Abstract book

3rd Dinaric Symposium on Subterranean Biology

9th - 10th April 2022

Trebinje, Bosnia and Herzegovina

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Table of contents

Foreword

Dear friends and admirers of the unique subterranean life of the Dinaric Karst, welcome to the 3rd Dinaric Symposium on Subterranean Biology!

The symposium is organized by the SubBioLab team of the University of Ljubljana (Department of Biology, Biotechnical Faculty) in collaboration with the Centre for Karst and Speleology from Sarajevo, and will be held in Trebinje, Bosnia and Herzegovina, on 9th and 10th April 2022.

The symposium will be organized as a final event of the SubBIOCODE project, financed by the Critical Ecosystem Partnership Fund. The project area encompasses Trebišnjica River catchment in Southeastern Bosnia and Herzegovina. Trebinje, Trebišnjica River and the wider area surrounding it played an important role in the history of speleobiology. It represents a large part of the Southern Dinaric subterranean biodiversity hotspot (together with the Western Montenegro and Dalmatia). In addition, Vjetrenica cave is one of the richest localities in the world considering the number of subterranean species. Unfortunately, this exceptional subterranean biodiversity hotspot area is highly threatened by human activities. Therefore, there is no better place than Trebinje to address various questions regarding raising level of knowledge and conservation of subterranean biodiversity in the Dinaric region.

Following the successful 1st and 2nd Dinaric Symposia, held in Zagreb (Croatia) and Postojna (Slovenia), respectively, we are welcoming the participants to the the 3rd Dinaric Symposium! We hope it will establish new and deepen the existing networks between scientists and amateurs, interested in rich subterranean biodiversity of the Dinaric region. This includes a wide array of topics, related to subterranean world - systematics, biogeography, evolution, ecology, biodiversity, conservation, etc.

Last but not least. I guess there is no better place to express gratitude to Ivana Grujić from the Museum of Herzegovina, which offered us a venue for the Symposium, than doing it here.

Teo Delić, Chair of the Organizing Committee

Critical Ecosystem Partnership Fund (CEPF)*

Since 2012 CEPF has been supporting biodiversity conservation projects in the Mediterranean. Freshwater ecosystems are one of our top priorities, and we are proud to invest in research on subterranean biodiversity. With its iconic Dinaric mountain range and adjacent subterranean habitats, the Balkans are home to many endemic species, many still unknown to us but crucial for the functioning of fragile subterranean ecosystems. The long-term conservation of this unique biodiversity, and biodiversity in general, has to rely on local and national environmental actors, civil society, including academia and private enterprises. We are witnessing immense pressures on biodiversity, mainly due to unsustainable planning of large infrastructural projects driven by short-term financial gains and without adequately considering the well-being of local communities. In parallel, we are glad to see an increase in underground research and conservation capacities in Bosnia and Herzegovina and other Balkan countries. The future of Dinaric and Balkan subterranean habitats depends not only on the governmental decision-makers but also on us - biologists, speleologists, conservationists, donors - and it is our collective responsibility to keep caves dark and their future bright.

Pierre Carret, CEPF Grant Director

*The Critical Ecosystem Partnership Fund (CEPF) empowers people in developing and transitional countries to protect the world's biodiversity hotspots—some of the most biologically richest yet threatened ecosystems that are vital to humanity.

By providing grants to civil society—nongovernmental, private sector and academic organizations—CEPF implements conservation strategies that are developed with local stakeholders. These investments are especially important because the hotspots are home to millions of people who are impoverished and highly dependent on nature for survival.

The fund is a joint program of l'Agence Française de Développement, Conservation International, the European Union, the Global Environment Facility, the Government of Japan and the World Bank.

As one of the founding partners, Conservation International administers the global program through a CEPF Secretariat.

Find out more at www.cepf.net

Supporting future research and conservation of subterranean fauna in Southeastern Bosnia and Herzegovina – the case of SubBIOCODE project

The Dinarides are a global hotspot of subterranean biodiversity, which is being threatened in many of its parts. This is especially notable in the Southeastern Dinarides, where a combination of ambitious economic development plans and incomplete knowledge of subterranean species richness largely coincide. Recognising the need for action, the Critical Ecosystem Partnership Fund is supporting the project "**SubBIOCODE - Development of New Tools for Rapid Assessment of Subterranean Biodiversity in Bosnia and Herzegovina**" from September 2019 to May 2022, targeting the Trebišnjica River Catchment (https://subbiocode.net/). The project is led by SubBioLab (Biotechnical Faculty, University of Ljubljana) and the partner organisation Centre for Karst and Speleology (Sarajevo). It aims to improve knowledge and methodology for the assessment of subterranean biodiversity and to transfer this know-how to the local population and decision makers. Here, we provide a brief overview of the key project achievements through the end of March 2022.

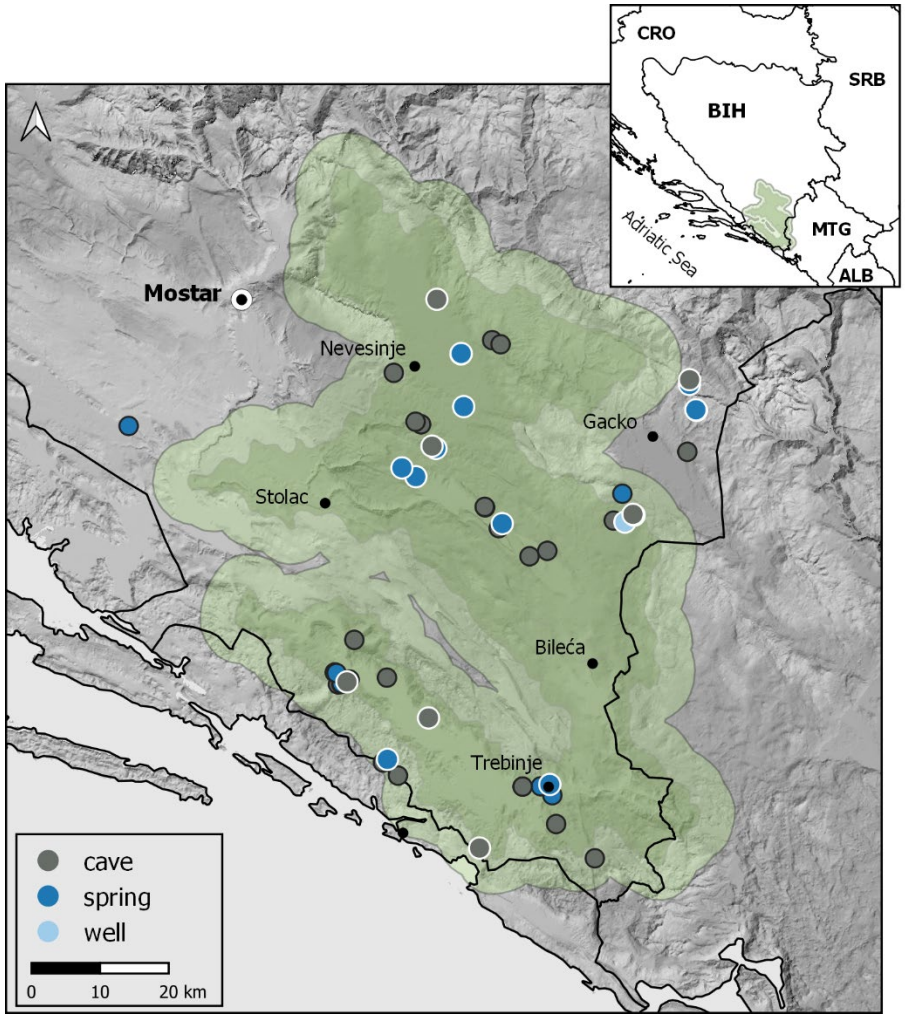

Figure 1. SubBIOCODE project area (green), with localities studied in 2021 and 2022. Circles with black edge refer to localities that already had some data, circles with white edge refer to localities investigated for the first time during this project. Dark green area marks Trebišnjica river catchment, while lightergreen presents additional 5 km buffer belt**.**

A large part of the project activities is dedicated to compiling existing data and collecting new datasets from less explored parts of the region. Field expeditions were organised in 2021 and 2022 to explore caves and springs, in many of which fauna was studied for the first time. Samples collected on these trips, as well as samples from some previous studies, were subject of molecular analyses to obtain diagnostic DNA sequences that can be used for future species identifications - the so-called DNA barcoding approach.

One of the main goals of the project was to organise the existing and new data and feed the information back into the region - to local stakeholders, decision makers and relevant authorities, researchers, and anyone interested in the region's subterranean biodiversity. We have developed an online SubBIOCODE Database that contains information on the occurrence of subterranean animals, provides molecular DNA barcodes for many species, and includes conservation information. The website also enables a citizen science approach for reporting new observations from the region - with a form on the same web interface. This database is the first open-access source of data on subterranean biodiversity in the region and a rare case of all information being offered in one open-access platform.

To facilitate conservation of important subterranean sites and species, we are compiling a list of important sites along with the IUCN Red List status assessments or reassessments for at least 15 aquatic species. In this way, we hope to highlight even more the importance of conservation and the threats to subterranean fauna.

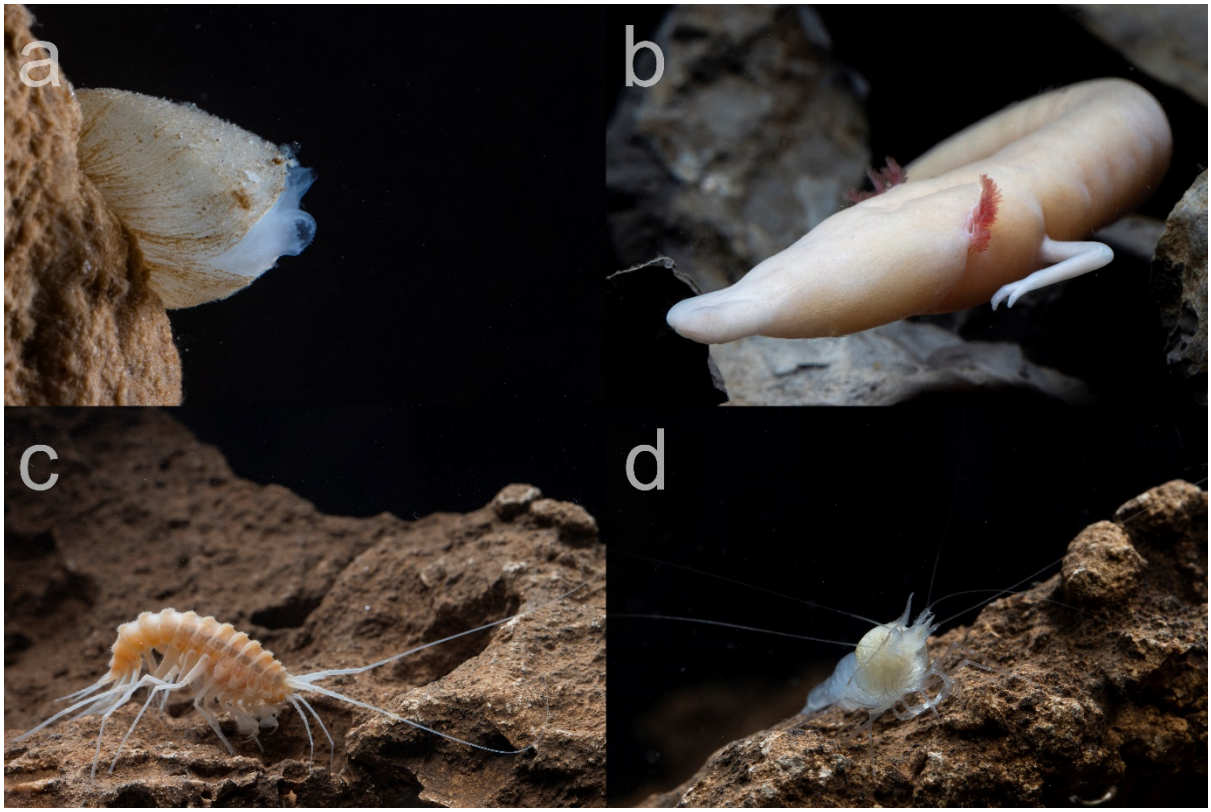

Figure 2. Some representatives of the aquatic subterranean species, present in the SubBIOCODE project area: **a** – Kuscer's cave clam *Congeria kusceri*, **b** – Olm *Proteus anguinus*, **c** – Balkan cave amphipod *Niphargus balcanicus*, **d** – new species of cave shrimp, discovered during the project (all photos: Teo Delić).

To engage the local community, students, naturalists, and raise public awareness, we organized a series of activities. During the 2020 covid pandemic travel restrictions, we provided online lectures on the subsurface diversity of the Dinarides. In early 2022, we hosted three promising students from BIH for a one-month internship at SubBioLab's facilities in Slovenia. We also established protocols for field sampling and molecular work to train future researchers in the region. We organised a workshop for students in Trebinje, where participants gained hands-on experience in sampling caves and springs and managing samples after fieldwork. A selection of high quality macrophotographs of subterranean animals has been prepared and shall be made available to interested stakeholders in the region. The 3rd Dinaric Symposium on Subterranean Biology in Trebinje is currently being organised and will help to establish and strengthen links between researchers of subterranean fauna in the region.

During the project, we have strengthened existing and established new collaborations with stakeholders and students in the region, provided conservation-related data to policy makers, and made the basis for collaborations with various scientists across Europe. Not only have we improved basic knowledge on the occurrences of subterranean species, but also discovered many new species for science. Last but not least, the activities were carried out in collaboration with interested local stakeholders, who are crucial for the implementation as well as for a long-term continuation of the project results – in order to preserve and protect this exceptional world heritage for future generations.

Maja Zagmajster[1], Špela Borko[1], Gregor Bračko[1], Teo Delić[1], Cene Fišer[1], Anja Kos[1], Anja Pekolj[1], Ester Premate[1], Behare Rexhepi[1], Peter Trontelj[1], Valerija Zakšek[1], Jasminko Mulaomerović[2]

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Committees of the 3rd Dinaric Symposium on Subterranean Biology

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Program of the 3rd Dinaric Symposium on Subterranean Biology

9th April 2022
Museum of Herzegovina, Trebinje

8.00 Registration

9.45 Welcome word
Teo Delić, Chair of the Organizing Committee
Ivana Grujić, Director of Museum of Herzegovina

10.00 Supporting future research and conservation of subterranean fauna in Southeastern Bosnia and Herzegovina – the case of SubBIOCODE project
***Zagmajster**, Borko, Bračko, Delić, Fišer, Kos, Pekolj, Premate, Rexhepi, Trontelj, Zakšek, Mulaomerović.*

10.30 – 11.00 Coffee break

Session 1: From field observations to database managing (Chair: Maja Zagmajster)

11.00 The contribution of biology students' research camps to the knowledge on subterranean fauna in the Western Balkans
***Premate**, Delić, Fišer, Polak, Rexhepi, Škufca, Zagmajster*

11.20 20 years of underwater cave research in Herzegovina
***Balázs**, Lewarne*

11.40 Cave spiders of Bosnia and Herzegovina
Komnenov

12.00 Observational data on subterranean freshwater taxa coming from a touristic cave
***Vuica**, Jalžić*

12.20 A review of the cavernicolous millipedes (Myriapoda, Diplopoda) from the Caucasus
Antić, Turbanov

12.40 SubBIOCODE Database – web interface to facilitate access to data on subterranean biodiversity in Southeastern Bosnia and Herzegovina
***Kos**, Di Batista, Delić, Zagmajster*

Lunch 13.00 -14.30

Session 2: New approaches to studies of long known taxa (Chair: Marko Lukić)

14.30 Setting up the laboratory invertebrate facility - good and bad practices
***Kuharić**, Lukić, Bedek, Grgić, Jovović, Rožman, Bilandžija*

14.50 Explosive evolution of pseudoscorpions in Croatia
***Hlebec**, Harms*

15.10 Recent progress in barcoding water mites (Acari, Hydrachnidia) of the Western Balkans
*Jovanović, **Pešić***

15.30 Research of *Delminichthys ghetaldii*, semi-cave fish of Dinaric karst
***Vucić**, Jelić*

15.50 Possible ways of stygobiotic gastropod dispersal
Grego

16.10 Hidden diversity of the cave centipede *Lithobius stygius* Latzel, 1880 (Chilopoda: Lithobiomorpha: Lithobiidae) in the Northwestern Dinarides
***Kos A.**, Delić, Kos I., Zagmajster*

16.30 - 17.00 Coffee break & Poster session 1

Session 3: Testing species hypotheses and distributional patterns (Chair: Dragan Antić)

17.00 *Typhlogammarus mrazeki*, a holodinaric species broken apart
***Rexhepi**, Fišer, Jalžić, Delić*

17.20 Spatial genetic structure of five sympatric cave spider species
***Pavlek**, Gauthier, Tonzo, Bilat, Arnedo, Alvarez*

17.40 Resolving the phylogeny of Anthroherponina with notes on new species of specialized hygropetricolous genera
***Delić**, Lohaj, Brestovanský, Čáha, Jalžić*

18.00 Zoogeographical borders of the Dinaric karst based on Leptodirini beetle's distribution
Polak

18.20 Subterranean evolution of the tribe Bythinini (Pselaphinae) in the Balkan Peninsula
***Mizerakis**, Hlaváč, Delić, Jalžić, Faille*

19.00 Guided tour through the center of Trebinje

20.00 Evening reception

10th April 2022
Museum of Herzegovina, Trebinje

Session 4: Back to the basics – on coping with subterranean conditions (Chair: Martina Pavlek)

9.00 Olm lineages native to Bosnia and Herzegovina in the framework of parallel evolution of troglomorphy across the Dinaric Karst
Recknagel*, Zakšek, Trontelj*

9.20 The role of phenotypic plasticity in cave colonization of the genus *Proasellus*
Bedek*, Lelas, Bilandžija*

9.40 Acanthocephalan-induced phenotypic alterations of *Asellus aquaticus*
Benko*, Fišer, Kostanjšek*

10.00 *Telestes karsticus* – a cave adapted fish in the Dinaric Karst
Bilandžija*, Čupić, Lukić, Gračan, Marčić*

10.20-11.00 Coffee break & Poster session 2

11.00 Ommochrome pigments in surface isopods (Isopoda, Crustacea) and their loss in cave-adapted albino relatives
Jovović*, Figon, Bilandžija*

11.20 A conceptual solution to the problem of subterranean vicariance and dispersal
Trontelj

11.50 Concluding remarks, coffee break and field excursion

Poster session

An overview of the biospeleological research in wider area of Barać caves
Bregović, Mišerić, ***Sudar,*** *Bedek, Ćukušić, Čupić, Dražina, Hlebec, Pavlek, Rožman*

Recent biospeleological research in the Žumberak – Samobor hills Nature Park
Dražina*, Šepčević, Kermek, Bedek, Čupić, Hlebec, Pavlek, Kuharić, Kirin*

Testing the utility of eDNA metabarcoding for studying groundwater biodiversity
Grgić, Kuharić, Kajan, Dražina, Orlić, ***Bilandžija***

Unexpected diversity of *Niphargus boskovici* species complex inferred from mitochondrial DNA
Kuna*, Rexhepi, Kos, Zagmajster, Fišer, Delić*

Centipedes (Chilopoda) from Bosnia and Herzegovina in the collection of the Museum für Naturkunde Berlin
Mitić*, Ašćerić*

Snail *Spelaeoconcha paganettii* Sturany, 1901 - A new finding in Western Herzegovina
Mulaomerović*, Dževlan, Hasanspahić*

Including data on subterranean species distribution into a common Nature Conservation Information System in Slovenia – challenges and opportunities
Pekolj*, Kos, Fišer, Zagmajster*

Špilja na Gradini kod Premanture cave, the first systematic biospeleological study
Ružanović, Kuharić, Cindrić, ***Jagić***

Eupolybothrus (*Parapolybothrus*) *herzegowinensis* (Verhoeff, 1900), a poorly-known Dinaric centipede (Myriapoda: Chilopoda: Lithobiomorpha)
Stojanović, Rađa

Range sizes and conservation of *Niphargus* species (Amphipoda) occuring in Trebišnjica river catchment in Southeastern Bosnia and Herzegovina

Vasić*, Kos, Fišer, Zagmajster*

Inventory of fauna in the Ljubačevo cave near Banja Luka, BIH
Vulić*, Delić, Zagmajster*

Abstracts

Oral communications

and

Posters

Oral Communication

A review of the cavernicolous millipedes (Myriapoda, Diplopoda) from the Caucasus

Antić, Dragan[1,2]*; Turbanov, Ilya[3,4]

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[2]Serbian Biospeleological Society, Novi Sad, Serbia
[3]Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Yaroslavl Region, Nekouz District, Borok, Russia
[4]Cherepovets State University, Vologda Province, Cherepovets, Russia

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The Caucasus is one of the 36 world's biodiversity hotspots. In addition to nine climate zones, more than 6400 plant and about 740 vertebrate species, this area also hosts a large number of invertebrates with many endemics. In recent years, with the significant increase in the number of discovered and described cave animals, the Western Caucasus has become recognized as the potential subterranean hotspot too. This region includes not only the deepest caves known in the world so far, but also some very interesting cave creatures, and millipedes are no exception. Up to now, there are about 170 diplopod species known from the Caucasus (ca 85% endemics), from over 50 genera (ca half endemic), 15 families and eight orders. In terms of cave millipedes of the Caucasus, until recently only a few species have been described from the region. During the last decade, the number of presumably troglobiontic millipedes in this area has tripled, and includes 32 cavernicolous species from 12 genera, six families and four orders. The largest number of cave-dwellers are in the families Julidae and Anthroleucosomatidae with 15 and 12 likely troglobiontic representatives, respectively. The family Blaniulidae contains two, while the remaining families, viz., Glomeridae, Glomeridellidae and Trichopolydesmidae have, so far, only one representative each in the Caucasian underground. Of special interest is the julidan genus *Leucogeorgia* Verhoeff, 1930, which includes 16 species, 14 of which are troglobionts. In addition to characteristic troglobiomorphic features, such as body depigmentation and eye reduction, as many as half of these species have gone a step further. The shortening of the body, the elongation of the legs and tarsal claws, as well as the high modification of the head and mouthparts enable these animals an amphibious way of life—they were observed to occasionally enter cave waters, probably for filtering food.

Oral Communication

20 years of underwater cave research in Herzegovina

Balázs, Gergely[1]*; Lewarne, Brian[2]

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It is often overlooked that to be able to carry out any speleobiology research, first we need to find the habitats. Cave exploration is a demanding activity in general, but it is even more challenging when the passages are filled with water. The Caudata Cave Research Group was formed almost 20 years ago with the aim of carrying out systematic underwater cave research in Eastern Herzegovina in Bosnia and Herzegovina, to provide information on cave biology, hydrology, and geology. Our activity in the area was initiated by the Devon Karst Research Society in cooperation with Zelena Brda Caving Club from Trebinje to help the research program named “A joint strategy for the protection of the endangered underground endem *Proteus anguinus* and its natural karst habitat in the Trebišnjica River Basin” or as we call it the Proteus Project. Over the years we’ve organized more than 40 cave diving expeditions and visited, discovered and rediscovered numerous caves and made considerable progress in many locations while tried to collect as much information on subterranean habitats as possible. While our primary aim is to provide solid background for ongoing and future scientific activities for the project and for anyone who is interested, we’ve successfully developed and tried out various methods and carried out researches on our own.

Oral Communication

The role of phenotypic plasticity in cave colonization of the genus *Proasellus*

Bedek, Jana[1]*; Lelas, Luka[2]; Bilandžija, Helena[1]

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Bilandžija, Helena: hbilandz@irb.hr

Diverse adaptations have evolved in animals living in the peculiar environment of caves. It has already been shown that high plasticity in response to darkness may be an important factor in the evolution of cave-adaptive traits in the Mexican tetra, *Astyanax mexicanus* (De Filippi, 1853) but it is unknown whether phenotypic plasticity plays a role in cave colonization in general. To address this question, we selected *Proasellus coxalis* (Dollfus, 1892), a widespread asellid, as a model organism. It is a surface relative of *P. anophtalmus* (Karaman, 1934), which has several cave-adapted lineages. It also inhabits caves (e.g., *P. c. lucifugus* Deeleman-Reinhold, 1965) and may form subterranean populations (e.g., *P. c. nanus* Sket, 1991). We first compared *P. coxalis* to *P. anophtalmus* to understand what adaptations evolve during cave colonization in this genus. Next, we randomly divided adults (G0) collected in nature into two cohorts, exposing one group to complete darkness (DD) and the other group to a normal light-dark photoperiod (LD) as a control. We established breeding colonies and obtained the first generation (G1) in both species and second generation (G2) in *P. coxalis* under both experimental conditions. LD and DD cohorts of *P. coxalis* differ in both gene expression and phenotype. 3024 genes showed differential expression in the DD and LD groups after four months in the experiment. We also sequenced *P. anophthalmus* after 4 months under the DD and LD conditions for comparison, and there were only 481 differentially expressed genes. At the phenotypic level, we observed many differences between DD and LD *P. coxalis*, including body pigmentation, growth rate, fecundity, and survival. Our results indicate that phenotypic plasticity may be an important mechanism leading to adaptation to cave environments in general.

Oral Communication

Acanthocephalan-induced phenotypic alterations of *Asellus aquaticus*

Benko, Grega[1]*; Fišer, Žiga[1]; Kostanjšek, Rok[1]

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Acanthocephalans are obligatory endoparasites that often alter the phenotype of their invertebrate intermediate host to facilitate trophic transmission to their final vertebrate host. *Acanthocephalus anguillae*, a widespread parasite of European freshwater fishes and isopods *Asellus aquaticus*, was recently discovered also in Postojna-Planina Cave System (Slovenia) parasitizing olms (*Proteus anguinus*) and cave populations of *A. aquaticus*. This setting offers a unique opportunity to investigate potential fine-tuning of parasitic manipulations to the specifics of the highly divergent subterranean environment where some common phenotypic alterations lose functionality, but others might gain it. We measured three behavioral (activity, thigmotactic sheltering, photophobia) and one morphological (pigmentation) trait of infested and uninfested isopods of both sexes from one surface (Planina Polje) and one subterranean (Pivka Channel of Planina Cave) ecomorph population. All behaviors were quantified from one-hour video-recordings via video-tracking isopod's movement in empty or customly modified (half-sheltered / half-illuminated) Petri dishes. To quantify pigmentation, we photographed individuals against a grayscale standard and averaged the body area pixel values. Isopod activity was not altered by parasite infestation, but surface isopods were significantly more active than subterranean ones. Contrarily, infested isopods of both ecomorphs spent significantly less time sheltering and were significantly less photophobic than uninfested ones. The reduction of sheltering seemed more pronounced in subterranean isopods. We observed no differences between the sexes in either behavior. Finally, pigmentation of infested surface isopods was significantly darker than of uninfested ones, especially so in males, but subterranean isopods were always completely depigmented. Taken together, we demonstrate that *A. anguillae* manipulates sheltering and photophobia of both intermediate host ecomorphs, but also pigmentation of surface isopods. It seems phenotypic alterations are not particularly fine-tuned to the subterranean environment and its hosts, and likely still reflect the parasite's surface origin. Future investigations might reveal the opposite pattern in other traits.

Oral Communication

Telestes karsticus – a cave adapted fish in the Dinaric Karst

Bilandžija, Helena[1,2]*; Čupić, Mateo[1]; Lukić, Marko[1,2]; Gračan, Romana[3]; Marčić, Zoran[3]

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Animals that are not fully troglomorphic are often neglected in cave biology studies. However, they can provide important insights into the early stages of cave colonization. This is especially true when a surface population is also present and available for comparative studies. Although fishes are not uncommon in caves of the Dinaric Karst, it is generally believed that there are no cave-adapted fishes in this region. Several genera of fishes, such as *Delminichtys*, *Telestes*, and *Phoxinellus*, are known to be associated with caves, as they have been reported to retreat to caves during dry periods and spend several months underground until favorable conditions return. Data on such populations and their occurrence in caves are sparse, but during recent intensive surveys of Sušik Cave, Velika Kapela Mt, Croatia, cavers have frequently encountered fish. Sušik cave is a sinkhole of the small stream inhabited by a single endemic fish species, *T. karsticus*. Therefore, we set out to investigate whether *T. karsticus* lives permanently in Sušik Cave, how large the cave population is, and whether it exhibits any cave-adaptive characteristics. Our results show that *T. karsticus* lives in Sušik Cave throughout the year and its population is estimated to be several hundred individuals. Moreover, we found morphological and physiological characteristics that clearly distinguish it from the neighboring surface population. Therefore, *T. karsticus* forms a permanent cave population and the presence of a conspecific surface population makes it a potentially important model system for understanding the initial adaptive processes that occur during cave colonization.

Poster

An overview of the biospeleological research in wider area of Barać caves

Bregović, Petra[1]; Mišerić, Ivana[1]; Sudar, Natalija[1]*; Bedek, Jana[1]; Ćukušić, Anđela[1]; Čupić, Iva[1]; Dražina, Tvrtko[1,2]; Hlebec, Dora[1,2]; Pavlek, Martina[1,3]; Rožman, Tin[1]

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In 2020 and 2021, biospeleological surveys were carried out in the wider area of Baraćeve špilje (eng. Barać caves), in cooperation with The Public Institution for the Management of Protected Natural Values on the Territory of the Municipality of Rakovica. The main goal was to make an inventory of the subterranean fauna in selected caves, and to conduct a two-year biospeleological monitoring of the Gornja Baraćeva špilja cave. We have undertaken eight field trips, one in each season (spring, summer, autumn and winter) over two years. In addition to the inventory of subterranean fauna, we measured microclimatic conditions, photographed interior of the caves and cave entrances, and collected, identified, and photographed fauna. In 2020, we investigated Božićeva špilja, Dumenčića špilja, Donja Baraćeva špilja and Gornja Baraćeva špilja caves, while in 2021 research was conducted in Velesova Jama, Jama pod Guvnom, Kunina špilja, cave system Ponorac-Jovina caves and biospeleological monitoring of Gornja Baraćeva špilja continued. With this research, we obtained the first lists of subterranean fauna for selected caves. By systematically studying the Gornja Baraćeva špilja cave, we updated its list of fauna which now contains 46 taxa—13 taxa more than were recorded in the literature before we started our research. The species *Machaerites pavleki* Hlaváč & Jalžić, 2010 was found for the first time in thirteen years, both in Donja Baraćeva špilja and in Gornja Baraćeva špilja. This two-year research was of great importance because it shows that interesting new fauna can be found even in well-studied caves with systematic approach.

Oral Communication

Resolving the phylogeny of Anthroherponina with notes on new species of specialized hygropetricolous genera

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One of the most illustrative examples of morphological variability in subterranean animals is presented by the cave-adapted subtribe Anthroherponina (Coleoptera: Leiodidae). In addition to highly troglomorphic, Anthroherponina also includes ecologically ultra - specialized hygropetricolous genera, namely *Hadesia, Croatodirus, Kircheria, Nauticiella* and *Velebitodromus*. These genera inhabit the cave hygropetric – a special subterranean habitat where water, constantly running down the cave walls, enables existence of microbial communities. To access this unusual food source, hygropetricolous beetles share mouthparts with widened maxillae and mandibles, and dense setose maxillipeds, which are used for scraping and filtering of fine organic matter. Recent exploration of hardly accessible, deep-phreatic caves of the Dinaric Karst, enabled us to access the new populations of specialized filter feeders. Among them, also the representatives of the iconic hygropetricolous genus *Hadesia* and syntopically distributed, but elusive *Nauticiella*. Up to now, the monophyly of Anthroherponina was questioned by employment of both, morphological and molecular data. However, all analyses hitherto were based on a subset of genera and lack resolution on their phylogenetic relations. In addition, previous studies uncovered existence of hardly distinguishable *Hadesia* species. Therefore, we collected all Anthroherponina genera missing in previous studies, tested the monophyly of the group, and set the groups evolutionary development into paleogeographic context by reconstructing a multilocus calibrated phylogeny. In addition, we studied the taxonomic structure of the newly assembled *Hadesia* samples using uni- and multilocus molecular species delimitation. As a result, monophyly of Anthroherponina, which developed along with the orogeny of the Dinarides, turned out to be an artefact due to the morphological convergence of hygropetricolous beetles. In addition, we discovered two new species of *Hadesia* and an additional, third species of *Nauticiella*. Finally, the new results raise more questions on our understanding of evolution in subterranean beetles and their adaptation to special ecological niches.

Poster

Recent biospeleological research in the Žumberak – Samobor hills Nature Park

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Croatian Biospeleological Society and Biology Students Association – BIUS conducted biospeleological research at the Žumberak – Samobor hills Nature Park in 2020 and 2021. From a geotectonic point of view, this protected area is very interesting – it is situated at the contact zone between structural complexes of the Inner Dinarids and central Dinaric carbonate platform. The massif continues in southeast Slovenia under the name Gorjanci. More than 150 caves and pits are known from this massif (including the Slovenian part). Most of the biospeleological data concerning Žumberak and Samobor hills are scattered in technical reports, occasional publications or in abstracts of scientific conferences. Inventory of biodiversity of two groups (Coleoptera and Amphipoda) are more represented in scientific papers. Nevertheless, all literature data indicate diverse and unique subterranean fauna. This was also confirmed by our research, in which we recorded numerous taxa of Gastropoda, Arachnida, Crustacea, Myriapoda, Collembola and Coleoptera. From a speleological and biospeleological perspective several caves stand out: Provala (2161 m long and 55 m deep) and Bedara (1593 m long and 113 m deep) as the largest speleological objects in the Nature Park, together with Drobovnik and Pušina. All four caves have both dry passages and flowing water parts, with diverse troglobiotic and stygobiotic fauna. Drobovnik is a type locality for endemic coleopteran species *Machaerites curvistylus* Nonveiller & Pavićević, 2001. It is also very important to mention the Jamina pit, in which a dense population of Natura 2000 species *Leptodirus hochenwartii* Schmidt, 1832 was recorded. Thus, this pit needs to be regularly monitored. Furthermore, it is necessary to systematically investigate other, smaller caves and pits of Žumberak – Samobor hills Nature Park, which are also faunistically interesting.

Oral Communication

Possible ways of stygobiotic gastropod dispersal

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The subterranean invertebrates including Gastropoda are frequently considered as evolutional relicts trapped and isolated in their hypogean environment with limited ability of dispersal. However, in most cases we do not have enough hydrogeological paleohydrological, climatological and paleographical information to understand the level of their isolation in space and in time. Recent molecular studies on genus *Hauffenia* revealed a large dispersal capability as a result of adaptation to different type of hypogean habitat with different food preference. Similar larger dispersal ability was proven in the genus *Montenegrospeum* driven by different, still not fully understood preferences and events. In contrast, the current data on genera as *Kerkia, Bracenica* or *Lanzaia (Plagigeyeria)* support the "island" theory, and it is hypothesized that their limited dispersal capabilities are derived from their narrow habitat preference resulting in a higher species radiation. No universal distribution/dispersal pattern can be formulated as a general rule for all stygobiotic gastropoda. It's not surprising knowing the high diversity of Dinaric stygobiotic gastropoda that comprises 233 presumably valid, and about 30 still undescribed species from 26 genera and four families. Each genus has its own, sometimes very different rules, of habitat preference and dispersal/adaptation capabilities, which could be frequently different within the species associated in one genus.

Poster

Testing the utility of eDNA metabarcoding for studying groundwater biodiversity

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Subterranean habitats are among the least explored environments on our planet and their fauna remains inadequately known. Fortunately, the rapid development of methods in modern biology can help expand our understanding of cave biodiversity. We tested the utility of environmental DNA (eDNA) to identify subterranean communities in Plitvice Lakes National Park. This is a complex karst area with many rivers, streams, lakes, and springs, but with limited access to groundwater. We combined classical sampling of cave fauna in the broader region of the Park with 18S and COI metabarcoding of 2 caves and 9 springs in or near the Park. The eDNA metabarcoding approach identified a much greater diversity of species and groups than classical sampling methods. Most interestingly, using eDNA, we detected a cave leech (identified as *Croatobranchus mestrovi*), a cave bivalve (*Congeria* sp.), and fishes of the genus *Telestes,* none of which were previously reported from this region. If confirmed, these results will greatly improve our understanding of the distribution of these species and help us in our conservation efforts. However, the metabarcoding approach did not detect species that we had collected with classical sampling, and it had a significant amount of noise and potential contamination. We can conclude that eDNA metabarcoding is a powerful tool for characterizing subsurface communities, especially in areas where sampling is limited, but it must be done with multiple controls and the results must be interpreted very carefully. In the future, the utility of this method will continue to increase as the databases and bioinformatics tools used to identify sequences become more developed.

Oral Communication

Explosive evolution of pseudoscorpions in Croatia

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Dinaric Karst is a global biodiversity hotspot containing a plethora of endemic taxa that evolved in this glacial refugium of the southeastern Europe. Phylogenetic approaches revealed deep genetic divergences between species and populations in many lineages that occur in these karst habitats. This genetic diversity is mostly caused by a complex palaeographic history, habitat heterogeneity and extraordinary climatic variation. Pseudoscorpions are one of the most diverse arachnid lineages in Croatia with 144 described species. Genera *Neobisium* and *Chthonius* are especially diverse. Many species have been described from Dinaric Karst in the last 30 years. The present state of taxonomy is highly problematic because many species descriptions are poor, type specimens lost or part of private collections, and type localities are often vague. We morphologically examined 2197 specimens mostly collected in collaboration with Croatian Biospeleological Society and sequenced two genes, *COI* for 502 specimens and *28S* for 96 specimens. Our results show correlation between morphological variability and genetic diversity within pseudoscorpion species in the Dinaric Karst and form the baseline for a sound taxonomic framework needed for studies that focus on biogeography or evolutionary patterning. Preliminary *COI* barcoding data (part of the project *DNA barcoding of Croatian faunal biodiversity*, funded by Croatian Science Foundation) indicate a high level of intraspecific variability and the presence of several cryptic and/or undescribed species and one undescribed genus (family Chernetidae). The results so far highlight the need for testing the validity of species described from the Dinaric Karst using a multi-gene phylogenetic approach and specimens from type localities. Furthermore, it is necessary to identify hotspots of subterranean biodiversity at the genetic level, thereby facilitating the protection of endemic pseudoscorpion species and their subterranean habitats.

Oral Communication

Recent progress in barcoding water mites (Acari, Hydrachnidia) of the Western Balkans

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Water mites are a significant component of freshwater ecosystems inhabiting a wide range of aquatic habitats, including lotic, lentic, interstitial and temporary waters. The research of water mites has a relatively long tradition in the Balkan, resulting in the presence of about 400 species, of which approximately one-fifth are inhabitants of subterranean habitats. Recently, in the framework of the "DNA-Eco" project, DNA barcode library for water mite species fauna of Montenegro was formed. The latter publicly released reference library includes DNA barcodes for 326 specimens of water mites belonging to 99 morphologically identified species (55% of Montenegrin fauna). The sampling campaign done in 2021 enlarged the existing barcode reference library with new sequences of specimens collected in Serbia, North Macedonia, Croatia and Bosnia and Herzegovina, increasing the number of BOLD available barcodes to almost 500, which is a solid basis for creating a comprehensive library of water mite DNA barcodes of the Western Balkan region.

Oral Communication

Ommochrome pigments in surface isopods (Isopoda, Crustacea) and their loss in cave-adapted albino relatives

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Cave-adapted animals are an excellent model for studying the molecular mechanisms underlying the convergent evolution of various traits such as pigment loss or albinism. Characterizing the nature of biological pigments in phylogenetically closest surface relatives is the first step that must be taken to understand this phenomenon. To date, the only information on the mechanisms of pigment regression in troglobites has been published for melanin pigment, while the mechanisms of ommochrome loss remain largely unknown. Ommochromes are tryptophan derivatives and generally a less studied class of biological pigments found exclusively in protostomes including crustaceans. Our study was focused on surface and cave crustaceans of the order Isopoda. Using a biochemical approach to extract and detect ommochromes in conjunction with liquid chromatography coupled to mass spectrometry (UPLC-MS), we identified for the first time and to the best of our knowledge ommochromes in three groups of isopods: Sphaeromatidae (Flabellifera), family Trichoniscidae (Oniscidea), and genus *Proasellus* (Asellota). Using the same method, we quantified the precursors of ommochrome synthesis in both surface and cave representatives to determine which step of the biosynthetic pathway may be defective in albino cave dwellers. In general, our results show an accumulation of tryptophan in cave dwellers, suggesting that the disruption of ommochrome synthesis is likely at the very beginning of this anabolic cascade. Our data suggest that isopods are a good group to study the precise molecular mechanisms underlying albinism as a consequence of ommochrome deficiency. To address this issue, we will apply RNA-Seq and assemble *de novo* transcriptomes for several pairs of surface-cave relatives. We plan to compare transcriptomic data for candidate genes to understand expression changes and/or possible inactivating mutations that may be responsible for albinism in different isopod lineages.

Oral Communication

Cave spiders of Bosnia & Herzegovina

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Dinarides are recognized as a global hotspot of subterranean biodiversity. The diversified geomorphology, paleogeography and historical climate have resulted in a remarkable range of different underground habitats in Bosnia & Herzegovina. Despite the fact that cave spiders in Bosnia & Herzegovina have been subject of study by several authors in the past 120 years, they still remain heavily understudied. As a result, the knowledge about this group is still scarce and far from being complete. Out of the total 165 spider species known from the country, and based on critical analysis of literature and author's unpublished data, the cave spiders of Bosnia & Herzegovina are represented by 45 taxa from 7 families: Agelenidae, Anapidae, Dysderidae, Leptonetidae, Linyphiidae, Nesticidae, Pholcidae and Tetragnathidae. Cave adapted, the so called troglobiont, rich genera include *Centromerus*, *Folkia*, *Kryptonesticus*, *Mesostalita*, *Parastalita*, *Rhode*, *Stalagtia*, *Stalita*, *Sulcia* and *Troglohyphantes,* the last genus being the most numerous among troglobitic spiders.

Oral Communication

Hidden diversity of the cave centipede *Lithobius stygius* Latzel, 1880 (Chilopoda: Lithobiomorpha: Lithobiidae) in the Northwestern Dinarides

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Centipedes present top predators in terrestrial ecosystems, with some species living exclusively in subterranean habitats. The first described cave centipede, *Lithobius stygius* Latzel, 1880, was discovered in Postojnska jama, Slovenia. The species has since been reported from all over the Balkans, often based on vague morphological determinations. The latter, coupled with the complex ontogeny of centipedes, resulted in an unresolved species-level taxonomy and establishment of the *L. stygius* 'umbrella term'. Here we present the study on genetic diversity of individuals belonging to *L. stygius* s.str., a group which we defined as the monophyletic clade of populations including the individuals from the type locality. We used material from Southern Slovenia (Northwestern Dinarides) and acquired sequences for two mitochondrial and one nuclear gene fragment for altogether 64 individuals from 34 caves. We performed COI species delimitation and haplotype network analyses and inferred the multi-locus phylogeny of the group. The results revealed that *L. stygius* s.str. is not uniform, but a complex of several cryptic species. Furthermore, during the fieldwork we discovered specimens morphologically similar to *L. stygius* that are only distantly related to *L. stygius* s.str., and possibly present a new species to science. Our analyses open many unresolved questions regarding phylogenetic relations within the *L. stygius* complex of species. To fully understand those relations, analyses of samples from whole distribution area of the species complex is needed, which may reveal even higher cryptic diversity. The study presents the basis for further research on centipede diversity in the Dinarides and offers a rare insight into the phylogeny of overlooked and widely spread ground predators.

Oral Communication

SubBIOCODE Database – web interface to facilitate access to data on subterranean biodiversity in Southeastern Bosnia and Herzegovina

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Reliable and accessible information on species distributions is needed for planning of research and conservation activities, which is true also for the Dinarides with exceptionally high subterranean biodiversity. The project SubBIOCODE, supported by CEPF, is taking place in the Trebišnjica river catchment. A vital component of the project is the goal to improve the knowledge and access to data on subterranean biodiversity in the project area, to facilitate its use in research and conservation. Data access has been enabled by developed web interface SubBIOCODE Database (http://db.subbiocode.net), covering the project area. Information is taken from a source database, SubBioDB, managed by SubBioLab (Biotechnical Faculty, University of Ljubljana). Source database is set on MSSQL relational database management system, while data are being transformed and transferred to SubBIOCODE Database as a client with a frontend application. Implemented authorisation layer enables access to exact positional data of the localities to registered users only. SubBIOCODE Database contains data from published sources and fieldwork, conducted by the SubBioLab. In March 2022, the database contained at least 5710 records (taxon-locality-source), but new data are being added constantly and will continue to be updated via established connection with the source database. The SubBIOCODE Database currently includes over 600 localities, many of which are equipped also with photographs of the sites. Presentation of taxa includes information on conservation statuses and, whenever available, DNA sequences, useful as barcodes for molecular identification of a certain species. An important part of the web interface is the invitation to the interested public, to report on interesting observations of subterranean animals via online form on the database web page. The concept developed in SubBIOCODE Database could be easily extended, implemented in other regions or interlinked with existing databases that lack data on subterranean biodiversity and thus presents an excellent opportunity for collaborations.

Oral Communication

Setting up the laboratory invertebrate facility - good and bad practices

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Many aspects of the biology of cave animals cannot be understood simply by *in situ* observations. This is especially true for comparative evolutionary and developmental studies. For example, for studying physiological and behavioral adaptations or the role of phenotypic plasticity in the evolution of adaptations, animals must be kept under controlled laboratory conditions. For this reason, we have established an animal facility in our laboratory where we plan to culture cave species from different taxonomic groups and their close surface relatives. The available literature to help us design the facility and care for the animals in the initial stages was sparse. Fortunately, many colleagues in cave biology contributed with advice and assistance. Here we report our findings to provide some guidelines for other researchers in similar endeavors. We will briefly discuss good and bad practices related to animal care - housing, water treatment, small terrarium design, different types of food, and breeding. We will also address general protocols necessary for keeping different species closely together - decontamination of equipment and tools and quarantine. To date, we have successfully established breeding colonies of four different cave/surface species pairs and several others have survived in our facility for many months, if not years, including planarians, crustaceans, and mollusks.

Poster

Unexpected diversity of *Niphargus boskovici* species complex inferred from mitochondrial DNA

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More than 30 % of approximately 450 *Niphargus* species originates from the subterranean biodiversity hotspot in the Dinaric Karst. Despite the immense diversity, new species from the Dinarides are being described on a yearly basis. In addition, employment of molecular techniques revealed that some of the widely distributed species in fact comprise of morphologically indistinguishable species, the so-called cryptic species. Among species with relatively large distributional range is also a small species, *Niphargus boskovici*, described from the epikarst habitats of Bjelušica cave in Popovo polje. During fieldwork conducted mainly within the project SubBIOCODE, we collected numerous samples from the Central and Southern Dalmatia, Herzegovina and Western Montenegro. Many of the collected specimens resembled *N. boskovici,* therefore, we tested the taxonomic structure of the *N. boskovici* species complex. We amplified COI barcoding fragment in 163 specimens from 43 localities. The acquired sequences were aligned into a central COI dataset counting more than 1500 *Niphargus* sequences, and subjected to species delimitations. The results surprisingly identified 33 putative species. The nominal species, *N. boskovici*, is apparently narrow endemic and was found only in the type locality, Bjelušica, and another nearby locality, Vjetrenica. The vast majority of specimens resembling *N. boskovici* form two large, geographically slightly overlapping, phylogenetic lineages. All putative species are nested within the Southern Dinaric niphargid radiation. The newly discovered species increased species richness of the entire clade by 61% and pinpoint a deep gap in our knowledge of epikarst fauna in the region. The discovery of yet undetected subterranean species richness in this 'biodiversity hotspot within the hotspot' emphasizes the need for inclusion of additional nuclear markers and employment of multilocus species delimitation, that will fully resolve the taxonomic structure of this species complex.

Poster

Centipedes (Chilopoda) from Bosnia and Herzegovina in the collection of the Museum für Naturkunde Berlin

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The present work lists the type and non-type centipedes (Chilopoda) from Bosnia and Herzegovina held in the Myriapoda collection of the Museum für Naturkunde Berlin, one of the largest and most significant collection of its kind in the world. Twenty-three currently valid species (15 Lithobiomorpha, 2 Scolopendromorpha, 6 Geophilomorpha) of this collection represent almost 30% of the whole centipede fauna of Bosnia and Herzegovina (81 taxa). Type material comprises 11 valid species or junior synonyms, all described by the pioneering myriapodologist Karl-Wilhelm Verhoeff between 1895 and 1901: *Clinopodes carinthiacus* (Latzel, 1880) = *Geophilus flavidus trebevicensis* Verhoeff, 1898; *Eupolybothrus (Leptopolybothrus) tridentinus* (Fanzago, 1874) = *Lithobius (Polybothrus) leptopus brölemanni* Verhoeff, 1900; *Eupolybothrus (Parapolybothrus) herzegowinensis* (Verhoeff, 1900) = *Lithobius (Polybothrus) herzegowinensis* Verhoeff, 1900; *Geophilus bosniensis* Verhoeff, 1895; *Geophilus cribelliger* Verhoeff, 1898; *Geophilus cribelliger* Verhoeff, 1898 = *Geophilus cribelliger antecribellatus* Verhoeff, 1898; *Geophilus pusillifrater* Verhoeff, 1898 = *Geophilus pusillus pusillifrater* Verhoeff, 1898; *Lithobius (Lithobius) matulici* Verhoeff, 1899 = *Lithobius (Oligobothrus) matulicii* Verhoeff, 1899; *Lithobius (Sigibius) apfelbecki* Verhoeff, 1900 = *Lithobius (Oligobothrus) apfelbecki* Verhoeff, 1900; *Lithobius (Sigibius) reiseri* Verhoeff, 1900 = *Lithobius (Oligobothrus) reiseri* Verhoeff, 1900; and *Pleurogeophilus herzegowinensis* (Verhoeff, 1901) = *Geophilus herzegowinensis* Verhoeff, 1901. Among these centipedes, only one species (*L. matulici*) might be considered as troglobiont, as it has only been found in caves and never in surface (epigean) habitats. However, this unique collection is highly important, not only because of its great size, but also because it goes far towards filling one of the largest gaps in our knowledge of the centipede fauna of the Balkan Peninsula. There remains, of course, much to be done on the centipedes of Bosnia and Herzegovina. Future investigations will probably bring to light a great number of new forms, particularly among the cave-dwelling lithobiomorphs.

Oral Communication

Subterranean evolution of the tribe Bythinini (Pselaphinae) in the Balkan Peninsula

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The unique karstic subterranean environment of the Balkan Peninsula has proven to be a world biodiversity hotspot for beetles, as it is illustrated by the tribe Bythinini (Staphylinidae: Pselaphinae), with high diversity and endemism. In the present project, the diversity and evolution of the tribe is being investigated through an integrative approach, by implementing molecular and morphological tools. So far, 96 taxa of 26 genera from 11 Pselaphinae tribes (among which 72 taxa of 14 genera for Bythinini) have been molecularly analyzed via mitochondrial and nuclear markers, giving some interesting preliminary phylogenetic insights. Bythinini appears to form a monophyletic tribe with regard to all included outgroups and is split into two major clades. One clade contains exclusively subterranean taxa, including the species-rich *Tychobythinus* genus and 6 strictly cavernicolous genera. The other major clade mainly comprises two species-rich epigean genera, *Bythinus* and *Bryaxis*, but includes a few cave adapted taxa and genera. Various clade clusters are congruent biogeographically and hypogean isolation repeatedly impacted the evolution of the tribe. Lastly, multiple convergent morphological adaptations related to colonization of the subterranean environment were identified within Bythinini taxa and lineages. The results of this study will set the base for various taxonomic revisions and descriptions, provide the first molecular phylogenetic reconstruction of the tribe and shed light into multiple questions regarding modalities of colonization, speciation and evolution of hypogean taxa.

Poster

Snail *Spelaeoconcha paganettii* Sturany, 1901 - a new finding in Western Herzegovina

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Spelaeoconcha paganettii Sturany, 1901 is the monotypic, meaning the only, representative of the family *Spelaeoconchidae*. After the first discovery on the Island of Korčula (Croatia), according to which the species was described, the shells of this small snail species were found at several other sites in Dalmatia and Bosnia and Herzegovina. The species representatives are quite rare and usually found under the collapsed rocks in the caves. However, an empty shell of a *Spelaeoconcha* specimen was also found on a forest slope opposite the main entrance to the "Vrelo Bosne" nature reserve near Sarajevo. The finding enables hypothesizing that other habitats might also be suitable for this species. Currently, three subspecies of *Spelaeoconcha* are known: *S. paganettii paganettii*, *S. paganettii polymorpha* and *S. paganettii alphonsi,* and an intermediate form *S. paganettii polymorpha-alphonsi,* showing morphological characters of both subspecies. All three subspecies are narrowly endemic and exhibit a disjunct distributional range. Recently, a representative of *Spelaeoconcha* was discovered at the source of the river Vrioštica in Vitina, which springs from a small cave. Unfortunately, only one empty shell was found, belonging to the subspecies *S. paganettii polymorpha* A. J. Wagner, 1914. This is the first finding of this genus in Western Herzegovina and it raises further questions regarding our knowledge on the distribution of this rare monotypic family, potential reasons for it and its biology in general. The species was found during research on freshwater and spring snails within the project "Distribution, population, and status of threats to the biodiversity of freshwater snails of the family Hydrobiidae" which was financially supported by the Critical Ecosystem Partnership Fund (CEPF).

Oral Communication

Spatial genetic structure of five sympatric cave spider species

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The cave habitat has driven strong constraints in organisms that thrive therein, and caused the acquisition of troglomorphic traits such as the loss of eyes or depigmentation. Because of the limited connectivity between potential habitats, and the inability to use the surface for dispersal, organisms strictly associated with caves might demonstrate population dynamics similar to those species found in islands. In this study we compared the population genetic structure of five cave spider species with different ecology and biology from different caves across the north Dinarides. Namely, two depigmented and anophthalmic Dysderidae species *Parastalita stygia* (Joseph, 1882), and *Stalita pretneri* Deeleman-Reinhold, 1971, which were never collected outside caves, and three *Troglohyphantes* species, *T. croaticus* (Chyzer, 1894), *T. excavatus* Fage, 1919, and *T. kordunlikanus* Deeleman-Reinhold, 1978, which exhibit different levels of troglomorphism and dependency on cave habitat (*T. excavatus* and *T. kordunlikanus* can be found in surface habitats). Additionally, all *Troglohyphantes* species spin webs, while all Dysderidae are wandering active hunters. We applied a hybridization-capture museomics approach (i.e., HyRAD) to extract and capture DNA from 177 historical samples. By comparing the population genetic structures among the five species, and by studying isolation by distance, we identified contrasted genetic structures related to the biology and ecology of each species. We detected several common breaks in gene flow for all species except *T. kordunlikanus*, some of which can be explained by geographic and geologic features.

Poster

Including data on subterranean species distribution into a common Nature Conservation Information System in Slovenia – challenges and opportunities

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Prerequisites for proper planning of nature conservation actions are reliable, accessible, updated and critically assessed data on species and habitats distribution. In addition, quick access to existing nature conservation documents is needed for the effective implementation of nature conservation laws and regulations. Subterranean habitats are an important part of our environment but are mostly overlooked in environmental and spatial planning. Biological inventories of subterranean habitats are challenging and time-consuming, species are difficult to find, and identifications require expert knowledge. Many species are endemic, having narrow distribution ranges, and are therefore more endangered than species on the surface. Subterranean biodiversity is an important part of the natural heritage. Subterranean species and habitats have formal protection statuses, binding the state for efficient conservation actions. In 2021, a four-year project Life NarcIS - Life NAture Conservation Information System (LIFE19 GIE / SI /000161, co-funded by the EU Life Programme) has started, with the aim to develop a common information system, that will combine biological data, nature conservation data and documents from initially 11 different databases/sources. The central national information system is intended to optimize and facilitate spatial planning, enhance implementation of national and EU legislation, and improve nature conservation in practice. The system will also contain data on subterranean species in the country. It will include the data from SubBioDB, an integrative database on the distribution of subterranean species, managed by SubBioLab at University of Ljubljana. SubBioLab' involvement is manifold. Among other activities, it will take over taxonomic checklists for subterranean taxa and prepare and standardize distributional data. An important part of the project is also to inform and educate the next generation of biologists to ensure future data influx and successful transition from current working practices to data-informed nature conservation.

Oral Communication

Zoogeographical borders of the Dinaric karst based on Leptodirini beetles distribution

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Extending over approximately 60,000 km^2 the Dinaric karst is the largest continuous karstic landscape in Europe. It is positioned in the so-called Dinaric direction between the Pannonian Basin in the northeast and the Adriatic Sea in the southwest part. The transition towards the Alps on the northwest and the Albanian mountains towards the southeast is not very distinct. Depending on their geographical, geological, tectonic or orogenetic approach, different authors define the Dinaric karst slightly differently. Thus, the exact external borders of the Dinaric karst, especially on its south edge are not defined yet. The zoogeographical units of the different animal group distribution usually depend on geological, or paleo-environmental events. In our study we tested the phylogenetic origin of the Leptodirini beetles, a group of beetles where the great majority of taxa express strictly subterranean adaptations and are mostly narrow endemic. In study, most of the Balkan genera compared to the taxa from other European regions, were phylogenetically and zoogeographically tested. We found that within isolated European karstic massifs, independent evolutionary radiations led to morphologically distinct yet genetically related groups of species. In the Dinaric karst live and partly overlapping representatives of two different generic clades. As exclusively Dinaric we defined the genera of the North Dinaric and the South Dinaric clades. These two clades represent two phylogenetically independent evolutional radiations. In the central part of the Dinaric Karst, these two clades slightly overlap. With the help of the geolocation of taxa localities and cartographic distribution display of the phylogenetically proven Dinaric genera, we precisely determined the protentional zoogeographical boundaries of the Dinaric karst area.

Oral Communication

The contribution of biology students' research camps to the knowledge on subterranean fauna in the Western Balkans

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Students' research camps organized by Biology Students' Society from the University of Ljubljana, Slovenia, have been running for over thirty years. About ten-days long events are primarily intended for undergraduate students who want to focus on selected group of organisms and want to broaden their knowledge obtained during the regular study curriculum. During the camps organized in Slovenia and other Western Balkan countries, students gain experience in field work, data handling and reporting, as well as learn about species diversity and identification. The group dedicated specifically to subterranean fauna has constantly been a part of these camps for over a decade. As mentors, we participated at nineteen camps all together, fourteen in Slovenia and five in other countries. In this contribution, we present the overview of data collected during the camps and evaluate their contribution to the knowledge on subterranean fauna in the Western Balkans. Data was taken from the SubBioDB, the largest database on subterranean fauna of the Western Balkans, managed at SubBioLab). In total, we surveyed over 270 localities, most of which were caves (62%), followed by springs and wells. In most surveys (77%), we found at least one troglobitic species. Many localities (66%) were surveyed for the first time, so our findings importantly improved the knowledge on many species' distributions and ecology, including discoveries of species new to science. Besides that, gathered information is valuable also in conservation of subterranean species and habitats. The samples collected during the camps were included in the analyses in over thirty scientific and professional publications. Considering these implications, the impact of research camps reaches far beyond their main educational intention to pass the expertise to younger generations. Students and researchers from universities worldwide can gain a lot by organizing or supporting such camps, which successfully combine education, research and fun.

Oral Communication

Olm lineages native to Bosnia and Herzegovina in the framework of parallel evolution of troglomorphy across the Dinaric Karst

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The olm is the largest subterranean animal native to the Dinaric Karst and considered its top predator. Phenotypically, it lacks pigmentation and eyes, and has an elongate body, snout and appendages. However, research within the last three decades has shown that the image of a phenotypically uniform widely distributed species is wrong. The discovery of the black olm forms only a part of this new view; cave phenotypes with different degrees of troglomorphism appear to have evolved multiple times independently. These distinct cave phenotypes are present in geographically and phylogenetically distinct lineages, that can be considered separate species. On the one hand, this shows the extraordinary power of convergent evolution: the repeated origin of similar phenotypes, in similar environments, but completely independently. On the other hand, this means that the previously thought widely distributed singular species is now separated into geographically isolated species, sometimes with extremely small distributions. This includes two species native to Bosnia and Herzegovina (BIH), one known only from five locations in Bosanska Krajina. Here we focus on these two phenotypically distinct species and their molecular diversity, putting them into the context of other olm species and their transitions to cave life. While one species has the widest distribution and highest diversity among all species, the species endemic to BIH has a comparatively low diversity. This low diversity could be explained in part by the limited number of sites currently accessible for sampling this species. In any case, there is a positive association between molecular diversity and range size. While more locations may be discovered in the future, it is clear that even with potentially new discoveries this species' range is comparatively small and requires conservation actions for its future protection.

Oral Communication

Typhlogammarus mrazeki, a holodinaric species broken apart

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Employment of molecular techniques revealed that cryptic species are a common phenomenon within subterranean environments. A plethora of species, exhibiting the so-called holodinaric distribution pattern were shown to consist of morphologically identical, yet distinct species, including representatives of amphipod genus *Niphargus,* shrimp *Troglocaris,* or clam *Congeria*. Herein the presence of cryptic species was tested in another holodinaric species, subterranean amphipod *Typhlogammarus mrazeki*, distributed from Gorski kotar (Croatia) to Skadar Lake (Montenegro). *T. mrazeki* is an outlier among Dinaric amphipods in several aspects. It is the bulkiest among all Dinaric subterranean amphipods, and belongs to the gammarid clade, although it does not have any known surface relatives. It is an ecological specialist that inhabits unusual ecological niches, including fast-running waters and the cave hygropetric. It is morphologically variable; however, the attempts to revise its taxonomy using morphology were not successful. Here, we revised its taxonomic structure by combining both morphological and molecular approaches. We analyzed 80 specimens from 21 localities. We amplified mitochondrial and nuclear markers to i) delimit potential cryptic species, ii) position *T. mrazeki* into gammaridean phylogeny, and iii) disentangle the origin of the group. Unilocus species delimitations using COI suggested that *T. mrazeki*, in fact, consists of three to five distinct species. Alternative species scenarios were tested within the multilocus delimitation framework, which gave higher support to the three species scenario; Southern Dinaric, Northern Dinaric, and the species from Mt. Biokovo. In the next step, we scanned the morphology of a few individuals and selected 9 morphological characters that showed variation among individuals. While we found high morphological variation within the population and within species, no morphological trait could be used as a potential diagnostic character. The time-calibrated multilocus phylogeny shows that *Typhlogammarus* comprises a monophylum with *Acubogammarus* and *Metohia*, all from Dinaric regions. *Typhlogammarus* lineage split from *Echinogammarus* around 36 mya, implying an old, pre-modern age Dinarides origin of Dinaric subterranean gammarids. The speciation events within *Typhlogammarus* are younger and correspond to the onset of the Pleistocene, 2.5 mya.

Poster

Špilja na Gradini kod Premanture cave, the first systematic biospeleological study

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The cave Špilja na Gradini kod Premanture is the southernmost cave on the Istrian peninsula, Croatia, located in the Upper Kamenjak significant landscape. It is a part of the Natura 2000 network of protected areas, protected under the Habitats Directive of the European Union as a habitat type classified as “caves not open to the public.” The cave is of simple morphology, consisting of one canal which is 102 m long and 22 m deep. It is a type and, as far it is known, the only locality for a troglophilic isopod subspecies *Androniscus roseus histrianorum*. The first systematic biospeleological study that included microclimate measurements (air and soil temperature, and relative humidity) of Špilja na Gradini kod Premanture was conducted in 2016. Then 27 taxa of invertebrates were documented, including four troglobionts and ten troglophiles. The cave was divided into three parts, covering different habitats and fauna structures, and a monitoring proposal was given. Biospeleological monitoring started in 2021 when troglophilic spider *Metellina merianae* was recorded for the first time in the locality, raising the total number of invertebrate taxa to 28. Microclimatic conditions in the cave remained unchanged compared to 2016 measurements. Although most taxa in the cave are troglophilic, the most numerous taxon recorded in 2021 was troglobiontic isopod *Alpioniscus strasseri.* A decline of both, Collembola and the endemic *A. roseus histrianorum* populations, was documented in 2021. Further monitoring of the habitat and the species populations is essential and will be continued in collaboration with the Kamenjak Public Institution.

Poster

Eupolybothrus (*Parapolybothrus*) *herzegowinensis* (Verhoeff, 1900), a poorly-known Dinaric centipede (Myriapoda: Chilopoda: Lithobiomorpha)

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Due to an extraordinary number of endemic and unique subterranean animals, the Dinaric karst is renowned as one of the world's most famous subterranean hotspots. As one might assume, the subterranean realm of this region is inhabited primarily by various groups of arthropods, both aquatic and terrestrial. As the third largest group of terrestrial arthropods, myriapods are among the most common animals in the underground of the Dinaric region. This primarily refers to the millipedes (Diplopoda), with a large number of troglobiontic and troglophilic taxa. However, a number of subterranean centipedes (Chilopoda) are also found here. They are mostly troglophiles, but also include a few striking troglobionts. In fact, the Dinarides are one of the richest regions in subterranean centipede diversity. Among the recorded centipedes from Dinaric subterranean habitats, the most common are species from the genus *Eupolybothrus* Verhoeff, 1907, which includes some of the largest species of Lithobiomorpha. Twenty of the approximately 30 described species in this genus have been reported from the Balkan Peninsula, with as many as 15 known from the Dinarides (six endemics). One of the poorly-known troglophiles amongst them is *E. herzegowinensis* (Verhoeff, 1900). This species was described based on specimens collected outside the caves in Bosnia and Herzegovina, Croatia and Montenegro (Dubrovnik, Herceg Novi, Kotor, Mt. Orjen and Trebinje). Afterward, it was reported very few times (from Albania and southern Croatia), both in caves and epigean habitats. Here, we report finding of this species in the underground part of the Diocletian's aqueduct channel in Split (Croatia). In total, two males were collected from this ancient Roman tunnel for water supply, in May and October 2021. All morphological characters relevant for species identification as well as the list of Dinaric species are presented in detail on the poster.

Oral Communication

A conceptual solution to the problem of subterranean vicariance and dispersal

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Ranges of subterranean species exceeding a single geotectonic unit of contiguous porous substratum present scientific and practical problems. A range discontinuity can arise through a number of processes and at different stages of the evolutionary history of a subterranean species. These processes are essentially vicariant or dispersalist, and can take place at the surface before colonization, within the subterranean realm after colonization, or as a combination of both. Hence we have an additional level of complexity, which is why in subterranean biology biogeographical problems cannot be addressed by simply applying the standard toolbox of epigean biogeography. As a solution, some authors have proposed a differential treatment of processes related to colonization and those taking place after colonization, but this has not been echoed in the work of other speleobiologists. In the 2000 edition of Ecosystems of the world – Subterranean ecosystems, William Humphreys wrote: "confusion arises because the distinction is not clearly drawn between the process of regional vicariance and those resulting in the movement of species into the hypogean realm". The veil of confusion has never been truly lifted. In this contribution, a conceptual framework is proposed that discriminates between exogenous processes that take place at the surface or during invasion, and endogenous processes that take place in the subterranean realm and are governed by its specific conditions. Mixed biogeographical hypotheses are less parsimonious and more difficult to test than purely epigean and purely hypogean ones. As in biogeography in general, corroboration can be gained from phylogenetically independent taxa with same or similar distribution patterns. In subterranean biology, this presents a challenge of its own, because of the paucity of distantly related taxa with coinciding ranges. Areas of high biodiversity, such as the Dinaric Karst, offer a testing ground for new hypotheses of subterranean biogeography.

Poster

Range sizes and conservation of *Niphargus* species (Amphipoda) occuring in Trebišnjica river catchment in Southeastern Bosnia and Herzegovina

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Most subterranean taxa have smaller distribution ranges than their surface relatives, due to the specifics of the environment they live in. Species with smaller range sizes are generally more vulnerable to disturbances, including anthropogenic threats. Knowledge on species' distribution is important information when setting conservation strategies. The goal of our study was to analyze the distribution of the selected subterranean crustacean taxa from SubBIOCODE project region, covering Trebišnjica river catchment, and to consider their inclusion into existing protection schemes. We analyzed distribution of 11 species/species groups of the amphipod genus *Niphargus*, occurring within the SubBIOCODE project area. Data were retrieved from SubBioDB, a database managed by SubBioLab (Biotechnical Faculty, University of Ljubljana). After checking and correcting the spatial position of localities, the distribution of each taxon was mapped, and range sizes were estimated as maximum linear extent (MLE) between the occurrence points, using QGIS analysis tools. For the area within the Republic of Srpska, we examined how many localities fall into areas with protection status. Our research shows that some species of *Niphargus* in the project region are highly endemic. Four species analyzed are known only from the SubBIOCODE project area. Among these, two species, *N. zavalanus* and *N. factor*, are known from two localities each. Six out of 11 species (54.5 %) had MLE less than 100 km, another species had MLE less than 200 km (9.1 %), whereas MLE of four species exceeded the limit of 200 km (36.4 %). As we studied only morphologically recognizable species/species complexes, we cannot rule out that ranges of actual species would be smaller. None of the records from Republic of Srpska originated from the protected sites. The high endemicity, combined with low protection of *Niphargus* species, indicates the need for the conservation of subterranean fauna in the region.

Oral Communication

Research of *Delminichthys ghetaldii*, semi-cave fish of Dinaric karst

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Popovo Polje is a karst field located in the south of Bosnia and Herzegovina. The double water regime (summer dry and winter flood) was originally active in Popovo polje, until the 1970s and the channeling of Trebišnjica River. *Delminichthys ghetaldii* (Steindachner, 1882) is an endemic species of southern Croatia and Bosnia and Herzegovina. In Bosnia and Herzegovina, this species inhabits water bodies (streams and underground streams) of Popovo Polje and the surrounding area. The genus *Delminichthys* Freyhof et al., 2006 includes four species found only in a few spatially small locations in Croatia and Bosnia and Herzegovina. All four species of the genus, including *D. ghetaldii,* migrate underground in certain parts of the year (usually at low water levels). Therefore, the mentioned species can be considered a stigophilic, semi-cave animal. It is currently recognized as a very rare and endangered freshwater fish. According to the Red Book of Freshwater Fish of Croatia, it is considered an endangered (EN) and is protected by the Nature Protection Act. Since 1996, it has been on the IUCN Red List in the category of sensitive species (VU). It is still a relatively little-known species that requires more complete knowledge of biology and ecology, and in particular its distribution. In order to determine the distribution, number and state of the populations of *D. ghetaldii*, a total of 23 locations were investigated by combining electrofishing and speleodiving. Study covered sites on the Croatian coast, Popovo, Mokro, Ljubomirsko, Dabarsko, Fatničko, and Gatačko Polje. The research showed a significant reduction in known distribution area and reduced populations. Morphological analyses and genetic analysis on COI mtDNA marker showed very low diversity of populations within *D. ghetaldii* species. eDNA method has been developed for further research and monitoring of this species.

Oral Communication

Observational data on subterranean freshwater taxa coming from a touristic cave

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Southern Dinarides bear an extremely rich and endemic subterranean fauna, including both, terrestrial and aquatic components. One of the often overlooked localities, rich in peculiar freshwater aquatic taxa, is the cave Jama u Predolcu in Metković (Croatia). Due to its position, with the entrance opening in the background of the family house, the cave itself has been known for ages. Unfortunately, the proximity of the settlement also represented the main threat to the cave, as it served as a local depot for a long time. This was changed only recently, when the unique touristic potential of the cave, offering a direct insight into subterranean life of the two cave lakes, was recognized. A decade ago, restauration of the natural habitat started by cleaning the cave and concluded by installation of the visitor platform and establishing an observation center "*Congeria*". The center includes a series of cave water filled aquaria, where visitors can directly observe the only known subterranean mussel, *Congeria*, the only subterranean polychete, *Marifugia cavatica*, and diverse subterranean crustacean community, including isopod *Trogloaega*, cave shrimps *Troglocaris* or *Niphargus* amphipods. Lately, some efforts to quantify the activities of the unique cave mussel by measuring their traveling distance were made. Opposing to what one would expect from a sedentary animal, *Congeria* mussels were shown to be mobile, relocating up to a few centimeters per month. Unpurposely, while observing *Congeria*, we witnessed proliferation of *Marifugia cavatica*, which overgrown the aquaria glass and their inner plastic parts (pump, filter, etc.). By presenting our observational data, which present one of the rare insights into lifestyles of unique freshwater subterranean taxa, we stress out the lack of knowledge on basic biology of the Dinaric subterranean animals, and support further efforts in collecting similar observational data.

Poster

Inventory of fauna in the Ljubačevo cave near Banja Luka, BIH

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The Ljubačevo cave is located within a quarry in the village of Ljubačevo, municipality of Banja Luka, only 14 km from the city center. It was discovered in 2004, during quarry's rock excavation, when the entrance to this underground system was opened by blasting. Hereafter, due to opening of an artificial entrance, the rest of the cave (southern part) has been declared an IUCN category III – Natural monument in 2008. The Ljubačevo cave consists of four halls and several galleries rich in speleothems, with a total length of 338 meters. Few studies on fauna of the cave were published, with no systematic research of its biodiversity. In March 2019 and December 2021/January 2022, we conducted sampling of fauna, with the use of visual inspection and pitfall traps. Material was identified to the greatest possible level based on photographs (2019) or examination of collected individuals (2022). For some of the latter, we also used molecular approaches for species identifications. Altogether, at least 31 different taxa were found in the cave, of which six were troglobionts. Compared to published research, our sampling revealed the presence of 14 additional taxa. Particularly interesting were the findings of subterranean beetles *Parapropus* sp. (Leiodidae) and *Neoduvalius opermanni* (Carabidae), which increased their distribution range. We found a group of previously unrecorded bat species, *Miniopterus schreibersii*, increasing the number of bat species known to occur in the cave to eight. Our work presents the first attempt of systematic data collection for the Ljubačevo cave and contributes importantly to the knowledge on the distribution patterns of Dinaric subterranean taxa.

Muzej Hercegovine, the Symposium venue

The Museum of Herzegovina in Trebinje was established in 1952. The first excavation was conducted in 1957 at the church of St Peter in Zavala. In 1966, it published the first publication under the name Trebinje 1 of Vasilije Korac. In 1975, it initiated the journal Tribunija. It has about 1.500 m² of space at its disposal, with about 800 m² dedicated for exhibitions.

Permanent displays:
- Dučić's collection with valuable artistic exhibits (72 exhibits of stone plastic, 14 paintings of domestic and foreign painters, tapestry, mosaic, Japanese embroidery, Jovan Dučić's orders, his ambassadorial clothes and his m–anuscripts);
- Permanent ethnological display Folk life and culture of Serbs in East Herzegovina in the second half of the 19th century and the first half of the 20th century;
- Permanent archeological display with worthy exhibits from the Stone Age to middle age;
- Memorial exhibition of the paintings of Atanasije Popović – 43 paintings of famous painter, gift of painter 's brother Uros Popović to town Trebinje;
- Bequest of painter Milena Šotra - 24 paintings with motifs of Herzegovina and Itinerary along Russia and 15 graphic arts of painter's brother, famous graphic artist Branko Šotra;
- Bequest of academic painter Milorad Ćorović – 57 paintings;
- Bequest of writer and sculptor Radovan Ždral – 122 sculptures made of semi-precious stone.
Apart from these permanent displays and bequests, there are 400 paintings, graphic arts, sculptures and other objects in the fund of Museum of Herzegovina. Annex on Ušće (Lastva),

bequest of Dubravka Kujačić in which exhibitions, promotions and concerts are held, is also part of Museum of Herzegovina.
The museum also has a considerable collection of library material (the library with technical books from different areas of science, culture and art).

Apart from permanent displays and bequests, occasional exhibitions, poet recitals, promotions of books and music concerts are held in the Museum of Herzegovina.

Text source: http://www.trebinje.rs.ba/?subpg=muzej; http://www.trebinjeturizam.com/en/kultura_obrazovanje.php