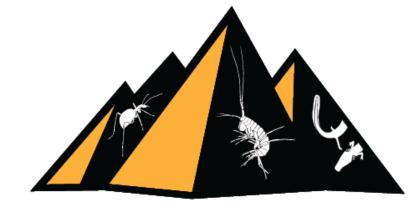
# Abstract book



## **2**<sup>nd</sup> **Dinaric Symposium** on Subterranean Biology

18<sup>th</sup> - 19<sup>th</sup> October 2019 Postojna, Slovenia

Abstract book

18<sup>th</sup> – 19<sup>th</sup> October 2019, Postojna

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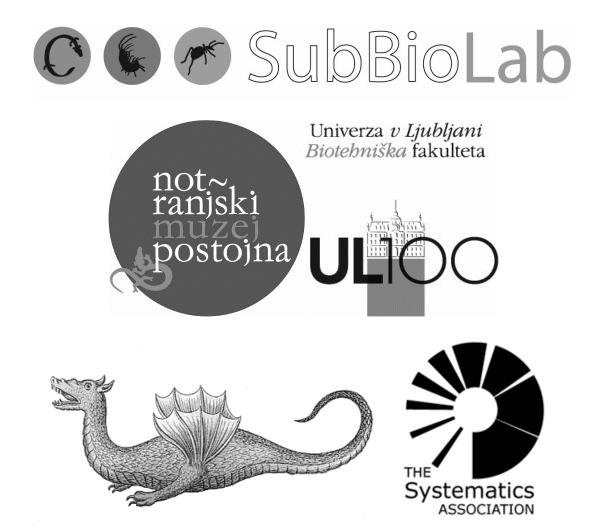
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#### Foreword from the coordinators

Dear friends and admirers of the unique subterranean life of the Dinaric Karst, welcome to the 2<sup>nd</sup> Dinaric Symposium on Subterranean Biology!

The Symposium is jointly organized by the team of University of Ljubljana (SubBioLab, Department of Biology, Biotechnical Faculty) and Notranjska Museum Postojna. It will be held on 18th and 19th of October 2019 in Postojna, Slovenia.

Postojna is considered as "the cradle of speleobiology". It is a place where the whole story of uncovering the specialized, cave-adapted life forms begun following to the discovery of the blind cave beetle *Leptodirus hochenwartii* in 1832.

Now, almost 200 years later, we try to follow the idea set on the 1<sup>st</sup> Dinaric Symposium in Zagreb (2016), to establish and deepen the networks between scientists and amateurs, interested in rich subterranean biodiversity of the Dinaric Karst. This includes a wide array of topics, related to subterranean world - systematics, biogeography, evolution, ecology, biodiversity, conservation, etc.

The organizers are grateful to the following sponsors and supporters: Systematic Association (1500 €), International Society for Subterranean Biology, and Youth Hostel Postojna, which offered accommodation for participants at reduced price.

Most of the funds was allocated to cover the costs of lunch, coffee breaks, and fees and travel costs for colleagues from countries with limited funds.

Teo Delić, Chair of the Organizing Committee

#### Committees

#### Organizing committee

Teo Delić, chair Slavko Polak Maja Zagmajster Špela Borko Ester Premate Žiga Fišer Valerija Zakšek Simona Prevorčnik Cene Fišer Peter Trontelj

#### Scientific committee

Maja Zagmajster (SI) Helena Bilandžija (CRO) David C. Culver (USA) Cene Fišer (SI) Dragan Antić (SRB Peter Trontelj (SI)

#### Program of the 2<sup>nd</sup> Dinaric Symposium on Subterranean Biology

18<sup>th</sup> October 2019 Notranjski muzej Postojna

- 8.30 Registration
- 9.45 Opening ceremony

#### Session 1: Faunistics and historical overview (Chair: A. Komerički)

- 10.00 The Balkan Peninsula one of the world's major hotspots of troglobitic millipedes (Myriapoda: Diplopoda)
   Antić, Makarov
- 10.15 *Proteus* 330 years since Valvasor's description *Aljančič*
- 10.30 New subterranean leptodirine leiodid beetle (Coleoptera: Leiodidae: Cholevinae: Leptodirini) taxa from the Dinarides *Ćurčić, Vesović, Pavićević, Mulaomerović, Antić, Bosco, Marković, Petković, Rađa*
- 10.45 Cave spiders of Montenegro *Komnenov*
- 11.00 11:30 Coffee break (Poster Session)

#### Session 2: Threats and Conservation of Subterranean Fauna (Chair: H. Bilandžija)

- 11.30 Type localities and conservation of subterranean beetles in Slovenia *Zagmajster*, *Premate*, *Bregović*, *Delić*, *Fišer C*.
- 11.45 Clean Underground: a bottom-up model for building a national cave protection system *Novak*
- 12.00 Microbial and parasitic associations of proteus (*Proteus anguinus*) *Kostanjšek*
- 12.15 Monitoring of cave habitats and fauna: a case study from Croatia **Dražina**, Miculinić, Komerički, Bedek, Bregović, Jalžić, Kutleša, Lukić, Pavlek, Kuharić, Rožman
- 12.30 Global Red List assessments of Dinaric cave invertebrate fauna *Komerički, Deharveng*

#### 13.00 – 14.30 Lunch

#### Session 3: Diversification and diversity in the Dinaric Karst (Chair: T. Delić)

- 14.30 A synopsis of *Alpioniscus* (*Illyrionethes*) recent research in the Dinaric Karst *Bedek*, *Gottstein*, *Taiti*
- 14.45 Hidden radiation: Niphargus in the Dinaric Karst **Borko**, Jalžić, Fišer C.
- 15.00 Molecular phylogeny of the subterranean genus Verhoeffiella (Collembola, Entomobryidae)
   Lukić, Delić, Pavlek, Deharveng, Zagmajster
- 15.15 The story of Dinaric cave-dwelling dysderid spiders *Pavlek*, Adrian, Gasparo, Arnedo

15.30 – 16:00 Coffee break (Poster session)

## *Session 4: Adaptation and differentiation to/in subterranean habitats* (Chair: M. Zagmajster)

- 16.00 Functional ecology of the genus Niphargus
   Fišer C, Altermatt, Copilas-Ciocianu, Delić, Fišer Ž, Jemec-Kokalj, Kralj-Fišer, Premate, Trontelj, Zagmajster, Zakšek
- 16.15 Trophic differentiation of *Niphargus* amphipods *Premate*, *Touchet*, *Borko*, *Delić*, *Malard*, *Simon*, *Fišer C*.
- 16.30 Asellids (Isopoda, Pancrustacea) of the World: a useful case study for understanding eco-evolutionary processes during the transition from surface water to groundwater *Malard*, *Lewis, Francois, Eme, Konecny-Dupré, Simon, Saclier, Grison, Lefébure, Douady*
- 16.45 Phenotypic plasticity as a mechanism of cave colonization and adaptation *Bilandžija*, Hollifield, Steck, Meng, Ng, Koch, Gračan, Ćetković, Porter, Renner, Jeffery
- 17.00 General activity and boldness of a freshwater isopod after transition from surface to cave environment
   *Fišer Ž., Kralj-Fišer, Balázs, Horváth, Herzeg\*, Fišer C\* (\*co-senior author)*
- 17.30 Notranjski Muzej Postojna tour, guided by Slavko Polak

#### 19<sup>th</sup> October 2019 Notranjski muzej Postojna

#### Session 5: Closing session (Chair: D. Antić)

- 9.00 Cave Cadaster of the Republic of Croatia as a tool for biospeleological research **Bedek**, *Đud, Komerički, Katušić*
- 9.15 Understanding species richness patterns of subterranean beetles in the Western Balkans through environmental variables, species distribution ranges and body sizes **Bregović**, Zagmajster
- 9.30 Limb regeneration in captive born juvenile of the European blind cave salamander *Bizjak Mali*, *Dolenc Batagelj*, *Gnezda*, *Kanduč*, *Sessions*
- 9.45 Expeditionary approach to biospeleological research example from Croatia *Kuharić*, *Kirin, Sudar*
- 10.00 Dispersal of subterranean amphipods during marine regressions *Delić*, *Stoch, Borko, Flot, Fišer C.*
- 10.00 Coffee break, Fieldtrip...Lunch at Rakov Škocjan

Evenning: Dinner organized at "Gostilna ob kaminu", Tržaška 88, Postojna

#### **Poster session**

Allozymatic studies of *Pterostichus fasciatopunctatus* (Creutzer, 1799) (Coleoptera, Carabidae): an innovative method to trace underground sections of hydrological basins. *Bonivento, Colla, Restaino* 

Speological organization Antroherpon Brestovanský, Čáha, Brestovanská

Mali Šibenik - great wonders of the underground Čuček, Belak, Pavlek, Dražina

Biospeleological research in the Kornati National Park **Čuković Malenica**, Kutleša, Bedek, Bregović, Dražina, Lukić, Pavlek, Rožman

1<sup>st</sup> Croatian National Report on Article 17 of the Habitats Directive for cave species and habitat type for the period 2013–2018 **Dud**, Bedek, Cindrić, Gambiroža, Katušić, Zadravec

A first insight into subterranean Dinaric Trechini (Carabidae: Trechinae) phylogeny *Faille, Lohaj, Jalžić, Delić* 

Exploring bats in a tunnel through Volujica hill (Bar, Montenegro) *Ivanović, Jovićević, Mulaomerović, Hasanspahić* 

Preliminary result of research in "Tomina jama", an anchialine cave, Neretva River Estuary, Croatia

Jalžić, Bishop Pierce, Cukrov, Nakić, Omanović, Cukrov, Sudar, Vuica

Biospeleological research of subterranean beetles in the higher plateau of National Park Paklenica Jalžić, Šarc, Sudar, Kuharić, Bregović

Mandible morphometry predicts diverse trophic niches in Leiodidae beetles *Klenovšek, Novak, Janžekovič, Polak, Kozel* 

The first species of the genus *Nemaspela* from Montenegro *Kozel*, *Delić*, *Novak* 

Review of bat research in Bosnia and Herzegovina *Mulaomerović* 

Diatoms – invisible residents of Resavska Cave (eastern Serbia) Nikolić, **Popović**, Subakov Simić, Vidaković, Krizmanić

Phototrophic biofilms in Serbian caves: exploration and features *Popović*, Krizmanić, Vidaković, Karadžić, Nikolić, Pećić, Subakov Simić

*Lithobius (L.) lapidicola* Meinert, 1872 (Chilopoda: Lithobiomorpha: Lithobiidae) – the most common troglophilic centipede from southwestern Bosnia and Herzegovina *Stojanović, Komerički, Antić* 

Developing new tools for rapid assessment of subterranean biodiversity in Bosnia and Herzegovina

Zagmajster, Borko, Delić, Fišer C., Fišer Ž., Mulaomerović, Prevorčnik, Trontelj, Zakšek

# Abstracts

# Oral communications and Posters

#### Oral Communication

#### Proteus - 330 years since Valvasor's description

#### Aljančič, Gregor<sup>1\*</sup>

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In his fundamental work Die Ehre des Herzogthums Krain [The Glory of the Duchy of Carniola] published 330 years ago, Janez V. Valvasor, one of pioneers of karstology, presented a short description of proteus (Proteus anguinus Laurenti 1768) - the first published record of a cave animal. Five years earlier, while Valvasor was returning from one of his study travels across the Duchy of Carniola (present-day central Slovenia), people from the area of Vrhnika told him about the Lintvern, a mysterious spring from which the water flows only twice a day. The next day, 25 June 1684, Valvasor followed this lead and visited the spring: its periodic flow seemed to have inspired a local legend of a dragon living in the hill behind the spring. Namely, when the groundwater is rising up to its throat, the dragon has to move causing the water to flow from the spring, which happens twice a day. As a proof, Valvasor was told about the finds of young dragons washed-out after heavy rain. Among many eyewitnesses, Valvasor also interviewed the Vrhnika's postal master Hofmann, who described the dragon he collected two years ago and displayed it to the public. Valvasor finally concluded, that the creature in question was, of course, not a mythological creature, but an animal shorter than a span, similar to a lizard – in sum, just an earthly reptile, which can be occasionally found here and there. Valvasor had never seen the animal or heard about it anywhere else, although visited several proteus localities. In fact, proteus has never been confirmed at the Lintvern nor it is likely to harbour the species. It seems that locals naively linked both phenomena: an exceptionally rare type of intermittent spring was explained by finds of proteus washed-out from many springs around Vrhnika, just tree kilometers away.

## The Balkan Peninsula – one of the world's major hotspots of troglobitic millipedes (Myriapoda: Diplopoda)

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The Balkan Peninsula is characterized by numerous unique and relict cave animals, both aquatic and terrestrial. This region is known as one of the world's major subterranean biodiversity hotspots. One of the Balkan's main groups of terrestrial arthropods, rich in troglobites, are millipedes. So far, there are about 150 described troglobitic or presumably troglobitic millipede species and 35 genera endemic to this region, altogether represented by approximately 50 genera, 13 families and five orders. Moreover, endemism concerns not only the generic or species level, as there are also three endemic chordeumatidan subfamilies inhabiting the Dinaric region, viz. Acherosomatinae, Biokoviellinae and Macrochaetosomatinae. The largest number of troglobitic species belong to the order Chordeumatida (57 species in 21 genera and six families), followed by Polydesmida (40 species from 13 genera and three families), Julida (30 species, eight genera and one family), Glomerida (16 species, four genera and two families) and Callipodida (one species). Endemic Balkan genera are particularly numerous in the order Chordeumatida, with 18 genera, while Polydesmida, Julida, Glomerida and Callipodida are far less diverse, with nine, six, one and one endemic genus, respectively. Such an extraordinary rich fauna of subterranean millipedes, as presently estimated in the Balkan Peninsula, is far from completely known. During the last decade alone, a large number of new taxa have been described. The complex and long geological history, coupled with palaeoclimatic changes, resulted in a great subterranean biodiversity in the Balkans, a region which indeed merits the term of one of the main biodiversity hotspots of endogean fauna globally.

#### Cave Cadaster of the Republic of Croatia as a tool for biospeleological research

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The Cave Cadaster of the Republic of Croatia is the culmination of a long history of cave research and organized gathering of data on caves. It was established by the State Institute for Nature Protection (at present Ministry of Environment and Energy), which provides the financial and technical support and coordination, in collaboration with numerous Croatian caving clubs, which provide verified data. Currently, 27 caving clubs, along with Croatian Geological Survey, are involved in the Cadaster, which now comprises of 2448 verified caves and 424 caves awaiting verification. This is still less than 30% of all explored caves in Croatia, assuming that the estimate of 10.000 explored caves is correct. The Cadaster was a response to the vast knowledge on caves in Croatia, scattered in numerous archives of caving clubs and thousands of publications of both speleological and biospeleological nature. The need to organize the data, resolve countless synonyms, verify decades old information and make them available to the caving community as well as the Croatian Nature Protection Sector and public institutions dealing with protecting life and property led to its creation in 2015. Generalized data are available to public through web portal of NPIS – Bioportal (http://www.bioportal.hr/). High quality data is a priority, maintained by a complex methodology for data collection. This data will provide the basis for biospeleological analyses along with faunistic data compiled under CroFauna database. Both databases are a part of the Nature Information Protection System, and will be available to expert community for research and conservation planning.

#### A synopsis of Alpioniscus (Illyrionethes) recent research in the Dinaric Karst

Bedek, Jana<sup>1\*</sup>; Gottstein, Sanja<sup>2</sup>; Taiti, Stefano<sup>3,4</sup>

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The southern European subterranean genus *Alpioniscus* consists of two subgenera: Alpioniscus s. s. and Illyrionethes. In the Dinaric Karst 19 nominal troglobiotic species of Illyrionethes have been recorded, seven described in last two years. Molecular analyses using two mtDNA (16S rRNA and COXI) and a nuclear gene (H3) fragments have been performed, together with a detailed taxonomic study on the Dinaric species. Dinaric species grouped into three distinct lineages: strasseri-, heroldi- and magnus-lineage; which follow a northwest-southeast direction and have overlapping distributions. The strasseri-lineage was thoroughly sampled and highly supported by all phylogenetic methods employed, so a detailed morphometric analysis was performed on the species of this lineage. The analyses were carried out on the male dataset to identify and clarify new differentiating characters, and to distinguish similar species with higher precision. Besides sexually dimorphic characters of male 7<sup>th</sup> pereopod and 1<sup>st</sup> and 2<sup>nd</sup> pleopods, body size and other characters were analysed. Due to observed differences in body size between species and populations, ratios are considered adequate to eliminate the size influence, and were statistically analysed. A high variability in non sexual characters was observed. On the other hand sexually dimorphic characters showed statistically significant differences between species. As a result, novel morphological characters (ratios of length and hump ending point of male pereopod 7<sup>th</sup> carpus; length and concavity turning point of male 1<sup>st</sup> pleopod exopod) are proposed for future species identifications of the *strasseri*-lineage species.

#### Phenotypic plasticity as a mechanism of cave colonization and adaptation

Bilandžija, Helena<sup>1,2\*</sup>; Hollifield, Breanna<sup>1</sup>; Steck, Mireille<sup>3</sup>; Meng, Guanliang<sup>4,5</sup>; Ng, Mandy<sup>1</sup>; Koch, Andrew<sup>6</sup>; Gračan, Romana<sup>7</sup>; Ćetković, Helena<sup>2</sup>; Porter, Megan<sup>3</sup>; Renner, Kenneth<sup>6</sup>; Jeffery, William<sup>1</sup>

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A widely accepted model for the evolution of cave animals posits cave colonization by surface dwelling ancestors followed by the acquisition of adaptive traits over many generations. However, the speed of cave adaptation in some species is difficult to reconcile with this conventional viewpoint, suggesting the importance of alternative mechanisms operating over shorter timescales. To address these mechanisms, we used Astyanax *mexicanus*, a teleost with multiple populations of conspecific cave-dwelling morphs (cavefish, CF) and an ancestral-like surface dwelling morph (surface fish, SF). We exposed SF to completely dark (dark/dark = D/D) conditions during early development and compared them to siblings placed on a standard photoperiod (light/dark = L/D). Numerous altered traits were identified in D/D SF compared to L/D SF at both the gene expression and phenotypic levels, including changes related to endocrine signaling, circadian rhythms, metabolism (especially in lipids), eye anatomy, pigmentation, metabolic rates, starvation resistance, and neurotransmitter levels. Remarkably, most of these alterations mimicked CF phenotypes. Our results indicate that many cave-related traits can be induced by darkness, the main feature of subterranean habitats, and can appear within a single generation by phenotypic plasticity. In the next generation, plasticity can be enhanced and traits further refined. The initial plastic responses are random in adaptive outcome but may determine the subsequent course of adaptive evolution. Our study suggests that phenotypic plasticity contributes to the rapid evolution of cave-related traits, enabling a successful transition of A. mexicanus to dark cave habitats. Future studies using Dinaric cave fauna will address whether plasticity is a general phenomenon in the colonization and adaptation to cave environments.

#### Limb regeneration in captive born juvenile of the European blind cave salamander

Bizjak Mali, Lilijana<sup>1\*</sup>; Dolenc Batagelj, Katja<sup>2</sup>; Gnezda, Primož<sup>2</sup>; Kanduč, Katarina<sup>2</sup>; Sessions, Stanley<sup>3</sup>

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Urodele amphibians are unique among adult vertebrates in the ability to regenerate structurally complete and functional limb. According to the old records Proteus anguinus can regenerate tail fin and the limbs. Occasionally, regeneration resulted in extra or missing digits or other deformities. In adult Proteus it took more than 1 year after wound healing for the limb to begin to regenerate. A recent egg-laying event by captive Proteus anguinus at the Postojna Cave provided the opportunity to implement "optimal artificial" (OA) conditions for raising this vulnerable amphibian species in captivity. After three years under OA conditions, 92% of the juveniles continue to thrive. These captive-raised juveniles allow us to observe the dynamics of their growth and behavior. At 1.5 years the juveniles began to exhibit aggressive behavior, and limb injury happened during the fight, which allowed us to observe the process of limb regeneration. Five days following the injury, the damaged portion of the limb died and detached followed by wound healing that was completed within one week. The limb stump was initially swollen and inflamed. A blastema formed after 6 weeks and full regeneration took almost 1 year. The regenerate was first a smaller replica of the original limb which than continued to grow over the next few months. After 1.5 years the regenerate has almost reached the size of the original limb. Overall, limb regeneration in *Proteus* is similar to other salamanders but occurs at a much slower rate. This slow rate of regeneration in *Proteus* is probably due to its large genome size (approx. 47.9 Gb of DNA) which, in combination with lower temperature, slows the rate of cellular processes. As far as we know, this is the first time that limb regeneration has been described chronologically in a Proteus juvenile.

# Allozymatic studies of *Pterostichus fasciatopunctatus* (Creutzer, 1799) (Coleoptera, Carabidae): an innovative method to trace underground sections of hydrological basins. First results

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For over a decade, the Adriatic Society of Speleology has undertaken systematic studies of hydrological basins. The Karst offers a remarkable variety of such case studies, as it lies at the crossroads of the Isonzo, Timavo and Danube basins. The past biological samplings in the caves through which the Timavo-Reka river flows were carried out irregularly, with some surveys done more than 10 years ago. For about a year, authorized biomonitoring was systematically resumed through the aforementioned caves (Jama Sežanske Reke, the cave of the Cava Marmor and the Škocjan Caves in Slovenia, together with the Luftloch, Trebiciano abyss, and Martel cave in Italy). Research has begun on the invertebrate fauna present which is yielding interesting results, also as regards the presence or not, of subsoil waters and the specific hydrological basin to which these belong. The study of the population genetics has begun through the analysis of allozymes of beetles of the species Pterostichus fasciatopunctatus, both those recently captured and those preserved for more than 100 years in Trieste's Natural History Museum. This method, innovative in terms of beetles, has thrown up interesting correlations between the allelic variants present in the caves and the caves' catchments, correlating the corresponding genetic types identified with the various river basins. Progress was made on the "mystery" of the Martel cave (cadastral number 144 VG) near Prosecco (Trieste), using old Museum specimens of Pterostichus fasciatopunctatus. Other interesting results also emerged from the biological survey conducted in the cave of Cava Marmor, in Slovenia.

#### Niphargus in the Dinaric Karst

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Subterranean genus *Niphargus*, counting over 400 described species, comprises one of the most speciose clades of freshwater amphipods in the World. Its distribution ranges from Ireland to Iran. With approximately 30% of described species, the genus diversity peaks in Dinarides, the subterranean diversity hotspot. 180 years after the very first described dinaric *Niphargus* species, and after decades of intensive studies, genus' overall diversity in this region is still not known. Cryptic species are common and accurate species identification is more often than not impossible without molecular data. In past years, molecular studies have shown several taxonomically yet unresolved species complexes. With aim to inventory and address the question of criptycity of *Niphargus* in the Dinarides, we dug into the SubBio Lab collection, and systematically sequenced all samples collected over the last decades. So far, we sequenced COI fragment from 892 specimens, from 424 localities. For 83% of localities we did not have previous molecular data. Preliminary results show that the extent of hidden diversity is unexpectedly high, despite extensive work that was already done in this area.

### Understanding species richness patterns of subterranean beetles in the Western Balkans through environmental variables, species distribution ranges and body sizes

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Species richness of subterranean beetles in the Western Balkans shows a distinct pattern of two hotspots: one in the northwestern and the other in the southeastern part of the region. We assembled the spatially equipped dataset of over 430 species of subterranean beetles in the Dinarides and Eastern part of Southern Calcareous Alps, mapped them onto 20x20 km quadrat grid and tested the importance of different factors in forming the species richness patterns (SRPs). First, we used environmental variables to test the importance of three hypotheses (productive energy, habitat heterogeneity and historical climate stability) in explaining SRPs. Second, we tested the importance of species range sizes, using species maximum linear extent as a measure of range size. Third, we linked the importance of species body sizes to shaping overall SRPs. We have shown that even though the relative importance of environmental factors differed among taxonomic groups of subterranean beetles, it was habitat heterogeneity that received the biggest support, followed by historical climate stability. Despite the large proportion of single site species, it was the common (large ranged) species that contributed more than rare (small ranged) species to forming the overall SRPs. When species were separated in classes according to body sizes, richness patterns of both small and large species were related to overall SRPs. Inclusion of the range of body sizes per quadrat in the model using environmental variables significantly increased the amount of explained variation in species richness. This suggests that range of different body sizes could be regarded as a surrogate for number of different spatial niches within the quadrats. We have shown that both extrinsic and intrinsic factors should be studied in investigating the overall SRPs. Such understanding of complexity of factors influencing SRPs should be considered in improving conservation strategies of subterranean habitats in the whole region.

#### **Speological organization Antroherpon**

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The presented contribution briefly introduces to the history, activities and some results of a renewed Speleological organization Antroherpon (Praha, Chech Republic). The group incorporates both amateurs and scientists, who operate a wide range of speleological activities. In a recent time, it is mainly biospeleological exploration of caves in the Czech Republic, but also in foreign countries. Beetles (especially Carabidae, Leiodidae, Staphylinidae), spiders, pseudoscorpions and harvestmen belong to the main groups of interest. Some members are specialists from scientific institutes, e.g. the Czech Entomological Society, Czech Arachnological Society or Institute of Soil Biology.

### New subterranean leptodirine leiodid beetle (Coleoptera: Leiodidae: Cholevinae: Leptodirini) taxa from the Dinarides

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As a result of exploration of biodiversity in subterranean habitats of the Dinarides in recent years, seven leptodirine leiodid taxa (six species and one subspecies), new to science, were described in 2018. Delimitation of the new taxa is based on a detailed observation and comparison of all relevant morphological features (28 different morphological measurements and ratios), but, above all, the structure of genitalia. The genus Adelopidius Apfelbeck, 1907 currently comprises 10 species, including the three recently described: Adelopidius hrustovacensis Ćurčić, Pavićević & Mulaomerović, 2018, A. ljubacevoensis Ćurčić, Pavićević & Mulaomerović, 2018 and A. pougarjensis Ćurčić, Pavićević & Rađa, 2018. These inhabit mountainous areas in Bosnia and Herzegovina. The genus Pholeuonopsis Apfelbeck, 1901 now includes 16 species and six subspecies of both, troglobitic and endogean beetles, ascribed to the three subgenera: Pholeuonopsis s. str., Scotosites Knirsch, 1929 and Silphanillus Reitter, 1903. Two most recently described species from western Serbia are Pholeuonopsis (Pholeuonopsis) tarensis Ćurčić & Pavićević, 2018 and P. (P.) lupi Ćurčić & Pavićević, 2018. Pholeuonopsis taxa are distributed in a wider Dinaric area (Serbia, Montenegro and Bosnia and Herzegovina). The genus Leonhardia Reitter, 1901 comprises totally six species (including newly described Leonhardia sebesicensis Ćurčić, Pavićević & Mulaomerović, 2018) and five subspecies of both cave-dwelling and endogean leiodid beetles, distributed in montane areas of central and southwestern Bosnia and Herzegovina. The genus Apholeuonus Reitter, 1889 contains totally five species and nine subspecies (most recently described subspecies is Apholeuonus nudus ledenjacensis Ćurčić, Pavićević & Antić, 2018). Apholeuonus taxa are known from caves situated in mountainous areas in both, central and eastern Bosnia and Herzegovina. Certain Dinaric karstic regions of Bosnia and Herzegovina and Serbia still remain unexplored. Thus, the findings of new leptodirine leiodid taxa may be expected in the future as well.

#### Mali Šibenik - great wonders of the underground

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"Mali Šibenik - veliko bogatstvo podzemlja "(Mali Šibenik- great wonders of the underground) was a project that has been conducted during two expeditions in 2018. It was organised by the Breganja Association in conjunction with the SAK Ekstrem-Speleological Club from Makarska and the city of Vrgorac. The project was carried out in the hills above Vrgorac. During the expeditions, 21 new caves were discovered, thirteen of which have been speleologically investigated and eleven caves were surveyed. A great number of caves have shown a high biodiversity of subterranean fauna and pointed out a need for further exploration of this area. Although the deepest explored cave in this project is Bokšića Golubinka (-50.6 m), the more interesting fauna has been found in shallower caves. For diplopods and spiders, the most significant caves are Jama Malog Ulaza, Golubinka u Gaju, Antipatična, Rođendanska Jama, Ošlja Jama i Ančića Velika Golubinka in which potentially new species have been found. Results of this project were presented via a poster exhibition, the "Mali Šibenik- veliko bogatstvo podzemlja "at Croatian anannual Speleogathering in Ogulin (2018). Also, some educational presentations for high school students and interested public were held in the city of Vrgorac. The main goal of these presentations was to raise environmental awareness among general public, especially the local youth.

#### **Biospeleological research in the Kornati National Park**

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During a two year period (2017-2018) first systematic biospeleological research in the Kornati National Park was conducted. Besides being protected as a national park, Kornati archipelago, with the area of 217 km<sup>2</sup> and a total of 89 islands and reefs, is also part of Croatian Natura 2000 network (site code HR4000001). Subject of interest of this study were two habitat types protected under the Habitats Directive: Caves not open to public (code 8310) and anchialine caves with terrestrial habitats as part of Submerged or partially submerged caves (code 8330). Animals were collected from 28 caves and three wells in spring and autumn. All explored caves were relatively small (up to 40 m length), the deepest one having 70 m vertical extent. Altogether 60 taxa from 12 taxonomic groups (Palpigradi, Araneae, Scorpiones, Isopoda, Amphipoda, Diplopoda, Collembola, Diplura, Orthoptera, Coleoptera, Hymenoptera (Formicidae), Psocoptera) were identified. Most diverse were spiders (Araneae) with 19 and springtails (Collembola) with 15 species. Ten taxa were classified as troglobionts. During these research one new species for science was described (isopod Alpioniscus drazinai Bedek, Gottstein & Taiti, 2019), while three potentially new species (Mesostalita sp., Troglopedetes sp., Onychiuroides sp.) and one potentially new genus (form diplopod family Trichopolydesmidae) wait for further analyses. The number of recorded troglobionts was relatively high, given the fact that most of explored caves were dry and small in size.

#### Dispersal of subterranean amphipods during marine regressions

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Fluctuations of sea level have been considered as a major force shaping distribution patterns of terrestrial and freshwater fauna. Dispersal routes established during marine regressions and terminated during marine transgressions, yielded an interplay of dispersal and vicariant events. We tested the interchangeable role of dispersal and vicariance using a clade of subterranean amphipods of the genus *Niphargus*. The clade is distributed across the Western Balkans and the Apennine peninsula, the so called trans-Adriatic distribution. We assumed that the clade originated in the Balkans, and during one or more marine transgressions colonized the emerging Apennine peninsula, followed by vicariant speciation. We tested this hypothesis using multilocus calibrated phylogeny, and modelling of ancestral ranges. First, we analyzed the taxonomic structure of the trans-Adriatic clade using unilocus species delimitation. The clade, counting 101 putative species, indeed originated in the Balkans and dispersed independently across the Adriatic basin multiple times. The first two independent dispersal events were related to the establishment of two little known land bridges between the Balkans and the southern Italian peninsula, approximately 13 Ma. The third dispersal event corresponded to Messinian Salinity Crisis (Miocene, 5.9-5.3 Ma), when an Italian clade colonized an archipelago in the Eastern Adriatic Sea. The last dispersals presumably took place during Pleistocene Adriatic regressions and included between-island dispersal and colonization of islands from the Balkan Peninsula. All dispersal events were best explained by a biogeographic model hypothesizing jump dispersal, which is in agreement with relatively short time frames enabling dispersal via land bridges. We conclude that trans-Adriatic distribution reflects an unexpected and complex history of dispersal-vicariance events, which significantly increased species diversity of the clade.

#### Monitoring of cave habitats and fauna: a case study from Croatia

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A two-year research projects in the Krka National Park and Paklenica National Park were conducted aiming to test different methods for cave habitats and fauna monitoring in order to develop monitoring protocols for different species, show caves and cave habitats. Altogether, we investigated 16 caves and 28 target species from the following taxa: Serpulidae, Araneae, Isopoda, Amphipoda, Decapoda, Diplopoda, Chilopoda, Collembola, Diplura and Coleoptera. Here, we will present the results from the monitoring of fauna in three caves from the Krka National Park (Stara jametina, Miljacka II and show cave Oziđana špilja), and the show cave Manita peć in Paklenica National Park. We used two different methods: (1) invertebrate census of the whole cave (in Ozidana špilja), and (2) invertebrate census on plots (in Stara jametina and Miljacka II). The first method is suitable for small caves. For this method, it is best to divide the cave into 3-5 zones and conduct a "minimumtime census", with a predefined number of people and the amount of time per zone. The second method is applicable for large caves. Again, it is necessary to define the number of people and the amount of counting time per plot. In Manita peć, we combined these two methods: the cave was divided into three zones and plots per zone (in triplicates) were established. With this approach, we were able to collect quantitative data sets and estimate the total abundance of the fauna as well as the abundance of individual taxa. Furthermore, this monitoring scheme is replicative and suitable for long-term monitoring of both fauna and cave habitats. A detailed species inventory of the investigated cave and the trained researchers that can identify species on sight, without collecting them, are crucial prerequisites for the successful implementation of the abovementioned methods.

## 1<sup>st</sup> Croatian National Report on Article 17 of the Habitats Directive for cave species and habitat type for the period 2013–2018

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We present the 1<sup>st</sup> Croatian National Report to the EU Commission on the progress and implementation of Article 17 of the Habitats Directive during the period 2013-2018. The main objective of the Directive is to maintain and restore a favourable conservation status for habitat types and species of community interest. Conservation status (CS) is the overall assessment of a habitat or a species status, within a Member State's biogeographical or marine region. The CS of a species is evaluated through range, population and habitat, while habitat types are assessed through their range, area, structure and functions, as well as future prospects. The reports also include information on the main pressures and threats as well as conservation measures. Here we present the reports for habitat type Caves not open to the public (8310), cave species Leptodirus hochenwartii (4019), Proteus anguinus (1186), Congeria kusceri (4065) and Congeria jalzici (6922). Our evaluations show that only Leptodirus hochenwartii has a favourable conservation status in all three biogeographical regions. The CS for Congeria kusceri was assessed as unfavourable-inadequate, while for Congeria jalzici and Proteus anguinus it was assessed as unknown. The CS in the Alpine and Mediterranean biogeographical regions for habitat type Caves not open to the public was assessed as unfavourable-inadequate, but as unfavourable-bad in the continental region. The highest registered negative impact on C. kusceri were agricultural activities, whereas C. *jalzici* and *P. anguinus*, as well as habitat type Caves not open to the public are endangered by hydropower infrastructure, the deposition of waste and discharge of urban wastewater. Although we recognized no high impact pressures or threats for L. hochenwartii, illegal collecting, with medium impact, is still a substantial pressure.

#### A first insight into subterranean Dinaric Trechini (Carabidae: Trechinae) phylogeny

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Western Balkan's Dinaric Karst bears one of the richest subterranean communities worldwide, and has long ago being recognized as a subterranean biodiversity hotspot. Extensive proportion of this unique subterranean diversification has being attributed to anophthalmous beetles, belonging to two major families: Leiodidae (Cholevinae) and Carabidae (Trechinae). While the patterns of spatial distribution in the Dinaric Karst's beetles have being well documented and rigorously tested, knowledge on their origin and phylogenetic relatedness remains restricted. To answer this question, we assembled a molecular dataset including 15 genera of Trechini occurring in the Dinaric karst, amplified DNA for 4 molecular markers (two mitochondrial and two nuclears), and set it in a Western Palearctic Trechini molecular framework. Our preliminary results show that the Dinaric Karst Trechini fauna does not share a single origin. Contrarily, it presumably belongs to at least seven different and only distantly related lineages. While some of the lineages seem to be geographically confined, the others exhibit peculiar connections with species occurring in distant geomorphological units in Western and Northern Mediterranean Sea. Despite the compelling results, this analysis represents a humble first step of a running project aiming at unveiling the natural history of the Dinaric Karst subterranean Trechini, and only enhances the need for their further sampling and thorough molecular analysis.

#### Functional ecology of the genus Niphargus

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Understanding the function of traits is a cornerstone of evolutionary ecology. Function is an outcome of a concerted action of one or more heritable traits, such as morphology, physiology or behavior, and is critically linked with an organisms' performance and fitness. The relationships between heritable traits, their function and organisms' fitness are essentials of adaptive biology, whereas organisms' performance, realized through specialized functions, is central to community ecology. Here we review studies on the genus Niphargus to 1) identify functionally relevant traits, 2) revise the functional correlates of these traits, 3) identify the tradeoffs they are subjected to, and 4) identify and discuss main research gaps. Virtually all Niphargus live in subterranean environments. Despite being eyeless, some of these species living at the subterranean-surface boundary detect light and show a strong photophobia, a presumed habitat choice mechanism. Body size is a complex trait; its upper size is controlled by void size. Comparative correlational studies suggest that it positively and negatively correlates with fecundity and metabolic demands, respectively. Appendages, such as antennae and pereopods, have sensory and locomotory functions. Aesthetascs-bearing antennae could be important in chemoreception, although the evidence for this claim is merely anecdotic. Pereopods' lengths relate to locomotion. Correlational analyses imply that the large species walking in upright posture with long pereopods move faster than short-legged species that crawl on their sides. Field observations suggest that the appendage length is controlled by water flow velocity. Gnathopods are important for grooming, feeding and digging. Although observations are mostly lacking, species with different gnathopods tend to co-occur more frequently than species whose gnathopods are similar in shape. The mouthparts have not been studied. Finally, a series of species in the Dinaric Karst, living together with olm, display strong pleonal spines. According to correlational analyses, these spines might have an antipredatory function. Our review identified two major fields in need of further investigation. First, the functional evidence for most traits is indirect, correlational or qualitative. Second, the heritability of the traits has not been studied. We conclude with a proposal for systematic experimental work, and development of a database of functional traits.

### General activity and boldness of a freshwater isopod after transition from surface to cave environment

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General activity and boldness (a tendency towards risky behaviour) are fitness-related personality traits affecting multiple behaviours and are expected to shift towards a new optimum when populations adapt to novel environments like caves. The traditional speleobiologist's hypothesis that general activity should decrease in a nutrient-poor cave environment to save energy has more recently been challenged by evidence from cavefish which display increased general activity that presumably enhances their food-finding success. On the other hand, boldness of cave animals has received little attention, but is expected to increase due to the reduced predation pressure in caves. We tested these two hypotheses using four surface-cave population pairs representing independent cave colonisations of the freshwater isopod Asellus aquaticus, an emerging model organism for studies of adaptive evolution in caves. We recorded one hour videos of behaviour in a familiar environment of 30 well-fed males and females per population. To control for differences in the natural light regime of surface and cave populations, we randomly assigned individuals into two groups that were acclimatized to darkness and diurnal light cycle prior recording in the same light conditions. Using video-tracking we extracted variables describing general activity (total path, total time moving, average and maximal speed) and measured boldness as the proportion of time spent in the inner arena zone. Preliminary analyses suggest that cave individuals had lower general activity in two population pairs and higher variability at the bold end of the shy-bold continuum in three population pairs. Additionally, light made cave individuals more active, while surface individuals were less active and shyer than in darkness. These results partly support the traditional expectation of selection for reduced general activity in a food-scarce environment, while the pattern observed in boldness suggests a relaxed selection for shyness in an environment with few predators.

#### Exploring bats in a tunnel through Volujica hill (Bar, Montenegro)

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In the mid-October of 2018, as a part of explorations bat fauna od Možura hill in the Municipalities of Bar and Ulcinj, an artificial underground tunnel under Volujica hill object was also explored. The tunnel is located near the southernmost houses in the city of Bar, and was drilled in order to drain the excessive water of river Rikavac that flooded the city of Bar during heavy rainfall. The tunnel is almost 2 km long and its dimensions are about 4 m width and about 5 m height. During our research, a smaller amount of water was flowing through the tunnel. Plastic bottles and branches in the ceiling of the tunnel show that the tunnel is filled with water to the top during heavy rainfall. By using the hand-held bat detector Echo Meter Touch 2 for an Android mobile phone (Wildlife acoustics) and analysis with BatSound 4.0 software (Petterssoon Elektronik AB) for recording, we have registered the following species: *Rhinolophus hipposideros, Rhinolophus ferrumequinum, Pipistrellus pipistrellus kuhlii, Myotis myotis / Myotis blythii, Miniopterus schreibersii.* 

### Biospeleological research of subterranean beetles in the higher plateau of National Park Paklenica

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Collaboration between "Paklenica National Park" Public Institution and Croatian biospeleological society resulted in the implementation of the project "Biospeleological research of subterranean beetles in the higher plateau of National Park Paklenica". The project was conducted in four pits in the area of Buljma, Struge and Počiteljski vrh (National park Paklenica) during the spring and summer of 2018. The main aim of the research was inventarization of subterranean beetles. Besides collecting subterranean beetles, microclimate parameters were measured, namely the temperature of the substrate, air temperature and relative humidity. Also the fauna, interior of the caves and cave entrances were photo documented. Biological material was collected by hand (tweezers and exhaustors) and by traps. Analysis of the collected specimens and literature data revealed that this area is species rich in troglobiotic beetles, with 7 species recorded. Two species belong to the family Carabidae, and five species to the family Leiodidae. Narrow-necked blind cave beetle–*Leptodirus hochenwartii* Schmidt, 1832) –which is on the species list under the Habitats directive of the ecological network Natura 2000, is present in two localities in this research area.

#### Preliminary result of research in "Tomina jama" an anchialine cave, Neretva River Estuary, Croatia

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Tomina jama cave was explored by Baštinik (Metković), the Croatian Biospeleological Society, Ruđer Bošković Institute, Pennsylvania State University and Society for karst research – Phreatic in 2016 and 2017. Currently, it is the only known anchialine cave in the river Neretva estuary. Tomina jama is a typical anchialine cave system with a freshwater lens at the water surface and seawater layer beneath, explored up to 58 m depth with the last 25 m submerged. Physical and chemical properties of the water, including salinity, dissolved oxygen, temperature and Ph were measured. Concentration of redox sensitive trace metals, namely Mo and U, was shown to fall along the depth profile, due to removal of these elements in anoxic waters at the bottom. In the anoxic waters at the bottom, increased concentrations of Fe and Mn were observed, indicating formation of reduced Fe and Mn minerals. Several stygobionts, including Serpulids Marifugia sp. and the crustaceans Monolistra sp., Troglocaris sp. and Niphargus sp., were discovered in the upper freshwater layer. Most notable is the finding of the endemic serpulids, *Marifugia* sp., in an anchialine cave. Prior to this discovery, Marifugia have only been found in freshwater caves along the Dinaric Karst. Preliminary DNA results indicate that this population represents a new species for science. Below the halocline, the cave walls and the bottom were lined with a significant bacterial mat, collected for future microbiome analyses. Both, discovery of Marifugia sp. populations and the significant bacteria mats require further thorough research. This cave system is the farthest inland of any Croatian anchialine cave, with significant communities in both, fresh and saltwater layers. In addition, the cave may serve as a natural laboratory for studying redox sensitive trace metals biogeochemistry.

#### Mandible morphometry predicts diverse trophic niches in Leiodidae beetles

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Leiodidae is a non-predatory beetle family with over 4,000 known species living worldwide and inhabiting habitats from leaf litter to the deepest caves. These widely distributed and closely related species, feeding on fungi and various decomposing organic matter available in the habitat, pose a challenging opportunity to study the trophic niche diversification in non-predatory beetles from the surface to the subterranean habitats. The core idea of this study was that adaptation to different habitats includes diversification of the mouthparts, which mirrors differences in the trophic niches. We therefore anticipated differences in the size and shape of the mouthparts in beetles inhabiting different habitats. For this purpose, we analysed 18 leiodid species belonging to the subfamily Cholevinae (16 from Slovenia, 1 from Bosnia and Herzegovina and 1 from Italy) from four habitat types: leaf litter, epikarst, deep subterranean terrestrial habitats, and deep subterranean hygropetric. Here we present morphometric outcomes on the mandible only. Digital images of mandibles of 59 specimens were taken under a microscope, and synchroscopy images were provided for the analysis. Twenty-eight landmarks were digitized on the right mandible. Landmark coordinates of the mandibles were aligned using Generalized Procrustes Analysis. A Principal Component Analysis (PCA) was used to summarize and explore patterns of variation among the specimens in the morphometric space. We identified four clearly separated size classes in the studied leiodid mandibles. The PCA revealed clear distinction among groups of species belonging to the deep subterranean habitats and leaf litter habitats. The deep subterranean groups have long, narrow mandibles, while the litterdwelling group has short, robust mandibles with a wide base.

## Global Red List assessments of Dinaric cave invertebrate fauna

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The IUCN Red List of Threatened Species is widely recognized as the most comprehensive, objective global approach for evaluating the conservation status of plant and animal species. Its purpose is to provide information and analyses on the status, trends and threats to species in order to inform and catalyze action for biodiversity conservation. Currently, there are 105 732 species globally assessed, of which 173 are invertebrate taxa inhabiting subterranean habitats of the Dinaric Karst. These include taxa across only five groups: Bivalvia (1), Branchiopoda (2), Decapoda (7), Orthoptera (9) and Gastropoda (154). The earliest assessments date back to 1999, while most have been assessed from 2010 to 2018. In 2009, Croatia produced the first Red Book of Cave Fauna, providing national assessment for 186 invertebrate taxa. Dinaric Karst is estimated to host more than 900 species of obligate subterranean fauna, and the number is constantly increasing, as new species are described each year. Considered one of the world hot spots for cave biodiversity, the Dinaric Karst is home to some unique aquatic cave animals (Eunapius subterraneaus, Congeria spp., Velkovrhia enigmatica, Marifugia cavatica) which are highly sensitive to changes in their environment. Although Dinaric countries have various formal levels of protection of cave habitats and species, a large proportion of this biodiversity has not been evaluated, and is without any protection. Including these species in the IUCN Red List would contribute to their visibility in global conservation and provide opportunities for fundraising for their research and conservation. Prioritizing their assessments according to known and imminent threats is crucial to achieve abovementioned goals.

## **Cave spiders of Montenegro**

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Spiders are one of the most diverse animals present in the caves in the Dinarides. Despite this fact, the cave spiders of Montenegro still remain heavily understudied. Based on critical analysis of literature and unpublished data by the author, the cave spiders of Montenegro are represented by 33 species and 2 subspecies (troglobites and troglophiles) from 8 families: Agelenidae – 7, Anapidae – 1, Dysderidae – 6, Leptonetidae – 4, Linyphiidae – 10, Nesticidae – 2, Pholcidae – 2 and Tetragnathidae – 3. The genus *Troglohyphantes* is the most numerous among troglobitic spiders with 4 species. Troglobitic spiders are present in five families: Agelenidae – 1, Dysderidae – 5, Leptonetidae – 2, Linyphiidae – 5 and Nesticidae – 2.

## Microbial and parasitic associations of proteus (Proteus anguinus)

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Despite long history of scientific research on Proteus several aspects of its ecology and biology, including its microbial and parasitic associations, remain understudied. Relatively scarce data on parasites in *Proteus* comprise descriptions of specialized protozoans, trematodes and recently described acantocephalans, as well as several less specific descriptions of other parasites. Despite their potentially detrimental effects on the host, frequent occurrence of parasites in otherwise healthy Proteus indicate effectiveness of host's defense mechanisms which, under normal circumstances, keep parasites in their tissues under control. Similar balance can be observed in microbial community inhabiting the skin of Proteus. The skin community is predominated by several groups of environmental bacteria facilitated by selective microenvironment of the skin. The composition of skin bacterial communities appear stable and comparable between individuals from different unpolluted locations, whereas a considerable shift in skin community was observed in animal exposed to fecal and organic pollutants. Together with relatively frequent occurrence of opportunistic infections of Proteus in captivity, changed composition of normal skin microbiota under pollution indicate an increased susceptibility to pathogens and parasites of Proteus, when exposed to sub-optimal environment. In that view, we can only speculate on its susceptibility to pathogens under moderate, yet constant pressure of pollutants in their natural environment which, besides direct threats by acute pollution and habitat loss, represents yet another threat to this charismatic underground amphibian and its fragile underground ecosystem.

## The first species of the genus Nemaspela from Montenegro

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Nemaspela (Opiliones: Nemastomatidae) is the genus of exclusively troglobiotic harvestmen species. To date, eight species inhabiting caves in the Crimea, Caucasus and Balkan Peninsula are known. In September 2014, a new Nemaspela species was found in the Njegoševa pećina cave in Montenegro, which is the first species of the genus in the country and the second one in the Balkans. It resembles recently described Nemaspela ladae Karaman, 2013 from Bosnia and Hercegovina. Both species are of comparable size and have similarly shaped pedipalps. However, the new species has slightly more robust chelicerae and clearly different genital morphology. According to the glans morphology it namely seems much more closely related to N. sokolovi (Ljovuschin & Starobogatov, 1963) and N. abchasica (Ljovuschin & Starobogatov, 1963) from the Caucasus. We speculate that N. ladae and the new Nemaspela species originate from two Nemaspela lineages.

## Expeditionary approach to biospeleological research - example from Croatia

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The Croatian Biospeleological Society (CBSS) is an NGO, gathering biologists, cavers and scientists of different research fields. Its goals are scientific research, conservation and protection of cave habitats and cave fauna. In order to conduct biospeleological research in highly interesting and challenging karst areas, CBSS decided to organize biospeleological expeditions in collaboration with caving club Breganja, and local caving clubs. This resulted in two rather different expeditions. The first expedition was in 2017 on Biokovo Mt (Dalmatia, Croatia) and focused on deep caves which also require intense speleological research. Caves of Biokovo Mt are well known; more than 400 caves have been registered and it is estimated that over 1000 exist. Approximately one-third of them have been biologically investigated, but the deep caves provided new insight into its highly diverse subterranean fauna. During the expedition, fauna was collected in 26 caves, of which 5 deep caves (>250 m) and 7 caves were surveyed (mapped), 2 of which were deep caves. Ten new species for science have been found from the following groups: Hirudinea, Araneae, Opiliones, Isopoda, Chilopoda, Collembola, Diplura and Coleoptera. The second expedition was in 2019 at the Pelješac peninsula (Dalmatia, Croatia), which is a highly karstified area. From Pelješac only 60 caves were known until 2019, while biospeleological research was sporadic. During the expedition 20 new caves have been discovered, 40 caves were biologically investigated, while 26 were surveyed (mapped). Preliminary taxonomic analyses suggest the discovery of 1 new coleopteran genus, and 9 new species from the groups: Isopoda, Araneae, Coleoptera and Collembola. In both expeditions a total of 79 people participated, and 36 are cavers without special interest in biospeleology. Having so many cavers during field research allowed both biologists and cavers to concentrate on their own interests. The results of this speak for themselves.

# Molecular phylogeny of the subterranean genus *Verhoeffiella* (Collembola, Entomobryidae)

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Subterranean genus Verhoeffiella occurs in five discontiguous karstic regions throughout Europe: Dinarides, Jakupica in North Macedonia, South-eastern Calcareous Alps, Southern Catalonia and Cordillera Cantabrica. This disjunct distribution challenges the monophyly of the genus and questions the scenarios of its origin and colonization. In the molecular phylogenetic framework we tested the monophyly of Verhoeffiella and explored its relationship with the surface and presumably related genus Heteromurus. Further, using species delimitation methods and time divergence estimation, we explored hidden diversity and temporal diversification of the genus. Our results show that both genera, Verhoeffiella and Heteromurus, are paraphyletic, while species delimitation methods revealed overlooked diversity and increased molecular divergence. We identified 79 MOTUs of Verhoeffiella in Dinarides, which is seven times more than the number of nominal species. In addition, we identified more than 30 MOTUs of troglophilic species Heteromurus nitidus. When placed on the time scale, events triggering the diversification were linked to the Messinian Salinity Crisis and Pleistocene climatic shifts. The combination of this extensive subterranean diversification and close evolutionary links with epigean relatives makes Verhoeffiella an exceptional case within the subterranean fauna of Dinarides.

## Asellids (Isopoda, Pancrustacea) of the World: a useful case study for understanding ecoevolutionary processes during the transition from surface water to groundwater

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The asellids (Asellidae, Isopoda, Pancrustacea) are one of the rare family of metazoans containing both a great number of obligate groundwater and surface water species. This makes it an ideal case study for understanding eco-evolutionary processes taking place during the transition to groundwater habitats. However, this understanding requires worldwide sampling and phylogenetic analyses for obtaining multiple independent pairs of surface and subterranean asellid species. These species pairs are then to be used in comparative analyses as true replicates of the ecological transition from surface water to groundwater. Assembling such a comparative data set requires tremendous efforts and resources: these include worldwide sampling, intensive identification and sequencing work, skills in morphology and DNA based taxonomy, phylogeny and bioinformatics, and new software tools for sharing information among multiple collaborators. Here, we provide an overview of the progress made over the last ten years in the following areas: knowledge of species richness and distribution patterns, understanding of phylogenetic relationships among taxa, and changes in species traits during the transition from surface water to groundwater using comparative analyses. We end up our presentation by introducing a new laboratory information management system for optimizing data acquisition through collaborative exchanges and information sharing.

2<sup>nd</sup> Dinaric Symposium on Subterranean Biology

#### Poster

## Review of bat research in Bosnia and Herzegovina

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The first mention of bats in Bosnia and Herzegovina comes from a Bosnian-Turkish dictionary (1631/1632) called Makbuli arif (also known as Potur Shahidi), written by Muhamed Hevaji Uskufi. The first finding of a bat, though as a fossil, comes from 1892, when a Miocene stone bat from the salt plant in Tuzla was given as a present to the mineral collection of the National Museum in Sarajevo. According to the inventory book of this museum, the first live specimen that was included in the collection, was Pteropus, which was killed at Podveležje near Mostar in 1898. The first native species, Nyctalus noctula, was collected by the curator of the National Museum Otmar Rajzer from a field excursion in the vicinity of Sarajevo in 1887. Later, from 1908-1925, other associates brought bats to the museum collection. In the first mammal list of the collection of the National Museum, curator S. Bolkay (1924) lists only five bat species, even though other species have been found in the collection by that time. In the second list, Bolkay (1926) examined the collection more carefully and listed 11 species all together. According to the Catalogus faunae Jugoslaviae, B. Đulić and Đ. Mirić (1967), there were 17 known species in BIH. In 2006, M. Zagmajster and colleagues reported all known records on bats in BIH from the literature and own research, and listed already 27 species. In recent years, more intensive bat research has been carried out, including regular winter monitoring of caves. This resulted in the discovery of four new species recorded for the country: Myotis brandtii, M. daubentonii, M. dasycneme and M. alcathoe and raising known number of bat species to 31.

## Diatoms - invisible residents of Resavska Cave (eastern Serbia)

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Diatoms (Bacillariophyta) are widespread microorganisms that were found in different aquatic ecosystems, but also in other habitats, such as stone substrates exposed to air. There diatoms thrive with other phototrophic organisms (cyanobacteria and algae) if they have sufficient sunlight, water and nutrients. Phototrophic organisms penetrate into tourist caves where artificial lights support their growth. This community in the vicinity of lights, so called lampenflora, includes bacteria, cyanobacteria, algae, fungi, mosses and lichens. Despite cave diatoms in Serbia have been studied several times, the knowledge about this group is scarce. Epilithic diatoms were collected by scraping biofilms from the entrance (two sampling sites) and the inside (four sampling sites) of Resavska Cave (eastern Serbia). Since the Resavska Cave is famous show cave, higher diversity of all phototrophs, not only diatoms is expected because of artificial light, but also due to the presence of the tourists that can introduce different microorganisms from the outside environment. A total of 24 diatom species from 10 genera were identified. The most frequent and abundant genera are Humidophila (12 species) and Sellaphora (3 species), while others (Cyclotela, Fallacia, Diadesmis, Luticola, Nitzschia, Orthoseira, Simonsenia, Tryblionella) count one to two species. This cave is mostly populated by aerophytic and cosmopolitan species such as Humidophila contenta, Humidophila paracontenta and Humidophila perpusila that were present at the entrance and inside the cave. Beside of Humidophila representatives, species Fallacia insociabilis, Diadesmis biceps, Sellaphora nigri and Sellaphora saugerresii were registered inside the cave in low abundance. Inside the cave, at sampling site with dripping water Cyclotella sp. and Luticola sp. were documented. Entrance of the cave includes ten species which were recorded inside as well, except for the Humidophila aerophila which was found only at the entrance.

## Clean Underground: a bottom-up model for building a national cave protection system

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"Clean Underground" is a volunteer project of the Zagreb Speleological Association that deals with the problem of karst underground waste landfills. Since 2015, we gathered data from 26 Croatian caving organizations into a unique national cave pollution database (publicly available at www.cistopodzemlje.info). We documented 783 polluted sites revealing the magnitude of this nation-wide problem for the first time. Recognizing the unique diversity of underground fauna and the importance of groundwater aquifers of the Dinaric karst, we have extensively used this data as a starting point for testing different approaches to karst protection. Initially our efforts were oriented mostly inwards (to the caving milieu) as we organized clean-up campaigns and lectures for experts, legislators and educators alike. Soon we realised that all the media coverage and positive hype in the caving community failed to make a real contribution toward solving the problem. Instead, we expanded our efforts and started cooperation with the Ministry of Environment and Energy, which resulted in our data being included in the national and regional waste management plans. The locations of polluted caves are also documented on the Bioportal, the official nature protection geographic information system. This way, the project "Clean Underground" has become a well-known source of information for cave protection for both NGOs and governmental institutions. Project data is used to implement relevant conservation documents for underground fauna, e.g. the national olm protection action plans. Turning our gaze to future generations of karst protection leaders, we have recently implemented our project into the national high-school programme. "Clean Underground" will be discussed as an example of successful karst protection initiated by a small group of motivated enthusiasts. The main purpose of this talk is to inform the community of our work and consider the possibilities of expanding our concepts to the regional level.

## The story of Dinaric cave-dwelling dysderid spiders

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It all began here in Postojna, with the discovery of the first cave-dwelling spider in the world - dysderid Stalita taenaria Schiödte, 1847. Since then, 21 more cave-dwelling species of the family Dysderidae were described, belonging to seven genera and two subfamilies: Stalagtia and Folkia in the Harpacteinae, and Rhode, Stalita, Mesostalita, Parastalita and Stalitella in the Rhodinae subfamily. Almost all species show high level of cave adaptation and are narrow endemics. The taxonomy of these groups, including their generic status, is based exclusively on morphology and is a matter of debate since there has been no explicit, quantitative phylogenetic hypothesis about the relationships within the family so far. To resolve taxonomy and to understand the origins and diversification of Dinaric cave dysderids, a necessary first step was a thorough taxonomic sampling of the region. Based on that, we performed a target multi-locus phylogenetic analysis, combining mitochondrial and nuclear genes. Additionally, we explored species boundaries using several species delimitation methods, and estimated divergence time combining fossil and biogeographic node calibrations. We uncovered a major, previously overlooked diversity at both species and genera levels, especially within Harpacteinae which show a high level of cryptic diversity. Cave lineages showed a common pattern of long stem branches, which may indicate high extinction levels during the evolutionary history of these groups. We hypothesize that Miocene climatic changes may have played a crucial role in shaping the extant diversity of these cave-dwelling spiders.

## Phototrophic biofilms in Serbian caves: exploration and features

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Phototrophic biofilms can be found in different habitats, including subterranean ones, such as caves. In caves, they thrive in the presence of daylight at the entrances, or in the vicinity of artificial light inside, and are usually very heterogeneous. For the purpose of this study, biofilm sampling from stone substrata was performed in 15 caves (mostly entrances) to examine biofilm characteristic and features, as well as to record the main phototrophic groups. Cyanobacteria, Bacillariophyta and Chlorophyta were the main phototrophs recorded. Cyanobacteria were, according to morphological characteristics, additionally divided into coccoid, simple trichal and heterocytous forms. Biofilms were sorted according to the degree of development and moisture (taking into account free seeping water and water bound in extracellular polymeric substances (EPSs)). Other parameters, namely the water content and content of organic/inorganic matter were determined too. According to the principal component analysis (PCA), Bacillariophyta and Chlorophyta were usually dominant in thin biofilms: while Chlorophyta were connected with dry thin biofilms, Bacillariophyta were abundant in thin biofilms constantly moistened by seeping water. Cyanobacteria dominated in thick and EPSs rich biofilms that also were positively correlated with light intensity and higher values of chlorophyll a. Biofilms were also sorted according to colour, where certain colours or colour assemblages corresponded to whole divisions, cyanobacterial groups or even only one taxon. Chlorophyta rich biofilms were mostly green, only few of them yellow and orange, while those where Cyanobacteria accounted as the most abundant, were very colourful (red, purple, blue, yellow, brown), for which usually only one species was responsible. Scanning electron microscopy (SEM) has additionally helped us to distinguish different biofilm types and their morphology better and enabled detailed analysis of distribution, shape, size and orientation of biofilm constituents. The biofilm rich in Cyanobacteria with calcified filaments occurred as particularly interesting for SEM exploration.

## Trophic differentiation of Niphargus amphipods

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The amphipod genus Niphargus is ecologically diverse, living in virtually all types of subterranean aquatic habitats. On a local scale, different Niphargus species regularly cooccur. The co-occurring species often differ in microhabitat preferences and morphology, suggesting that these species evolved different ecological niches. The ecology of individual species, and differences in ecological niches are poorly understood. In the present study, we focused on trophic niche differentiation of species occurring within the same community and compared the trophic position of species from different localities. Previous studies suggested that *Niphargus* can feed on various food sources. Besides that, appendages involved into feeding, i.e. gnathopods, show a substantial between-species variation, suggesting that appendage morphology might correlate with feeding ecology. We sampled five caves, in which 2-3 species of *Niphargus* regularly co-occur. We measured carbon (C) and nitrogen (N) isotope ratios of animals and of the different basal resources including sedimentary biofilm and particulate organic matter, assessed the differences in isotope compositions among species, and estimated the trophic position of individuals and species. The results suggest that i) co-occurring species differ in feeding ecology, where ii) some species apparently occupy different trophic levels, whereas iii) species of the same trophic level apparently exploit different food sources. Additionally, iv) co-occurring species differ in shape and/or size of gnathopods, and v) the size of gnathopods seem to correlate with species' trophic position. Put together, current results show that co-occurring species tend to differ in their trophic niches.

# *Lithobius* (*L*.) *lapidicola* Meinert, 1872 (Chilopoda: Lithobiomorpha: Lithobiidae) – the most common troglophilic centipede from southwestern Bosnia and Herzegovina

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The troglobites, troglophiles and trogloxenes represent the basic ecological categories among cave animals, defined mostly by the level of morphological adaptation to subterranean environments. Usually, the interests of biospeleologists decline from troglobites to trogloxenes; from the most specialized cave animals to the accidental strays in underground habitats. The troglophiles are somewhere in between, but probably represent the pivotal group for understanding the origin and recent distribution of many "true" subterranean species. Within this group, we can define species found only in caves (but still morphologically less specialized), but also species, which prefer the cave habitats but are at the same time widespread outside the caves. One of the species from the last mentioned category is widely distributed European centipede Lithobius (L.) lapidicola Meinert, 1872. The genus Lithobius Leach, 1814 is one of the largest and most complex centipede genus, whose representatives are, among Chilopoda, the most common inhabitants of caves in Europe. Here we present new data on centipedes from 14 caves in Bosnia and Herzegovina, collected mainly during the last decade by the members of the Croatian Biospeleological Society. Of the total 72 collected Lithobius specimens, as many as 63 were L. lapidicola, recorded in 12 caves. The results suggested that L. lapidicola from the southwestern Dinarides displays a strong affinity to the cave environments. There are no other literature reports of such troglophilic preferences of this species. In addition to L. lapidicola, the samples contained two troglobites – L. matulicii Verhoeff, 1899 (collected from four caves) and L. troglomontanus (Folkmanová, 1940) (one cave); as well as one troglophilic and two trogloxenic species, respectively: Harpolithobius anodus (Latzel, 1880), Theatops erythrocephalus (C. L. Koch, 1847) and Strigamia transsilvanica (Verhoeff, 1928).

## Type localities and conservation of subterranean beetles in Slovenia

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Type localities are sites where type specimens of species descriptions derive from. There is no doubt that such localities are of high value in taxonomy, but it is less clear how important are these sites as conservation targets. Subterranean species typically have small distribution ranges, with many known even from only one site. For such single site species, type localities should be conserved with priority. But, when subterranean species are known from more sites, or have large ranges, conservation value of type localities can be questioned. Using the data on species of subterranean beetles from Slovenia, we checked how vulnerable are type localities to potential threats, how well they present species' distributions, in what extent they fall into species rich areas and whether they are already included into different nature conservation schemes. About one third of subterranean beetles' type localities in Slovenia are species' single sites. Type localities, compared to other known caves in the surroundings, are easily accessible and therefore more vulnerable to anthropogenic disturbances. Type localities of broadly distributed species are at different distances from centroids of species ranges, and they do not adequately represent species distribution. All 20x20 km quadrats with high species richness of subterranean beetles (having at least 14 species) in Slovenia contain at least one type locality, but less than half of all type localities are spatially positioned within such quadrats. About half of type localities are included in the network of Special Areas of Conservation under European Union's Habitats Directive. We suggest that type localities can have conservation importance in taxa with small ranges, and as such should be regarded as one of multiple criteria to develop subterranean biodiversity conservation plans.

## Developing new tools for rapid assessment of subterranean biodiversity in Bosnia and Herzegovina

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Dinarides in the Western Balkans are known as a global hotspot of subterranean biodiversity. Despite this, subterranean fauna of the region is highly endangered. This is dramatically true for parts of the region where an unfortunate combination of ambitious economic-developmental plans and incomplete knowledge on subterranean species richness coincide. Therefore, there has never been a greater need for a rapid assessment of subterranean biodiversity, involvement of local stakeholders in conservation activities and facilitating access to existing knowledge to authorities and wider community in the region. This urge has been recognized also by the international foundation "Critical Ecosystem Partnership Fund" (CEPF), which financially supported a project entitled "Developing new tools for rapid assessment of subterranean biodiversity in Bosnia and Herzegovina" (September 2019 - May 2022). The project will be run by partners from Slovenia and Bosnia and Hercegovina, and will actively include local stakeholders, like cavers and nature conservationists. Activities will target CEPF – predefined Catchment Management Zones in the wider Trebišnjica River Basin, and will be directed toward selected subterranean animal groups. We aim to fill the knowledge gap in species inventories and distributions, and develop a DNA barcoding system for routine species identification. In addition, we will evaluate the status of selected subterranean taxa according to the IUCN Red List criteria. We will organize the data in a database that will be available to local stakeholders and authorities. In order to transfer the knowledge, increase local capacities and raise the awareness in the region, we will organize workshops, student internships and different public events. All activities will be conducted in close collaboration with interested local stakeholders, who are of crucial importance for local implementation, as well as for a longterm continuation of the project outcomes.

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