2016 International Conference on Subterranean Biology
13 – 17 June 2016
Fayetteville, Arkansas, United States of America

Organized under the Auspices of the
International Society for Subterranean Biology

Abstracts

(in alphabetical order by names of presenters)
(an asterisk indicates presenting author)

Special Session: Subterranean Microbial Systems

Banaay, Charina Gracia B.
cbbanaay@up.edu.ph

Microbial indicators of air and water quality in a tropical cave.

Abris, Mattheus Imcon[1]; Palanca, Mishael Grace[1]; De Leon, Marian P.[2];
Banaay, Charina Gracia B.*[1,3]

[1] Environmental Biology Division, Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, Laguna, Philippines
[2] Museum of Natural History, University of the Philippines Los Baños, Laguna, Philippines
[3]Faculty of Management and Development Studies, University of the Philippines Open University, Los Baños, Laguna, Philippines

The Philippines has an extensive cave system owing to its geologic history. However, as the resident human population and tourism expand, this unique ecosystem is becoming increasingly threatened by anthropogenic influences. The extent of these human impacts as well as the suitability of certain ecosystems for human use can be monitored through measurement of certain microbiological parameters. Cultivable microbial groups were used as quality indices for air and water habitats in the Cacupangan Cave, Pangasinan in Luzon, Philippines. Air quality was measured by determining the Index of Microbial Air Contamination (IMA) through a passive sampling procedure using settle plates for enumeration of fungal spores in the air. Water quality was measured by determining the Most Probable Number of coliforms through a multiple tube fermentation technique. Results showed that air quality ranges from good to very good based on the IMA in fungal colony forming units per hour per plate. Genera identified are *Aspergillus*, *Penicillium*, *Cladosporium*, *Mucor*, *Fusarium*, *Geotrichum*, and *Alternaria*. Water quality, on the other hand, is low because of high coliform content. Coliforms may have come from bats as well as epigean sources such as soil, vegetation, flowing water, and humans that enter the caves. Aside from *E. coli*, genera of bacteria identified were *Klebsiella*, *Enterobacter*, *Shigella*, *Alcaligenes* and *Pseudomonas*. Continuous regular monitoring of this ecotourism site is important for its sustainable management.

Barjadze, Shalva
shalva.barjadze@iliauni.edu.ge

Character systems and criteria for species diagnosis in *Plutomurus* (Collembola, Tomoceridae), with description of two new species from Georgian caves (Caucasus).

Barjadze, Shalva*[1]; Baquero, Enrique[2]; Soto-Adames, Felipe[3]; Giordano, Rosanna[3]; Jordana, Rafael[2]

[1]Institute of Zoology, Ilia State University, Tbilisi, Republic of Georgia
[2]Department of Environmental Biology, University of Navarra, Pamplona, Navarra, Spain
[3]Department of Entomology, University of Illinois at Urbana-Champaign, Champaign, Illinois, United States

The genus *Plutomurus* Yosii, 1956 comprises 27 species living in soil and caves in Asia (13 species), Europe (10 species) and Western North America (4 species). Four species of *Plutomurus* are known from Georgia: *P. abchasicus* Martynova, 1969 (soil), *P. birsteini* Djanashvili and Barjadze, 2011 (cave), *P. kelasuricus* Martynova, 1969 (cave) and *P. ortobalaganensis* Jordana and Baquero, 2012 (cave). Two new species of the genus *Plutomurus*-one from Prometheus and Satsurblia caves and one from Satevzia Cave are described, illustrated and differentiated from other morphologically closely related species. Analysis of the morphology of type series of a new *Plutomurus* species from Satsurblia Cave shows that high levels of variation in number of teeth in the unguis, unguiculus and mucro and number of dental spines render these characters useless for diagnostic purposes. Instead, it is proposed that the dorsal chaetotaxy provides stable characters for species diagnosis. Analysis of DNA sequences for the COI and 28S genes is congruent with species level groups delimited using chaetotaxy, and thus provide additional support for chaetotaxy as the most reliable morphological character system to diagnose species in the genus *Plutomurus*. It is thus suggested that the dorsal macrochaetotaxy of all available type materials of *Plutomurus* species should be examined to confirm their validity. A key to species of the genus *Plutomurus* found in Georgia is provided, which for the first time includes characters of the macrochaetotaxy.

Bastos-Pereira, Rafaela
rafaelabastospereira@gmail.com

Brazilian subterranean amphipods with notes on their ecology and conservation.

Bastos-Pereira, Rafaela*; Ferreira, Rodrigo Lopes

Study Center on Subterranean Biology, Biology Department, Federal University of Lavras. Lavras, Minas Gerais, Brazil

A literature review was conducted in order to list the Amphipoda species known for caves in Brazil and evaluate the impacts to which the caves are subjected based on reports present in the papers. Collections were also conducted in caves in North, Northeastern and Southeastern Brazil to search for new species, to obtain more individuals of already described species with few individuals and to describe their habitats. In the laboratory, the specimens were dissected and mounted on permanent slides. Identification was made under a stereomicroscope with the aid of the few taxonomic papers available in the literature. The results showed that five amphipod families are recorded from Brazilian caves: Artesiidae, Hyalellidae, Bogidiellidae, Seborgidae and Mesogammaridae. The two richest families are Hyallelidae (six stygobionts, two stygophiles and two undescribed species, one of them expanding the distribution of the family by more than 1700 km) and Artesiidae (6 described and 1 undescribed species, all of them stygobiotic). Both Bogidiellidae and Seborgidae are represented by one species (*Megagidiella azul* and *Seborgia potiguar*, respectively). For Mesogammaridae, one species is already described (*Potiberaba porakuara*), but eight new populations were found. The new populations may consist of potentially new species, considering their geographical distance and relative isolation given the limited dispersal ability of these amphipods. Furthermore, there is a potentially new family in the state of Minas Gerais. These crustaceans are potentially threatened by the land use and water removal, since most of them inhabit arid regions in which the groundwater is the unique source of water for human survival and supply for their crops. The discovery of new troglomorphic species contributes significantly to the conservation of subterranean habitats, especially in Brazil where the legislation restricts the full protection to caves with maximum relevance: those with some unique, rare troglobiotic species.

Bastos-Pereira, Rafaela
rafaelabastospereira@gmail.com

Stygobiotic isopods (Styloniscidae) from Brazilian caves: a huge hidden biodiversity.

Bastos-Pereira, Rafaela*; Souza, Leila Aparecida; Ferreira, Rodrigo Lopes

Study Center on Subterranean Biology, Biology Department, Federal University of Lavras. Lavras, Minas Gerais, Brazil.

Styloniscidae is a family of isopods divided into four subfamilies, two of them found in Brazil (Styloniscinae and Iuiuniscinae), with all species found in caves. Styloniscinae consists of more than 40 species, with four in Brazil: *Pectenoniscus angulatus, Spelunconiscus castroi, Xangoniscus aganju* and *X. odara*. Iuiuniscinae was recently described and is monotypic with the species *Iuiuniscus iuiuensis*. The present work compiled data on stygobiotic Styloniscidae from Brazil and recorded new species. Collections were made in 15 caves from four municipalities in Minas Gerais state: Pains, Itacarambi, Montes Claros and São João da Ponte. Specimen collections from these caves at random intervals from 2009 to 2015 showed that styloniscids were found in three situations: i) permanent water in travertines or puddles with isopods observed throughout the year; ii) short-term intermittent water bodies with isopods observed every year only during the rainy season; and iii) long-term intermittent water bodies with isopods only observed in periods with extremely high rainfall. In general, density of isopods was low. Overall, 16 potentially new species were found and all were stygobiotic: ten in Pains, three in Itacarambi, two in Montes Claros and one in São João da Ponte. These species are being described. The ten species from Pains deserve special attention since they are found within a radius of less than 15 km, which makes the biospeleological province of Arcos-Pains-Doresópolis a center of diversity for such isopods. Considering these new species, the number of styloniscids known from Brazilian caves will increase from five to 21. Furthermore, such species have a crucial role on the conservation of caves and surroundings considering the current Brazilian legislation. Investigations on their phylogeny and biogeography are urgently needed to understand the colonization and speciation processes of Styloniscidae in Brazil.

Symposium: Cave Animals at the Frontiers of Modern Biology

Bilandžija, Helena
hbilandz@irb.hr

Evolution of melanin pigment regression in cave animals.

Bilandžija, Helena

Department of Biology, University of Maryland, College Park, Maryland, United States; Department of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia; Croatian Biospeleological Society, Zagreb, Croatia

Many cave-adapted animals have lost melanin pigmentation but the underlying molecular and evolutionary mechanisms are unknown. Here we report experiments addressing the evolution of albinism in cave animals. First, applying the melanogenic substrate assay to diverse albino cave animals from different phyla, we have shown that melanin synthesis is always blocked at its first step. In several cave populations of teleost *Astyanax mexicanus oca2* is the single gene responsible for albinism, and its knockdown in surface fish abolished melanin synthesis and increased L-tyrosine and the catecholamine (CAT) dopamine. In addition, we found that albino cave *Astyanax*, bivalves, planthoppers, and polychaetes show increased CAT levels compared to their most closely related surface relatives. These results suggest that albinism may be advantageous for increasing the CAT synthesis pathway by providing additional L-tyrosine. Second, we have also tested the possibility that interference with melanin synthesis is beneficial for energy conservation. Using gene silencing techniques and membrane-inlet mass spectrometry, we found that interruption of melanin synthesis in *Astyanax* surface fish embryos results in lower oxygen consumption, supporting the energy conservation hypothesis. Third, we have performed experiments to address our finding that diverse albino cave animals retain L-DOPA positive melanoblast-like cells. It is known that arthropods use melanin in their innate immune response, and we discovered that several albino cave arthropods retain the ability to synthesize melanin after an immune challenge, suggesting a benefit of conserving undifferentiated melanoblasts. In addition, we have observed that *Astyanax* melanophores or L-DOPA positive melanoblasts accumulate around a wound site, and treatment with a chemical agent that kills these cells affects wound repair, suggesting that the melanin synthesizing cells cannot be completely lost because they also function in innate immunity. It is concluded that multiple mechanisms may work together to control the evolution of albinism in cave animals.

Poster Session

Bilandžija, Helena
hbilandz@irb.hr, hbilandz@umd.edu

Adaptive differences between dopamine-related locomotor activity in cave and surface dwelling *Astyanax mexicanus.*

Shoen, Ezra; Jeffery, William; Bilandžija, Helena*

[1] Department of Biology, University of Maryland, College Park, Maryland, United States

Many behavioral differences have evolved between cave and surface dwelling *Astyanax mexicanus*. Here we explore locomotor activity and its relationship to dopamine between these two subgroups. Three different assays were used to quantify locomotor activity between cave and surface *Astyanax mexicanus* by varying the size and shape of a swimming arena. The number of times that fish crossed a line drawn in the center of each arena during a specific time period was recorded. Our results show that the independently evolved Pachon and Tinaja cavefish populations have increased activity levels compared to conspecific surface dwelling fish. To test the hypothesis that cavefish have increased activity levels because of the need to be constantly searching for food in nutrient depleted cave environments, we studied the differences in activity between fish that had been fed varying amounts. We found that fish fed more have decreased activity confirming the hypothesis and suggesting that increased locomotor activity is adaptive in cavefish. Recently we discovered that Pachon cavefish have higher dopamine levels than surface fish. To understand the effect of increased dopamine levels on locomotor activity we tested different fish populations in arenas containing exogenous dopamine. The addition of dopamine increased the activity of Pachon cavefish, Tinaja cavefish, and surface fish. In order to understand the effects of dopamine on the activity levels, we explored the effects of the dopaminergic agonist quinpirole and the antagonist haloperidol on locomotor activity. The agonist and antagonist resulted in increased and decreased activity levels in cavefish respectively but had little or no effects on surface fish, confirming that activity changes in cavefish are related to dopamine. Our future goal is to understand the causes and mechanisms for the differences in locomotor activity and their relationship to dopamine levels in cave and surface *Astyanax*.

Borko, Špela
borko.spela@gmail.com

Deep cave fauna – fact or fiction?

Borko, Špela*; Delić, Teo; Trontelj, Peter

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

Recent advances in speleology, sports and equipment have allowed speleobiologists to explore the fauna of even the deepest caves in the world. Titles like “The deepest living animals” have made the news in popular media, reaching a wider audience than any other speleobiological research before. Yet, does good promotion of our science automatically imply good science? How justified is the assumption of a unique fauna deep inside a limestone massif that is fractured and opened to the surface in all directions? According to the Giachino-Vailati model, a deep section of a vertical cave is equivalent to the distal part of a long horizontal cave at the same altitude. Indirect influence from high-altitude entrances (cold air dropping, precipitation, low organic input) might nevertheless cause some heterogeneity inside the massif. We tested the hypothesis of a unique deep cave fauna by surveying 10 caves of various depth and at various altitudes at the karst massif of Trnovski gozd, Slovenia. Across the vertical extent of more than 800 meters we collected 10.000 specimens from over 60 mostly terrestrial taxa, some of them new to science. Faunal assemblages from different depth zones formed three distinct groups: 1–inner parts of the massif; 2–high-altitude entrance parts; 3–caves at lower altitudes or with specific geomorphology. Assemblages of the first group started already at about 50 meters of depth and were not restricted to the deepest caves. When looking only at deep vertical caves, a few species were found exclusively in the deepest zones. In other caves, however, the same species occurred close to the surface. We conclude that vertical distance from the cave entrance is probably not as important in determining the qualitative and quantitative composition of subterranean communities as suggested by some recent publications.

Boyd, Olivia F.
boydo@oregonstate.edu

Geographically structured genetic diversity in the cave beetle *Darlingtonea kentuckensis* Valentine 1952 (Coleoptera: Carabidae: Trechinae).

Boyd, Olivia F.*[1]; Johnson, Jarrett[2]; Philips, T. Keith[2]

[1]Department of Integrative Biology, Oregon State University, Corvallis, Oregon, United States
[2]Department of Biology, Western Kentucky University, Bowling Green, Kentucky, United States

Analysis of DNA sequence data from a subunit of the mitochondrial gene cytochrome c oxidase (COI) revealed geographically structured genetic diversity among populations of the cave carabid beetle *Darlingtonea kentuckensis*. Relationships between putative geographical barriers to subterranean dispersal and the boundaries of genetically distinct groups were tested at two scales using hierarchical analyses of molecular variance (AMOVA). Five genetic distance clusters emerged from an unrooted analysis of all 81 individuals; AMOVA revealed high FCT and low FSC among three of the five clusters. Low Mantel correlations supported these clusters as independent of isolation by distance. The *a priori* landscape features considered in this study were not predictive of the genetic breaks among these three clusters, which are genetically distinct despite their close geographic proximity, suggesting that unidentified barriers to gene flow have promoted divergence among populations from closely grouped caves.

Symposium: Cave Animals at the Frontiers of Modern Biology

Carlini, David B.
carlini@american.edu

Speleotranscriptome profiling casts light on differential expression and polymorphism in cave and surface populations of the amphipod *Gammarus minus*.

Carlini, David B.

Department of Biology, American University, Washington, District of Columbia, United States

Gammarus minus, a freshwater amphipod living in the cave and surface streams in the eastern United States, is an excellent model for investigating the genetics and evolution of troglomorphic traits. Multiple pairs of genetically related, physically proximate cave and surface *G. minus* sister populations exist which exhibit a high degree of intraspecific morphological divergence. The morphology, ecology, and genetic structure of these populations are well characterized, yet the genetic basis of morphological divergence remains largely unknown. RNA-Seq was conducted on one pair of morphologically distinct sister populations inhabiting surface and cave habitats (four individuals from each population) and used to identify genes that were significantly upregulated or downregulated in the cave population, as well as genes that exhibited differential levels of polymorphism in the two populations. Of the ~104,000 transcripts identified in the *de novo* transcriptome assembly, 551 were found to be significantly downregulated in the cave population, and 1517 were significantly upregulated in the cave population, indicating a roughly three-fold enrichment of cave-upregulated genes. The average level of nucleotide diversity across all transcripts was significantly lower in the cave population. An asymmetry in variation was also apparent within the cave population, where the average nucleotide diversity of cave-downregulated transcripts was 75% that of the cave-upregulated transcripts, a highly significant difference. Cave-downregulated transcripts were also 3.5x more likely to be highly diverged from their surface homologs than cave-upregulated transcripts, suggesting that the genes encoding these downregulated transcripts have been the targets of selection in the cave population. Dozens of transcripts exhibiting differences in levels of expression and/or sequence variation between the cave and surface populations were identified. The expression patterns and sequence variation in one such transcript, encoding the light-dependent DNA repair protein dipyrimidine photolyase, will be discussed in detail.

Poster Session

Carlson, Brian M.
bcarlson@wooster.edu

The unusual suspects: Genetic analysis reveals candidate genes potentially underlying altered activity profiles in the blind Mexican tetra, *Astyanax mexicanus.*

Carlson, Brian M.*[1]; Gross, Joshua B.[2]

[1]Department of Biology, College of Wooster, Wooster, Ohio, United States
[2]Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio, United States

Cave-adapted species display a wide array of morphological, physiological and behavioral alterations that have accompanied their colonization of these extreme environments. The perpetual darkness, stability and isolation of cave ecosystems have made these organisms attractive systems in which to study the consequences of life without light and the strategies that allow species to survive and even thrive in such environments. *Astyanax mexicanus*, the blind Mexican tetra, has found particular favor as a natural model system for studying regressive evolution and cave adaptation due to the fact that this species is tractable in a lab setting, includes ~30 different cave populations and consists of both a derived, cave morphotype and an extant surface morphotype, which can be readily hybridized. In this study, we investigated the genetic underpinnings of differences in locomotor activity between the surface and Pachón cave forms of *Astyanax mexicanus.* An automated, video-based system was used to assay the activity of each member of an F_2 surface x Pachón hybrid pedigree over the course of 24 hours. Subsequent analysis of the data generated revealed the presence of multiple quantitative trait loci (QTL) associated with metrics for levels of overall activity, as well as spatial components of locomotor activity patterns. Available genomic and transcriptomic data were then leveraged to screen genes found in the genomic intervals underlying these QTL and generate a set of potential candidates for further study. Our results highlight several genes that may play a role in mediating observed changes in locomotor activity and related behaviors. Interestingly, while our results support the candidacy of a number of genes, they do not suggest that differences in the patterns of behavior observed in this study are the results of alterations to members of the core circadian clockwork, teleost multiple tissue opsins or melanopsins, as has described in other species.

Symposium: Conservation of Subterranean Fauna and Habitats

Colatskie, Shelly
Shelly.colatskie@mdc.mo.gov

A working relationship between the Missouri Department of Conservation and caving organizations.

Colatskie, Shelly*; Elliott, Anthony

Missouri Department of Conservation, Powder Valley Conservation Nature Center, 11715 Cragwold Road, Kirkwood, Missouri 63122, United States

With over 7000 known caves, many springs, and with an unknown number of abandoned quarries and mines in Missouri, the Missouri Department of Conservation cannot manage all of the unique karst resources alone. We rely on multiple caving organizations (individual grottos, Cave Research Foundation and Missouri Speleological Survey) as well as individuals to help conduct dye tracing, cave mapping, biological inventory and management of the state's karst ecosystem. Since most karst features are on private land, we would not know much about this unique ecosystem without their help. In addition to conducting this work on private land, we rely on these individuals to conduct the above activities on governmental lands.

Poster Session

Coleman, William T.
willcoleman@txstate.edu

Dynamics of detectability: Insights from high-frequency sampling at a stygobiont hot-spot, Edwards Aquifer, Texas.

Coleman, William T.*[1]; Hutchins, Benjamin T.[1, 2]; Schwartz, Benjamin F.[1]

[1]Department of Biology, Texas State University, San Marcos, Texas, United States
[2]Texas Parks and Wildlife Department, Austin, Texas, United States

The Edwards Aquifer in Texas supports abundant stygobiont biodiversity. Extending over 400 km along the Balcones fault zone, the aquifer serves as habitat for >50 described stygobitic species, including four federally endangered species: the Texas blind salamander (*Eurycea rathbuni*), Austin blind salamander (*Eurycea waterlooensis*), Peck's cave amphipod (*Stygobromus pecki*), and the Comal Springs dryopid beetle (*Stygoparnus comalensis*). Due to habitat inaccessibility, little is known about the ecology of stygobionts in the Edwards Aquifer, including their life history, population sizes, distributional ranges, trophic dynamics, and interspecific interactions. A flowing artesian well on Texas State University campus has been used to collect and study aquifer organisms for over a century, and it is the type locality for a number of species. Since 2013, the well has been consistently sampled with a 100 µm net for intervals ranging from 24-72 hours with collections ranging from 200 to >1300 individuals per sample. Species collection data was analyzed alongside hydrological data including aquifer discharge and storm events. While there were shifts in species composition after storm events, temporal variance in species richness and abundance were not explained by hydrological fluctuation. Including undescribed stygobion species, sampling efforts have yielded over 30 different species from the artesian well, illustrating the site's importance as one of the most diverse stygobiont sites known. This occurrence data extends the range of multiple stygobionts in the Edwards Aquifer and prompts discussion of the ecology and biogeography of inconspicuous aquifer fauna.

Cooper, Steven John Baynard
steve.cooper@samuseum.sa.gov.au

Regressive evolution of beetles from the subterranean archipelago of Western Australia: insights from comparative transcriptomics.

Cooper, Steven John Baynard*[1,2]; Tierney, Simon Martin[1]; Hyde, Josephine Charlotte Anne[1]; Saint, Kathleen Margaret[2]; Bertozzi, Terry[1,2]; Austin, Andrew Donald[1]; Humphreys, William Frank[3]

[1]Australian Centre for Evolutionary Biology and Biodiversity, and School of Biological Sciences, The University of Adelaide, Adelaide, South Australia, Australia
[2]Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, South Australia, Australia
[3]Terrestrial Zoology, Western Australian Museum, Welshpool, Western Australia, Australia

The 'calcrete islands' of central Western Australia contain a suite of stygobiotic dytiscid beetle species, the majority of which have independently evolved from surface ancestors over millions of years, that provides a unique comparative system for exploring the genomic changes that accompany regressive evolution. A key question is whether long-term evolution in an aphotic environment leads to pseudogenisation (accumulation of mutations that result in gene inactivation) of genes that are associated with regressed phenotypes such as vision and pigmentation. Here we present transcriptome data from next generation sequencing (NGS) of mRNA from three stygobiotic and two related surface dytiscid species and focus on 45 genes involved in photo-transduction and eye pigmentation (henceforth referred to as 'eye genes'). These analyses revealed that four genes (UV opsin, a non-visual ciliary opsin, a visual arrestin *Arr1* and the myosin III gene *ninaC*) were transcribed exclusively in surface species, but not in stygobiotic species. However, the vast majority of 'eye genes' (39 out of 45) were transcribed in at least one of three stygobiotic species we targeted. There are two possible explanations for these results: either 1) purifying selection is maintaining 'eye genes' in a functional state or 2) there has been insufficient time for destructive mutations to accumulate under a neutral process of evolution. Notably, several of the 'eye genes' sequenced from stygobiotic species appear to have insertion/deletion mutations that lead to premature stop codons in the translated protein product. We are currently validating our assemblies to confirm these are cases of incipient transcribed pseudogenes.

Poster Session

Culver, David C.
dculver@american.edu

The presence of troglomorphic species in Sistema Muévelo Rico, a large cave with limited dark zones in Quintana Roo, México.

Mejia-Ortiz, Luis M.[1]; Culver, David C.[2*]; Pipan, Tanja[3]; Martínez, Christian O.[1]; Sprouse, Peter[4]

[1] Biospeleology & Carcinology Lab, Sustainability Development Division, University of Quintana Roo, Cozumel, Mexico
[2]Department of Environmental Science, American University, Washington, District of Columbia, United States
[3]Karst Research Institute at ZRC-SAZU, Postojna, Slovenia
[4]Zara Environmental, Manchaca, Texas, United States

Sistema Muevelo Rico is a large cave, with 1152 m of passage and at least ten entrances and skylights. Few places in the cave reach a zone of complete darkness. Nevertheless, there is a stygobiotic and troglobiont fauna present in the non-dark zones. Stygobionts include the caridean shrimps *Typhlatya mitchelli* and *T. pearsei*, and the mysid *Antromysis cenoticola.* Troglomorphic species (and likely troglobionts) include members of the Amblypigi, Araneae, Hemiptera, Opilionida, Orthoptera, Scorpiones, and Scutigeromorpha. The presence of stygobionts is not surprising, since the cave provides, via several pools, access to the regional groundwater and its fauna. The presence of troglomorphic terrestrial species indicates that they can survive in a photic environment, albeit one with low light intensity. Some eyed predatory species are also present, including Hymenoptera (Formicidae), Scorpiones, and Scutigeromorpha. Extensive data on temperature and light intensity indicates a daily and seasonal cycle for each.

Special Session: Biology of Subterranean Crustaceans
(Sponsored by The Crustacean Society)

Cunanan, Dianne J.
djcunanan@up.edu.ph

Phylogeography, haplotype diversity and niche differentiation among freshwater crab *Sundathelphusa* species (Decapoda: Brachyura: Gecarcinucidae) in the subterranean habitat of Quezon, Philippines.

Cunanan, Dianne Jaula*; Husana, Daniel Edison

Environmental Biology Division, Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, College, Laguna, Philippines

Sundathelphusa species is a semi-terrestrial freshwater crab known to inhabit diverse habitats. Although record shows that some species of this genus are occupying the same territory (e.g. in Bohol Island, Philippines), it is still unusual and obscure. In this study, 16 freshwater crab samples were collected from various microhabitats (i.e. inside and outside the cave) in Nilulubugan Cave in Quezon Province. The species relationship and haplotype diversity were determined through analysis of the 16S mitochondrial DNA sequences of *Sundathelphusa* samples. Upon closer examination and investigation, morphological and molecular evidence revealed four species co-inhabiting a single cave. Phylogenetic analysis revealed two major lineages with four unique haplotypes. Each clade corresponds to a population of each species occupying distinct habitats inside the cave. This includes (1) water- and guano-filled potholes in a huge elevated rock boulder; (2) travertine flowstone with clear flowing water from seep; (3) rock crevices along the banks of subterranean river; and (4) a subterranean stream, which connects to the surface. Phylogenetic evidence shows that there is no sharing of haplotypes among the four populations studied, indicating genetic isolation despite overlapping of populations inside the cave. Different species of freshwater crab *Sundathelphusa* were found to thrive together in a single cave in Quezon Province. These findings suggest that multiple species coexistence in a small territory is possible when there is niche differentiation.

Poster Session

Deharveng, Louis
Dehar.louis@wanadoo.fr

Conservation of cave invertebrates: the role of the new IUCN-SSC Cave Invertebrate Specialist Group.

Deharveng, L.*[1]; Whitten, T.[2]; Khela, S.[3]

[1]Museum national d'Histoire naturrelle, Paris, France
[2]Fauna & Flora International, Cambridge, United Kingdom
[3]Red List Authority for the CISG group, Cambridge, United Kingdom

Founded in 2014, as part of the IUCN Species Survival Commission, the ***Cave Invertebrate Specialist Group*** is an informal group of 80 scientists specialized in the fields of invertebrate taxonomy, biospeleology, limestone caves and karst biodiversity. The group's objectives are:

- To conserve karst and non-limestone subterranean habitats, and fill-in one of the largest gaps in conservation biology – that of cave and subterranean invertebrates.
- To carry out conservation assessments for the IUCN Red List to protect species and their habitats.
- To encourage comprehensive baseline biodiversity surveys, in particular to determine the proportion of unknown biodiversity yet to be discovered.
- To assist mining, tourism and other companies with access to specialists capable of conducting Environmental and Social Impact Assessments, Biodiversity Action Plans and site management plans.

Many of the invertebrates living permanently in caves are highly range-restricted endemics, incapable of dispersing to other sites. Some have evolved to become blind, wingless, pigmentless, with very slow metabolism and very low reproductive rates. Others have adapted to life in bat or swiftlet guano, which is a highly patchy and fragile resource. Most are very susceptible to changes in their habitats and hence vulnerable to extinction. Subterranean systems can be threatened by water extraction, exploitation of birds' nests and guano, light, waste and chemical pollution, building at entrance and inside caves, cultivation of mushrooms, hunting of bats, and major land use changes on the land above the cave. Disturbance from high levels of uncontrolled tourism, recreational use and subsequent water pollution and littering can also cause severe degradation of the cave environment.

Extraction of limestone for the production of cement aggregates and lime is one of the main threats to limestone-restricted biodiversity. Quarrying affects the integrity of caves, fissures and groundwater systems through dust, blasting shock, changes in hydrology, water pollution and direct destruction. Species confined to a small limestone area, often a single cave or hill, are at high risk of extinction from this kind of threat, as shown by recent examples in southern Vietnam (Hon Chong hills) and the Malay Peninsula (Kanthan hill). Limestone-restricted biodiversity is particularly vulnerable to impacts from extraction because the habitats are impossible to restore.

If participants are interested in joining the group they are invited to contact the authors.

Special Session: Biology of Subterranean Crustaceans
(Sponsored by The Crustacean Society)

Delić, Teo
teo.delic@bf.uni-lj.si

Thermal adaptation, a new driver of ecological speciation in subterranean fauna.

Delić, Teo*; Trontelj, Peter; Fišer, Cene

Subterranean Biology Lab, Department of Biology, University of Ljubljana, Ljubljana, Slovenia

In northern latitudes, the distribution of subterranean taxa has been strongly affected by historical climatic oscillations. According to the predominant view, their restricted geographical distribution is a consequence of their low dispersal ability and thermal stability of the subterranean environment, which selects against thermal adaptability. Because of their small ranges, subterranean species offer little opportunity for in-situ testing of thermal adaptation. The exceptions are species spanning wide altitudinal ranges, but these are exceedingly rare, especially among stygobionts. One of them is *Niphargus stygius*, a subterranean amphipod from the north-western Dinaric Karst and the Julian Alps. Reaching from the high Alps to the sea level, its populations face mean annual temperatures from 0 to 14 °C. *N. stygius* has a complex phylogeographic structure comprising four morphologically cryptic species that evolved during the Pleistocene, and have a parapatric distribution with a few cases of sympatry and even syntopy. Bayesian Skyline plots indicated modest population expansion during the last 20,000 years, which is the period since the end of the Last Glacial Maximum. Ecophysiological measurements of respiration rate and catalase specific activity showed different stress response when exposed to different temperatures, suggesting that the cryptic species are thermally adapted to different thermal optima. In contrast with the traditional view that presumes species decimation during Pleistocene glaciations, our results imply that these climate changes in altitudinally structured habitats contributed to the emergence of new subterranean species through the process of thermal adaptation.

Dumnicka, Elzbieta
dumnicka@iop.krakow.pl

Distribution and diversity of stygobionts in Poland.

Dumnicka, Elzbieta*; Galas, Joanna

Institute of Nature Conservation, Polish Academy of Sciences, al. A. Mickiewicza 33, 31-120 Krakow, Poland

The studies of invertebrate fauna in subterranean waters of Poland started at the end of XIX century and they are being continued by zoologists and hydrobiologists. Wells, caves, interstitial waters, and springs situated mainly in the mountains and uplands in the southern part of the country have been sampled; sometimes stygobionts have been also found among benthos during hydrobiological studies. On the basis of the published data and our own studies approximately 80 stygobiotic invertebrate species have been recorded. This number includes 45 species of Hydracarina found almost exclusively in interstitial waters (hyporheic and alluvial sediments). Amphipoda are represented by 11 species or subspecies, mainly from the genus *Niphargus*, described from wells in Tatra Mountains.

The remaining stygobionts belong to oligochaetous clitellates (10 species) and ostracods (8 species) while Tricladida, Polychaeta, Copepoda and Gastropoda are represented by only one species from each of these groups. The highest diversity was found in Tatra Mountains where subterranean waters were studied the most intensively. Some stygobionts such as *Niphargus tatrensis, Enchytraeus dominicae* and *Atractides latipes* have been found in many locations, but approximately 30 species are known from singular locations.

Among stygobiotic taxa described till now from Poland nine seem to be endemic. The actual number of stygobionts is not certain because the taxonomic status of some of them is not clear. This is due to findings of singular, sometimes atypical individuals (*Trichodrilus spelaeus*, *Niphargus cf. inopinatus*) or due to recent taxonomic revisions (for example of the family Enchytraeidae).

The diversity of stygobionts in Poland appears quite high, despite the glaciation of almost all the territory, but distinctly smaller than that in unglaciated European countries. The majority of species have been found in interstitial waters, perhaps because it was a habitat easy to re-colonize.

Special Session: Subterranean Microbial Systems

Engel, Annette Summers
aengel1@utk.edu

Prevalence of microbial taxonomic groups to specific subterranean habitats may shed light on ubiquity of microbial function in cave ecosystems.

Engel, Annette Summers

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

The physical and chemical nature of a habitat influences the taxonomic and functional diversity of organisms within an ecosystem. The subterranean ecosystem of a cave is described as having complete darkness, strong gradients of organic carbon and nutrient energy, and potentially dynamic environmental conditions, such as pH, oxygen, and salinity. A statistical evaluation of microbial taxonomic diversity and putative functional diversity from previously studied cave habitats was done to address the ubiquity or rarity of certain microbial taxonomic groups to environmental conditions, habitat constraints, or biogeography of caves. Data from carbonate caves, both from cave walls and from flowing and ponded water bodies, as well as from sulfidic streams and aquifers, and lava tubes were compared. Distinct microbial groups were associated with subaerial (e.g., Actinobacteria, Alphaproteobacteria) versus aquatic cave habitats (e.g., Bacteroidetes, Betaproteobacteria), as well as with sulfidic conditions (e.g., Epsilonproteobacteria, Gammaproteobacteria). Some taxa were more prevalent from lava tubes (e.g., Nitrospira, Acidobacteria). In some cave habitats, usually proximal to the surface, organic carbon and nutrient contents are abundant, and metabolic processes associated with organic matter decomposition, specifically heterotrophy and chemoorganotrophy, appear to sustain the ecosystems. However, in where water and air movement into and through the subsurface becomes restricted, microbial activities associated with nutrient acquisition from minerals and water, and inorganic carbon fixation in the absence of photosynthesis, serve as the energetic base to these ecosystems. Because the subterranean habitat may be hundreds of thousands to millions of years old, biological activities and overall biodiversity found may have resulted in endemic communities that are specially adapted to the subsurface, although knowledge of actual genetic details related to endemism for microbes are currently lacking.

Symposium: Conservation of Subterranean Fauna and Habitats

Fenolio, Danté
dante@anotheca.com

Threats to the conservation of stygobionts.

Fenolio, Danté

Conservation & Research, San Antonio Zoo, San Antonio, Texas, United States

Salamanders and fishes are among the few vertebrates that have successfully colonized and exploited subterranean waters, demonstrating flexibility in behavior and in physiology. A far greater number of invertebrates have invaded subterranean waters and these invertebrates show the same remarkable and flexible characters. Combined, this assemblage is referred to as stygobionts or animals that live in groundwater. In spite of the challenges to living below ground, subterranean biodiversity "hotspots" for stygobionts have been identified around the globe. Regions of high subterranean species diversity include southern China, southern Mexico, and regions of Brazil, India, Thailand, Europe, Australia and North America. However, our knowledge of the ecology and life history of many subterranean groundwater inhabitants is poor because of the limits and challenges of studying organisms in these habitats, particularly aquifers. These limitations often times inhibit conservation work as basic parameters, such as population size and range, remain elusive. What we do know is that groundwater is regularly harvested at a rate faster than rainfall can replenish the resource. We also know that groundwater is being contaminated on a scale not previously experienced and that limestone mining is removing subterranean habitats, worldwide, on a wholesale basis. Conservation efforts of stygobionts will require the protection of subterranean and corresponding surface habitats if we are to conserve any of the extant biodiversity. Clean groundwater harvested at a renewable rate is the only solution to a healthy future for both stygobiotic wildlife and humans.

Ferreira, Rodrigo Lopes
drops@dbi.ufla.br

Brazilian troglomorphic fauna: besides raising the knowledge, are we contributing to their conservation?

Ferreira, Rodrigo Lopes*; Souza-Silva, Marconi

Study Center on Subterranean Biology, Biology Department, Federal University of Lavras. Lavras, Minas Gerais, Brazil.

In the last 10 years, inventories carried out in Brazilian caves have shown a great number of new species. Currently, there are only 120 cave species described for Brazil, from which 76 are troglobitic and 44 are stygobitic. However, at least 800 undescribed troglomorphic species are known for Brazilian caves. The great amount of recently discovered new species and their high degree of troglomorphism indicate that the events of climatic changes in Neotropics, even not so severe as in temperate regions, could have led to the isolation of subterranean lineages. Furthermore, other mechanisms of isolation (e.g. oceanic regressions), might have led to the evolution of many lineages of subterranean fauna in Brazil. Although different models of evolution of troglobites have been proposed for Brazilian caves, it seems that different processes have led to isolation and speciation, depending on several parameters, highlighting the paleoclimate and prevailing lithology of each region. Increasing the studies on Brazilian cave fauna has contributed not only to increase the number of known troglomorphic species, but also to reveal the first hotspots of subterranean biodiversity in South America. However, although many species have been discovered, their conservation is not ensured. Until 2008, the law protected all Brazilian caves, but currently some Brazilian caves can be destroyed by different anthropogenic activities. In order to define which caves can be suppressed and which should be preserved, categories that define the status of each cave (based on biological and geological parameters) were created. To assure the preservation of a cave in Brazil, it is necessary, from the biological point of view, that it possesses at least one endemic, troglobitic or rare species. Therefore, the description of new species endemic to caves became urgent for the conservation of these environments, and thus, their inhabitants.

Ferreira, Rodrigo Lopes
drops@dbi.ufla.br

Iron ore plateaus in the Amazon forest: hotspots of subterranean biodiversity?

Ferreira, Rodrigo Lopes

Study Center on Subterranean Biology, Biology Department, Federal University of Lavras. Lavras, Minas Gerais, Brazil

Ferriferous formations are certainly among the most unknown ecosystems of the world, especially considering their subterranean components. In Carajás region (Pará state, Brazil), extensive iron ore plateaus comprising superficial ferruginous breccia (canga formations) cover the banded ferriferous formations. In such rocks, there are several interconnected small spaces, called canaliculi, trespassing huge extensions of the canga formation. Such canaliculli also connect macro-caves occurring on each plateau. Brazilian ferruginous caves certainly stands out regarding their high richness of troglobitic species, especially when compared to caves inserted in other lithologies. A study conducted in the Carajás region revealed that caves located in a given unit (plateau) are highly similar regarding the troglobitic fauna, indicating the existence of a species flow among the macro-caves of the same plateau, although the dissimilarity observed in caves of different units is remarkable, what demonstrates that there is no displacement of troglobites among plateaus. Therefore, troglobitic species that exist in a given plateau are completely different from those that exist in other plateaus of Carajás region. Thus, each plateau in that area may eventually represent a great and unique cave formed by macro-galleries interconnected by huge systems of canaliculi. In this sense, since all the investigated plateaus possess more than 20 troglobitic species, each one could be considered as a hotspot of subterranean biodiversity. However, since Brazilian laws do not protect other subterranean habitats than macro-caves, such huge systems are extremely endangered, since parts of each plateau are frequently destroyed, especially due to iron ore exploration. The question that arises is whether the remaining areas preserved from each plateau are enough to protect the integrity of the troglobites. Therefore, it is urgent that Brazilian government also start to protect other subterranean habitats, which can be of great importance for the conservation of many species.

Special Session: Biology of Subterranean Crustaceans
(Sponsored by The Crustacean Society)

Fišer, Žiga
fiser.ziga@gmail.com

Searching for reproductive barriers between sympatric surface and subterranean ecomorphs of *Asellus aquaticus.*

Fišer, Žiga*; Trontelj, Peter

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

The origin of subterranean species has intrigued biologists ever since Darwin first called them 'wrecks of ancient life' in his monumental work *The Origin of Species*. However, evolutionary mechanisms that constitute the reproductive isolation between surface and subterranean species remain largely unexplained. Previous work has demonstrated that speciation is possible despite permanent contact between populations, but invested almost no effort in recognizing explicit reproductive barriers opposing gene flow. To unveil these, traits evolved through adaptation to the subterranean environment should be investigated for their contribution to the total reproductive isolation. We chose three behavioral traits, i.e. response to light, shelter seeking and resistance to water current, and conducted behavioral experiments to test their function as reproductive barriers between several surface and subterranean populations of a freshwater isopod *Asellus aquaticus*. The results show that response to light differs between both types of populations but also between different subterranean populations and could act as a weak reproductive barrier where subterranean isopods are more photophobic than surface isopods. On the other hand, shelter seeking behavior turned out to be similar in surface and subterranean populations and as such cannot reduce cross-ecomorph mating. Preliminary results suggest that subterranean isopods are able to resist stronger water currents than surface isopods, which could provide a strong isolating barrier due to the inability of surface isopods to persist underground during seasonal floods. Our results imply that there is not a single mechanism responsible for speciation of subterranean populations, but rather that reproductive isolation is complex and composed of several ecologically-driven premating components.

Fišer, Žiga
fiser.ziga@gmail.com

A subterranean hybrid zone between two young stygobiont species in secondary contact.

Konec, Marjeta; Fišer, Žiga*; Prevorčnik, Simona; Trontelj, Peter

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

Hybrid zones between distinct groups of individuals are natural evolutionary experiments providing insight into the process of speciation and mechanisms of reproductive isolation. Although hybrid zones are widespread across different taxa and various environments, hitherto not a single example of a hybrid zone between two obligate subterranean species has been described. A unique natural setting of the Planina Cave (Slovenia) holds conditions where a subterranean hybrid zone could exist. This cave is formed by two separate, large subterranean rivers that meet inside the cave. Each river hosts its own distinct troglomorphic population of the freshwater isopod *Asellus aquaticus*. The two populations differ genetically, morphologically, and behaviorally. At the confluence and downstream of it, animals from both populations are regularly found together. By microsatellite genotyping we identified a small portion of them as potential hybrids. Their hybrid origin was confirmed by comparing them to F1 hybrids reared in the laboratory, and to in-silico generated F1, F2 and backcrosses. The presence of a few second-generation hybrids revealed that the F1 generation is at least moderately fertile under natural conditions. However, the small percentage of F1 and even smaller share of second-generation hybrids suggest that some form of isolating barrier keeps the two subterranean populations from fusing. The persistence of genetic independence of the two recently evolved subterranean *Asellus* populations might be due to extrinsic (ecological) or intrinsic (genomic) causes. This case demonstrates that cave populations are not only fast to evolve in terms of adaptation and isolation from their still present surface ancestors, but can also acquire some sort of reproductive isolation between each other relatively quickly.

Special Session: Biology of Subterranean Crustaceans
(Sponsored by The Crustacean Society)

Fong, Daniel Wu
dfong@american.edu

Variation in phototactic behavior among surface and subterranean gammarid and crangonyctid amphipod species from different habitats.

Fong, Daniel Wu*[1]; Wanner, Maria J.[2]

[1]Department of Biology, American University, Washington, District of Columbia, United States
[2]Department of Biology, Gettysburg College, Gettysburg, Pennsylvania, United States

A phototactic behavior index, normalized from -1.0 for complete light avoidance to +1.0 for complete light preference with 0.0 indicating photo-neutrality, was scored from five to six individuals each of six surface, non-troglomorphic and nine subterranean, troglomorphic populations and species of gammarid and crangonyctid amphipods. The behavior of each individual was video-recorded in an arena with a light/dark choice versus no choice in a trial, with four trials conducted for each individual. There was extensive variation among individuals within each population but no population was photo-positive. In the species *Gammarus minus* two surface populations were photo-negative (-0.464 and -0.445), while two other surface and two troglomorphic populations were photo-neutral (-0.119, -0.068, -0.016 and -0.031). The most troglomorphic *Gammarus*, *G. cohabitus*, was strongly photo-negative (-0.786). Among crangonyctids, two surface populations of *Cangonyx shoemakeri* and two troglomorphic populations of *Stygobromus emarginatus* were photo-neutral (-0.082, -0.061, +0.033 and -0.041), while one population each of four troglomorphic species was photo-negative, *Crangonyx antennatus* (-0.434), *Stygobromus allegheniensis* (-0.378), *S. leensis* (-0.582) and *S. tenuis potomacus* (-0.631). Although photo-neutrality was expected and even photo-sensitivity was documented in troglomorphic species with highly reduced to absent eyes, photo-neutrality among surface species with well-developed eyes was unexpected. There is also little concordance between light avoidance behavior and habitat in terms of probability of light exposure. Our results indicate a need to reevaluate the dogma of evolution from surface ancestors with well-developed and therefore highly functional eyes to cave species with degenerated eyes and thus blind. The evolution of optic structure and function and the evolution of extra-optic photosensitivity and function may follow two independent paths governed by different evolutionary processes in different lineages. Detailed examinations of optic structures and function as well as extra-optic structures and their function from a phylogenetic perspective are warranted.

Friedrich, Markus
friedrichwsu@gmail.com

Adult lens cuticle deposition in a microphthalmic cave beetle.

Friedrich, Markus*[1,2]; Kulacic, Jasmina[1]

[1]Department of Biological Sciences, Wayne State University, Detroit, Michigan, United States
[2]Department of Cellular Biology and Anatomy, Wayne State University, Detroit, Michigan, United States

Troglobionts exhibit a consistent suite of dramatic regressive traits including the strong or complete reduction of the visual system. An example of extreme eye reduction (microphthalmy) is the troglobiotic small carrion beetle *Ptomaphagus hirtus* of Mammoth cave. In this species, the typical compound eye of surface insects is reduced to a minute single-chambered eye (eyelet) that is characterized by a pronounced single lens, covering approximately 130 photoreceptors and 70 accessory cells. Studying the development of the *P. hirtus* eyelet in comparison to compound eye development, we have found that the formation of lens cuticle is dramatically delayed compared to the timing of lens development in surface insects. In the latter case, lens formation is fully completed by beginning of the adult stage. In *P. hirtus*, however, the deposition of lens cuticle does not seem to initiate in the pupa. The majority of the lens forms during the first 11 weeks of adulthood, increasing in depth from approximately 1.8 um to 7.5 um. In one year old adults, the lens measures on average 9.6 um in depth.

To the best of our knowledge, *P. hirtus* represents the first animal example in which the development of the lens is delayed into the adult stage. We speculate that this dramatic modification evolved due to reduced purifying selection on the visual performance of young adult animals.

Symposium: Cave Animals at the Frontiers of Modern Biology
Keynote Address

Friedrich, Markus
friedrichwsu@gmail.com

Cave animals at the dawn of speleogenomics.

Friedrich, Markus

Department of Biological Sciences and Department of Cellular Biology and Anatomy, Wayne State University, Detroit, Michigan, United States

Many animal groups have been successful in diversifying into one of the most extreme habitats on earth: the light-secluded and energy-poor environments of caves. These transitions from surface to cave habitats have been associated with spectacular changes in morphology, physiology, and life history. Triggered by the advent of high throughput sequencing, recent years have seen a new generation of studies shedding light on the genetic basis of cave adaptation by exploring transcriptomes or whole genome sequence information. I will review the first lessons learned and speculate on things yet to come.

Special Session: Biology of Subterranean Crustaceans
(Sponsored by The Crustacean Society)

Gabriel, Ana Eugenia
ana.e.gabriel@okstate.edu

Environmental DNA for monitoring and detection of rare and endangered cavefish and cave crayfish in the Ozark Highlands.

Gabriel, Ana E.*[1]; Van Den Bussche, Ronald A.[1]; Brewer, Shannon K.[2]; Stark, Richard[3]; Niemiller, Matthew L.[4]; Fenolio, Dante B.[5]

[1]Oklahoma State University, Stillwater, Oklahoma, USA
[2]U.S. Geological Survey, Oklahoma Cooperative Fish and Wildlife Research Unit
[3]U.S. Fish and Wildlife Service
[4]Illinois Natural History Survey, Prairie Research Institute, University of Illinois Urbana-Champaign
[5]San Antonio Zoo

Caves and karst subterranean aquifers are common in the Ozark Highlands ecoregion of northern Arkansas, southern Missouri, and northeastern Oklahoma. These groundwater habitats are home to a unique and important biodiversity, but many species are difficult to monitor and study due to the complexity and other challenges associated with sampling groundwater habitats. Many groundwater aquifers in this region are threatened by a variety of anthropogenic activities, including groundwater exploitation, pollution, and habitat degradation, which has resulted in conservational concerns for several endemic, troglomorphic species, including some listed as federally threatened. Surveys of these species have traditionally relied on visual census methods, which are often unreliable due to low detection and habitat disturbance. New monitoring methods employ the use of environmental DNA (eDNA). As organisms interact in their environment, trace genetic material (eDNA) is shed or excreted and can be detected in soil, sediment, and water samples. This noninvasive technique offers a cost-effective supplement to visual surveys. Here, we propose to develop an eDNA-based detection and monitoring approach for several threatened groundwater species, including *Troglichthys rosae* (the Ozark Cavefish) and the crayfishes *Cambarus. tartarus, C. setosus,* and *C. aculabrum.* Preliminary studies have revealed the need for more stringent methods than by the amplification of eDNA using PCR as individual species of crayfish cannot be distinguished from one another. To overcome this, we have designed study parameters that will distinguish differences between eDNA samples based on melting curve analysis, pinpoint variations between species, and allow exclusion of false-positives. This study will benefit conservation and management efforts for these target species.

Gladstone, Nicholas
ngladsto@vols.utk.edu

Cave snails of East Tennessee and Alabama.

Gladstone, Nicholas*; McKinney, Michael; Douglas, Daniel

Earth & Planetary Sciences, University of Tennessee, Knoxville, Tennessee, United States

Despite their abundance and diversity in many subterranean ecosystems, gastropods remain a relatively unstudied group with very little documentation of the taxa and their natural history found in these habitats. Here we report on the aquatic and land snails collected in 39 different cave systems located in 20 counties of Tennessee and Alabama, The collections were conducted from 2013-2015 by Matthew Niemiller and several colleagues, and included 98 individual snails from both twilight and dark zone habitats. For our initial study of these collections, we have tentatively identified individuals from a wide range of micro- and macrosnails, including species of*: Inflectarius inflectus, Patera appressa, Triodopsis sp., Carychium clappi, Gastrocopta armifera, Glyphyalinia indentata, Pomatiopsis lapidaria,* and *Punctum minutissimum.* Several other species are also present in the collections but are still under study. Most of the collected species seem to be facultative inhabitants of the subterranean systems, and many of them are common and widespread in terrestrial habitats. As many cave systems are naturally vulnerable to an array of anthropogenic impacts, these findings emphasize the knowledge gap concerning these animals and the need for future study of gastropods in hypogean environments.

Gorički, Špela
goricki.spela@gmail.com

Searching for black *Proteus* (*Proteus anguinus parkelj*) in karst groundwater with the help of eDNA.

Gorički, Špela*[1]; Stanković, David[1,2]; Năpăruș-Aljančič, Magdalena[1]; Snoj, Aleš[3]; Aljančič, Gregor[1]

[1]Tular Cave Laboratory, Society for Cave Biology, Kranj, Slovenia
[2]Department of Life Sciences, University of Trieste, Trieste, Italy
[3]Department of Animal Science, Biotechnical faculty, University of Ljubljana, Ljubljana, Slovenia

The black *Proteus* (*Proteus anguinus parkelj*) forms a very small and endangered population of the only European subterranean amphibian. Since its discovery in 1986 until the most recent sighting in 2012, the black *Proteus* has been documented at only four sites in extreme Southeastern Slovenia. They are situated within a 2 by 2.5 km maximum aerial distance from each other. Nearby in the same geographic area, but presumably in a different geological formation, the nominate troglomorphic white *Proteus* (*Proteus anguinus anguinus*) has been spotted at about 10 sites (springs and caves), several of which have never been verified by experts. Since the habitat of *Proteus* is inaccessible to man, we developed an environmental DNA-based assay of spring water samples to test for its presence. Employing TaqMan qPCR chemistry with specific probes and primers we (1) conducted a systematic inventory of *Proteus* presence in the region, (2) verified certain historic records of white *Proteus*, (3) determined the maximum span of the black *Proteus* range to the East and South, and (4) tested for possible co-occurrence of the two subspecies. We detected black *Proteus* eDNA at five new sites. In one of these we found both black and white *Proteus* eDNA syntopically. These results are consistent with known hydrogeological patterns, and suggest a possible contact or parapatry of the subspecies. eDNA data can therefore, when used together with information from phylogeographic and population genetic analyses, broaden our knowledge of species evolution and systematics, as well as help establish efficient conservation measures through updated distribution maps of the species.

Special Session: Biology of Subterranean Crustaceans
(Sponsored by The Crustacean Society)

Gottstein, Sanja
sanja.gottstein@biol.pmf.hr

Microhabitat selection of subterranean amphipods in the Western Balkan peat bog.

Gottstein, Sanja*[1]; Brigić, Andreja[1]; Kerovec, Mladen[1]; Ternjej, Ivančica[1]

[1]Department of Biology, Faculty of Science, University of Zagreb, HR-10000 Zagreb, Croatia

Peatlands are extremely rare, small in size and highly fragmented habitats in the Western Balkans. Nowadays, they are critically endangered mainly due to the change in drainage regimes, abandonment of traditional land management practices and climate change, resulting in rapid vegetation succession. Subterranean amphipods, as an important part of this unique aquatic environment, have been sporadically studied in the Western Balkans peatlands. With an aim to elucidate the limiting environmental factors for amphipod micro-spatial distribution, four types of aquatic microhabitats with three gradients of light, in the Croatian largest and oldest Đon močvar peat bog were studied. Amphipods were sampled using a benthos net (0.0625 m^2) with the mesh size of 500 μm during one vegetation season. Species composition and structure differed significantly between the studied microhabitats, most likely due to their isolation and specific hydrology of the environment. In addition, physico-chemical water parameters, especially temperature and pH, have played an important role as an environmental barrier for species occurrence in the study area. All microhabitats were dominated by *Synurella* sp. Wrzesniowski, particularly deep water holes surrounded by *Sphagnum* spp. L. mosses. Also, an unexpected large local biodiversity of *Niphargus* spp. Schiödte were discussed. In order to investigate possible between-species avoidance, allotopic and syntopic occurrences were observed. High microhabitat overlapping of specific species was determined. The current study has revealed the threats and ecological status of the Đon močvar peat bog, highlighting the necessity of active conservation measures. Additionally, based on our results, we propose a general conceptual model for peatlands, as groundwater dependent ecosystems, in Croatia.

Symposium: Cave Animals at the Frontiers of Modern Biology

Gross, Joshua
grossja@ucmail.uc.edu

Genetic analysis of craniofacial changes in blind Mexican Cavefish, *Astyanax mexicanus.*

Gross, Joshua

Department of Biological Sciences, University of Cincinnati, 312 Clifton Court, Rieveschl Hall Room 711B, Cincinnati, Ohio 45221-0006, United States

Blind-cave dwelling organisms commonly evolve a series of phenotypes that are diminished ("regressive") or expanded ("constructive") as a consequence of life in total darkness. Certain phenotypes are not easily characterized as either regressive or constructive, including modifications to the cranial skeleton. For instance, the dermal bones encircling the eye have evolved a number of extreme morphological changes, including fusions and fragmentations. Our prior research indicates that certain regions of the cavefish genome are associated with fragmentation of the largest circumorbital bone, the third suborbital bone ("SO3"). Interestingly, the genetic signals we detected for SO3 fragmentation were asymmetrically distributed. That is, we only observed significant QTL signals when evaluating the right, but not left, side of the cranial skeleton in our experimental F_2 pedigree. The genetic and cellular basis for fragmentation remains unknown, however, prior research demonstrated that sensory neuromasts are densely populated directly superficial to this bone. We therefore reasoned that the development and/or maintenance of sensory neuromasts may interfere with normal development of the SO3 bone. To evaluate this, we performed a comprehensive survey of the left and right sides of numerous representative *Astyanax* cavefish collected from across the Sierra de El Abra region of northeastern Mexico. Our results indicate that cranial neuromasts are more numerous, denser and more evenly distributed across the SO3 bone in all cave forms compared to surface fish. Qualitative evidence identified a dorsal "stream" of superficial neuromasts directly situated along the posterior SO3 margin. This work suggests a relationship exists between the developmental positioning and maintenance of sensory neuromasts and the SO3 bone that underlies them. Moreover, the pattern of left-right asymmetry observed in SO3 fragmentation is mirrored by similar asymmetry of cranial neuromasts. This may indicate that bone fragmentation occurs as a potentially indirect consequence of higher neuromast density in the cranial region.

Hahn, Hans Jürgen
hjhahn@uni-landau.de

Global warming – where are the refugia for cold-stenothermous stygofauna?

Spengler, Cornelia; Hahn, Hans Jürgen*

University of Koblenz Landau, Institute for Environmental Sciences, Fortstr. 7, 76829 Landau, Rheinland-Pfalz, Germany

Since the pliocene, stygofauna in central Europe has adapted to lower temperatures. Our data from all parts of Germany imply a critical upper limit at around 14 °C for most stygobiontic species. In some areas, for example in the Upper Rhine Plateau, the warmest region of Germany, groundwater temperatures naturally range between 11 °C and 13 °C, but in most part of Central Europe, temperatures are lower. For the Upper Rhine stygofauna, temperature was found to be – beside organic matter - the main parameter shaping subsurface communities.

Taking in consideration a predicted increase of the annual air temperature of at least 2 °C until the end of this century, a groundwater warming up to 14 °C and more has to be suspected. The consequence might be a strong loss of subterranean biodiversity. With this background the question has become evident, if groundwater warming will have spatially inclusive and comprehensive effects or if there will remain some cold refugia.

The Upper Rhine Plateau is strongly industrialized, and groundwater is commonly used for cooling purposes. Temperatures of those anthropogenicly affected groundwaters often reach or even exceed 20 °C. Amazingly, we found in this area groundwater communities and species with temperature optima lower than 10 C – which is significantly lower than both the annual air and the natural groundwater temperature. These findings can be explained by the very special hydrological conditions of the Southern Upper Rhine Plateau and the adjacent Black Forest, creating cold islands in a warm groundwater landscape – and potential refugia for cold-stenothermous stygofauna.

Symposium: Conservation of Subterranean Fauna and Habitats

Halse, Stuart
stuart.halse@bennelongia.com.au

Challenges and rewards of subterranean fauna environmental impact assessment.

Halse, Stuart

Bennelongia Environmental Consultants, 5 Bishop Street, Jolimont, Western Australia 6014, Australia

Much of the focus of subterranean fauna conservation has been on the occurrence of species in caves, where the cave and its fauna form an easily recognized conservation unit. However, in many parts of the world rich subterranean fauna communities occur across substantial parts of the landscape in alluvium, calcrete, weathered rock and other geologies. These communities may be threatened by activities that include mining, water supply and agriculture. The major impacts of mining are excavations of large open mine pits and the frequent de-watering of aquifers in which these pits sit. Water supply impacts include abstraction of groundwater for irrigation, industrial processes and potable water. Agricultural impacts include application of pesticides and increases in groundwater salinity. The key information requirements for assessing impacts on subterranean fauna revolve around what species are present, their ranges and the habitats they occupy. In theory this information should be straightforward to collect but the complexity and three-dimensional structure of subterranean habitats makes it difficult. Information on the structure and distribution of subterranean habitats comes from the drill logs of a small number of holes that are more usually widely spaced than the spatial pattern of habitat change. Faunal sampling via these holes is inefficient and it is rare to collect more than 70% of the estimated number of species present. Most species are undescribed and the collection of them for assessment often represents the first records of the species. A high proportion of species are known from a single record and the depth and habitat in which the species occurred prior to collection are almost always unknown. Despite the challenges involved in conducting impact assessments with limited information, the process forms an important step in improved subterranean fauna conservation and has led to vastly improved knowledge of subterranean fauna taxonomy and ecology.

Harms, Danilo
danilo.harms@bennelongia.com.au

Biodiversity in complex subterranean systems: a tale of arachnids in arid Western Australia.

Harms, Danilo*; Halse, Stuart; McRae, Jane; Scanlon, Michael; Curran, Michael

Bennelongia Environmental Consultants, 5 Bishop Street, Jolimont WA 6014, Australia.

The Pilbara region of Western Australia is rich in subterranean invertebrates. Little is known about the distributions of troglofauna in this extremely arid region, although current research suggests troglofauna species in the Pilbara typically have small ranges that are constrained by geological or topographic barriers. Other factors affecting ranges must include the unique biology of subterranean species and the highly complex underground "matrix" they inhabit. Short-tailed whipscorpions (Schizomida) are arachnids that are distantly related to scorpions and spiders. Most schizomid species occur in moist leaf litter in tropical forest ecosystems where they prey on other invertebrates. In contrast to their usual habitat, the Western Australian schizomid fauna is almost exclusively subterranean and may comprise more than a hundred species; most of which are currently undescribed.

Some previous research on schizomids in the western Pilbara suggested species are mostly allopatric and confined to isolated mesas in an otherwise flat landscape. It was proposed this is a result of multiple independent colonisations from the surface since the Miocene, some 15 million years ago. However, the observed species patterns in low, geologically more complex ranges in the central Pilbara are markedly different and diverse communities occur with few obvious geological or topographical features that might constrain ranges or promote allopatric speciation.

We used a dated molecular phylogenetic approach, reconciled with distribution mapping and habitat analyses, to reconstruct the evolutionary history of 13 schizomid species within an 80,000 ha area in the central Pilbara. We examined species ranges, phylogenetic relationships and ages, and the relative contribution of surface colonisation versus subterranean speciation events.

As well as providing a snapshot of subterranean biodiversity in the Pilbara, our results highlight the importance of sampling design and sample size in determining species boundaries, especially in groups that are difficult to sample and naturally occur at low abundance.

Hart, Pamela
Pamelabeth.hart@gmail.com

Shape variation within the Southern Cavefish, *Typhlichthys subterraneus* (Percopsiformes: Amblyopsidae).

Hart, Pamela*; Burress, Edward; Armbruster, Jonathan.

Department of Biological Sciences, Auburn University, Auburn, Alabama, United States

The Southern Cavefish, *Typhlichthys subterraneus* Girard 1859, is one of the most fascinating stygobionts of the Amblyopsidae because of undescribed diversity contained within the species. Previous molecular analysis suggests the presence of at least 10 distinct lineages in the Southeastern United States. Shape variation for this group had not been quantified previous to this study. We studied the morphological variation within the Southeastern populations of the Southern Cavefish using geometric morphometric analysis. Initial analyses of geometric morphometric data uncovered allometry, thus an allometric correction was implemented to recover size-independent morphospace for both the dorsal and lateral views. We then examined if morphological variation corresponded to aforementioned putative genetic lineages as well as aquifer association.

Poster Session

He, Qiufang
hqfeddy@hotmail.com

Bacterial calcium carbonate precipitation in Shi Jiangjun Cave, Guizhou province, China

He, Qiufang*[1]; Jiang, Jianjian[1]; Liu, Ziqi[2]; Lü, Xianfu[1]; Zhao, Ruiyi[1]

[1]School of Geographical Sciences, Southwest University, 400715, Chongqing, China
[2]Institute of South China Karst, Guizhou Normal University, 550000, Guiyang, China

Calcite (calcium carbonate) in caves usually forms as a chemical precipitate, although microbes can be involved in the formation, too. To investigate the role bacteria have on calcium carbonate precipitation, bacteria were isolated from calcium carbonate-rich water collected from Shi Jiangjun Cave. More than 30 strains were isolated using different culture media, and most of the bacteria belonged to the genera *Nocardia* and *Bacillus*. Crystal mineralogies and structures were analyzed in the liquid cultures using X-ray diffraction analyzer (XRD) and scanning electron microscope (SEM). Bacteria grown on B-4 medium without Mg produced calcium carbonate crystals of calcite, vaterite mixed with calcite, and vaterite alone. Differences in mineralogy were related to increases in medium pH. Bacteria grown on B-4C medium containing Mg generated only calcite. SEM analyses revealed that the newly formed calcium carbonate crystals were irregular hexagonal polytypes. Cylindrical, square, and hemispherical crystals were rare. Bacteria shaped holes on calcium carbonate crystals surface indicated the existence of microbes in the calcium carbonate depositing process.

Poster Session

Helf, Kurt Lewis
Kurt_Helf@nps.gov

Sampling cave crickets (*Hadenoecus subterraneus*) using strip adaptive cluster sampling.

Helf, Kurt Lewis*[1]; Philippi, Tom[2]

[1]National Park Service, Southeast Region Inventory and Monitoring Division, Cumberland Piedmont Network, Mammoth Cave, Kentucky
[2]National Park Service, Natural Resources Stewardship Science, Inventory and Monitoring Division Support Office, Fort Collins, Colorado

Adaptive cluster sampling is a strategy for sampling rare or clustered populations that can provide more precise estimates for population means and totals relative to other designs. Adaptive sampling allows the surveyor to respond in real-time by increasing their sampling efforts where clusters of observations of interest are found. Cave crickets roost on walls and ceilings of irregular passageways near cave entrances. Even if those surfaces are mapped, they don't correspond to a simple surface where random quadrats are easy to define. Further, cave crickets aggregate into clumps; and most random quadrats will be empty. We defined a sample frame as rings of cave wall surface, perpendicular to a baseline down the middle of the cave passage, indexed by distance along that baseline. In summer 2015, I performed the first full implementation of a long-term population monitoring protocol using strip adaptive cluster sampling, a 2-stage extension of standard adaptive cluster sampling designs, to estimate population sizes of cave crickets at selected cave entrances in Mammoth Cave National Park. We used a generalized random tessellation stratified sampling scheme to draw distances along the baseline, so that the monitoring data would be sensitive not only to changes in cricket abundance, but also to shifts in roosting locations toward or away from the cave entrances.

Hollifield, Breanna
bbhollifield@comcast.net

Understanding the colonization of caves: Effects of constant darkness on the surface form of *Astyanax mexicanus.*

Hollifield, Breanna*; Bilandžija, Helena; Jeffery, William

Department of Biology, University of Maryland, College Park, Maryland, United States

The fish *Astyanax mexicanus* is able to survive in sunlight as well in the depths of caves concealed in complete darkness. Those living in light are called surface fish and those in the dark are called cavefish. However, on occasion surface fish have been encountered living in caves, and the cavefish are descended from surface fish ancestors that originally colonized caves. It is not clear what adaptations the surface fish colonizers had to make in order for their progeny to survive in complete darkness for many generations and eventually evolve into cavefish. The purpose of this research is to understand the effects of darkness on surface fish by studying a suite of dark-related physiological traits and changes on a molecular level. The study groups were surface fish born at the same time and raised separately for 6 months, one-half kept in completely dark conditions and the other half kept in normal 14-hour light/10-hour dark conditions. All other factors were kept identical between the two groups of fish. Our results showed that darkness had an effect on the expression of genes involved in the hypothalamic-pituitary-adrenal (HPA) axis, which controls basal and stress-related homeostasis and energy storage and expenditure. Furthermore, our research has shown that darkness causes increased stress measured by cortisol levels. Future investigations will be conducted to determine if any other physiological and molecular differences occur between the two groups of fish using a genome wide approach. The results suggest that neuroendocrine changes in the HPA axis related to stress and energy budgeting may be one of the early physiological effects of constant darkness on the surface form of *Astyanax mexicanus*.

Symposium: Patterns of Subterranean Biogeography and Biodiversity

Humphreys, William F.
Bill.Humphreys@museum.wa.gov.au

Patterns on patterns: The rise and rise of Australian subterranean biodiversity.

Humphreys, William F.

Department of Terrestrial Zoology, Western Australian Museum, Locked Bag 49, Welshpool DC, Western Australia 6986, Australia; School of Animal Biology, University of Western Australia, Crawley, Western Australia 6009, Australia.

The ancient stable landscape of Australia is largely eroded flat and has several shield regions emergent from the sea since before the Palaeozoic. Deep history events are hypothesised to have significantly influenced the biogeography of the subterranean fauna of Australia. Namely, tectonic stability of the continent; absence of widespread glaciation since the Permian; latitudinal extent of the continent with mean annual temperature range from ca 8-28 C; spreading aridity, now affecting 70% of the continent, since establishment of the Antarctic Circumpolar Current; scarcity of karst relative to Laurasian landmasses; past connections with Gondwana; and the close connection of the North West Shelf with Tethys. The emerging diversity of subterranean fauna in Australia is associated with a broad range of habitats, both traditional and novel, carbonate karst (Devonian and Tertiary), volcanic, groundwater and lacustrine calcretes, alluvium, fractured rock, and goethite pisolites associated with Precambrian banded iron formations and their derivatives. In addition the aquatic regimes range from anchialine systems to continental waters ranging from fresh to marine salinities. Recently, there has been a greatly increased awareness of biodiversity in Australian subterranean fauna, often informally through molecular studies, which greatly exceeds taxonomic knowledge. I will set the discussion of the biogeography of the better known higher taxonomic groups against this schema. The background information on biodiversity leads to discussion of ancient freshwater anchialine fauna with Tethyan or Gondwanan affinities, arid Tertiary orogenic karst fauna with relict wet forest affinities, the periglacial fauna of Tasmania, high biodiversity in the Pilbara craton with freshwater Gondwanan affinities some constrained by drainage basins, subterranean islands of the Yilgarn arid zone, and total fauna turnover in contiguous regions in need of a coherent hypothesis.

Symposium: Conservation of Subterranean Fauna and Habitats

Humphreys, William F.
Bill.Humphreys@museum.wa.gov.au

Conservation of subterranean species and habitats in Australia.

Humphreys, William F.*[1,2]; Humphreys, Garth [2,3]

[1]Department of Terrestrial Zoology, Western Australian Museum, Locked Bag 49, Welshpool DC, Western Australia 6986, Australia; School of Earth and Environmental Sciences, University of Adelaide, South Australia 5005, Australia.
[2]School of Animal Biology, University of Western Australia, Crawley, Western Australia 6009, Australia.
[3]Biota Environmental Sciences Pty Ltd, PO Box 155, Leederville, Western Australia 6903, Australia.

Australian subterranean fauna is highly diverse but knowledge of its subterranean ecology is sparse. Subterranean species occur in classic karst settings in both tropical and temperate areas but is most diverse in the arid zone, especially in non-karstic landscapes on shield regions. The fauna is recently discovered so taxonomy lags far behind species discovery. The estimated diversity keeps rising, for example, ca 916 species of troglofauna and stygofauna reported from a small proportion only of the Pilbara. Conservation is set in a federal context with seven regional jurisdictions overlain by the national Environment Protection and Biodiversity Conservation Act (EPBC). Each jurisdiction has from 1 to 4 pieces of legislation bearing on fauna protection but none meets the best practice for threatened species protection, many are seriously deficient and many are not implemented effectively. We will draw on Western Australia because it comprises one third of the continent, is where subterranean fauna is best known, and where is most focus in regulatory and management sense. Western Australia has an outdated Wildlife Conservation Act 1950 providing basic protection. All native species of plants and animals are nominally 'protected' from direct but not indirect 'taking', the latter being the major issue including land clearance, mining, water pollution, declining groundwater. EPBC listed species and communities have recovery plans, management plans are prepared for National Parks, major development projects are subjected to various levels of environmental impact assessment and approved projects may have legally enforceable Ministerial Conditions attached, and, finally, litigation by entities with standing. We present examples of the contribution of how ecological knowledge is injected into these processes, including survey and taxonomy on risk assessment, experiments in karst informing methodology, ecosystem studies informing recovery plans, management plans, and environmental impact assessment in groundwater and terrestrial contexts, and biogeography in establishing protected areas.

Husana, Daniel Edison
dmhusana@up.edu.ph

Phylogeography of crab genus *Sundathelphusa*: history of extensive migration, cave colonization and refugia in the Philippines.

Husana, Daniel Edison*[1]; Haga, Takuma[2]; Kase, Tomoki[3]

[1]Environmental Biology Division, Institute of Biological Sciences, University of the Philippines Los Baños, Laguna 4031, Philippines
[2]Toyohashi Museum of Natural History, 1-238 Oana, Oiwa-cho, Toyohashi, Aichi 441-3147, JAPAN
[3]Invertebrate Paleontology Division, National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba City, Ibaraki 305-0005 Japan

Sundathelphusa is a tropical crab that strictly completes its entire life cycle in the freshwater habitat. This species cannot tolerate saltwater hence hampering its dispersal ability leading to occupancy of smaller territories compared to other terrestrial and marine crabs. But this study has found that this crab inhabits many areas in the entire archipelago including subterranean habitats that harbor species with true troglomorphic characters. To determine the phylogeography of this genus, samples were collected from diverse habitats in various islands in the Philippines. We also reviewed records of the paleoclimatic and geologic history of the Southeast Asian region. Tissue samples were isolated from the muscle of ambulatory legs for molecular analysis. Results of morphological examination and analysis of mitochondrial 16S rRNA gene revealed the phylogenetic relationships as well as the position of cavernicolous species in the genus *Sundathelphusa*. It shows the widespread territory and speciation occurrences that coincide with the geologic and paleoclimatic events in the history of the Philippines. Owing to their semi terrestrial habits, this species group seems to be one of the most successful colonizers of this archipelago, invading every suitable habitat that could provide the requirements for their existence. This is the only freshwater crab that successfully colonized the freshwater cave habitats and exploited various resource niches that may have influenced the evolution of the characters of a true troglobite, a troglophile and a trogloxene from the surface species lineage. The restricted mobility of *Sundathelphusa*, coupled with the paleoclimatic and geologic history of the Philippines may have contributed to the high diversity of this crab in the archipelago.

Symposium: Patterns of Subterranean Biogeography and Biodiversity

Hutchins, Benjamin T.
ben.hutchins@tpwd.texas.gov

Biodiversity, stability, and trophic complexity in the Edwards Aquifer, United States: The influence of chemolithoautotrophy on stygobiont community structure.

Hutchins, Benjamin T.*[1,2]; Engel, Annette Summers[3]; Nowlin, Weston H. [2]; Schwartz, Benjamin F.[3,4]

[1]Texas Parks and Wildlife Department, Austin Texas, United States
[2]Texas State University, Aquatic Station, Department of Biology, San Marcos, Texas, United States
[3]University of Tennessee, Department of Earth and Planetary Sciences, Knoxville, Tennessee, United States
[4]Edwards Aquifer Research and Data Center, Texas State University, San Marcos, Texas, United States

Chemolithoautotrophy can subsidize or replace allochthonous food sources in some subterranean ecosystems. The presence of autochthonous production has important implications for niche specialization and evolution of higher trophic levels. We examined spatial variation in the isotopic composition of basal food resources and consumers, food web structure, stygobiont species diversity, and chromophoric organic matter (CDOM) across a geochemical gradient in a large and complex groundwater system, the Edwards Aquifer in Central Texas (USA). Mean $\delta^{13}C$ values of stygobiont communities become increasingly more negative along a gradient of photosynthetic organic matter (OM) sources near the aquifer recharge zone to chemolithoautotrophic OM sources closer to the freshwater-saline water interface (FWSWI) between oxygenated freshwater and anoxic, sulfide-rich saline water. Stygobiont species richness declined with increasing distance from the FWSWI. Bayesian mixing models were used to estimate the relative importance of photosynthetic OM and chemolithoautotrophic OM for stygobiont communities at three biogeochemically distinct sites. The contribution of chemolithoautotrophic OM to consumers at these sites ranged between 25% and 69% of total OM utilized and comprised as much as 88% of the diet for one species. In addition, the food web adjacent to the FWSWI had greater trophic diversity when compared to the other two sites. Our results suggest that diverse OM sources and in-situ chemolithoautotrophic OM production can support complex groundwater food webs and increase species richness. Chemolithoautotrophy has been fundamental for the long-term maintenance of species diversity, trophic complexity, and community stability in this subterranean ecosystem, especially during periods of decreased photosynthetic production and groundwater recharge that have occurred over geologic time scales.

Symposium: Conservation of Subterranean Fauna and Habitats

Hutchins, Benjamin T.
ben.hutchins@tpwd.texas.gov

Conservation status of stygobionts in Texas, United States.

Hutchins, Benjamin T.

Texas Parks and Wildlife Department, Austin, Texas, United States

Conservation status assessments for rare species can be an important tool to 1) prioritize allocation of limited resources for conservation and management, 2) identify common factors threatening multiple taxa, 3) identify data gaps, and 4) highlight vulnerable and overlooked taxa. Using NatureServe methodology, conservation status assessments were performed for all sixty-four stygobiontic invertebrates known from Texas, U.S.A: a large, geographically diverse state harboring an interesting assemblage of stygobionts of both freshwater and marine origin. Thirty-six species (56%) were identified as imperiled or critically imperiled and only a single species was identified as apparently secure. Small-range endemism was the most important factor affecting conservation ranks, and 50% of species occupy an area less than or equal to 5 km^2. Despite substantial pressures on groundwater resources throughout the state, threats facing most species were of low or medium severity. Threats varied by aquifer and habitat and were greatest for species in shallow habitats in areas of intense urbanization. Species richness was greatest in the Edwards Aquifer where geology and regulation for federally protected species function to reduce threat severity despite increasing urbanization. Additional protective measures are needed in the adjacent Edwards-Trinity and Trinity Aquifers, which have lower levels of species richness, but fewer water quantity and quality protections. Beetles and snails were the most imperiled taxa, but insufficient data precluded rankings for several species of mites, ostracods, and copepods. Additionally, undescribed stygobionts are known from throughout the state, but are unrankable because of insufficient data. Stygobionts throughout the world face comparable threats, and levels of imperilment calculated for Texas stygobionts are probably not unusual. If so, stygobionts, although not a monophyletic taxon, should be included among the most imperiled of freshwater organisms.

Symposium: Cave Animals at the Frontiers of Modern Biology

Jeffery, William
Jeffery@umd.edu

Homocystinuria in Cavefish: Molecular analysis of an *Astyanax* eye QTL reveals the role of cystathionine ß-synthase in eye degeneration.

Jeffery, William

Department of Biology, University of Maryland, College Park, Maryland, United States

Cavefish eye degeneration is a multigenic trait represented by quantitative trait loci (QTL) spread throughout the *Astyanax* genome. The identity of the responsible genes within the eye QTL, the nature of the underlying mutations, and the roles they play in eye development and degeneration are unknown. We identify here a mutated gene in a QTL on *Astyanax* chromosome 21 that is involved in cavefish eye degeneration. According to the draft cavefish genome sequence, the chromosome 21 QTL contains 24 protein-coding genes. We found that 21 of these genes are expressed during the critical period of cavefish eye degeneration: 15 genes showed no change, 3 genes were upregulated, and 3 genes were downregulated in surface fish relative to cavefish embryos. Analysis of gene expression in F1 hybrids suggested that one of the downregulated genes, *cystathionine ß-synthase a* (*cbsa*), harbors a *cis*-acting mutation. Based on genome wide ATAC sequencing and open chromatin analysis, a putative enhancer in a large *cbsa* intron is a strong candidate for this mutation. The *cbsa* gene, which is expressed at high levels in the lens and optic cup in surface fish but not cavefish embryos, encodes the CBS enzyme that is responsible for conversion of homocysteine to cysteine in the transsulfuration pathway producing glutathione, the major antioxidant in the eye. Human CBS mutations result in toxic homocysteine accumulation and lead to homocystinuria, a hereditary disease with debilitating effects on the eye. Similarly in *Astyanax*, knockdown of the surface fish *cbsa* gene caused defects in eye development, and ELISA showed that homocysteine is elevated in cavefish embryos during the critical period of eye degeneration. The results suggest that the cavefish regressive eye phenotype resembles CBS-related homocystinuria and may be caused in part by breakdown of the defense system protecting the eye against chemical and oxidative stress.

Special Session: Biology of Subterranean Crustaceans
(Sponsored by The Crustacean Society)

Jenson, Aubri
aaj49@txstate.edu

Molecular and morphological analysis of *Stygobromus sp.* near San Marcos, Texas.

Schwartz, Benjamin[1,2]; Nice, Christopher[1]; Jenson, Aubri*[1]

[1]Department of Biology, Texas State University, San Marcos, Texas, United States
[2]Edwards Aquifer Research and Data Center, San Marcos, Texas, United States

Subterranean environments impose unique selective pressures leading to convergent troglomorphic adaptations that make it difficult to distinguish one species from another by morphology alone. The possible presence of unrecognized, cryptic species among the amphipod genus *Stygobromus* was investigated through morphometric analysis within and among species identified using taxonomic keys (Holsinger, 1966, 1967, 1974, 1980) for comparison with results from molecular genetic sequencing. The Edwards Plateau in south-central Texas is known for its high biodiversity of stygobitic fauna, making it an ideal location for a study of this kind. 120 individuals of five species (*S. bifurcatus, S. flagellatus, S. longipes*, *S. pecki*, and *S. russelli*) from nine springs in south-central Texas were dissected, mounted, and measured for fifteen morphological parameters such as total body length, antenna lengths, telson length and width, etc. A total of 392 individuals from 21 site locations were analyzed for genetics using mitochondrial DNA and ITS (Inter-Transcribed Spacer of ribosomal RNA Gene). Preliminary results suggest that the morphology-based taxonomic keys do not accurately reflect genetic diversity, particularly within S. *flagellatus*, and that revisions are warranted where significant differences in morphological traits exist.

Special Session: Biology of Subterranean Crustaceans
(Sponsored by The Crustacean Society)

Keany, Jenna
jk2592a@student.american.edu

Investigating the physico-chemical niche of obligate subterranean amphipods in shallow subterranean waters of the DC metro area.

Keany, Jenna*[1]; Culver, David[1]; Knee, Karen[1]; Fong, Daniel[2]

[1]Department of Environmental Science, American University, Washington, District of Columbia, United States
[2]Department of Biology, American University, Washington, District of Columbia, United States

Washington, D.C. is home to a remarkable assemblage of troglomorphic amphipods and isopods living in shallow subterranean habitats that emerge at seepage springs, or "seeps". Species include amphipods (*Crangonyx* and *Stygobromus*) and isopods (*Caecidotea*). One species, *Stygobromus hayi*, is on the endangered species list and is endemic to the district and another is soon to be listed; however, little is known about their habitat preferences or their distribution. Seepage springs were sampled in national park lands in the DC metropolitan area for rare subterranean fauna, soil morphology, and water quality indicators such as nitrates, phosphates, radon, pH, DO, and conductivity. More than 100 seeps have been found in Southeast, D.C., with a high concentration (more than 5 per hectare) in some parts of Shepherd Parkway. Especially noteworthy is the presence of seepage springs dominated by *Crangonyx* amphipods, a previously unknown community type. This study points to the potential importance of small parks as reservoirs of biodiversity in low-socioeconomic communities.

Kirchner, Sandra
sandra.kirchner@nhm-wien.ac.at

As above, so below? Testing for gene flow between cave and surface-dwelling populations of *Garra barreimiae.*

Kirchner, Sandra*[1,2]; Sattmann, Helmut[3]; Plan, Lukas[4]; Krenn, Harald[1];
Victor, Reginald[5]; Haring, Elisabeth[1,2]; Kruckenhauser, Luise[2]

[1]Department for Integrative Biology, University of Vienna, Althanstraße 14, 1090 Vienna, Austria
[2]Central Research Laboratories, Natural History Museum, Burgring 7, 1010 Vienna, Austria
[3]Third Zoological Department, Natural History Museum, Burgring 7, 1010 Vienna, Austria
[4]Department for Geology & Paleontology, Natural History Museum, Burgring 7, 1010 Vienna, Austria
[5]Department of Biology, Sultan Qaboos University, Al Khoudh, Muscat 123, Oman

Garra barreimiae is a cyprinid freshwater fish endemic to the southeastern Arabian Peninsula, where it inhabits regularly desiccating water bodies. A hypogean population with troglomorphic traits found in the Al Hoota cave system in northern Oman is considered as conspecific with the normally developed surface morphotypes. Normally the fish live in quiet cave pools but after heavy rain the cave is flushed and limited contact with the surface population is likely. Therefore *G. barreimiae* is a perfect organism to study the effects of life in complete darkness and mechanisms of adaption.

The DNA-study of Kruckenhauser et al. (2011) based on a section of the mitochondrial control region showed that specimens from the cave population are genetically differentiated from the adjacent surface populations. Some surface individuals possess haplotypes characteristic for the cave population and most of these individuals display intermediate phenotypes. These results suggest a quite recent separation of the cave population. For a better understanding of the evolution of the troglomorphic phenotype it is crucial to know if and to what extent gene flow between these morphotypes occurs. Hence, we investigated individuals from two troglomorphic cave populations (including Al Hoota), one cave with constant water outflow giving connection to the surrounding surface water body and two surface sites. In order to estimate potential gene flow between cave and surface populations we utilized 19 variable microsatellite markers designed specifically for *G. barreimiae* (Kirchner et al. 2014). The results show that both caves inhabited by troglomorphic fish are clearly separated from the surface populations. Some of the specimens with intermediate phenotypes possess a hybrid genotype, while others are genetically clearly of cave origin. For the latter, it can be assumed that cave fish were washed out during floods and the intermediate phenotype is a result of phenotypic plasticity induced by light exposure.

Kováč, Ľubomír
lubomir.kovac@upjs.sk

Traits of terrestrial subterranean biota of the Western Carpathians (Central Europe) are affected by productivity of above ground ecosystems.

Elhottová, Dana[1]; Kováč, Ľubomír*[2]; Nováková, Alena[3]; Chroňáková, Alica[1]; Mock, Andrej[2]; Krištůfek, Václav[1]; Mulec, Janez[4]; Lukešová, Alena[1]; Ľuptáčik, Peter[2]; Parimuchová, Andrea[2]; Papáč, Vladimír[5]; Miklisová, Dana[6]; Fenďa, Peter[7]; Jászay, Tomáš[8]; Košel, Vladimír[9]

[1]Biology Centre ASCR, v. v. i., Institute of Soil Biology, České Budějovice, Czech Republic
[2]Department of Zoology, Institute of Biology and Ecology, Faculty of Science, P. J. Šafárik University, Košice, Slovakia
[3]Institute of Microbiology of the CAS, v. v. i., Prague, Czech Republic
[4]Karst Research Institute ZRC SAZU, Postojna, Slovenia
[5]State Nature Conservancy SR, Slovak Caves Administration, Rimavská Sobota, Slovakia
[6]Institute of Parasitology SAS, Košice, Slovakia
[7]Department of Zoology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia
[8]Šarišské múzeum, Bardejov, Slovakia
[9]Hornádska 24, 821 07 Bratislava, Slovakia

During 2010–2012 a complex survey of terrestrial biota was carried out in nine karst caves in the Western Carpathians, Slovakia. Geographic coverage, size of the cave and microhabitat variability were used as principal criteria for the selection of sites. Cave microorganisms were investigated using: (1) complex analysis of sediments *in situ* using profiles of phospholipid fatty acids (PLFA) and DNA analyses of communities (CARD–FISH), (2) analysis of speleoaerosol by active aeroscopy and by sedimentation technique, (3) RIDA count tests for bacteria detection, (4) various isolation techniques including direct isolation, dilution plate method, washing technique, and bait-technique, (5) identification of isolated microbiota (MIS Sherlock system, analysis of 16S rRNA gene). Cave arthropods were surveyed by pitfall trapping, extraction of organic material (wood, leaves, bat guano) in high-gradient apparatus and direct hand collection of individuals.

Principal component ordination plot of phospholipid-derived fatty acids (PLFA) profile in cave sediments segregated microbial communities of high-mountain and plateau karst caves.
Cave entrance elevation, internal air temperature and geographic location played an important role in the distribution of arthropods. The Slovak and Aggtelek Karst and adjacent southern karst areas have higher species diversity and number of arthropod troglobionts in contrast with karst areas of the caves located in the central Western Carpathians.

Higher species diversity and quantitative parameters of cave biota communities was observed in caves with higher air temperature and content of nutrients in cave sediments, partly associated with the presence and amount of bat guano deposits. Traits of biotic communities in the Western Carpathian caves are associated with productivity of above ground ecosystems that is higher in deciduous forests in the southern areas compared with predominantly coniferous forests in the karst areas of the central Western Carpathians. The overall number of troglobiotic arthropod forms is, however, mostly not affected by this relationship.

Kozel, Peter
peter.kozel@zrc-sazu.si

Synchronous ceiling-floor pitfall trapping allows study of microdistribution and habitat preferences of terrestrial subterranean fauna in caves.

Kozel, Peter*[1,3]; Pipan, Tanja[1]; Culver, C. David[2]; Šajna, Nina[3]; Polak, Slavko[4]; Novak, Tone[3]

[1]Karst Research Institute ZRC SAZU, Postojna, Slovenia;
[2]Department of Environmental Science, American University, Washington, District of Columbia, United States
[3] Department of Biology, Faculty of Natural Sciences and Mathematics, University of Maribor, Slovenia
[4] Zavod Znanje, OE Notranjska Museum Postojna, Slovenia

Biotic inventory is fundamental in biological and ecological studies of habitats. In subterranean biology and ecology, besides observations and manual collection, baited pitfall trapping, which allows relatively easy sampling of the fauna, has been one of the most frequently used methods. Detailed analyses of the faunal distributions and dynamics within the cave microhabitats have still been sparse and usually focused on particular species. In order to study differences in faunal distributions and dynamics with respect to variation of the environmental circumstances in different microhabitats, we carried out a one-year monthly sampling using two parallel sets of pitfall traps, an upper and a lower one, in a small horizontal karstic cave. Environmental parameters were measured at the same time along the cave. Both the shallow (epikarst) and the deep subterranean species were recorded. We found three distinctive cave sections with respect to the upper and the lower faunal composition. In the entrance and the inner sections, the species richness was significantly higher on the floor, while in the middle section these two communities were comparably rich in species. As expected, the number of the non-troglobiotic species decreased, while the number of troglobiotic species increased from the entrance towards the inner sections. These different, simultaneous responses of the same fauna directly refer to different qualities of the environmental parameters on a small scale within a cave. The presented methodology thus helps to provide more complete information on the faunal distribution and dynamics within a cave. Due to conspicuous differences between the two pitfall trap sets we conclude that differences between microhabitats within caves have been generally underestimated in species richness research. This subject deserves much more attention in designing studies on the subterranean biology and ecology at least in the horizontal caves in the future.

Poster Session

Kruckenhauser, Luise
Luise.Kruckenhauser@nhm-wien.ac.at

The fauna of the Al Hoota Cave in Northern Oman.

Kruckenhauser, Luise*[1]; Reginald, Victor[2]; Sattmann, Helmut[3]

[1] Central Research Laboratories, Museum of Natural History, Burgring 7, 1010 Vienna, Austria
[2] Department of Biology, College of Science, Sultan Qaboos University, Oman
[3] Third Zoological Department, Museum of Natural History, Burgring 7, 1010 Vienna, Austria

The Al Hoota Cave System has a length of 5 km and several connected subterraneous lakes, it is situated in the in the Western Al Hajar Mountains of the Northern Oman. Due to its exceptional wealth of dripstones and numerous underground lakes, it was developed as a show cave from 2001 – 2006. The cave is of Plio-Pleistocene age, roughly one to three million years old.

The most sensational faunal element of the Al Hoota cave is a population of *Garra barreimiae* FOWLER & STEINITZ, 1956 which was discovered in 1980. *G. barreimiae* is a cyprinid fish endemic to the southeastern Arabian Peninsula. Individuals of the cave population exhibit a typical troglomorphic phenotype with no pigmentation and lack of externally visible eyes. Surface populations of this species live in the surrounding wadis. Genetic analyses showed that the cave population is genetically isolated from the surface, but there is also an indication for geneflow from the cave to the surface (Kruckenhauser et al. 2011). Some of the individuals we collected on the surface exhibit an intermediate phenotype, with eyes and pigmentation reduced at different stages (see also abstract by Kirchner et al.).

Other vertebrates which could be found in the Al Hoota cave system are the Egyptian fruit bat (*Rousettus aegyptiacus*), the Muscat mouse-tailed bat (*Rhinopoma muscatellum*), and a gecko species of the genus *Ptyodactylus* most probably *P. orlovi*.

Two species which has been described from specimens from the Al Hoota Cave are the Al Hoota Hunting Spider, *Spariolenus secundus* JÄGER, 2006 from the family Sparassidae and the gastropod *Gulella protruda* NEUBERT & FRANK, 1996 from the family of Streptaxidae.

Also several beetles can be found in the waterbodies of the cave, one species *Hydraena putearius* JÄCH & DIAS, 2000 has been described in the Oman.

Lavoie, Kathleen
lavoiekh@plattsburgh.edu

Long term population trends of biota in White Cave, Mammoth Cave National Park (2003-2016).

Gilmore, Terrence[1]; Lavoie, Kathleen[*1]; Helf, Kurt[2]; Poulson, Thomas[3]

[1]Biological Sciences, State University of New York College at Plattsburgh, Plattsburgh, New York 12901
[2]Cumberland-Piedmont Network, National Park Service, Mammoth Cave, Kentucky 42259
[3]318 Marlberry Circle, Jupiter, Florida, United States

White Cave is a small (135 m) remnant trunk passage that once connected to Historic Mammoth Cave. White Cave has the richest biota in MCNP. We have conducted a yearly visual census of the first 90 m of White Cave beginning in 2003. Our results show extreme fluctuations in populations probably related to surface weather. The dominant species in the cave, *Hadenoecus subterraneus* cave crickets, are known to be affected by weather as they must leave the cave to forage. Cave crickets are a keystone indicator species. Many other species depend on inputs of energy from cave crickets as guano, eggs, and carcasses. Cricket populations were high in 2003, reached exceptional lows from 2004-06, then fluctuated with another low in 2009, followed by a steady increase to a record high in 2015. Trogloxenic camel crickets (*Ceuthophilus stygius*) and salamanders (*Eurycea lucifuga*) were highest in the early 2000s and show a steady decline to the present. While always low, no bats have been censused in White Cave since WNS arrived in MCNP in 2014. The pattern in numbers of predatory *Meta* cave spiders closely follows the numbers of cave crickets. Reproduction by the cave crickets is shown by comparing the ratio of the two smallest size classes of crickets to the two largest size classes. Total numbers of larger crickets is greater closer to the entrance, while numbers of smaller crickets is fairly steady up to 80 m into the cave. Large crickets normally exceed small crickets by 3-10x more, but in 2004 and 05 small crickets were at normal levels, but the great reduction in larger crickets flipped the ratio. The extreme variations in our long term data show the importance of repeated observations over time to monitor the health of keystone species.

Poster Session

Lavoie, Kathleen
lavoiekh@plattsburgh.edu

Absence of antibiotic producing bacteria, but high levels of antibiotic resistance in bacteria from Great Onyx Cave, Mammoth Cave National Park, Kentucky.

Rumabika, Tania; Bawa, Anissa; Whitney, Aaryn; deOndarza, Jose; Lavoie, Kathleen*

Biological Sciences, State University of New York College at Plattsburgh, Plattsburgh, New York 12901

Great Onyx Cave in Mammoth Cave National Park is the only major cave on Flint Ridge that has yet to be connected to the 400 mile long Flint Ridge-Mammoth Cave System. It has a small natural entrance and a blockhouse with an airlock entrance for access of tourists when it was a commercial cave starting in 1916. Extending across the entire width of Flint Ridge, the middle parts of the cave extend under the sandstone caprock. This results in dryer conditions suitable for the deposition of gypsum, which range from scattered areas to complete crusts to gypsum flowers. Poulson noted the relative absence of organisms in the gypsum areas, especially the lack of *Hadenoecus subterraneus* cave crickets, describing it as "The Great Kentucky Desert." If the Great Kentucky Desert hypothesis is accurate, then the stress of gypsum might also apply to microorganisms. One hypothesis for when bacteria produce antimicrobials is that they do so when stressed or in competition with other microbes for scarce resources, as would be the case in a nutrient-limited cave environment. We sampled soils in Great Onyx Cave, in areas with differing amounts of gypsum, and isolated culturable bacteria. We used three methods to isolate antibiotic producing bacteria from the cave, including soil crumb, soil crumb with indicator bacteria, and cross streak plates with indicators. No antibiotic producers were detected. In contrast, we found high levels of antibiotic resistance from our pure cultures. Testing them against seven antibiotics using disk-diffusion, all 23 isolates were resistant to three or more antibiotics. Three were resistant to all seven antibiotics tested. There was no effect of distance into the cave, soil moisture, or the presence or amount of gypsum. We suggest that the Great Kentucky Desert hypothesis does not extend to culturable bacteria.

Lewis, Julian J.
lewisbioconsult@aol.com

Improving outcomes and modifying policies with evidence-based research on the karst of the Hoosier National Forest.

Lewis, Julian J.*; Lewis, Salisa L.

Lewis and Associates LLC, 17903 State Road 60, Borden, Indiana, United States

Both subterranean and surface environments of the karst of the Hoosier National Forest have been researched over the last twenty years for the purpose of enhancing conservation management practices. Cave bioinventory projects have demonstrated a diverse subterranean fauna inhabiting caves of the Hoosier National Forest, including numerous endemic, globally rare species. Beyond routine karst management, this knowledge of the karst fauna played a critical role in diverting a catastrophe: construction of Interstate 69 planned to traverse the Tincher Karst Special Area. Although easements for construction had already been obtained, when the Indiana Department of Transportation was made aware of the presence of the rare cave fauna inhabiting the area, the path of the highway was moved. Subsequently, in 2015 we turned our attention to evaluate sinkholes in the national forest. Assessments of sinkholes in 12 management tracts included the analysis of the physical environment (e.g., sink dimensions, geologic horizon, soil type), micro-climate, plant community and fauna. Preliminary results demonstrated that the micro-climate of the sinkhole floors (mean depth 2.0 meters) differed from that of the rim, e.g., with summer sampling temperatures depressed by a mean of 4.8 degrees C. and relative humidity elevated by a mean of 22.7%. Regional Forester Sensitive Species, like the pseudoscorpion *Kleptochthonius griseomanus* or millipede *Conotyla bollmani*, were found in 76% of sinkholes, Exotic species, like the terrestrial isopod *Trachelipus rathkei* or millipede *Oxidus gracilis*, were present in 72%. Species like *Kleptochthonius griseomanus* previously known only from caves were discovered inhabiting sinkhole floors. Populations of several species of spiders, like *Gladicosa pulchra* or *Phrurolithus singulus* and the millipede *Cleidogona unita*, were discovered that are currently known in Indiana only from sinkholes.

Symposium: Patterns of Subterranean Biogeography and Biodiversity

Lukić, Marko
marko.lukic@hbsd.hr

Disjunct distribution of terrestrial troglobiotic species in Europe: the case of Collembola.

Lukić, Marko*[1,2,3]; Delić, Teo[3]; Zagmajster, Maja[3]; Bedos, Anne[4]; Deharveng, Louis[4]

[1]Ruđer Bošković Institute, Zagreb, Croatia
[2]Croatian Biospeleological Society, Zagreb, Croatia
[3]Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
[4]Muséum National d'Histoire Naturelle, UMR7205 CNRS/MNHN, Paris, France

Most species rich cave-dwelling groups in Europe share a similar pattern of distribution. At continental scale, a ridge of highest subterranean biodiversity was recognized at mid-latitudes, where a few hotspots containing numerous troglomorphic species can be found. These subterranean species mostly have narrow distributional ranges, limited to individual karst areas, often known even from a single cave. Yet, there are few taxa where disjunct geographical distributions in remote karst areas were documented. While such patterns have been studied and evaluated in some stygobionts, there are very few studies focused on terrestrial taxa. The latter present a challenge in understanding such patterns, having limited dispersal abilities when compared to many aquatic counterparts. We present three cases of disjunct distribution ranges among subterranean collembolan genera which match also those of a few other terrestrial arthropods. Genera *Tritomurus* (Tomoceridae), *Ongulonychiurus* (Onychiuridae) and subgenus *Heteromurus* (*Verhoeffiella*) (Entomobryidae) have highly troglomorphic species recorded in Dinaric and northern Pyreneo-Iberian karst regions, within hotspots of the European subterranean biodiversity ridge, more than 1000 km apart. These cases of disjunct geographical distributions are defined mostly on the basis of comparative morphological descriptions of the genera and species. We discuss the pattern in the light of new molecular studies conducted on *Heteromurus* (*Verhoeffiella*) which revealed some indications of polyphyly. Special attention shall be given to the striking morphological convergences of species living in remote karst areas.

Lyon, Anastasia
anastasia.lyon@centre.edu

Two to three genes control scleral ossification in blind *Astyanax mexicanus* cavefish.

Lyon, Anastasia*[1]; Krutzler, Amanda J.[2]; Gross, Joshua B.[2]; O'Quin, Kelly E.[1]

[1]Department of Biology, Centre College, Danville, Kentucky, United States
[2]Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio, United States

Astyanax mexicanus are Characin fish with a cave fish morph and a surface fish morph that are able to successfully interbreed, which provides us with an evolutionary-genetic model of cave adaption. These two morphs have evolved numerous differences in eye size, pigmentation, and scleral ossification. The focus of this research is the sclera, the tough outer wall of the eye, and the patterns of scleral ossification in cavefish-surface fish hybrids. Our previous research determined that two to three genes likely influence the presence/absence of scleral ossicles. In this study, we expanded the sample size of our previous analysis in order to (1) confirm the two to three gene model and (2) detect quantitative trait loci (QTL) responsible for scleral ossification. In order to answer these two questions, we assessed photographs of surface fish and cavefish F2 hybrids for the presence/absence of zero (0), one (1), or two (2) scleral ossicles. We then analyzed their distribution among the F2 via a X^2 test and used a genetic linkage map of over 100 microsatellite markers to identify the location of QTL responsible for scleral ossification. We found that scleral ossification follows a recessive threshold model of inheritance because the majority of individuals sampled had one or more scleral ossicles. While the X^2 results were consistent with a two-locus model of threshold inheritance for the loss of scleral ossification, the QTL analysis identified three QTL on linkage groups 4 and 18. Scleral ossification remains a complex trait with limited knowledge behind its genetic characterization. This study provides insight into the number and location of genes controlling the formation of scleral ossicles in *Astyanax mexicanus*, which may be applied to human ocular diseases such as *phthisis bulbi*.

Ma, Li
mal2@ucmail.uc.edu

Molecular analysis of melanophore lineage genes in cavefish depigmentation.

Ma, Li*[1]; Stahl, Bethany[1,2]; Adams, Hannah[1]; Gross, Joshua[1]

[1]Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio, United States
[2]Department of Biological Sciences, Florida Atlantic University, Jupiter, Florida, United States

Depigmentation is one of the most common features evolving in cave animals. The melanin synthesis pathway has been well studied in vertebrates, providing a rich source of candidate molecules for analysis. For instance, melanin synthesis involves the transport of L-tyrosine substrate into the melanosome, the conversion of L-tyrosine to L-DOPA and L-DOPA to L-DOPAquinone by the rate-limiting enzyme tyrosinase (*Tyr*). This process leads to the production of black eumelanin pigment from L-DOPAquinone via a sequence of downstream reactions promoted by the melanogenic enzymes tyrosinase, tyrosinase-related protein 1 (*Tyrp1*), dopachrome tautomerase (*Dct* or *Tyrp2*), and others. To understand the molecular mechanisms underlying the evolution of depigmentation in *Astyanax* cavefish, we specifically evaluated expression of the *tyrosinase* gene family including *Tyr*, *Tyrp1a*, *Tyrp1b*, as well as the genes *Dct* and *Pmela* gene. We first evaluated expression level differences in mRNA level using RNA-seq analysis, and then performed whole-mount *in situ* hybridization on early stage embryos of surface fish and Pachón cavefish. Our results indicate that all genes are down-regulated in Pachón cavefish embryos which have fewer cells expressing these genes compared to surface fish. *In situ* analyses revealed distribution of pigmentation cells principally on the head and flank areas. Knockdown of these genes using morpholinos in zebrafish embryos resulted in variable melanosome defects. The differential expression levels we observed for *Dct, Tyr, Tyrp1a, Tyrp1b* and *Pmela* reveal that melanosomes are generated and activated during melanin synthesis, but do so at very different rates and levels between surface and cave forms. Altogether, our results suggest that multiple steps and genes in the melanin synthesis pathway have been targeted during the evolution of depigmentation in cavefish.

Symposium: Patterns of Subterranean Biogeography and Biodiversity
Keynote Address

Malard, Florian
florian.malard@univ-lyon1.fr

A macroecological take on European groundwater biodiversity patterns.

Eme, David[1,2]; Zagmajster, Maja[3]; Delić, Teo[3]; Douady, Christophe[1];
Fišer, Cene[3]; Flot, Jean-François[4]; Galassi, Diana M.P.[5]; Konecny-Dupré, Lara[1];
Marmonier, Pierre[1]; Stoch, Fabio[5]; Zakšek, Valerija[3], Malard, Florian*[1]

[1]UMR 5023 LEHNA, University of Lyon 1, CNRS, ENTPE, Villeurbanne, France
[2]Department of Life and Environmental Sciences, University of Iceland, Reykjavik, Iceland
[3]Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
[4]Evolutionary Biology & Ecology, Université Libre de Bruxelles, Brussels, Belgium
[5]Department of Life, Health & Environmental Sciences, University of L'Aquila, L'Aquila, Italy

Earlier coarse-grained studies of groundwater biodiversity patterns in Europe documented a northward monotonic decrease in species richness and a concomitant increase in the size of the geographic range of species. These patterns have long been mainly attributed to the disproportionate extinction of narrow-ranging species at higher latitudes during cold Pleistocene climate and to limited postglacial colonization in northern Europe. We revisited these patterns and their explanation using 21,700 point occurrence data for 1570 obligate groundwater species of crustaceans and a total 2350 COI sequences for 263 species of Aselloidea (Isopoda) and Niphargidae (Amphipoda). We challenged the view that Pleistocene climatic variation alone drives European groundwater biodiversity patterns by testing also for the roles of productive energy and spatial heterogeneity using morphologically distinguishable species or COI-based species as currencies for analysis. We found that species richness exhibited a hump-shaped latitudinal pattern whereas range size increased abruptly above a latitudinal threshold of 43° N. Species richness variation was best explained by productive energy and spatial heterogeneity whereas Pleistocene climatic variation was by far the best predictor of range size. Using COI-based species instead of morphologically distinguishable species as units of analysis strengthened our findings.

Martin, Lindsey
martinlm@terpmail.umd.edu

The relationship between differential anesthesia tolerance and melanin pigment development in cave-adapted and surface *Astyanax mexicanus.*

Martin, Lindsey*; Bilandžija, Helena; Soueidan, Sara; Jeffery, William

Department of Biology, University of Maryland, College Park, Maryland, United States

A recent hypothesis for the evolution of albinism in *Astyanax* cavefish has postulated the existence of a tradeoff between pigmentation and behavioral changes linked by catecholamine (CAT) levels. However, the previous research did not identify any behavioral differences between surface and cave-dwelling *Astyanax* that occur during the critical period of larval pigment development. During a recent screen for behavioral or physiological differences linked to pigment development, we noted a difference in anesthesia tolerance. Anesthesia tolerance was tested by exposing fry to ice water or the chemical anesthetic tricaine methanesulfonate (MS-222) and calculating the average time to unconsciousness, based on motion detection. The results showed that albino cavefish larvae treated with anesthesia remained conscious significantly longer than pigmented surface fish larvae. We discovered that the difference in anesthesia tolerance begins between 3 and 4 days post fertilization, during the height of pigmentation in surface fish. Further testing showed that differences in anesthesia tolerance also persisted into adulthood: adult albino cavefish treated with anesthesia remained conscious significantly longer than pigmented adult surface fish. The results suggest a negative correlation between anesthesia tolerance and melanin pigmentation in *Astyanax*, which is also known to occur in humans. Additional tests on anesthesia tolerance in conjunction with pharmacological treatments will be done in *Astyanax* to determine whether anesthesia tolerance is based on changes in CAT levels in developing neural systems. Because cave-adapted and surface populations of *Astyanax mexicanus* are thus far the only natural model system for studying anesthesia tolerance this research could help elucidate the general mechanism of anesthetic action.

Poster Session

Mojaddidi, Hafasa
hafasa.mojaddidi@students.dominican.edu

Developmental and morphological comparisons in surface and cave embryos of the crustacean *Asellus aquaticus.*

Mojaddidi, Hafasa*; Protas, Meredith

Department of Natural Sciences and Mathematics, Dominican University of California, San Rafael, California, United States

Cave-dwelling animals have long served as excellent model systems to understand pressing questions in evolutionary biology. Species adapted to subterranean environments often share phenotypic characteristics such as the reduction or complete absence of eyes, lessened pigmentation and enhanced sensory systems. In order to understand the evolution of these morphological changes, this study is using an invertebrate model system, the freshwater isopod crustacean, *Asellus aquaticus* to examine the crucial timeframe in embryonic development in which morphological differences occur. Specifically, our goal is to discover whether phenotypic differences between adult cave and surface dwelling individuals including pigmentation, ocular structures, and antenna length, are established in embryogenesis. We recorded when pigmentation and ocular structures first appear in surface embryos and found that cave embryos never show pigmentation or any external ocular structures. In addition, we are measuring appendage length at hatching and the survival rate post hatching without nutrition in both populations. These experiments allow for a better understanding of the evolution of cave characteristics and create a resource for further embryonic work and analysis in the emerging model system, *Asellus aquaticus.*

Special Session: Subterranean Microbial Systems

Moldovan, Oana Teodora
oanamol35@gmail.com

Microbiological monitoring in Romanian show caves.

Bercea, Silviu[1]; Nastase-Bucur, Ruxandra[1]; Kenesz, Marius[1];
Constantin, Silviu[2]; Moldovan, Oana Teodora*[1]

[1] Department of Cluj, Emil Racovitza Institute of Speleology, Cluj-Napoca, Romania
[2] Department of Geospeology and Palentology, Emil Racovitza Institute of Speleology, Bucuresti, Romania

Microbiological monitoring is not a common tool used in show cave management although it is a good indicator for the state of human impact. Microbiological monitoring test kits (RIDA Count, R-Biopharm AG, Germany) were used as a handy tool to indicate the bioaerosol concentration in different parts of the four cave systems. Water and swab samples were also simultaneously collected to observe the direct impact of fluctuating human presence over speleothems and shallow water pools. The test plates revealed the total bacterial count, yeasts and molds count, and clinically important human-associated groups, like *Enterobacteriaceae* and coliform bacteria, with specific indicators for *Escherichia coli*. Atmospheric parameters were registered, including temperature, airflow, CO_2 values, particle count, and relative humidity. The tourist traffic flow was also monitored and indicated the same pattern in all five caves: a constant ascent of the number of visitors from March to August, followed by a steep decline. The overall number of microbial colonies was higher during the active tourist season. Higher numbers of yeasts and molds tended to be from nearer to the cave entrances, except for one of the caves, which had intense bat activity and very large openings. Samples showed human impact well outside the visited areas of the caves. Human pathogens were present on monitored surfaces and in the cave water, creating a need to warn visitors not to touch speleothems or drink from any source of water related to the cave, as they represent a potential biohazard. 16S rRNA gene sequencing is being performed on samples for taxa identification.

Symposium: Conservation of Subterranean Fauna and Habitats

Moore, Patrick Ryan
patrickmoore555@gmail.com

Home range and habitat use of foraging *Myotis grisescens* from five maternity sites in northern Arkansas using aerial tracking.

Moore, Patrick Ryan*; Morris, Keith; Rolland, Virginie; Risch, Thomas Stephen

Department of Biological Sciences, Arkansas State University, Jonesboro, Arkansas 72467, United States

Gray bats (*Myotis grisescens*) were listed as endangered in 1976 because of declining populations resulting from cave disturbance. The Gray Bat Recovery Plan recommends further study on foraging habits and home range. Yet, little data exist partly because gray bats have large home ranges, making ground-based tracking methods problematic. Accordingly, our objective was to assess gray bats' foraging habits using aerial tracking. In 2014-2015, five maternity sites in Arkansas were harp-trapped, and 112 adult reproductive female gray bats were radio-tracked from a Cessna 182 Skylane, gathering 1,293 time-independent locations from June 15-July 15. Fixed-kernel density with least square cross validation was used to determine home range (95% of locations) and core-foraging area (50% of locations) of a sub-sample of 42 individuals with ≥15 independent locations. Minimum Convex Polygon (MCP) was also used for comparative purposes with past published studies. In 2014, mean 95% home range was 362.2 km² (SE= 24.9 km²), and 50% core-foraging was 83.2 km² (SE= 25.2 km²). In 2015, mean 95% home range was 57.9 km² (SE=8.6 km²), and 50% core-foraging was 13.0 km² (SE= 2.2 km²). Each individual used 1-4 core-foraging areas. A compositional analysis revealed that female gray bats traveled and foraged over water (creek, river, or pond) more than expected based on percent availability across the landscape when compared to other habitat types. With such large home ranges, management strategies for gray bats should go beyond protecting roost sites to include waterways and riparian areas for travel and foraging on sensitive aquatic insect species.

Nair, Parvathi
p_n22@txstate.edu

Estimating the trophic ecology of aquatic invertebrate using stable isotopes.

Nair, Parvathi*[1]; Nowlin, Weston[1]; Diaz, Pete[2]

[1]Department of Biology, Texas State University, San Marcos, Texas, United States
[2]US Fish and Wildlife Service, San Marcos, Texas, United States

Spring- and groundwater-associated organisms often exhibit restricted geographic ranges, high levels of endemism, and sensitivity to changes in habitat and water quality. In addition, it is thought that many subterranean habitats are resource poor, leading to simple food webs populated by generalist species exhibiting little functional diversity. However, some spring- and groundwater-associated organisms exist in transition zones between subterranean and surface habitats and frequently co-occur with surface-species, potentially competing for resources. We examined patterns in resource and habitat use in the invertebrate community found in the Comal Springs ecosystem in central Texas, USA. We assessed the resource use of several spring/subterranean species which are thought to occupy the transition zone between subsurface and surface habitats (i.e., *Stygobromus pecki*, *Stygoparnus comalensis*, and *Heterelmis comalensis*) using stable isotopes of carbon (^{13}C) and nitrogen (^{15}N). We also assessed the food resource use of several co-occurring surface species that occupy these transition zones. Isotopic mixing model results indicated that there was a substantial amount of niche-partitioning between spring/subterranean-associated and surface-species in Comal system. Both *H. comalensis* and *S. comalensis* derived >70% of their diet from terrestrial-derived organic matter (OM) whereas closely-related surface invertebrate consumers largely derived most of their diets from algal-based resources. Isotope data also indicated that *S. pecki* was the apex predator in the Comal invertebrate food web, likely consuming both surface and subsurface prey. Data also showed that *S. pecki* switched to an alternate basal resource food chain depending upon spatial habitat heterogeneity. However, *H. comalensis* and *S. comalensis* dependence on terrestrial OM did not vary across the system, indicating that their populations may be affected by changes to aquifer levels, spring flows, and removal of riparian vegetation that could lead to decreased inputs of terrestrial OM.

Symposium: Conservation of Subterranean Fauna and Habitats

Niemiller, Matthew L.
cavemander17@gmail.com

Conservation of amblyopsid cavefishes (Percopsiformes: Amblyopsidae) of the Ozark Highlands and Interior Low Plateau karst regions, USA.

Niemiller, Matthew L.

Illinois Natural History Survey, Prairie Research Institute, University of Illinois Urbana-Champaign, Champaign, Illinois, United States

Amblyopsid cavefishes are a small family of freshwater fishes that includes six stygobiotic species that occur in the Ozark Highlands (two species) and Interior Low Plateau (four species) karst regions of the eastern United States. All species are of conservation concern; however, range size, abundance, and potential impacts of threats vary greatly among these cavefishes. Four species are included on the IUCN Red List, and two species, *Troglichthys rosae* and *Speoplatyrhinus poulsoni*, are federally threatened and endangered under the U.S. Endangered Species Act, respectively. Here I review the conservation status and major threats facing each stygobiotic amblyopsid, including two species that have recently been recognized or described: *Typhlichthys eigenmanni* and *Amblyopsis hoosieri*. I also discuss conservation measures that have been proposed or implemented and offer recommendations for conservation and management of these unique but imperiled cavefishes.

Niemiller, Matthew L.
cavemander17@gmail.com

Predicting the occurrence of cave-inhabiting fauna based on features of the surface environment.

Culver, David C.[1]; Christman, Mary C.[2]; Doctor, Daniel H.[3]; Niemiller, Matthew L.*[4]; Weary, David J.[3]; Young, John A.[5]; Zigler, Kirk S.[6]

[1]Department of Environmental Science, American University, Washington, District of Columbia, United States
[2]Departments of Biology and Department of Statistics, University of Florida, Gainesville, Florida, and MCC Statistical Consulting LLC, Gainesville, Florida, United States
[3]U.S. Geological Survey, Reston, Virginia, United States
[4]Illinois Natural History Survey, Prairie Research Institute, University of Illinois Urbana-Champaign, Champaign, Illinois, United States
[5]U.S. Geological Survey, Leetown Science Center, Kearneysville, West Virginia, United States
[6]Department of Biology, The University of the South, Sewanee, Tennessee, United States

One of the most difficult fauna to study *in situ* is the obligate cave fauna because of the difficulty of sampling. Cave-limited species display patchy and restricted distributions, but it is often unclear whether the observed distribution is a sampling artifact or a true restriction in range. Further, the drivers of the distribution could be local environmental conditions, such as cave humidity, or they could be associated with surface features that are surrogates for cave conditions. If surface features can be used to predict the distribution of important cave taxa, then conservation management is more easily obtained. We examined the hypothesis that the presence of major faunal groups of cave obligate species could be predicted based on surface features. Georeferenced records of cave obligate amphipods, crayfish, fish, isopods, beetles, millipedes, pseudoscorpions, spiders, and springtails within the area of Appalachian Landscape Conservation Cooperative in the eastern United States (Illinois to Virginia and New York to Alabama) were assigned to 20 x 20 km grid cells. Habitat suitability for these faunal groups was modeled using logistic regression with twenty predictor variables within each grid cell, such as percent karst, soil features, temperature, precipitation, and elevation. Models successfully predicted the presence of a group greater than 65% of the time (mean=88%) for the presence of single grid cell endemics, and for all faunal groups except pseudoscorpions. The most common predictor variables were latitude, percent karst, and the standard deviation of the Topographic Position Index (TPI), a measure of landscape rugosity within each grid cell. The overall success of these models points to a number of important connections between the surface and cave environments, and some of these, especially soil features and topographic variability, suggest new research directions. These models should prove to be useful tools in predicting the presence of species in understudied areas.

Nowlin, Weston H.
wn11@txstate.edu

Ecological and consumer-driven nutrient recycling in a subterranean aquatic community.

Nowlin, Weston H.*[1]; Loney, Lauren[1]; Hutchins, Benjamin[2]; Schwartz, Benjamin F.[3]

[1]Department of Biology, Aquatic Station, Texas State University, San Marcos, Texas, United States
[2]Texas Parks and Wildlife Department, Austin, Texas, United States
[3]Edwards Aquifer Research and Data Center/Department of Biology, Texas State University, San Marcos,Texas, United States

In all ecosystems, and aquatic ecosystems in particular, metazoans have direct and indirect impacts on the nutrient cycling. The assimilation and recycling of nutrients by animals can have wide-ranging effects on ecosystem processes, affecting nutrient availability, rates of primary production, and species composition. Although numerous studies have examined the role of consumer-driven nutrient cycling have been conducted in lake, ocean, stream, and riverine ecosystems, relatively little is known about the ecology and nutrient cycling dynamics of subterranean ecosystems. In addition, it is thought that obligate subterranean organisms should exhibit a suite of adaptations and life-history traits consistent with living under low food (energy) availability, such as relatively low metabolic rates, slow growth rates, and increased lifespan when comparted to their surface (i.e., epigean) counterparts. We examined nitrogen (N) and phosphorus (P) excretion rates and body stoichiometry [carbon (C), N, and P content] of stygobiont invertebrates from a site located within the Edwards Aquifer in central Texas, USA. Over a one-year period, we collected approximately 30 stygobiont taxa, making the site one of the most speciose stygobiont locations in the world. Nutrient excretion trials were conducted on live animals in the field and we found that stygobiont mass-specific N and P excretion rates (ug nutrient/mg DM/h) declined with body size (mg DM). We also compared excretion rates of three common stygobiont species to analogous surface –dwelling taxa and found that when we accounted for differences in body size, excretion rates were not substantially different from those of surface taxa. We additionally found that there was significant variation in N and P content among stygobiont species, but that stygobiont species did not systematically differ from analogous surface species in their N and P content.

Oliveira, Marcus Paulo Alves
marcuspbr@gmail.com

Are cave sampling methods effective to assess subterranean fauna richness?

Oliveira, Marcus Paulo Alves*; Ferreira, Rodrigo Lopes

Department of Biology, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil

Species richness is the most common environmental variable to describe the diversity and is the basis of many ecological models of community structure. However, the richness recorded in a given location may vary depending on the collection methods used. Considering the diversity present in the caves and the lack of information about the techniques used to access it, the present study compared the efficiency of collection methods most commonly used in caves in sample the richness of these environments. For this study, we chose caves in three distinct lithologies: iron ore, limestone and quartzite. Each cave was divided into sampling quadrants, where three manual collection methods were evaluated (method of plotting specimens, time/area collection method and manual collection by exhaustion). Regarding the passive capture methods, Winkler extractors and pitfall traps were used when possible. In addition, we evaluated the composition of species differs between the sampling techniques and the efficiency of Randomization quadrant sampling method. The method of plotting specimens showed a higher number of species and was more efficient for ferruginous and quartzite caves. For the limestone caves, the time/area collection method showed to be more efficient. The composition of species found in active and passive capture methods differed in all sampled caves. Randomization quadrant sampling showed low efficiency when using conventional methods of manual collection. According to the results, we suggest using the specimen plotting method in studies designed to assess terrestrial fauna richness in cave environments. The standardization of sampling in future groundwater fauna studies are extremely important to generate genuinely comparable data, allowing to assess general patterns of subterranean community structuring.

Oliveira, Marcus Paulo Alves
marcuspbr@gmail.com

Choosing the richness estimator that best fit your sampling method.

Oliveira, Marcus Paulo Alves*[1]; Oliveira, Thami Gomes[2]; Ferreira, Rodrigo Lopes[1]

[1]Department of Biology, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil
[2]BioEspeleo Consultoria Ambiental, Lavras, Minas Gerais, Brazil

Estimators of species richness are alternatives to characterize the local biodiversity, facing the limitations of complete fauna inventories. An useful estimator should attend the following cirteria: 1) Presents estimated median richness as closer as possible of local real richness; 2) Show lower variation among estimated values for a same method; 3) Should not produce estimates that widely differ from others estimators. Here we evaluated the results of seven richness estimators (ACE, ICE, Chao 1, Chao 2, Jacknife 1, Jacknife 2, Boostrap and Michaelis-Menten) for sampling methods commonly used in cave inventories. For this study, we chose nine caves which was divided into sampling quadrants, where three manual collection methods were employed (method of plotting specimens, time/area collection method and manual collection by exhaustion). Once the number of species collected in the manual collection by exhaustion can be considered the fauna limit that can be assessed locally, it was chosen as control in the evaluation of richness estimators. We calculated the median value of each estimator considering all caves studied. When the value is closer to 1, the better the estimator. We used Kruskal-Wallis test to evaluated if there were differences between the estimators and Tukey test for pairs of estimators when some alteration was observed. The ICE richness estimator was the most appropriated for the lowest categories of time in the time/area sampling method. Jackknife 2 was the best in estimating species richness for highest time periods in the time/area sampling method and for the method of plotting specimens. Bootstrap was inefficient to estimate cave fauna richness and underestimate the number of species in all caves. Our results show that, for best results in estimating cave fauna, it is important consider the sampling method applied when will choose the richness estimator.

Symposium: Cave Animals at the Frontiers of Modern Biology

O'Quin, Kelly E.
kelly.oquin@centre.edu

The evolution of scleral ossification in the Mexican Cavefish (*Astyanax mexicanus*).

O'Quin, Kelly E.*[1]; Doshi, Pooja[2]; Lyon, Anastasia[1]; Hoenemeyer, Emma[1];
Yoshizawa, Masato[3]; Jeffery, William R.[2]

[1]Biology Program, Centre College, Danville, Kentucky, United States
[2]Department of Biology, University of Maryland, College Park, Maryland, United States
[3]Department of Biology, University of Hawaii at Manoa, Honolulu, Hawaii, United States

The sclera is the tough outer covering of the eye. In some fishes, reptiles, and birds, the sclera is reinforced with a ring of hyaline cartilage or bone that forms from scleral ossicles. Currently, the evolutionary and genetic basis of scleral ossification is poorly understood, especially in teleost fishes. We assessed scleral ossification among the eyed (surface) and eyeless (cave) morphs of the Mexican tetra (*Astyanax mexicanus*). Although surface fish have bony sclera similar to other teleosts, the ossicles of cavefish generally do not form. We assessed scleral ossification in cavefish from multiple populations and used ancestral character state reconstructions to determine how many times scleral ossification has been lost. We then confirmed this result by assessing complementation of scleral ossification among the F1 hybrid progeny of two cavefish populations. Finally, we quantified the number of scleral ossicles present among surface x cave F2 hybrids and used this information to identify quantitative trait loci (QTL) responsible for scleral ossification. We found that the loss of scleral ossification is common – but not ubiquitous – among *Astyanax* cavefish, and that this trait has been convergently lost at least three times, which is confirmed by the presence of wild-type, ossified sclera among the progeny of our complementation cross. We observed a strongly skewed distribution of scleral ossicles among the surface x cave F2, suggesting that scleral ossification is a threshold trait with a complex genetic basis. Quantitative genetic mapping identified a single QTL for scleral ossification on *Astyanax* linkage group 1. We estimate that the threshold for this trait is likely determined by at least three genetic factors which may control the severity and onset of lens degeneration in cavefish. We conclude that complex evolutionary and genetic patterns underlie the loss of scleral ossification in *Astyanax*.

Orndorff, William David
Wil.Orndorff@dcr.virginia.gov

Conserving cave invertebrate fauna in Virginia (USA).

Orndorff, William David

Virginia Department of Conservation and Recreation, Natural Heritage Program, Richmond, Virginia, United States

Conservation of cave-limited invertebrate fauna is a complex and difficult task for which a coherent methodology is sorely needed, as illustrated in the state of Virginia (USA). As of 2013, 168 described, cave limited species were documented, with a few dozen additional, undescribed species. Of these, only the Lee County cave isopod (*Lirceus usdagalun*) and the Madison Cave isopod (*Antrolana lira)* are protected under the US Endangered Species Act, which both 1) prohibits "take" of individuals (including habitat disruption) and 2) provides a gateway to funding, including land protection. Funding for conservation of these two species exceeds that expended on Virginia's other cave-limited species combined, supporting ecological, genetic, and inventory investigations for both species and extensive land conservation in the Cedars Natural Area Preserve for *L. usdagalun*. Most of Virginia's other cave limited species are highly endemic, with most classified as imperiled (G2) or critically imperiled (G1) under Natureserve's Global Conservation Status Rank system. Few are protected through land conservation or by state endangered species laws. Two additional state Natural Area Preserves and several private conservancies protect all or portions of cave systems rich in cave-limited invertebrates, as a result of the fortunate coincidence of sporadic state funding, generous benefactors, and willing sellers. Many karst systems with rich invertebrate fauna remain unprotected. Prioritization of conservation efforts for cave-limited invertebrates, by karst system, should be based on 1) diversity of cave-limited species rather than rarity or legal status and 2) environmental integrity of the system. Conservation of high-ranking systems should be pursued, with a goal of long-term protection of their watersheds. Under private land ownership, this approach requires establishing and maintaining relationships with landowners and managers to facilitate responsible stewardship and establishment of permanent land protection measures through easement or acquisition.

Parimuchová, Andrea
andrea.parimuchova@gmail.com

Contrast between phenotypic and genetic variability in *Protaphorura janosik* (Hexapoda, Collembola) from the Western Carpathians.

Parimuchová, Andrea*[1]; Žurovcová, Martina[2]; Miklisová, Dana[3];
Paučulová, Lenka[1]; Kováč, Ľubomír[1]

[1]Department of Zoology, Faculty of Science, Institute of Biology and Ecology, P. J. Šafárik University, Moyzesova 11, 040 01, Košice, Slovakia
[2]Institute of Entomology, Czech Academy of Sciences and Faculty of Biological Sciences, University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic
[3]Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, 040 01, Košice, Slovakia

The cave-adapted collembolan *Protaphorura janosik* Weiner, 1990 is among the most widespread and abundant troglobiotic forms in the Western Carpathians, Central Europe. Owing to limited dispersal ability in subterranean environment, its populations are effectively isolated in different karst areas.

We investigated both morphological and genetic variability of five populations from the central Western Carpathians. Totally, 40 individuals were sampled per population, 20 for morphological and 20 for molecular study. Analysis of 10 measurable or countable morphological traits by Non-metric multidimensional scaling (NMS) showed slight separation of neighboring localities, however, clear geographic pattern was not evident among distant populations. In the contrary, genetic analysis, based on sequences of the part of mitochondrial COI gene, showed different pattern. Although only eight haplotypes out of 88 sequences were detected, their distribution among populations pointed toward their high genetic differentiation. Only one haplotype was shared by two populations (Nová Kresanica and Mylna caves), while all the others were unique to different populations. Phylogenetic tree, Principal Coordinates Analysis (PCoA) as well as results of Mantel test showed a significant correlation of the geographic and genetic distances, suggesting thus an ongoing process of isolation by distance. However, the existence of cryptic species is not confirmed so far.

Paterson, Audrey Tarlton
apaters1@utk.edu

Niche bacterial and archaeal community compositions as indicators of ecosystem processes and health in Bahamian and Mexican anchialine caves.

Paterson, Audrey T.*[1]; Iliffe, Thomas M.[2]; Bracken-Grissom, Heather[3]; Pérez-Moreno, Jorge L.[3]; Porter, Megan[4]; Gonzalez, Brett C.[4]; Engel, Annette Summers[1]

[1]Department of Earth and Planetary Sciences, University of Tennessee, Knoxville, Tennessee, United States
[2]Department of Marine Biology, Texas A&M University at Galveston, Galveston, Texas, United States
[3]Department of Biological Sciences, Florida International University-Biscayne Bay, North Miami, Florida, United States
[4]Marine Biology Section, University of Copenhagen, Copenhagen, Denmark

Karstic anchialine systems require conservation attention, as diverse, often endemic, macrofaunal species assemblages have been described. Improvements to conservation efforts can be done with the inclusions of microbial and macrofaunal community identification and characterization of ecological drivers impacting community structure and function. For instance, although chemolithoautotrophic microbial taxa are known to be responsible for nutrient availability in many anchialine systems, overall community assemblages and structures are relatively understudied, particularly with respect to niche partitioning across the marked morphological, hydrological, and geochemical gradients. Here, we describe the bacterial and archaeal mat communities from vents on the floor of Cenote Crustacea, cave walls of Cenote Bacteria in the Yucatan Peninsula, Mexico, and the cave wall below the halocline in Magical Blue Hole, Abaco, The Bahamas. When compared to previously studied sulfidic subsurface systems, these new anchialine communities showed similarity to other cenotes and deep sulfidic karst aquifers. Magical Blue Hole consisted predominantly of Chlorobi (45-56% relative abundances), Chloroflexi (13-14%), and Deltaproteobacteria (~8%), all of which are taxa associated with sulfur metabolism. Proteobacteria that are putative sulfur-oxidizers (e.g., Gammaproteobacteria and Epsilonproteobacteria) were less abundant in Magical Blue Hole but were present in Cenote Crustacea and Cenote Bacteria. These data indicate niche specificity for some microbial groups and demonstrate the need for systematic characterization of anchialine cave systems, including zonation and environmental gradients that may be linked to system health. These results can be used for predictive ecosystem modeling of macrofaunal and microbial communities, which can improve conservation efforts of these ecosystems through time.

Poster Session

Pavlek, Martina
mpavlek@irb.hr

Flying deeper underground? A new troglobiotic chironomid (Diptera) from the Lukina jama – Trojama cave system in Croatia.

Andersen, Trond[1]; Baranov, Viktor [2]; Hagenlund, Linn Katrine[1]; Ivković, Marija[3]; Kvifte, Gunnar Mikalsen[1,4]; Pavlek, Martina*[5]

[1]Department of Natural History, University Museum of Bergen, University of Bergen, Bergen, Norway
[2]Institute for Freshwater Ecology and Inland Fisheries, Berlin, Germany
[3]Department of Zoology, Division of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia
[4]Department of Zoology, Institute of Biology, University of Kassel, Kassel, Germany
[5]Department of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia, and Croatian Biospeleological Society, Zagreb, Croatia

The Lukina jama – Trojama cave system, situated in the Northern Velebit National Park in Croatia, is the 14th deepest cave in the world and the deepest of the Dinaric Karst. Speleological research of this cave system started in 1992. Since then several expeditions were undertaken and biospeleological surveys resulted in altogether 54 animal taxa, 32 of which are true cave dwellers. Maybe the most extraordinary among them is the discovery of several females of a pale Chironomidae belonging to the subfamily Orthocladiinae collected in a chamber at -980 m. Next to the typical troglomorphic features like pale color, very long legs and strongly reduced eyes, in contrast to all other cave Diptera, it has retained large wings and appears to be capable of flying. All these features potentially make it the first flying troglobiont worldwide. Morphologically it doesn't fit within any described genus and shares some characteristics with the genera of "Metriocnemini" and "Orthocladiini" tribes. Molecular phylogenetic analysis also confirms its unique position and groups it with the genera *Tvetenia*, *Cardiocladius* and *Eukiefferiella* in the "tribe Metriocnemini". So far only females were collected making this species potentially parthenogenetic, which is not unusual among chironomids and is most often found in extreme or isolated habitats. This significant discovery confirms the position of the Dinarids as a highly important hotspot of subterranean biodiversity in general.

Perina, Giulia
g.perina@ecu.edu.au

Remarkable biodiversity of a neglected group of stygofauna: Bathynellidae (Bathynellacea, Crustacea) in the north of Western Australia.

Perina, Giulia*[1,2]; Huey, Joel[2]; Camacho, Ana[3]; Horwitz, Pierre[1]; Koenders, Annette[1]

[1]Centre for Ecosystem Management, Edith Cowan University, Joondalup, Western Australia, Australia
[2]Western Australian Museum, Welshpool, Western Australia, Australia
[3]Museo Nacional de Ciencias Naturales (CSIC), Dpto. Biodiversidad y Biología Evolutiva, Madrid, Spain

In the past decades the number of subterranean taxa discovered in Australia increased consistently thanks to environmental and biological studies conducted, often associated with mining surveys. Subterranean fauna have received increasing attention especially in Western Australia, where the government has legislative responsibility to protect the environment. The north of Western Australia, and especially the Pilbara region, have become a hot spot for underground fauna. Different studies on troglo-stygofauna revealed interesting patterns of distribution that reflect the climate and hydro-geological history of the craton.

Bathynellidae occur in most of the Australian aquifers, but very little is known about them since they have conservative morphology and fragile exoskeleton, which makes their manipulation and study very difficult. Therefore the aid of molecular techniques is used to support and define species-genera boundaries and their phylogenetic relationships.

I will present results on Bathynellidae in the north of Western Australia. Morphological and molecular data show a substantial variability, new genera and species in the family are discovered, and fascinating relationships are unfolded. A better understanding of the biodiversity and distribution of Bathynellidae will improve the knowledge of the delicate subterranean ecosystem, and consequently the management and monitoring of the underground resources.

Perroni, Gustavo Pisa
gustavo.perroni@carste.com.br

First note about Schizomida in Minas Gerais.

Perroni, Gustavo Pisa*; Soares, Gustavo Araujo

CARSTE Ciência e Meio Ambiente, Belo Horizonte, Minas Gerais, Brazil.

The order Schizomida (Petrunkevic, 1945) is composed by small arachnids, whose major morphological features are robust pedipalps, a pair of chelicera with two segments, four pairs of legs - being the first one elongated and whip-like – and an elongated abdomen with a short terminal flagellum. The group consists of 274 species, distributed in 46 genera and two families: Protoschizomidae and Hubbardiidae. Its distribution is limited to most of the tropical and subtropical regions of the world, and occurrence associated with burlap, soil and, for some species, subterranean habitats. In Brazil, Schizomida is a faunal group relatively unknown. Most species found in the country were described in the last 10 years as a result of terrestrial arthropods inventories in places previously poorly sampled, such as caves and other subterranean habitats. Currently, 13 Schizomida species have been already described in Brazil, distributed in the states of Bahia, Ceará, Paraíba, Pernambuco, Rio Grande do Norte, Rio de Janeiro, São Paulo, Tocantins and in the Amazon basin. In a recent speleological study, during exploratory activities in the cave called Gruta da Gameleira - urban area of the city of Vazante, Minas Gerais, Brazil – 22 schizomids were observed, being three of them found in the twilight zone and the other 19 individuals found in the dark zone. Although this finding suggests presence of local Schizomida population, further studies are still needed, since collection of observed individuals was not done due to lack of sampling permit. Complementary studies may elucidate whether these individuals consist of a new species or belong to described ones. Thereby, it is reported the first evidence of Schizomida for the State of Minas Gerais and it is recommended further explorations in the cave in order to perform collections of specimens for identification.

Phillips, John G.
John-phillips@utulsa.edu

Uncovering divergent lineages and phylogeographic structure in an obligate cave-dwelling Salamander (*Eurycea spelaea*).

Phillips, John G.*[1]; Fenolio, Dante B.[2]; Emel, Sarah L.[1,3]; Bonnett, Ronald M.[1]

[1]Department of Biological Sciences, University of Tulsa, 800 South Tucker Drive, Tulsa, Oklahoma 74104, United States
[2]San Antonio Zoo, San Antonio, Texas 78212, United States
[3]Department of Biology, Temple University, Philadelphia, Pennsylvania 19122, United States

The biology of many cave-dwelling organisms (troglobites) is poorly known due to their cryptic lifestyles and habitat inaccessibility. Some widespread troglobites whose molecular phylogenies have been examined display geographic genetic structure due to low dispersal rates and their highly fragmented habitat. However, an insufficient amount of phylogeographic studies of troglobites exist. This is particularly important given the potential for unrecognized cryptic species within troglobitic taxa, which are commonly imperiled by anthropogenic hazards such as land development, water pollution, and climate change. The Grotto Salamander (*Eurycea spelaea*) is endemic to the Ozark Plateau and its adults are confined to life in caves. There is only one currently recognized species of Grotto Salamander, but recent work has revealed high levels of genetic diversity, indicating the presence of cryptic lineages (three highly divergent lineages across the Ozarks). To further investigate the fine scale genetic diversity and geographic structure within *E. spelaea*, we have employed next-generation sequencing techniques and compared the observed patterns to mitochondrial divergence. Using a sequence capture method, we have successfully collected data for over 500 nuclear loci across *E. spelaea*, which have been used to construct a phylogenetic hypothesis for the *E. spelaea* species complex. We have used the phylogeny to identify evolutionary significant lineages within Grotto Salamanders.

Polak, Slavko
slavko.polak@notranjski-muzej.si

Phylogeny and systematic of the enigmatic Anthroherponina (Leptodirini, Cholevinae, Leiodidae, Coleoptera).

Polak, Slavko*[1]; Delić, Teo[2]; Trontelj, Peter[2]

[1]Notranjska museum Postojna, Institute Znanje Postojna, Kolodvorska cesta 3, SI-6230 Postojna, Slovenia
[2]Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

A significant part of subterranean coleopteran biodiversity can be attributed to a single large Coleopteran tribe, the Leptodirini. Evolutionary radiation of the group resulted in a wide range of morphologies and ecological specializations. The most renowned of the Leptodrini subtribes, the Anthroherponina, consists of morphologically highly evolved and ecologically specialized genera geographically limited to the Dinaric Karst. The group comprises the genera *Anthroherpon*, *Leptomeson*, *Parantrophilon*, *Hadesia*, *Remyella*, *Croatodirus*, *Velebitodromus*, *Nauticiella* and *Kircheria*, all sharing morphological traits like the highly troglomorphic leptodiroid or scaphoid body shape, the point of insertion of antennae, and spoon-shaped claws. Despite numerous similarities that have been traditionally interpreted as synapomorphies, the affinities of some of the genera have been questioned. Several characters are associated with the specialized ecology these genera, making them prone to convergence and parallelism, and the monophyly of the whole subtribe questionable. To test the monophyly of the Anthroherponina, we inferred multilocus molecular phylogenies based on three mitochondrial and three nuclear gene sequences for most of the presently known genera. Within a wide array of potential in- and outgroup taxa, the genera currently assigned to the Anthroherponina clearly emerged as polyphyletic. This finding once again demonstrates the prevalence of morphological convergence in subterranean organisms not in the form of general troglomorphies, but as a consequence of adaptation to finer-scaled ecological niches within the subterranean realm.

Porter, Megan Linnay
mlporter@hawaii.edu

Cuticular hydrocarbon analysis of cave versus surface Hawaiian planthoppers.

Porter, Megan Linnay*[1]; Yew, Joanne [2]

[1]Department of Biology, University of Hawai'i at Mānoa, Honolulu, Hawaii, United States
[2]Pacific Bioscience Research Center, University of Hawai'i at Mānoa, Honolulu, Hawaii, United States

Improved chemosensory capacities are commonly cited among the suite of characters associated with troglomorphy. One potential correlate with improved chemosensory capacity is an enhanced mode of chemical communication among individuals. Particularly important in subterranean habitats, pheromones play crucial roles in mate discrimination and stimulation. In many insects, cuticular hydrocarbons act as pheromones involved in courtship behavior. However, few studies have investigated the chemical diversity of cuticular compounds in cave-adapted species, or their behavioral contexts. In this study we investigate the potential chemical signals present in the cave-adapted planthopper *Oliarus polyphemus* (Cixiidae). We characterize the cuticular hydrocarbon diversity in *O. polyphemus* using gas chromatography mass spectrometry (GC-MS). For comparison, we also analyze the cuticular hydrocarbons of two surface cixiid species. Because diet is tied to the composition of cuticular hydrocarbons, we expect to see differences between surface and cave planthopper species, which feed on different plants and/or different parts of the same plants (roots for hypogean species, leaves and stems for epigean species). However, continuing studies will allow us to begin to assess if there are any unique pheromones or novel functions for chemical signals evolved by cave-adapted species.

Poulson, Thomas L.
tomandliz05@gmail.com

Food limitation is necessary to explain elaborated troglomorphy in some species.

Poulson, Thomas L.

318 Marlberry Circle, Jupiter, Florida, United States

I critique the Culver and Pipan hypothesis that absence of light is necessary and sufficient to explain patterns of troglomorphy. This is probably correct for reducing troglomorphy, like vestigial eyes and pigment. But I suggest that elaborated troglomorphy, like metabolic economies and life history, are most selected when there is striking food limitation. I test the hypotheses that there is increasing elaborated troglomorphy but convergent reduced troglomorphy. 1. With increasing body size, organ system complexity, and costs of doing business from flatworms and snails to crayfish and fish and from detritivore to predator. 2. With increasing food limitation within and among caves from chemo-autotrophic systems (e.g. Movile) to distance from guano piles (especially southeast Asia) to very open caves with continual allochthonous food in-wash (e.g. dissected tower karst) to new lava tubes, to north temperate caves that are recent with little surface karst development, and to deep phreatic caves isolated from surface input. 3. Organic eutrophication favors troglophiles and trogloxenes. 4. Natural and manipulated fecal types with differing food rigor (e.g. raccoon vs. cricket).

Poulson, Thomas L.
tomandliz05@gmail.com

Some troglobite traits are spandrels and not adaptations.

Poulson, Thomas L.

318 Marlberry Circle, Jupiter, Florida., United States

I suggest that a number of traits of troglobites could be spandrels sensu Gould and Lewontin. For each example I consider and attempt to test the alternative hypotheses of adaptive value and side effects of reducing or elaborating troglomorphys that may not have adaptive value. Possible examples I will discuss are: 1. Photo-tactic (sic) photo-kinetic behavior in cavefish and a beetle; 2. Shovel-shaped heads of fish and salamanders; 3. Vibration attraction behavior (VAB) in *Astyanax* cavefish; 4. Loss of circadian rhythmicity of activity and metabolism; and 5. Fragile body build with long and thin appendages in many troglobites.

Poulson, Thomas L.
tomandliz05@gmail.com

Sixty years of changing views of a very senior biospeleologist.

Poulson, Thomas L.

318 Marlberry Circle, Jupiter, Florida, United States

By using multiple hypotheses I have not been trapped in any favorite hypothesis. I have changed my views and developed and tested new hypotheses. I have continued to use caves as models to study ecological and evolutionary processes (Poulson 1963, Poulson and White 1969). I also still use a large variety of approaches form functional morphology and physiological ecology to agonistic behavior and food finding adaptations. I continue to consider rigor, variability, and predictability of abiotic and biotic agents of natural selection (Poulson and Culver 1969). Aquatic and terrestrial species the aquatic troglobites experience greater food rigor and variability and lower predictability and so the constant cave paradigm is relative at best. For example aquatic troglobites have greater metabolic economies and longer lives. And rare good and bad times have especially strong effects. I also compare both reducing and elaborating troglomorphy in troglophiles and troglobites that have been isolated for increasing evolutionary time especially among Amblyopsid cavefish, Linyphiid spiders, Rhaphidophorid "crickets," and Carabid beetles. In all of my studies I have had new insights from studying over several decades the same species in a few caves that have local differences in abiotic features, especially food input. Finally I am always observing the species natural history which leads to unexpected discoveries.

Powers, Amanda
krutzlaa@mail.uc.edu

The evolution of craniofacial shape change in the blind Mexican Cavefish.

Powers, Amanda*; Davis, Erin; Kaplan, Shane; Gross, Joshua

Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio, United States

The blind Mexican Cavefish, *Astyanax mexicanus*, has undergone extensive morphological changes, such as eye loss and pigmentation regression, following colonization of numerous caves in northeastern Mexico. Extant surface-dwelling forms of this species provide powerful comparisons between ancestral and derived morphologies. Several populations of cavefish are completely eyeless, which has had a dramatic effect on the shape and positioning of cranial bones. Further, cavefish harbor dramatic lateral asymmetries in their skull, such as premature bony fusions present on one side of the face and not the other. Using geometric morphometrics to capture shape variation, we performed a global shape analysis of the chondrocranium and osteocranium in juveniles and adults across surface fish, cave x surface hybrids and two geographically and phylogenetically distinct cavefish populations (Pachón and Tinaja). In juveniles, each population segregated into significantly distinct groups with respect to cartilaginous skull shape ($p<0.05$). Intra-individual shape asymmetry was found at the level of individual bones in adults, such as the opercle bone in hybrids ($p=0.0015$). We also found segregation of shape by performing an inter-individual analysis across adult surface fish, cavefish, F_1 hybrids and F_2 hybrids. By removing the landmarks outlining the eye, we found that the shape of the eye orbit is not the only factor that differs between the surface and cavefish skull ($p<0.0001$). This result may highlight other shape changes, such snout shape and jaw width, which evolved as a foraging mechanism in the dark caves. This work reveals dynamic changes to the craniofacial skeleton, over the course of life history, which likely arose in response to the extreme environmental pressures of the cave environment.

Symposium: Cave Animals at the Frontiers of Modern Biology

Protas, Meredith
Meredith.protas@dominican.edu

Developmental and genetic analysis of eye and pigment loss in the cave isopod *Asellus aquaticus.*

Mojaddidi, Hafasa[1]; Klein, Emily[2]; Trontelj, Peter[3]; Protas, Meredith[1]*

[1]Department of Natural Sciences and Mathematics, Dominican University of California, San Rafael, California, United States
[2]Department of Biology, Whitman College, Walla Walla, Washington, United States
[3]Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

There are still many gaps in our understanding of the genetics and developmental biology of cave dwelling animals. Only a few cave dwelling species have two necessary features that allow for embryological and genetic studies: a surface and cave morph that can interbreed and produce fertile offspring and the ability to raise and breed the animals in the laboratory. We have been developing one such system- the isopod crustacean, *Asellus aquaticus*. Previously, we had established the means to set up genetic crosses and map regions responsible for particular traits in one particular cave population. Now, we are examining another population occupying a different channel of the same cave, which is thought to have evolved independently from the other channel. We are investigating the genetic basis of the cave characteristics in this new channel population. In addition, we have developed methods to obtain embryos and perform comparative embryological studies. By tracking the development of these individuals, we have observed that the lack of eyes and pigmentation are already established in embryonic development in this species. Furthermore, we have identified specific embryonic time points when pigmentation and eye phenotypes diverge in cave and surface embryos. We used this information to harvest samples of particular embryonic stages for comparative transcriptomics between cave, surface, and hybrid embryos. This information will increase the genetic resources available for *Asellus aquaticus* and will help us to understand the genes and pathways responsible for the morphological differences between the cave and surface populations.

Prous, Xavier
xavier.prous@vale.com

Drivers of iron ore cave biodiversity.

Jaffé, Rodolfo[1]; Prous, Xavier*[2]; Giannini, Tereza Cristina[1]; Imperatriz-Fonseca, Vera Lúcia[1]; Maurity, Clóvis[1]; Oliveira, Guilherme[1]; Zampaulo, Robson[2]; Brandi, Iuri[2]; Siqueira, José Oswaldo[1]

[1]Vale Institute of Technology - Sustainable Development. Belém - PA, Brazil.
[2]Vale S/A - Management of environmental licensing and speleology, Belo Horizonte, Minas Gerais, Brazil.

Caves in iron ore tend to have higher species richness when compared to other lithologies caves, whether troglomorphic species or not. However, there is a knowledge gap regarding the main drivers of iron ore cave biodiversity. We perform a large-scale quantitative study relating iron cave biodiversity indicators to different cave characteristics. We analyzed data for 844 iron caves from different speleology reports for the Carajás region (Brazil). We found significant spatial autocorrelation in species richness across most of the analyzed spatial scales. On the other hand, presence of rare troglobites, presence of troglobites, and presence of bat populations only showed significant spatial autocorrelation at small and intermediate spatial scales. In all cases spatial autocorrelation decreased with increasing spatial scale, and did not differ from the random expectation when reaching the maximal extent of our study area. No spatial autocorrelation was detected in the residuals of our final models. Cave area and the presence of organic material and water reservoirs were found to be key predictors of cave biodiversity. Specifically, species richness increased with cave area, and this increase was more pronounced in caves containing guano. Species richness was also higher in caves containing detritus, roots and bat populations, but lower in caves with water reservoirs than in caves without them. Larger caves were also more likely to contain troglobitic species, rare troglobites, and bat populations. However, caves with water reservoirs were less likely to contain rare troglobites. The results suggest that iron cave must have connectivity between them and should not be viewed as independent units, but as part of a connected landscape.

Poster Session

Prous, Xavier
xavier.prous@vale.com

Variation of temperature and air relative humidity in four iron ore caves in Brazil.

Prous, Xavier*[1]; Pietrobon, Thadeu[1]; Callux, Allan[1]; Dutra, Georgete[1]; Lot, Carlos[2]; Brandi, Iuri[1]

[1]Vale S/A - Management of environmental licensing and speleology, Belo Horizonte, Minas Gerais, Brazil
[2]Ativo Ambiental Projetos Sustentáveis, Belo Horizonte, Minas Gerais, Brazil

The understanding of the cave ecosystem is directly associated with climate and its influence on cave fauna. Despite their importance, climate studies are scarce and mostly on limestone caves. Iron ore caves are smaller and more superficial than limestone ones, besides, their climate and its influence on cave biodiversity is still unknown. Here, we present a first insight into climatic aspects of four iron ore caves. Monitoring of temperature and relative humidity was carried out for 21 months, with records taken every 30 minutes in sensors and data loggers installed at the entrance and every 150 m² of the internal area of the caves. Two cavities were smaller and we installed one sensor in each. In the other two caves we installed 3 and 4 sensors respectively. The 13 sensors recorded more than 900.000 temperature (°C) and relative humidity (%RH) data. Spectral analysis of the data shows temperature oscillations linked to annual, diurnal and semi-diurnal patterns, even in the deepest parts of the larger caves. The average of thermal amplitude at the entrance of the caves was of 9.0°C. Inside, the average of thermal amplitude was of 9.3°C in smaller caves and 4.1°C in larger caves. In the deeper chambers of the two larger caves, the sensors recorded a small temperature variation of approximately 1°C. Inside the smaller caves, the temperature and relative humidity variation were similar to external variations, with higher temperatures records in the dry season (maximum 31.2°C, 45,5% RH) and lower in the rainy season (minimum 22.0°C, 100% RH). In the deepest parts of the larger caves, the temperature and relative humidity variation were congruent, with the highest temperature records in the highest humidity and lowest temperatures in the smallest humidity. Studying climate in iron ore caves is a step towards understanding its influence on biodiversity.

Symposium: Patterns of Subterranean Biogeography and Biodiversity

Reboleira, Ana Sofia Pereira Serrenho
sreboleira@ua.pt

Portugal - the emergence of a new hotspot of subterranean biodiversity in Europe.

Reboleira, Ana Sofia P. S.

Natural History Museum of Denmark (Zoological Museum), University of Copenhagen, Universitetsparken 15, DK-2100 København Ø, Denmark.

Along Europe, subterranean hotspots of biodiversity are mainly concentrated in in the Balkans and southern France. Although it has been hypothesized that a ridge of high subterranean biodiversity occurs in Europe at mid-latitude (ca 42º/48º) coinciding with temperate areas of high productivity and cave density, within the Iberian Peninsula, only the Pyrenees and the Spanish Cantabrian area appear along this ridge.

Worldwide, the definition for subterranean biodiversity hotspot is based in the presence of more than 20 troglobiotic or stygobiotic species per cave. The cave-dwelling fauna from Portugal was traditionally classified as poor, but recent years of intense biological prospections revealed a surprisingly high diversity. New biodiversity patterns and biogeographic hypotheses are enlightening the richness and affinities of Lusitanian subterranean faunas.

The southernmost part of the country, the Algarve, hosts nowadays the richest massif of Portugal, with a considerable amount of relicts and the promise of its increment with future standardized sampling efforts. After this study a question is launched and answered: do we have a new cave-dwelling diversity hotspot in Western Europe?

Reboleira, Ana Sofia Pereira Serrenho
sreboleira@ua.pt

"Troglobiário" – a cave lab building bridges between citizens and science.

Reboleira, Ana Sofia P. S.*[1,2]; Fernandes, Maria Jesus[3]; Martins, Olímpio[3]

[1]Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark
[2]Departamento de Biologia & CESAM, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, portugal
[3]Parque Natural das Serras de Aire e Candeeiros, Instituto de Conservação da Natureza e das Florestas, I.P., Portugal

"Citizens and science" become a central recent concern for nature conservation, particularly relevant for cryptic and unknown ecosystems – such as caves. Located in the heart of Estremenho massif in central Portugal, the cave "Algar do Pena" is opened to public since 1997. This constitutes an innovative model for the sustainable use of the subterranean environment implemented by the Serra d'Aire e Candeeiros Natural Park (ICNF), where accurate scientific information is offered to the visitor.

The increasing public interest in cave biology in Portugal lead to the construction of a subterranean laboratory, inaugurated in 2014, where living cave animals can be observed by a wide variety of visitors of Algar do Pena, the so-called "Troglobiário".

The reduction of visitation impacts were implemented in delimiting daily visitors capacity, reduction of allochthonous source of matter input, the use of removable stainless structures for visitors and the use of light sources that inhibit photosynthesis. A monitoring station recovers daily the levels of CO_2, air and rock temperature, and air hygrometry. Multimedia technologies are also implemented to support the biophysical interpretation of the cave.

The facility receives around 3500 visitors/year, starting from six years old. The main target is focus on public school, primary and secondary education and interest groups. The visitation has particular impact in terms of the public perception about cave and karst environmental conservation issues, as well as in the scientific and technological culture of citizens.

This presentation will provide an overview of the implementation of this subterranean lab and about the impact of the facility visit a bridge between citizens and science regarding the peculiar subterranean ecosystem.

Reboleira, Ana Sofia Pereira Serrenho
sreboleira@snm.ku.dk

Unsafe sex – interesting interactions between cave inhabitants.

Reboleira, Ana Sofia P. S.*; Enghoff, Henrik

Natural History Museum of Denmark (Zoological Museum), University of Copenhagen, Universitetsparken 15, DK-2100 København Ø, Denmark

Most cave inhabitants belong to the phylum Arthropoda that can be parasitized by a group of obligate fungal ectoparasites, the Laboulbeniales. These fungi have two basic types of specialization: a high specificity for a host species and a preference for growth in restricted areas of the host body.

The subterranean ecosystem combines important properties which are favourable for the development of Laboulbeniales: successive generations of adult hosts overlapping in time, stable populations, and high humidity. We have studied two models of interaction pattern between arthropods and Laboulbeniales, using morphological, genetic and ecological approaches. The endemic millipede *Lusitanipus alternans* forms stable year-round populations in caves of central Portugal. We estimated that 64% of the specimens are parasitized by two Laboulbeniales species that occupy different positions on the host: *Diplopodomyces lusitanipodos* is distributed especially on the legs but also more or less randomly along the body, whereas *D. veneris* grows on copulatory organs (gonopods) of males and around female gonopores, indicating that it is transmitted by direct contact of the hosts during copulation. In the same caves, a unique case of fungus-insect-vertebrate hyperparasitism has been discovered in bat flies *Penicillidia* spp parasitized with Laboulbeniales species of the genus *Arthrorynchus*, and we can shed the first lights on this phenomenon.

We present an overview of interesting features of parasitism and hyperparasitism, including the first evidence of indirect transmission in caves, where nevertheless copulation between hosts plays a major role in Laboulbeniales transmission.

Rendoš, Michal
michal.rendos@gmail.com

Organic carbon content as substantial factor affecting diversity and vertical distribution of Collembola on forested scree slopes.

Rendoš, Michal *[1]; Raschmanová, Natália [1]; Miklisová, Dana [2]; Mock, Andrej[1]; Ľuptáčik, Peter[1]; Kováč, Ľubomír[1]

[1]Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafárik University, Košice, Slovakia
[2]Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia

The impact of temperature and edaphic parameters (pH, organic carbon content) on the structure and vertical distribution of Collembola along a depth gradient were studied on three forested scree slopes differing in type of bedrock and topography. Collembola were collected using subterranean traps at depths of 5 to 95 cm from the soil surface. A total of 69 collembolan species were identified, among them 28 trogloxenes, 21 subtroglophiles, 17 eutroglophiles and 3 troglobionts. Most of the trogloxenes were exclusively bound to the uppermost level of the depth gradient and they occurred deeper very rarely. With few exceptions, the majority of subtroglophiles and eutroglophiles had tendency to be distributed across the entire depth gradient. Troglobionts showed a reversed pattern of distribution, with significant predominance in the lower half of the depth gradient. Moreover, they occasionally occurred closer to the surface, at the levels completely filled with the soil. Among the soil parameters monitored, organic carbon content had the greatest impact on species composition and richness. The activity of subterranean species, both eutroglophiles and troglobionts, correlated with organic carbon. The north-exposed limestone scree slope with sediment layers rich in organic carbon had the most diverse community structure and showed a distinctly higher number of eutroglophiles and troglobionts. The south-west facing volcanic scree slope revealed slightly higher values of temperature and distinctly lower species richness compared with the other two sites. The present study implies that in forested scree deposits with well-developed upper organic horizons microarthropod communities have obvious vertical stratification. This study was supported by grant 1/0199/14 from the Slovak Scientific Grant Agency VEGA and by grant VVGS-2014-228 of P.J. Šafárik University in Košice.

Poster Session

Roenfeldt, Rachel
rachelroenfeldt@gmail.com

Diet expansion in a cave adapted *Eurycea*.

Roenfeldt, Rachel[1]*; Fenolio Dante[2]; Slay, Michael[3]; Hammond, Shea[4]; Soares, Daphne[5]

[1]Department of Ecosystem Science and Management, Texas A&M University, College Station, Texas, USA
[2]San Antonio Zoo, San Antonio, Texas, USA
[3]The Nature Conservancy, Fayetteville, Arkansas, USA
[4]U.S. Fish & Wildlife Service, Ozark-Plateau National Wildlife Refuge, Colcord, Oklahoma, USA
[5]Biological Sciences, New Jersey Institute of Technology, Newark, New Jersey, USA

Salamanders are traditionally understood as having a carnivorous diet. However, *Eurycea spelaea* has been shown to ingest bat guano. Here we examine the effect of a strict bat guano diet on salamander weight to test the hypothesis that there has been a shift in food preference during cave invasion. Our results show that, although bat guano contains similar nutritional caloric values as live prey (amphipods), *E. spelaea* was not able to maintain weight with a bat guano diet alone. We suggest that coprophagy is used by salamanders as a way of complementing their nutritional needs, and that cave adaptation has not shifted their food preferences but has instead broadened it.

Symposium: Cave Animals at the Frontiers of Modern Biology

Rohner, Nicolas
nro@stowers.org

Astyanax mexicanus as a natural model for metabolic adaptation.

Aspiras, Ariel[1]; Tabin, Cliff[1]; Rohner, Nicolas[*2]

[1]Department of Genetics, Harvard Medical School, Boston, Massachusetts, United States
[2]Stowers Institute for Medical Research, Kansas City, Missouri, United States

Understanding the genetic basis of adaptation has broad implications not only for a basic understanding of evolution, but also for human pathologies given that many human diseases are a consequence of mis-adaptation to modern societies. The emerging model system *Astyanax mexicanus* has become an important cavefish species to address adaptation to extreme environments due to its unique ecology and the availability of genetic tools and genomic resources.

We have previously shown that cavefish acquired impressive metabolic adaptations such as hyperphagia (increased appetite), starvation resistance and altered feeding behaviors to cope with the typically nutrient deprived cave environments. Here, we have focused on the insulin transduction pathway and its role on glucose and fat homeostasis in cave and surface forms of *A. mexicanus*. Interestingly, we detected very low insulin levels in cavefish (compared to surface fish) partially due to lower numbers of beta-insulin producing cells in the pancreas. In addition, we observed strong insulin resistance in cavefish when administered with ectopic insulin. Despite the consequential elevated and highly fluctuating blood glucose levels, cavefish live long and healthy lives, probing the question whether they have acquired mechanisms allowing them to cope with extreme nutritional levels.

Taking advantage of the newly available genome of *Astyanax mexicanus,* we identified mutations in the insulin receptor of cavefish. Importantly, the same mutations were found in cases of insulin resistant patients in human populations. Our findings in independently derived cavefish populations suggest that cavefish are inherently insulin resistant, potentially as an additional strategy to acquire better starvation resistance. We are currently using genome editing to functionally test these and other candidate mutations in zebrafish and cavefish itself to study in detail the molecular mechanisms underlying the metabolic adaptation of cavefish to the extreme and nutrient poor environments.

Sawicki, Thomas R.
thomas.sawicki@famu.edu

Physiochemical differences in water sources within De Leon Springs, Florida, and their potential effect on cave biota.

Sawicki, Thomas R.[*1]; Stine, Michael[2]; Long, Richard A.[3]

[1]Department of Biological Sciences, Florida A & M University, Tallahassee Florida, United States
[2]Department of Mathematics, Physics, and Biological Sciences, North Florida Community College, Madison, Florida, United States
[3]Department of Biological Sciences, Florida A & M University, Tallahassee Florida, United States

In 2005, Terrance Tysall of the Cambrian Foundation collected a new species of stygobitic amphipod in the genus *Crangonyx* from Wekiwa Springs, Blue Springs and De Leon Springs in central Florida. In Blue Springs and Wekiwa Springs, specimens were collected in fissures located outside of the caves; however, in De Leon Springs, specimens were collected swimming in and around white filamentous microbial mats, presumed to be the sulphur oxidizing bacterium *Beggiatoa*, within the cave.

Starting in the summer of 2012, expeditions to these locals to collect additional amphipod specimens commenced. After 3.5 years of intensive effort, no new specimens of this amphipod species have been found at any of the three locations, but significant changes in the quality and quantity of the microbial mats within De Leon Springs were observed. The white filamentous bacterial mats that were initially 10-20 cm thick, and covering the walls, ceilings, and even the sediments on the cave floor, appeared to thin—exposing bare rock in places—and have transitioned from white to brown mats in most regions of the cave. The white bacterial mats became largely restricted to a single vent from which water flows into the cave at a high velocity. Starting in January, 2014, physiochemical data (e.g. pH, conductivity, dissolved oxygen, temperature, and depth) have been recorded using a sonde. Significant differences in pH and conductivity between the vent water and the water in the rest of the cave have been observed. Bacterial mat and water samples were collected for comparison of the white and brown mats by community fingerprinting and 16S rDNA sequencing.

Siemensmeyer,Tobias
siemensmeyer@groundwaterecology.de

The effects of well type and quality on sampling of stygofaunal communities.

Siemensmeyer,Tobias*[1,2]; Schwenk, Klaus[2]; Hahn, Hans Jürgen[1]

[1]Institute for Groundwater Ecology IGE GmbH, University of Koblenz Landau, Fortstr. 7, 76829 Landau, Rheinland-Pfalz, Germany
[2]Institute for Environmental Sciences, University of Koblenz Landau, Fortstr. 7, 76829 Landau, Rheinland-Pfalz, Germany

Monitoring wells represent the most common and most frequently used method to sample groundwater fauna. However, only little is known about the response of groundwater communities to different well construction types and well qualities. Despite and because of this gap in knowledge, the construction type and condition of wells are rarely considered for groundwater ecological studies.

Surface water intrusion is the main environmental factor determining groundwater assemblages, since surface water provides food and oxygen for subterranean fauna. Thus, insufficiently built or damaged wells, which are stronger influenced by surface water, were suspected to be characterized by a more abundant and more diverse fauna with stygoxenous elements. Vice versa, properly sealed wells with a reduced surface water influence, should display an impoverished fauna.

In order to determine the impact of wells on groundwater ecological studies and biomonitoring, we analyzed faunal data from wells in two different areas, Baden-Wurttemberg in Southern Germany and Berlin in Northern Germany. In both areas we found a strong impact of well type and quality on groundwater communities. Furthermore, significant differences between Southern and Northern Germany groundwater communities were found, due to the different regional hydrogeological characteristics.

Symposium: Conservation of Subterranean Fauna and Habitats

Simon, Scott
ssimon@tnc.org

Building a constituency for karst conservation.

Simon, Scott*; Slay, Michael E.

[1]Arkansas Field Office, The Nature Conservancy, 601 North University Avenue, Little Rock, Arkansas, United States

The Nature Conservancy has helped conserve more than 320,000 acres of natural lands and waters in Arkansas. The Arkansas program currently owns or manages 49 nature preserves encompassing more than 31,000 acres and has ongoing cooperative projects with public and private landowners on more than 35,000 acres. These sanctuaries are found in every corner of the state. Globally, the Conservancy has conserved more than 15 million acres in the United States and has helped conserve more than 102 million acres in Latin America, the Caribbean, Asia, Africa, and the Pacific. Natural resource agencies are in increased competition for funding with many other societal needs (education, health care, prisons, technology, etc.). Having a broad constituency for your agency or organization is critical in maintaining support for natural area programs and conservation in general. In Arkansas, large scale restoration projects provide examples of how consensus is developed across a wide range of stakeholders. A brief description of two Ozark projects, stream restoration in Little Red River watershed and pine-oak woodland restoration in the Boston Mountains, demonstrate how industry, municipalities, and agencies collaborate to protect and restore unique habitats. Finally, karst conservation examples in northwest Arkansas and northcentral Arkansas are discussed to highlight how conservation investments can be leveraged for maximum species benefit.

Soares, Gustavo
gustavo.soares@carste.com.br

Troglomorphic fauna sampling methods in canga formations, Minas Gerais State, Brazil.

Soares, Gustavo*; Andrade, Renata; Perroni, Gustavo

Instituto do Carste, Belo Horizonte, Minas Gerais, Brazil

Although macro caves are currently the best studied subterranean habitats, smaller spaces have assumed a great significance for subterranean biology. Among known subterranean environments, the shallow subterranean habitats (SSH) are characterized for being aphotic, near to the surface and able to harbor specialized species for subterranean life. In the Brazilian iron formations, the superficial layer of canga that covers the iron ore deposits can be considered an important example of SSH. This type of rock features high porosity with a large number of small channels, providing the potential to maintain fauna populations, exclusive or not, of subterranean environments. The present study aims to present the inventory methods of subterranean fauna present in canga, as well as the first results on the troglomorphic fauna of Minas Gerais State. The methods selected were: (1) Leaf Litter Trap - perforated container having in its interior humidified and sterilized leaf litter as substrate for fauna colonization; (2) Drip Pools - apparatus used for collection of organisms found in canga conglomerates and carried by water to the interior of cavities; and (3) MSS-trap - apparatus developed for shallow subterranean spaces, inserted in artificial holes in canga. Although the proportion of registered troglomorphic is low, its presence in these spaces has been regularly verified. The MSS-trap proved to be the most favorable method for collecting these organisms, among which have been recorded the Collembola genera *Pararrhopalites*, *Pseudosinella* and *Trogolaphysa*, and organisms with greater body size, such as the Pseudoscorpiones genus *Pseudochthonius* and the Aranae family Caponidae. All these taxa were already described as present in the macro caves of the studied areas. These results attest the efficiency of the chosen methods, as well as confirm the existence of troglomorphic species in canga, and the potential of this environment for maintenance of subterranean populations.

Souza, Maysa Fernanda Villela Rezende
mvillelabio@yahoo.com.br

Updated records of troglomorphic palpigrades in Brazil.

Souza, Maysa Fernanda Villela Rezende*; Ferreira, Rodrigo Lopes

Study Center on Subterranean Biology, Biology Department, Federal University of Lavras, Lavras, Minas Gerais, Brazil

Palpigradi is a group extremely rich in Brazil, with several new species being discovered in recent years, especially inside caves. Most of them belong to the genus *Eukoenenia* and some present troglomorphic features. The troglomorphims in this group consist mainly of increases in body size, elongation of the appendages and the increase in the number of blades that form the lateral organs. Despite of the big number of troglomorphic species occurring in the country, only four species are already described: *Eukoenenia maquinensis*, *E. sagarana* and *E. virgemdalapa* from Minas Gerais State and *E. spelunca* from Espírito Santo State. Therefore, the aim of this study is to report the distribution of troglomorphic Palpigrades in Brazil. These records are from inventories of cave fauna carried out over the past fifteen years by the team of Study Center on Subterranean Biology (Federal University of Lavras). The surveys included caves located in sixteen Brazilian states (Minas Gerais, Goiás, Espírito Santo, Bahia, Rio de Janeiro, Mato Grosso, São Paulo, Paraná, Santa Catarina, Rio Grande do Sul, Sergipe, Ceará, Rio Grande do Norte, Alagoas, Pernambuco and Tocantins). It was also included material deposited in other collections. It was found at least twelve new troglomorphic *Eukoenenia* species: seven from Minas Gerais, three from Bahia, one from Goiás and one from São Paulo. Furthermore, there are two additional records for Bahia: one new species that has elongated appendages and fits in *Allokoenenia* diagnosis and another new species that potentially belongs to a new genus, since it features a flagellum with a quite unique morphology. Most of the new species was found only in one cave and is represented by few individuals. It is important to highlight that all species are under description process, which is essential for the conservation of these rare and endemic taxa.

Poster Session

Souza, Maysa Fernanda Villela Rezende
mvillelabio@yahoo.com.br

Advances in the knowledge regarding troglomorphisms in Palpigradi: new tools for cave conservation.

Souza, Maysa Fernanda Villela Rezende*; Ferreira, Rodrigo Lopes

Study Center on Subterranean Biology, Biology Department, Federal University of Lavras, Lavras, Minas Gerais, Brazil

Palpigradi are small arachnids found mainly in soil, leaf litter and caves. Thus, the most common troglomorphisms are not applicable to palpigrades, since all species present anophthalmia and depigmentation. In this group, number of blades in the lateral organs and the value of ratios basitarsusIV/tibiaIV and propeltidium/basitarsusIV have been considered the most useful troglomorphic traits. However, such index has not been suitable to categorize all the Brazilian species. Accordingly, our objective was to detect which morphometric attributes are more important to differentiate edaphic from troglobitic palpigrades. For this purpose, 120 adult specimens belonging to 39 species of *Eukoenenia* from South America and Europe were measured. The morphometric attributes used were the relative lengths (ratio between the length of the segments and total length of the appendages related to the propeltidium) of pedipalp, leg I and leg IV. The ratio basitarsus/tibia of leg IV was also used. For the analysis, the species were classified in four categories: troglobitic (Tb); troglomorphic, collected inside caves (Tm); edaphic (E) and edaphomorphic, collected inside caves (Ec). The discriminant analysis showed a significant difference between all the groups. The morphometric attributes that contributed significantly to differentiate them were the relative lengths of tarsus2 and tarsus3 of pedipalp and relative length of leg 1. Pairwise comparisons were conducted and only the difference between E and Ec was not significant. Furthermore, the F-test showed higher values for troglobitic and troglomorphic species, indicating that they have longer tarsus 2 and tarsus 3 on the pedipalp and longer leg I. This work is the first to test statistically the morphological differences between edaphic and troglobitic palpigrades and to provide new useful characters to detect troglomorphisms, what is essential to preserve these species and their habitats.

Souza-Silva, Marconi
marconisilva@dbi.ufla.br

Brazilian vs. Slovenian aquatic subterranean biodiversity: the case of Areias and Postojna-Planina cave systems

Ferreira, Rodrigo Lopes[1]; Souza-Silva, Marconi[1*]; Fišer, Cene[2]; Zagmajster, Maja[2]; Prevorčnik, Simona[2]; Sket, Boris[2]

[1]Center of studies on Subterranean Biology, General Zoology sector, Department of Biology, Federal University of Lavras, Minas Gerais, Brazil
[2]Department of Biology, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia.

Biodiversity patterns and environmental conditions in subterranean aquatic habitats show considerable global scale variations. We studied abiotic factors and diversity of benthic macroinvertebrates in two sinking rivers flowing in two different parts of the globe: Brazilian Areias (ACS) and Slovenian Postojna-Planina (PPCS) cave systems. These complex cave systems harbor about 6 and 60 aquatic troglobionts, respectively. In winter and summer 2012, we sampled approx. 5.8 km (ACS) and 11.5 km (PPCS) long river stretches, at 12 (ACS) and 17 (PPCS) equidistant subterranean sampling stations. We measured various physical and chemical parameters of water and used Surber Sampler for collecting benthic macroinvertebrates. We registered 48 morphotaxa (1 stygobiotic) in ACS and 71 (24 stygobiotic) in PPCS. Benthic abundance and richness were higher in PPCS than in ACS and season-dependent: highest abundance in summer at ACS (1109 individuals) and PPCS (18377 individuals) and highest richness in summer at ACS (30 morphotaxa) and in winter at PPCS (58 morphotaxa). Community composition and abundance were changing along both rivers, yet at different pace, evenness and morphotaxa turnover were higher in ACS than in PPCS. Using DistLM, the best predictors of species composition in ACS were found to be dissolved oxygen in summer and water flow velocity in winter. In PPCS, the best predictors were nitrate concentration in winter and biochemical oxygen demand in summer. The highest total carbon and total organic carbon levels were measured in ACS. Besides the expected differences between both river systems due to their distinct environmental, historical and faunistic backgrounds, we have also found mutual similarities: season-dependent community structure, decline in species richness downstream of the river sink (except in ACS in winter) and a tendency of aquatic troglobionts to be present and more abundant in the deeper parts of the cave system.

Stephen, Charles Donald Robert
czs0021@auburn.edu

Species delimitation and phylogeography of *Hesperochernes* (Pseudoscorpiones: Chernetidae) from karst regions of the southeastern United States.

Stephen, Charles Donald Robert*[1]; Niemiller, Matthew Lance[2]; Bond, Jason Edward[1]

[1]Department of Biological Sciences, Auburn University, Auburn, Alabama, United States
[2]Illinois Natural History Survey, Prairie Research Institute, University of Illinois Urbana-Champaign, Champaign, Illinois, United States

The karst regions of the southeastern United States form a subterranean biodiversity hotspot. Troglobiotic pseudoscorpions are exceptionally diverse here and tend to have highly restricted distributions. An exception to this pattern is *Hesperochernes mirabilis* (Chernetidae). The distribution of this species spans all major karst regions east of the Mississippi River, being approximately bound in the south and east by the Appalachian Mountains and in the north by the Ohio River. *Hesperochernes holsingeri* is the only other described congener within this region; its distribution is limited to two geographically proximate caves. Epigean *Hesperochernes* are not known from within the distribution limits of *H. mirabilis*. The goals of the present research are to test species limits in *Hesperochernes*, predict ancestral and current distributions, and to suggest the timing of *Hesperochernes* adopting a hypogean lifestyle. We extensively sampled caves throughout the southeastern United States. For molecular sequencing, we selected individuals representing each geographically disjunct population, each field-recorded ecological association, and each cluster identified by morphometric analyses. Phylogenetic and phylogeographic approaches inferred (1) monophyly of *Hesperochernes* with respect to its epigean sister taxon *Illinichernes* and other epigean chernetid taxa within the prescribed region; (2) monophyly of *H. mirabilis* and *H. holsingeri* relative to epigean and hypogean sister species; (3) monophyly of *H. mirabilis* across sampled populations; (4) haplotype networks of *H. mirabilis* and *H. holsingeri* populations; (5) current and ancestral distribution extents of hypogean *Hesperochernes*; and (6) timing of *Hesperochernes* invading caves. Using the cohesion species concept, we integrated ecological, molecular, and morphological data to test the null hypothesis that *H. mirabilis* populations form one species. We present initial results from our analyses and suggest an ecological hypothesis for the distribution of *Hesperochernes* within this region.

Symposium: Conservation of Subterranean Fauna and Habitats

Sutton, Michael
haganandsutton@gmail.com

Documenting Missouri cave biology – from Ruth Hoppin to Missouri Cave Database.

Sutton, Michael*[1]; House, Scott[2]

[1]Cave Research Foundation, 5544 CR204, Annapolis, Missouri 63620, United States
[2]Cave Research Foundation, 1606 Luce Street, Cape Girardeau Missouri 63701, United States

The documentation of Missouri cave wildlife began in 1889 when amateur speleologist Ruth Hoppin collected eyeless crayfish and eyeless fish from caves in SW Missouri. These were described and published by Walter Faxton and C. H. Eigenmann as, respectively, *Cambarus setosus* and *Amblyopsis rosae*. Records of cave bats and stygobiotic salamanders appeared soon after due to the efforts of two other amateur enthusiasts – T.S. Powell and Luella Agnes Owen. Records continued to accumulate slowly – Leslie Hubricht collected crustaceans up to 1943, while Peck and Lewis documented cave life of the Perry County sinkhole plain in the mid-1970s, but the first widespread catalog of cave fauna was Gene Gardner's 1975-1985 survey of caves in public ownership. The accumulated records were available, but scattered throughout the literature. In 1998, the Missouri Department of Conservation hired Bill Elliott as the state cave biologist. Elliott developed Cave Life Database on an Access platform, which made the published records available in one source, and he also added unpublished records from field surveys and research projects. Following Elliott's retirement in 2012, the database was no longer supported. Since it had become almost indispensable to cave biologists and land managers, and since the original Access platform was often difficult to query, personnel from Cave Research Foundation and Missouri Speleological Survey revived the database by incorporating it into the Missouri Cave Database, which runs on the equally powerful but more user-friendly FileMaker Pro. The result is a versatile tool which contains over 21,000 records documenting 1100 taxa, including 600+ cave adapted species, and is one of the most comprehensive such databases in the US. Plans are underway to extend the coverage to neighboring states, especially Arkansas. The capabilities of the database in performing complex searches and sorts, the generation of distribution maps, etc. will be demonstrated.

Special Session: Biology of Subterranean Crustaceans
(Sponsored by The Crustacean Society)

Švara, Vid
vid.svara@gmail.com

Integrative taxonomy of cryptic subterranean Amphipods (Niphargidae: *Niphargus*) from Dinaric Karst.

Švara, Vid*[1]; Delić, Teo[1]; Coleman, Charles Oliver[2]; Fišer, Cene[1]

[1]Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
[2]Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany

With over 300 described species, *Niphargus* is the most species rich genus of freshwater amphipods. The genus shows a high ecological and morphological diversity. *Niphargus* amphipods are important as top invertebrate predators in the Dinaric Karst. They also represent an evolutionary phenomenon with exceptionally large body size for lower geographic latitudes. The most fascinating species belong to the cave-lake ecomorphs with large body size and raptorial appendages. Nevertheless the species inventory and distribution of cave-lake *Niphargus* ecomorphs remains incompletely studied. The main problem of genus taxonomy is due to high intra specific variation and small between species differences, in addition to general problems of taxonomy, such as small sample sizes due to species rarity. Besides that, many so called morphologically cryptic species typically remain undescribed and neglected in biodiversity research, which should be improved at least in charismatic and ecologically important species complexes such as cave-lake ecomorphs of genus *Niphargus.* Species delineation methods using molecular markers unveiled that species diversity of many cave species, including *Niphargus*, is highly underestimated.

Therefore I will present a case study of a taxonomically extremely demanding *Niphargus* species complex of cave-lake ecomorphs endemic to Dinaric Mountains. In our research we combined morphological analyses with multilocus species delimitation methods and ecological modeling, and we show that the species complex *Niphargus arbiter-Niphargus salonitanus* contains seven new species that are named, diagnosed, and discussed within broader biodiversity context.

Symposium: Patterns of Subterranean Biogeography and Biodiversity

Taiti, Stefano
stefano.taiti@ise.cnr.it

Cave-dwelling terrestrial isopods (Crustacea, Oniscidea) from Southeast Asia: a review.

Taiti, Stefano*[1]; Cardoso, Giovanna Monticelli[1,2]

[1]Istituto per lo Studio degli Ecosistemi, Consiglio Nazionale delle Ricerche, Florence, Italy
[2]Instituto de Biociências, Laboratório de Carcinologia, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

Until 10 years ago, very little was known about cave-dwelling terrestrial isopods from Southeast Asia, including southern China. Only 21 species (6 troglobionts, 1 stygobiont, 14 troglophiles) in the families Styloniscidae, Philosciidae and Armadillidae were recorded from southern China (Yunnan, Guangxi and Guangdong) (5 spp.), Vietnam (1 sp.), Burma (1 sp.), Thailand (4 spp.), Laos (1 sp.), Malaysia (4 spp.), and Indonesia (Sumatra, Java and Sulawesi) (5 spp.). No species were known from caves in Cambodia and the Philippines, despite their large karst systems. In recent years several species have been described from caves in southern China, mainly from karst areas in Guangxi and Guizhou. Of particular interest was the discovery of four troglobiotic and stygobiotic species of the genus *Troglоniscus* (Styloniscidae), previously known only from one troglobiotic species. In the last years collecting activities in many karst areas of Southeast Asia have got a huge increase due to several expeditions mainly led by L. Deharveng (Paris), M. Tian (Guangzhou) and P. Beron (Sofia). A large number of terrestrial isopods have been collected from caves in southern China, Vietnam, Malaysia, Laos, Cambodia, Philippines and Indonesia. Several new troglomorphic species are about to be described in the genera *Papuaphiloscia*, *Burmoniscus*, *Paraphiloscia* (Philosciidae), *Exalloniscus* (incertae sedis), *Hybodillo*, *Sumatrillo*, *Troglodillo*, *Dryadillo*, *Chelomadillo* and *Spherillo* (Armadillidae). All the troglobiotic Oniscidea from Southeast Asia are endemic to single caves or karst areas. *Troglоniscus* is the only genus known exclusively from caves, while all the other species of Philosciidae and Armadillidae belong to genera largely distributed in that area with many species occurring in epigean environments.

Taylor, Steven J.
sjtaylor@illinois.edu

Biogeography of subterranean California.

Elliott, William R.[1]; Reddell, James R.[2]; Rudolph, D. Craig[3]; Graening, G.O.[4]; Briggs, Thomas S.[5]; Ubick, Darrell[5]; Aalbu, Rolf L.[5]; Krejca, Jean K.[6]; Taylor, Steven J.*[7]

[1]914 Bannister Drive, Jefferson City, Missouri, United States
[2]Curator Emeritus of Cave Invertebrates, Texas Natural History Collections, The University of Texas at Austin, Texas, United States
[3]Southern Research Station, USDA Forest Service, 506 Hayter Street, Nacogdoches, Texas, United States
[4]Department of Biological Sciences, California State University, Sacramento, 6000 J Street, Sacramento, California, United States
[5]Department of Entomology, California Academy of Sciences, San Francisco, California, United States
[6]Zara Environmental LLC, 1707 FM 1626, Manchaca, Texas, United States
[7]Illinois Natural History Survey, Prairie Research Institute, University of Illinois at Urbana-Champaign, Champaign, Illinois, United States

California's karst is comprised of small outcrops of marble or limestone. Additionally, lava and ash flows, tens of thousands of mines, and hundreds of sea caves on the coast and islands provide habitat for subterranean life.

Here, we summarize efforts to compile all biological records for subterranean sites in California, with some records dating back to 1863. Early 20th century archaeologists and paleontologists surveyed caves containing Pleistocene deposits, discovering various new extinct and extant species. Since 1975, the rate of discovery of new species has accelerated with funded projects in most of the important cave areas. The nine authors' field work across the state is being prepared for a forthcoming monograph.

At least 3,318 caves are known in California, of which 30% have been biologically sampled. We cover 1,285 of these sites, including 683 caves, 170 lava tubes and ash flows, 133 sea caves, 137 groundwater sites, and 162 mines. The accompanying map depicts 500 localities of all five types in eleven regions.

At least 1,294 species are recorded from the states' subterranean sites, including 104 terrestrial troglobites, 12 stygobites, and 29 phreatobites, for a total of 145 obligate subterranean species. The richest regions for obligate subterranean species are Sierra Nevada North (53 species in 203 sites), Sierra Nevada South (27 in 57 sites), Klamath and Marble Mountains (16 in 26 sites), and Lava Flows North (10 in 78 sites). The high number (72) of single-site endemic species is indicative of the insular distribution of karst and the many river systems cutting across the Sierra Nevada. Sierra Nevada North has 18 single-site endemics, Sierra Nevada South has 16, and Klamath and Marble Mountains together have 11. In contrast, Lava Flows North only has 5 single-site endemics. The lava flow conotylid millipede *Plumatyla humerosa* has the largest troglobite range (194 km).

Throneberry, Jason
Jason@arkansasheritage.org

Current status and habitat use of the Foushee cavesnail, *Amnicola cora* (Hydrobiidae) in Foushee Cave, Independence County, Arkansas.

Throneberry, Jason*[1], Slay, Michael E.[2]; Taylor, Stephen J.[3]

[1]Arkansas Natural Heritage Commission, 323 Center Street, Little Rock, Arkansas, United States
[2]Arkansas Field Office, The Nature Conservancy, 601 North University Avenue, Little Rock, Arkansas, United States
[3]Illinois Natural History Survey, University of Illinois at Urbana-Champaign, 1816 South Oak Street, Champaign, Illinois, United States

The Foushee cavesnail, *Amnicola cora* Hubricht 1979 (Gastropoda: Neotaenioglossa: Hydrobiidae), is a single-site endemic stygobiont found in Foushee Cave, Independence County, Arkansas. Because little information was available concerning this species, a project was initiated in 2007 to establish baseline data on habitat use and population size. Sampling trips occurred during late spring and summer months to minimize disturbance to hibernating gray bats (*Myotis grisescens*) and at monthly intervals to minimize in-stream trampling of cavesnails. We established 25 sampling locations along the first ~1,000 m of cave stream and counted snails that occurred within a 0.05 m^2 quadrat placed haphazardly at each location. To characterize habitat use, we quantified snail position on substrate and measured water depth, flow, and substrate proportions. Sampling occurred during 12 visits from 2007 to 2014. The surveys yielded a significant difference ($p < 0.001$) between the numbers of snails among habitat types, with riffles having more snails than pools. Also, there was a strong preference ($p < 0.001$) for cobble and rock substrate. The quadrat census project spurred additional conservation efforts by several Arkansas state agencies. The potential for groundwater impacts to the cave system was assessed with funding from Arkansas Game and Fish Commission, and this funding was used to delineate the recharge boundary, characterize vulnerability, and document point hazards. Following the recharge delineation, a landowner parcel assessment was conducted to determine number and size of parcels that overlay the cave system. This information was then used by Arkansas Natural Heritage Commission to identify landowners interested in selling property and several land acquisitions were completed. Following these acquisitions, over 80% of land recharging groundwater to Foushee Cave is part of a new Arkansas state natural area.

Symposium: Patterns of Subterranean Biogeography and Biodiversity

Tian, Mingyi
mytian@scau.edu.cn

Diversity and distribution of cavernicolous ground beetles (Insecta: Coleoptera: Carabidae) in China.

Tian, Mingyi*[1]; Faille, Arnaud[2]

[1]Department of Entomology, College of Agriculture, South China Agricultural University, 483, Wushanlu, Guangzhou, 510642, China.
[2]Zoologische Staatssammlung - Münchhausenstraße 21, 81247 Munich, Germany.

China limestone landscapes are the largest in the world, and so are the caves. Does China hold the most diverse cave-dwelling fauna of the world? The answer is probably "Yes". For example, 124 species of ground beetles, one of the most emblematic groups of terrestrial invertebrates in subterranean habitats, have been recorded from southern China's karsts. Among them, 110 species belong to Trechini, 8 to Platynini, 3 to Clivinini, 2 to Bembidiini, and 1 to Ozaenini. The most diverse and morphologically modified beetles are Trechini, which include also the most troglomorphic species. Characters of trechine diversity in southern China's karsts are as follows: high generic diversity; all species narrow endemics; very rarely sampled in the caves; highly modified in morphology; subtropical lineages; and relict species. Majority of the cave trechine beetles in China occur in Guizhou Province and Guangxi Zhuang Autonomous Region, known so far by 71 species in 23 genera, more than half of the total numbers of genera (53.5%) and species (64.6%) respectively recorded in mainland China. Hotspots for cavernicolous trechine beetles in China documented so far are the Maolan—Mulun karst, a small area across southern Guizhou and northern Guangxi; the Huoyan karst, in Wulongshan Geopark, Longshan County, northwestern most Hunan Province; and the Du'an karst, northern Guangxi.The magnitude of this high biodiversity has been only recently disclosed. Many karsts remain un-surveyed, and prospects for new discoveries are extremely high.

Symposium: Cave Animals at the Frontiers of Modern Biology

Tovar, Ruben U.
rut676@utulsa.edu

Crossing to the dark side: the South Central Texas *Eurycea* clade as a novel subterranean model system for the study of evolutionary developmental biology.

Tovar, Ruben U.*[1]; García, Dana M.[2]

[1]Department of Biological Science, The University of Tulsa, Tulsa, Oklahoma, United States
[2]Department of Biology, Texas State University, San Marcos, Texas, United States

The south central Texas *Eurycea* clade exhibits a continuum of karst salamander phenotypes. The Texas blind salamander (*E. rathbuni*) is considered a stygobiont because it completes its life cycle in the aquatic subterranean habitat of the Edwards Aquifer where it lives in perpetual darkness. Consequently, *E. rathbuni* exhibits a broad head, gracile limbs, limited pigmentation and highly reduced eyes. In contrast, the Barton Springs salamander (*E. sosorum*) is epigean and is endemic to surface habitats; it exhibits small robust limbs, pigmentation, and well developed eyes. To determine the sequence of events during development that lead to widely disparate ocular outcomes and to gain insights into the molecular mechanisms responsible various stages of embryos were obtained from the two species (*E. sosorum* and *E. rathbuni*), and sectioned using a Shandon Cryotome at 10μm. Five developmental stages of *E. sosorum*, and six developmental stages of *E. rathbuni* were identified and labeled for *pax6* and *shh*. Expression of *pax6* and *shh* were detected using immunohistochemistry, and images were obtained using an Olympus FV-1000 scanning confocal microscope. Expression of Pax6 and Shh proteins is observed in both morphotypes represented by *E. rathbuni* and *E. sosorum*. *Eurycea sosorum* maintained expression of *pax6* and *shh* through embryogenesis and into a juvenile stage. Decreased labeling of Pax6 protein was observed during later stages of *E. rathbuni* development, while Shh protein labeling was increased in a select subset of cells surrounding the brain and eye. Interestingly, these results parallel *pax6* and *shh* expression in two morphotypes explored in *Astyanax mexicanus*, suggesting convergent evolution of the developmental mechanisms that lead to the development of vestigial eyes.

Special Session: Biology of Subterranean Crustaceans
(Sponsored by The Crustacean Society)
Keynote Address

Trontelj, Peter
peter.trontelj@bf.uni-lj.si

The origin of niphargids revisited and tested at the continental scale.

Trontelj, Peter*; Moškrič, Ajda; Verovnik, Rudi; Fišer, Cene

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

Throughout the history of subterranean biology few problems have received as much attention as those of biogeography and origin of troglobiotic species. At the same time, few big questions have remained as poorly answered as those of whence and how the major and most widespread subterranean lineages evolved. One of those lineages is the mega-diverse Western Palearctic amphipod genus *Niphargus* with some associated taxa. Until now, global scenarios explaining its evolution have failed due to the lack of known close non-troglobiotic relatives, high endemism, and the sheer number of species. However, recent development in multi-locus molecular phylogenetics, relaxed molecular clocks and probabilistic reconstruction methods for ancestral traits and distributions has enabled us to propose a set of testable hypotheses about the biogeographic and evolutionary history of niphargids. A surprising sister relationship to the brackish and marine pseudoniphargids suggests a marine origin for the niphargids. We reject the recently proposed model of a single marine-to-freshwater transition in north-western Europe with subsequent dispersal over the continent, in favor of multiple origins from Tethyan and Paratethyan coastal interstitial waters. Our multiple-marine-origin hypothesis is further supported by two independent lines of evidence. First, inferred ancestral morphologies of major *Niphargus* clades consistently point to small-bodied interstitial forms. Second, most major clades contain halotolerant representatives, suggesting saltwater was their ancestral habitat. Our findings agree surprisingly well with traditional speleobiological biogeographic hypotheses (marine origin of continental stygofauna, active colonization of groundwater, poor dispersal). On the other hand, the frequently held view of multiple invasions of the subterranean world by surface ancestors has to be replaced by a quite different one – that of a common subterranean ancestor, albeit a marine one. This makes it much easier to understand why lack of eyes and depigmentation are found in every single one among hundreds of niphargid species.

van den Berg-Stein, Susanne
vandenberg@groundwaterecology.de

StygoTracing - a biological tracing method for underground waters.

van den Berg-Stein, Susanne[1]; Schwenk, Klaus[2]; Hahn, Hans Jürgen[1]

[1]Institute for Groundwater Ecology IGE GmbH, University of Koblenz Landau, Fortstr. 7, 76829 Landau, Rheinland-Pfalz, Germany
[2]Institute for Environmental Sciences, University of Koblenz Landau, Fortstr. 7, 76829 Landau, Rheinland-Pfalz, Germany,

Hydrological exchange processes, in particular groundwater-surface water-interactions, are essential for groundwater ecosystems and for the quality insurance of drinking water. These hydrological dynamics are assessed using various techniques, for example chemical tracers. However, the application is associated with a number of disadvantages, such as potential disturbance of natural habitats, dilution effects (low recovery rate), etc. Furthermore, to record the reoccurrence of the tracers, you have to be in the right spot at the right time.

Here, we present a promising alternative technique to trace hydrological processes using biological tracers. We use a population genetic approach, which allows for estimating the similarity of individuals and populations and thus the connectivity in natural and man-made systems (e.g. water supply networks). This “StygoTracing” approach was applied to uncover the origin of surface water leakage into subsurface waters in the framework of a drinking water quality assessment for a water supplier. Currently, we are extending this approach to characterize hydrodynamic processes in catchments and water protection areas, and are searching for representative catchments and appropriate biotracer species across Central Europe.

Special Session: Subterranean Microbial Systems
Keynote Address

Wang, Hongmei
wanghmei04@163.com

Geomicrobiology study in Heshang Cave, central China.

Wang, Hongmei*; Yun, Yuan; Man, Baiying; Zhou, Jianping

State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan, 430074, P R China

Microbial biota and their potential function in different dark, oligotrophic habitats in Heshang Cave, including sediment, weathered rocks, bat guano, and dripping waters, were conducted via 16S rRNA sequencing and functional gene analysis coupled with isolation (culture-dependent) techniques. *Penicillium* dominated all culturable fungal communities from cave sediments, weathered rocks, and bat guanos, although β-diversity indices indicated significant compositional and abundance differences between fungal communities. Culture-independent sequencing indicated a high specificity of bacterial communities in each individual habitat. Specifically in cave sediments, phyla included Chloroflexi, Nitrospirae, Gemmatimonadetes, and Firmicutes, but Actinobacteria dominated the weathered rocks and Proteobacteria dominated the dripping waters. Potential nitrification rate analysis coupled with the quantification of *amoA* genes and crenarchaeol analysis strongly indicated potential contributions of ammonia-oxidizing archaea to nitrification in Heshang Cave. Our results answer fundamental questions about the subsurface microbial world, such as “who are they and what they are doing?”

Symposium: Conservation of Subterranean Fauna and Habitats
Keynote Address

Watson, Cyndee
Cyndee_Watson@fws.gov

Managing the spread of *Pseudogymnoascus destructans* and conserving bats threatened by White-nose Syndrome in North America.

Coleman, Jeremy T. H.[1]; Reichard, Jonathan D.[1]; Geboy, Richard[2]; Kocer, Christina[1]; Watson, Cyndee[3]*

[1]U.S. Fish and Wildlife Service, Hadley, Massachusetts, USA
[2]U.S. Fish and Wildlife Service, Bloomington, Indiana, USA
[3]U.S. Fish and Wildlife Service, Austin, Texas, USA

White-nose syndrome (WNS) is an infectious disease, caused by the fungus *Pseudogymnoascus destructans (Pd)*, which is responsible for decimating hibernating bat populations in eastern North America. WNS continues to spread and now has been confirmed in 7 North American bat species in 27 states and 5 Canadian provinces. The fungus infects torpid bats resulting in physiological and behavioral impacts, often leading to mortality. Corresponding population declines exceeding 90% have been documented at many hibernacula. *Pd* was likely recently introduced to North America and it has been documented on numerous bat species across Europe and Asia. Coordinated plans in both the U.S. and Canada provide the framework for a comprehensive North American response and working groups have been established to address the research and management needs for affected bats. The U.S. Fish and Wildlife Service is the lead federal agency coordinating the response to WNS in the U.S., and since 2008 the agency has allocated over $32 million to the response. The Service has provided funding for important research that has led to promising disease treatment and management strategies, as well as advancements in our understanding of bat hibernation physiology, population dynamics, disease ecology, and general bat behavior. Collaboration between the many groups engaged in the WNS response remains critical to continue the considerable advances we have made in our understanding of this disease and conserving bats.

Watson, Cyndee A.
Cyndee_Watson@fws.gov

How the Endangered Species Act protects subterranean fauna in central Texas.

Watson, Cyndee A.

United States Fish and Wildlife Service, Austin Ecological Services Office, Austin, Texas, USA

The protection of caves and subterranean fauna has typically been a challenge in rapidly urbanizing areas. This is the case in the central Texas area. There are sixteen species of federally endangered terrestrial karst invertebrates and two aquatic invertebrates that occur in caves, karst, and springs near Austin and San Antonio, Texas. Since these species are federally endangered, they are afforded protection under the Endangered Species Act. As a result, recovery plans have been completed, preserves have been established, many habitat conservation plans have been finalized, and several section 10(A)(1)(b) permits have been issued by the United States Fish and Wildlife Service to protect these species. While these conservation tools are in place, there are still constant challenges such as the rising cost of land, funding, willing sellers, finding adequate undeveloped land around a cave to protect, and lawsuits. Here, we will give a brief history of what has been done to protect these species while dealing with many conservation challenges.

Weitowitz, Damiano Claudio
weitowid@roehampton.ac.uk

Investigating the distribution determinants of groundwater animals at the UK scale.

Weitowitz, Damiano Claudio[1]; Maurice, Louise[2]; Robertson, Anne[1]

[1]Department of Life Sciences, Whitelands College, University of Roehampton, London, United Kingdom
[2]British Geological Survey, Maclean Building, Wallingford, Oxfordshire, United Kingdom

Geological habitats harbour unique assemblages of obligate subterranean (stygobite) organisms, which contribute significantly to global biodiversity. However, the relative importance of factors controlling stygobite distributions is still debated and no UK-scale investigation has been carried out to date. Our aim was to explore to what extent geology or other environmental parameters determine the observed faunal distribution patterns. Using a novel groundwater habitat typology based on hydrogeological and –chemical characteristics, we examined the distribution pattern of stygofauna in England and Wales. Long-term survey data was pooled from several sources to provide the most complete UK stygobite dataset to date. Stygobite abundance and diversity differed significantly between geological habitats, with the most complex communities found in karstic habitats. Fractured rocks, such as mudstones, harboured significantly lower stygobiont diversity and abundance. Although the quality of geological habitats appears to be important in determining species distributions, other factors such as dispersal may be equally important. Species like *Niphargus kochianus* and *Niphargus fontanus* occur mainly in karstic habitats and may be excluded from adjacent habitats via geological control, such as in Lower Greensand where pore spaces may be too small to live in. Other species, such as *Microniphargus leruthi* and *Niphargus aquilex* were found to be habitat generalists, probably using hyporheic corridors for dispersal. *N. glenniei*, a local generalist, may be limited to south-west England due to the presence of mudstone barriers preventing dispersal. The chemical conditions species were found in illustrate the nature of their different habitats and indicate that some species may be more tolerant to fluctuating environmental conditions (e.g. oxygen or temperature) than others. Overall, our results indicate that distribution patterns are the result of many interacting factors and that the determinants may vary by species.

Worsham, McLean
biolyth@txstate.edu, mworsham@bio-west.com

The effect of selection on the phenotype of response to light in subterranean, epigean, and interstitial Crangonyctidae.

Worsham, McLean*[1]; Nair, Parvathi[1]; Nowlin, Weston[1]; Gibson, Randy[2]; Schwartz, Benjamin[1]

[1]Texas State University, Department of Biology, Aquatic Station, San Marcos, Texas 78666, United States
[2]U. S. Fish and Wildlife Service, San Marcos Aquatic Resources Center, 500 East McCarty Lane, San Marcos, Texas 78666, United States

Amphipods in the Crangonyctidae occupy a variety of epigean, interstitial, and subterranean habitats across much of North America. The subterranean species usually have vestigial eyes or are without eyes while the surface species have well developed compound eyes. Despite being eyeless, subterranean amphipods in other families have been shown to have the capacity to detect and respond to light. We studied the response of four subterranean eyeless species, one interstitial species with reduced eyes, and one epigean species with eyes. We found that two of the four subterranean species not only responded to light but also found exposure to light to be stressful. We also found that the interstitial species became stressed by light. In an attempt to explain these results we tested two hypotheses; the first hypothesis suggests that phylogenetic nearness best explains response to light while the second hypothesis suggests that selection of the various habitats best explains response to light. We found that habitat best explained the variation in our results. To rationalize these findings, we postulate that selection in the environment drives the various species to converge on phenotypes best suited to their particular habitat. We attribute these findings to selection because only the species with reduced or absent eyes that live near the surface and thus likely regularly encounter surface habitats invested effort in avoiding direct light which is not surprising considering being found above the surface is almost certainly an evolutionary dead end for organisms that cannot actively avoid predation; nor should a subterranean species expect to find a mate in a surface habitat and thus procreate.

Symposium: Cave Animals at the Frontiers of Modern Biology

Yoshizawa, Masato
yoshizaw@hawaii.edu

Adaptation through changes of behavioral and morphological traits in Mexican Cavefish.

Yoshizawa, Masato*[1]; Settle, Alexandar[1]; Macaspac, Christian[1];
Fernandes, Vania[1]; Yoshida, Mina[1]; Keene, Alex[2]

[1]Department of Biology, University of Hawai‘i at Mānoa, Honolulu, United States
[2]Department of Biological Sciences, Florida Atlantic University, Boca Raton, United States

Cave animals have been evolving under no visual information and with different food sources from surface environment. Accordingly, cave animal evolved multiple traits including enhanced sensory system and shifted physiology in nervous systems. *Astyanax mexicanus* is a model cave animal with sighted surface-dwelling and blind cave-dwelling forms, whose surface ancestors were separated in caves 1 - 5 million years ago. Here we describe multiple cave associated behaviors, adaptation, and their sensory basis. First, cavefish (but not many surface fish) exhibit vibration attraction behavior (VAB), or the ability to swim toward the source of a water disturbance in darkness. Individual fish expressing this VAB showed a significant advantage in foraging comparing with the fish without VAB. VAB is mediated by mechanosensory superficial neuromasts (SN), which are enhanced in number and sensitivity in cavefish. Endothelin signaling regulates the cranial SN number through the intramembranous bone formation. The treatment of Endothelin inhibitor, BQ123, in one month old-surface fish, successfully enhanced the cranial SN number but didn't increase VAB level, suggesting that the sensory enhancement may be not enough for the evolution of adaptive foraging behavior. Secondly, cavefish lost their sleep and gained hyper activity through evolutionary process. The treatment of human psychiatric drugs, Fluoxetine (selective serotonin reuptake inhibitor) and Clozapine (inhibitor for multiple serotonin and dopamine receptors) but not Naltrexone (inhibitor for opioid receptors), significantly recovered sleep and reduced the activity in cavefish, suggesting that, in certain parts, cavefish behavior can be based on the overlapped neural pathways with human patients. Finally, we are using genotyping-by-sequencing technology to genome wide association mapping for VAB, SN, sleep and hyper activity. We will discuss some candidate genes for these traits, which will provide the bases of a detailed evolutionary example for cave-type behaviors.

Poster Session

Yun, Yuan
yunyuan2009cug@gmail.com

Both pH and organics shape the bacterial communities across various habitats in a karst ecosystem in central China and their implications.

Yun, Yuan*[1]; Wang, Hongmei[1, 2]; Man, Baiying[1]; Xiang, Xing[1];
Zhou, Jianping[1]; Qiu. Xuan[1]; Duan, Yong[1]

[1]State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan 430074, P. R. China
[2]Laboratory of Basin Hydrology and Wetland Eco-restoration, China University of Geosciences, Wuhan, 430074, P. R. China

The impact of environmental conditions on microbial distribution across complex habitats is poorly understood. To address this issue, samples were collected from overlying soils, dripping waters, sediments, and weathered rocks from a karst cave in central China exposed to increasing acid rainfall. 16S rRNA genes were sequenced using an Illumina platform. A high level of specificity for microbial communities was observed for the individual habitats, despite geographical location similarities. The pH, total organic carbon (TOC), and potassium content significantly explained the distribution of bacterial communities. This is the first demonstration that pH is one of the most important environmental factors shaping the bacterial communities across different cave habitats. The wide occurrence of heterotrophic bacteria in the cave habitats was explained by TOC. Future changes in microbial communities in karst ecosystems are inevitable, and these changes will result from the variation in the amount of monsoonal precipitation and acid rainfall in central China. Microbial contributions to the cave carbon cycle will be affected, such as increase, in the future due to the acidification of the alkaline system. Our work helps to decipher the scenario of response in a cave ecosystem undergoing global change.

Zagmajster, Maja
maja.zagmajster@bf.uni-lj.si

The neglected subterranean biodiversity hotspot under threat: can we protect the aquatic interstitial fauna of the Sava River in the Balkans (Europe)?

Zagmajster, Maja*; Sket, Boris; Remškar, Anja; Prevorčnik, Simona

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

Aquatic interstitial habitats along watercourses receive far less scientific and conservation attention than caves, despite harboring numerous exclusively subterranean invertebrates. Many of them are bound to interstitial subterranean habitats only, and can display narrow distribution ranges. Consequently, they are potentially more prone to extinction due to poor river management practices and water pollution. In the Balkans, many rivers are about to be dammed as the hydropower plants (HPPs) are considered to be "green energy". This applies also to the Sava River, harboring one of the world's richest interstitial faunas. Although the latter is greatly endangered by the existing and planned HPPs, it is completely ignored in the environmental impact assessments of all construction works and no potential mitigation measures are given. Based on the example of Slovenia, there are also other impediments that hinder interstitial fauna protection:1) Temporally and spatially deficient data: studies from the 1980's revealed 29 stygobionts from limited area of the Sava river only, while data from other river sections and periods are lacking. 2) Lack of taxonomists for the minute, diverse and endemic taxa. 3) Inadequate implementation of legislation. In 2015, we conducted the systematic sampling of the Sava River in Slovenia, using Bou Rouch pump at several sites. Our preliminary results show high interstitial biodiversity at two previously unexplored sites near the Slovenian-Croatian border, where we recorded 17 different stygobiotic taxa. Some are listed under the Slovenian and/or **IUCN Red list** of threatened species, some present the first records for the country, and some are new, yet undescribed species**.** Despite their importance and uniqueness, these sites seem to be doomed to vanish. Unfortunately, the presented interstitial fauna conservation issues are not bound to state borders and can apply worldwide.

Poster Session

Zagmajster, Maja
maja.zagmajster@bf.uni-lj.si

Steps toward understanding the drivers of terrestrial subterranean biodiversity on a regional scale

Bregović, Petra[1,2]; Zagmajster, Maja*[1]

[1]Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
[2]Croatian Biospeleological Society, Zagreb, Croatia

Drivers of biodiversity patterns in subterranean habitats are poorly known, especially in the terrestrial domain. This is most likely due to lack of extensive and quality data on species distribution as well as on potential explanatory variables. Dinarides in SE Europe are a global subterranean biodiversity hotspot, with data on subterranean species being gathered in the extensive georeferenced database on distribution of subterranean species (SubBio Database). Additionally, many spatially defined environmental datasets had recently become available, offering a unique opportunity for in depth studies of biodiversity drivers. In the Dinarides, subterranean beetles are the richest and best studied group of terrestrial subterranean animals, and can be regarded a model group for terrestrial subterranean fauna. In the first step, we checked the three main hypotheses on the drivers of species richness. In our models the biggest importance for shaping the observed species richness pattern of subterranean beetles was expressed by habitat heterogeneity, followed by historical climate stability. Somewhat unexpectedly, the importance of productive energy turned out lowest, which is probably due to small variation of productivity within the region. A closer look to the two main taxonomic groups of subterranean beetles revealed some differences in most important drivers of species richness, implying the need to consider also the influence of biological traits. So, in the next steps, we collected the information on species body sizes. Body size can be regarded a simple surrogate for species characteristics, like its spatial niche, or its dispersal possibilities. Dispersal limitation is one of the major constraints influencing species range, so adding body size should help in better understanding the species richness and range size patterns.

Symposium: Patterns of Subterranean Biogeography and Biodiversity

Zigler, Kirk S.
kzigler@sewanee.edu

Long-term cave adaptation and diversification in the *Ptomaphagus hirtus*-group (Coleoptera: Leiodidae: Cholevinae).

Leray, Vincent L.[1]; Zigler, Kirk S.*[2]; Friedrich, Markus[3]

[1]American University, Washington, District of Columbia, United States
[2]University of the South, Sewanee, Tennessee, United States
[3]Wayne State University, Detroit, Michigan, United States

Round fungus beetles (Coleoptera: Leiodidae) have colonized caves on numerous occasions worldwide. The *hirtus*-group species assemblage of the genus *Ptomaphagus* (Leiodidae: Cholevinae) stands out for its highly endemic, cave-dwelling representatives in the southeastern United States, which exhibit troglomorphism including the loss of hind wings and extreme reduction of eyes. Morphological and biogeographic data were previously interpreted to suggest the *hirtus*-group diversified through a sequence of speciation and cave colonization events within the past 350,000 years. Our molecular analysis of 2300 nucleotides from three mitochondrial regions for all 19 species of the *hirtus*-group paints a different picture. Most notably, molecular clock estimation reveals that most speciation events in the *hirtus*-group date from 3-10 million years ago, characterizing the *hirtus*-group as an example of long-term cave adaptation. Twelve species of the *hirtus*-group are known from the southern Cumberland Plateau in Tennessee and Alabama. This clade originated ~7 mya and vicariance related to the erosion and fragmentation of the Cumberland Plateau likely contributed to its diversification.

Zigler, Kirk S.
kzigler@sewanee.edu

Reproductive seasonality in *Nesticus* (Araneae: Nesticidae) cave spiders.

Carver, Linnea M. [1]; Perlaky, Patricia[2;], Cressler, Alan[3]; Zigler, Kirk S.[1]*

[1]University of the South, Sewanee, Tennessee, United States
[2]Raccoon Mountain Caverns, Chattanooga, Tennessee, United States
[3]Atlanta, Georgia, United States

Spiders of the family Nesticidae are members of cave communities around the world, with cave-obligate (troglobiotic) species known from North America, Europe, Asia and the Indo-Pacific. A radiation of *Nesticus* (Araneae: Nesticidae) in the southern Appalachians includes ten troglobiotic species. Many of these species are of conservation interest due to their small ranges, with four species being single-cave endemics. Despite conservation concerns and their important role as predators in cave communities, we know little about reproduction and feeding in this group. We addressed this knowledge gap by examining populations of two species on a monthly basis for a year. We made further observations on several other species and populations, totaling 671 individual spider observations, more than doubling reported observations of reproduction and feeding in troglobiotic *Nesticus*. Female *Nesticus* carry egg sacs, facilitating the determination of the timing and frequency of reproduction. We found that *Nesticus* exhibit reproductive seasonality, carrying egg sacs from May through October, with a peak in frequency in June. These spiders were rarely observed with prey; only 3.3% (22/671) of individuals were observed with prey items. The frequency at which prey items were observed did not vary by season. Common prey items were flies, beetles and millipedes, with troglobiotic species comprising approximately half of all prey items observed. This result represents a greater proportion of troglobiotic prey than has been reported for various troglophilic spiders. Our findings shed light on the life history of troglobiotic *Nesticus* and on their role in cave ecosystems but further work are necessary to support effective conservation planning for many of these rare species.

Index to Authors & Coauthors