20th International Conference on Subterranean Biology

Postojna, Slovenia
Aug 29 - Sept 3, 2010

ABSTRACT BOOK

20th International Conference on Subterranean Biology

Postojna, Slovenia

29 August – 3 September 2010

Organized under the auspices of the International Society for Subterranean Biology by:

- Biotehniška fakulteta – Univerza v Ljubljani
- Inštitut za raziskovanje krasa ZRC SAZU
- Notranjski muzej Postojna

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Foreword

Welcome to the 20th International Conference on Subterranean Biology, and welcome to Postojna, "the cradle of speleobiology"! The organizers have strived to provide the essentials for a professionally successful and personally inspiring and gratifying scientific meeting.

At this occasion we wish to point to a few specifics of this event and draw a formal outline of the conference organization. It has been exciting for the organizers to observe how the usual frame of former International Symposia of Biospeleology or Subterranean Biology was disintegrating, compelling us to search for new solutions. Not because we thought something was wrong with previous meetings, but because we saw it as a sign of our beloved métier maturing into a full-grown interdisciplinary science. Encouraged by Valerio Sbordoni at the 2009 Council meeting in Verona, we decided to structure the meeting by special symposia offered in advance rather than simply sorting the submitted abstracts by topics. Some of these symposia, for example *Superficial Subterranean Habitats* and *Microbiology and Geomicrobiology*, turned out to be very popular, pointing to the current hot topics of subterranean biology. Some symposia had to be coined post hoc in order to embrace all the received contributions. Among those, *Phylogeography and Phylogeny* is the most populated, reflecting a trend that has started more than a decade ago. Also noteworthy is the appeal of taxonomically oriented special symposia with *Subterranean beetles* taking the lead, followed by *Subterranean aquatic annelida*. We are especially pleased with the latter as annelids have been neglected in their role as a major component of stygofauna for too long. Unfortunately, the *Cave Fish Symposium* did not receive the expected attention, and the few contributions had to be relocated to other symposia.

When the number of received oral contributions approached 80, we knew there was no way around having two sessions running in parallel. This means an end to the familiar, peaceful, single-room style of meetings. While there are obvious advantages to it, it is simply impossible to fit that many talks successively into four days. The solution is a hybrid between a day of introductory plenary lectures, two days of special symposia running in parallel, and a last day with selected common presentations introducing new concepts, models and methods in subterranean biology. Further details are evident from the program itself. The two lecture rooms in which the parallel symposia will be held are next to each other, so swapping symposia will be easy. This is a somewhat experimental solution, but we hope to have found an optimal compromise between the limited time available and the growing number of presentations.

The number of posters is about twice as high as at previous biospeleological meetings. In order to have enough time to look at all posters and discuss them with their authors, there will be two poster sessions, scheduled for Tuesday and Thursday after the Special Symposia. Posters will remain displayed throughout the entire meeting.

Other events include a visit to Postojna Cave on Monday evening for all participants and separate visits to the Vivarium Proteus for the first half of the participants on Tuesday and the second half on Thursday. Name lists of the Tuesday and Thursday groups will be displayed at the conference announcement board. The mid-conference excursion will be on Wednesday and is described in detail in the Conference Program brochure. The conference will end with the social dinner on Friday evening at Jamski dvorec. All these events are included in the conference fee.

We are inviting you to join us, to present your contribution, participate in the discussions, and enjoy this celebration of Subterranean Biology together. Thanks to all of you – the growing Subterranean Biology Community from 24 countries – there are 108 lectures and 68 posters, two documentaries, 9 special symposia and an extra theme to choose from.

The Organizing Committee of the 20th ICSB

SCIENTIFIC PROGRAM

SUNDAY 29 AUG : Karst Research Institute

17:00 and ongoing **Registration**

19:00 **Welcome reception sponsored by the Municipality of Postojna**

Brief welcomes will be given by the Mayor of Postojna, Mr. Jernej Verbič, and the head of the Karst Research Institute at ZRC SAZU, Dr. Tadej Slabe - Opening of the exhibition "The Proteus among us", prepared by Notranjska museum Postojna.

MONDAY 30 AUG : Jamski dvorec – Luka Čeč Hall

8:00 and ongoing **Registration, poster hanging**

9:00 **10:00** **Opening ceremony**

Mr. Matjaž Berčon	Director of Turizem Kras, Conference Host
Prof. Giuseppe Messana	President of the International Society of Subterranean Biology
Dr. Roko Žarnić, Minister	Slovenian Ministry of the Environment and Spatial Planning

10:00 **10:50** **Opening lecture**

Sket, B.	Subterranean fauna of the wider Dinaric area – from first discoveries to a global hotspot and its up-to-date biological evaluation

10:50 **11:20** **Coffee break**

Introductory lectures to special symposium: Superficial Subterranean Habitats

Chair: P. Marmonier

11:20	12:00	Culver, D. C.*, Pipan, T.	Shallow subterranean habitats – gateway to the subterranean realm
12:00	12:20	Hahn, H. J.*, Bork, J., Schmidt, S. I.	What is groundwater? A new approach, and what this means to fauna
12:20	13:00	Humphreys, W.*, Guzik, M., Bradford, T., Cooper, S., Leijs, R., Watts, C., Austin, A.	Groundwater calcretes: sheets of subterranean habitat scattered on an Archaean landscape

13:00 **14:00** **Lunch**

Introductory lectures to special symposia: Microbiology and Geomicrobiology; Adaptation, Development, Physiology; Annelida; Countdown 2010 and Conservation

Chair: O. Moldovan

14:00	14:20	P Paoletti, M.G., Beggio, M., Dreon, A. L., Pamio, A., Gomiero, T., Brilli, M., Toniello, V., Concina, G. , Dorigo, L., Concheri, G., Squartini, A., Summers Engel, A.	A newly discovered cave foodweb: the *Cansiliella* story
14:20	15:00	Jeffery, W. R.	Pleiotropic tradeoffs between constructive and regressive traits during troglomorphic evolution
15:00	15:20	Martinez-Ansemil, E.*, Sambugar, B.	Annelida, an often neglected component of groundwater ecosystems

MONDAY 30 AUG : Jamski dvorec, continued

15:20	15:40	Eberhard, S.	Impacts of climate change on stygofauna in southwest Western Australia
15:40	16:10	Coffee break	
		Introductory lectures to special symposia: Patterns and Processes in Subterranean Biodiversity; Subterranean Beetles - *Chair: S. Gottstein*	
16:10	16:30	Porter, M. L.*, Culver, D. C.	Tethyan distribution of stygobionts: fact or fiction
16:30	16:50	Zagmajster, M.*, Sket, B.	Biodiversity pattern and distribution ranges of terrestrial troglobionts in the northwestern Balkans
16:50	17:10	Schuldt, A., Drees, C., Drescher, N., Schäfer, K., Assmann, T.*	What determines subterranean ground beetle diversity in the West Palaearctic? A macroecological approach using country-based distribution data (Coleoptera: Carabidae)
17:10	17:50	Casale, A.	From *Anophthalmus schmidti* to molecular phylogenies: past and present in the knowledge of subterranean carabid beetles (Coleoptera: Carabidae)
17:50	**19:00**	**Break, poster hanging**	ISSB Council Meeting
19:00	**20:00**	**Visit to Postojna Cave**	

TUESDAY 31 AUG : Jamski dvorec

8:00 and ongoing **Registration, poster hanging**

		NANOS ROOM		PIVKA ROOM	
		Superficial Subterranean Habitats *Chair: D. C. Culver*		**Phylogeography and Phylogeny** *Chair: C. J. Douady*	
8:30	8:50	Pipan, T.*, Culver, D. C., Simon, K. S.	Organic carbon in aquatic shallow subterranean habitats	Kornobis, E.*, Pálsson, S.	Phylogeny of Crangonyctoidea: taxonomic status and origin of groundwater amphipods, endemic to Iceland, based on two nuclear genes
8:50	9:10	Marmonier, P.*, Navel, S. , Piscart, C., Chauvet, E.	Particulate organic matter breakdown in shallow interstitial habitat of a rural stream	Leijs, R.	Evolution of chiltoniid amphipods from subterranean and surface habitats in Australia
9:10	9:30	Gottstein, S.*, Žgane, K., Cetin Krnjević, V., Popijač, A.	Life history traits of the epigean populations of *Niphargus dalmatinus* (Crustacea: Amphipoda) along the Cetina River, Croatia	Abrams, K.*, Guzik, M., Cooper, S., King, R., Austin, A.	Systematics and phylogeography of Australian Parabathynellidae (Crustacea: Bathynellacea)
9:30	9:50	Fong, D. W.*, Kavanaugh, K. E.	Population dynamics of the stygobiotic amphipod crustacean *Stygobromus tenuis potomacus* and isopod crustacean *Caecidotea kenki* at a single hypotelminorheic habitat over a two-year span	Baratti, M.*, Messana, G., Filippelli, M., Sket, B.	New biogeographical and phylogenetic data about the genus *Sphaeromides* and its relatives (Crustacea: Isopoda: Cirolanidae)
9:50	10:10	Meleg, I. N.*, Fiers, F., Kelemen, B., Popescu, O., Moldovan, O. T.	Heterogeneous copepod distribution in different groundwater habitats from Northwestern Romania	Verovnik, R.*, Stoch, F., Sket, B.	Phylogeny of the Western taxa of the genus *Monolistra* (Crustacea: Isopoda: Sphaeromatidae)

TUESDAY 31 AUG : Jamski dvorec, continued

10:10	10:30	Papi, F.*, Pipan, T., Culver, D. C.	Ecological studies of an epikarst community in Alpine cave Snežna jama na planini Arto: preliminary results	Lukić-Bilela, L.*, Pleše, B., Bruvo-Mađarić, B., Imešek, M., Bilandžija, H., Četković, H.	The mitochondrial genome analysis of the unique cave dwelling sponge *Eunapius subterraneus* Sket & Velikonja, 1984 (Porifera: Spongillidae)
10:30	**11:00**	**Coffee break**			
		Superficial Subterranean Habitats, continued		**Phylogeography and Phylogeny, continued - *Chair: M. Porter***	
11:00	11:20	Juberthie, C.	Mesovoid shallow substratum (MSS)	Allegrucci, G.*, Trucchi, E., Sbordoni, V.	Patterns of speciation in *Dolichopoda* cave crickets (Orthoptera, Rhaphidophoridae)
11:20	11:40	Novak, T.*, Perc, M., Janžekovič, F.	Candidates for superficial subterranean habitats and epikarst among terrestrial cave inhabiting species	Mugue, N. S.	Caucasus and vicinity: comparative phylogeography of Ponto-Caspian and subterranean crustaceans
11:40	12:00	Oromí, P.*, López, H., Manhert, V.	Diversity and allopatric distribution in the Canarian MSS: a case study in an old island	Jugovic, J.*, Prevorčnik, S., Blejec, A., Sket, B.	Linking molecular phylogeny to morphological evolution in *Troglocaris* (Crustacea: Decapoda: Atyidae)
12:00	12:20	Růžička, V.	Central European spiders adapted to life in subterranean habitats	Zakšek, V.*, Sket, B., Trontelj, P.	Phylogeography of the cave shrimp *Troglocaris* s. str.: a basis for a comparative phylogeography of Dinaric subterranean fauna
		Patterns and Processes in Subterranean Biodiversity - *Chair: M. Zagmajster*		**Phylogeography and Phylogeny, continued**	
12:20	12:40	Eberhard, S.*, Stevens, N., Perina, G., Bell, P.	Troglofauna in the Pilbara region, Western Australia – Patterns in diversity and distribution, and sampling considerations for conservation assessment	Palandačić, A.*, Zupančič, P., Matschiner, M., Snoj, A.	Genetic evidence of subterranean migration of imotska gaovica *Delminichthys adspersus* (Pisces: Cyprinidae)
12:40	13:00	Halse, S.	Distribution patterns of different groups of troglofauna in the Pilbara region, Western Australia: are arachnids the most restricted troglofauna?	Strecker, U.*, Wilkens, H.	Problems of taxonomy in Mexican *Astyanax*
13:00	**14:00**	**Lunch**			
		Patterns and Processes in Subterranean Biodiversity, continued		**Systematics and Faunistics *Chair: R. Verovnik***	
14:00	14:20	Berkhoff, S.E.*, Matzke, D., Fuchs, A., Bork, J., Hahn, H. J.	Recording the stygofauna of the federal state of Sachsen-Anhalt, Germany	Sidorov, D. A.	Pseudocrangonyctidae (Crustacea: Amphipoda) and its forming pathways
14:20	14:40	Moldovan, O. T.*, Meleg, I. N., Perşoiu, A.	Habitat fragmentation and its effects on groundwater populations	Semenchenko, K. A.	Water mites (Hydrachnidia) from interstitial habitats of the Russian Far East and their relationship with faunas of adjacent lands

TUESDAY 31 AUG : Jamski dvorec, continued

14:40	15:00	Bork J.*, Fuchs A., Barufke K. P., Hahn H. J.	Nine years of long-term stygofauna monitoring in Southwest Germany	Eberhard, S., Moulds, T.*	Review of the subterranean biodiversity of the Nullarbor plain, Southern Australia
15:00	15:20	Stein, H.*, Berkhoff, S.E., Matzke, D., Hahn, H. J.	Spatial distribution patterns of faunal groundwater communities across Germany	Deharveng, L.*, Tian, M., Li,Y., Bedos, A.	Invertebrate biodiversity of the Guangxi caves (Southern China)
15:20	15:40	Stoch, F.*, Gasparo, F.	Regional species richness and diversity patterns of obligate cave-dwelling fauna in the Classic Karst in Italy	Drescher, N., Loos, J., Levanony, T., Dayan, T., Schuldt, A., Schäfer, K., Assmann, T.*	Unexpected rich terrestrial subterranean fauna in Israel: first results from the inventory of 13 caves
15:40	16:00	Lopes Ferreira, R.	Hidden biodiversity: recent advances and perspectives in Brazilian subterranean biology	Subhash Babu, K. K, Bijoy Nandan, S.*	The hypogean fauna of selected ecosystems of Kerala, India with two new records
16:00	**16:30**	**Coffee break**			
		Patterns and Processes in Subterranean Biodiversity, continued		**Systematics and Faunistics, continued**	
16:30	16:50	Bedek, J.	Data analysis of spatial distribution of cave terrestrial isopods (Isopoda: Oniscidea) in Croatia	Kováč, L.*, Ľuptáčik, P., Papáč, V., Mock, A., Mourek, J.	Contribution to morphology of palpigrade *Eukoenenia spelaea* (Peyerimhoff, 1902) and its distribution in the Western Carpathians
16:50	17:10	Maurice, L.*, Robertson, A., Bloomfield, J., Allen, D.	Spatial variations in stygobiont distributions in the English Chalk	Mock, A.	Terrestrial isopods and millipedes in Slovak caves: results of long-term exploration
17:10	**17:30**	**Short documentary**	Mlinar, C.	The aquatic life of the Postojna -Planina Cave System	
17:40	**19:00**	**Poster Session I**			
19:10	**20:00**	**Visit to Vivarium Proteus for first group**			

WEDNESDAY 1 SEP : MID-CONFERENCE EXCURSION

THURSDAY 2 SEP : Jamski dvorec

8:00 and ongoing **Registration**

		NANOS ROOM		PIVKA ROOM	
		Countdown 2010 and Conservation of Subterranean Life - ***Chair: G. Beltram***		**Adaptation, Development, Physiology** ***Chair: W. R. Jeffery***	
8:30	8:50	Souza Silva, M.*, Parentoni Martins, R., Lopes Ferreira, R.	Conservation of cave invertebrates and study of impacts on caves located in the Brazilian Atlantic rain forest	Šustr, V.*, Nováková, A., Lukešová, A., Vošta, O.	Feeding biology of the cave isopod *Mesoniscus graniger* (food preference and digestive enzymes)

THURSDAY 2 SEP : Jamski dvorec, continued

8:50	9:10	Muriel-Cunha, J.*, Cardoso, N. A., Martinelli Filho, J. E., Gutembergue, G., Albino, U.	Biospeleological research in the Amazon: the case of Planaltina cave in the ecoregion Xingu-Tapajós, Brazil	Vittori, M.*, Žnidaršič, N., Štrus, J.	The gland -piliferous organs of *Titanethes albus* (Crustacea: Isopoda)
9:10	9:30	Dravec, L., Kostelić, B.*, Mandić, A.	Protection of speleological objects in the region of Istria through the European Union projects	Lipovšek, S.* , Novak, T., Janžekovič, F., Pabst, M. A.	Role of the fat body in the cave crickets *Troglophilus cavicola* and *T. neglectus* (Rhaphidophoridae, Saltatoria) during overwintering
9:30	9:50	Ozimec, R.*, Polak, S., Bedek, J., Zakšek, V.	Biospeleological component of the project KUP (Karst Underground Protection) in Istra Peninsula	Antolinc, E.*, Janžekovič, F., Perc, M., Novak, T.	Cold-hardiness in Central European troglophiles and trogloxenes
9:50	10:10	Ipsen, A.	How efficient are the non invasive protection measures in the Segeberg Cave in Northern Germany for the population of bats and subterranean beetles	Trajano, E.*, Herrero, J.C.H., Menna-Barreto, L.	Chronobiological studies on Brazilian subterranean fishes: a summary and new data on locomotor activity rhythms under light-dark cycles
10:10	10:30	Susac, R. A. J.*, Anderson, J., Moulds, T. A.	Comparisons of subterranean biodiversity from the West Kimberley Karst, Australia	Aljančič, G.	Fifty years of Tular Cave Laboratory
10:30	**11:00**	**Coffee break**		**Coffee break**	
		Countdown 2010 and Conservation of Subterranean Life, continued *Chair: S. Eberhard*		**Adaptation, Development, Physiology, continued**	
11:00	11:20	Dvorščak, K.	History of the presentation of the proteus (*Proteus anguinus*) in Postojna Cave	Karaman, I.*, Ozimec, R.	New long-legged cave-dwelling representatives of the Balkan genus *Cyphophthalmus* (Opiliones, Cyphophthalmi, Sironidae) and the question of functional significance of troglobite appendage elongation
11:20	11:40	Goater, S.*, Gardner, A., Knott, B.	Are stygofauna really protected in Western Australia?	Zacharda, M.	Troglomorphisms in Rhagidiidae (Acari: Prostigmata): are they all a morphological clock of adaptation ?
		Countdown 2010 and Conservation of Subterranean Life, continued		**Subterranean Aquatic Annelida** *Chair: E. Martínez-Ansemil*	
11:40	12:00	Beltram, G.* , Sket, B.	Subterranean habitats as wetlands of international importance	Martínez Garcia, A.*, Kvindebjerg, K., Worsaae, K.	Annelid diversity in anchialine systems: unique adaptations and functional morphology of *Protodrilus* n. sp. to the cave environment of La Corona lava tube (Canary Islands, Lanzarote)

THURSDAY 2 SEP : Jamski dvorec, continued

12:00	12:20	Reboleira, A. S. P. S.*, Gonçalves, F., Oromí, P.	Subterranean biology of mainland Portugal: historical review and new insights	Dumnicka, E.	Stygobiotic oligochaetes in Poland with remarks on their occurence and distribution in Central Europe
12:20	12:40	Semikolennykh, A.* , Rakhleeva, A., Poputnikova, T.	An environmental impact assessment of spent calcium carbide disposal in caves and mines	Achurra, A.*, des Châtelliers, M. C., Rodriguez, P.	Molecular and morphological analyses reveal the presence of two species in the stygobiont oligochaete *Troglodrilus galarzai*
12:40	13:00	Fajdiga, B., Stupar, M.*	Subterranean protection starts on the surface	Giani, N., Sambugar, B.*, Martínez-Ansemil, E., Martin, P., Schmelz, R.	Groundwater oligochaetes (Annelida, Clitellata) of Slovenia
13:00	14:00	Lunch		Lunch	
		Microbiology and Geomicrobiology ***Chair: R. Fowler***		**Evolution and Systematics of Subterranean Beetles - *Chair: A. Casale***	
14:00	14:20	Silva Taylor, E. L.* , Lopes Ferreira, R., Aparecida de Resende Stoianoff, M.	Microbiological study for a management plan in a touristic cave in Brazil	Giachino, P. M.	From the cavernicolous to the subterranean concept: past and present in Leptodirinae (Coleoptera, Cholevidae)
14:20	14:40	Nováková, A.	Cave microscopic fungi as food source for caves inhabiting springtails and some microfungal records	Vrezec, A.*, Kapla, A.	The influence of aboveground invasions on the diversity and distribution patterns of subterranean carabids (Carabidae)
14:40	15:00	Tkavc, R.*, Sonjak, S., Gunde-Cimerman, N.	Entomopathogenic fungi associated with two troglophilic moths *Scoliopteryx libatrix* L. and *Triphosa dubitata* L.	Fresneda, J., Grebennikov, V. V., Ribera, I.*	The geographic and phylogenetic limits of Leptodirini
15:00	15:20	Hermosin, B., Nováková, A., Jurado, V., Laiz, L., Porca, E.*, Rogerio, M. A., Sanchez-Moral, S., Saiz-Jimenez, C.	Microbial Observatory of Spanish Caves: assessing the origin of fungal outbreaks	Polak, S.*, Trontelj, P.	Suprageneric systematics of leptodirine beetles (Leiodidae, Cholevinae): molecular verus morphological characters
15:20	15:40	Brannen, K. M.*, Summers Engel, A., Birdwell, J. E.	Creating humic matter indices for the interpretation of ecosystem energetics	Friedrich, M.*, Rai, P., Barret, R., Daines, B., Chen, R.	The blind cave beetle that isn't: histological, behavioral and molecular evidence of functional photoreceptors in *Ptomaphagus hirtus*
15:40	16:00	Mulec, J.*, Walochnik, J.	Airborne microorganisms and relation to atmospheric parameters in big cave systems (Postojnska jama, Slovenia)	Hauser, B.	Leo Weirather (1887-1965) revisited
16:00	16:30	Coffee break		Coffee break	

THURSDAY 2 SEP : Jamski dvorec, continued

		Microbiology and Geomicrobiology, continued		Evolution and Systematics of Subterranean Beetles, continued	
16:30	16:50	Pašić, L.*, Kovče, B., Sket, B., Herzog-Velikonja, B., Porca, E., Jurado, V., Saiz-Jimenez, C.	Diversity of microbial communities colonizing the walls of a karstic cave in Slovenia	Perreau, M.	What does palaeontology reveal on the radiation of Leiodidae, Cholevinae and their colonisation of the subterranean biotopes?
16:50	17:10	Zakotnik, T., Mulec, J., Turk, V., Avguštin, G., Stres, B.*	Composition and activity of bacterial microbial communities in the Postojna cave sediments: are the microbes in 700 000 years old sediments still active?		
17:10	**17:30**	**Short documentary**	Lukić, M.*, Bedek, J.	Behavior of Cave Fauna	
17:40	**19:00**	**Poster Session II**			
19:10	**20:00**	**Visit to Vivarium Proteus for second group**			

FRIDAY 3 SEP : Jamski dvorec – Luka Čeč Hall

8:00	and ongoing	**Registration**		
8:40	**9:00**	**In Memoriam**		
			Marmonier, P.	Janine Gibert (1945-2009)
			Latella, L.	Sandro Ruffo (1915-2010)
		New Models, Methods, and Concepts for Subterranean Biology - *Chair: R. Leijs*		
9:00	9:40		Douady, C. J.*, Malard, F., Konecny, L., Morvan, C., Colson-Proch, C., Calvignac, S.	A new phylogenetic framework to decipher evolutionary processes involved in groundwater
9:40	10:00		Morvan, C.*, Konecny, L., Malard, F., Douady, C. J.	Is stygobiont diversification a consequence of extrinsic factors?
10:00	10:20		Malard, F.*, Konecny, L., Magniez, G., Douady, C. J.	The large distribution ranges of northern stygobiotic species of *Proasellus* (Isopoda): a test of cryptic diversity
10:20	10:40		Avguštin, G.*, Gramc, S., Bizjak Mali, L., Bulog, B., Ambrožič Avguštin, J.	The structure and diversity of the microbial community inhabiting the hind gut of the olm (*Proteus anguinus*)
10:40	**11:10**	**Coffee break**		
		New Models, Methods, and Concepts for Subterranean Biology, continued		
		Chair: E. Aden		
11:10	11:30		Prevorčnik, S.*, Trontelj, P., Sket, B.	Rapid re-invasion and evolution following the mysterious disappearance of Racovitza's *Asellus aquaticus cavernicolus* (Crustacea: Isopoda: Asellidae)
11:30	11:50		Konec, M.*, Trontelj, P.	Microsatellites as new tools to study the evolution of subterranean crustaceans

FRIDAY 3 SEP : Jamski dvorec, continued

11:50	12:10	Fowler, R.	Quantitative Real-Time PCR as a tool for the quantification and characterization of microorganisms in caves and karst aquifers: phytoplankton, lampenflora, bacterial communities, and fecal source tracking
12:10	12:30	Moškrič, A.*, Trontelj, P., Fišer, C.	A bioinformatic quest for phylogenetic resolution: adding new genes to the Niphargus supermatrix
12:30	12:50	Trajano, E.	Source *versus* sink populations concept applied to the Schiner-Racovitza classification of subterranean organisms
13:00	14:00	Lunch	
		New Models, Methods, and Concepts for Subterranean Biology, continued	
		Chair: S. Halse	
14:00	14:20	Slay, M. E.*, Fong, D. W.	Preliminary estimates of species detection probabilities for North American troglobionts
14:20	14:40	Turjak, M.*, Trontelj, P.	A new tree-based method for the quantitative analysis of phylogenetic character patterns: a case study with *Niphargus* (Amphipoda; Crustacea)
14:40	15:00	Flot, J. F.*, Bauermeister, J., Dattagupta, S.	*Niphargus* amphipods and their *Thiothrix* ectosymbionts in Frasassi (central Italy): a tale of multiple invasions and host specificity
15:00	15:20	Fišer, C.*, Trontelj, P.	Adaptive morphology of subterranean amphipod communities
15:20	15:40	Lopes Ferreira, R.	Translocation of cave fauna in Brazilian iron ore cave
15:40	16:00	Coffee break	
16:00	**16:30**	**Conference close**	
		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	

List of poster presentations

Adaptation, Development, Physiology	
Aden, E.	Eye development in the cave fish *Garra barreimiae*
Aljančič, G., Prelovšek, M*.	Does *Proteus* detect and react to a sudden rise of water conductivity which indicates incoming flood?
Bernabò, P., Jousson, O., Lencioni, V., Latella, L.	Heat Shock Response in the leptodirins *Neobathyscia mancinii* and *Neobathyscia pasai*
Bizjak Mali, L.*, Talaber, I., Žibert, U., Bulog, B.	Oogenesis in *Proteus*: Stages of oocyte development
Jugovic, J.*, Prevorčnik, S., Aljančič, G., Sket, B.	The shrimp rostrum between phylogeny and adaptation
Konec, M.*, Bulog, B.	Three -dimensional reconstruction of the inner ear of *Proteus anguinus* (Amphibia: Urodela)
Lukić, M.*, Houssin, C., Deharveng, L.	Extreme troglomorphy in a new species of cave springtail, *Tritomurus* sp. nov., from Croatia (Collembola: Tomoceridae)
Ľuptáčik, P.*, Šustr, V.	What we know about *Pantelozetes cavaticus* (Acari, Oribatida), notes on distribution, ecology, food preference and morphology
Simon, L., Mermillod-Blondin, F., Malard, F., Lécuyer, C., Fourel, F., Douady, C. J.*	Trophic niche of two subterranean isopod species along a parapatric boundary in pre-Alps and Jura Mountains (France): a preliminary field study using stable isotopes
Trajano, E., Pavani, M. P.	Related and yet different: behavioral differences between troglobitic heptapterid catfishes, *Pimelodella kronei* and *Rhamdia* spp.
Countdown 2010 and Conservation of Subterranean Life	
Crnčević, M., Ivanišin Kardum, K., Sudarević, N.*	Conservation education of cave and subterranean biodiversity: Dubrovnik underground tales
Manconi, R., Cadeddu, B.*, Stocchino, G.A., Pansini, M., Pronzato, R., Ledda, F.D.	Porifera checklist and database of Mediterranean marine caves
Manconi, R., Ledda, F. D. *, Stocchino, G. A., Casale, A., Grafitti, G.	Working for the candidate Orosei Marine Protected Area (Central-East Sardinia): on a benthic community from a subterranean estuary in a karstic coastal cave
Năpăruş, M., Aljančič, G.* , Oštir, K.	Design of a GIS database to monitor possible threats to the habitat of *Proteus anguinus* (Amphibia: Proteidae). A case study of a highly vulnerable population of *P. a. parkelj* in Bela krajina, Slovenia
Susac, R. A. J.*, Zakrzewska, B.	Management of a declining watertable at Yanchep National Park, Western Australia; for the benefit of subterranean biology
Šturm, S., Sedmak, A.*, Zorman, T., Peric, B.	Technical illustrations and application: Škocjan Caves, Velika Dolina cross section
Torres-Talamante, O.*	Food webs in Mexican Carribean Caves
Evolution and Systematics of Subterranean Beetles	
Bognolo, M.*	The genus *Aphaobius* Abeille de Perrin, 1878 (Coleoptera, Cholevidae, Leptodirinae)
Jalžić, B., Bregović, P.*	The edge of the range of genus *Anthroherpon* in Croatia
Njunjić, I.*, Pavićević, D.	Diversity of troglobitic beetles (Insecta, Coleoptera) of Krivošije area (Orjen Mt, Montenegro) in the scope of the recent biospeleological investigations
Ortuño, V. M., Gilgado, J. D.*, Sendra, A.	Update of the knowledge of the Ibero-Balearic hypogean Carabidae (Insecta: Coleoptera): faunistics, biology and distribution
Pavićević, D.*, Ozimec, R.	First finding of the troglobitic genus *Seracamaurops* (Coleoptera, Staphylinidae, Pselaphinae) for Croatia
Rizzo, V.*, Comas, J., Fadrique, F.,	Evolution and phylogeny of the subterranean genus *Troglocharinus* (Coleoptera,

Microbiology and Geomicrobiology	
Borda, D.*, Mulec, J., Nastase-Bucur, R.	Bat guano – a potential biohazard agent of caves in the temperate zone?
Buffoni Roque da Silva, L.*, da Costa Maia, N., Silva Taylor, E. L., Batista, L. R., Lopes Ferreira, R., Gomes Cardoso, P.	Evaluation and morphological identification of tannase-producing cave fungi
Buzzacott, P. L.*, Buckley, D. Waterworth, P.	Chemoautotrophic microbial mantle prevalence in Murra El Elevyn: catastrophic decline or seasonal fluctuation?
Krištůfek, V., Chroňáková, A., Mulec, J.*	The heavy metal content in bat guano heaps in karst caves
Nirlane da Costa, P., Gonçalves de Melo, A.*, Batista, L. R., Silva Taylor, E. L., Lopes Ferreira, R., Gomes Cardoso, P.	Identification of tannase producing fungi species in Brazilian caves
Nováková, A.,Brad, T., Moldovan, O. T., Hillebrand, A.	Microscopic fungi isolated from several caves in Romania
Porca, E.*, Jurado, V., Nováková, A., Saiz-Jimenez, C.	Origin and development of a fungal outbreak in Castañar de Ibor Cave, Spain
Silva Taylor, E. L.*, da Silva, D. M., Ferreira Terra, M., Batista, L. R., Lopes Ferreira, R.	Microbiologic study in a Brazilian cave: biodiversity, biotechnological potential and toxin production
Patterns and Processes in Subterranean Biodiversity	
Hazelton, E. R.*, Hobbs III, H. H.	Effects of glaciation on the distribution of troglomorphic biota in Ohio, USA
Latella, L.*, Verdari, N., Gobbi, M.	Distribution and frequency of cave-dwelling terrestrial arthropods in two spatially closed karst areas of the eastern Italian Prealps
Lips, J., Bedos, A., Kaufmann, B., Rahmadi C., Deharveng, L.*	Arthropods of guano in Santo caves (Vanuatu)
Lopes Ferreira, R.*, Souza Silva, M.	The cave lithology determining the structure of the cave invertebrate communities in the Brazilian Atlantic Rain Forest
Mihevc, A., Paul-Istrate, V., Moldovan, O. T.*, Constantin, S.	First results on subfossils in cave sediments from Slovenia and Romania
Rampini, M., Di Russo, C., Cobolli, M.	The cave crickets of the Eastern Mediterranean area: a contribution to the study of Balkan and Anatolian Rhaphidophoridae diversity
Souza Silva, M.*, Parentoni Martins, R., Lopes Ferreira, R.	Trophic dynamics of the coarse particulate organic matter (CPOM)in a tropical limestone cave
Zagmajster, M.*, Kadiš, A., Golob, K., Fišer, C.	Range sizes in subterranean amphipods of the genus *Niphargus*
Phylogeography and Phylogeny	
Bilandžija, H.*, Podnar, M., Jalžić, B., Patarčić, I., Tvrtković, N., Četković, H.	Phylogeny and phylogeography of the cave bivalve *Congeria kusceri*, with an outline for its endangerment in Croatia
Fišer, Ž. *, Moškrič, A., Fišer, C.	A molecular test for *Niphargus krameri* (Crustacea: Amphipoda) intraspecific diversity
Kornobis, E.* , Pálsson, S., Kristjánsson, B. K., Svavarsson, J.	Molecular evidence of the survival of subterranean amphipods (Arthropoda) during Ice Ages underneath glaciers in Iceland
Schepetov, D. M.*, Mugue, N. S., Ljovushkin, S. I.	On molecular phylogeny of *Niphargus* from the West Transcaucasus
von Rintelen, K.*, Page, T. J., Cai, Y., Roe, K., Kuhajda, B. R., Iliffe, T., Hughes, J., von Rintelen, T.	Living in the dark: phylogeny of atyid freshwater shrimps reveals multiple cave invasions
von Rintelen, K., Page, T. J.,Cai,	Colonization and subterranean speciation in atyid freshwater shrimps from Maros

Subterranean Aquatic Annelida	
Cukrov, M.*, Manconi, R., Cukrov, N., Jalžić, B., Despalatović, M.	Biodiversity in anchialine caves: first record of the tubeworm *Ficopomatus enigmaticus* (Annelida, Polychaeta)
Zakšek, V.*, Sket, B., Trontelj, P.	Phylogeography of the unique cave tube worm *Marifugia cavatica* (Polychaeta: Serpulidae)
Superficial Subterranean Habitats	
Porter, M. L.* , Culver, D. C., Pipan, T.	Molecular diversity of epikarst copepods from John Friends Cave, Maryland, USA
Rendoš, M.*, Mock, A., Luptáčik, P.	First observation of terrestrial arthropods in superficial subterranean habitats in Slovakia: vertical distribution, seasonal dynamics and temperature
Schmidt, S. I.*, Kreft, J. U., Avramov, M., Griebler, C., Hahn, H. J., Humphreys, W. F.	Is there actually enough (import of) carbon in(to) the groundwater system to support the microbial and faunal numbers that we see?
Systematics and Faunistics	
Esmaeili, S. R.*, Sari, A.	Niphargids of Iran with focus on the Zagros Mountains
Lana, E.*, Isaia, M.	Subterranean arachnids of the Western Italian Alps (Arachnida: Araneae, Opiliones, Palpigradi, Pseudoscorpiones)
Geoffroy, J.-J.*, Iorio, E.	The French soil - and cave-dwelling centipedes (Chilopoda): updated checklist and distribution in mainland France, Corsica and Monaco, with emphasis on subterranean fauna, conservation purposes and regional biodiversity
Komerički, A.*, Ozimec, R.	Faunistic and biogeografic characteristics of the centipedes (Chilopoda) in Croatia with special review on the genus *Eupolybothrus* (Lithobiidae)
Manconi, R.*, Ledda, F. D., Stocchino, G. A., Grafitti, G.	Biogeographic patterns of lithistids (Demospongiae) from Mediterranean marine caves
Manconi, R., Ledda, F. D., Stocchino, G. A., Grafitti, G.*	Is the geographic range of the palaeoendemic sponge *Petrobiona massiliana* (Porifera: Calcarea) restricted to the central-northwestern Mediterranean Sea?
Mori, N.*, Meisch, C., Brancelj, A.	Biodiversity of ostracods (Ostracoda, Crustacea) in groundwater habitats of Slovenia
Oarga, A.*, Schiller, E., Perşoiu, A., Šebela, S., Mulec, J.	Contribution to the ecology of Copepoda in sulphidic karst springs (Žveplenica – Dolenja Trebuša, Slovenia)
Ozimec, R.*, Karaman, I. M., Tulić, U., Pavičević, M., Lukić-Bilela, L.	Biospeleological research of Pećina na Vrelu Mokranjske Miljacke Cave in Bosnia and Herzegovina
Papáč, V.*	Collembolan communities (Hexapoda, Collembola) in karst and basalt caves of central Slovakia (Western Carpathians)
Perreau, M.*, Faille, A.	Advances in the knowledge of subterranean Staphylinidae of Morocco: the genus *Apteranillus* Fairmaire (Staphylinidae, Aleocharinae, Lomechusini)
Sendra, A., Moldovan, O. T., Ballesteros, B. J., Domínguez-Sánchez, J. A., Teruel, S., Urios, G., Jaume, D., Reboleira, A. S. P. S.*, Gilgado, J. D.	Discovery of stygobiotic crustaceans in boreholes at the Deep Jurassic aquifer of El Maestrazgo (S.E. Spain)
Sidorov, D. A.*, Pankov, N., Krasheninnikov, A. B.	A *Bactrurus*-like subterranean amphipod (Crangonyctidae) from the Ural Mountain karst region
Stocchino, G. A.*, Sluys, R., Manconi, R., Casale, A., Marcia, P., Grafitti, G., Cadeddu, B., Corso, G., Pala, M.	Triclads from Italian groundwaters (Platyhelminthes, Tricladida)
Vahid, A.*, Esmaeili, S. R., Ali, F.	Diversity and distribution of subterranean species in karst areas of Iran
Višňovská, Z.	Species diversity and distribution of aquatic Crustacea in caves of Slovakia (Central Europe, Western Carpathians)
Weigand, A. M., Jochum, A.*, Slapnik, R., Klussmann-Kolb, A.	A 21st Century identity for an old snail condemned to darkness – Barcoding *Zospeum* (Pulmonata, Ellobioidea, Carychiidae)
Wessel, A.*, Mühlethaler, R., von Rintelen, K., von Rintelen, T.,	First record of a root community in Southeast Asia: cave-dwelling planthoppers

Opening lecture

Subterranean fauna of the wider Dinaric area – from first discoveries to a global hotspot, and its up-to-date biological evaluation

Boris Sket

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First troglobiotic animals were described from Slovenia (amphibian *Proteus,* Laurenti 1768; beetle *Leptodirus*, Schmidt 1832); freshwater interstitial fauna discovered in Macedonia (Karaman, 1932). Postojna-Planina Cave System remained an arena of research. Some important discoveries: repeated immigrations of a species; morphologically different populations of it within the system; fauna succession and clinal variability within some species along an allogenous subterranean river; deeper invasion of epigean species after organic pollution. Some interesting species will be presented according to their distribution patterns. Interstitial fauna of Black Sea Drainage closely related to that of Adriatic drainage and both different of the Aegean drainage. **European distribution.** *Asellus aquaticus* (Isopoda) and *Synurella ambulans* (Amphipoda: Crangonyctidae) with some troglobiotic races in Dinaric karst (DK). Genus *Niphargus* (Amphipoda: Niphargidae) with over 110 species in DK. **Transdinaric distribution** (from DK to E and W). *Troglocaris* (Decapoda: Atyidae) with members in Caucasus, *Sphaeromides* (Isopoda: Cirolanidae) with members in Bulgaria, French species of both not related to Dinaric ones. *Zospeum* (Gastropoda: Carychiidae) Dinarides – Pyrenees; *Delaya bureschi* (Oligochaeta: Haplotaxidae) Slovenia – Bulgaria; *Monolistra* (Isopoda: Sphaeromatidae), DK and Southern Calacareous Alps. **Holodinaric pattern** (between Kras-Carso and SE Hercegovina). Subgenus *Troglocaris* s.str., *Marifugia 'cavatica'* (Polychaeta: Serpulidae), *Proteus 'anguinus'*, genus *Titanethes* (Isopoda: Trichoniscidae), *Velkovrhia enigmatica* (Cnidaria: Bougainvilliidae). All molecularly studied elements exhibit splitting into races or species within the area. Complementary **NW and SE merodinaric patterns** best represented by geographically vicariant genera of Coleoptera, also of hydrobioid Gastropoda and others; leptodiroid habitus developed only in DK. **Paralittoral merodinaric pattern** along NE Adriatic coast, but its elements absent in the Kvarner Golf, i.e. historically grounded. Here originated some of the first ecological data on anchihaline caves: presence of a disoxic layer; presence of troglobionts in illuminated layers if surface competitors are absent; withdrawal of predators of tender Thermosbaenacea into the disoxic layer. **Narrower endemic distribution patterns.** Seldom species bound to recent river drainages, like

Microlistra spp. (Isopoda: Sphaeromatidae); distribution of the related *Monolistra coeca* and most others supposed to be defined by paleodrainages. *Niphargobates orophobata* (Amphipoda: Niphargidae) known only from one point. Narrow areas also: the sponge *Eunapius subterraneus* (Porifera: Spongillidae), bizzare leech *Croatobranchus mestrovi* (Hirudinea: Erpobdellidae), an undescribed terrestrial planarian etc. Rich is the fauna of epizoic Turbellaria Temnocephalida. The troglobiotic **species density** probably the highest in the world. Up to 21 terrestrial Coleoptera species in a 20 x 20 km area. For 20.000 km^2 of Slovenia, 241 aquatic and 189 terrestrial troglobionts registered. For Postojna-Planina Cave System alltogether 99 species. New species are still being found; a non-troglomorphic race of *Proteus* found only in 1990s; it is very instructive with its being troglobiotic and by its position in the phylogenetic tree. The interstitial and karst underground fauna is **endangered** from the surface: by hydrotechnical works, by industrial, urbane, agrotechnical pollution. Extremely rich *Proteus* populations were destroyed by pollution, unique huge *Marifugia* and *Congeria* colonies by regulating the surface river beds. Protection is efficient only against innocuous researchers.

SYMPOSIA

1. Superficial Subterranean Habitats

Oral presentation - invited plenary lecture:

Shallow subterranean habitats – gateway to the subterranean realm

David C. Culver* [1], Tanja Pipan [2]

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[2] Karst Research Institute ZRC-SAZU, Titov trg 2, 6230 Postojna, Slovenia

We define shallow subterranean habitats (SSHs) as aphotic environments within 10 m of the surface and with species limited to subterranean environments. We review four such habitats—shallow interstitial habitats, seeps (hypotelminorheic), epikarst, and milieu souterrain superficiel (MSS). For each habitat type, we review information on environmental variability (especially detailed temporal temperature profiles), species composition, and general aspects of morphology of stygobionts and troglobionts in the habitat. The sites reviewed showed temperature variation throughout the year although variation was less than that of surface sites. Many showed seasonal and daily variation as well. Epikarst drips were the least variable and seeps the most variable. Numbers of troglobiotic and stygobiotic species in SSHs ranged from seven in seeps near Washington, DC and MSS sites in southern France to 14 in epikarst drips in Županova jama in Slovenia. Most SSH sites also had species apparently specialized for these habitat types, as well as generalist species. An analysis of the subterranean amphipod genus *Stygobromus* indicated that species from epikarst and seep sites showed no differences in the level of troglomorphy compared to cave species in the same lineages. These results suggest that the primary selective factor in the evolution of troglomorphy is darkness, not lack of food or seasonality. SSHs hold considerable promise as repositories of subterranean biodiversity and as evolutionary laboratories for the study of adaptation.

Oral presentation:

Population dynamics of the stygobiotic amphipod crustacean *Stygobromus tenuis potomacus* and isopod crustacean *Caecidotea kenki* at a single hypotelminorheic habitat over a two-year span

Daniel W. Fong*, Karen E. Kavanaugh

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We monitored the population size of the stygobiotic amphipod, *Stygobromus tenuis potomacus*, and of the stygobiotic isopod, *Caecidotea kenki*, at the resurgence of a

hypotelminorheic habitat for two years at an average sampling interval of 10 days. Surface abundance of *S. tenuis* ranged from zero to 35 around a mean of 12.02, while *C. kenki* ranged from 10 to 101 around a mean of 44.86. Water temperature varied from 9.4 to 17.8 °C about a mean of 14.15 °C, while depth varied from 2 to 14 mm about a mean of 6.10 mm. Temperature was uncorrelated with depth. Abundances of the two species were also uncorrelated. Abundance of *S. tenuis* showed a significant decrease with increasing temperature (R^2 = 0.51), and a weak increase with increasing depth but a decrease beyond a depth of 8 mm (R^2 = 0.23). Abundance of *C. kenki* showed a weak increase with increasing temperature with a decrease beyond 15 °C (R^2 = 0.28), and no dependence on depth (R^2 = 0.03). We conclude that *C. kenki* is a specialist of the surface habitat immediately adjacent to the resurgence of hypotelminorheic water, and that *S. tenuis* is adapted to the colder subterranean water of the hypotelminorheic. When surface temperature is low, *S. tenuis* may actively move to the surface to forage. We suggest that this movement may be associated with lower temperatures because at higher temperatures the quality and quantity of resources may be insufficient to offset metabolic losses.

Oral presentation:

Life history traits of the epigean populations of *Niphargus dalmatinus* (Crustacea: Amphipoda) along the Cetina River, Croatia

Sanja Gottstein* [1], Krešimir Žganec [1], Vesna Cetin Krnjević [2], Aleksandar Popijač [1]

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Very little is known about the epigean niphargid autecology. The aim of this study was to establish the life history traits of the endemic amphipod *Niphargus dalmatinus*, which regularly inhabits springs, epigean streams and rivers in Middle Dalmatia. Three replicate samples were collected once a month with benthos net in the period from August 2004 to August 2005 at ten study sites located in the upper, middle and lower reaches of the Cetina River and its tributaries. The highest population density of the species was recorded on two study sites in hypocrenal zone, where the mean water temperature reached 9.7°C with the narrow range of 7.3-12.5 °C. Ovigerous females were recorded year-round at the main spring and in the upper course, but were more numerous during spring (March, April, May) and autumn (October) months. The sex-ratio (males vs. females) was in favour of males almost throughout the all study year. Mean number of eggs was 30 for 100 analysed females, but one female carried a

maximum of 111 eggs. There was no correlation between fecundity and female body size (total body length). A better insight into the life history strategy of *N. dalmatinus* will help to interpret the distribution patterns, population structures, and the coexistence with other crustacean species.

Oral presentation:

What is groundwater? A new approach, and what this means to fauna

Hans Jürgen Hahn* [1], Jörg Bork [1], Susanne I. Schmidt [2]

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On the 18th International Symposium on Biospeleology in Cluj, we asked the question, whether all water that is called "groundwater" is real groundwater. Following a hydrological approach, we concluded that much of the so-called groundwater is actually hyporheic water. Furthermore, we argued for distinguishing subsurface water by the origin of the organic matter – either from the saturated or from the unsaturated zone. In the last years, we modified this idea and applied it to several data sets from Korea and Germany. The results are promising, indicating that groundwater communities at these sites 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). However, there are many gaps of knowledge and open questions left, which should be discussed along this presentation.

Oral presentation - invited plenary lecture:

Groundwater calcretes: sheets of subterranean habitat scattered on an Archaean landscape

William Humphreys* [1], Michelle Guzik [2], Tessa Bradford [2], Steve Cooper [2, 3], Remko Leijs [3, 4], Chris Watts [3], Andrew Austin [2]

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[2] Australian Centre for Evolutionary Biology and Biodiversity, School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, SA 5005, Australia
[3] South Australian Museum, North Terrace, Adelaide, SA 5000, Australia
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Groundwater (phreatic) calcretes are the main, often the only, habitat for aquatic troglobionts through much of arid Australia. Originally considered as homogeneous habitats, calcretes are proving to be complex in structure and

enigmatic in origin and timing. Essentially, they form thin patches of suitable troglobiont habitat overlaying the Archaean shield in a climatically challenging region largely lacking surface water and where they support diverse aquatic troglobiont communities. The aquatic troglobionts include Gondwanan elements (e.g., Spelaeogriphacea and some Parabathynellidae and Candoninae) as well as late Tertiary colonisers (e.g., Dytiscidae, Oniscidea). Groundwater flow in the Yilgarn is confined to broad palaeodrainage systems incised in the Archaean basement which forms the watersheds. This restricts groundwater connectivity between catchments but may not eliminate faunal exchange owing to the low gradients in the landscape. The calcretes were deposited as thin sheets, generally ~10-20 m thick, from groundwater near the base level salt lakes (playas). The southern part of the Western Shield comprises the Yilgarn where six major palaeovalleys are beaded with salt lakes and their associated groundwater calcretes that each form a geologically discrete unit. Biologically the calcretes form subterranean islands in the desert. The stygiobionts are mostly endemic each to a single calcrete irrespective of whether the species may be air breathing (Dytiscidae), or not (Amphipoda), or whether they are interstial (Parabathynellidae). The groundwater between the calcretes occurs in a matrix unsuitable for aquatic troglobionts. Chemical sedimentation of calcretes from groundwater flow progresses through the combined effects of evaporative concentration, groundwater level fluctuations and selective ion-exchange reactions. Together, these result in complex porosity and permeability zones that may account for the fine scale phylogeography of the aquatic troglobionts. Gene flow does occur through the length of a single large calcrete (scale 10^4 m) although the populations are not panmictic. No detailed fine scale hydrology has been conducted on calcretes but molecular genetic research reveals fine-scale differentiation with isolation by distance that supports the presence of heterogeneous subterranean landscape even within small spatial scales (10^2-10^3 m), and that different species of aquatic troglobionts, even of sympatric sister species, do not have a genetically concordant response to this spatial heterogeneity.

Oral presentation:

Mesovoid shallow substratum (MSS)

Christian Juberthie

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The MSS was discovered by Juberthie et al. (1980, 1981) in the French Pyrenees, then in 1981 in Carpathian Mountains in Romania (Juberthie et al.). At the same time Ueno described in Japan the Upper Hypogean Habitat. In 1986, Oromi et al. described from Canary Islands a Volcanic MSS type found also in Hawaii by Howarth. The MSS is present in mountains of the temperate zone. The MSS is

recorded at least from: Canary Islands, Spain, France, Italy, Austria, Czech Republic, Slovenia, Romania, Bulgaria, Greece, Turkey, Japan, Taiwan, China, Hawaii. The MSS is located beneath the last mineral horizon of soil and the compact bedrock. The more frequent MSS is composed of a network of small voids in screes covered by soil. When a soil has covered the scree, a climatic subterranean habitat, similar to the climate of caves is generated and a new MSS is available for colonization. The difference consists in the greater range of seasonal temperature variations. In karstic areas the MSS lies at the foot of carbonated cliffs; it can be connected with caves. The second type is very superficial cracks of the rocks, covered by soil. The third type is the Volcanic MSS in scoriaceous layers or in some types of lava flow, or in combination of the first and the second. The MSS extends the subterranean habitat. It is present in karstic areas but mainly in other types of rocks, without caves. Trophic resources consist in organic matters introduced by meteoric waters, and soil animals which penetrate passively or actively. Two fauna communities inhabit the MSS: one specific to the MSS, and the other composed of selected soil dwellers. The specific community is composed of troglophile and troglobiotic species, the same as in caves, or specific to MSS. Dominant group, the Coleoptera Trechinae and Leptodirinae: around 120 troglobitic species, and 46 genera. Also found are: Isopoda, Pseudoscorpiones, Araneae, Chilopoda, Diplopoda, Collembola, Campodea, Blattaria, Orthoptera, Diptera. In Pyrenees, a scree on a slope of a glacial valley was formed at the end of the last glacial period, from 24000 to 12000 BP, the genesis of the MSS began 12000-13000 years ago when climate changed, and a soil covered the scree. It was colonized by Coleoptera *Aphaenops* and *Speonomus* migrating from small populations surviving in limestone caves in the neighbouring karstic massif. The MSS is really a permanent subterranean habitat similar to caves.

Oral presentation:

Particulate organic matter breakdown in shallow interstitial habitat of a rural stream

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Particulate organic matter is the major source of energy for most low-order streams, but a large part of this litter is buried within bed sediment during floods and thus become poorly available for benthic food webs. The fate of this buried litter is little studied. We tested two methods to study litter breakdown: large litter bags (15 x 15 cm) filled with *Alnus glutinosa* leaves buried with a shovel at 20 cm

deep and metallic cylinders (1.5 x 8 cm) pushed at 20 cm deep inside the river sediment using a mobile mini-piezometer. Bags and cylinders were retired of the sediment after 7, 14, 28, 53 days. We tested these two methods in six stations within different land-use contexts (from forest to intensive agriculture) and with different sediment grain sizes. Breakdown rates were slightly different between the two methods. In the large bags, k varied between 0.0011 and 0.0188 d^{-1} (i.e. 32% to 62% of biodegradation). In the cylinders, k varied from 0.0015 and 0.0049 d^{-1} (i.e. 24% to 42%). Breakdown rates measured with large litter bags were negatively correlated with a decrease in oxygen concentrations between surface and buried bags and positively correlated with both the percentage of coarse particles (20-40 mm) in the sediment and benthic macroinvertebrate richness. Breakdown rates measured in the cylinders were correlated with the land-use around the station and the concentrations in nutrient in the river. In conclusion, (i) the cylinder method integrates large scale ecological characteristics of the river rather than local feature of the sediments and (ii) the vertical exchanges between surface and hyporheic water play a crucial role in litter breakdown and organic matter recycling.

Oral presentation:

Heterogeneous copepod distribution in different groundwater habitats from northwestern Romania

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The mixture of soil, epikarstic and hypogean fauna, as inputs and outputs of the vadose zone offers the possibility to understand the complex structure of this heterogeneous ecosystem, by studying the structure of its populations from two different habitats: fissures network and pools. Relationships between the copepod assemblages and the habitat characteristics from the vadose zone in caves from the Pădurea Craiului Mountains (northwestern Romania) are emphasized based on nine environmental parameters. The aims of the present research are to: 1. identify the dissimilarities between copepod communities from the vadose zone within and between caves and in the two different habitats: voids and pools; 2. depict spatial and temporal trends in heterogeneous copepod distribution in drips and pools along a vertical gradient in the vadose zone, in relationship to the environmental features at small spatial scale. Canonical Correspondence Analysis was used to

explore the relationship between the copepod species and the environmental features. Vegetation cover was the most important factor influencing copepod diversity and abundance. Precipitation and the amount of drips were related to epigean species, while the electrical conductivity seemed to be related indirectly to hypogean species. Pools on limestone harbored a more diverse and abundant fauna than those on clay; the hypogean species prefer mainly the pools on limestone. Genetic analyses are in progress, extraction and PCR protocols are optimized for harpacticoid populations.

Oral presentation:

Candidates for superficial subterranean habitats and epikarst among terrestrial cave inhabiting species

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Since 1977, sixty-two (62) caves and artificial tunnels, mostly in central and northern Slovenia, have been systematically investigated for their environmental characteristics and terrestrial faunas. Among more than 450 species found, two species groups outstand for their disagreement with the general statement that, in accordance with their adaptation to the hypogean environments, organisms progressively exhibit troglomorphoses from shallow towards deep habitats. The first such group is represented by a dozen of troglomorphic species which are rare in caves, thus these are not their preferable habitats. The second one consists of a few troglophilic species with a moderate troglomorphic appearance, which found stable and relatively abundant populations also in some places deep inside caves. Both groups are assumed to enter caves either from stone and gravel accumulations, like the superficial subterranean habitats (SSH) or from the epikarst. In this contribution we present the way of detecting the two origins of such species with respect to their distributional pattern within caves. For this purpose we used the following three groups of data collected in the field and in the laboratory. 1) The distances from the entrance and from the surface, respectively, indicating the general distribution pattern within the upper few tens of meter within the subterranean domain. 2) The presence of populations deep inside some larger caves providing evidence of the ability of a species to live in these habitats and/or, in case of inhabiting various cave sections, its relatively euryecious response within the range of various hypogean habitats. 3) A moderate cold-hardiness to temperatures below –3 °C serving as a measure of estimating either a temporary contact of a species with freezing habitats or its incomplete adaptation to deep, meteorologically stable hypogean habitats with temperatures constantly above 0°C. We first analyze the general types of distribution of 18 dominant species within

the investigated caves addressing to the interpretation of their preferred habitats. We comment on the presence of troglomorphic and some other species in the caves.

Oral presentation:

Diversity and allopatric distribution in the Canarian MSS: a case study in an old island

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The terrestrial hypogean fauna in the Canary Islands had been studied mainly in the four western islands, with relevant results on its high diversity and island speciation. Conversely, in the eastern islands this subject had been hardly accomplished because of the apparent worse conditions of their caves, either very dry (Lanzarote and Fuerteventura) or very scarce (Gran Canaria). Indeed, only 5 among the 124 adapted species so far know in the archipelago were described from the eastern islands, three of them from Gran Canaria. Our current sampling in the MSS of Gran Canaria has shown that the absence of caves does not imply an extremely poor fauna. A special type of pitfall traps has been designed for sampling the MSS continuously for a long time, with minimal disturbance of the habitat once the system is stabilized. 12 traps distributed in 6 different locations have been working during the last five years with some intervals, and have shown an unexpected diversity of subterranean arthropods. Pseudoscorpions have resulted particularly diverse in the MSS with 18 species, six of them new to science and five of them being more or less troglomorphic. These species have an allopatric distribution within the island, probably due to geological boundaries that have favoured local speciation. A similar but less strict allopatric distribution has been found in different species of the genera *Oromia* (Col., Curculionidae) and *Symploce* (Blat., Blattellidae). As a whole, the subterranean species from Gran Canaria have a lesser degree of troglomorphism than their vicariants in the younger island of Tenerife, either belonging to the same or to closely related genera.

Oral presentation:

Ecological studies of an epikarst community in Alpine cave Snežna jama na planini Arto: preliminary results

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The subterranean fauna in Alpine and Pre-Alpine caves has been little studied. Therefore we investigated the epikarst fauna from caves which are found in higher elevations in the Slovenian Alps and in the Italian Prealps and we compared these results with findings from Slovenian Dinaric karst. In the Alpine ice cave, Snežna jama na planini Arto, in north central Slovenia, five sampling sites were monitored for fauna and physical and chemical parameters in percolation water in a period of one year. Temperature, conductivity, discharge and pH in water were measured in monthly sampling. DOC, total hardness and concentration of various ions (calcium, chloride, nitrite, sulphate and phosphate) were determined at less frequent intervals. The entrance of the cave is covered by ice and the temperature inside the cave never exceeds 4 °C due to alpine climate and high elevation. These environmental conditions are reflected in the fauna. Sampling sites chosen in the permanent ice, were without fauna. Striking results were from the sampling site in the area with well developed moonmilk, where we would expect more diverse fauna due to the microbial communities of moonmilk. However, the fauna was impoverished. In other sampling sites invertebrates from seven different taxonomic groups were found, including abundant Copepoda and Amphipoda.

Oral presentation:

Organic carbon in aquatic shallow subterranean habitats

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Organic carbon is likely to be an important limiting factor in shallow subterranean habitats (SSHs). Data on dissolved organic carbon (DOC) for interstitial, epikarst, and hypotelminorheic habitats are reviewed. The best studied of these is the epikarst. In Organ Cave, West Virginia (U.S.A.), DOC in epikarst drips averaged 1.10 ± 0.15 mg C/L over the course of the year. In Postojna Planina Cave System, Slovenia, DOC in epikarst drips averaged 0.70 ± 0.04 mg C/L over the course of the year. While this is at least five times lower in concentration than water entering the caves through sinking streams, it plays a vital role because it is more

ubiquitous in the caves and forms the basis for the biofilm. Specific UV absorbance (SUVA) at 254 nm, an estimate of aromatic C content and an indicator of dissolved organic matter composition, was significantly lower in drips than in sinking streams and cave streams. In studies of the Rhône and its tributaries, Marmonier et al. report DOC values averaging between 1.9 and 3.5 mg C/L. Lower values were reported for smaller streams, increased depth, and increased lateral distance from the river. For the first time, we report on values for hypotelminorheic habitats, which average 3 mg C/L.

Poster presentation:

Molecular diversity of epikarst copepods from John Friends Cave, Maryland, USA

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Epikarst habitats are ecologically important reservoirs of stygobiotic fauna. While a number of studies have investigated the biodiversity of these habitats, few have employed molecular tools. In this study, we investigated the molecular diversity of epikarst copepods from John Friends Cave, Maryland USA. Previous studies of epikarst copepod biodiversity from this cave identified eight species. Copepods from dripwaters in 6 different locations throughout the cave were collected in September 2008 and preserved in 100% ethanol. In order to investigate the molecular diversity found in the epikarst habitat of this cave, individual copepods were used to PCR amplify a ~650bp region of the mitochondrial gene cytochrome oxidase I (COI). Sequences were obtained from 37 individuals, representing five different drips within the cave. Based on sequence similarity, the individuals analyzed represent three different species. In all cases, sequences from a single species were >98% similar, while sequence similarities among the three species ranged from 66-78%. Based on sequences available in public databases (e.g. GenBank), two of the species are most closely related to harpacticoids from the family Cletopsyllidae (86%), while the third species is represented by a single sequence that is most closely related to cyclopoids from the family Cyclopidae (88%). Among the harpacticoids sampled so far, one of the species was found in 4 of the 5 drips and the second in 2 of 5 drips. The ability to use molecular tools to identify the copepod diversity within a drip offers the potential for long term monitoring of epikarst fauna and the tools for investigating the connectivity of the epikarst habitat.

Poster presentation:

First observation of terrestrial arthropods in superficial subterranean habitats in Slovakia: vertical distribution, seasonal dynamics and temperature

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After some studies aimed on the ecology of superficial subterranean habitats (MSS) realised in other parts of Europe we used series of pitfall traps plugged in 110 cm plastic tubes for investigation of subterranean invertebrate communities in the Čierna hora Mts., Western Carpathians. The study plot was situated in steep limestone scree slopes covered by linden-maple forest in the valley Malý Ružínok (NNR Sivec) about 500 m a. s. l. Three tubes with 10 traps each placed in the depth from 5 to 95 cm (every 10 cm) under the surface were installed here during one year (Sept. 2008-Nov. 2009). The traps with 4% formalin were checked monthly. Temperature was recorded continually by dataloggers along all tubes. Sampled fauna was counted and identified on the species or on the higher taxa levels. More then 26,000 individuals were found: arthropods and a few specimens of gastropods and earthworms. Eudominant Collembola (67.5%) were followed by Acarina (15.5%), insect larvae (7.5%), Diptera (5.1%) and Coleoptera (1.2%), all the groups were captured along the entire depth gradient. Opiliones (3 spp.), Oniscidea (5 spp.), Diplopoda (9 spp.), Chilopoda (6 spp.) and Formicoidea (3 spp.) were studied in detail. The majority of the representatives live on or closely under the surface here. But we found also rare subterranean taxa (isopod *Mesoniscus graniger*, millipede *Mecogonopodium carpathicum*). The arthropods from other groups were infrequent and mostly at the surface, but some of them were living also deeper (e.g. Aphidinea or Hymenoptera) feeding on tree roots or as carnivores. A high degree of similarity of arthropod communities in caves and MSS promises good possibilities to collect rare cavernicoles in MSS. Such type of MSS is important as refugium for relic fauna. Animals with large body, or those more sensitive to gently unstable microclimate, or those with low competition ability are not dwelling in MSS. The depth of the trap is not crucial, the habitat has specific climate regime almost up to the surface here. Activity of invertebrates is forced by seasonal climate changes and for faunistic studies the end of spring time is the most convenient period. But it is not interrupted during winter or summer. Dynamic microclimate without extremes of the above surface atmosphere is more stable deeper in the debris. The study was supported by the grant Vega 1/0139/09.

Oral presentation:

Central European spiders adapted to life in subterranean habitats

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Many species of macroarthropods have colonized various types of subterranean habitats. These are, for example, voids in soil layers, clastic river and slope sediments, stony accumulations, young volcanic deposits, old sedimentary and metamorphic bedrock, lava tubes in consolidated lava flows, and typical pseudokarst and karst caves. Morphological adaptations of arthropods to life in subterranean habitats can be subdivided into edaphomorphisms and troglomorphisms. Edaphomorphisms, i.e., adaptations to life in subsurface interior voids in soil are usually manifested as body diminishing and sometimes also vermiform elongation, shortening of appendages, reduction or rearrangement of chaetotaxy and sensory organs. In contrast, troglomorphisms, i.e., adaptations to life in relatively large spaces, are characteristic by elongation of appendages, and hypertrophy of chaetotaxy and sensory organs. Depigmentation, desclerotization, and reduction of eyes are common for both these groups of adaptations. In Central Europe, we register some of these adaptations in eighteen species of spiders, and eight of them are representatives of the genus *Porrhomma*. They inhabit leaf litter, ant's nests, deep soil layers, void systems under soil surface, scree voids, and caves. Some of them are specialised to only one exclusive type of subterranean habitat, in contrast some others were recorded in several types of subterranean habitats. *Bathyphantes eumenis buchari* inhabits exclusively deep scree layers. *Porrhomma profundum* was recorded exclusively in caves. *Porrhomma microps* was recorded in leaf litter, deep soil layers and caves. *Porrhomma myops* has edaphomorphic populations in voids of deep soil layers, and troglomorphic populations in scree voids and caves. Hotspots of subterranean biodiversity, such as Postojna–Planina Cave System, harbour highly specialized, fascinating creatures that we can encounter at the end of their long-term subterranean evolution. On the contrary, temperate latitudes of the northern hemisphere lying in the former Pleistocene periglacial zone harbour invertebrates at the very beginning of their underground evolution. These subterranean habitats are natural laboratories in which we can study early phases of underground evolution of troglobionts.

Poster presentation:

Is there actually enough (import of) carbon in(to) the groundwater system to support the microbial and faunal numbers that we see?

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Groundwater fauna and microorganisms (both prokaryotic and eukaryotic) use organic material, dead or alive, as food source. The groundwater food web is most likely bottom-up regulated since resources are usually so low that they are probably limiting, and in many cases resources might be sparse enough to only support just the lowest trophic level, namely microorganisms. This might be part of the explanation why fauna occurs in such a patchy pattern. The system being probably bottom up-regulated also raises other questions, such as: how much resource is needed to cover at least the maintenance energy in microbes, then how much resource is needed for microbial growth, and how much microbial growth is needed for protists and for sediment fauna to establish populations? And how much resource leads to which complexity of the food web? We tackle these questions first by testing whether our data contradict or support the hypothesis of the 10% energy efficiency ratio between subsequent trophic levels in groundwater. We chose one of the very few field sites from which detailed faunal and microbiological data are available, the Rur/Erft site described recently by Stein et al. in 2010, to test this hypothesis.

SYMPOSIA

2. Patterns and Processes in Subterranean Biodiversity

Oral presentation:

Data analysis of spatial distribution of cave terrestrial isopods (Isopoda: Oniscidea) in Croatia

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This paper presents data analysis of spatial distribution of cave terrestrial isopods in Croatia. Data were analyzed through UTM grid map of Croatia (10 x 10 km) and macroregions according to Roglić, 1974, taking into account endemics and adaptation to cave habitats.

In Croatia 129 species and 32 subspecies of terrestrial isopods have been recorded to date, 58 species and 18 subspecies being endemic to Croatia. More than 9000 caves have been registered in Croatia, but only for 211 caves (~2.3%), and 14 different habitat types literature data were gathered. Altogether, with data from collections, this paper presents records from 502 (~5.6%) caves, 5 mines and 19 different habitat types. Among cave taxa, 34 species and 7 subspecies have been registered, belonging to five families and six subfamilies. The family Trichoniscidae and subfamily Trichoniscinae are the most abundant. Out of 22 Croatian endemic taxa 13 (59%) belong to the subfamily Trichoniscinae. Out of 20 troglobitic species, 15 (75%) belong to the subfamily Trichoniscinae. The most represented seemed to be the genus *Alpioniscus* with 10 species, followed by the genus *Androniscus* with 9 taxa. Out of 814 Croatian 10x10 km UTM squares only 149 (~16 %) have one or more cave species of terrestrial isopods and most of them are scattered all over the Dinaric region in Croatia. The UTM plot VL41 presents extreme with 6 species, belonging to the Northern Croatian Littoral macroregion. The UTM plots BN71, YH03 and YH13 have 5 species, belonging to the Southern Croatian Littoral macroregion. Out of 41 cave taxa in Croatia the Southern Croatian Littoral macroregion has 27 (~66%) and the Northern Croatian Littoral macroregion has 14 (~34%). Out of 22 endemic cave taxa in Croatia, the Southern Croatian Littoral mesoregion has 15 (~68%), and among them, 2 are widespread and 14 are endemic to that region. Out of 79 Croatian islands and 523 small islands there are records of terrestrial isopods for 9 islands. Seven islands have their own endemic species (10 species). The island of Mljet presents the extreme with 3 endemic species. Out of 41 cave taxa in Croatia, all are distributed in the Dinaric karst area, with just 3 troglophilic species distributed outside the Dinaric karst area. There are no Croatian endemic taxa outside the Dinaric karst area.

Oral presentation:

Recording the stygofauna of the federal state of Sachsen-Anhalt, Germany

Sven E. Berkhoff* [1], Dirk Matzke [2], Andreas Fuchs [1], Jörg Bork [1], Hans Jürgen Hahn [1]

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In 2008 and 2009 stygofauna of the federal state Saxony-Anhalt was sampled from a total of 78 representative monitoring wells. The goal of this study was an initial survey of the groundwater fauna. Additionally, distribution patterns of fauna and their relations to biogeographic, geological and hydro-chemical particularities were analysed. Faunal distribution patterns were evaluated at three different spatial scale levels (biogeographic level, landscape level, site specific level). The large scale distribution patterns of fauna were clearly influenced by biogeography. At landscape level, the major natural geographic units and the "Georegs" (combination of major natural geographic units and aquifer types) were well reflected by the groundwater fauna. On the local scale, the hydrologic exchange, in particular the influence of surface water to groundwater was identified as a crucial factor for the composition of groundwater communities.

Oral presentation:

Nine years of long-term stygofauna monitoring in Southwest Germany

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Since 2001, groundwater fauna as well as microbiological and hydro chemical data are being sampled annually from 43 groundwater bores in Baden-Württemberg, Southwest Germany. Faunal and abiotic data provide long-term information on the ecological conditions of the bores investigated. It seems that changes in faunal communities are related to changes in quite different abiotic environmental parameters, indicating that changes in faunal communities allow for inferences on environmental changes or stability. The use of stygofauna thus offers interesting perspectives as an additional tool for long-term groundwater monitoring. With

this background, we will present some general results, together with several examples both for stable and unstable wells.

Oral presentation:

Troglofauna in the Pilbara region, Western Australia – Patterns in diversity and distribution, and sampling considerations for conservation assessment

Stefan Eberhard*, Nick Stevens, Giulia Perina, Peter Bell

Subterranean Ecology, Scientific Environmental Services, Unit 8/37 Cedric Street, Stirling, 6021, Western Australia

Discovery of diverse terrestrial invertebrate assemblages in subterranean habitats associated with iron-ore bearing rocks in the Pilbara region has driven a spate of troglofauna surveys as part of pre-mining environmental impact assessment. We present the results from recent surveys undertaken by mining companies which contribute new understanding towards this remarkable hidden element of biodiversity in Western Australia's arid regions. Patterns in the systematic composition, species richness and abundance of troglofauna assemblages collected from mining exploration drill holes are described. The systematic composition of the assemblages includes arachnids (Araneae, Pseudoscorpionida, Schizomida, Palpigrada), insects (Diplura, Thysanura, Coleoptera, Hemiptera, Blattodea), myriapods (Diplopoda, Chilopoda, Symphyla, Pauropoda) and crustaceans (Isopoda). Species distribution patterns, which ranged from regionally widespread to highly localised short-range endemics, were not necessarily concordant with geologic habitat discontinuities. Among the taxa which exhibited morphological modifications to subterranean life (troglomorphy), such as loss of eyes and pigment and elongation of appendages, their degree of specialization varied, and a proportion of troglomorphic taxa were more typically associated with soil, plant roots or leaf litter, as opposed to deep subterranean habitats. The emerging patterns and characteristics of the subterranean assemblages have important ramifications for interpretation of ecological survey data, and the conservation assessment of 'troglofauna'. We identify some key issues involved with survey and assessment of troglofauna, and highlight future challenges in this rapidly developing research field.

Oral presentation:

Review of the subterranean biodiversity of the Nullarbor plain, Southern Australia

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The Nullarbor Plain covers an area over 200000 km^2 and is one of the largest areas of continuous exposed karst in the world. Scientific documentation of the caves and biological collections commenced in the late 19th century although much of this earlier information on caves and cave fauna has remained scattered in scientific journals, unpublished reports, museum collections, speleological databases and private records. This has hindered integration and coherent assessment of the region's karstic and subterranean biodiversity values, needed for informed conservation management of this highly significant karst area. The purpose of this study was to compile an inventory of caves and karst features, and develop a preliminary characterization of subterranean biodiversity values, including knowledge gaps and future research needs. Presently, more than 687 caves and nearly 3000 other karst features (dolines, blowholes, rock shelters, etc) have been recorded, of which approximately 200 have had biological collections. The compiled database of biological collections comprised nearly 2000 occurrence records of 309 provisional taxa belonging to 134 families. Invertebrates comprised 90% of these records, with bats and birds representing the remainder. The most well represented invertebrate taxa were arachnids (157 taxa), followed by insects, crustaceans, and myriapods. The overall taxonomic resolution was low, with less than one-half (49%) of the fauna identified to species level, however, the obligate subterranean fauna known to date comprises at least 26 species in 19 genera. Stygobionts are conspicuously absent from most Nullarbor caves despite the presence of large saline lakes in about a dozen caves. Stygobionts with marine affinities have been recorded from caves on the Roe Plain, a portion of the Nullarbor karst which was subject to a marine transgression in the Pliocene-Early Pleistocene. To assist with setting conservation priorities, the caves were assigned a preliminary biological importance ranking based on a combination of obligate species richness, total species richness, and cave length. This study identified major gaps in taxonomic knowledge, geographic sampling coverage, and reservation status for biologically important caves, and highlighted the need for further systematic surveys.

Oral presentation:

Distribution patterns of different groups of troglofauna in the Pilbara region, Western Australia: are arachnids the most restricted troglofauna?

Stuart Halse

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The Pilbara region of Western Australia hosts very diverse subterranean communities under its arid surface. Recent sampling has shown that the diversity of troglofauna in the region matches that already documented for stygofauna and that the region truly is a subterranean fauna hotspot. Groups such as schizomids seem to be particularly diverse but cockroaches, beetles, bugs, silverfish, bristletails, pauropods, spiders, pseudoscorpions, palpigrads, centipeds and millipedes are also represented by many species. Sampling to date has been focused in hard rock geologies, where troglofauna inhabit the crevices created by weathering. One of the outstanding characteristics of troglofauna occurrence in the Pilbara is that almost all species are found in the subterranean landscape mosaic, rather than in caves. However, rather than being a unique pattern of occurrence, this may be a general pattern globally, with the focus on caves and subterranean fauna reflecting ease of access rather than unique occurrence. One implication of troglofauna species occurring in the landscape matrix rather than caves is that, where the matrix is a widespread habitat, at least some troglofauna species might be expected to be widespread also. Recent surveys in the Pilbara have provided the opportunity to test this hypothesis and showed that some species are, indeed, widespread. However, species ranges appear to be at least as strongly influenced by phylogeny as by distribution of their habitats, with insect groups usually more widespread than arachnids. A cautionary note in relation to determining species ranges is that defining what constitutes a troglofauna species is often difficult because DNA divergence between populations of the same subterranean species may be very high compared with the patterns in related surface species, which are usually used as abenchmark. Region-scale sampling, however, provides a much better basis for interpreting genetic and morphological variation than site-specific work. One important challenge for troglofauna survey work is to devise efficient methods of sampling alluvium and softer substrates at depth. Only after this has been done are we likely to develop a proper understanding of troglofauna distributions.

Poster presentation:

Effects of glaciation on the distribution of troglomorphic biota in Ohio, USA

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Glaciation events are thought to have impacted cavernicoles by displacing, extirpating, or, in the case of stygobionts, possibly expanding species' habitats exponentially and allowing for larger geographic ranges. The distribution of cave-adapted fauna in Ohio offers evidence for this theory. Glaciers covered approximately two-thirds of Ohio during the Wisconsin ice-age and their boundaries are well-documented, covering both carbonate and non-carbonate (sandstone, conglomerate) cave-bearing bedrock. Recent investigations in the caves and springs of Ohio have resulted in a better understanding of the distribution and species of cave-adapted fauna inhabiting the subterranean environs of the state. More than 260 caves and springs in 31 counties in Ohio were surveyed for biota between 2007 and 2009, specimens were preserved in 70% ethyl alcohol (95% for arachnids), and subsequently identified. Combining this information with data from previous collections, fifteen cave-adapted species were identified from Ohio: eight troglobionts and seven stygobionts. Troglobionts are represented by four species of carabid beetles, three species of arachnids, and two pseudoscorpions. Stygobionts include four species of amphipods and three species of isopods. Of these troglomorphs, 11 are endemic to Ohio and seven are new to science. Most cave-adapted species were found in isolated populations (site-specific endemics and often only a single cave-adapted species per cave). However, up to four species were found syntopically. Although troglobiotic species were more numerous, stygobiotic species had a greater distribution as has been documented elsewhere in the United States (eight troglobionts found in nine caves in six counties vs. seven stygobionts found in 22 caves in eight counties). Troglobionts were confined to counties contiguous with unglaciated counties (found not more than 58.77 km from most recent glacial boundary (Wisconsin, Pleistocene), and only one occurrence was documented outside of a contiguous five-county region. Stygobionts were documented much farther inside of the most recent glacial extent, as far as 135.53 km from this boundary. These results support the findings of biospeleological research in other regions of the United States as they relate to regional distribution patterns of terrestrial and aquatic cave-obligate species.

Poster presentation:

Distribution and frequency of cave-dwelling terrestrial arthropods in two spatially closed karst areas of the eastern Italian Prealps

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The purpose of our research is to describe the spatial distribution of cave dwelling terrestrial arthropods in two geographically closed karst areas (Monte Baldo 398 km^2 and Monti Lessini 1403 km^2) of the eastern Italian Prealps. Our aims were to test the influence of the: a) number of sampled caves, b) cave's geographic location and c) cave's elevation, on the troglobiotic and endemic species richness. A total of sixty caves (Baldo = 17; Lessinia = 43) and forty-one species (Baldo=6; Lessinia = 35) have been analysed. To avoid the positive correlation between the species richness and the number of caves sampled in each areas, Lessinia has been divided in three cells with the same size of Baldo and with a comparable number of caves (Lessinia_1 = 14, Lessinia_2 = 15, Lessinia_3= 14). We calculated species accumulation curves based on Mau Tau values to compare sampling completeness at all different cell sizes. Local richness has been estimated by using the incidence-based coverage (ICE) species richness estimator. The ANOVA test has been computed to evaluate differences in the frequency of endemic species in the different cells. A mean of 11.9 additional species was expected to be found in each of the three Lessinia cells, but not in the Baldo area (exp. sp = 6.46) as indicated by the ICE estimator. Both the troglobiotic and endemic species richness is not influenced by the elevation of the caves. The frequency of endemic species is significant higher in the Baldo area compared with the Lessinia's cells (ANOVA test: $F_{3,59}=16.92$; $p<0.0001$). On the basis of these results the influence of the Quaternary glacial dynamics at local scale on the spatial distribution of troglobionts and on the frequency of endemic species is analysed and discussed.

Poster presentation:

Arthropods of guano in Santo caves (Vanuatu)

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The caves of Santo island in Vanuatu were biologically surveyed in September 2006 by the karst team of the expedition "Santo 2006". Focus was placed on guano habitats, which were present and rich in Arthropod species in most of the surveyed caves. Both free and standardized samplings were performed in 26 different guano caves, and at several guano sites in some caves. The diversity of arthropods guano communities was characterized by three features: 1) a low alpha-diversity; 2) inside each community hyperdominance of one or a few species; 3) unexpectedly high variation in species composition among sites. Guanobionts represented a much richer community than troglobionts in the studied area, with several species strictly limited to caves.

Oral presentation:

Hidden biodiversity: recent advances and perspectives in Brazilian subterranean biology

Rodrigo Lopes Ferreira

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In recent years, inventories of cave fauna carried out across diverse regions of Brazil have shown a great number of new species, many of which belong to new genera, families and even suborders. In the last five years, at least 300 troglobiotic species were discovered in Brazilian caves. Many of these species are highly troglomorphic. During past decades, researchers thought that the Neotropical region would harbor few troglobionts due to the low climatic severity in the tropics during the glacial maxima in comparison to temperate regions. The main model that assumes the isolation and evolution of species in underground systems in the world is based on these climatic changes occurring during distinct glacial maxima. However, the great amount of new species recently discovered in Brazilian caves and their high degree of troglomorphism indicates, that the events

of climatic changes in Neotropics, even if not so severe as in temperate regions, could have led to the isolation of subterranean lineages. Or, alternatively, other mechanisms of isolation (e.g. parapatric speciation, oceanic introgressions and regressions), might have led to the evolution of many lineages of subterranean fauna in Brazil. Furthermore, especially for terrestrial troglobionts, it seems that there is a geographic belt in northeastern Brazil (from SW to NE) in which troglobiotic species are concentrated. This belt eventually can represent the area in which many ancient populations had first become isolated due to the separation of the continuous evergreen tropical forest that used to exist in the area during the last glacial maximum, when the Amazon forest and the Brazilian Atlantic forest were connected. The Neotropical region is also characterized by the huge diversity of higher taxonomic groups of subterranean animals, what makes it especially interesting for ecological studies.

Poster presentation:

The cave lithology determining the structure of the cave invertebrate communities in the Brazilian Atlantic rain forest

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Only limestone and a few arenitic, ferruginous and granitic caves had their invertebrate communities studied. The present study aimed to compare the structure of invertebrate communities associated with carbonatic, magmatic, siliciclastic and ferruginous caves. Significant differences related to richness were observed when comparing pairs of caves: siliciclastitic and carbonatic, ferruginous and siliciclastic, magmatic and siliciclastic, and magmatic and ferruginous. Significant differences in relative abundance were observed between ferruginous and siliciclastic caves, and magmatic and siliciclastic caves. Ferruginous caves had the highest richness of troglomorphic species. Total richness of invertebrates was significantly positively correlated with cave length regardless of lithology. Relative richness and relative diversity of invertebrates in siliciclastic caves was positively related with the altitudinal variation. Total species richness of invertebrates was significantly positively corrrelated with environmental stability in siliciclastic caves. Total abundance of invertebrates was significantly positively correlated with environmental stability in carbonatic caves. Cave lithology determines clear differences in richness, abundance, and diversity of subterranean invertebrate communities. Ferruginous caves have the highest richness, and fauna composition

most different from caves of other lithologies. Linear development of caves in different rock types causes difference in amount of species. Cave size should always be considered along with lithology when different caves are biologically evaluated.

Oral presentation:

Spatial variations in stygobiont distributions in the English Chalk

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New groundwater ecology studies are underway in the UK to sample the many geologies and areas in which the groundwater ecology is largely unknown. More detailed studies are also being carried out to investigate the local geological and hydrogeological controls on the distribution of groundwater fauna. One area of focus is the English Chalk. Stygobites have been known in Chalk groundwaters from sporadic records for more than 150 years, but there have been no systematic investigations of their distribution, and the spatial variability of stygobites in the English Chalk is largely unknown. New studies have been carried out in recent years in the Chalk of Southern England. In Berkshire 19 boreholes in two catchments (total ~ 400 km^2) were sampled at maximum and minimum water level conditions. The hydrogeology of these boreholes is well known (the detailed chalk stratigraphy, the location of inflowing fractures to the boreholes, whether the boreholes contain upward or downward vertical flow, and the nature and size of the voids through which the water flows from borehole imaging data). The boreholes are in a range of topographical situations (valley and interfluve) with variable depths to the water table, and they are at varying distances from surface karst features. Results from these boreholes are used to investigate whether there are local geological and hydrogeological controls on the distribution of groundwater fauna, and whether there are variations between low and high water table sampling. In addition sampling at different depths in the boreholes was carried out to investigate whether stygobionts live on the bottom or in the water column. Results from the Berkshire study are compared to those from other areas to investigate any regional differences in groundwater fauna in the Chalk.

Poster presentation:

First results on subfossils in cave sediments from Slovenia and Romania

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Cave sediments preserve unaltered a broad range of proxies that can be used for past climate change reconstruction. Subfossil remains from cave sediments can give indications on sediments origin, conditions for deposition and paleoenvironment. Samples of suspended sediments from six caves (one unroofed) were taken from Slovenia and Romania. Fragments of aquatic (oligochetes, watermites, copepods, cladocerans) and terrestrial (insects) invertebrates were identified at group level. Paleomagnetic properties of sediments, vertebrate fossils and invertebrate subfossils were used as proxies in an integrated study about cave sediments. The presence of the subfossils is explained in the frame of the paleoenvironmental and paleoclimatic context and is supporting the proposed hypotheses about time and conditions for sediment deposition and paleogeographic evolution.

Oral presentation:

Habitat fragmentation and its effects on groundwater populations

Oana Teodora Moldovan* [1], Ioana N. Meleg [1], Aurel Perşoiu [2]

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The subterranean unsaturated zone of the karstic areas is a fragmented landscape. The maze of more or less connected voids harbors simple communities of mixed surface and subterranean species. The hypogean crustaceans are dominant in almost all communities of eight stations sampled monthly in a cave from north-western Romania. Water stable isotopes and flow rates were used to understand the morphological organization of the subterranean habitats. Communitiey structure, synchrony, temporal and spatial structures and variation at different geographical levels are discussed in evolutionary and conservation terms.

Oral presentation:

Tethyan distribution of stygobionts: fact or fiction

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The Tethyan Seaway was a circumtropical sea that closed with the closing of the Mediterran about 15 million years ago and is commonly invoked as an explanation for the distribution of stygobionts. In order to test this hypothesis, we examined the distribution of 72 stygobiotic genera of Crustacea, exclusive of Isopoda and Amphipoda. Using Paleomap™ for ArcGIS™, we plotted distributions by tectonic plate. We divided the resulting distributions into three categories: (1) Single region distributions which are consistent with but not necessarily evidence for a role for the Tethyan Seaway; (2) Tethyan Seaway distributions, consisting of a least two regions; and (3) distributions not consistent with the Tethyan Seaway. A total of 29 genera were in the first category and 41 were in the third. Surprisingly, only three genera were in the second category. Of the 29 distributions from a single Tethyan region, 9 were Mediterranean, 16 were Caribbean, three were Australian, and one was Indian. There were a variety of 41 non-Tethyan distributions, including Pacific Islands. There may be several explanations for the apparent lack of importance of the Tethyan Seaway. It may have closed before many species colonized subterranean habitats, or dispersal may be important. Our study suggests that a new paradigm for the historical biogeography of subterranean organisms is in order.

Poster presentation:

The cave crickets of the Eastern Mediterranean area: a contribution to the study of Balkan and Anatolian Rhaphidophoridae diversity

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Two genera of Rhaphidophoridae cave crickets are widespread in the Southern Europe and Asia minor, inhabiting caves of the Mediterranean area. At the end of the last century 22 species belonging to the genus *Dolichopoda* Bolivar, 1880 and 12 species belonging to the genus *Troglophilus* Krauss, 1879 were reported in literature for the caves of the Eastern Mediterranean area. Both genera are components of the parietal community, and important vectors of trophic energy

within the caves. Morphologically these two genera differ in the number of spines on the hind tibiae and in the shape of genitalia. On the basis of their ecology and of some morphological traits, i.e. appendages elongation and body coloration, *Dolichopoda* species show an higher degree of cave adaptation than *Troglophilus*. Most of the oriental *Dolichopoda* species are concentrated in continental and insular Greece; the other species are limited to the Balkans, Anatolia and Caucasus. The number of *Troglophilus* species is significantly lower: 7 species from Balkans, 3 from Anatolia and 2 from Aegean islands. Our recent researches carried out in these areas allowed us to identify and to describe 11 new species of *Dolichopoda* and 3 new species of *Troglophilus*. Other new taxa are still uncertain, needing further investigations. These are the cases of *Dolichopoda* specimens from Diktaion Antron (Crete) and from Korician Antron (Beotia) and the *Troglophilus* specimens collected in two Albanian caves and on Mljet island (Dalmatia). The results of our work point out the richness of species of both genera in these regions, 48 out the 65 total species, supporting the hypothesis of a common oriental origin for both genera, whose centre of dispersal was placed on the former Aegean plate. The preliminary analysis based on some morphological traits (e.g. epiphallus in *Dolichopoda* and X tergite in *Troglophilus*) suggest a clear divergence of the Caucasian-Anatolian species from the Balkanic-Hellenic taxa. The separation of these two groups of species in both genera could be interpreted as the result of some important geological events that occurred in this area during the late Miocene (e.g. rising of Anatolian plateau and formation of Mid-Aegean Trench).

Poster presentation:

Trophic dynamics of the coarse particulate organic matter (CPOM) in a tropical limestone cave

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The temporal budgets of the input, retention and use by invertebrates of the coarse organic matter were evaluated in a tropical limestone cave. These budgets are essential for the understanding of the trophic dynamics in this environment, which is poor regarding invertebrate species and food resources. Primary resources are roots that emerge from the bottom of the hypogean stream and provide shelter and food for numerous invertebrate's species. In addition, these microhabitats are distinct from those provided by the sediment. Detritus penetrates only through the

stream in lower quantities in the dry season, contrary to what happens in the rainy season. However, higher transport and leaching energies in the rainy season prevent detritus retention. During the rainy season, flood flows work as a force that destabilizes the processes of retention, biological colonization and detritus processing in the stream. In the terrestrial environment, bat feces was the main secondary resource available for the invertebrates; the constant production of this resource influences the structure and distribution of invertebrates. Unfavourable temperature conditions and, especially, low humidity in the soil promote low consumption rates of plant detritus. The cave functionality depends directly of the alochtonous food resources. Organic matter is transported in pulses by water flows and bats. Water can transport litter, while bats release feces. Both are extremely epigean-dependent processes of the dry season conditions and intact with the surrounding epigean vegetation.

Oral presentation:

Spatial distribution patterns of faunal groundwater communities across Germany

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Faunal distribution patterns and community structure in groundwater ecosystems are shaped by certain key factors working on different spatial scales. 1) On a large scale the faunal assemblages differ according to the biogeographic region. 2) On a regional scale the community structure of faunal groundwater organisms is shaped by regional particularities, mainly the type of aquifer. 3) On a local scale the strength of groundwater-surface water interactions and consequently the allochthonous input of nutrients and oxygen into the aquifer shapes the subterranean communities. The reliability of this hierarchical concept was tested combining and analysing data that were obtained by several groundwater studies across Germany. In general, the first results fit well with that concept, although large scale distribution pattern were not always in accordance with the biogeographical regions.

Oral presentation:

Regional species richness and diversity patterns of obligate cave-dwelling fauna in the Classic Karst in Italy

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Local and regional species richness of the obligate cave-dwelling fauna of the Classic Karst in Italy (about 200 square kilometers, over 3,070 caves known up to now), a well-known subterranean biodiversity hotspot, was assessed using: (1) bibliographic data (about 410 papers published between 1819 and 2009); (2) unpublished data collected by the Authors during the last 30 years; (3) data collected during a monitoring program (carried out in 2008-2009) using standardized sampling techniques applied to 28 caves. A database including the distribution of 382 species in 223 caves was assembled; 121 species (32%) were classified as obligate subterranean (33 of them being terrestrial, 88 aquatic); 105 species were considered endemic or subendemic to the Classic Karst. Based on species accumulation curves and jackknife 1, Chao2, bootstrap, and incidence-based coverage (ICE) estimators, we concluded that 82% of all species inhabiting the karstic area have been recorded so far (94% of terrestrial troglobiotic species, 75% of stygobiotic species). During the recent monitoring program (based on 28 caves out of the 223 surveyed), 45% of the whole regional fauna was collected, including 8 stygobiotic species new to Science. Notwithstanding the difficulty in assessing species richness of obligate cave-dwelling fauna because of the highly localized distributions of several species, results allowed (1) to assess the relative contribution to total species richness of terrestrial and groundwater (vadose and phreatic) fauna, dissecting regional diversity into alpha and beta components; (2) to analyze the contribution of historical and ecological determinants in shaping spatial patterns of subterranean biodiversity across the region; and (3) to assess the conservation value of species and caves, mapping priority areas for biodiversity conservation in the Karst.

Oral presentation:

Biodiversity pattern and distribution ranges of terrestrial troglobionts in the northwestern Balkans

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Beetles represent about half of terrestrial subterranean species in the northwestern Balkans (Dinarides and parts of Southern Calcareous Alps). Due to a large number of published records as well as a relatively resolved taxonomy they serve as a model group for studies of regional biodiversity patterns of terrestrial troglobionts. Two areas of high species richness have been identified, one in northwest (in Slovenia) and the other in southeast of the region (extending over southeastern Bosnia and Herzegovina, southeastern Croatia and western Montenegro). In this study, we analysed the range sizes of troglobiotic beetles, common distribution patterns and how they relate to the observed biodiversity pattern. We utilised a dataset of 371 troglobiotic species from 1,857 localities and covered the study area with a 20x20 km grid. Troglobiotic beetles have small ranges, 37% of them being known from one cave only (single site endemics). With additional studies it can be expected they can be found in more caves, yet their ranges may remain restriced – in our dataset 52% of species occur within the area of one 20x20 km grid quadrat and only 7 species are known from more than 100 caves and 20 grid quadrats. Some overlap among quadrats with many single site endemics and high species richness is apparent in the southeastern part of the Dinarides, yet numbers of single site endemics were not generally correlated to non-endemic species richness. When maximal range sizes were compared, about 84% of species had them smaller than 50 km and only 5% of species larger than 150 km. Those few species having large distribution ranges may be complexes of separate species currently recognized as subspecies. We further analysed species range similarities using clustering techniques. The ranges of troglobiotic beetle species show common distribution patterns. The northwestern and southeastern merodinaric patterns overlap with two areas of highest species richness, both having different species composition.

Poster presentation:

Range sizes in subterranean amphipods of the genus *Niphargus*

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Molecular taxonomy revealed highly incomplete taxonomy in several groups, where existing species names frequently cover many yet undiscovered species. For aquatic troglobionts it has been suggested that species with distributions exceeding 200 km are probably a complex of species and should be taxonomically revised. On the other hand, narrow distribution ranges in subterranean species have been observed, with large numbers of its extremes – single site endemics. Many taxonomic groups, also in aquatic troglobionts, express high level of single site endemism. Within the amphipod genus *Niphargus*, distributed in the western Palaearctic, it has been estimated that about half of the species are known from the type locality only. Should this proportion be accepted as valid and expected, or should this be regarded as a result of lack of studies and insufficient sampling in the areas where single site endemics occur? To approach this issue, we used the dataset on about 13% of all the species of the genus *Niphargus*, which were included in our study based on two criteria. First, species had a well supported taxonomy, the variation of which has been revised by molecular characters or by easily diagnosed autapomorphic traits. Second, we selected the species from locally well explored areas. We mapped the distributions of 43 species, and calculated the maximum extent of their ranges. Only three species are known from single localities, all from the Balkans. Of five species known from two localities, the smallest distance among them was less than 1.5 km and the largest over 120 km. About 60% of the species had the maximum extent of the range less than 100 km, and about 78% of the species less than 200 km. Extent of nine species (21%) exceeded the 200 km limit, with three extremes: *N. kolombatovici* on the Balkans having the 350 km distance, *N. aggetelekiensis* in central Europe over 550 km and undescribed species within "*N. virei*" complex extending over 600 km in eastern France. This shows that aquatic troglobionts with large distribution ranges do exist. On the other hand, there are only few single site endemics, indicating that most of the single site species presently known in *Niphargus* are not true and other localities can be expected.

SYMPOSIA

3. Phylogeography and Phylogeny

Oral presentation:

Systematics and phylogeography of Australian Parabathynellidae (Crustacea: Bathynellacea)

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The order Bathynellacea is an ancient group of subterranean aquatic (stygobitic) crustaceans which inhabit various groundwater habitats. Previous molecular and morphological research has revealed a striking diversity of species and remarkably high levels of short-range endemism within the bathynellacean family Parabathynellidae of arid Western Australia. This study is the first to utilize DNA sequence data to explore the higher level phylogenetic relationships amongst Australian parabathynellid taxa and examine their distribution throughout the continent. Sequence data was generated from a region of the mitochondrial DNA cytochrome oxidase 1 gene and nuclear 18S gene. The results suggest that genera are largely monophyletic and revealed numerous undescribed taxa. They also provide evidence for high levels of endemism in other regions of 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 conservation 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.

Oral presentation:

Patterns of speciation in *Dolichopoda* cave crickets (Orthoptera, Rhaphidophoridae)

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This study focuses on the phylogenetic relationships among ninety percent of known *Dolichopoda* species (44 out of 49); primarily a Mediterranean genus, distributed from eastern Pyrenees to Caucasus. A total of 2490 base pairs were sequenced corresponding to partial sequences of one nuclear (28S rRNA) and three mitochondrial genes (12S, 16S and COI). A relaxed molecular clock,

inferred from Bayesian analysis was applied to estimate the divergence times between the lineages using well dated palaeoevents of the study areas. Molecular substitution rates per lineage per million years were also obtained for each analysed gene. Based on the nearly complete species phylogeny, temporal patterns of diversification were analysed using Lineage-Through-Time plots and diversification statistics. Alternative hypotheses about the colonization of western Mediterranean by *Dolichopoda* species were tested by means of Approximate Bayesian Computation analysis and by comparing the degree of discordance between species trees and gene trees under four plausible biogeographic scenarios. Both phylogenetic reconstruction and results from the biogeographical hypotheses test suggested that the current distribution of *Dolichopoda* species has been essentially shaped from the palaeogeographic and climatic events occurred in the Mediterranean region, starting from Late Miocene up to the Plio-Pleistocene. Our results suggest that the current distribution of *Dolichopoda* can be explained by a combination of both vicariance and dispersal events, with many processes occurring in ancestral epigean populations before the invasion of the subterranean environment.

Oral presentation:

New biogeographical and phylogenetic data about the genus *Sphaeromides* and its relatives (Crustacea: Isopoda: Cirolanidae)

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Sphaeromides spp. are among the most remarkable aquatic troglobionts. The putative genus exhibits a trans dinaric distribution, with a pronounced diversity in the Dinaric karst, but occurring also outside it, in France and Bulgaria. We succeeded to sample the genus from ca 17 localities throughout its range (except in Bosanska Krajina). A molecular analysis was conducted using mtDNA genes (16S rRNA and COXI) at two levels: 1) to infer the phylogenetic relationships of the *Sphaeromides* species with the other subterranean cirolanid taxa, and 2) to investigate the phylogeography of the *S. virei* populations in the Dinarides and its relationships with the other *Sphaeromides* species (*S. raymondi* and *S. bureschi)*. The 16S tree, including available GenBank cirolanid sequences, show that *Sphaeromides*, as conceived now, is a polyphyletic assemblage. *S. raymondi* from France is a sister taxon to the eastern *Sphaeromides* spp. plus some American genera (*Cirolanides* and *Antrolana* from USA and *Speocirolana* and *Sphaerolana*

from Mexico). The populations of the *S. virei* comprise until now the two subspecies *S. v. virei* and *S. v. mediodalmatina*. *S. virei* occurs only along the eastern Adriatic coast, but always in pure fresh water, while *S. mediodalmatina* is limited to the central inland parts of Dalmacija. In the phylogenetic reconstruction, *S. virei* is again a monophylum, probably representing separate genus, inhabiting the Dinaric and western Balkan (or Stara planina) karst. The COI and the 16S trees show that *S. virei* may be regarded as at least two distinct species, since the supposed subspecies *S. virei mediodalmatina* is a very distinct phyletic line and beside that it occurs in one cave syntopically with *S. v. virei*. These molecular investigations sustain new evidences about the genus *Sphaeromides,* which should be supported by a complete morphological analysis of the genus.

Poster presentation:

Phylogeny and phylogeography of the cave bivalve *Congeria kusceri*, with an outline for its endangerment in Croatia

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Congeria kusceri Bole 1962 is the only stygobitic bivalve in the world. It is also the only surviving species of the genus *Congeria* which was widespread in the tertiary. Its current distribution is quite disjunct. Mitochondrial 16S rRNA and COI and nuclear ITS and 18S rRNA markers were employed to examine the position of the genus within the family Dreissenidae as well as to infer the relations between different populations. Our results support *Congeria* being the third extant genus of the family Dreissenidae as previous studies suggested. Within *Congeria*, two main phylogeographical groups were found: one encompassing Markov ponor (Lika region) and Suvaja (northern Bosnia) populations, and another comprising all southern Dalmatian and Hercegovinian populations. Uncorrected genetic distances (P) between them were up to 1.7%, and 8.8%, for the 16S rRNA and the COI gne, respectively. In contrast to the relatively low level of genetic divergence between populations within second group (maximal P values being 0.5% for 16S rRNA and 1.4% for COI), genetic distances between Markov Ponor and Suvaja amounted to 1.0% and 4.3% for 16S rRNA and COI, respectively. These results are in congruence with biogeographical data since Markov ponor and Suvaja are several hundred kilometres away and hydrologically isolated from the rest of the

localities. The species is recorded in a total of fifteen localities in Croatia, but at most sites only shells were found. Live populations were documented in only five sites, but the surveys in 2008 revealed that the two populations disappeared. In addition to being strictly protected by the Croatian law, the species is listed in the Annexes II and IV of the Habitats Directive. Nevertheless, two out of three remaining populations could be facing serious destruction if not extinction in the near future. All this led to the enlistment of *Congeria kusceri* in the Red list of Croatian cave fauna in the IUCN category CR.

Poster presentation:

A molecular test for *Niphargus krameri* (Crustacea: Amphipoda) intraspecific diversity

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The taxonomic research on amphipods in the *Niphargus krameri* species complex has had a long history, making it an educative example of an intensely studied taxon whose taxonomy kept unfolding proportionally with the amount of invested work. The first record dates to 1935 when Schnellenberg described *N. puteanus* ssp. *krameri*. S. Karaman raised it to the species level in 1954. Based on a minor morphological variation, he additionally described *N. krameri* f. *spinulifemur* and *N. krameri* ssp. *timavi*. Thirty years later, in 1984, G. Karaman identified autapomorphic traits in both subspecific taxa and raised them to a species level. Moreover, G. Karaman also reported on coexisting populations of *N. krameri* and *N. spinulifemur* inhabiting northern Istra Peninsula and Italian Venezia Giulia lacking crossbreeds. More recently, we distinguished three distinct, geographically well-delimited morphs of *N. krameri* s.str. that can be identified on a basis of setal patterns on gnathopods in adult specimens. Despite the congruence between morphological and geographic data, no taxonomic conclusions were made in that study. Here we approached the problem using molecular data. Newly obtained 28S rDNA (nuclear) and COI (mitochondrial) sequences for 19 specimens from 11 localities were aligned with published sequences and subjected to a phylogeny test. Results show a clear separation of specimens into two strongly supported clades that match with geographical division and agree, to some extent, with the distribution of setal patterns. It is evident that gene flow between some populations is restricted, probably due to poor dispersal abilities in a geologically diverse environment, which supports the idea of *N. krameri* being in the course of speciation and perhaps also morphological differentiation. All datasets justify hypothesizing a new species distributed across W and N Istria.

Oral presentation:

Linking molecular phylogeny to morphological evolution in *Troglocaris* (Crustacea: Decapoda: Atyidae)

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Ever since first species of *Troglocaris* (Dormitzer 1853) was described the taxonomy of the genus has been unresolved. Only after the phylogenetic reconstruction (COI, 16S rRNA and 28S rRNA) of the putative *Troglocaris* taxa from three separated distribution areas – from the Dinarides (Western Balkan Peninsula), S France and Caucasus – their phylogenetic relationships were revealed. The only French species was relocated into a new genus as *Gallocaris inermis* Sket & Zakšek 2009, being more closely related to the epigean *Dugastella valentina* (Ferrer Galdiano 1924) than to its supposed congeners. The taxa from the other two areas constitute a monophylum comprising the Caucasian *Xiphocaridinella* Sadovsky 1930, and three Dinaric subgenera: sg. *Troglocaris* (= *Troglocaris* s. str.), sg. *Spelaeocaris* Matjašič 1956 and sg. *Troglocaridella* Babić 1922. Eleven species and phylogroups of the Dinaric subgenera were morphometrically analysed. The accordance of morphology and molecular data is demonstrated by multivariate statistical analyses. Although already a set of non-sexual characters enables distinct separation of all subgenera, optimal accordance of morphological and molecular data is achieved by the consideration of sexual characters in adult males. At the subgeneric level, both phylogenetic subclades of the subgenus *Spelaeocaris* are morphologically recognized, together with most of their species. In *Troglocaris* s. str., however, only a combination of numerous characters can separate phylogroups to some extent. A few characters, inappropriate for the multivariate statistics support the separation additionally. While the majority of the morphometric characters seem to be a subject of a phylogenetic patrimony, rostral characteristics and body size may be a result of adaptation. Eventually, the molecular approach remains the most appropriate for a reliable determination of the most *Troglocaris* s. str. species and phylogroups.

Oral presentation:

Phylogeny of Crangonyctoidea: taxonomic status and origin of groundwater amphipods, endemic to Iceland, based on two nuclear genes

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Two new endemic species of subterranean freshwater amphipods, *Crangonyx islandicus* and *Crymostygius thingvallensis* were recently discovered in groundwater underneath porous lava fields in Iceland. We recently demonstrated that *Crangonyx islandicus* survived the repeated glaciations periods of the Ice Age in sub-glacial refugia. This species is widespread over the active volcanic zone and presents unique morphological and meristical features compared to other *Crangonyx* species and might represent a new genus. *Crymostygius thingvallensis*, defines a new family, is rare and has mainly been found in lake Thingvallavatn. These two species belong to the Crangonyctoidea super family, which has representatives both in North America and on the Eurasian continent. In order to understand where the species come from and to confirm their taxonomic status we have sequenced nuclear genes (18S rRNA and 28S rRNA, about 3000 bp per individual) from the two species from Iceland and from species from North-America, Europe and Asia. A comparison of the gene sequences to published sequences of other amphipod species resulted in phylogeny comprising 10 genes and a total of 21 species. On the taxonomic side, the phylogenetic analyses supports that the two species from Iceland are truly unpreviously described species. Furthermore, no species of Crangonyctoidea appeared closely related to *C. thingvallensis*. An early divergence from the other species of the group is observed, confirming its monotypic family status. The *Crangonyx* genus is polyphyletic and *C. islandicus* is clearly distinct from the other *Crangonyx* species, and may thus define a new monotypic genus. *Crangonyx* species from Europe appeared more closely related to the *Stygobromus* and *Bactrurus* genus than with the other *Crangonyx* species. These findings clearly highlight the need for a taxonomic revision of the group. On the phylogeographic side, *C. islandicus* is more closely related to other *Crangonyx* species from North America which supports the hypothesis of an ancient colonization trough groundwaters contacts between Greenland and Iceland during the early formation of the island. These two endemic species might therefore be the oldest inhabitants of Iceland.

Poster presentation:

Molecular evidence of the survival of subterranean amphipods (Arthropoda) during Ice Ages underneath glaciers in Iceland

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Two endemic groundwater arthropod crustacean species, *Crangonyx islandicus* and *Crymostygius thingvallensis*, were recently discovered at the mid-Atlantic volcanic island of Iceland. The extent of morphological differences from closest relatives, endemism, the geographic isolation of Iceland and its complete coverage by glaciers 21,000 years ago, suggests that these two species have survived glaciation periods in a sub-glacial refugium. Here we provide a strong support for this hypothesis by an analysis of mitochondrial genetic variation within *Crangonyx islandicus*. Our results show that the species is divided into several distinct monophyletic groups, found along the volcanic zone in Iceland, which have been separated by 0.5 to around 5 million years. The genetic divergence between groups reflects geographic distances between sampling sites, indicating that divergence occurred after the colonization of Iceland. The genetic patterns and the dependency of genetic variation on distances from the tectonic plate boundary and altitude, point to recent expansion from several refugia within Iceland. This presents the first genetic evidence of a multicellular organisms, as complex as crustacean amphipods, which have survived glaciations underneath an ice sheet. This survival may be explained by geothermal heat linked to volcanic activities, which may have maintained favorable habitats in fissures along the tectonic plate boundary in Iceland during glaciations.

Oral presentation:

Evolution of chiltoniid amphipods from subterranean and surface habitats in Australia

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Stygobitic chiltoniid amphipods recently have been found in subterranean habits such as caves, aquifers, and underflow of rivers and creeks in a number of geographic areas in Australia (Yilgarn WA, Flinders Ranges and Eyre Peninsula SA and Musgrave Ranges NT). All the discovered subterranean species are de-pigmented and blind. Additionally, chiltoniid amphipods are also common in surface waters of the temperate zone of southern Australia (SW Western Australia, South Australia, Victoria and Tasmania) as well as in mound springs in the Great Artesian Basin and sinkholes in the SE of South Australia. Presently, six species in this group have been described: *Phreatochiltonia anopthalma* (subterranean) and *Austrochiltonia dalhousiensis* (surface), both from Dalhousie Springs; *Arabunnachiltonia murphyi* and *Wangiannachiltonia guzikae* from the Lake Eyre mound springs and *A. australis* and *A. subtenuis* from surface waters in Victoria and South Australia. The latter two species have been reported to have a wide distribution across entire southern Australia.

Molecular analyses of mitochondrial and nuclear genes of subterranean and surface taxa collected from the entire geographical range of this group shows a number of interesting patterns: (1) There is no evidence for a wide spread distribution of *A. australis* and *A. subtenuis*. (2) There are numerous undescribed, morphological cryptic surface species that often are restricted to individual catchments. (3) There are a number of well defined subterranean clades of which its species are confined to different geographical areas. (4) There are also clades that each contains a mix of species from inland areas such as the Yilgarn and the Great Artesian Basin as well as from the coastal limestone aquifers of Eyre Peninsula.

A dated phylogenetic tree, palaeogeographic and climatic data are used to address several evolutionary questions: (1) To what extent did the palaeogeography and historical climates shape the current distribution of the amphipod species and clades? (2) What triggered the evolution of subterranean species in the different areas?

Oral presentation:

The mitochondrial genome analysis of the unique cave dwelling sponge *Eunapius subterraneus* Sket & Velikonja, 1984 (Porifera: Spongillidae)

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Although sponges posses plain morphology (absence of organs and tissues) and their phylogenetic position is at the base of the kingdom Metazoa several studies showed that sponges have strikingly complex genomes. Furthermore, their gene content and functional repertoire are more related to their orthologs in human than to either *Drosophila melanogaster* or *Caenorhabditis elegans* counterparts, so studies of these living fossils are important in terms of evolution and phylogeny. Sponges are mainly marine but a few taxa within Demospongiae (suborder Spongillina) live in freshwater. Cave fauna stands out among all other freshwater biota owning peculiar species which are usually distinct from that of surrounding freshwater habitats. *Eunapius subterraneu*s Sket & Velikonja, 1984, is the only stygobitic member of the suborder Spongillina, therefore it was a challenge to elucidate the origin of this unique sponge by obtaining the complete mitochondrial genome data. Hopefully, more freshwater sponge mitochondrial DNA sequences will be available so that the phylogeny of this group could be completely resolved and progress in studies of biology, evolution, biodiversity and efficient conservation will be possible.

Oral presentation:

Caucasus and vicinity: comparative phylogeography of Ponto-Caspian and subterranean crustaceans

Nikolai S. Mugue

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We will present our data on molecular phylogenetics of large Ponto-Caspian groups of crustaceans (pontogammarids, corophiids and mysids), and subterranean genera *Niphargus* and *Troglocaris* from caves and springs of the Caucasus. High ecological plasticity of the modern Ponto-Caspian taxa, its extraordinary success in

dispersion in the European fresh waters and in colonization of new environments may give a clue for understanding of peculiar and enigmatic distribution of European subterranean crustaceans. A freshwater lake on the Caucasus coast of the Black Sea (lake Abrau) and several lakes along the Turkish coast of the Marmara Sea contain relic ponto-caspian fauna and provide examples of successful invasion of both potamophilous and limnetic taxa in the water bodies elevated above the sea level. The hypothesis of paleo-Pontocaspian origin of some subterranean genera has testable conclusion: the genetic diversity of (mostly unexplored) niphargid fauna from the Caucasus and the Turkish coast of the Black Sea should be higher than in the rest of the area of the genus distribution.

Oral presentation:

Genetic evidence of subterranean migration of imotska gaovica *Delminichthys adspersus* (Pisces: Cyprinidae)

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Several springs and accompanying subterranean waters of Dinaric karst are populated by endemic small-sized leuciscinae fishes vernacularly called "gaovice". On the basis of morphological and ecological characters, they used to be classified into a single genus *Phoxinellus* Heckel (1843); however, according to recent molecular investigations four of them have been reclassified into a new genus *Delminichthys*: *D. adspersus*, *D. ghetaldii*, *D. jadovensis* and *D. krbavensis*. *D. adspersus*, inhabiting Imotsko polje, occupies the most fragmented habitat and forms several apparently separated populations without obvious surface connections. As "gaovice" sustain long-term subterranean life, a question emerges whether they could maintain species gene flow via subterranean migrations. To answer this question, genetic structure of several geographically separated populations of *D. adspersus* have been studied using mitochondrial and microsatellite DNA analysis. By applying several population genetic programs, we determined at least three main groups of *D. adspersus*, which turned out as genetically unified with only slight gene flow between the western and south-eastern group, while the third group appeared as completely isolated population. On the other hand, gene flow was detected among several subpopulations within each group, e.g. Red Lake population and other nearby subpopulations in the western group. Taking into account spatial distribution of *D. adspersus* and known facts about hydrological network of Imotsko polje, the observed genetic outcome can only be explained by subterranean communication between geographically

separated populations of *D. adspersus*. According to our knowledge, this is the first proven evidence of subterranean migration of surface fishes.

Poster presentation:

Colonization and subterranean speciation in atyid freshwater shrimps from Maros karst, Sulawesi

Kristina von Rintelen [1], Timothy J. Page [2], Yixiong Cai [3], Daisy Wowor [4], Andreas Wessel [1], Björn Stelbrink [1], Thomas Iliffe [5], Thomas von Rintelen* [1]

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The evolution of troglobionts has fascinated scientists since Darwin's time. A high number of cave-dwelling animals are well studied today, among these several freshwater organisms, e.g. fishes or crustaceans. The freshwater shrimp family Atyidae (Crustacea, Decapoda, Caridea) also has several subterranean representatives worldwide. Whereas the troglobiotic atyids from Australia and Europe have already been comprehensively studied with morphological and molecular methods, such data for Southeast-Asian shrimps is still largely lacking. From the Indonesian island Sulawesi, situated within the biogeographic hotspot area Wallacea, more than 46 species in four genera are known, the majority from the genus *Caridina*. One of these genera (*Marosina*) and approximately fifty percent of all species are endemic to the island. Two genera (*Caridina* and *Atyopsis*) have epigean representatives, while the other two (*Marosina* and *Parisia*) exclusively occur in subterranean rivers in Maros karst, southwestern Sulawesi. The genus *Marosina* comprises only two species, *M. longirostris* and *M. brevirostris*. Comprehensive collections of *Marosina* and *Caridina* from several caves of Sulawesi in 2007 and 2009 were studied with morphological and molecular methods. A phylogeny assessed from mtDNA revealed two independent cave colonizations: Cave-dwellers with reduced eyes in the genus *Caridina* derived from epigean ancestors from the island. In contrast, the troglobiotic genus *Marosina* evolved within the caves independently and may have been derived from a widely distributed and anchialine cave dweller. In this genus, there are further hints of subterranean speciation into the two species known today.

Poster presentation:

Living in the dark: phylogeny of atyid freshwater shrimps reveals multiple cave invasions

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Freshwater caridean shrimps (Crustacea, Decapoda) occur in all biogeographic regions bar Antarctica, but are in general among the less well studied groups of decapod crustaceans. This might not be surprising regarding the fact that the majority of shrimp-like decapods are found in marine environments. Freshwater taxa only account for approximately a quarter of all described Caridea and are numerically dominated by the two families Atyidae and Palaemonidae. At present, the Atyidae contain 42 extant genera. The vast majority of species are described within the genus *Caridina*, which is widely distributed throughout the Indo-West Pacific. Atyid shrimps are abundant in various freshwater habitats worldwide including cave systems (freshwater and anchialine). There are many cave-dwelling species that are well-adapted to subterranean life, e.g. by strongly reduced eyes and lack of pigmentation. Previous molecular studies using Atyidae mainly from Europe and Australia already gave interesting insights into the evolution and biogeography of cave adapted shrimps. Data from Asia, a hotspot of atyid diversity, was lacking so far. A molecular phylogeny including 34 of the 42 living genera, based on mitochondrial and nuclear genes (16S, 28S, H3), revealed at least six independent clades comprising subterranean and often landlocked taxa. These results suggest multiple independent cave colonizations of atyid freshwater shrimps worldwide. Parallel, the phylogeny implies new insights into the systematics of these shrimps, e.g. on subfamily level.

Poster presentation:

On molecular phylogeny of *Niphargus* from the West Transcaucasus

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Of all European subterranean invertebrates, the genus *Niphargus* Schiödte 1849 (Crustacea, Amphipoda) had traditionally attracted the attention of most researchers, being the largest one among freshwater amphipods, with over 300 described species and subspecies. The majority of species were described in the middle of the 20th century, but much was left undiscovered. Further problems occurred to interrelation of ongoing studies on Caucasus and Crimea, due to the lack of material and information exchange between Soviet and European scientific community. Many species from Transcaucasus were described as closely related to European ones, yet the validity of these may be doubted, as traits used for description were not completely overlapping. In-region taxonomic relations were not clear, too. In our research we try to understand the formation of the genus *Niphargus* on the Caucasus by using of molecular phylogenetic analyses. Samples from nine locations along Transcaucasian shoreline were taken and identified by morphological means and processed for further research. We used H3 and 28S nuclear and COI mitochondrial molecular markers for our study to get reliable data on different taxonomic resolution. As a result we succeeded in resolving existing uncertainties the taxonomic relations of *Niphargus smirnovi* Birstein 1952 and *Niphargus stygius* Schiödte 1847 (*Niphargus stygius latimanus* Birstein 1952, *Niphargus stygius pseudolatimanus* Birstein 1952 and *Niphargus stygius longidactylus* Birstein 1952). What has been referred to as *N. stygius* subspecies should be treated as subspecies of *N. smirnovi*, and appears to be completely separate from the real *N. stygius*. Furthermore, not yet having samples of all species known to inhabit the Caucasus we can already be sure that niphargids inhabiting this region are polyphyletic and their invasion to the territory and following speciation was of the step-by-step type.

Oral presentation:

Pseudocrangonyctidae (Crustacea: Amphipoda) and its forming pathways

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At present the subterranean amphipod fauna of the Far East remains insufficiently investigated. In this region a complex of stygobiont amphipods of the endemic family Pseudocrangonyctidae consists of two genera: *Pseudocrangonyx* and *Procrangonyx*. The subterranean freshwater amphipod genus *Pseudocrangonyx,* which includes 15 described and several undescribed species, is widespread in eastern China, the Korean Peninsula, the Japan Archipelago, the continental part of the far-eastern Russia and Sakhalin Island and Kamchatka Peninsula. The species of *Pseudocrangonyx* inhabit various subsurface streams, deep groundwater aquifers, and cave reservoirs connected with groundwater table; whereas species of *Procrangonyx*, as a rule, are confined to deep groundwater aquifers and are restricted to areas surrounding the semi-enclosed East Sea. Presently, three species are known in the genus *Procrangonyx*. The morphological and ecological differences between *Pseudocrangonyx* and *Procrangonyx* were studied, and a preliminary scenario of the evolutionary history of Pseudocrangonyctidae is proposed, based on phylogenic and biogeographic considerations. Supported by: Russian Foundation for Basic Research grant 09-04-98544.

Oral presentation:

Problems of taxonomy in Mexican *Astyanax*

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The neotropical characid fish *Astyanax*, which has colonized Middle and North America at the end of Pliocene, has gained considerable interest in evolutionary research. It occurs in all freshwater habitats providing sufficient temperature conditions like rivers, creeks, stagnant pools, cenotes, and lakes. Within two restricted karst areas in Mexico it has even developed eye and pigment reduced cave forms. The various surface populations show considerable meristic and morphometric variation. This is probably the reason why a large number of species and subspecies have been described in the past. However, they were very poorly defined and their taxonomy is still a matter of research. Molecular studies of the cave populations showed that they have descended from multiple invasions of surface *Astyanax* at different times being the cause of different phylogenetic age. It

is assumed that this is due to climatic change during Pleistocene. It was furthermore found that genetic introgression is a relatively frequent event in the cave populations. We performed a molecular study using mtDNA sequences and microsatellite data of several surface and the cave populations. Based on these markers several different haplotype lineages and microsatellite clusters could be discriminated. However, the clusters of the two markers were not congruent. Furthermore, the distribution of the clusters is mostly not correlated with geographic barriers. Such distribution pattern cannot be explained by deep vicariant events but rather reflects random colonization events. The taxonomic status of Mexican *Astyanax* populations is discussed.

Oral presentation:

Phylogeny of the western taxa of the genus *Monolistra* (Crustacea: Isopoda: Sphaeromatidae)

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The exclusively subterranean genus *Monolistra* is predominantly distributed in the Dinarides; however some species penetrate westwards along the Southern Limestone Alps in Italy, reaching western Lombardia and Ticino in Switzerland. In order to highlight the phylogenetic relationships of the westernmost taxa, combined sequences of 28S, 16S and 12S r-DNA fragments in total length of pruned sequences of 1832 bp were analysed using Bayesian inference. The phylogram is characterised by three main clades with all taxa inhabiting the Alpine region representing a well supported (93%) monophylum. The branching order within the Alpine clade in the phylogram indicates a stepwise differentiation of Alpine species mainly in direction from West towards East. This is well exemplified by the well supported (100%) sister species relationship of two westernmost taxa, *M. (T.) pavani* and *M. (T.) boldorii bergomas*. All but one taxon in this clade belongs to the subgenus *Typhlosphaeroma*. Namely, the *M. coeca julia*, which is nested within Alpine clade, is morphologically more similar to species of the supposed plesiomorphic subgenus *Monolistra*, characterized by developed uropods. Geographically *M. coeca julia* inhabits the transition area, including parts of the Alpine and the Dinaric karst. On the other hand, *M. (T.) racovitzai* is the only representative of the *Typhlosphaeroma* subgenus in the Dinaric clade, forming a well supported monophylum with the species of all the other subgenera also limited to the Dinarides. This species and the species of the subgenus *Microlistra* are the most recent invaders at the NW edge of the Dinarides, with very little or

no genetic differentiation among populations; however their distribution area does not override the western border of the Carso/Kras in Italy. Given the surprisingly large genetic divergence observed, the taxonomic rank of some taxa needs to be revised.

Poster presentation:

Phylogeography of the unique cave tube worm *Marifugia cavatica* (Polychaeta: Serpulidae)

Valerija Zakšek*, Boris Sket, Peter Trontelj

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Marifugia cavatica (Polychaeta: Serpulidae), the freshwater cave tube worm, is distributed in groundwaters along the Dinaric Karst on the Western Balkan Peninsula. With its large distribution range it contradicts the generalized upper range limit of about 200 kilometers for macro-stygobionts. Two independent gene fragments were sequenced and analyzed to reveal its phylogeographic structure. A 386 bp long fragment of mitochondrial cytochrome b (cytb) and a 700 bp fragment of 28S rDNA were amplified and sequenced for 44 *Marifugia* specimens from most of its range. The results of phylogenetic analyses showed that *M. cavatica* is composed of four distinct and geographically well defined phylogroups: Western, Eastern, Southern and Southeastern. Both, mitochondrial and nuclear data supported the same phylogroups, although the relationships between them remain unresolved. The Western phylogroup includes populations distributed in the Italian Carso, southwestern Slovenia and the northeastern Istra Peninsula; the Eastern phylogroup contains populations from southeastern Slovenia trough Croatia to Bosanska Krajina in Bosnia and Herzegovina; the Southern phylogroup is limited to the cave Vjetrenica, and the Southeastern in the cave Obod at Fatničko polje (both Bosnia and Herzegovina). Divergence between them was high even at the amino acid level of the cytb. We found no indication of gene flow between phylogroups. A molecular clock calibrated on *Marifugia* fossils suggested that the phylogroups separated about 8 million years ago. In comparison to the phylogeographic structure of other aquatic subterranean taxa from the Dinaric Karst (*Troglocaris* s. str., *Proteus anguinus*, *Asellus aquaticus*), *Marifugia* shows both concordant and discordant groupings. We explain the discordance by ecological differences during the life-cycle caused by its microscopic, conditionally planktonic larva.

Oral presentation:

Phylogeography of the cave shrimp *Troglocaris* s. str.: a basis for a comparative phylogeography of Dinaric subterranean fauna

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Cave shrimps *Troglocaris* s. str. (sensu Sket & Zakšek, 2009) are distributed along the Dinaric Karst from Italy to southern Herzegovina, displaying the so called holodinaric distribution pattern. Within its large range (about 600 kilometers) we tested it for possible unknown diversity and phylogeographic structure. Its detailed phylogeographic structure was revealed by analyzing two mitochondrial gene fragments (COI and 16S) and nuclear one (ITS2) for more than 250 specimens along its entire range. The results of phylogenetic analyses and several different phylogeographic approaches congruently revealed six phylogroups (species): Western, Eastern, Adriatic, Soča, Istra and *T. bosnica*. All recognized phylogroups are geographically well defined and allopatric. Only in Istra (Istra Peninsula, Croatia), representatives of two phylogroups (Western and Istra) were co-occurring. We used this phylogeographic structure, covering nearly the entire range of the Dinaric Karst, as basis for a comparative phylogeographic study of holodinaric subterranean taxa: the European cave salamander *Proteus anguinus* and the cave tube worm *Marifugia cavatica*. All three, although taxonomically so distant from each other, similary show high levels of genetic differentiation. Their ranges are usually small and rarely exceed 150 kilometers (except of the Adriatic phylogroup in *Troglocaris*). Furthermore, there is substantial agreement between the geographic extent of the phylogroups, most markedly between *Troglocaris* and *Proteus*, while the cave tube worm shows a somewhat different pattern in the inner part of Dinarides. We explain these differences as a possible consequence of its different life history and dispersal abilities.

SYMPOSIA

4. Subterranean Aquatic Annelida

Oral presentation:

Molecular and morphological analyses reveal the presence of two species in the stygobiont oligochaete *Troglodrilus galarzai*

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Troglodrilus galarzai (Giani & Rodriguez, 1988) is a stygobiont oligochaete species (Tubificinae, Clitellata, Annelida) in a monospecific genus. Its geographical distribution appears restricted to the south western Europe, and the populations known up to date occupy two well separated regions, namely northern Iberian Peninsula (Ereñozar and Gorbeia karstic units) and south eastern France (gallery of Montgelas and Crotot cave). A previous morphological study revealed some morphological differences between Iberian and French populations but concluded that they were not substantial to separate two species. More recently, we conducted a molecular analysis of two populations of the species (Gorbeia and Montgelas) using 16S rDNA and COI gene sequences and we have combined these results with a new detailed morphological analysis of all known populations. The obtained genetic distances between Gorbeia and Montgelas populations were 17.8-18.1% for COI sequences and 9.2-10% for 16S sequences, which together with mutual exclusivity of the haplotypes, supported the hypothesis of the presence of two cryptic species in *T. galarzai*. The new morphological study is mainly based on the reproductive system and grouped together Ereñozar + Gorbeia populations and Montgelas + Crotot populations. We found no overlap between Iberian and French populations for some measurements related with the penial sac and the spermathecal bulb, as well as key differences on the shape of the penial sheath between French and Spanish populations. Considering both the molecular results and the differences on the morphological characters between Iberian and French populations, we suggest the presence of two species in *T. galarzai*.

Poster presentation:

Biodiversity in anchialine caves: first record of the tubeworm *Ficopomatus enigmaticus* (Annelida, Polychaeta)

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Ficopomatus enigmaticus (Fauvel, 1923) is a sedentary polychaete which has been found worldwide inhabiting coastal brackish waters, lagoons and estuaries of warm temperate areas of both hemispheres. This tubeworm builds calcareous tubes on any hard substrate (Serpulidae) with distinctive collar-like rings at irregular intervals and is relatively easy to identify. It is an efficient suspension-feeder, very tolerant and physiologically well adapted to temperature and salinity variations, eutrophic conditions and low dissolved oxygen content. Here we report on the first record of *F. enigmaticus* in two anchihaline caves which are located along the Mesozoic coastal carbonate rocks of the Mediterranean Sea, namely the Orljak cave in the Krka River Estuary (Croatia, E-Adriatic) and the Bue Marino cave in the Gulf of Orosei (Sardinia, W-Tyrrhenian Sea). The Orljak cave has an entrance *ca.* 50 m from the coast without direct connection with the Krka Estuary. The cave is 23 m deep and 90 m long with two pools. The Bue Marino cave with entrance above the sea level and direct connection with marine water has a well developed underground drainage. An almost horizontal profile and several pools characterises the three branches of this karstic complex with a total length *ca.* 20 km. In Orljak cave salinity ranges from 2 to 8 in winter, and from 7 to 13 during summer. Water temperature varies from 15 to 17 °C in summer and from 11 to 13 °C in winter. Salinity values in the Bue Marino range from 28.4-32.3 in summer to pure freshwater during winter floods, and water temperatures at the surface were 19-20 °C in summer. *F. enigmaticus* inhabits, in both caves, totally dark zones on rocky walls or submerged parts of speleothemes, of surfaces water layers until 1 m depth. The density is low with solitarily growing individuals, contrasting the typical gregarious growth of the species. Environmental conditions confirm the well known extreme adaptability of the species, also to live in the dark, and suggest its ability to survive, although intermittently, in freshwater. R.M. was supported by the Italian *Ministero dell'Ambiente* e della Tutela del Territorio e del Mare (MATTM), Ministero dell'Istruzione, Università e Ricerca (MIUR), and the EU program INTERREG Sardinia-Corsica-Tuscany on Biodiversity.

Oral presentation:

Stygobiotic oligochaetes in Poland with remarks on their occurence and distribution in Central Europe

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Despite that oligochaetes are very common in subterranean waters and usually form significant part of the benthic community in this habitat, knowledge concerning their diversity, ecological requirements as well as the distribution is only fragmentary.
The stygobiotic and stygophilic species from the families Lumbriculidae, Naididae (former family Tubificidae) and Enchytraeidae were found in subterranean waters of Central Europe. Lumbriculidae are represented by genus *Trichodrilus*; particular species were found in various kind of subterranean waters, including the springs. From the family Naididae only a small number of species is known. They belong to subfamilies such as Tubificinae, Rhyacodrilinae and Phallodrilinae, which has origin in a different aquatic environment. Family Enchytraeidae (mainly genera *Cernosvitoviella* and *Enchytraeus*) seems to be the most common in subterranean waters of Central Europe, but enchytraeids of this environment were studied almost exclusively in Hungary and Poland. The number of stygobionts or even the existence of such species among enchytraeids is not known because some of the species described from cave waters have wider distribution and the others are similar to surface species (aquatic or terrestrial). The diversity of stygobiotic oligochaete species is higher in karst areas than in regions of other geology due to the concentration of biospeleological studies in karst localities. Some of stygobionts have wide distribution, eg. *Trichodrilus cernosvitovi*, known from West- and Central Europe, the others are found in a few localities or even seem to be endemic for one karst region. In Central Europe the knowledge concerning stygobiotic species diversity and distribution is highly insufficient and more studies in subterranean aquatic environment are needed.

Oral presentation:

Groundwater oligochaetes (Annelida, Clitellata) of Slovenia

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Historical data on the biodiversity of oligochaetes inhabiting ground waters of Slovenia depict a fauna of approximately 50 species, 20 of which are stygobionts. In the last decades, new researches in Slovenian ground waters enabled us to study into more depth the oligochaete fauna of this environment. The studied material resulted from three main sources: a campaign in Slovenian caves conducted by Fabio Stoch, a large collection of groundwater fauna made available to us by Boris Sket, and samples collected during the European project PASCALIS. The data derived from the examination of this large amount of material enabled us to broaden the faunistic spectrum of oligochaetes of Slovenia, as well as to show that the oligochaete biodiversity in Slovenian ground waters is a substantial fraction of the European one. Endemic and very rare species constitute a remarkable proportion of the stygobiont oligochaete fauna. Among these, the genera *Rhyacodriloides, Cernosvitoviella, Parvidrilus, Trichodrilus* and *Haber* are some of the most outstanding taxa. In Pajsarjeva Cave, which was sampled many times over the past 15 years, the sporadic presence of some species, as well as the high rate of presence of stygobionts lead us to formulate a hypothesis about the relationship between the faunal data and the hydrogeology of the cave.

Oral presentation:

Annelid diversity in anchialine systems: unique adaptations and functional morphology of *Protodrilus* n. sp. to the cave environment of La Corona lava tube (Canary Islands, Lanzarote)

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After crustaceans, annelids are the second most diverse animal group in anchialine caves. Despite little attention paid to this group in early studies, the evidences of its high diversity has been recorded from several anchialine environments during the last 20 years, especially regarding interstitial species. Many of these records correspond to offshore forms inhabiting offshore habitats resembling caves, but stygobiotic species have also been described. Although all endemic, these stygobiotic taxa show in fact very diverse morphological adaptations. They range from typical interstitial species, morphologically similar to their offshore relatives, to highly modified meiofauna taxa with unique adaptation to drifting life style in the still anchialine water column at the caves. This holds also for several animals belonging to otherwise predominantly interstitial annelid families, such as Nerillidae and Protodrilidae. We here explore the unique functional morphology of *Protodrilus* n sp., an endemic species from La Corona lava tube (Lanzarote, Canary Islands). Musculature, nervous system, adhesive glands and ciliation were investigated by immunostaining and CLSM, SEM, TEM and LM. Motility, feeding activity and behavior are described from *in situ* and laboratory observations on live specimens (including video recording). These observations are compared to similar studies conducted in other species of the genus from coastal interstitial habitats with adaptations to the turbulent upper zone of the seafloor. The habitat of each species was characterized by measuring organic matter content, chlorophyll, salinity and sediment structure. Other cave species of interstitial annelids were compared to *Protodrilus* n. sp., emphasizing adaptive convergences among different lineages. These convergences are discussed in terms of the habitat of the species and compared to offshore relatives, in order to test the applicability of the current concept of troglomorphisms in Annelida. The role of historical and ecological processes on the origin of these taxa is briefly discussed.

Oral presentation:

Annelida, an often neglected component of groundwater ecosystems

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About 350 species and 100 genera represent the presently known contribution of the annelids to the groundwater fauna. Stygobionts account for about 1/3 of these species. The bulk of groundwater annelids are oligochaetes, and only a few hirudineans and polychaetes are found. Despite their frequency and richness, annelids are often ignored in the studies of groundwater diversity. This is partly due to their size, often quite small - so that they might be overlooked in the researches, and partly to the fact that their identification is difficult. We present a synthesis of the current state of knowledge about annelids, on their diversity, patchwork of endemicity and regional differences, and we underline their contribution to the diversity of the subterranean aquatic fauna. In the last decade, investigations of the groundwater fauna led to the discovery of a fauna with characteristic elements (such as the naidides of marine lineage and the stygobiont family Parvidrilidae), and of freshwater taxa showing an adaptive radiation in subterranean environments (such as *Rhyacodrilus* and *Trichodrilus*), or relicts of an ancient fauna (such as *Rhyacodriloides*). Extensive studies like the European PASCALIS Project allowed to prove the high species richness of subterranean annelid fauna of some European regions (namely Italy and Spain) and to examine the environmental gradients driving the distribution patterns of stygobiotic annelid assemblages. Habitat structure, water chemistry, anthropogenic pressure and historical factors are shown to influence the biodiversity patterns. In annelids, adaptations to the subterranean environments can be attempted by several different mechanisms, such as the body size reduction, the shifting or the asymmetrical bending of some genital organs, or the cyst formation in order to survive to habitat constraints.

SYMPOSIA

5. Countdown 2010 and Conservation of Subterranean Life

Oral presentation:

Subterranean habitats as wetlands of international importance

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The Ramsar Convention on wetlands (Iran, 1971) is amongst the oldest environmental conventions. It deals with globally threatened ecosystem types that are present also in karst. In 1990's, the Convention included karst and other subterranean hydrological systems as a wetland type and developed criteria for their inclusion in the List of Wetlands of International Importance. The main goal of including subterranean wetlands in the Ramsar List is to assist the conservation and wise use of subterranean wetland functions and values and thus implementation of Ramsar principles and strategic guidelines. In general terms, many "living" karst areas are wetlands, surface or subterranean. Both, direct or indirect development pressures are increasing and threatening ground waters and subterranean biota. Appropriate management, including conservation and sustainable use, is crucial to maintain the functions and values of the interacting karst surface and subterranean hydrological systems in the whole catchment area and to prevent or mitigate threats to karst wetlands. The Ramsar Convention can help on the one hand by fostering conservation and wise use of subterranean wetland systems in general, and on the other, by ensuring that examples of the most characteristic karst wetlands are considered and added to the List to conserve their values and characteristics, including unique and endemic biodiversity and specific hydrology. Guidelines based on cave fauna could be elaborated. Two examples from the Dinaric karst in Slovenia are examined for the purpose. Škocjanske jame are a karst underground water cave system developed in the area of Kras (i.e. the "classical" Karst). The main hydrological characteristics are the extremely high fluctuations of ground water level, moving water currents fed by rainwater, and pools of stagnant water. A typical example of a complex surface and subterranean karst wetland is the karst catchment area of the Ljubljanica River, including a series of intermittent lakes on karst poljes and water caves with underground rivers (Križna jama, Postojnsko-planinski jamski sistem) well representing the interaction and interdependency between the surface and subterranean wetlands belonging to the common hydrographical systems.

Poster presentation:

Conservation education of cave and subterranean biodiversity: Dubrovnik underground tales

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The example of field research, as a basis of activity of natural history museums and of the management of protected natural areas, is used in order to create educational publications for children of preschool and school age on the subject of protection of karst phenomena and subterranean biodiversity of the Dubrovnik region. For this purpose the results of interdisciplinary workshops for children were used to review their interests. The workshops consisted of lectures, movie screenings, use of speleological equipment, field work – cave visiting, and finally, as the summary, a creative element, the life-size reconstruction of a cave addressing the aspects of subterranean fauna. The workshop results pointed out themes of greater interest such as field research, use of speleological equipment and subterranean fauna. The idea is to adapt educational publications in accordance to the age group they are meant to appeal to. The publications will contain information regarding karst phenomena, legislation, protected natural areas and subterranean biodiversity. Also, for each of the species the publications are to contain the data relating to their distribution, their biotope, the threat to species as well as a visual depiction of them. Selected were those species that are described in the Red Data Books of the Republic of Croatia according to IUCN categories of threat, listed in legislation with a particular attention on the species significant to the Dubrovnik region. On the example of the story about explorers of the Dubrovnik caves, with the emphasis on the significance of the scientific research for the purpose of conservation of subterranean biodiversity, the aim is to create educational publications that will be both, a guide for the youngest Dubrovnik Natural History Museum visitors as well as a "Homework".

Oral presentation:

Protection of speleological objects in the region of Istria through the European Union projects

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Region of Istria, Department for Sustainable Development, Department for Nature and Environmental Protection

In order to protect sources of potable water, prevent their pollution, and reduce the harmful anthropogenic impact on karst relief in general, the Region of Istria

began planning on a project which would with the financial assistance of EU funds assure better and safer management of the speleological facilities and environment in general. Most of the territory of Istria is situated on limestone rocks. Most of speleological objects represent the direct connection between surface and underground water flows so their protection presents logical and very important activity for the entire community. The project "Underground Istria" is one of the most successful projects financed by the European Union which had its main objective in improvement of the speleological facilities status and their protection. Within the project 9 speleological objects were cleaned and sanitized, the database of speleological objects was created, together with numerous workshops and lectures for the local population and high school students in order to introduce the community especially young people with the importance of caves and pits as karst phenomena in the Istria. Considering that sustainability of karst is possible only by conduction of continuous scientific research and monitoring of their status Region of Istria applied a new "karst Underground Protection" project on the cross-border cooperation call Slovenia-Croatia 2007–2013 as logical continuation of previously mentioned "Underground Istria". Numerous new activities are planned within the two years of duration of KUP project and one of them are subterranean biology researches and the education of speleologists about the possible more scientific approach to the caving. Project will assure better cooperation and common operating of Croatian and Slovenian regional and state institutions involved in environment protection together with recognition of areas of natural landscape and the geographical particularities of the karst relief in Istrian peninsula. Many other activities with an objective of preventing further pollution of karstic aquifer are also proposed and should be implemented during KUP project. Complete inventory and proposed way of managing the speleological objects within the project area should assure their sustainability and reconstruction of an old abandoned school building in the village of Vodice in municipality of Lanišće and its conversion into so called "Speleo house" should provide the further development of the caving in Istria.

Oral presentation:

History of the presentation of the proteus (*Proteus anguinus*) in Postojna Cave

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The presentation of the proteus to the public and the development of Postojna Cave as a show cave are closely connected. The proteus (*Proteus anguinus*) is without a doubt one of the most charismatic animals from the point of view of the interpretation of the natural phenomena of the subterranean world. The history of

the presentation of the proteus goes back two centuries. Many visitors came to Postojna to see the proteus, not the cave – in the days before the discovery of its inner sections. It is also interesting to follow the history of the ways in which the proteus has been presented – since through its interpretation we gain an insight into the attitude of contemporary visitors and the cave management towards this subterranean creature. The first researchers shared the fundamental satisfaction of entering uncharted territory (like Luka Čeč when he discovered the cave) when they observed the proteus in the natural environment of the Black Cave. The occasional visitors from the beginning of the 19th century took part in romantic, mystery-filled visits to which a certain amount of prestige was often attached. These visits were complemented by the looting of stalactites and other cave formations and the purchase of a 'human fish', as the proteus was known. Mass tourism began to develop with the arrival of the railway, and in particular after the Second World War: a visit to Postojna Cave and the proteus became a programmed dramatic presentation where nothing was left to chance. A visit to the cave ceased to be a natural experience and became an urban, stage-managed event. The proteus was a constituent part of the visit, so the need for 'urbanisation' of the cave has also been reflected in the pools in which specimens of proteus have been presented in Postojna Cave over the last 50 years. Today, urban requirements have given way to nature protection regulations and legislation. First and foremost are conditions for presenting cave-dwelling animals to the public. The route of a standard visit to Postojna Cave is largely unchanged. Visitors do, however, have the opportunity of a more in-depth individual experience – either of specific sections of the cave system or of cave fauna. The trends that point to a future interpretational approach include the search for authenticity and the desire for exclusive experiences. There is also a yearning for the fundamental satisfaction of the original discoverers – that of seeing and experiencing something genuine, such as seeing animals in their natural environment. As managers of the cave, we are therefore looking for ways to bring the cave fauna as close as possible to different sections of the public, using modern interpretation methods and tools, and in the most sustainable manner possible.

Oral presentation:

Impacts of climate change on stygofauna in southwest Western Australia

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Southwest Western Australia has experienced an unparalleled climate shift since the mid 1970's, characterised by reduced rainfall which has contributed to

declining groundwater levels. Climate modelling attributes part of this change to atmospheric greenhouse gases, and predicts the drying trend will increase over coming decades. Groundwater pools and streams in limestone caves at Yanchep and the Leeuwin-Naturaliste region are habitat for assemblages of stygofauna associated with tree roots which grow in the cave pools and streams. Because of the declining water levels, these *Aquatic Root Mat Communities* were listed as Threatened Ecological Communities (TECs) (status Endangered) under the Federal Environmental Protection and Biodiversity Conservation Act. A study of the Leeuwin-Naturaliste caves and dependent stygofauna communities characterised their ecological relationships with hydrology, vegetation, rainfall, climate and other potential threatening processes. Radiometric dating and stratigraphic leveling of sediments were used to reconstruct a history of groundwater changes in Jewel Cave spanning the Early Pleistocene to Present. The lowest palaeo groundwater levels were recorded near the end of the Pleistocene (ca. 12000 BP), followed by generally elevated levels through the Holocene. Molecular genetic evidence from two species of crustaceans endemic to Jewel Cave suggests that the stygofauna survived *in situ*, the low groundwater levels experienced in the Late Pleistocene. In the last five years however, groundwater in Jewel Cave has declined below the lowest recorded Pleistocene limit, and all known occurrences of its stygofauna community have disappeared, and are presumed extinct. Recovery Plans prepared for the Leeuwin-Naturaliste and Yanchep TECs have met with limited success. Faced with a continued drying climate trend in southwest Western Australia, the future outlook for survival of the Leeuwin-Naturaliste communities, and other stygofauna in shallow limestone aquifers, is less than optimistic. The impact of a drying climate in this region is compounded by increasing extractive demands on groundwater resources associated with urbanisation in the Perth Basin. The coastal limestone aquifers, which occupy a narrow linear band and provide the most prospective habitat for stygofauna, are also most impacted by urban developments, reduced water quality and contamination, and potentially saltwater intrusion caused by pumping or sea level rise. Recently the Augusta-Margaret River Tourism Association (AMRTA) has instigated measures to control and manage the groundwater decline in Lake Cave, by harvesting rainfall to supplement groundwater recharge and sustain the cave lake, which is a major ecotourism drawcard. In tandem with this, a major study is underway to understand the hydrology and stygofauna in Lake Cave, with the ultimate goal of developing management strategies for coping with climate change.

Oral presentation:

Subterranean protection starts on the surface

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The Slovenian legislation ensures the establishment of protected areas with several laws, among which The nature conservation Act (since 1999) provides a legal framework and effective mechanism for planning and management of protected areas. The first initiatives for karst conservation were connected with the protection of caves. Some clearly defined suggestions for cave protection have been given (Badiura and Brinšek 1908, Pirc 1911) defining the necessity of protection of cave fauna and dripstone. The protection of underground caves with interesting cave fauna and flora was stated in the third part of Spomenica (1920) which represents the first Slovenian nature conservation program. Considering the lithology of Slovenia, with the majority of the bedrocks having carbonate origin, the majority of large protected areas are obviously also linked with carbonate surface. Protected areas of Slovenian karst landscape presents three quarters of all protected areas in the country which indicates the great nature conservation value of karst. A legal step towards the protection of all caves was defined, when the parliament declared The Cave protection act in 2004. This act defines protection and restricts the use of caves; it determines the protection regimes, protection measures and other rules of behaviour in caves. It also defines the minimal standard which each cave should fulfil to achieve a status of natural heritage. Generally speaking every cave whose length exceedes 10 meters is automatically given the status of natural heritage. Since 2004 some of the most important cave habitats are protected as a part of the European ecological network Natura 2000. An important legislation for cave conservation has thus been enacted, but it is still necessary to attain a more complex protection of the karst underground areas and thereby cave habitats. The problem of the protection of the karst underground can not be solved only on the basis of the conservation legislation. Mostly the problems of endangerment are linked with the pollution on the surface. Thereby, to achieve the protection of karst subterranean areas it is necessary to protect the surface within range of underground water. The system for the complex conservation should be based on the concept of interaction of all spheres of activity on the surface; urbanism, industry, agriculture, traffic, waste water purification.

Oral presentation:

Are stygofauna really protected in Western Australia?

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The protection of ecological communities within ground water, as distinct from groundwater-dependent surface ecosystems, is relatively novel in water resource management policy. Given the international focus on protection of ground water communities, it is pleasing to note that Australia, in particular Western Australia (WA), is considered among the scientific community as world leading in its recognition of the need to protect groundwater resources and their dependent ecosystems through water resource policy. In WA, there is considerable regulatory focus currently on the protection of groundwater-dependent stygofauna as competing priorities to develop groundwater resources for human use begin to be realised. Accordingly, in the past 10 years, collaboration between speleologists, scientists, and government institutions has been matched by increased regulatory focus - requiring commercial and industrial proponents to fund surveillance programs to inform proposals undergoing Environmental Impact Assessments (EIA) by the Environmental Protection Authority (EPA). Yet, contrary to external perceptions, the collaboration has not been without its difficulties due to inconsistent application of the EPA guidance by administrative authorities and a general uncertainty over a proponent's legal obligations towards protecting stygofauna. During the years 2005 – 2009 I investigated whether the regulatory framework in WA, ostensibly designed to protect stygofauna, really achieves that objective – specifically in the context of abstraction of ground water for the town of Exmouth in northern WA. Particularly, I sought to unravel the confusion over legal and regulatory responsibilities towards stygofauna conservation and clarify the true statutory protection afforded to these animals under national and WA State laws. Here, I discuss: (i) an overview of historic and present-day Commonwealth and WA State legislation providing directly or indirectly for protection of stygofauna; (ii) inconsistencies and/or limitations of administrative and policy documents used to regulate stygofauna conservation; and, (iii) avenues for improving links between regulatory, scientific and societal groups to better protect stygofauna in Australia.

Oral presentation:

How efficient are the non invasive protection measures in the Segeberg Cave in Northern Germany for the population of bats and subterranean beetles

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The natural monument Kalkberg, which includes the Segeberger Cave, the gypsum hill and the Small Segeberg Lake, is intensively influenced by human. If measures of protection are necessary, the Kalkberg Stadium, the show cave and the cave as a hibernating place for more than 22000 bats and the habitat for the endemic cave beetle *Choleva septentrionis holsatica* must be considered. During the last three years such measures of protection and the modernization of the lighting with participation of experts were planned in the underground gypsum show cave, in cave passages not open for the public, and on the overground rock faces of the hill. Most of these measures are already realised. Thus it was possible to minimize the effects of primarily planned geotechnical measures. The focus was on non-invasive techniques to assure to protect the ecosystem.

Poster presentation:

Porifera checklist and database of Mediterranean marine caves

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Conservation measures and protection planning of marine caves are promoted by the EU Habitat Directive. Porifera represent one of the main taxa in cave-dwelling sessile benthic assemblages. In this framework we report on a preliminary biodiversity inventory of sponges from Italian caves, based on the literature review. New data from recent faunistic surveys carried out in some submerged karstic caves of southern Italy (Sardinia and Sicily) are also reported. This contribution is the starting point for the creation of a Porifera database for Mediterranean marine caves. The work was supported by Italian MATTM and PRIN-MIUR, EU project Interreg III Sardinia-Corsica-Tuscany, Fondazione Banco di Sardegna and Regione Autonoma Sardegna.

Poster presentation:

Working for the candidate Orosei Marine Protected Area (central-east Sardinia): on a benthic community from a subterranean estuary in a karstic coastal cave

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The Orosei Gulf is presently candidate for the establishment of a new Marine Protected Area. Along the coastal karst, the Bue Marino Cave represents one of the most attractive units of natural heritage needing particular regard in terms of biodiversity conservation for its diversified endemic fauna (mainly Arthropoda) from both the terrestrial and freshwater habitats. The cave is characterised by the presence of a subterranean river flowing along a necklace of large subaerial freshwater/brackish water pools up to the cave entrance. Here we report on the discovery of a conspicuous sessile filter-feeders assemblage in a totally dark myxohaline pool. The community structure seems to be based on *taxa* displaying adaptive strategies (cryptobiosis by resting bodies; euryhalinity) to survive in extreme environmental conditions (intermittent freshwater aquifer activity/marine ingression). The research was supported by Italian Ministero dell'Ambiente (MATTM, Studio degli ambienti di grotte marine sommerse (Codice Habitat 8330) nelle Aree Marine Protette di Pelagie, Plemmirio e Capo Caccia), Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MIUR-PRIN), EU Interreg III Sardinia-Corsica-Tuscany, Fondazione Banco di Sardegna and Regione Autonoma Sardegna.

Oral presentation:

Biospeleological research in the Amazon: the case of Planaltina cave in the ecoregion Xingu-Tapajós, Brazil

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There are more than 1000 not yet systematically studied caves in the Northern region in Brazil. The Pará State has at least 467 caves registered by the National Center for Study, Protection and Management of caves (CECAV). Two decades ago five caves from Speleological Sandstone Province Altamira-Itaituba were

studied by Trajano´s group and among them the Planatilna cave appears to be the biggest sandstone cave in Brasil (1500m of length) and is characterized by high populational densities if compared with the other Brazilian caves. Planaltina shows a great production of guano dispersed by massive bat colonies which depends upon the neighborhood Amazon forest resources. We carry out several expeditions in order to evaluate the biodiversity status and populational aspects of the Planaltina cave fauna. Samples were also collected for microbiological analyses from guano/soil/river bed/wall from cave; ph, temperature were registered. The population of some macroinvertebrates was counted along seven transects. The average pH and average temperature were 4 and 26 °C, respectively. The number of colonies formed by microrganism per gram was from 10^4 to 10^7, the highest density from guano and lowest from riverbed. Among vertebrates, characiform fish (*Erythrinus erythrinus*, Erythrinidae) showed various levels of pigmentation. The size of cricket population was estimated at 11264 individuals with an average density of 1.37 ind./m^2, and the size of cockroach population at 3075 with 0.37 ind./m^2. These numbers were lower than 22 years ago. The great level of actual deforestation in the regioned by the construction of the Rodovia Transamazônica could be considered a source of great environmental impact to the caves in the area. Therefore, the biodiversity conservation of those subterranean habitats is in a fragile equilibrium with the integrity of the Amazon forest; its equilibrium is been threatened by the deforestation front and now by projects of construction of several dams in the Ecoregion Xingu-Tapajós.

Poster presentation:

Design of a GIS database to monitor possible threats to the habitat of *Proteus anguinus* (Amphibia: Proteidae). A case study of a highly vulnerable population of *P. a. parkelj* in Bela krajina, Slovenia

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The population of pigmented, black *Proteus (Proteus anguinus parkelj* Sket & Arntzen, 1994) is, by no doubt, the most distinguished of all *Proteus* populations. Due to its most limited habitat in the karst hinterland, namely only three springs in Bela krajina, SE Slovenia (less than 50 km^2), even a local pollution could have a devastating impact on the whole population. Since it was discovered in 1986, a considerable amount of data on geology, hydrology and geomorphology has been gathered; also, the presence and accumulation of heavy metals and other pollutants, from agriculture and industries, has been well documented. All this

data should be put together in a coherent database, based on the local hydrogeological conditions where *P. a. parkelj* lives, as well as on the identification of those areas of natural and anthropogenic conditions, affecting its habitat. The goal of this study is to produce a customized GIS data model in order to examine and organize the physical aspects of the hydrogeological system of the habitat. It will comprise the existing processes and objects in the karst landscape, leading to the accurate mapping of the area where *P. a. parkelj* is present. This model is designed first in a conceptual scheme, developed with the help of UML (Unified Modeling Language) – having a high flexibility to be further integrated within the GIS software (ArcGIS 9.3.1 will be used in our case). The model will provide a useful analytical tool to better understand the habitat of *P. a. parkelj* and to provide the framework to model surface and subsurface events that could influence its population. This could guide nature conservation actions against arising threats in the future.

Oral presentation:

Biospeleological component of the project KUP (Karst Underground Protection) in Istra Peninsula

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Nearly 70% of the Istra Peninsula, belonging to Croatia, Slovenia and Italy, is situated on limestone rocks, showing typical Karst character with underground water flow and many Karst phenomena. More than 2000 caves are known in the karstic parts of Istra. Caves are inhabited by many endemic, rare, endangered and protected animal species, as: *Istriana mirnae, Niphargus echion, Thaumatoniscellus speluncae, Eupolybothrus obrovensis, Verhoeffodesmus fragilipes, Troglochthonius doratodactylus, Leptodirus hochenwartii, Croatodirus bozicevici, Prospelaeobates vrezeci, Pauperobythus globuliventris, Proteus anguinus* ssp. n. still not systematically explored and evaluated. Unfortunately, due to many reasons, caves and, especially water cave habitats together with their fauna are endangered. Within the project KUP, financed by OP IPA Slovenia-Croatia programme, lead by the Region of Istria with the *Natura Histrica* as Croatian and ZRC SAZU – Karst Research Institute, Postojna, as Slovenian partner, adequate protection of Istrian Karst with biospeleological research and accompanied activities is envisaged. The goal is to evaluate the diversity and core populations of troglobionts; to recognize and register potentially new underground species; to define and evaluate ecological conditions of selected habitats, 6 in Slovenia and 6 in Croatia; to educate local

population on the importance of underground fauna and its protection together with their environment; to educate speleologists about cave fauna in a way of popular science and to publish scientific and popular articles about cave fauna in Istra. The overall objective of the two year project, started in 2010, is protection and improvement of the caves as a phenomenon in the border area of Istra region between Slovenia and Croatia. The implementation of the project will provide cross-border cooperation of institutions responsible for Karst research and monitoring, as well as its improvement. The greatest value of project is the establishment of a joint supervision of the Karst area, which extends to the territory of both countries, and also establishing a biospeleological data base for the Istra region.

Oral presentation:

Subterranean biology of mainland Portugal: historical review and new insights

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In Portugal more than 3000 caves and several types of subterranean habitats are known, but until today the subject of biological studies have been mostly the caves and the freshwater aquifers. A historical review of the Portuguese hypogean fauna since the first written record in 1870 until today has been carried out, concluding that 19 troglobionts and 62 stygobionts are known. The knowledge of the subterranean fauna in the karstic areas was scarce and mainly based on studies made during the last century. Though this subterranean fauna has been considered moderately poor, there are several highly interesting hypogean species considered as relicts. Since 2006, a new biological prospection has been accomplished in more than 30 caves of 13 different karstic regions, which revealed an interesting cave fauna and the existence of new hypogean species of high scientific value. During 2009 the cave fauna and environmental parameters were monitored seasonally in 15 caves distributed along 500 km and comprising 9 different karstic regions. The present communication provides the interim results on their biodiversity and seasonal activity, reporting the discovery of 17 new troglobionts and a clear bloom in troglobiont abundance during the spring. Our results confirm that the terrestrial hypogean fauna on karstic regions is ascribed to two subterranean main biogeographic districts: the Lusitanic covering the major part of Portugal, and the Baetic whose greatest extent is in Spanish Andalusia but also includes the Portuguese Algarve to the west. Severe problems of reduction of the subterranean habitat as a result of limestone quarries and the impact of human pollution can

lead to the extinction of this important biodiversity. The protection measures for subterranean species are clearly insufficient and there is an urgent need to set rank priorities for conservation, since the resources are not enough to protect hypogean spaces in karstic regions.

Oral presentation:

An environmental impact assessment of spent calcium carbide disposal in caves and mines

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We studied the environmental impact of wastes derived from calcium carbide, which is widely used for generating acetylene in industry and speleology. It was shown that spent carbide is toxic for biota and harmful to cave ecosystems and the surrounding environment. The toxic components of spent carbide waste were found to include calcium hydroxide, strontium and polycyclic aromatic hydrocarbons. The toxicity of spent carbide declined only slowly over time, with toxicity still present in 13-year-old samples. Spent carbide should be disposed of with great care to ensure that it cannot be disseminated into natural water systems.

Oral presentation:

Conservation of cave invertebrates and study of impacts on caves located in the Brazilian Atlantic rain forest

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The Brazilian Atlantic rain forest extends over great extensions and includes discrete caves. Through historic (295 caves) and present data of 103 caves, the study is aimed to evaluate the knowledge on invertebrates cave fauna of the Atlantic Forest, to evaluate the main antropic changes, and propose a methodology to evaluate the biological relevance and vulnerability of caves. Historical data indicate presence of 208 troglomorphic species, contrasting with 95 species found in this study. The similarity observed between the cave invertebrate communities was not higher than 30%. The anthropogenic changes were deforestation (40%), mining (15%), inorganic waste (13%), trample (12%),

touristic usage (10%), constructions (10%), graffiti (8.5%), exotic species (8.5%) religious usage (7.5 %). Vulnerability level was considered extreme for 7% of the caves, high for 38%, average for 26%, and low for 29%. Among the caves visited in this study, 47% need emergent conservation and management plans. The karst areas classified as richer in troglomorphic species were considered to be of major priority for conservation actions. Such areas correspond to the limestone caves of the Chapada Diamantina (9 spp.), the limestone caves of the Serra do Ramalho (7 spp.), the limestone caves of the Parque Nacional Cavernas do Peruaçu (10 spp.), the limestone caves of the Rio Pardo karst (18 spp.), the quartzite caves of Parque Estadual Serra de Ibitipoca (9 spp); the ferrugineous caves of the quadrilátero ferrífero (68 spp.), the limestone caves of the south of São Paulo state and northeast of Paraná state (62 spp.) and the limestone caves of the Serra da Bodoquena (9 spp.). The caves sampled up to the present moment have presented heterogenous ecological conditions (trophic structure, endemism, number of invertebrate species, and impacts registered). Such heterogeneity justifies the necessity of urgent and more detailed studies, including as many caves as possible (located in the Atlantic Forest), for a better understanding of underground biodiversity, subsidize plans of use and actions of management and conservation.

Oral presentation:

Comparisons of subterranean biodiversity from the West Kimberley Karst, Australia

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The Devonian Reef Complexes of the West Kimberley, in northern Australia, contain numerous endemic communities of relictal fauna including both troglomorphic and stygobitic representatives from several key groups. These fauna are contained within a diverse range of habitats, which have only recently begun to be systematically investigated for subterranean biology, mainly due to the remote nature of the karst. Several distinct limestone ranges which have been investigated include the Napier Range; and to successively lesser extents, the Oscar, Geikie, Lawford, Laidlaw, Hull and Pillara Ranges. Access to areas of the karst that maintain elevated humidity during the 'Dry Season', when surveys can be safely performed, have been a limiting factor for representative sampling success. This paper will use the biological diversity and endemism from Tunnel Cave, which includes four endemic species to highlight the need for further collecting and research in this important Australian karst region. The endemic fauna of Tunnel

Cave currently includes *Cheridiidae cheridium* (Pseudsoscorpion), *Bamazomus hunti* (Schizomid), *Kimberleydillo waldockidae* (terrestrial isopod) and *Tainisopidea*, *Tainisopis sp.* (aquatic isopod). This cave is subject to high seasonal visitation during the Dry Season due to its large size and historical importance. As a result there are several cave management issues with regards to subterranean biodiversity. These include, trampling of habitat, rubbish and food being left in the cave, and disturbance to bat populations, which reside in the cave. These issues will also be discussed in relation to the cave's significant subterranean biodiversity values. The diversity of Tunnel Cave will also be compared at a broad scale with the subterranean ecology from other sites nearby in the Napier Range. The recent progress that has been made is hoped to further inspire continued investigation into this vastly unexplored region.

Poster presentation:

Management of a declining watertable at Yanchep National Park, Western Australia; for the benefit of subterranean biology

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In 1997 Jasinska documented the biodiversity and ecology of cave streams from Yanchep National Park (YNP); revealing an extremely rich root mat ecosystem, containing Short Range Endemic species and Gondwanan relicts. These cave streams of the Swan Coastal Plain, are driven by the hydraulic head of the Gnangara Water Mound and are now severely depleted, as the watertable has dramatically lowered at a regional scale. This has been attributed to reduced rainfall, increased human abstraction rates and broad scale catchment interception from pine tree monocultures. These factors, being beyond the immediate control of managers at YNP, have resulted in various rescue strategies to be implemented over the course of this continuing water decline. The most recent and currently ongoing of these strategies, involves the mass pumping of water for the creation of localized artificial water mounds, to allow for cave stream recolonisation. This latest strategy has encountered a plethora of implementation problems and has been from the onset, a contentious subject regarding the sustainability of resource use towards this elusive goal. Despite this the project is progressing and will undoubtedly continue to inspire debate regarding the value of our resources, both natural and anthropogenic into the future. We seek to highlight the ecological changes that have occurred in YNP in context of the water regime and the measures undertaken to preserve cave stream ecology.

Poster presentation:

Technical illustrations and application: Škocjan Caves, Velika Dolina cross section

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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. In this regard, a very important task is raising visitors' awareness of the importance of protecting endangered and protected plant and animal species. We therefore need to properly interpret scientific data and findings, and adapt and present this information so that it can easily be understood by the general public. In our case, we decided to use technical illustrations to present some of the most important species, with an emphasis on the aboveground and subterranean karst world. So far, twenty-seven stygobiotic and troglobiotic organisms have been discovered in Škocjan Caves. Epikarst fauna (i.e., Copepoda) is particularly abundant. Moreover, numerous troglophiles 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). A total of twenty-three students and thirteen lecturers and mentors participated in our project; activities included workshops in Škocjan and at the Academy of Fine Arts and Design (University of Ljubljana), and the Kaverljag Workshop. Over fifty illustrations were produced, illustrating ten troglobiotic and three troglophile organisms. These organisms have been incorporated into the illustration of the Velika Dolina cross-section with part of Škocjan Caves. The illustrations are accompanied by short texts explaining individual habitats, from karst forests, dry karst meadows, and steep cliffs to the bottom of dolines and the subterranean world. The illustrations are an attractive tool for interpreting the area's natural heritage and biodiversity. This manner of presenting natural heritage is attractive and technical enough for people of all ages. Younger visitors are introduced to the plant and animal species, and visitors seeking more information can read the names of interesting species, learn about their habitats and ecology. One of the main goals of the publication is to raise awareness about the wealth and diversity of flora and fauna, as well as their vulnerability.

Poster presentation:

Food webs in Mexican Carribean Caves

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Anchialine ecology is needed for both decision-making and conservation strategies establishment of these vulnerable ecosystems. The Mexican Caribbean coast requires special attention due to explosive urban expansion in response to tourism development. On the other hand anchialine research is young and quite challenging so food webs studies are scarce in anchialine systems and the lack of baseline make comparisons difficult. Stable isotopes are a powerful tool and an informative starting point to elucidate the origin of organic matter and diets in anchialine ecosystems. Along with stable isotopes, nutrients concentrations, pH and dissolved oxygen will help to elucidate trophic dynamics between pristine caves and those with an anthropogenic signal. Historic and recent stable isotope studies in Mexican Caribbean coast show variation within and among species, and between different areas, suggesting variation in the sources of organic matter input into the anchialine systems. Results from an anchialine cave in Mexico show huge variations. Atyid shrimp show variations of d13C >15 ‰ and d15N >10 ‰, amphipods show variation of d13C 9 ‰ and d15N >10 ‰. Diet assessment using gut content and mixing equations for stable isotopes, indicates that omnivory is a strategy in Remipedia. The same cave shows the world's highest abundances of Remipedia (Crustacea). Remipeds censuses have been conducted in 2001, 2007, 2009 and monthly during 2010.

SYMPOSIA

6. Microbiology and Geomicrobiology

Poster presentation:

Bat guano – a potential biohazard agent of caves in the temperate zone?

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In temperate climatic zone in Europe insectivorous bats often roost in natural underground cavities. Big summer colonies composed of several thousands individuals are not very frequent. Still, in some caves bats produce relatively large quantities of guano. Guano represents a suitable milieu for growth and propagation of different organisms. The first literature documentation in Europe with reliable identification of a human pathogen, fungus *Histoplasma capsulatum* from bat guano from a cave is dated in 1966 (Topolnița Cave, Mehedinti County, Romania). In this cave the average temperature is 11.5 °C, and 13.7 °C in Guano Chambers. The colonial bat species in Topolnita Cave are *Rhinolophus ferrumequinum, R. euryale, Miniopterus schreibersii, Myotis myotis/oxygnathus*). In agreement with some indications, *H. capsulatum* might be present also in other caves in Romania, for example in Adam Cave, placed near by Topolnița Cave, which is a thermal influenced cave with an average air temperature of 27 °C and colonized with the same bat species. Compared to endemic areas in the Americas, in Europe the incidence of histoplasmosis originating from cave was never studied in details. This can be attributed to several reasons: (i) absence of huge bat colonies in Europe, (ii) low cases of identified histoplasmosis as its symptoms can be easily misinterpreted and are ranging from simple mild flu-like till fatal, (iii) low awareness among physicians of cave-associated histoplasmosis and lack of epidemiological studies linked to histoplasmosis emerging from underground environments in Europe, and (iv) insufficient awareness among cavers and other cave visitors. In this study the relevant literature on histoplasmosis incidence in Europe and the potential use of molecular biology to identify *H. capsulatum* without its cultivation were reviewed, and guidelines to avoid contacts with airborne pathogens in the underground were prepared. Furthermore, results on microbial quantification and potential biohazard of airborne microorganisms in the "suspicious caves" are presented.

Oral presentation:

Creating humic matter indices for the interpretation of ecosystem energetics

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Cave and karst ecosystem energetics are an important factor in understanding the microbial processes in aquifers. Determining the flux of dissolved organic matter (DOM), a source of organic carbon for heterotrophic microorganisms within an aquifer, from the surface into the subsurface is one method of tracking ecosystem energetics. The relative contributions of different sources of DOM can be evaluated using excitation-emission matrix (EEM) fluorescence spectroscopy since no photodegradation of OM occurs in cave and karst waters. This method can characterize DOM at natural, low abundance levels, and can differentiate recalcitrant DOM (e.g., humic substances) from more biologically labile material (e.g., proteins, peptides, and amino acids). Consequently, in karst environments, the influence of surface derived (allochthonous) DOM can be distinguished from autochthonous DOM produced by microbial chemolithoautotrophy. Karst environments are heavily influenced by surface water recharge and allochthonous DOM. Autochthonous OM in karst is poorly understood because it is unclear how microbes contribute to DOM types and abundances, as either primary DOM sources or during degradation processes of allochthonous material. It is important to differentiate allochthonous from autochthonous humic matter for the interpretation of cave and karst ecosystem energetics. Autochthonous humic matter is often overshadowed by allochthonous matter; meaning allochthonous humic matter fluoresces brighter than autochthonous matter. In this study, our aim was to create an index of humic/fulvic acids and protein (tryptone) mixtures to help differentiate the overshadowing effects of the brighter humic substances by using difference mg/L concentrations of tryptone, Suwannee River Fulvic Acid (SRFA), and Pony Lake Fulvic Acid (PLFA), and different mixtures of these standards. Although the SRFA and PLFA standards mixed in different ratios with the protein tryptone will be less complex than natural cave waters, we will be able to see if there is any substantial fluorescence overshadowing of tryptone by SRFA and PLFA. This index, coupled with a detailed analysis of microbial communities in specific subsurface environments could allow for a greater understanding of microbial processes and metabolisms within the subsurface.

Poster presentation:

Chemoautotrophic microbial mantle prevalence in Murra El Elevyn: catastrophic decline or seasonal fluctuation?

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The Nullarbor Plain of Western Australia is a plateau of horizontal Eocene and Miocene karst, home to numerous extensive caves flooded with brackish water. In 1999 chemoautotrophic microbial mantles were recorded in Murra El Elevyn, and samples collected in Weebubbie and Warbla caves. The temperature in Murra El Elevyn was 23.7 degrees Celsius, and in nearby Tommy Graham's cave it was 23.1 degrees. One year later return visits were made to Weebubbie and Warbla caves, and divers reported limited re-growth of 1-2cm where samples had previously been collected. In September 2009 microbial mantles were again recorded in abundance in Murra El Elevyn cave, hanging 20-30cm long underneath ledges and covering the rubble below. The temperature was recorded to have fallen to 18.9 °C. On a return visit six months later, after the dry Australian summer, divers found substantially fewer mantles, the largest of which was a mere 2cm long. Water temperature was 19.3 °C in Murra El Elevyn and 23.4 °C in Tommy Graham's cave. Meanwhile, the mantles at Weebubbie and nearby Olgolwin caves remained abundant. Given the rapid decline over six months in the prevalence of microbial mantles in Murra El Elevyn alone, we postulate two potential scenarios. Firstly, that localised falling average water temperature has transformed Murra El Elevyn into an inhospitable environment (e.g. altered water chemistry), causing the catastrophic demise of microbial mantles in that cave. Alternately, with notably different rain-driven dissolved nutrient ingress to Weebubbie and Warbla caves, the otherwise morphologically similar mantles in Murra El Elevyn have evolved an annual, seasonally regulated lifecycle and are, thus, relatively faster growing than has been observed in other Nullarbor caves. Further research is underway to monitor this previously unreported phenomena and to establish which, if either of these possibilities, is likelier the cause.

Poster presentation:

Identification of tannase producing fungi species in Brazilian caves

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Caves are environments that might favor the development of microorganisms due to their environmental characteristics. However, cave microbiology is still poorly studied in Brazil. The biodiversity of subterranean microbiota is still unknown, as well as the biotechnological importance of such organisms. Understanding the importance of preserving cave microbiota, as well as its potential use, may function as an important tool for cave conservation. Tannase (tanino acil) hidrolase EC 3. 1. 1.20 is an extracellular inducible enzyme of great biotechnological interest. It hydrolyses ester and depside bonds present in gallotannins, complex tannins and gallic acid esters. It is mainly used in beverage, tea, fruit juice and food processing. This enzyme is also of environmental importance since it acts as a hydrolyzing agent in cleaning up the highly polluting tannin from the effluent of leather industry. Micorganisms are important source of tannase, such as species from the Aspergillus and Penicillium genus. The objective of this study was to search for isolates of biotechnological interest (tannase producers) present in caves located in the Brazilian biome named Caatinga (Brazilian xeric shrubland). With this information it is possible to provide new tools that might be used for conservational purposes. The enzymatic production (tannase) by filametous fungi was tested in specific media. The tannase producers were identified up to the species level. They were isolated in specific media, incubated for seven days and then identified according to their macroscopic and microscopic morphological characteristics. From all the isolates tested, nine tannase producing species were identified: six *Aspergillus* species (*A. tubingensis*, *A. pumiceus*, *A. japonicus*, *A. tamarii*, *A. foetidus* and *Aspergillus* sp.) and three *Penicillium* species (*P. oxalicum*, *P. corylophilum* and *P. sclerotiorum*). Only few of these species have already been reported as Tannase producers. This result provides important information pointing caves as environments holding species of biotechnological potential. The identification of these species highlights the need of more studies concerning cave microbiota and the importance of preserving these environments.

Oral presentation:

Microbial observatory of Spanish caves: assessing the origin of fungal outbreaks

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The last decade has seen a progressive colonization of visitable caves by microorganisms. The caves of Lascaux, Montignac, France, and of Castañar de Ibor, Cáceres, have suffered outbreaks of *Fusarium solani*, a fungus that has also been found in the air and sediments of the Doña Trinidad Cave, Ardales, Málaga. This work sets the bases for the creation of a Cave Microbiological Observatory for controlling the fungi present in the ecosystem and the timely detection of outbreaks that could compromise the integrity of the cave and any cave paintings present. At the same time, it is intended to study the relationships between the fungal community and cave inhabitants (insects, rodents, etc.), as many of the fungi present are parasites, and use the inhabitants to enter the cave and disperse in it. The execution of this study entails monitoring of the cave air, and the use of molecular techniques such as the amplification of gene sequences of 18S and ITS ribosomal RNA to identify the members of the fungal communities. Knowing the processes of spore dispersion and modelling it taking into account air currents and temperature gradients, the eventual colonization of different materials by microbial communities, the trophic nature of the latter, and the relationships between the different inhabitants of the cave, should enable the design of a control strategy to guarantee its conservation.

Poster presentation:

The heavy metal content in bat guano heaps in karst caves

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Compared to surface habitats, caves are frequently nutrient-poor. The main source of carbon in caves originates from percolation water, sinking streams and droppings of cave animals. Bat guano is one of the most important food sources

for cave invertebrates; however data on ecology and its degradation are still poor. Fresh bat guano contains a large quantity of chitin residues as fragmented and non-fragmented butterfly/mosquito scales, insect wings, hairs of bats and pollen. Recent studies of guano heaps from Domica Cave (Slovak Karst, Slovakia) showed that fresh bat guano (0-11 years old) had low pH (3.2) and contained high concentrations of heavy metals (Cd, Cu, Zn). Bat guano (with and without harboring an active bat colony) from two caves from Slovenia (Škocjanske jame I, II and Predjama) were used as reference material for guano from Domica Cave. All caves are populated by the same insectivorous bats *Miniopterus schreibersii* and in Domica Cave by *Rhinolophus euryale* in addition. Guano without an active colony of bats had higher pH (4.5 in Škocjanske jame I and 6.1 in Predjama) compared to the fresh guano in Škocjanske jame II (pH 3.5). Guano samples (layer 0-5 cm) from Domica Cave, Škocjanske jame I, II and Predjama contained (in mg per kg): 207 - 795 Cu, 167 – 1360 Zn, 0.81 – 11.8 Cd, 0.2 – 1.8 As, 2 – 48 Cr, 2 – 25 Pb and 0.3 – 0.5 Hg. Values some of these heavy metals in the guano samples exceeded EU limits for agricultural soils (EC Document 86/278/EEC): Cd 3-4 times, Cu 2-6 times and Zn 4 times. Extremely high amounts of heavy metals in guano can be a reason that chitinolytic activity of microorganisms in guano in many caves is inhibited or even stopped and thus guano remains preserved in caves for a long period.

Oral presentation:

Airborne microorganisms and relation to atmospheric parameters in big cave systems (Postojnska jama, Slovenia)

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Air represents an important habitat and intermediate stage in propagation of microorganisms. Airborne microbes and influences of atmospheric parameters to their distribution, seasonal variability and counts were studied in the Postojnska jama cave system. The study included measurement of temperature, relative humidity, CO_2, air pressure, total dust concentration, DNA concentration, and cultivation of airborne microbes on group specific media (bacteria, fungi, algae, amoebae) and subsequent identification of free-living amoebae (FLA). In the cave atmosphere, the highest variations of atmospheric parameters were attributed to CO_2 and dust concentrations, 3-times or 2-times higher in the summer period. Similarly, using a depositional sampling method, higher DNA concentrations were detected in summer compared to winter e.g. 68 ng DNA/cm^2 and 42 ng

DNA/cm^2, respectively. Airborne microorganisms were sampled using an Air Sampler Mas-100 (Merck). Inside the cave system bacterial viable counts were more stable throughout the year (34-41 cfu/m^3) compared to airborne fungi (40-195 cfu/m^3). Air flow from cave exteriority brings inside the cave viable algal propagules, as expected the highest number was detected in the cave entrance (1-4 cfu/m^3). R squared statistics was applied to explain the proportion of variability in microbial count vs. atmospheric parameters. Each atmospheric parameter individually did not contribute significantly (max<0.54) to the global trend of microbial counts. Air in huge cave systems like Postojnska jama is subjected to mixing due to natural air and river flow, and tourist activities caused by tourist trains and various walking tours in the cave. However, bioaerosol analyses out of the main cave passage revealed existence of more stable atmospheric conditions and microbial counts throughout the year. Sampling with the impactor of maximum 1 m^3 of air revealed no FLA, but when depositional sampling of open Petri plates was adopted, similar dynamics of airborne FLA was observed compared to other microbial groups; with the peak in the summer period. The most prevalent FLA were acanthamoebae, hartmannellids and vahlkampfiids. All acanthamoebae were genotyped and almost all isolates belonged to genotype T4. Other amoebozoans found very frequently were mycetozoans, mostly dictyostelids.

Oral presentation:

Cave microscopic fungi as food source for caves inhabiting springtails and some microfungal records

Alena Nováková

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Records of saprotrophic microfungi are reported from a number of Czech, Slovak, Romanian and Spanish caves. Of them, several species represent coprophilous fungi occurring on various types of animal excreta such as bat guano and bat droppings, marten or dormouse excrements or isopod and diplopod faeces (*Penicillium glandicola*, *P. vulpinum*, *Chrysosporium speluncarum*, *Chaetomium brefeldii*, and *Phycomyces nitens*). Additional coprophilous species, *Coemansia aciculifera*, was isolated from cave sediments. *Botryosporium longibrachiatum* was isolated from the frog carcass in the Domica Cave system (Slovakia). Rarely reported microfungal species *Dimargaris bacillispora* was found repeatedly in collembolan rearings on the cave sediment from the Domica Cave system and the Punkva Caves (Czech Republic), but also on dead isopod *Mesoniscus graniger* in laboratory rearing and from *C. aciculifera* growth after one month exposition of agar disc with *C. aciculifera* colony on the cave sediment in the Domica Cave. Sixteen microfungal species isolated from cave sediment of the Domica Cave

(*Paecillium lilacinum*, *Clonostachys rosea* f. *rosea*, *Cladosporium herbarum*, *Mucor dimorphosporus*, *Absidia glauca*, *Coemansia aciculifera*, *Talaromyces flavus*, *Myxotrichum deflexum*, *Mortierella* sp., *Isaria farinosa*, *Doratomyces stemonitis*, *Oidiodendron cerealis*, *Fusarium solani*, *Trichosporon* cf. *pullulans*, and *T. dulcitum*) were used in food preference test with four collembolans, *Folsomia candida*, *Heteromurus nitidus*, *Hypogastrura aequepilosa* and *Orthonychiurus rectopapillatus*. The test was carried out in 20 cm Petri dishes covered with damp layer of Plaster of Paris. The food was offered in form of agar discs cut from 7 days old microfungal colonies, each disc having been put into a separate sector. Twenty individuals of each collembolan species were then placed into the central part of Petri dish. The presence of springtails on the food and their grazing activity were recorded daily for a period of 10 days. At the beginning of the experiment, some fungi (e.g. *T. dulcitum, T.* cf. *pullulans* and *C. aciculifera*) were significantly preferred, while several species were ignored completely (*D. stemonitis, T. flavus*, and *C. rosea* f. *rosea* by *O. rectopapillatus*; *T. polysporum and C. herbarum* by *F. candida*; and *Mortierella* sp. by *H. nitidus*). *D. stemonitis*, *O. cerealis* and *I. farinosa* were preferred by some springtails only in advanced stages of the experiment. Nevertheless, there were strong differences in food preferences among individual collembolan species.

Poster presentation:

Microscopic fungi isolated from several caves in Romania

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Saprotrophic micromycetes were investigated in several caves located in the Bihor Mountains, the Padurea Craiului Mountains, and in the Dobrogea region. Airborne microfungi from outdoor and cave air were studied from the point view of colony-forming unit (CFU) numbers and species diversity. In addition, samples of cave sediment and other substrates such as bat guano, animal excreta, and visible microfungal colonies were collected from all visited caves. There were marked differences in CFU numbers among individual caves, the highest CFU numbers having been estimated in the Fânaţe Cave, probably due to the extensive bat colonization. In the Urşilor Cave, differences in CFU numbers were found between of airborne microfungi isolated during the day (tourist time) and those isolated during the night.

Oral presentation:

A newly discovered cave foodweb: the *Cansiliella* story

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Cansiliella is a troglobitic beetle of the Bathysciinae (now Leptodirinae), endemic in a few hydrologically active carbonate caves of the Prealps in the regions Veneto and Friuli in northeastern Italy. It has a modified feeding apparatus with hoe-shaped mandibles, spoon-shaped galeas, and the third article of labial palps small and bearing short sensillae. This beetle is not attracted by conventional baits such as meat, cheese, carrion or excrements as the majority of Leptodirinae. It has been mostly observed deambulating under a thin layer of percolating water on walls covered by moonmilk (a carbonate speleothem of marzipan-like consistency). We have studied *Cansiliella*'s behavior and its foodweb in Bus de la Foos cave for three years. It spends most of its time underwater on moonmilk formations; self-cleaning behavior also takes a considerable amount of time and is possibly associated to feeding. The beetle is also feeding directly underwater on the moonmilk surface. Bacteria from the water, the moonmilk, the gut of the beetle, and from its body surface have been identified. Carbon and Nitrogen isotope analyses and other nutrient profiles suggested that the nutrition of the beetles could be, for the most, composed of hindgut-borne heterotrophic bacteria subsisting on dissolved organic carbon and dissolved inorganic nitrogen. Additional intakes of nutrients can be related to the browsing of matter from the moonmilk surface and from the self-preening activities.

Oral presentation:

Diversity of microbial communities colonizing the walls of a karstic cave in Slovenia

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Some karstic cave systems host microbial communities that consist of distinct, small, white, pink, yellow and gray colonies. Visible to the naked eye, these colonies cover cave walls and are strewn with light-reflecting water droplets. Recently, we have assessed the total diversity of prokaryotes in these multicolored colonizations by establishing small subunit rRNA diversity in samples from Pajsarjeva jama, Slovenia. Furthermore, we have determined the major components of microbial communities constituting yellow and gray cave colonizations by studying environmental samples obtained in caves in Slovenia, Spain and Czech Republic in a comparative manner. These communities studied were diverse, with members of eight bacterial phyla detected in samples, while members of *Archaea* were not recovered. The abundant phylotypes belonged to *Proteobacteria*, *Actinobacteria* and *Nitrospira*. The high number of clones most closely related to environmental 16S rRNA gene clones showed the broad spectrum of unknown and yet to be cultivated microorganisms inhabiting these cave systems.

Poster presentation:

Origin and development of a fungal outbreak in Castañar de Ibor Cave, Spain

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Caves are extremely prone to deterioration from human-induced energy perturbations. It has been demonstrated that uncontrolled or mass visits is one of the factors most detrimental in the conservation of cave art. The mere presence of a group of visitors for some minutes before a panel of rock paintings can cause temperature and humidity in the cave to vary more than during the whole annual cycle under natural conditions. These disturbances, together with those derived from organic matter generated by the visitors, plus that coming in from outside, lead to a progressive alteration of the microenvironment and of the cave ecology.

A little-studied aspect is the impact of organic matter on the ecosystem of a cave. Recently we have had the opportunity to study the effects of an accidental release of organic matter in the Cave of Castañar de Ibor, the activation of the microorganisms present, and the production of a fungal outbreak, similar to that originated nine years ago in the Lascaux Cave, and to suggest the means of tackling and controlling this invasion. The closure of the cave, together with environment-friendly measures, including the use of products that (unlike commercial biocides) did not leave residues in the cave, has minimized the fungal outbreak. The studies made in the cave throughout one year of closure, and the struggle against the fungal colonization, are described.

Poster presentation:

Evaluation and morphological identification of tannase-producing cave fungi

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Caves have peculiar environmental characteristics which provide favorable conditions for the development of some organisms, such as fungi. There are almost no studies on cave microbiota in Brazil. Much information on cave microbiology is being lost with the continuous suppression of caves in the country. The lack of knowledge and studies in this area highlights the potential of finding new fungi species or even isolates of biotechnological interest. Such findings could strengthen conservational actions for this environment. One of the areas that has been growing in the country is the study of fungi capable of producing enzymes of biotechnological potential. One of these enzymes is the Tannase (tannin acyl) hydrolase. This enzyme catalyses the hydrolysis reaction of the ester bonds present in the hydrolysable tannins and gallic acid esters. It is produced by plants and microorganisms. It is largely used in the production of instantaneous tea, acorn liquor and of gallic acid. This acid is an important compound for the synthesis of antibacterial drugs used in the pharmaceutical and food industries. Tannase is also used as clarifying agent in some drinks (wines, juices and coffee flavored drinks). The *Aspergillus* genus has been widely used for tannase production. The objective of this study was to isolate tannase-producing filamentous fungi from Brazilian

caves in the *Caatinga* biome. The isolation of fungi was done in PDA (Potato Dextrose Agar) media with 0.2% of tannic acid for 5 days at 28 °C. Screening was performed in media containing tannic acid (only carbon source). The growth was analyzed in 3, 5 and 7 days. The tannase-producing isolates were identified to genera level. These isolates belonged to 5 different genera: *Aspergillus*, *Penicillium, Fusarium, Rhizopus* and *Cladosporium*. A total of 386 from the 544 fungi isolated produced tannase, representing 70.96% of the samples. The fungi presenting significant growth will be submitted to specific enzymatic activity and species identification. The results obtained in this study highlight the biotechnological potential of cave microorganisms and the need of more studies concerning cave microbiology and its applicability.

Poster presentation:

Microbiologic study in a Brazilian cave: biodiversity, biotechnological potential and toxin production

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The hypogean environment is a peculiar system and the habitat of many organisms. Microorganisms are an important part of this subterranean system. They may be associated with ecological and geological processes in this environment. These organisms may also produce many substances, such as enzymes and toxins. These substances may be of great biotechnological importance or offer risks. There are almost no studies on the microbiological biodiversity, their importance and potential in Brazilian caves. The objective of this study was to access the microbiological diversity in the aphotic zone of a cave located in northeastern Brazil. The toxigenic and biotechnological potentials of these microorganisms were tested with the objective of understanding better the potential and risks offered by cave microorganisms. The isolates were obtained through the exposure of Petri dishes containing Dychloran Glycerol (DG-18) Agar and DRBC (Dichloran Rose Bengal Chloramphenicol) media for 20 minutes in the cave. After this proceeding, the Petri dishes were incubated for 7 days at 25 °C. The isolates were purified, identified and tested on their toxin (aflatoxins, ochratoxins) and enzymatic (amylase, protease, lipase and pectinase) production. The enzymatic activity was obtained through semi-quantitative

analyses. The toxin production was analyzed through a Thin-layer Chromatography of Plugs from agar cultures. A total of 17 species were identified among the 58 isolates obtained in the cave: *Aspergillus* (13 spp), *Penicillium* (2 spp), *Mucor* (1 sp), *Cladosporium* (1sp). Enzyme producing fungi were confirmed for lipase (21 isolates), amylase (22 isolates) and protease (16 isolates). Some species presented high biotechnological potential. A total of 6 isolates produced ochratoxin A (*A. ochraceus* , *A. sclerotiorum, A. niger, Aspergillus* sp and *A. sulphureus*) and 1 isolate (*Aspergillus flavus*) produced aflatoxin (B1 and B2). It was also possible to identify a possible new species of *Aspergillus* (*Asergillus* sp). The results highlight the need of more microbiological studies in subterranean environments in order to know the subterranean microbiological biodiversity, the biotechnological potential of cave microorganisms and the risks they might be offering.

Oral presentation:

Microbiological study for a management plan in a touristic cave in Brazil

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Caves are stable environments with characteristics favoring the development of microorganisms such as the filamentous fungi. However, there is a lack of studies on cave microbiology. *Histoplasma capsulatum* is one of the many fungi found in caves. This is a pathogenic species which may cause a lung disease named histoplasmosis. *H. capulatum* is a great concern to cave visitors due to its association with bat guano. Brazilian caves have been historically used for ecological and religious tourism. The presence of pathogenic microorganisms may cause serious health problems. The development of studies associated with management plans before opening a cave for tourism is of great importance. The present study is part of a management plan already approved for a touristic cave in Brazil. The objectives of this study were to verify the presence of possibly pathogenic fungi in the cave, verify a possible influence of tourism on the microbiota, and elaborate a management plan including microbiological analyses for a touristic cave. For the isolation of *H. capsulatum*, Petri dishes containing

Brain-Heart Agar and Saburaud media were placed along the cave and then incubated at 37 °C and 25 °C respectively. Soil, guano and suspicious material were also sampled for direct inoculation and dilution methods in Brain-Heart Agar and Saburaud (37 °C and 25°C) media. Petri dishes containing DRBC (Dichloran Rose Bengal Cloraphenicol) and Saburaud were also exposed along the cave for the isolation of other fungi and CFU(colony formation unit) analyses. No colonies of *H. capsuluatum* were identified by any of the methods. A total of six possibly pathogenic, toxin producers and/or allergenic species were identified. These species belonged to the genera *Aspergillus* (*A. japonicus* and *A. ochraceus*), *Cladosporium* (*C. cladosporioides* and *C. herbarum*) and *Fusarium* (*F. oxysporum* and *F. solani*). It was also possible to observe a significant variation of CFU in some sampling points during the intense tourism transit. A new visiting route was also suggested at the end of this study. The present study highlights the importance of studies on the underground microbiota, its biodiversity and the inclusion of microbiological studies in the management plans of touristic caves.

Oral presentation:

Entomopathogenic fungi associated with two troglophilic moths *Scoliopteryx libatrix* L. and *Triphosa dubitata* L.

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Entomopathogenic fungi are very important ecological factors for they influence the insect population size, diversity and distribution and play an important role in nutrient cycling in hypogean environments. Imagos and imago cadavers of troglophilic moths *Scoliopteryx libatrix* and *Triphosa dubitata* were sampled in 10 different limestone caves in Slovenian karst. From aseptically isolated intestine of sacrificed imagos we couldn't isolate any fungi. From imagos' cadavers entomopathogenic fungi were isolated and identified on the basis of macro- and micromorphology and on the basis of nucleotide sequence of the ITS1-5,8S rRNA-ITS2 region. From cadavers three different fungal species were isolated: *Beauveria bassiana*, *Isaria farinosa* and *Lecanicillium fusisporum*. Fungal species *Beauveria bassiana* was isolated most frequently, although it wasn't ever isolated from *Scoliopteryx libatrix* and *Triphosa dubitata* before. The population structure of isolated strains of the species was analysed by amplified fragment length polymorphism (AFLP). We found out that the majority of the strains had a low rate of polymorphism and that strains isolated from *Scoliopteryx libatrix* cadaver group together regarding sampling location, while strains isolated from *Triphosa dubitata* imago cadavers were in contrast very different.

Oral presentation:

Composition and activity of bacterial microbial communities in the Postojna cave sediments: are the microbes in 700 000 years old sediments still active?

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A sedimentation gradient ranging from present time to 700 000 year old sediments according to Th/U dating was sampled in Postojna cave protected area. A relationship between the sediment age, microbial activity and current microbial community structure were explored. Long-term physical-chemical parameters served as explanatory variables in the redundancy analyses (CCA coupled RDA) for the identification of environmental parameters explaining the largest variability in the structure of microbial communities and in their specific activity. Whole microbial community DNA was successfully extracted from all samples and used in microbial community structure assessment by profiling of the genes for bacterial 16S rRNA. A special protocol for the detection of molecular contamination was developed and used consistently throughout the analyses. The specific activity of microbial communities present in these sediments was assessed through incorporation of [^{14}C]-leucine in microbial biomass. Due to highly oligotrophic environmental conditions, extensive positive and negative controls were used to ensure high signal-noise ratio. A highly sediment-age related decay in microbial activity was observed. In addition, clone libraries containing genes for bacterial 16S rRNA that were prepared from the three samples exhibiting highest signal-noise ratio, were analyzed at various taxonomic levels and related to other published descriptions of cave and soil microbial communities. The results show highly consistent but complex microbial community structure in the sediments of varying age.

SYMPOSIA

7. Adaptation, Development, Physiology

Poster presentation:

Eye development in the cave fish *Garra barreimiae*

Elke Aden

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Eye degeneration is a general evolutionary tendency shown in many animal groups that are adapted to dark environments. Inside the Hoti Cave located in the Jabal Akhdar mountains in Oman lies a subterranean lake, which is home to the blind fish *Garra barreimiae*. Outside the cave, *Garra barreimiae* is a common species of eyed epigean surface fish. The eyes of the blind cave fish and of its epigean ancestor have been studied comparatively. The eye structure of the surface fish is a typical teleost eye. Parts of the eye of the cave fish show hypertrophy. Individuals compensate the lack of a lens in various ways with extreme growth of different parts of the eye. For instance, the anterior eye chamber is often filled out with a derivative of the annular ligament, and as a result the pupil no longer transmits light. The eye rudiment of the *Garra barreimiae* is not sunken in the orbit as is the case with other cave fish species. From these examples we can conclude that the adult cave fish population is very heterogeneous with regard to the size and structure of the eye rudiment. To understand how this diversity evolves, the ontogenetic eye development was investigated. During early ontogeny the differentiation of the eye of hypogean *Garra barreimiae* is similar to the epigean one. All major structures are present. However, the lens remains in the embryonic stage, and no lens differentiation occurs. It disappears altogether at the age of three months. Initially there is a complete laminated retina with typical opsin expression in the photoreceptor cells. But opsin expression remains at a low level and ceases at the age of four months. On the other hand, other retinal strata like the amacrine cell layer are well differentiated in juvenile cave *Garra* and remain functional for longer than the photoreceptor cells. In adult animals, on the other hand, no evidence of functional retina cells could be detected.

Oral presentation:

Fifty years of Tular Cave laboratory

Gregor Aljančič

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Tular is a natural cave, which was formed by a local stream in the Sava river Pleistocene conglomerates in Kranj, Slovenia. It was first mentioned already in 1689 by the famous naturalist J. V. Valvasor. Later, a subspecies of a cave beetle, *Anophthalmus miklitzi ssp. staudacheri* has been described from this cave. In 1944 it was partly walled into an air-raid shelter for the nearby factory. In 1960, with the support of the Biological Institute at the Medical Faculty in Ljubljana, and

through the help of the town of Kranj, the cave was turned into a laboratory by speleobiologist Marko Aljančič (1933–2007), who populated it with the European cave salamander, *Proteus anguinus* (Amphibia: Urodela). It is the only cave laboratory in Slovenia and – apart from the cave laboratory in Moulis, France – the only place with succesful breeding of this endangered cave amphibian in captivity. Since 2002, a colony of the dark pigmented subspecies, *Proteus anguinus parkelj* is also studied in this laboratory. In the laboratory, the ecology and behaviour of *Proteus*, mainly its breeding, are studied. Considerable effort was put in the fieldwork – observing *Proteus*' behaviour, surveying environmental parameters of the habitat, verifying the old data on its presence and documenting new localities. Another important subject is the study of the history of research of *Proteus*. Owing to this interest, the laboratory has put together an extensive library on this species. The laboratory also raises the public awareness of *Proteus* as the symbol of the Slovene natural history, with special emphasis on nature conservation. Since the beginning, one of the missions of Tular was to establish a breeding colony, which could reintroduce *Proteus* back to a destroyed habitat. This idea became most urgent after the discovery of an extremely vulnerable and rare *P. a. parkelj* in SE Slovenia, where even a local pollution could destroy the entire population. Occasionally, the laboratory serves as a sanctuary for injured *specimens* that were washed out of their subterranean habitat.

Poster presentation:

Does *Proteus* detect and react to a sudden rise of water conductivity which indicates incoming flood?

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The European cave salamander, *Proteus anguinus* (Amphibia: Urodela) is restricted to its aquatic cave habitat. However, during seasonal flooding, some individuals are washed out of their subterranean environment. In fact, this was the only way to obtain *Proteus* through the early decades of its research. Also, many new localities were discovered on the account of this, obviously not rare, phenomena. Though this may be considered as a highly hazardous way of *Proteus* to disperse into new habitats, it is clear that all these individuals present a constant loss for their population. There are several suggestions how *Proteus* could directly detect the coming flood and search for shelter (sensing sudden rise of water level/ increase of water current, detecting changes of sound owing to the higher water level/ percolating water, sensing the changes of temperature/ chemistry of water, etc.), though non of them were observed or tested. Recently, another possibility was proposed by M. Prelovšek. Precise measurements of several physical and chemical

parameters of cave water have revealed a significant rise in electrical conductivity shortly after first rain, up to several hours before a substantial rise of the water level. Namely, the rain above the cave squeezes the old, saturated water from the aquifer. A fast response of local infiltration of precipitation is followed by higher but later flow of water from a distant but larger catchment area, which actually brings the flood, and a rapid decrease of electrical conductivity. On the basis of a short preliminary behavioural test, performed in the Tular Cave Laboratory, it was not possible to conclude with certainty whether *Proteus* is sensitive to the changes of electrical conductivity in the range that appears in a cave water prior the flood. Further laboratory investigation, supported by observation in nature is needed.

Oral presentation:

Cold-hardiness in central European troglophiles and trogloxenes

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Cold–hardiness is the ability of organisms to survive shorter or longer exposure to temperatures lower than those causing their body fluids to freeze. Trogloxenes, characterized as not adapted, and troglophiles not completely adapted to hypogean, thermo-stable environments, have not lost their ability to withstand freezing, while troglobionts presumably did. We hypothesized that troglophiles are less cold-hardy than trogloxenes, which are better adapted to unstable epigean environments, including freezing. We assumed that cold-hardiness can serve as one of relevant measures to discuss the degrees of adaptation to hypogean environments. In our investigation, 25 trogloxene and troglophile species temporary or permanently inhabiting central European caves, and three troglobiont reference species were tested for their cold resistance. The specimens for the analysis were collected in winter and summer, if present in caves, otherwise once a year in either of these seasons. We measured their supercooling points (SCPs) within a precise thermostatic cooling chamber, starting at –2.0 °C, and stopped at –12.0 °C, which no individual sustained. The specimens were exposed to the experimental temperature for 24 hrs and afterwards they were hold for 48 hrs in a refrigerator at 2.0 °C. The procedure was carried out consequently at 1.0 °C lower temperatures until reaching the SCP. As expected, troglophiles are generally less cold-hardy than trogloxenes. The SCP values differed much with respect to the species and developmental stages, while the differences between winter and summer individuals were negligible. The resistance to cold is not sex–dependant.

Poster presentation:

Heat Shock Response in the leptodirins *Neobathyscia mancinii* and *Neobathyscia pasai*

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The response to increasing temperature in two cold stenothermal leptodirins, *Neobathyscia mancinii* Jeannel and *Neobathyscia pasai* Ruffo (Coleoptera, Cholevidae, Leptodirinae) was evaluated as lethal temperature (LT_{100} and LT_{50}) and as expression of a family of heat shock proteins (the constitutive form HSC70 and the inducible form HSP70). Adults of the two species were collected in the Damati Cave and Tana delle Sponde Cave (Veneto Province, NE-Italy) and stressed by direct short-term heat shocks (1h, from 25 °C to 31 °C). The expression of the HSP70 family was performed by qPCR on organisms stressed at 25°C (= maximum temperature at which all the tested organisms were alive), 28 °C (= LT_{50}) and 29 °C (= sub-lethal temperature). In both species, HSC70 level was constant with increasing temperature, whereas a significant increase of HSP70 (= Heat Shock Response) was observed, significantly higher in *N. pasai*. This could be due to their different in-cave distribution: *N. pasai* colonizes the cave entrance, where the temperature is more variable (= 5-18 °C), whereas *N. mancinii* is confined to the internal part of the cave where the temperature remains constant (= 9.8 °C). These results highlighted for the first time the occurrence of a Heat Shock Response in cave insects and suggest that the intensity of this response might be correlated to the adaptation to the environment.

Poster presentation:

Oogenesis in *Proteus*: Stages of oocyte development

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In comparison with the other amphibians *Proteus* has very extended reproductive period, continuing 30 years or more and it has an extremely long reproductive cycles, that is to say *Proteus'* female lays eggs at intervals of 6 years. Sexual maturity is also acquired very late, with males maturing earlier than females, appears in 11 year-old males and 15 year-old females at 11-12 °C. Opposite to the knowledge of

reproduction, little is known about *Proteus* oogenesis; a process of differentiation and maturing of the oocyte, resulting in the mature ovum capable of ovulation. To gain a better understanding and elucidation of the *Proteus* reproductive cycles, we have begun with studies of its ovary and oogenesis. Ovaries of 30 females of *Proteus anguinus anguinus*, with snout-vent lengths of 225 – 270 mm were examined by light and transmission electron microscopy. The gonad samples used in this research were taken from animals that had been collected in previous years (from 1972 to 2009) for other research purposes. Animals had been collected during different seasons and from different locations of the subterranean karstic system of Slovenia. The morphology of the *Proteus* ovary, the stages of oocytes and characteristics of developmental oocytes were determined. The oocytes were divided into five stages based on size, colour and histology. The most predominant oocytes in the ovaries of *Proteus* beside the oogonium are previtellogenic oocytes (stage I and II). Oogonium and previtellogenic oocytes are a constant stock of oocytes for growth. In a few cases, early vitellogenic oocytes (stage III and IV) and only in two cases late vitellogenic oocytes (stage V) were encountered. Stage V oocytes are the most mature oocytes found in the ovary of *Proteus*. No mature (postvitellogenic) oocytes or ova were found in the materials that were available. In majority of ovaries examined a degenerating vitellogenic oocytes or atretic bodies occurred too. Furthermore, we found that larger and heavier females have more mature oocyte stages and also that vitellogenic oocytes occur in ovaries independently of seasons, therefore *Proteus* females could lay eggs at any period of the year.

Oral presentation - invited plenary lecture:

Pleiotropic tradeoffs between constructive and regressive traits during troglomorphic evolution

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The adaptive benefits of regressive troglomorphic traits, such as eye and pigment degeneration, are difficult to identify in cave animals. We have approached this problem by studying the developmental and molecular relationships between several different constructive and regressive traits. In the cavefish *Astyanax mexicanus*, *sonic hedgehog (shh)* overexpression along the embryonic midline is responsible for eye degeneration. Subsequently, *shh* overexpression spreads to the cavefish oral-pharyngeal area. Cavefish have constructive oral-pharyngeal traits, including larger mouths and more taste buds, relative to surface dwelling *Astyanax*. A tradeoff between constructive oral pharyngeal development and optic degeneration has been demonstrated by conditional *shh* overexpression in surface *Astyanax* embryos, which increases mouth and taste bud development at the

expense of eyes via pleiotropic Shh signaling. Cavefish lack melanin synthesis in regressed pigment cells due to loss-of-function mutations in *oca2*, which normally regulates the supply of L-DOPA precursor during melanin synthesis. The block in cavefish pigmentation occurs at a metabolic branch point in which L-tyrosine is normally converted either (1) to L-DOPA, DOPAquinone, and melanin by tyrosinase or (2) to L-DOPA, dopamine, and related catecholamines by tyrosine hydroxylase and other enzymes. A similar block in the initial step of melanin synthesis has evolved independently in the cave plant hopper *Oliarus polyphemus* and other diverse cave animals. In *Astyanax* cavefish, the benefit of lost melanin pigment appears to be the production of excess L-DOPA and its derivative dopamine by the second alternative pathway, which promotes constructive development of dopaminergic neurons and enhances the magnitude of adaptive feeding behavior. We conclude that the evolution of beneficial constructive traits could have driven regressive traits via developmental tradeoffs encoded in pleiotropic genes, which adapt cave animals to life in darkness.

Poster presentation:

The shrimp rostrum between phylogeny and adaptation

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Cave shrimps of the subgenus *Troglocaris* (Crustacea: Decapoda: Atyidae), exhibit high variability in rostral length and dentition. In the shrimp populations that are co-occurring with its amphibian predator *Proteus anguinus*, longer rostra armed with more numerous teeth are recorded. These shrimps are also larger than the ones living in the presumably *Proteus*-free environment. Discrepancies between the molecularly established phylogenetic relations and distributions of rostral length, as well as body size, directed our search towards possible environmental influences and possible defence mechanisms of cave shrimps. Although there are some exceptions, the common use of the rostral length is disputable in the diagnoses of the Atyid taxa. In preliminary laboratory observations no successful frontal attack of *Proteus* was recorded on shrimps with long rostra. Also, a handling time of *Proteus* feeding on shrimps with long rostra was longer.

Oral presentation:

New long-legged cave-dwelling representatives of the Balkan genus *Cyphophthalmus* (Opiliones, Cyphophthalmi, Sironidae) and the question of functional significance of troglobite appendage elongation

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During recent biospeleological research of the Velebit Mountains (Croatia), performed by members of Croatian biospeleological Society (CBSS), new taxa of the genus *Cyphophthalmus* (Opiliones, Sironidae) have been found. New taxa inhabit all regions of Velebit Mountains regularly in ca. 100 m deep cave habitat. New *Cyphophthalmus* taxa are characterized by very elongated appendages compared with some closely related troglobitic species from the same genus and troglobitic sironids generally. There are some endogean representatives of the same genus with elongated appendages too. This fact opens a question about functional significance of appendage elongation in troglobites, as a rule. Is it an adaptation or not? Specimens of new *Cyphophthalmus* from North and Middle Velebit show some morphoanatomical differences compared with specimens from South Velebit. But molecular analyses show no differences between the two taxa considered to be two different subspecies. Due to the fact that all collected specimens are females and that their *receptacula seminis* are not well sclerotized, we assume that the taxa are parthenogenetic.

Poster presentation:

Three-dimensional reconstruction of the inner ear of *Proteus anguinus* (Amphibia: Urodela)

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From serial histological sections (paraffin-embedded, 10 μm) of the otic region, a three-dimensional model of the left and right inner ear in the non-pigmented subspecies of *Proteus anguinus* was built. Sensory epithelia and the perilymphatic system were also reconstructed. A three-dimensional model of the right inner ear of the pigmented subspecies of *Proteus anguinus* was built from serial semi-thin sections (2.5 μm). Those were made from the isolated organ. All sections were photographed. The freeware program Reconstruct was used for reconstruction. It

turned out to be useful. It enables all crucial steps in reconstruction: import of pictures, alignment, tracing and generating the three-dimensional model. The semi-thin sections were easier to align, because the sides of the block were still seen and served as fiducial marks. Three-dimensional models were accompanied by pictures of sections in order to present the detailed anatomy of the inner ear. This enabled us to confirm previous results and describe the anatomy of inner ear in the pigmented subspecies of *Proteus anguinus*. The membranous labyrinth turned out to be shorter in the pigmented subspecies. The description is based on a single individual organ; therefore it must be confirmed by additional research.

Oral presentation:

Role of the fat body in the cave crickets *Troglophilus cavicola* and *T. neglectus* (Rhaphidophoridae, Saltatoria) during overwintering

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The cave crickets *Troglophilus cavicola* and *T. neglectus* are the most widely distributed European species of the family Rhaphidophoridae. In both, the life cycle spans two years. They overwinter in caves where *T. cavicola* undergo sexual maturation, while *T. neglectus* do not. This non-feeding period is appropriate for a comparative study of the fat body role, especially in the energy supplying lipid and glycogen metabolism. Optical and TEM cytological, and biochemical methods were applied. The fat body was studied at the beginning (November), in the middle (January) and at the end of overwintering in younger and older nymphs, and adults. Overwintering in warmer microhabitats, *T. cavicola* was expected to use lipids and glycogen more extensively than *T. neglectus*. In all individuals of both species, the fat body was composed of about 40 oval ribbons and consisted of two principal cell types: adipocytes and urocytes. Adipocytes are characterized by a large quantity of storage lipid droplets, glycogen rosettes and protein granula, and urocytes by glycogen rosettes and urate granula. Both undergo gradual structural changes. *T. cavicola* use glycogen continuously, but stop using lipids after the middle of overwintering, while the use of these substances is inverse in *T. neglectus*.

Poster presentation:

Extreme troglomorphy in a new species of cave springtail, *Tritomurus* sp. nov., from Croatia (Collembola: Tomoceridae)

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The family Tomoceridae includes 133 species in 16 genera. In the caves of Europe, Eastern Asia and northern America there are about 30 troglobiotic species. However, few species exhibit strong morphological adaptations to cave life. The most remarkable in this respect is *Tritomurus falcifer* Cassagnau 1958. We present from Biokovo Mt. in Croatia a second highly troglomorphic species, *Tritomurus* sp. nov. *Tritomurus* sp. nov. was collected from -170 to -430 meters in Amfora jama pit. All specimens were found in the thin water-film flowing on vertical walls or very close to it (hygropetric habitat). A number of caves were explored during the last years on Biokovo but *Tritomurus* sp. nov. was not found in any other cave, probably because cave hygropetric is practically inaccessible for investigation in most of them. Interestingly, the rare *Tritomurus falcifer* from Pyrenean caves of the Arbas massif, very similar morphologically to *Tritomurus* sp. nov., also lives in the hygropetric. Both have the ventro-apical labial brush particularly developed. This mouthpart modification recalls similar filtrating structures observed in other species of the cave hygropetric, and suggests special feeding habits. Both of these species has remarkable slender claw as an adaptation to cave life and walking in the hygropetric.

Poster presentation:

What we know about *Pantelozetes cavaticus* (Acari, Oribatida), notes on distribution, ecology, food preference and morphology

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Oribatid mites are common soil dwelling animals; together with springtails (Collembola) they dominate the soil mesofauna. Many of these surface species can be observed in caves. Only a few species show closer affinities to the cave

environment. *Pantelozetes cavaticus* (Kunst, 1962) appears to be the most abundant and frequent eutroglophilous oribatid mite in the region of Central Europe. Our contribution is focused on summarising published data on its distribution and presentation of new data from Central Europe. Distribution within the caves (photic, disphotic, aphotic zone) is discussed with new data on substrate preference. Potential food sources in the oligotrophic cave environment are assessed on the base of digestive enzymes detection. A variability of several morphological characters of species is noted in the final part of the contribution. The study was supported by the grant VEGA 1/0139/09.

Poster presentation:

Trophic niche of two subterranean isopod species along a parapatric boundary in pre-Alps and Jura Mountains (France): a preliminary field study using stable isotopes

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The influence of abiotic and biotic interactions in shaping the present-day distribution ranges of stygobiotic species has attracted very little attention essentially because distributional patterns have historically been interpreted as palaeogeographical imprints of the geographic range of putative epigean colonizers. Only very recently have some studies attempted to model species richness or distribution using abiotic predictors. In most groundwater studies, however, physical variables alone left a substantial amount of unexplained deviance. It is therefore necessary to investigate the role of biotic interactions in the distribution patterns of groundwater species. *Proasellus cavaticus and Proasellus valdensis* exhibit separate but contiguous distributions along the western margins of the Jura and pre-Alps mountains. The goal of this work is to determine whether these two parapatric species exhibit the same trophic niche in nearby groundwater systems, hence suggesting interspecific competition for food along the parapatric boundary. We thus determined the diet of both species in six caves located along the contact zone, using carbon and nitrogen stable isotopes. The contribution of the different food resources to *Proasellus* diet have been calculated using mixing model from their $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios. In the six caves, *P. cavaticus* and *P. valdensis* exhibit a similar diet, mainly constituted of bacteria attached on sediments (> 70%), while particulate organic matter contributes with a maximum of 30% to both species diet. This result indicates that interspecific competition for

food may be a structural factor of species distribution in groundwater ecosystems. Laboratory experiments are needed to test this hypothesis of competition by measuring the influence of the interactions between *P. cavaticus* and *P. valdensis* on their trophic efficiency. This work was developed within the framework of the DEEP research program.

Oral presentation:

Feeding biology of the cave isopod *Mesoniscus graniger* (food preference and digestive enzymes)

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Terrestrial isopod *Mesoniscus graniger* (Isopoda, Oniscidea), morphologically adapted to cave life, is abundant in caves of the Carpathians. Food preference of this animal was studied using preference tests, presence of digestive enzymes and field observations. Nine cultures of algae, ten of microscopic fungi including two of yeasts and one species of cyanophytes isolated from caves were offered as a food to isopods in five replicates of three variants of multiple-choice feeding preference tests arranged on Petri dishes. Presence of animals inside the sector with particular food, directly on the food, and distribution of faecal pellets were monitored. Direct consumption of microbial cultures was evaluated from macro-photos using PC image analysis. Isopods clearly prefer algae cultures (mainly *Protosiphon botryoides, Spongiochloris irregularis, Botrydiopsis intercedens* and *Stichococcus bacillaris)* over other microorganisms in laboratory tests. Only algae were consumed. The sectors containing another kind of food were visited to a lesser extent. Amylase, maltase, saccharase and trehalase prevailed in the digestive enzyme spectrum of *M. graniger*. This species is attracted to organic deposits in the field but its occurrence is not restricted to bat guano. The investigation showed the contrast between visible preference of some type of living cultures of microorganisms in laboratory and the non-specific consumption of cave sediment and dead organic material in the field. The preliminary results from food preference tests and digestive enzymes presence in several species of cave springtails and mites are compared and the influence of restricted food sources on the biodiversity and food web structure in the cave is discussed.

Oral presentation:

Chronobiological studies on Brazilian subterranean fishes: a summary and new data on locomotor activity rhythms under light-dark cycles

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The Brazilian subterranean ichthyofauna includes the largest number of species investigated with focus on rhythmicity. So far, 11 among 26 troglomorphic fishes have been studied under free-running conditions (DD) and light-dark cycles (LD 12:12 h): the characid *Stygichthys typhlops* and, among catfishes, the heptapterids *Pimelodella kronei*, *P. spelaea*, *Rhamdia enfurnada* and three *Rhamdiopsis* spp., the trichomycterids *Trichomycterus itacarambiensis* and two *Trichomycterus* spp., and the callichthyid *Aspidoras* sp. Regression of mechanisms of time control of the locomotor activity has been shown for all studied fishes, in a lower or higher degree paralleling that of reduction of eyes and pigmentation. Individuals of highly troglomorphic species, such as *S. typhlops* and *Rhamdiopsis* sp. from Campo Formoso, were arrhythmic under free-running conditions, whereas in less troglomorphic fishes (e.g., *R. enfurnada*, *P. spelaea*, *P. kronei*, *Rhamdiopsis* from Salitre Cave) part of the individuals retained significant circadian rhythms; interestingly, larger (probably older) individuals tend to present weaker rhythms. In general, the activity peaks were observed in the dark phases, indicating that these peaks correspond to a character state retained from the nocturnal ancestors. When submitted to LD cycles, locomotor activity was entrained in all studied individuals, except for *S. typhlops* fish (most specimens did not respond to this condition). Individual variation regarding the presence (or not) of residual oscillations when the fish returned to free-running conditions was observed in the studied species except for the highly specialized *S. typhlops* and *Rhamdiopsis* sp. The absence of such oscillations indicates a masking effect of LD cycles. Data on subterranean fishes provide good evidence for the hypothesis of evolutionary regression of time-control mechanisms involved in locomotor activity in troglobitic organisms, either affecting the oscillator(s) itself or due to uncoupling of the oscillator and its related function(s), supporting the hypothesis of external, ecological selection of circadian rhythms.

Poster presentation:

Related and yet different: behavioral differences between troglobitic heptapterid catfishes, *Pimelodella kronei* and *Rhamdia* spp.

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Species of *Pimelodella* and *Rhamdia* have adjacent placements within the Heptapteridae phylogeny, and are similar in general appearance, distribution and habitat preferences and food habitats - species of both genera are generalist carnivores, with *Rhamdia* tending to be more benthonic, reaching larger sizes and incorporating fish in their diet. Ecological studies on the Brazilian troglobitic *P. kronei* and *R. enfurnada* did not reveal any differences which could not be attributed to habitat specificities. However, important behavioral differences were observed in laboratory. Chronobiological studies indicated that, in aquaria, *P. kronei* exhibits higher levels of spontaneous activity, with shorter intervals without detectable locomotor activity (less than 30 min.), whereas *R. enfurnada* may remain stationary for hours; this may reflect differences in the epigean ancestors since large *Rhamdia* catfishes are sit-and-wait predators. *P. kronei* presents more organized and distinctive, therefore predictable, patterns of chemical communication at distance, more clearly related to size and sex (small individuals generally avoid water from cospecifics, larger males are usually attracted) than in *R. enfurnada*, in which reaction to cospecifics varied from indifference to attraction; when detected, such responses were delayed in relation to the observed for *P. kronei*, possibly as a correlate to the lower activity levels in *R. enfurnada*. A new troglomorphic *Rhamdia* species from Bodoquena karst area, NW Brazil, also studied, was intermediate in these aspects. Likewise, patterns of aggressiveness are distinct. Agonistic interactions are more frequent in *P. kronei* and include a richer behavioral repertoire, not leading directly to death, indicating a ritualized behavior. In opposition, such interactions, rarely observed in *R. enfurnada* kept in groups, are less defined but, when occurring (usually among larger individuals), they lead rapidly to casualties.

Oral presentation:

The gland-piliferous organs of *Titanethes albus* (Crustacea: Isopoda)

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Gland-piliferous organs, male-specific structures with numerous setae and pores, characterize many species in the family Trichoniscidae. These structures are found primarily in the cave-dwelling representatives of this terrestrial isopod family, most notably in the genera *Trichoniscus* and *Titanethes*. In different species, variously shaped organs can be found on the dorsal side of different body segments. Although these structures have been analyzed in some detail in the genus *Trichoniscus* by other authors, such an analysis in *Titanethes* is lacking, leaving many open questions concerning their function and origin. In *Titanethes albus*, a large troglobitic trichoniscid inhabiting the caves of northern Dinaric Karst, the gland-piliferous organs appear as paired bulges on the dorsal surface of the fourth pleonite in males but are absent in females and juveniles. Their external shape and porous nature have been described by several authors and it has been suggested that they are glands involved in reproduction. They have, however, received little attention since the early twentieth century. The aim of our study is to provide a more detailed analysis of the organs' microscopic anatomy. We examined the pleons of several *Titanethes albus* males from Planina Cave (Slovenia). Histological inspection revealed a great diversity of cuticular structures forming the external part of the organ. Several types of scales and bristles are found on the dorsal bulges and in their proximity. Each of the numerous pores, approximately 3 micrometers in diameter, is surrounded by a cuticular veil. Aggregations of large cells filled with granules are connected to the surface pores by channels. These granular cells occupy much of the pleon's volume. The surface structures and gland units of the organs in *Titanethes albus* appear similar to those reported for *Trichoniscus alexandrae*. Additional ultrastructural characterization will help us draw further conclusions concerning the organs' structure and function.

Oral presentation:

Troglomorphisms in Rhagidiidae (Acari: Prostigmata): are they all a morphological clock of adaptation?

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Psychrophilic rhagidiid mites are obligatory inhabitants of the mesovoid shalow substratum, subterranean voids in talus slopes and caves worldwide. They started their evolutionary processes of speciation and adaptation to life in subterranean habitats as early as the origin of these habitats. In the region of Prealps in the Mediterranean Basin the long-time persistence of fragmented subterranean habitats since the Pliocene or Miocene is consistent with long periods of post-colonization isolation and evolution of these mites. Their morphological adaptations to life in caves that have evolved under the similar selective pressures imposed by cave habitats, i.e., troglomorphisms, are often striking and well-discernible. In contrast, troglomorphisms in cave rhagidiids from Quaternary periglacial areas in central Europe and North America are only weakly discernible. These rhagidiids might have immigrated to cave refugia as late as at the beginning of, or during, the Quaternary glaciation. Tentatively, their troglomorphisms might reflect the history of immigration. Thus nowadays we can encounter rhagidiid mites with differently expressed traits of the derived troglomorphisms. In general, these similar morphologies are primarily the elongation of appendages and progressive development of sensory organs such as the increased length and number of the tactile setae and rhagidial solenidia on the tarsi and tibiae of the first two pairs of legs. However, individual suits of troglomorphisms are not uniform but species-specific. It is still in question whether all these differently expressed morphological adaptations are real troglomorphisms that positively correlate to a morphological clock, solely with time of the underground ancestral history of these troglobionts. Perhaps some of them also reflect specific adaptations to different specific subterranean niches, or feeding habits, i.e., not only differences in age. Molecular clock studies are the most suitable to resolve this problem and the troglomorphic representatives of the genera *Traegaardhia* and *Troglocheles* seem to be the most suitable for these purposes.

SYMPOSIA

8. Systematics and Faunistics

Poster presentation:

Diversity and distribution of subterranean species in karst areas of Iran

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Species diversity includes the entire range of species found on earth. In recent years a rapidly increasing amount of information is available about the fauna of caves and other subterranean habitats. The basis of available information on abundance of cave-dwelling species in Iran together with data obtained from recent studies, including observations on more than 30 karst areas, allowed for an assessment of the distribution and diversity of cave-dwelling species. The diversity of subterranean animals in Iran is not known well and for now only few species were accidentally reported. Cave localities including Ghoro-Ghale, Alisadr, Mozafar, Shirabad and Tadovan Caves are most important. In this study we found several species of cave-dwelling animals. The Iranian Cave-fish *Iranocypris typhlops* and *Paracobits smithi* are found in a well-like pool, the natural outlet of a subterranean limestone system of the Zagros Mountains in the Abe-Sirum Valley near Tange-Haft railway station in Lorestan Province, south-west Iran. The Gorganian salamander *Paradactylodon gorganensis* (Urodela, Hynobiidae) was found in the eastern part of the Elburz Mountains in Shirabad Cave of Golestan Province. Moreover, we encountered one large spider (Araneae: Sparacidae), one Pseudoscorpion, three species of lizard belong to family Gekkonidae (*Asacus elisae, Hemidactylus persicus* and *Asacus kermanshahensis*), two genera of crustacean (*Gammarus* and *Niphargus*), some species of insects and 14 species of Chiroptera including five species of *Rhinolophus* (*R. ferrumequinum, R. hipposideros, R. euryale, R. mehelyi* and *R. blasii*), three *Rhinopoma* (*R. microphyllum, R. hardwickeii* and *R. muscatellum*), one *Taphozous* (*T. perforatus*), three *Myotis* (*M. emarginatus, M. blythi* and *M. capaccinii*), one *Miniopterus* (*M. schreibersii*), one *Rousettus* (*Rousettus aegyptiacus*), one *Asellia* (*A. tridens*), *Plecotus* (*P. austriacus*) and one *Triaenops* (*T. persicus*). According to the criteria listed in the IUCN Red List Categories 2010, *Iranocypris typhlops* and *Paradactylodon gorganensis* are ranked as Vulnerable and critically endangered. One of the largest challenges regarding these species is disturbance of cave or karst habitat, especially by human activities. Almost all caves have been excavated extensively and vandalism is a major threat to species in caves.

Oral presentation:

The hypogean fauna of selected ecosystems of Kerala, India, with two new records

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Kerala is situated on the South West coast of India and is unique with the presence of several water bodies of varying size ranging from large lakes, backwaters, rivers, lagoons, inlets, reservoirs and ponds. In addition, there are also deep wells of varying kinds contributing to the water sources on the area. These wetlands and their associated ecotones on the south west coast India offer ideal habitats for the much undiscovered subterranean fauna of the region. In this context, it is also worth mentioning that no comprehensive scientific information is available on the hypogean fauna of Kerala. This paper attempts to give the status of selected hypogean fauna of Kerala and reports on two new records of fish from the area. The first report of a totally blind hypogean fish, *Horaglanis* was reported from India. Later in 1963, one Synbranchid eel was reported from Kottayam, Kerala and was named *Monopterus indicus*. In 1996, the Synbranchid eel, *Monopterus epeni* was reported from the same district of Kerala State. A cavernicole, Synbranchid eel called *Monopterus digressus* was also reported from the southern part of Kerala. In 2004, another Siluroid blind fish, *Horaglanis alikunhi* was reported from the central part of Kerala. All these observations on the subterranean fishes from Kerala were quite accidental and the information regarding their taxonomical and ecological details are still fragmentary. This contribution also describes two new species of fishes adapted to hypogean condition from central part of Kerala. They were collected from an old well at Irinjalakuda, Kerala. The species belong to the genus *Horaglanis* Menon and *Monopterus* Lacepede. The taxonomic descriptions of the two hypogean fishes have been discussed with that of the genus of the same species described earlier.

Oral presentation:

Invertebrate biodiversity of the Guangxi caves (southern China)

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Fast progress in the knowledge of Southern China cave biodiversity occurred during the last two decades, and accelerated since 2005. Recent efforts focused on the caves of Guangxi. The present paper lists all troglobitic invertebrates, described or undescribed species, that were recorded and collected so far in this province. To the impressive radiations of cave fish and cave beetles already documented, we can now add the radiation of cave *Sinella* among springtails and that of several families of millipedes. Several of the taxa encountered, including several of the most diversified ones, have no close relatives outside caves in the region, and can be qualified of relicts. Distribution patterns of the most remarkable groups of Guangxi troglobites are given and discussed. Geographical, ecological and taxonomical gaps in our knowledge are emphasized and future prospects are outlined.

Oral presentation:

Unexpected rich terrestrial subterranean fauna in Israel: first results from the inventory of 13 caves

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Israel's north is comparatively rich in karst formations but preliminary results of several authors indicate a poor terrestrial subterranean invertebrate fauna. A first detailed inventory of the terrestrial subterranean arthropod fauna from 13 caves ranging from Upper Galilee to the Judean Foothills revealed numerous species with clear troglomorphic features (e.g. prolonged extremities, reduced pigmentation and eyes). The troglomorphic species belong to 9 families of 7 orders. Of these, at least four species were represented exclusively in a single cave. Troglobites appeared among the orders Araneida, Pseudoscorpionida, Isopoda (Oniscidea), Coleoptera, Homoptera, Hymenoptera (Formicidae) and Chilopoda. The records of blind and depigmented representatives of Homoptera, typically found in tropical regions, and microphthalmic ants with distinct adaptations to a

subterranean life are perhaps the most spectacular ones. Several (if not most) of the discovered species with troglomorphic features seem to be new to science. The terrestrial cave fauna of Israel cannot be classified as poor in taxa any longer. This reclassification as a highly diverse fauna is also supported by records from the superficial underground compartment. In the light of these new results we discuss biogeographic aspects of the southern boundary of troglomorphic species in the Western Palaearctic and suggest the development of conservation action plans for the protection of this highly adapted and so far overlooked subterranean fauna in Israel.

Poster presentation:

The French soil- and cave-dwelling centipedes (Chilopoda): updated checklist and distribution in mainland France, Corsica and Monaco, with emphasis on subterranean fauna, conservation purposes and regional biodiversity

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According to field investigations and researches in collections conducted during the last period 1997-2009, this work provides an update on the checklist, taxonomic status and distribution of the class Chilopoda in France (s.l.). The area comprises three entities recognised by the Fauna Europaea Project: French Mainland, Corsica and Monaco. Lapses or inaccuracies in the presence of species in the territory have been updated. The proposed checklist reminds several recent synonymies, species recently collected in France or taxa recently described. The updated French centipede fauna comprises 140 taxa (136 species and 4 subspecies). Among them, 1 species belongs to the order Scutigeromorpha (<1% of the Chilopoda); 63 taxa (60 species and 3 subspecies) belong to the order Lithobiomorpha (45% of the Chilopoda); 9 species belong to the order Scolopendromorpha (6% of the Chilopoda); 67 taxa (66 species and 1 subspecies) belong to the order Geophilomorpha (48% of the Chilopoda). In order to improve the checklist, the presence in France or the validity of several taxa must be definitively confirmed. In addition, several species described from edaphic and subterranean compartments must be revised. On the ther hand, several species not formally listed in France but distributed in adjacent areas are strongly suspected to be collected in French Mainland in the near future, among them *Eupolybothrus excellens* (Silvestri), *Eupolybothrus tridentinus* (Fanzago), *Lithobius ambulotentus*

Demange & Serra, *Lithobius derouetae* Demange, *Lithobius nodulipes* Latzel, *Lithobius schubarti* Demange, *Geophilus pygmaeus* Latzel. Taxa still undescribed could also certainly be discovered from some poorly-known parts of the country, noteworthy from caves and subterranean systems (s.l.). The ecological and patrimonial status of species is specified, particularly regarding troglophilic and troglobiotic taxa among the chilopod community. Several highly troglomorphic taxa, linked with a high degree of endemism are selected and proposed as good candidates for major patrimonial interest and conservation measures, such as for instance *Lithobius cavernicola* Fanzago, *Lithobius cherpinedensis* Iorio, *Lithobius fagniezi* Ribaut, *Lithobius henroti* Demange, *Lithobius raffaldi* Iorio, *Lithobius scotophilus* Latzel, *Lithobius speluncarum* Fanzago, *Lithobius typhlus* Latzel, *Cryptops Umbricus umbricus* Verhoeff, *Cryptops umbricus lewisi* Iorio, *Geophilus persephones* Foddai & Minelli. Some of them could be selected for future UICN Red Lists.These results are included in the French Fauna database 'Fauna Gallica Myriapoda', to be forwarded to the 'Fauna Europaea' and to the SPN-INPN-MNHN databases.

Poster presentation:

Faunistic and biogeografic characteristics of the centipedes (Chilopoda) in Croatia with special review on the genus *Eupolybothrus* (Lithobiidae)

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This poster presents the fauna of centipedes in Croatia with a complete list of taxa, together with the detailed distribution and endemism for each taxon. Analyses of the centipede fauna have been performed according to all available references and systematic taxonomical survey of collections. All together 91 taxa of centipedes have been determined, of which 7 are endemic for Croatia. The largest number of taxa, 84.6%, belong to Mediterranean macroregion. Western-Panonian and Mountain macroregion are far behind with 48.3% and 46.1%, while the Eastern-Panonian macroregion has only 21.9% of centipede taxa recorded for Croatia. Centipedes of Croatian fauna belong to 4 superfamilies; Scutigeromorpha. Lithobiomorpha, Geophilomorpha and Scolopendromorpha. The superfamily Lithobiomorpha and genus *Lithobius* are the most abundant. Out of 91 Croatian taxa 50.5% belong to Lithobiomorpha, and 38.5% belong to genus *Lithobius*. Out of 91 taxa in Croatia, 37 are European endemics, 16 are cosmopolites, 6 are Mediterranean endemics, 5 are Eastern European endemics, 3 are Euromediterranean endemics and 1 is an Istrian endemic. The other taxa are European macroregional endemics and 7.8% of the total number of taxa are Croatian endemics. The genus *Eupolybothrus*, member of Lithobiidae family, is

represented by 9 species, of which all are present only in the Mediterranean macroregion. Specimens have been collected from 43 different cavernicolous and epigean localities, analyzed through UTM grid map of Croatia (10 x 10 km) and according to macroregions. Out of the 9 species, 3 are European endemics and are widely spread in Croatia, while Adriatic, Dinaric and Balkan endemics are each represented by 2 species. *E. leostygis* and *E. obrovensis* are troglobionts, found in Croatia only in a few caves, with a high degree of morphological adaptations to cave habitats. Other species are troglophiles, also found mainly in caves, but in epigean habitats as well.

Oral presentation:

Contribution to morphology of palpigrade *Eukoenenia spelaea* (Peyerimhoff, 1902) and its distribution in the Western Carpathians

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Palpigrades are primarily inhabitants of upper soil layers of tropical forests. Moreover, they occur in caves of the Northern Hemisphere where they are extremely rare. *Eukoenenia spelaea* (Peyerimhoff, 1902) is the only palpigrade species inhabiting the caves in the Western Carpathians (Slovakia, Hungary). Firstly it was described as *Koenenia vagvoelgyi* Szalay, 1956. Later, Dózsa-Farkas and Loksa (1970) made a redescription of the taxon and transferred it as subspecies to *Eukoenenia austriaca*. However, it clearly belongs to *E. spelaea*-complex that involves five subspecies with very vague taxonomic status since descriptions were made based on few specimens only and differential characters were not properly described. The present contribution is based on the detailed morphological study of a population from the Ardovská Cave in Slovakia. Unique collection of specimens allowed to study variability in the most important characters and to evaluate critically the subspecies status of the species. SEM electron-microscopy was used to study detailed morphological structures. At present 14 caves in the Western Carpathians are known to be inhabited by *Eukoenenia spelaea*, 17 in Slovakia and 4 in Hungary. It is the northernmost territory with distribution of palpigrades in the world. Paleogeological and paleogeographic data are used to explain distribution range of *E. spelaea* north to

Pannonian Basin. Feeding habit of palpigrades is unknown. Results from observations of the gut content using fluorescent stain are shortly discussed.

Poster presentation:

Subterranean arachnids of the Western Italian Alps (Arachnida: Araneae, Opiliones, Palpigradi, Pseudoscorpiones)

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The poster shows the results of five years of field work dedicated to the Subterranean arachnids of the Western Italian Alps. These results that have been recently published in the "Monographies" of the Natural Sciences Museum of Torino (NW-Italy), concern material collected by the authors with an intense field work (2005-2010) and a fruitful collaboration with several European arachnologists (among them: A. L. Schönhofer and E. Christian - harvestmen and palpigrads). Spiders represent the major order recorded in the study area, with *Meta menardi* and *Metellina merianae* being the most abundant, followed by *Nesticus eremita, Malthonica silvestris*, *Pimoa rupicola* and *Troglohyphantes lucifuga*. Most remarkable are the troglobiont species of the genus *Troglohyphantes* (*T. konradi, T. pedemontanus, T. lanai*) that also show, together with the troglophilic *T. bornensis, T. nigraerosae* and *T. pluto,* the most restricted distributions. Another interesting species is the troglobiont *Nesticus morisii*, only known from the type locality in the Maritime Alps. *Meta bourneti*, a troglophilic and markedly thermophilous species is confined, in NW Italy, to one cave in the province of Cuneo and a few caves in Susa Valley (province of Torino). Among opilionids, the most interesting taxa are *Holoscotolemon oreophilum,* three species of *Ischyropsalis*, and *Leiobunum religiosum.* Palpigrads represent the flagship of the work and of the entire arachnological fauna of the Western Italian Alps. According to current knowledge, the south-western part of the Alpine chain houses *Eukoenenia bonadonai* and *E. strinatii.* Several species of Pseudoscorpionids such as *Pseudoblothrus peyerimhoffi, P. ellingseni, Chthonius italicus, C. troglophilus*, and *Neobisium zoiai* deserve special attention for their pronounced troglomorphy and the restricted distribution. Scorpions and mites have not been considered, but an extremely specialized piedmontese species of *Troglocheles* (Prostigmata: Rhagidiidae) has been described recently by Miloslav Zacharda.

Poster presentation:

Biogeographic patterns of lithistids (Demospongiae) from Mediterranean marine caves

Renata Manconi *[1], Fabio D. Ledda [1, 2], Giacinta Angela Stocchino [1], Giuseppe Grafitti [1]

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Lithistid sponges recorded from marine dark karstic caves of the Mediterranean Sea are typically skiophilous and stygophilic/bathyphilic. They belong to the genera *Aciculites*, *Microscleroderma, Neophrissospongia*, *Neoschrammeniella, Discodermia* and *Gastrophanella* (families Scleritodermidae, Corallistidae, Theonellidae, Siphonidiidae). Geographic ranges of these ancient relic taxa show a peculiar spot-like pattern in the subtropical-tropical oceans from the Caribbean to New Caledonia. Worldwide records are reported on maps to highlight that Mediterranean lithistids belong to genera all characterised by a disjoined Tethyan distribution along the ancient margins of the Mesozoic Sea. The research was supported by Italian Ministero dell'Ambiente (MATTM, Studio degli ambienti di grotte marine sommerse (Codice Habitat 8330) nelle Aree Marine Protette di Pelagie, Plemmirio e Capo Caccia), Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MIUR-PRIN), EU Interreg III Sardinia-Corsica-Tuscany, Fondazione Banco di Sardegna and Regione Autonoma Sardegna.

Poster presentation:

Is the geographic range of the palaeoendemic sponge *Petrobiona massiliana* (Porifera: Calcarea) restricted to the central-northwestern Mediterranean Sea?

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Petrobiona massiliana living in dark marine caves is the single species of a monotypic genus belonging to the monotypic family Petrobionidae. It is a small skiophilous sponge (2-3 cm max size) peculiar for the ice-white colour and stony consistency (*petra* means rock in Latin). Rarely recorded, *P. massiliana* is a Mediterranean palaeoendemics apparently restricted to the central-northwestern basin. Despite several investigations it was never recorded in Spanish caves nor in

the Adriatic Sea, but it is known as fossil from Crete. Here we report on a recent census carried out in some insular karstic caves of southern Italy (Sardinia Sea, Ionian Sea, Sicily Channel) to evaluate the status of this protected species, its abundance and geographic range. The present contribution is focused also on the need to investigate the presence of this stygophilous sponge in caves of the northern Adriatic Sea and the eastern Mediterranean basin. The research was supported by Italian Ministero dell'Ambiente (MATTM, Studio degli ambienti di grotte marine sommerse (Codice Habitat 8330) nelle Aree Marine Protette di Pelagie, Plemmirio e Capo Caccia), Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MIUR-PRIN), EU Interreg III Sardinia-Corsica-Tuscany, Fondazione Banco di Sardegna and Regione Autonoma Sardegna.

Oral presentation:

Terrestrial isopods and millipedes in Slovak caves: results of long-term exploration

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Mountainous area of the Slovak Republic (Slovakia) in Central Europe (Western and partly Eastern Carpathians) is strewed with rather huge Mesozoic karst regions (more than 5000 caves). Terrestrial arthropods in the area were investigated from the second half of the 19th Century, including isopods and millipedes, but with limited successes for a long time. Up to the end of the 20th Century, the postulate of absence of local cavernicolous millipedes and terrestrial isopods was generally accepted, with exception of two eutroglophiles, *Mesoniscus graniger* (Isopoda) and *Allorhiscosoma sphinx* (Diplopoda). Nevertheless one troglobiotic millipede, *Typhloiulus polypodus*, was described from the Buekk Mountains in Hungary, as the most southern and rather isolated foreland of the W. Carpathians. Few years before the start of new millennium, other generation of biospeleologists started to investigate local caves (also in non-calcareous bedrock) and to co-operate in field with entomologists, specialized narrowly on beetles before. It has brought fruits, inter alia, 9 other cavernicolous millipedes and two isopods were found. They represent also higher taxa new for the region (e. g. millipede families Trichopolydesmidae, Anthroleucosomatidae and Brachychaeteumidae). One millipede was described as a new species (*Mecogonopodium carpathicum*). Description of two others from the families Haaseidae and Blaniulidae is in preparation. The taxonomy in some other cases is unclear at the moment. Biogeography and ecology of these arthropods are being studied on the fly. All of them are relicts but with various history and origin (probably from Miocene to Pleistocene) with relations to fauna of Southern

Carpathians or SE Alps and Dinarides or Atlantic Europe. The highest biodiversity of cavernicolous species is concentrated to karst areas of plain type in SE karst units of the W. Carpathians (Slovak/Aggtelek Karst, Muránska Plateau), obligate cave dwellers occur exclusively here. It seems their distribution is limited to old fluvial caves with allochthonous watercourses, transported organic material from surface. The bulk of specimens were found at/on wood material. Present state of knowledge of particular species will be discussed. The study was supported by the grant Vega 1/0139/09.

Poster presentation:

Biodiversity of ostracods (Ostracoda, Crustacea) in groundwater habitats of Slovenia

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The extensive sampling campaigns in the past few years in Slovenia provided new information about distributional patterns of many groundwater ostracod species. Many species known until now only from a single site or the type locality showed wider geographical distributions. The biodiversity and species composition of ostracods from different groundwater habitats and different geographical regions is compared and the updated checklist of recent Ostracoda is presented.

Poster presentation:

Contribution to the ecology of Copepoda in sulphidic karst springs (Žveplenica – Dolenja Trebuša, Slovenia)

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For many groups of animals sulphidic habitats represent an inhospitable environment. Žveplenica (46°5′38.21″N 13°50′20.04″E) sulphidic spring (Dolenja Trebuša, Slovenia) was investigated for faunal diversity and for environmental conditions. The sulphidic spring is situated only some 10 meters south of Dinaric oriented (NW-SE) Kobarid fault and 2.3 km south of regionally important Idrija fault in coarse-grained massive Upper Triassic dolomite with tectonic situation

typical for External Dinarides. The spring discharge was rather constant in the 2-year monitoring period ~1.8 l/min. Water temperature was 10.5 ± 0.2 °C, with pH of 7.56 ± 0.12, and specific conductance of 419 ± 9 µS/cm. Measurements at the spring orifice showed low concentrations of dissolved oxygen (0.13 mg/l). Dissolved sulphide concentration was 7.8 mg/l, sulphate 9.9 mg/l, and nitrate and ammonium was 0.0 mg/l. At the spring orifice filaments of microbial mat were attached in variable quantity. Dry weight of filtered water at the orifice was 2.7 mg/l. Water samples for stable isotope analysis were collected on a monthly basis. The constant $\delta^{18}O$ and $\delta^{2}H$ values in Žveplenica spring indicate long residence times of water in the underground. Invertebrate diversity was screened in different seasons. Seven different taxonomic groups were identified in the spring: Gastropoda, Oligochaeta, Aranea, Acarina, Cladocera, Copepoda and insects larvae. The most abundant group were copepods. In the sulphidic water *Bryocamptus echinatus luenensis*, *Bryocamptus zschokkei*, and *Paracyclops fimbriatus* were identified. Among the indentified copepods ovigerous females and different copepodit stages were present. These species are known to have wide ecological distribution, but little data exists on their presence in sulphidic habitats. Long residence time of water in the underground (>5 months) and stability of physicochemical parameters in Žveplenica spring suggest that these copepods which were found in every season were able to tolerate low oxygen and high sulphide concentrations. The results give us an interesting insight into copepod diversity and their ecology with respect to sulphidic karst habitats.

Poster presentation:

Biospeleological research of Pećina na Vrelu Mokranjske Miljacke Cave in Bosnia and Herzegovina

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Pećina na Vrelu Mokranjske Miljacke Cave with a length over 6800 meters is almost the longest cave in Bosnia and Herzegovina, measured thus far. The cave is located in the Romanija Mountain (near Mokro) on north edge of Dinarides, some 30 km east from capital Sarajevo. It is a hydrologically active complex cave, with one active channel, the main channel and lot of smaller lateral channels.

There are a few fossil channels with the largest one, near to the entrance of the cave. Very interesting findings of archaeological artifacts but also Pleistocene fauna were discovered: almost complete cranial skeleton of an adult male cave bear (*Ursus spelaeus* Rosenmüller & Heinroth, 1794) and cranial skeleton of beaver (*Castor fiber* Linnaeus, 1758*)*. During recent research at the International speleological camp in 2009, systematical biospeleological researches have been performed, including the use of water and terrestrial traps. A rich cave-dwelling fauna have been discovered for several groups: water and terrestrial snails (Gastropoda), spiders (Araneae), spring tails (Collembola), beetles (Coleoptera), but also very interesting taxa of tricladids (Tricladida), false spiders (Opiliones) and cicadas (Cicadomorpha), same as some parasitic fungi taxa. Among them, some taxa are considered to be a new species for science, most interesting new false spider genus, closely related to genus *Hadzinia* (Opiliones, Nemastomatidae). In future, research will proceed with further speleological but also biospeleological research combined with molecular genetic analyses.

Poster presentation:

Collembolan communities (Hexapoda, Collembola) in karst and basalt caves of central Slovakia (Western Carpathians)

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Distribution pattern of cave springtails within three geomorphological units in central Slovakia were investigated during the period 2005-2009: karst caves of the Muránska planina Plateau (MP) and Drienčanský Karst Region (DK), and non-karstic basalt caves in Cerová vrchovina Highland (CH). The study provides the first data on Collembola of volcanic caves in the Western Carpathians. More than 50 caves were examined and springtails were the predominating group of cavernicolous invertebrates. In total 88 species of Collembola were identified. The most diverse communities were detected in 31 monitored caves of the MP (64 species), followed by 15 caves of the DK (45 species) and 7 caves of the CH (21 species). Seven species are common in caves in all three studied units. Nine obligate cave species (troglobites) occurred limited to karst caves of MP (7 troglobites) and DK (4 troglobites). Other 13 taxa (e.g. *Mesogastrura ojcoviensis*, *Pygmarrhopalites pygmaeus*, *P. bifidus*, *Oncopodura reyersdorfensis*) were closely associated with cave environment. Four obligate cave species are new to science (*Pseudosinella* sp. 1, sp. 2, sp. 3 and *Megalothorax* sp. 1), probably endemic species of the Western Carpathians. *Pseudosinella* sp. 1 is known only from 5 caves of central MP exhibiting obvious troglobiomorphisms (e.g. extremely elongated claws). *Megalothorax* sp. 1 is known only from one cave in marginal part of MP.

Pronounced morphological adaptations are missing in *Pseudosinella* sp. 2 and 3 recorded in 2 caves of DK. Two psychrophilous species new to science were discovered in entrance parts of abysses in MP: *Plutomurus* sp. and *Supraphorura* sp. In the contrary, subterranean environment of CH supports diverse cave communities with absence of troglobites. Crevice basalt caves of this volcanic area are rather densely inhabited by troglophilous species such as *Heteromurus nitidus*, *Protaphorura armata* and *Pseudosinella thibaudi*. Moreover, *Pygmarrhopalites pseudoappendices* occurred in these caves representing eutroglophilous species occurring in Central Europe in: (1) mountains where it inhabits epigean habitats, and in (2) lower altitudes as cave-dweller. Other Collembola recorded in caves under study may be classified as epigean or edaphic, common in surface habitats and occasionally colonizing cave entrances.

Poster presentation:

Advances in the knowledge of subterranean Staphylinidae of Morocco: the genus *Apteranillus* Fairmaire (Staphylinidae, Aleocharinae, Lomechusini)

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The genus *Apteranillus* Fairmaire was introduced for an endogean species collected in the region of Tanger (Morocco): *Apteranillus dohrni* Fairmaire, 1854. Several species were subsequently described from North Africa, all endogean or cavernicolous, except one myrmecophilous. The genus *Antrosemnotes* was described by Scheerpeltz in 1935 for the troglobitic species *rotroui*, later downgraded to a subgenus of *Apteranillus*, then synonymized with it. Jeannel (1960) separated seven species living in Algeria and Tunisia in the genus *Apteranopsis*. Later, *Apteranopsis* increased of six endogean and cavernicolous species from Canary islands which were subsequently transferred to Athethini and *Apteranopsis* was downgraded to a subgenus of *Drusilla*. Finally, before the present work, the genus *Apteranillus* contained eight species, five endogean ones: *dohrni* Faimaire, 1854; *pueli* Peyerimhoff, 1907; *tressensi* Peyerimhoff, 1949; *championi* Bernauer, 1936; *peyerimhoffi* Fagel, 1954, and three troglobitic ones: *rotroui* Scheerpeltz, 1935; *ruei* Español, 1969; *minosianus* Lecoq & Quéinnec, 2005. One new species: *Apteranillus bichaini* in litt. has been discovered in Morocco during the Win-Timdouine 2008 speleological and biospeological expedition. Win-Timdouine is the longest subterranean river known in Africa. It is located under the Tasroukht Plateau, in the most oriental part of the Atlas chain, 60 kilometers

north-east of Agadir. Its subterranean course is seven kilometers long (13 km including affluents and ramifications). From this cave was already known the cave adapted Paederinae *Domene cantonsi* Español. During this expedition, other speleological objects were explored in the vicinity of the Taskroukht Plateau. In the cave Imi Ougoug (=Ifri Ouadou 1=grotte du vent) in Aksri, in the Aksri-Ankhout hydrogeological basin, 7 specimens of *A bichaini in. litt.* have been discovered. On this occasion, we redescribe the species of this genus and discuss their phylogenetic relationships.

Poster presentation:

Niphargids of Iran with focus on the Zagros Mountains

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Pervious studies on Iranian niphargids were limited to a single record of *Niphargus valachicus*. Despite many underground sources of water in Iran, especially at the Zagros Mountains, there are no further studies on hypogean amphipods associated with caves and springs with underground origin. The Iranian basin is a large triangular depression flanked by Elburz Mountains in the north and Zagros Mountains in the west. The Zagros Mountains extend diagonally from eastern Turkey to the north of the Persian Gulf and Pakistan border. The current survey aims to study members of the genus *Niphargus* in Iran taxonomically and phylogenetically. At the First step, the karst areas and springs in the west of Iran were located. The specimens were collected from several localities including: Dimeh spring in Chaharmahal-Va-Bakhtiari province, Brolan spring in Azarbaijan province, Sasan River in Fars province, Ghori-Ghale cave in Kermanshah province, Razbashi spring in Lorestan province and Ghaemshahr and Danial cave in Mazandaran province, Cheshmeh-Siah in Kohgiloyeh-Va-Boyerahmad Province. All species of the current study belong to the genus *Niphargus*. Drawings of the key characters were made using Camera Lucida on a compound microscope. Geographical distribution of all species is plotted around the Zagros Mountains. It seems there are at least three new species among the specimens collected from different water source around the Zagros Mountains. The main diagnostic characters of each species will be used in an illustrated key for niphargids of Iran.

Oral presentation:

Water mites (Hydrachnidia) from interstitial habitats of the Russian Far East and their relationship with faunas of adjacent lands

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Recently only four species of water mites in the genus *Wandesia* were known from the interstitial waters of the Russian Far East. During subsequent investigations of interstitial fauna some interesting new records were obtained. The genera *Amerothyasella, Stygomomonia* and *Uchidastygacarus* were reported from Russia for the first time and three new species in these genera were described. Two taxa of the genus *Feltria* (*F. aculeata* and *F. cornuta rossica*), found in superficial waters, are presumably also interstitial, as are the remaining representatives of the species-groups (*denticulata* and *cornuta*-group respectively), to which they belong. Two undescribed species in the genera *Chappuisides* and *Nudomideopsis* collected from river sediments at a depth of about 1 m are being investigated. It is the first report of these genera from Russia. The fauna of interstitial water mites from the Russian Far East is more closely related to those of Japan and North America. One of the above mentioned genera (*Amerothyasella*) is known only from the present territory and North America, the other one (*Uchidastygacarus*) is also widely distributed on the Japanese Archipelago. Two genera inhabiting exclusively interstitial waters (*Stygomomonia* and *Chappuisides*) have a Holarctic distribution. However, a majority of species belonging to these genera is known from Japan and North America, and a few from Europe. The other genera (*Feltria*, *Nudomideopsis* and *Wandesia*) are cosmopolitan. The first of these genera contains mainly superficial species, whereas the second and third contain interstitial species. Supported by: Russian Foundation for Basic Research grant 09-04-98544 and Far Eastern Branch of Russian Academy of Sciences grant 10-III-B-06-104.

Poster presentation:

Discovery of stygobiotic crustaceans in boreholes at the Deep Jurassic aquifer of El Maestrazgo (S.E. Spain)

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Random sampling of groundwaters through 100 to 350m-deep boreholes at the Deep Jurassic aquifer of El Maestrazgo, a region placed close to the Mediterranean coastline in the Spanish Levant, has rendered surprising results, including several stygobiotic crustacean species among Copepoda, Amphipoda, Isopoda and Decapoda. These discoveries have opened new perspectives to the biological study of deep karstic aquifers. More than ten years ago, the Spanish Geological Survey (IGME) at Valencia started to work on the delimitation, extension and connections of El Maestrazgo aquifer, and also on the hydrochemical characterization of the groundwater. This aquifer occupies more than 2,400 km^2 of carbonate rocks ranging from Jurassic to lower Cretaceous in age in the northern half of the province of Castellón. The aquifer reaches between 450 to 800 m in depth, showing high permeability derived from fissuration and karstification. A year ago, a team of biologists belonging to different research institutions from Spain, Romania and Portugal joined IGME hydrogeologists to study the relationship between the groundwater fauna and the spatial and temporal variation observed in several hydrochemical parameters. This was carried out sampling periodically at several depths in deep boreholes, including the freshwater-saltwater interface. The aquifer has few natural openings enabling sampling of aquatic fauna (subterranean rivers or springs), thus the use of deep boreholes is almost compulsory. Ten out of 68 boreholes were considered for biological sampling, which was carried out with two Nytex nets of 140µm mesh size and 25 and 10cm in diameter and 50 and 20cm length, respectively, both provided with a collection bottle. Until now we have recorded several sygobiotic

species of copepods, amphipods, isopods (*Thyphlocirolana* sp.) and decapods (*Thyphlatya miravetiensis*), also known to be present in several caves in the area.

Poster presentation:

A *Bactrurus*-like subterranean amphipod (Crangonyctidae) from the Ural Mountain karst region

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Recent biological inventory of caves and wells in the Ural Mountain karst region by N.N. Pankov and A.B. Krasheninnikov resulted in collections of crangonyctid amphipods from the cave lakes of Kungur Ice Cave, Babinogorskaya Cave, Ordinskaya Cave and driven wells in the Irgina River basin. The moderately stygomorphic animals are about 15–20 mm in length, white in color, and eyeless. Besides the above mentioned places amphipods were found in Metschka Cave that is the type locality of the poorly known *Crangonyx chlebnikovi*, which was described by E.W. Borutzky in 1928. The holotype was not designated properly but a syntype series was kept at the Zoological Museum of Moscow State University. Because the original description of the species was lacking several important details the correct generic assignment has remained problematic. Careful investigation of the recently collected materials leaves no doubt about the placement of this species in the family Crangonyctidae and a close morphological affinity with species of the genus *Bactrurus*. However, despite several similarities with *Bactrurus*, this species differs significantly in the shape of gnathopods and pleonal plates and an increasing number of pleopod retinaculi, and will therefore be designated as type-species of a new crangonyctid genus currently being described. The genus *Bactrurus* is endemic of the North American continent and widespread in subterranean groundwaters of the east-central United States. The finding of *Bactrurus*-like subterranean amphipod at the Ural Mountain karst region is a significant aspect in the understanding of Crangonyctidae evolutionary. Supported by the Russian Foundation for Basic Research grant 09-04-98544.

Poster presentation:

Triclads from Italian groundwaters (Platyhelminthes, Tricladida)

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Subterranean triclads from Italy include both stygobiotic and stygophilous species. The first record on triclads from Italian groundwaters is dated as far back as 1890 when Garbini reported the stygophilous species *Polycelis nigra* from two wells near Verona (north-eastern Italy). Up to now, among stygobiotic species, the genera *Dendrocoelum* (Dendrocoelidae), *Atrioplanaria, Phagocata* s.l. and *Polycelis* (Planariidae) are reported. Stygophilous species belong to the genera *Dugesia* (Dugesiidae), *Dendrocoelum* (Dendrocoelidae), *Polycelis* and *Crenobia* (Planariidae). Data on both stygobiotic and stygophilous species are mainly restricted to central-northern Italy and Sardinia. This contribution provides an account on the subterranean triclads from Sardinia with new records and a taxonomic synopsis on Italian taxa. Funds were provided by PRIN-MIUR and the EU project Interreg III Sardinia-Corsica-Tuscany. G. Stocchino acknowledges financial support from SYNTHESYS, a programme of the European Commission under the 6th Research and Technological Development Framework Programme "Structuring the European Research Area", which enabled GS to work at the Zoological Museum Amsterdam in November and December 2008 (grant number: NL-TAF 4717).

Poster presentation:

Species diversity and distribution of aquatic Crustacea in caves of Slovakia (Central Europe, Western Carpathians)

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The paper summarises the data from all available published sources (mainly of O. Štěrba, S. Hrabě, M. Straškraba, V. Košel, I. Hudec) and the author's own stygobiological research realized during 2002-2009. Slovakia is rich in caves (more than 5500), but relatively poor in stygobiotic or endemic crustacean species in comparison with karstic areas of southern Europe (mainly Balkan Peninsula). In

total, 27 Copepoda (belonging to families Cyclopidae, Canthocamptidae, Diaptomidae), 4 Amphipoda (Gammaridae, Niphargidae, Crangonyctidae), 1 Syncarida (Bathynellidae), 2 Ostracoda (Ilyocyprididae, Candonidae) and 9 Cladocera (Daphniidae, Chydoridae) species have been recorded from water habitats inside the Slovak caves up to date. Among them, 23 species can be considered as stygobiotic or stygophilic. Of the subterranean crustaceans, *Niphargus tatrensis* and *Bathynella natans* are typical inhabitants of the cave waters, such as pools, hyporheic interstitial (mostly *B. natans*), as well as running waters or springs flowing out of caves (mostly *N. tatrensis*) in various parts of the country. The Dinaric-Carpathian endemic amphipod *Synurella intermedia* is known from four Slovak caves, and another species, *Niphargus inopinatus*, only from the Bojnická hradná Cave near the Prievidza town. The common epigeic *Gammarus fossarum* tends to move in the hypogean environment. Its permanent and numerous populations showing a certain degree of reduction in eyes and pigmentation, were recorded in several underground streams (e. g. Brestovská Cave, Drienovská Cave, Milada Cave). Harpacticoida are represented mostly by members of the genera *Elaphoidella* (*E. pseudophreatica*, *E. phreatica*, *E. proserpina*), *Bryocamptus* (*B. zschokkei*, *B. echinatus*, *B. typhlops*, *B. spinulosus*), in some cases also by *Maraenobiotus vejdovskyi*, *Paracamptus schmeili* and *Epactophanes richardi*. Of the 15 cyclopid copepods, the most diversified genus is *Diacyclops* with its 6 taxa of languidoides-languidus group. Stygobiotic *Acanthocyclops venustus*, *Microcyclops rubellus* and stygophilic *Paracyclops fimbriatus* are also present. Ostracoda are known from several caves, but their taxonomic status is still unknown. Of all only two species have been identified: *Ilyocypris bradyi* from the Borová hora Cave within the Zvolenská kotlina geomorphologic unit and *Cryptocandona dudichi* from the Domica-Baradla cave system in the region of Hungary-Slovakia boundary zone. No stygobiotic Cladocera and Calanoida are known from Slovakia up to date. Findings of aquatic Isopoda from cave waters are absent.

Poster presentation:

A 21st Century identity for an old snail condemned to darkness – Barcoding *Zospeum* (Pulmonata, Ellobioidea, Carychiidae)

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Species identification through a short, homologous and ubiquitous stretch of nucleotides can be promising when classical taxonomy reaches its limits, e. g. through high morphological intraspecific variability or morphological stasis. DNA barcoding, i. e. delimitating species with a mitochondrial-encoded 650 bp fragment of the cytochrome c oxidase subunit 1 (CO1) gene, has shown to be especially suitable for species recognition. Suitability in this case means that intraspecific and interspecific genetic variability can be clearly separated. Here, we present a DNA barcoding approach to distinguish species in the troglobitic taxon Zospeum Bourguignat, 1856. These blind and colorless snails are endemic to the karst caves of central and southeastern Europe. Our investigation presents a DNA barcoding and scanning electron microscopic (SEM) debut for Zospeum (Pulmonata, Ellobioidea, Carychiidae) species collected in Slovenian and Croatian caves. The combination of minute size, the general paucity of clearly distinguishing interspecific conchological characters and the strong selective pressure associated with subterranean habitats make this taxon an ideal workhorse model to test the applicability of this method. Transferring this novel approach to other subterranean organisms can well reveal an enormous cryptic diversity otherwise hidden in the depths and vagueness of the dark.

Poster presentation:

First record of a root community in Southeast Asia: cave-dwelling planthoppers from Maros karst, Sulawesi (Hemiptera: Fulgoromorpha: Cixiidae: Bennini)

Andreas Wessel* [1, 2], Roland Mühlethaler [1], Kristina von Rintelen [1], Thomas von Rintelen [1], Björn Stelbrink [1], Ekkehard Wachmann [1], Hannelore Hoch [1]

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A systematic survey of Maros karst caves in summer 2009 revealed the first known terrestrial cave with roots in the dark zone and an associated fauna for Southeast Asia. Remarkably, this very first discovery of available resources for a root community in the region coincides with the finding of planthoppers as sap-sucking primary consumers. Planthoppers are common elements of root communities in different parts of the world. A total of more than 50 cave-dwelling species are known from Africa (incl. Madagascar), Australia, Latin America, and several oceanic islands. Two-thirds of the troglobiotic and troglophilic species belong to the Cixiidae as well as the newly discovered species from Sulawesi. The Maros cave planthoppers however, are the first representatives of the tribe Bennini ever recorded in a subterranean environment. The Bennini (about 100 species) are characterised by a unique feature – they possess very conspicuous lateral appendages each ending in a wax-covered sensillum. The precise function of these appendages and a possible role in orientation in the dark is unknown as in general the biology of this group is poorly studied. It is assumed that the ability of planthoppers to communicate by substrate vibrations is a prerequisite for the colonisation of cave environments. A well-studied example from Hawaii shows species-specific "song" patterns and revealed a complex pattern of subterranean speciation. The successful recording of vibrational signals from the Maros cave planthopper may open up a new model system for the study of the dynamics of subterranean evolution.

SYMPOSIA

9. Evolution and Systematics of Subterranean Beetles

Poster presentation:

The genus *Aphaobius* Abeille de Perrin, 1878 (Coleoptera, Cholevidae, Leptodirinae)

Marco Bognolo

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The genus *Aphaobius* was established in 1878 by Abeille de Perrin to segregate the species *Adelops milleri* (Schmidt, 1855). A few years later, the species *A. heydeni* was described by Reitter. After the first decade of the 20th century eleven new taxa were described by J. Müller. Further three subspecies of *A. milleri* were described in the ensuing decades by Mandl and then a new, remarkably distinct species was found and described by Pretner in 1963 as *A. muellerianus*. Only recently, new investigations led to the discovery of the new species *A. grottoloi* (Vailati, 2004). Following the latest revision (Bognolo & Vailati, 2010), the genus is composed of four species groups: *muellerianus*, *heydeni*, *kraussi* and *milleri*. The northern *kraussi* group is a cluster of species distributed on the left-hand side of the river Sava, which includes *A. mixanigi*, *A. angusticollis*, *A. knirschi*, *A. brevicornis* and *A. kraussi*. The southern *milleri* group is a cluster of species distributed on the right-hand side of the river Sava, which includes *A. forojulensis*, *A. grottoloi*, *A. lebenbaueri*, *A. miricae*, *A. kaplai*, *A. fortesculptus*, *A. robustus*, *A. kahleni*, *A. milleri*, *A. ljubnicensis*, *A. kofleri* and *A. alphonsi*. The *muellerianus* group and the *heydeni* group include one species each: *A. muellerianus* and *A. heydeni*. The overall distribution of the genus *Aphaobius* shows a combination of species with large distribution areas and other species known from a single site or a very restricted geographic area. The correlation of paleogeographic features and allopatric speciation is evident when considering the *kraussi* and the *milleri* species groups. In particular, the northern *kraussi* group is geographically located within the Southern Alps, whilst the southern *milleri* group is mainly located on the Outer Dinarides. In particular, in central Slovenia such groups have long been isolated due to the movement of respective plates which, according to plate tectonics, slid along the contact boundary represented by the Sava fault. To sum up, the speciation patterns of the genus *Aphaobius* show past isolation in the region between Ljubljana and Železniki, characterised by the complex paleogeographic evolution, as opposed to a high dispersal activity of the southern area, along the typical north-west to south-east orientation of limestones in the northern Balkan area.

Oral presentation - invited plenary lecture:

From *Anophthalmus schmidti* to molecular phylogenies: past and present in the knowledge of subterranean carabid beetles (Coleoptera: Carabidae)

Achille Casale

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The author presents an updated, tentative synthesis of the available knowledge of the main groups of carabid beetles (Geadephaga, or Caraboidea) represented in subterranean environments in different areas of the world. Caraboidea, with some families of Staphylinoidea, represent the almost totality of the subterranean Coleoptera. As generalized ground and mostly predaceous beetles, they became a successful group in all kind of soil and subterranean environments, and some of them show the most impressive examples of adaptation to hypogean life. In particular, three taxonomic groups will be stressed: 1. Scaritinae of the subtribe Reicheiina, with emphasis on the main questions concerning their global distributions, and their possible "adaptive radiation" in Sardinia; 2. Trechinae of the tribe Trechini, stressing the opportunity of a new taxonomic treatment of "phyletic lineages" supported by bio-molecular data; 3. Pterostichinae of the subtribes Molopina and Sphodrina, with emphasis on the Euro-Mediterranean genera, highly interesting from the biogeographic point of view. Some questions concerning other groups of subterranean carabids (Paussinae Ozaenini, Promecognathinae, Dryptinae Zuphiini) will be also recalled and debated. Funds were provided by PRIN-MIUR (Ministry of the University and Scientific Research, Italy) and the EU project Interreg III Sardinia-Corsica-Tuscany.

Oral presentation:

The geographic and phylogenetic limits of Leptodirini

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The tribe Leptodirini of the family Leiodidae includes one of the most extensive known radiations of subterranean beetles, with almost 900 species in ca. 240 genera. The highest diversity of the tribe is found in the Mediterranean basin, in particular in the north and east of the Iberian peninsula, Corsica and Sardinia, the southern Alps, Balkan peninsula, Romania and Southern Russia, the Caucasus, Middle East and Iran. The monophyly of the western Palaearctic Leptodirini is

well supported both from morphological and molecular characters, but there are a number of genera outside this geographical area that have usually been linked with Leptodirini based either on their general appearance or in some specific characters, but are of uncertain phylogenetic position. The recent finding of specimens of two of these genera (*Fusi* and *Sciaphyes*) by one of us (VVG) in the Siberian far East, and the accessibility to specimens of another (*Platycholeus*) allowed us a re-examination of their phylogenetic relationships, and a more precise delimitation of both the geographic and phylogenetic limits of Leptodirini.

Oral presentation:

The blind cave beetle that isn't: histological, behavioral and molecular evidence of functional photoreceptors in *Ptomaphagus hirtus*

Markus Friedrich* [1, 2], Puneet Rai [1], Ronald Barrett [2], Bryce Daines [3], Rui Chen [3]

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The small carrion beetle genus *Ptomaphagus* diversified into more than 50 species, which range from ancestral surface dwellers to facultative and obligatory cave inhabitants in the Southeast of the United States. One of the best-studied representatives is the troglobite *Ptomaphagus hirtus*, which is endemic to the cave system of Mammoth Cave National Park. *P. hirtus* adults are characterized by complete reduction of the hind wings and near complete reduction of the compound eye to a small lens patch. In his survey of North American cave animals, Packard (1888) was unable to detect photoreceptors or optic neuropils in sections of the adult head of *P. hirtus*, which led him to conclude that *P. hirtus* lacks visual senses. This assessment, however, is in conflict with the subsequent discovery that the specification of lens cells in the developing insect compound eye is dependent on inductive signals from differentiating photoreceptors. We therefore readdressed the question whether *P. hirtus* possesses a functional visual system. In serial sections of the adult head, we found organized cell clusters immediately beneath the lens patch cuticle. These cell clusters are separated from the head cavity by a basal membrane, which is penetrated by optic nerve like structure reminiscent of the organization of the retina in surface beetle species. Consistent with the presumed presence of photoreceptors, *P. hirtus* tested negatively phototactic in light versus dark choice assays. In addition, deep

sequencing of transcripts from *P. hirtus* adult head tissue recovered orthologs of genes, which are known to be specifically involved in phototransduction including opsins. In combination, these data suggest the presence of functional photoreceptors and the preservation of visual capacity in *P. hirtus*.

Oral presentation:

From the cavernicolous to the subterranean concept: past and present in Leptodirinae (Coleoptera, Cholevidae)

Pier Mauro Giachino

Settore Fitosanitario Regionale, Environment Park, Palazzina A2, Via Livorno 60, 10144 Torino, Italia

A brief overview of the evolution of the classification of Cholevidae Leptodirinae is given. It is based on experience acquired in the research of terrestrial subterranean fauna in Greece during the last 20 years. An interpretation of the relationship between fauna and the different spatial components of the Subterranean Environment is discussed. Attention is given to the definition of the subterranean environment in the Euro-Mediterranean area and of its limits, with some of its different components (fissure network of the bedrock, MSS and caves). This is compared to the endogean environment that is characteristic of the soil, and the use of the term "subterranean fauna" is restricted only to the hypogean habitat. Similarly, the faunas associated with the subterranean environment are defined, as well as the relationship between fauna and fissure network of the bedrock and the influence of bedrock type and tree cover on fissure network and MSS formation and colonization. Description of methodology and techniques used in the multi-annual research on the subterranean fauna in Greece and the result obtained concerning Leptodirinae beetles are presented.

Oral presentation:

Leo Weirather (1887-1965) revisited

Bernd Hauser

Museum of Natural History, Department of Arthropodology and Entomology I, PO box 6434, CH-1211 Geneva 6, Switzerland

As Egon Pretner's monograph on Leo Weirather finally goes to press in Innsbruck, the editor of this posthumous publication presents a short sketch of the life and work of Weirather, the extraordinary Tyrolean pioneer of biospeleological exploration in the Balkans, and explains the background to Weirather's use of "code names" (Decknamen) for his collecting sites. The speaker was, while assistant at the Zoological Institute in Innsbruck, in close contact with Weirather for some years, and tried in vain to safeguard his scientific legacy for Innsbruck

University. When Weirather passed away, the speaker was able to facilitate the deposit of Weirather's collection and unpublished works in the Geneva Natural History Museum, thereby avoiding their dispersal or loss. The commitment of Dr. Claude Besuchet, the famous beetle specialist of the Geneva Museum, actively supported by Prof. Villy Aellen, the then director and a well known biospeleologist, was of crucial importance in saving Weirather's legacy in 1966. As a responsible curator Besuchet did not content himself with making this interesting acquisition, but took the trouble to make this hidden treasure accessible to the biospeleologists of the world. He accepted the offer of the doyen of cave fauna research in the Balkans, Egon Pretner, to take on the Sisyphean task of deciphering Weirather's list of localities and lent him portions of the precious files over a number of years. Egon Pretner (1896-1982), who lived and worked in Postojna (Adelsberg), the birthplace of biospeleology and the site of this conference, was uniquely qualified for this arduous task. As a citizen of the Austro-Hungarian Monarchy he attended the German grammar school of Trieste, (his native town) and learned the same style of German shorthand (following the system of Gabelsberger, long since abandoned), as was used by Weirather. Later as citizen of the former Yugoslavia he had experience of the changes to place names that were imposed. Finally, having explored a huge number of caves himself, he had a tremendous topographic knowledge which helped him to translate Weirather's partly outdated names into modern nomenclature. He not only invested years in decoding hundreds of index-cards, but also drew up a synthesis: a sort of "Fauna endemica" that had been Weirather's unrealized dream. Pretner's magnum opus is a rare and illuminating example of scientific altruism! When he entrusted his manuscript of 168 typed pages in German, (Pretner 2010: Die Verdienste Leo Weirathers um die Biospeläologie, insbesondere Jugoslawiens, sein Höhlenkataster und seine Sammelplätze. – *Ber. nat.-med. Verein Innsbruck* 97 (in press) to the speaker for publication in Geneva in the mid-1970s, the political situation in Yugoslavia was starting its gradual deterioration. In the end, publication abroad became impossible without serious consequences for the author, who thus passed away without the satisfaction of seeing the results of all his heroic efforts in print. Subsequent changes to the hierarchy of the Geneva Museum meant that publishing the manuscript was no longer a priority. The unexpected appearance of a draft version of part of Pretner's monograph on Weirather, translated into English (Giachino & Lana, eds., 2006: Leo Weirather (1887-1965): Diaries of a biospeleologist at the beginning of the XX century. – *Fragmenta Entomologica* 37(2): 1-264) which increases its value, lent a new impetus to the publication of the complete work.

Poster presentation:

The edge of the range of genus *Anthroherpon* in Croatia

Branko Jalžić [1], Petra Bregović* [2]

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Although they have always attracted the attention of many biologists, subterranean beetles of genus *Anthroherpon* deserve to be still observed. The Republic of Croatia is the edge of their range, and no one has yet systematically analysed data on them. As part of the posters it will be presented the historical and recent data of these interesting beetles, as a result of re-survey of known sites from the existing literature (two caves: Špilja za Gromačkom vlakom, Močiljska špilja; one pit: Glogova jama). It will also be processed data from new sites (Kornjatuša jama) and will be compared with the nearest cave type locality in Bosnia ans Herzegovina. For now, the species recorded in Croatia are *Anhtroherpon apfelbecki apfelbecki* and *Anthroherpon matulici.* During this study the discovery of new taxa is possible. The poster will clearly display the distribution and taxonomy of the genus *Anthroherpon* in Croatia. There will be also a small note on the genus *Leptomeson*.

Poster presentation:

Diversity of troglobiotic beetles (Insecta, Coleoptera) of Krivošije area (Orjen Mt, Montenegro) in the scope of the recent biospeleological investigations

Iva Njunjić * [1], Dragan Pavićević [2]

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[2] Institute for Nature Conservation of Serbia, dr. Ivana Ribara 91, 11070 Novi Beograd, Serbia

Krivošije area (Orjen Mt.) belongs to the Dinarides karstic massif and represents one of the most important diversity hot spots of troglobiotic arthropod fauna. Despite the fact that Krivošije area is very well biospeleologically investigated, some very important new records of the troglobitic beetles, including new species and genera, have been noticed in recent years. The overview of all troglobiotic beetles of the mentioned area is given.

Poster presentation:

Update of the knowledge of the Ibero-Balearic hypogean Carabidae (Insecta: Coleoptera): faunistics, biology and distribution

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The Iberian Peninsula, because of its special location in southern Europe and its abundance and diversity of karst, has a large number of hypogean arthropods, among which, there is a notable presence of Carabidae. Often, new discoveries of exclusive subterranean taxa are added, which are listed in the very briefly discussed catalogues of the Ibero-balearic fauna. This procedure, that is correct with regard to general catalogues of Carabidae, seems to be insufficient for the hypogean species. This work updates all faunistic, biological and chorological information available on the Ibero-balearic hypogean Carabidae. Finally, according to the distribution of the lineages of the most representative of them, a regionalization of the Iberian Peninsula in biospeleologic districts is proposed. This biogeographic proposal is compared with others already known, which have been elaborated with the study of other groups of Arthropoda.

Poster presentation:

First finding of the troglobitic genus *Seracamaurops* (Coleoptera, Staphylinidae, Pselaphinae) for Croatia

Dragan Pavićević* [1], Roman Ozimec [2]

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During recent biospeleological research of Sniježnica Mt. (1234 m) in Konavle region near Dubrovnik, most south part of Croatia, performed by members of Croatian biospeleological Society (CBSS), new taxa of the genus *Seracamaurops* (Coleoptera, Staphylinidae, Pselaphinae) have been found. The new species was found in two caves, regularly in deep and cold cave habitat, under stones. The genus *Seracamaurops* is widespread in the southeastern part of the Dinaric region, till the reported finding known only for Bosnia and Herzegovina and for Montenegro. The genus *Seracamaurops* is divided into the subgenera *Seracamaurops* s.str. and *Cordiamaurops*. Including the new species, it counts altogether 13 species.

Oral presentation:

What does palaeontology reveal on the radiation of Leiodidae, Cholevinae and their colonisation of the subterranean biotopes?

Michel Perreau

Université Paris 7, IUT Paris Diderot case 7139, 5 rue Thomas Mann, 75205 Paris cedex 13, France

The chronology of the radiation of zoological groups can be inferred at least by three main methods: paleogeography, molecular genetics linked to a molecular clock hypothesis and palaeontology. For Leiodidae Cholevinae, and especially their subterranean adapted phyla, palaeogeography has been emphasised by Jeannel in several renown, but ancient contributions, and later by Giachino & al. (1993). Molecular genetics have been approached recently for Pyrenean Leptodirini species (Ribera & al., 2010), leading to evidences of monophyly, a reconstruction of the phylogeny, and an estimation of the chronology of radiations since the Eocene. The purpose of our presentation is to give an overview on recent investigations in the newly explored third way: palaeontology. Specimens of Cholevinae from several amber deposits of various ages are presented (Oligocene: Dominican Republic; Eocene: Baltic; Cretaceous: Myanmar...). The morphological investigations are enlightened using propagation phase contrast X-ray microtomography which allows a non invasive virtual dissection of specimen and a full comparison of external and internal structures (when preserved) with the extant fauna. The wide range of geological periods scanned, from lower Cretaceous to Oligocene, allows an investigation of the morphological changes compared with the extant fauna. These preliminary results will be confronted to the other approaches, emphasising the special instance of the Pyrenean subterranean fauna, which is so far the best known from other methods.

Oral presentation:

Suprageneric systematics of leptodirine beetles (Leiodidae, Cholevinae): molecular versus morphological characters

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Past attempts to understand the evolution and establish a phylogenetic system of the Leptodirini (Leiodidae, Cholevinae) were based on morphological characters. None of these attempts could satisfactorily explain the resulting morphological

and biogeographical patterns. Most authors concluded that modern molecular approaches are the only possible and legible way to solve the enigmatic Leptodirine phylogeny in the future. In the last years, we conducted a molecular phylogenetic study of 54 different genera of Leptodirini. We sequenced about 3.3 kbp from two mitochondrial (COI and 16S) and three nuclear gene segments (two pieces of 28S rDNA, Histone H3), and analyzed them using standard phylogenetic procedures. External and internal morphological characters used so far in the higher suprageneric classifications of leptodirines were cladisticly analyzed in combination with molecular data. Phylogenetic trees from different loci recovered a monophyletic origin of the studied leptodirines. Our results and those recently obtained by other authors suggest that most subterranean Leptodirini are geographically grouped. The most important and consistent result of the molecular phylogenetic reconstruction was the resolution of major lineages differing significantly from those recognized at present based on morphological characters only. The traditional suprageneric subdivision of leptodirines into Antroherponini (Antroherpona) and Bathysciini (Bathysciae) as well as subtribes Anthroherponina, Spelaeobatina, Bathysciina, Bathysciotina, Leptodirina and Pholeuonina are polyphyletic groups and have to be redefined or rejected. Since not all of the genera or genera-groups were molecularly tested, a more precise new systematics of the Leptodirini is not yet possible.

Poster presentation:

Evolution and phylogeny of the subterranean genus *Troglocharinus* (Coleoptera, Leiodidae, Leptodirini)

Valeria Rizzo* [1], Jordi Comas [2], Floren Fadrique [2], Javier Fresneda [3], Ignacio Ribera [1]

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Among Coleoptera, the tribe Leptodirini (Leiodidae, Cholevinae) includes some 240 genera and 1800 exclusively subterranean species that present morphologic and physiological characters related to the endogean habitat: blindness, depigmentation, typical "pholeunoid" or "batiscioid" morphologies, size reduction, or changes in physiology and life cycle. They are mainly distributed in the north side of the Mediterranean area, from the Iberian peninsula to the Middle East. Despite continuous attention from entomologists for the last two centuries, their phylogenetic relationships and evolutionary origin remain controversial. In this work we study the phylogeny and diversification of the genus *Troglocharinus*, a member of the recently identified monophyletic Pyrenean clade of Leptodirini,

largely corresponding to the traditional *Speonomus* series. The genus *Troglocharinus* presents a disjunct distribution, with twenty species distributed in the coastal ranges of Catalonia (Garraf, San Llorenç del Munt i Obac and Montserrat), and twelve in the pre-Pyrenees (Serra del Montsec de Rubies, Serra del Boumort, Alt Urgell and Serra de Lleràs), with a single isolated species in Alto Aragón. Due to the strong convergence of external morphological characters and the abundance and intraspecific variability of some taxa the taxonomy of the genus has been very unstable. We aim to establish a robust phylogeny to study the evolution of this extensive subterranean species radiation, and to provide a temporal framework for the diversification of various lineages and the colonization of the geographical areas in which they occur. For that purpose we use molecular phylogenies of six mitochondrial (cox1, cob, rrnL, trnL and nad1) and two nuclear (SSU and LSU) genes. Preliminary results using eleven species and seven subspecies show the respective monophyly of the Pyrenean and the coastal clades with a strong geographical structuring within each of them, suggesting the existence of multiple independent evolutionary lineages and the need of a deep taxonomic reordination of the genus.

Oral presentation:

What determines subterranean ground beetle diversity in the West Palaearctic? A macroecological approach using country-based distribution data (Coleoptera: Carabidae)

Andreas Schuldt, Claudia Drees, Nora Drescher, Katharina Schäfer, Thorsten Assmann*

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Potential impacts of historical and contemporary environmental conditions on the distribution of subterranean carabids in the western Palaearctic have been studied using species richness and environmental data on a country level. Regression models and variation partitioning showed a strong relationship between species richness and range in elevation. Potential effects of climatic variables, mainly those related to ambient energy input, were much weaker. These results are in contrast to conclusions from other studies regarding the determinants of distribution patterns of subterranean biodiversity. Historical climatic events (e.g. the distribution of permafrost grounds in Europe during the glacial periods) seem to have strong influence on present-day distribution patterns (especially on the northern limit of subterranean species) as already suggested (but not tested) by Holdhaus some decades ago. The (significant) decrease of subterranean diversity towards the southern Mediterranean region and the Sahara-Arabian desert belt is doubtlessly more difficult to explain. Especially the new findings from the Middle

East reveal the possibility of a bulk of not yet described species and indicate the need of further studies for a better understanding of distribution patterns of subterranean ground beetles.

Oral presentation:

The influence of aboveground invasions on the diversity and distribution patterns of subterranean carabids (Carabidae)

Al Vrezec*, Andrej Kapla

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The diversity of subterranean beetles in Slovenia appeares to be one of the highest on the globe. The studies were so far concentrated on the aspects of gamma and beta diversity of the group, but much less on the aspects of alpha diversity, which reflects actual coexistence of species in the same ecosystem. Carabids (Carabidae) form the most important terrestrial predator guild in underground ecosystems. In the study we focused on the diversity and spatial distribution patterns of coexisting subterranean carabids related to biotic and abiotic parameters. From the most Slovenian caves only one carabid species is known, but subterranean carabid assemblages (SCA) can hold up to five coexisting species. For the study we selected nine caves with SCA consisted from one to four species. The diversity of SCA compared to aboveground carabid (AC) assemblages was significantly lower, but did not change significantly from the entrance towards deeper parts of caves. However, the SCA diversity was significantly affected by the invasion of aboveground species, especially by invasion of AC. The AC negatively influenced the abundance and species richness of SCA. The abundance of AC significantly decreased towards from the entrance distant parts of caves, but was positively associated with higher amount of moisture and organic matter in the substrate. On the contrary, SC were more abundant at substrate with less moist and organic matter, which can act as areas free of competition or even predation by AC species. Among SC eight species were sampled in higher numbers, which were divided into three groups according to their spatial distribution patterns within caves: (1) entrance SC were most abundant at the entrance part of the cave (1 species), (2) deep SC were most abundant at the most distant parts of the cave from the entrance (2 species), and (3) intermediate SC with no specific preference found connected to the distance from the cave entrance (5 species). Among these groups, the AC significantly negatively affected only the abundance of entrance SC presented by *Laemostenus schreibersi*, the largest and the most abundant species in the SC guild.

SYMPOSIA

10. New Models, Methods, and Concepts for Subterranean Biology

Oral presentation:

The structure and diversity of the microbial community inhabiting the hind gut of the olm (*Proteus anguinus*)

Gorazd Avguštin* [1], Simona Gramc [1], Lilijana Bizjak Mali [2], Boris Bulog [2], Jerneja Ambrožič Avguštin [3]

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The amphibian salamanders known as "olms" inhabit the Karst underground systems along the Mediterranean Sea. Adapted exclusively to underground environment, which is denoted by complete darkness and lack of nutrients, they have developed an ability to survive for long periods without or with very little food. Since they are predators living mainly on avertebral pray, containing chitin, their microbial gut symbionts are most likely crucially involved in their nutrition. However, such possibilities have not yet been investigated intensely. Here we describe the first attempts to reveal the structure of the microbial community inhabiting of the olm's gut by traditional culturing and molecular biology approach. The gut contents of the animals from the "Planinska jama" underground Karst cave located in the South West of Slovenia were used for total microbial DNA isolation and subsequently the 16S ribosomal RNA genes were amplified using conserved bacterial and archaeal oligonucleotide primers. The randomly selected clones containing inserted amplicons were sequenced at Marogen Inc. Our efforts to amplify the acrhaeal 16S rRNA genes were not successful. The comparative sequence analysis of the bacterial part of the community displayed a rather unusual structure, however, with more than 80% of the retrieved sequences belonging to representatives of the bacterial phylum *Firmicutes* and within them to the genus *Peptostreptococcus* Incertae Sedis of the clostridial class. The rest of the sequences were assigned to *Firmicutes* too, mainly to the genus *Clostridium* and to unclassified *Clostridiales*. The closest hits in RDP databank were sequences from uncultured bacteria from the gut contents of various animals. Several pure cultures were already retrieved which posses chitinolytic activity. The specificity of the olm's gut microbiota structure coincides well with the uniqueness of its host, of the host's environment and nutritional particularity.

Oral presentation - invited plenary lecture:

A new phylogenetic framework to decipher evolutionary processes involved in groundwater

Christophe J. Douady*, Florian Malard, Lara Konecny, Claire Morvan, Celine Colson-Proch, Sebastien Calvignac

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The hidden and unexpectedly high diversity of living forms in groundwater is only beginning to emerge, following more than a century and a half of collection and identification of stygobionts (i.e. obligate groundwater species). Botosaneanu (1986) reported over 7000 obligate groundwater species worldwide, a number which is now an underestimate because new species are continuously being described and many await description. Although species inventories are far from being complete, biodiversity patterns emerged because of continued efforts in cataloging and mapping diversity at global, continental and regional scales. On the other hand process involved in groundwater colonization, adaptation and diversification have remained elusive to study. This is the direct consequence of two key factors. First, pattern-based approaches always suffer from the difficulty to link patterns to processes. Indeed, no single mechanism needs to explain a given pattern. Second, sampling constraints, organism rarity and fragility, and morphological convergence that plague taxonomic assignment are only few of the difficulties that groundwater biologists have to face. Thus, it comes as no surprise that subterranean biogeography has essentially developed on assumptions that still require more formal testing. As a first step toward process investigation we have developed within the framework of the DEEP research program a large phylogeny of the Aselloidea super-family with a special emphasis on one of the most diverse stygobiont genera: the genus *Proasellus*. Our phylogenetic inferences is based on three genes (two mitochondrial plus a nuclear one), includes 173 populations for about 90 species and subspecies. Taxonomic affinities between and within taxa as well as subsequent investigations are discussed.

Oral presentation:

Translocation of cave fauna in Brazilian iron ore cave

Rodrigo Lopes Ferreira

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An experimental translocation of part of the invertebrate community from an iron ore cave to an artificial cavity was carried out in Brazil. The artificial gallery was

exclusively built to receive individuals from the original cave. Such experiment was carried out since the original cave will be severely modified by archaeological research. The artificial cave was constructed trying to simulate the original conditions of the cave. Even the small channels in the rock that conform the cave were built through an external perforate machine. Plant species typically found in ferruginous outcrops were planted in hollows that were connecting the external environment with the gallery chamber. Inside these orifices, the root growth was stimulated with a supply of micronutrients and root hormones solution. Such procedure aimed to establish the trophic base in the artificial cave, which was, in the original cave, composed especially of roots of plants of the external vegetation. These roots were consumed by many invertebrate species in the original cave. A total of 57 invertebrate species have been translocated. Of these, 18 were considered to have high potential for the establishment of permanent populations in the gallery, 24 were considered as of low viability (even so, they could colonise), and for15 the potential could not be established since only one individual was translocated. After 5 years, 23 species have colonised the artificial gallery. Different species have shown distinct dynamics since their occupation of the new habitat. Those differences are probably related to their different life histories.

Oral presentation:

Adaptive morphology of subterranean amphipod communities

Cene Fišer*, Peter Trontelj

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Morphological evolution of subterranean species has been hitherto studied in relation to surface species. Still, closely related subterranean species co-exist at a number of sites. The stability of such communities implies niche-separation and thereby minimized competition. Consequently, co-existing species are expected to differ in their functional morphology. In this study we analyzed 16 niphargid communities consisting of minimally three species, both from caves and interstitial habitats. In 33% of the cave communities and 63% of the interstitial communities the species were more dissimilar than expected if communities were assembled by chance. We searched for parallel morphological differentiation independently occurring across communities, and for evidence for the adaptive value of morphological differences. In cave communities, Principal Component Analysis (PCA) clearly distinguished three eco-types, i.e. phreatic, lentic, and epikarstic species. The three habitats can be described by pore size and water velocity. Phreatic species are large and stout with elongated appendages. Species from streams are large and slender with short appendages, and species form crevices are

small and of various shapes and proportions. Covariance analysis of morpho-traits suggests that pore size affects evolution of body length, and water velocity affects the length of appendages. Interstitial communities consist of small and stout, small and vermiform, and larger and slender species. The third type may be opportunists, typically found also outside interstitial communities. Differences among species in this homogenous habitat cannot be explained by physical parameters, but the morphological types might differ in their trophic niche. To test this hypothesis, we compiled another set of measurements describing gnathopod shape as a surrogate for feeding ecology. Both datasets were separately subjected to PCA. In both datasets the first Principal Component explained over 90% of variation. First Principal Components from both datasets significantly correlated with each other. Slender community member with large gnathopods are presumable predators, while stouter species with feeble gnathopods are presumable microfeeders.

Oral presentation:

Niphargus amphipods and their *Thiothrix* ectosymbionts in Frasassi (Central Italy): a tale of multiple invasions and host specificity

Jean-François Flot*, Jan Bauermeister, Sharmishtha Dattagupta

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The sulfide-rich Frasassi caves in central Italy contain a rare example of a freshwater ecosystem supported entirely by chemoautotrophy. *Niphargus ictus*, the only amphipod species previously reported from this locality, was recently shown to host *Thiothrix* ectosymbionts on its cuticle. Whereas chemoautotrophic symbioses are widespread in the marine environment, this is the first instance of such a symbiosis to be reported from a freshwater ecosystem. Since the habitat of *N. ictus* is highly fragmented and is comprised of streams and lakes with various sulfide concentrations, we conducted a detailed study to examine the potential genetic diversity of this species within Frasassi. By sequencing one nuclear (ITS) and two mitochondrial (COI and 12S) regions, we found that four partially sympatric *Niphargus* clades are present in Frasassi. One of these clades corresponds to the published description of *N. ictus,* two others have since then been described as *N. frasassianus* and *N. montanarius*, and the fourth clade remains undescribed due to the scarcity of available specimens. Phylogenetic analyses of 28S ribosomal DNA (rDNA) sequences reveal that, among these four putative species, only *N. montanarius* and the fourth clade are closely related to each other. These results suggest that the Frasassi cave ecosystem was invaded independently by three different *Niphargus* lineages, one of which eventually split into two clades. Our

unexpected finding of distinct *Niphargus* species in Frasassi prompted us to look for *Thiothrix* symbionts on each of them. Scanning electron microscopy showed filamentous ectosymbionts on all three *Niphargus* species examined to date (*N. ictus, N. frasassianus* and *N. montanarius*), and their assignment to the genus *Thiothrix* was confirmed by sequencing 16S rDNA libraries. Phylogenetic analyses of 16S rDNA sequences reveal that *Thiothrix* ectosymbionts are not monophyletic. Moreover, some symbiotic *Thiothrix* lineages are found on more than one *Niphargus* species, which may indicate past lateral transfers. In spite of this, ARISA (Automated Ribosomal Intergenic Spacer Analysis) shows that the symbiotic communities associated with the three *Niphargus* hosts are distinct and highly host-specific, suggesting that ongoing symbiont transmission occurs chiefly from parent to offspring.

Oral presentation:

Quantitative Real-Time PCR as a tool for the quantification and characterization of microorganisms in caves and karst aquifers: phytoplankton, lampenflora, bacterial communities, and fecal source tracking

Rick Fowler

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PCR is a molecular tool to generate many copies of a specific DNA in a process called amplification, and quantitative Real-Time PCR (qRT-PCR) includes monitoring fluorescence during the PCR reaction and amplification process. In qRT-PCR, increase in fluorescence accompanies the accumulation of multiple copies of a target DNA fragment after successive cycles of PCR with specific primers. We use fluorescence measurements along with calibrated standards as a means to quantify specific sequences of DNA in complex mixtures of DNA extracted from the environment. Three examples are presented in detail: 1) DNA was extracted from cultures of *Chlorella* algae and from field samples along karstic surface streams. qRT-PCR with primers specific for *Chlorella* 18S rDNA was to measure concentrations of *Chlorella*, an indicator of phytoplankton abundance at cave springs or in surface channels. *Chlorella* is also a constituent of lampenflora, and qRT-PCR will soon be applied in lampenflora growth experiments; 2) Environmental DNA was extracted from cave sediments or artificial substrates and bacterial 16S rDNA was amplified with universal bacterial primers. Bacterial 16S rDNA concentrations were measured and bacterial community phylogenetic trees were derived for each study site; and 3) qRT-PCR of *Bacteroides* 16S rDNA is being investigated to quantify and identify fecal contamination sources. Further

analysis of the *Bacteroides* from different fecal samples illustrates that qRT-PCR coupled with other techniques can identify animal hosts responsible for point source fecal pollution in caves and karst aquifers.

Oral presentation:

Microsatellites as new tools to study the evolution of subterranean crustaceans

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The aquatic isopod *Asellus aquaticus* displays one of the most turbulent histories of cave invasion. Multiple lineages have invaded subterranean waters independently, sometimes even within a single cave, and have evolved various degrees of reproductive isolation and troglomorphy. Most work on the evolution of *Asellus aquaticus* conducted so far is based on mitochondrial DNA sequences. Mitochondrial DNA is inherited uniparentaly and is highly sensitive to demographic events such as bottlenecks. But the history of a single genetic unit might not reflect the history of the species. Another drawback is the fact that it does not represent a population as a whole. The data would show no migration if only males disperse among populations. Diversity among populations of *Asellus aquaticus* in the Karst region of Slovenia and NE Italy has also been assessed by RAPD genetic markers, which suffer from low reproducibility and the dominant nature of the marker. Microsatellites on the other hand are diploid, co-dominant markers that enable us to identify homozygous and heterozygous individuals. They have very high mutation rates, so they are useful for inferring recent evolutionary events. They enable us to estimate the amount of gene flow and the effective number of migrants with more certainty. In the end they might reveal the processes of recent and/or ongoing speciation, where other markers show no variation. We tested a set of newly developed microsatellite markers on three populations of *Asellus aquaticus* from the Ljubljanica drainage. Two troglobiotic and troglomorphic populations are from Planina Cave (Rak Channel and Pivka Channel), whereas the surface population is from Planina Polje, adjacent to the cave. So far, ten loci have been analyzed in subterranean populations, with seven of them also yielding positive results in the surface population. The results show that all three populations are significantly differentiated from each other. Pairwise Fst estimates are highest between the surface and the Pivka Channel population (0.8) and are also high (over 0.6) between the two cave populations (0.57). Expected heterozygosity and allele diversity are very similar in both cave populations. We interpret this strong structure and the apparent lack of gene flow in spite of the tight hydrological interconnectedness of all three populations as consequence of

ongoing speciation with ecological divergence. Our new results contradict the results obtained by mitochondrial DNA analysis and they seem to indicate stronger genetic isolation among parapatric populations. The results show the importance of employing different genetic markers and taking all of them into consideration.

Oral presentation:

The large distribution ranges of northern stygobiotic species of *Proasellus* (Isopoda): a test of cryptic diversity

Florian Malard* [1], Lara Konecny [1], Guy Magniez [2], Christophe J. Douady [1]

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Species distribution patterns within the genus *Proasellus* (Asellidae, Isopoda) typically retain the imprint of cyclical changes in climate and glacier extent that occurred during the Pleistocene. Southern regions (latitude <45°N) contain a high number of endemic species whereas northern regions harbor a few widely-distributed species that could have dispersed northward from southern refuges soon after the last glacial episode. However, there is a risk in considering widely-distributed species as a support for dispersal whereas they may in fact comprise unrecognized sibling species. In this study, we used a phylogenetic approach to test for the occurrence of cryptic diversity within two epigean species (*P. coxalis* and *P. meridianus*) and six stygobiotic species (*P. cavaticus*, *P. slavus*, *P. strouhali*, *P. synaselloides*, *P. valdensis* and *P. walteri*) showing large distribution ranges. First, the potential non monophyly of widely-distributed species was assessed from the topology of a *Proasellus* tree based on two independent mitochondrial loci (fragments coding for the COI protein and 16S rRNA). Second, the genetic divergence between potential cryptic sister species was compared to the divergence of known sister taxa. All species were monophyletic, with the exception of *P. slavus* and *P. nolli* which appeared to be synonyms. The epigean species *P. meridianus* and *P. coxalis* were highly homogenous with haplotypes almost identical over distances > 1000 km. The stygobiotic species *P. valdensis* and *P. slavus* also showed very little genetic diversity although their extent of occurrence was 150 and > 650 km, respectively. The four other stygobitic taxa all showed some genetic divergence but cryptic diversity did not appear to reduce the range size of *P. strouhali* (> 400 km) and *P. cavaticus* (> 1000 km). Our results effectively supported the occurrence of large northern distribution ranges among species of *Proasellus*, including within the stygobiotic fauna. Northern dispersal either via surface or subsurface pathways remains the most plausible scenario for explaining the present-day species distribution patterns within the genus *Proasellus*. This

research was conducted within the framework of the DEEP program (Disentangling Evolutionary and Ecological Processes shaping patterns of groundwater biodiversity).

Oral presentation:

Is stygobiont diversification a consequence of extrinsic factors?

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The striking features of groundwater biodiversity resulted in intensive debates about the origin and distribution of species. The prevalence of relicts naturally led to the idea that the great age and stability of many subterranean habitats protected species from the vicissitudes of climate through geological time whereas their epigean ancestors went extinct due to extreme climatic change. This paradigm undoubtedly influenced one of the most widely accepted models known as the "Glacial Relict" model (or Climatic Relict Hypothesis; CRH) and its derivatives (the regression model, the biphasic model of evolution, the two-step model and the three-step model). In all these conceptual models, specific events such as cold Pleistocene climate, marine regression, stream capture, and spring failure resulted in the ablation of surface populations, thereby unequivocally interrupting gene flow between epigean and hypogean populations. In these models, speciation is allopatric and diversification in groundwater is driven by extrinsic factors. In the seventies, the discovery of stygobionts in tropical regions where closely related species were still extant led to the proposal of an alternative model: the adaptive shift hypothesis (ASH). This model postulates that epigean and hypogean populations parapatrically diverge because of different selective pressures in surface and subterranean habitats. The ASH differs fundamentally from the CRH in that diversification in groundwater is a mostly continuous process depending on intrinsic factors linked to ecological adaptation in groundwater. Paleontology is the main method to evaluate diversification and its variation over geological times. However, fossil records of stygobionts are extremely scarce and morphological convergence is believed to be rampant. In this context, methods based on molecular data are promising alternatives for detecting temporal shifts in diversification rates, thereby enabling to tease apart the contribution of the CRH and ASH models. Using two of the most diverse genera of stygobiont, the isopods *Proasellus* and the amphipods *Niphargus*, we tested whether the speciation rate of stygobiotic taxa changed over time and if so, when and how did the changes occur. This work was developed within the framework of the DEEP and Biofresh research programs.

Oral presentation:

A bioinformatic quest for phylogenetic resolution: adding new genes to the *Niphargus* supermatrix

Ajda Moškrič*, Peter Trontelj, Cene Fišer

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The subject of our study is the genus *Niphargus* Schiödte (Amphipoda: Niphargidae) which is the largest genus of freshwater amphipods. It also represents one of the most diversified and well-studied group of aquatic troglobionts. Recent phylogenetic studies based on molecular (one nuclear and one mitochondrial locus) and morphological data of approximately one-third of all nominal *Niphargus* taxa revealed a number of smaller and geographically defined clades although relationships between them remain unsolved. In order to obtain the much needed, robust framework for the study of more fundamental problems of evolution, adaptation and adaptive radiation of this group we searched for novel, more informative and robust molecular markers. We used a bioinformatic approach combined with conventional PCR techniques. Our first step was a review of published alternative nuclear protein-coding regions that resolved other difficult phylogenies where standard universal markers had failed. We selected thirty-two potential nuclear regions and tried to amplify them using primers reported elsewhere. With genomic DNA as a template we successfully amplified four nuclear regions (glutamyl- and prolyl- tRNA synthetase, elongation factor 1-α, phosphoenolpyruvate carboxykinase and glucose phosphate isomerase). We also constructed several primers by scanning sequence databases and using bioinformatic tools and amplified two additional nuclear regions (glyceraldehyde-3-phosphate dehydrogenase and arginine kinase). Using DNA sequence data of these markers, a phylogeny of a subset of *Niphargus* species was constructed. Together with two mitochondrial genes, COI and ATP synthetase subunit β, our supermatrix currently contains approximately 5.000 base pairs. With the growing number of included gene sequences, both overall phylogenetic resolution and individual node support are increasing. The results of this preliminary study already show the potential of resolving power of nuclear protein-coding genes.

Oral presentation:

Rapid re-invasion and evolution following the mysterious disappearance of Racovitza's *Asellus aquaticus cavernicolus* (Crustacea: Isopoda: Asellidae)

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The completely depigmented *Asellus aquaticus cavernicolus* was first described by Racovitza (1925) from the cave Črna jama – a part of the Postojna Planina Cave System (PPCS, Slovenia). As Racovitza's description was rather deficient, a more detailed one was provided by Sket (1965). In the 1960s, however, the population found at the exact type locality (Črna jama) was highly heterogeneous in pigmentation. Therefore, Sket used specimens from the homogeneous, totally depigmented (sub)population from the adjacent downstream cave Planinska jama, also part of the PPCS. Four decades later, molecular population genetic and phylogeographic analyses revealed that the PPCS is inhabited by at least two distinct troglomorphic populations with very restricted, if any, recent gene flow: the upstream Črna jama and the downstream Planinska jama population. In a wider phylogeographic framework that included surface populations, it was shown that both populations result from independent invasions, the Črna jama being the younger one. Furthermore, extensive multivariate statistical analyses of morphometric characters revealed significant differences among recent (sub)populations from the PPCS, raising the question about the true identity of the 'original' *A. a. cavernicolus*. According to our newest morphometric analyses of Racovitza's type material, no recent (sub)population of the PPCS is identical to the type sample. So what could have happened to *A. a. cavernicolus* in an 80-years period? We propose two possible scenarios for morphological changes. The first one represents the traditional view of linear progression under directional selection. It implies that Racovitza's taxon has retained its identity but has undergone rapid phenotypic changes. The second corresponds to a more dynamic model of cave invasion considering the possibility of multiple successive and parallel events, as well as competitive interactions between old cave populations and new invaders. According to the second scenario, Racovitza's taxon was ultimately replaced by a population that has invaded the upstream parts of the PPCS somewhere in the time between Racovitza's (1925) description and Sket's (1965) re-description. The morphological evidence speaks in favour of the second scenario, implying that a few decades are sufficient for a new cave invasion and the corresponding troglomorphic changes to happen.

Oral presentation:

Preliminary estimates of species detection probabilities for North American troglobionts

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Because many troglobionts are considered rare, they may go undetected at sites even when present. Non-detection of present species introduces bias in state variables of interest, such as occupancy, habitat use, and reproductive success; therefore, it is necessary to use sampling methodologies that incorporate estimates of imperfect detection. Although this issue has been addressed for various surface fauna, few studies report detection probabilities for subterranean organisms. We surveyed 10 caves during the summer of 2008 (5 in Arkansas and 5 in West Virginia) for the presence of 19 troglobionts (8 in Arkansas and 11 in West Virginia). A single location in each cave was baited and monitored daily for 5 consecutive days. Species-specific detection data were analyzed separately using single-season occupancy models in Program PRESENCE version 3.0. Two models incorporating time constant and linear time effects relative to probability of detection were fit to the data and ranked according to Akiake Information Criterion for small sample sizes. Detection probabilities were estimated for 14 troglobionts (7 from each state), with estimates ranging from 0.24-1.0. Data was insufficient to model detection probabilities for 5 species. Only one species, the milliped *Pseudotremia fulgida*, was detected perfectly. For all species, time constant model had the greatest support (delta AICc values greater than 2) suggesting that detection probabilities did not vary across survey days. Due to small sample sizes, caution is needed for interpreting and extrapolating these results beyond the sampled caves. However, this study provides evidence that detection probabilities for troglobionts are less than perfect and supports the claim that estimating detection probabilities for troglobiotic species is needed.

Oral presentation:

Source *versus* sink populations concept applied to the Schiner-Racovitza classification of subterranean organisms

Eleonora Trajano

Departamento de Zoologia, Instituto de Biociências da Universidade de São Paulo, São Paulo, Brasil

One of the most interesting and useful recent concepts in speleobiology is the distinction between source and sink populations: a sink population, if cut off from all other migrants, eventually becomes extinct, whereas a source populations has excess production and continues to grow if isolated. Sink populations are habitat-level phenomena, corresponding to stranded groups of individuals in habitats less than suitable (in terms of space, food and other resources necessary for self-sustained, source populations). Therefore, their presence in such habitats is unpredictable. Cases of putative sink populations are known among stygobites observed in peripheral habitats such as intermittent pools fed by seeps in the vadose zone of caves (e.g., *Stygobromus emarginatus* amphipods in Organ Cave, USA; *Ituglanis epikarstikus* catfish in rimstone pools in São Mateus Cave, Brazil). Other examples include stygobitic *Trichomycterus* catfish in Lapa do Peixe, Brazil (source population is somewhere else in the cave system) and non-troglomorphic *Aegla* decapods in Barra Bonita Cave, Brazil (source population in epigean streams). When applied to the Schiner-Racovitza classification, this conceptualization leads to clearer and biologically meaningful definitions of the three categories classically recognized: 1. troglobites (stygobites) correspond to exclusively subterranean source populations, with sink populations possibly found in surface habitats; 2. troglophiles (stygophiles) include source populations both in hypogean and epigean habitats, with individuals regularly commuting between these habitats, promoting the introgression of genes selected under epigean regimes into subterranean populations (and vice-versa); 3. trogloxenes (stygoxenes) are instances of source populations in epigean habitats, but using subterranean resources (in the so-called obligatory trogloxenes, all individuals are dependent on both surface and subterranean resources). Sink populations do not fit the Schiner-Racovitza scheme, unless one considers stranded troglobitic or stygobitic individuals as part of the subterranean source population from which they originated (e.g., the above mentioned *Ituglanis* and *Trichomycterus* catfishes).

Oral presentation:

A new tree-based method for the quantitative analysis of phylogenetic character patterns: a case study with *Niphargus* (Amphipoda; Crustacea)

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We have developed a new method for calculating the probabilities of a character being synapomorphic for particular clades. It is based on the pattern of character states distribution among taxa, using tree topology as a computational framework representing only the hierarchical structure of the clades. In this case study we explored the phylogenetic patterns of morphological variability in the subterranean amphipod genus *Niphargus*. Traditional systematics of the genus has relied on general body shape, size and some combination of morphological characters to group the 300 or more taxa into infrageneric groups. We used a recent molecular topology to map the characters of interest. As expected, and as has already been shown earlier, we could confirm that the same morphological types have evolved in different clades independently, pointing to extensive parallel evolution in subterranean aquatic environments. Our approach enables a wide range of analyses and comparisons as it measures the probability of a given character being synapomorphic in a given monophyletic group. What makes it new and different is that the probability is calculated directly from the phylogenetic pattern, without having to rely on a particular model of evolutionary character transformation like a probabilistic substitution model or the parsimony principle.

Short documentaries on subterranean biology

Short documentary:

The aquatic life of the Postojna-Planina Cave System

Ciril Mlinar – Cic

Slovenian Museum of Natural History, Prešernova 20, SI - 1000 Ljubljana, Slovenia

The movie shows some of the characteristic cave animals from the deep phreatic waters of the Postojna-Planina Cave System. Known to harbor the most diverse subterranean fauna in the world, this place awakes in us both delight and a sense of responsibility as to its conservation. Not only the animals, also we humans depend critically on the quality of the karstic groundwater. The message the movie tries to convey is that the wonderful but fragile subterranean life depends on the same resources as the survival of our own species, and that these resources need to be conserved and protected. All scenes were filmed in natural underwater habitats. A special feature of the movie is a pregnant *Proteus* female – for the first time observed in the wild and for the first time caught on film.

Short documentary:

Behavior of cave fauna

Marko Lukić* , Jana Bedek

Croatian Biospeleological Society, Demetrova 1, 10 000 Zagreb, Croatia

For the last five years authors of this presentation have filmed macro scenes of cave fauna during biospeleological research in different parts of Croatia. All video material is filmed in situ using a Sony MiniDV camcorder DCR-HC1000. While observed and filmed many of species continued with their normal activities of feeding, exploring, moving around, mating, cleaning or interacting with another individual or species. Scenes chosen for this film show these interesting moments of cave life like: mating and feeding of *Alpioniscus*, entering a basin of stagnant water by *Titanethes*, feeding in Aranea, Chilopoda and Opiliones, grooming behavior in *Chthonius*, *Parastalita* and *Eupolybothrus*, and other interesting scenes. Macro filming of cave fauna discovers, in a unique way, interesting animals seen from different perspective in their natural environment.

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