than 20 thousand volumes of theological, historical, geographical and philosophical literature of the 15th to 19th centuries, written in 15 different languages.



### **Dobšiná**

- Small town, as a mining settlement it was first settled by Germans. The Turks destroyed the town in the years 1582 and 1584. It was renewed by fresh colonists, mostly miners, from different parts of eastern and northern Slovakia. The most valuable local product was copper.

### **Betliar castle**

## Slovak Paradise National Park (Slovenský raj)

- An important karstic area (197.63 km<sup>2</sup>) with famous gorges, waterfalls and caves.

## Dobšinská Ice Cave (Dobšinská ľadová jaskyňa)

- Length: 1,483 m (tourist path 475 m); Depth: 112 m. Dobšinská Ice Cave belongs among the most important ice caves in the world. Since 2000 it is inscribed in the UNESCO



**Dobšinská Ice Cave, entrance facility**

World Heritage list. As compared with known high-mountain ice caves Eisriesenwelt and Dachstein-Rieseneishöhle in Austrian Alps or with the Romanian Scărișoara Cave in Bihor Mts., which has the glaciated part at elevations of 1,100 to 1,120 m, the monumental glaciation of the Dobšinská Ice Cave persist for thousands of years at elevations only 920 to 950 m. The cave is formed in the Middle Triassic pale Steinalm and Wetterstein limestones of the Stratená Nappe, along the tectonic faults and interbed surfaces. The main part of the cave is represented by a giant cavity descending from the entrance to the depth of 70 m, which was formed by breakdown of rock columns between the passages formed by palaeoflow of the Hnilec River in several development levels. At present, the most of its volume is filled with glacier, sometimes up to the ceiling, by which it is divided into individual parts (Great and Small Hall, Ruffiny's Corridor and Ground Floor). Collapsed Dome (Zrútený dóm) is partly glaciated, and its edge reaches as far as under the Duča breakdown. Original oval shapes of river modelling are almost entirely destroyed by collapses and frost weathering. Dobšinská Ice Cave is a part of the Stratená Cave system (more than 23 km of corridors, 3rd longest cave system in Slovakia). National nature monument.

In the cave microscopic fungi were studied (NOVÁKOVÁ 2006). Few data on invertebrates were published in history, description of psychrophilous springtail *Hypogastrura crassaegranulata dobsinensis* and records of troglophilous millipede *Allorhiscosoma sphinx* and beetle *Choleva nivalis* were the most important (STACH 1949; GULIČKA 1975; RŮŽIČKA & VÁVRA 1993 in: KOVÁČ et al. 2006). In 2004 the microclimatic and speleobiological explorations were conducted. Low temperature is typical in both, glaciated and dry parts of the cave (-0.1 to + 3.5° C in growing period). Four species of troglotibiotic springtails were documented, *Protaphorura janosik* (numerous in glaciated parts of the cave), *Deuteraphorura kratochvili*, *Arrhopalites aggtelekiensis* and an undescribed species of the genus *Megalothorax*. Stygobiotic *Bathynella natans* was collected in pools while *Niphargus* sp. in the spring under the cave entrance (KOVÁČ et al. 2006). The cave is the most important wintering place of the whiskered bat (*Myotis mystacinus*) and Brandt's bat (*Myotis brandtii*) in the central Europe. From among 12 species detected in the cave important is the pond bat (*Myotis dasycneme*) and Natterer's bat (*Myotis nattereri*), which are the rarest bat species in Slovakia (UHRIN 1998; BOBÁKOVÁ 2002).



## Duča

- Great collapse and cave situated above the Dobšinská Ice Cave, part of the Stratenská Cave system.

## Museum of the Liptov Village, Pribylina

- The most attractive landmark of the Liptov region. It displays the typical folk architecture. The impetus behind the establishing of the museum was the extensive flooding caused by the construction of the Liptovská Mara dam. The most important cultural monuments were moved from eleven flooded communities. They now constitute the main exhibition of the museum. Entire original buildings were dismantled and moved to the museum, which was opened to public in 1991. It is the most recently opened out-door museum of Slovakia.

Precious samples of sacral and secular folk architecture are the Gothic-Renaissance manor house of Parížovce and an Early-Gothic church of the Virgin Mary from Liptovská Mara. The manor house is a fine example of some of the oldest landowners' architecture of the Liptov region and various architectural details including the Late-Gothic wall paintings will certainly attract the attention of the tourist. The church itself is much older, its foundations were constructed in the Romanesque style and laid down probably in the 12th century. It was rebuilt in the second half of the 13th century, later expanded, and in the 17th century the tower was added to it. The village school comprising also the teacher's apartment of Valaská Dubová and exhibition of old beehives in the orchard next to the local school will also intrigue the visitor. Within the museum complex there is a training centre offering samples of folk craft and arts (weaving, bobbin-lacing, wood, metal and leather-working, the manufacturing of wicker or straw baskets, etc.), as well as the typical folk costumes. Regular events organised in the museum are the Sunday of Shepherd-Ovenálie held last Sunday of May, Sunday of Children held in June, the Sunday of Compatriots in July, the Sunday of Bee-Keepers in August, the Day of St. Hubert in September, and Harvest Thanksgiving in October, as well as Liptov Christmas in December.

The museum also has a zoo that concentrates on farm animals and poultry including horse breeding where horse riding is possible.

## High Tatras National Park (Vysoké Tatry)

- Admirable, the highest mountain unit of entire Carpathians (Gerlachovský štít Peak, 2,654 m) with well developed subalpine and alpine habitats and with glacial relicts in fauna and flora. Famous are numerous tarns, lakes with glacial origin. Central part of the Tatras has granite bedrock (High Tatra Mts.) and the parts on eastern



**High Tatras**

and western margins, Belianske and Západné Tatry Mts., are mainly consisted of limestones. Karstic caves in the highest elevation (ca 2,000 m) are situated in western part of the High Tatras (Kresanica Mt.).

### Liptovský Mikuláš

- City of the Liptov region. Its situation on the eastern bank of the water dam Liptovská Mara and the vicinity of the Low Tatras, Západné Tatry Mts. and the Chočské vrchy Mts. have predetermined this city to become one of the most important tourist centres in Slovakia. Location of the Slovak Caves Administration and the Slovak Museum of Nature Protection and Speleology.

In 1677 the town became the seat of the Liptov province and provincial assemblies were held here regularly together with sessions of the provincial court of justice. Certainly a great deal of Slovaks are aware of the session of 1713 which sentenced to death the robber Juraj Jánošík who latter became the Slovak folk hero.

### Low Tatras Mts.

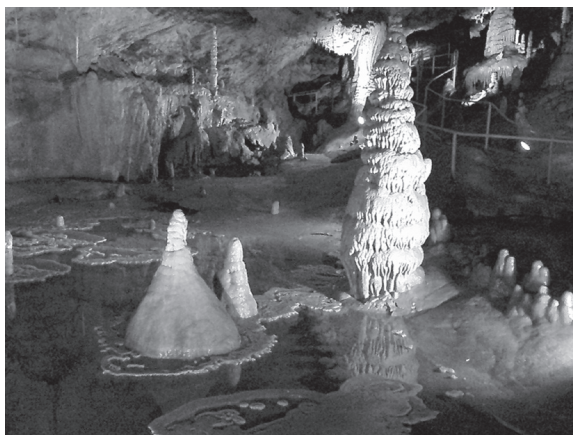
- The second highest mountain range in the Western Carpathians (Ďumbier Mt., 2,043 m) with both crystalline and limestone bedrock. North faced Demänovská Valley with the largest cave system in Slovakia (35.2 km).

### Demänovská Cave of Liberty (Demänovská jaskyňa Slobody)

- Length: 8,126 m (tourist path 2,150 m); Depth: 120 m. The national nature monument Demänovské Caves on the northern side of the Low Tatras Mts. represent a part of the longest cave system in Slovakia created in Middle Triassic Guttenstein limestones, exceeded 35 km. The Demänovská Cave of Liberty belongs among its dominating caves. It has been captivating the visitors by its rich flowstone fill of various colours, magical flow of underground Demänovka River as well as the charming pools for many years. It is the most visited show cave in Slovakia. The temperature in whole system in deeper zones varies from about 0 °C (Demänovská Ice Cave) to +7 °C.

Although the Demänovské Cave system is situated outside the zone of highest diversity of cavernicoles in the Western Carpathians, we consider it as an important speleobiological locality.

The history of local cave biological and groundwater research is long and it begins in 1870 with discovery and description of *Duvalius microphthalmus spelaeus* (“*Trechus spelaeus*”) by E. REITTER. The type localities of two troglotibiotic springtails *Pseudosinella pachti* RUSEK, 1961 and *Deuteraphorura kratochvili* (NOSEK, 1963) are situated here, too. Aquatic fauna was investigated by



**Demänovská Cave of Liberty**

HRABĚ (1942, 1954) and ŠTĚRBA (1964) (in: KOVÁČ et al., 2002). After more extensive zoological explorations in 2000 the list of known underground inhabitants was enlarged and comprehends such stygobionts as enigmatic turbellarian *Dendrocoelum carpathicum*, crustaceans *Niphargus tatrensis*, *Synurella intermedia hrabei*, *Acanthocyclops languidus*, *Paracyclops fimbriatus*, *Elapoidella phreatica* and terrestrial cavernicoles such as rhagidiid mite *Poecilophysis spelaea*, paligrade *Eukoenenia spelaea*, millipede *Allorhiscosoma sphinx*, springtail *Porotaphorura janosik*, etc. The discovery of *Eukoenenia spelaea* moves northernmost boundary of distribution range of Paligradi in Europe to 49° of northern latitude. Recently intensive microbiological investigations are conducted in caves of the Demänovská Valley. Several caves are important for hibernation of bats. In total, 13 species were observed, the most important are the Pustá Cave (12 spp.) and the Demänovská Ice Cave (11 spp.) The latter one is one of the most important hibernation spaces for Northern Bat (*Eptesicus nilssonii*) in Slovakia (VIŠŇOVSKÁ, 2007).

### **Levoča**

- The city is often considered the brightest jewel in the Spiš crown. It was first mentioned in an act of 1249 as *Leucha*. The community rapidly grew into a town with numerous privileges because of its position on the trade route *Via Magna*. Shortly after Levoča became the centre of German colonisation in the Spiš region. The driving force of the Levoča's development was trade which gradually acquired an international dimension. The citizens of Levoča traded with Kraków, the Hanseatic towns and even with Venice. The craftsmen of Levoča produced for the markets and fairs throughout Old Hungary and Poland. Later Levoča became the regional centre of the Renaissance and Humanism in Old Hungary. The local Evangelical lycée became an important centre of the Slovak national revival in the 19th century. The Levoča has small bastioned historical town built on the hill, reminiscent of Sevilla in Spain. The town is famous by the Late Gothic main altar in the St. Jakub church at a height of 18.6 m that is the highest of this kind in the world. Made of lime wood in 1507–1517 by Maestro Pavol of Levoča is of extraordinary high artistic value. Since 2009 the town is inscribed in the UNESCO World Heritage list. Neo-Gothic church of the Virgin Mary's Visitation is situated high above the town on the Mariánska hora Mt. Every year at the beginning of July Levoča becomes the meeting point of the largest pilgrimage in Slovakia.

### **Levočské vrchy Mts.**

- Flysh mountains with several pseudokarstic (sandstone) caves. The Cave under the Spišská Mt. is the longest one in Slovakia (over 720 m of corridors).

### **Spiš Castle (Spišský hrad)**

- Famous medieval castle partly in ruins with its area of more than 4 ha is one of the largest castle compounds in Central Europe. Construction of the medieval castle on a travertine hill dates back to the beginning of the 12th century. It became the seat of the head of Spiš region for many centuries. In 1780 the castle compound was destroyed by fire and gradually fell into ruin. In 1993 the Spiš Castle was included in the UNESCO list of monuments belonging to the world cultural heritage. At present there are museum collections placed in the castle.

In its vicinity numerous travertine phenomena are present, caves and xero-thermophilous sites including.

### **Branisko Mts.**

- Mountain ridge with crystalline bedrock and several small limestone units. The highway to Košice continues through the ridge via road tunnel.

### **Prešov**

- The third largest city in Slovakia (92,000 inhabitants), an important centre of Šariš region.

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**21st International Conference on Subterranean Biology  
2–7 September, 2012, Košice, Slovakia  
Abstract book**

Edited by Ľubomír Kováč, Marcel Uhrin, Andrej Mock & Peter Ľuptáčik

Published by Pavol Jozef Šafárik University in Košice, Slovakia

Publication year: 2012

Printed: 200 exemplars

Pages: 142

Typography: Marcel Uhrin

Cover design: Pavel Maria Smejkal

Printed by Equilibria, s. r. o., Košice

ISBN 978-80-7097-959-4



**Organized by:**



Pavol Jozef Šafárik University in Košice



Slovak Caves Administration



State Nature Protection of the Slovak Republic

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