

Cave Biology, Research Team  
Institute of Zoology,  
School of Natural Sciences and Medicine,  
Ilia State University

# 1st Caucasian Symposium on Subterranean Biology



## ABSTRACT BOOK

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Tbilisi, 16-17 June, 2023

# Program and Abstract Book of the 1<sup>st</sup> Caucasian Symposium on Subterranean Biology



ილიას სახელმწიფო უნივერსიტეტი

ILIA STATE UNIVERSITY

თბილისი 2023 Tbilisi

**Program and Abstract Book of the 1<sup>st</sup> Caucasian Symposium on  
Subterranean Biology**

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- Ilia State University, Tbilisi, Georgia
- Georgian Cave Researchers Society

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- Ľubomír Kováč (Pavol Jozef Šafárik University in Košice, Slovakia) Program of the 1<sup>st</sup> Caucasian Symposium on Subterranean Biology

# Program of the 1<sup>st</sup> Caucasian Symposium on Subterranean Biology:

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16<sup>th</sup> June, 2023

- 10:00 – 10:15 – **Levan Mumladze, Shalva Barjadze** - *Ceremonial opening of the symposium*
- 10:15 –13:55 – **Oral speech session 1 – Chair Dragan Antić**
- 10:15 – 10:45 – **Plenary keynote talk 1. Shalva Barjadze** – Cave dwelling invertebrate fauna of Georgia, Caucasus
- 10:45 – 11:05 – **Mariam Gogshelidze** – Integrative taxonomical investigations on the cavernicolous pseudoscorpions of Georgia
- 11:05 – 11:25 – **Arnaud Faille** – *Inotrechus* Dolzhansky & Ljovuschkin, 1989, an endemic genus of cave Trechini from Georgia (Coleoptera: Carabidae)
- 11:25 – 11:45 – **Shalva Barjadze** – Two new species of *Deuteraphorura* Absolon (Collembola: Onychiuridae) from the Georgian caves
- 11:45 – 12:05 – **Lika Chertoprud** – The little ones, hidden in the springs: crenobiotic Belgrandiellinae Radoman, 1983 (Mollusca: Hydrobiidae) from Georgia
- 12:05 – 12:35 – **Coffee break**
- 12:35 – 12:55 – **Jozef Grego** – Next work in progress on stygobiotic Gastropoda from Georgia further confirming the subterranean biodiversity hotspot status of Caucasus
- 12:55 – 13:15 – **Helena Bilandžija** - Lessons from recent and ongoing cave colonizations: an example of *Asellus aquaticus* (Crustacea: Asellidae)
- 13:15 – 13:35 – **Ľubomír Kováč** – Diversity and distribution of Palpigradi (Arachnida) in Georgia
- 13:35 –13:55 – **Marzia Rossato** – A rapid and accurate Minion-based workflow for tracking species biodiversity in the fields and caves

- **13:55 – 15:00 – Lunch**
- **15:00 – 17:50 – Oral speech session 2 – Chair Arnaud Faille**
- **15:00-15:30 – Plenary keynote talk 2. Dragan Antić** – Diversity of troglolithic millipedes (Myriapoda: Diplopoda) in the Caucasus
- **15:30 – 15:50 – William Jeffery** – Hypoxia and sonic hedgehog drive the evolution of heart asymmetry in cavefish
- **15:50 – 16:10 – Sophio Maglakelidze** – General overview of cave-dwelling bats in Georgia
- **16:10 – 16:30 – Mayvan M. Mehrafrooz** – Iranian regions of the Caucasus – the need to study underground ecosystems
- **16:30 – 16:50 – Ilya Turbanov** – Three new species of cave dwelling pseudoscorpions from the genus *Ephippiochthonius* Baier, 1930 (Arachnida: Pseudoscorpiones: Chthoniidae) from Abkhazia, Georgia (West Caucasus), with taxonomic comments on this genus
- **16:50 – 17:10 – Ivan Tuf** – Caucasian centipedes with special focus on cave fauna
- **17:10 – 17:30 – Tea Arabuli** – Oribatid mites (Acari: Oribatida) in the karst caves of Georgia (Caucasus)
- **17:30-17:50 – Marko Lukić** - Practical guide for building the *Astyanax* and cave invertebrate facility
- **18:10 – 19:30 – Dinner**

## 17<sup>th</sup> June, 2023

- **10:00 –13:00 – Oral speech session 3 – Chair Lubomír Kováč**
- **10:00 – 10:20 – Vangelis Mizerakis** – First phylogenetic insights into some subterranean Pselaphinae beetles (Coleoptera: Staphylinidae) of Caucasus (**online**)
- **10:20 – 10:40 – Marjan Komnenov** – Review of the cave-dwelling spiders of the Caucasus (**online**)

- **10:40 – 11:00 – Mert Elverici** – Subterranean spider (Arachnida: Araneae) biodiversity in the Caucasian part of Turkey and its environs under light of recent sampling
- **11:00-11:20 – Stefano Taiti** – Terrestrial isopods (Crustacea: Oniscidea) from Georgian caves (**online**)
- **11:20 – 12:20 – Coffee break + Lunch**
- **12:20 – 12:40 – Bella Babayan** – The study of bacteria isolated from fossil cave mud of Asni Cave of Armenia (**online**)
- **12:40 – 13:00 – Naia Modebadze** - Review of the genus *Nemaspela* Šilhavý (Opiliones: Nemastomatidae) in the Caucasus
- **13:00-16:00 – Excursion in Tbilisi Botanical Garden**

**PLENARY**  
**TALKS**





# CAVE DWELLING INVERTEBRATE FAUNA OF GEORGIA, CAUCASUS

**Barjadze Shalva<sup>1</sup>, Maghradze Eter<sup>1</sup>, Mumladze Levan<sup>1</sup>, Soto-Adames  
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Investigation of Invertebrates in Georgian karst caves started at the end of the 19th Century, and investigation of bats in caves did not start until the beginning of the 20<sup>th</sup> Century. Despite the early beginnings, both fields are still poorly investigated, considering that only about 12% of the 1500 caves registered in Georgia, have been explored biologically. So far, 553 invertebrate species belonging to 57 orders in 17 classes and 7 phyla have been recorded from 163 caves in Georgia. Of these 553 species, 131 are cavernicolous distributed across 97 caves: 60 are troglobionts and 71 species are stygobionts. Sixty-five species are single cave endemics. Family Zenkevitchiidae (Crustacea: Amphipoda) and 19 genera of cave adapted invertebrates are Georgia endemics, whereas 5 genera are endemic to the Caucasus. The most widespread cave dweller is *Trichoniscus aphoniscus* Borutzky, 1977, recorded in 13 caves. The species richness is highest in Kveda Shakurani Cave, with 18 species. Among the Georgian hypogean species, the springtail, *Troglaphorura gladiator* Vargovitsh, 2019, is the most troglomorphic. The largest number of the species (73) is known from Apkhazeti (=Abkhazia) region. Conservation issues associated with the Georgian cave adapted invertebrate species is discussed.

# DIVERSITY OF TROGLOBIOTIC MILLIPEDES (MYRIAPODA: DIPLOPODA) IN THE CAUCASUS

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The Caucasus ecoregion has long been known as one of the world's biodiversity hotspots with an exceptional number of endemic taxa. As an important group of soil organisms, millipedes are one of the best studied groups of arthropods in the Caucasus. So far, over 210 species have been recorded, 90% of which are endemic or subendemic to this ecoregion. The area is not only rich in epigeal millipedes, but is also characterised by an enviable number of presumed troglobionts or obligate cave-dwellers. In the last decade, the number of presumed trogllobiotic millipedes has increased considerably and has reached 34, or 15% of the total number. Of the 15 millipede families recorded in the region, six are characterised by cave dwellers. The families Julidae and Anthroleucosomatidae are by far the richest in the number of trogllobiotic species, with 15 and 14, respectively. The family Blaniulidae includes two, while the remaining three families, viz., Glomeridae, Glomeridellidae and Trichopolydesmidae, each have only one cave-dwelling representative. Caucasian caves harbour eight species of julids (seven from the genus *Leucogeorgia* Verhoeff, 1930 and the monospecific genus *Martvilia* Antić & Reip, 2020), which have gone a step further in their adaptation and have developed special modifications that enable them an amphibious lifestyle and filtering diet. One of these species, *Leucogeorgia profunda* Antić & Reip, 2020, found at a depth of -2204 m in Vervovkina Cave, is one of the deepest terrestrial animals ever recorded.

**ORAL**  
**PRESENTATIONS**

## ORIBATID MITES (ACARI: ORIBATIDA) FAUNA IN THE KARST CAVES OF GEORGIA (CAUCASUS)

**Arabuli Tea**<sup>1,2</sup>

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73 species of oribatid mite were registered in 27 karst caves of Georgia. Two new species of the soil dwelling mites were described from the Georgian karst caves, one species *Lucoppia nicora* described from Nikortsminda Cave by Djaparidze, 1986 and the second one - *Ghilarovus kvavadzei* described from Tsakhi Cave by Murvanidze, 2014. The oribatid mite fauna were investigated from 98 caves; the highest number of species was found in the Dzudzuana Cave (25 species), followed by Kidobana Cave with 17 species, and Navenakhevi Cave with 12 species. The most caves contain less than ten species of oribatid mites and only one species was registered in some caves. According the investigations conducted in karst caves two species - *Miracarus hurkai* Kunst, 1959 and *Epidamaeus pinguis* Kulijev, 1967 were new to the Caucasian oribatid mite fauna and *Dissorhina signata* (Schwalbe, 1989) was new records to the Georgian fauna.

# THE STUDY OF BACTERIA ISOLATED FROM FOSSIL CAVE MUD OF ASNI CAVE OF ARMENIA

**Babayan Bella**<sup>1,2</sup>, *Shahinyan Samvel*<sup>3</sup>, *Mikaelyan Aram*<sup>2</sup> & *Melkumyan Marina*<sup>1</sup>

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Microorganisms are the natural inhabitants of all the surfaces. They are an important components of Earth planet environment. There is the wide diversity of common bacteria and other microorganisms, which are ubiquitous such as like *Pseudomonas* genus representatives. Also, there are both producer and decomposer (reducer) bacteria which are specific for each separate type of ecosystems. Soil and wet surfaces of stones in caves world around are very specific media for growth of different microbes, including the extremophilic and chemoautotrophic organisms. Also, the pathogenic, opportunistic pathogenic and non-pathogenic representatives of fungi and bacteria, such as like some species of lichens were identified in different caves world around.

In current research the microbes from fossil samples of cave mud of Asni Cave and the surroundings of it were studied. During the research 8 strains of *Bacillus* genus were isolated from Asni Cave Church and the Urartian period Aq-ueduct near Afshar Village (N39° 47', Eo 44° 50', H 4284m). There were studied some characteristics of their morphology, physiology, genetics, antibiotic resistance, as well as xenobiotic biodegradation potential. Using different temperatures and the selective cultural media with 13 antibiotics, natural and synthetic derivatives of tartaric acid (6 cyclic and aromatic imides and complex amino-salts of tartaric acid) multi-drug resistant spore forming and non-spore forming microbes were identified. Among them there were identified both psychrophilic (6°C, 11°C, 22°C, 25°C) and thermophilic (65°C), while the mesophilic strains were not identified.

# LESSONS FROM RECENT AND ONGOING CAVE COLONIZATIONS: AN EXAMPLE OF *ASELLUS AQUATICUS* (CRUSTACEA: ASELLIDAE)

**Bilandžija Helena<sup>1,2</sup> & Bedek Jana<sup>1,2</sup>**

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Surface pigmented and eyed species are common in caves, but are largely overlooked by cave biologists. Furthermore, they are often referred to as accidentals in ecological classifications of the cave biota, further illustrating the lack of interest by researchers of cave fauna. However, these species may be key to unraveling the process of cave colonization, which is not yet well understood, since at least some of them do not live in caves by chance but are in a process of niche and habitat change. We studied the isopod crustacean *Asellus aquaticus* from Sušik Cave in Croatia and the adjacent Sušik Spring. Comparison between the two populations revealed that the cave population, despite its surface-like appearance, exhibited a number of adaptive changes, including lighter pigmentation, smaller eyes, longer antennae, etc. We have established laboratory colonies of these populations to gain a mechanistic understanding of the evolution of cave adaptations and the process of adaptation to caves. We found that maintenance in complete darkness in the laboratory is able to induce adaptive changes in surface populations without negatively affecting fitness, suggesting that phenotypic plasticity plays a role in cave colonization of *A. aquaticus*.

## **RHINOLOPHUS BLASII PETERS (CHIROPTERA: RHINOLOPHIDAE) IN GEORGIA**

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Blasius's horseshoe bat (*Rhinolophus blasii*) inhabits southern Europe, northern and eastern Africa and the Near and Middle East in Asia; it is characterized by a discontinuous range. In the 20th century, it was known from all countries neighboring Georgia, but has not been recorded here. The species was first recorded in Georgia in 2006 in Taroklde Cave near Zodi village, Chiatura municipality. The cave contained a male colony of 150-200 individuals. Subsequent studies (2007, 2014) showed that the colony was also replenished by male Mediterranean Horseshoe bats (*Rhinolophus euryale*). In 2006, Blasius's horseshoe bat was recorded in Prometheus Cave in the village of Kumistavi and in Gogolati cave near the village Gogolati, Ambrolauri municipality. Later on, *R. blasii* was also recorded near the village Chongari, near the town of Kutaisi, in Letsurtsume Cave in the village Letsurtsume, Chkhorotsku municipality and near the villages Chalovani, Sachkhere municipality. Near the villages Chognari and Chalovani, the species was recorded outside the cave, but together with *Rhinolophus ferrumequinum* and *R. hipposideros*, indicating the existence of unknown underground shelters nearby. This species is thought to inhabit karst caves in open landscapes, with shrubs, and low-growing broad-leaved forests, as well as in deserts and savannahs. All of our finds are confined to broad-leaved, high-wooded forests, with high humidity, sometimes with open spaces nearby. We have proposed to list the species in the new edition of Red List of Georgia, as being vulnerable.



# THE LITTLE ONES, HIDDEN IN THE SPRINGS: CRENOBIOTIC BELGRANDIELLINAE RADOMAN, 1983 (MOLLUSCA: HYDROBIIDAE) FROM GEORGIA

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The south-western Great Caucasus hosts a remarkable diversity of groundwater molluscan species. Among the great variety of hydrobiid snails inhabiting groundwaters of the South Caucasus, the endemic genus *Tschernomorica* Vinarski et Palatov, 2019 is of particular interest. Our study aimed to clarify the taxonomic position of the crenobiotic microsnails from the Western Georgia and conduct an integrative revision of this mollusks.

The material for this study was collected during field trips in Samegrelo, Imereti, Racha, Guria, and Adjara in 2014–2022. More than 1000 hydrobiid specimens were sampled in a large number of cave water bodies and spring outlets. Integrative taxonomy methods were used to investigate mollusks features and phylogenetic relationships between them.

Our analysis confirmed that the true biodiversity of the studied group exceeds earlier findings and revealed a presence of hidden local biodiversity within Western Georgia. As it turned out, *Tschernomorica* is a complex of at least 5 genera differing in their genital morphology and having the molecular support. Based on conchological, anatomical and genetic investigations, we describe three new genera and seven new species within the subfamily Belgrandiellinae Radoman, 1983, and provide their diagnostic features and distribution ranges. The data obtained allow us to more accurately estimate the time when the Caucasus ground waters were colonized by hydrobiid microsnails. The molecular chronogram indicates that the common ancestor of Caucasian representatives of this group diverged from its Balkan relatives about 5.63 Ma.

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# SUBTERRANEAN SPIDER (ARACHNIDA: ARANEAE) BIODIVERSITY IN THE CAUCASIAN PART OF TURKEY AND ITS ENVIRONS UNDER LIGHT OF RECENT SAMPLING

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Subterranean biodiversity in (Anatolia) Turkey is little known. Among spiders, despite a variety of taxa known to specialize in hypogean life, it is still being determined how well the current inventories represent the actual diversity. This is a fact for the Caucasian part of Anatolia as well. Aiming to shed light on this obscurity, we will summarize various aspects of spider diversity based on new data from 38 caves recently (2020-2022) sampled through direct intuitive sampling in the Caucasus and its environs, including the western parts of the Black Sea coast and northern parts of East Anatolia. Among the fresh material, 836 specimens collected from various zones correspond to 55 morphospecies. Per cave richness varies between 1 - 14, while communities with above six species accounted for caves with huge twilight zones (n = 4) and dominated by troglophiles. The largest cave systems harbored richer communities with 4-6 species (n = 9). More than 60% of the (morpho)species were unique to a particular cave, mainly among Linyphiidae (11 spp.), Leptonetidae (3 spp.), and Agelenidae (4 spp.). On the other hand, the following taxa were frequently represented (frequencies given in parentheses): *Aituaria borutzkyi* (18), *Metellina meriana* (16), *Lepthyphantes leprosus* (12), *Porrhomma convexum* (8), *Tegenaria percuriosa* (8) and *Hoplopholcus sensu lato* (17). An incidence (frequency) based analysis suggests a high level of coverage (0.77) among the studied caves. In contrast, almost all populations are new records for the caves or karstic regions studied, indicating the lack of knowledge in the knowledge.

# INTEGRATIVE TAXONOMICAL INVESTIGATIONS ON THE CAVERNICOLOUS PSEUDOSCORPIONS OF GEORGIA

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As of 2023 altogether fifty-two pseudoscorpion species are known from Georgia. Eight of these species were reported from cavernicolous habitats. Only four species from the above mentioned ones are considered as troglobitic: *Globochthonius satapliaensis* (Schawaller & Dashdamirov, 1988); *Neobisium* (*Blothrus*) *birsteini* (Lapschoff, 1940); *N.* (*Heoblothrus*) *sakadzhianum* Krumpál, 1984, and *N.* (*Blothrus*) *verae* (Lapschoff, 1940).

We use an integrative taxonomical approach (including morphology, morphometrics, and DNA barcoding) to explore taxonomical diversity in case of Georgian cavernicolous pseudoscorpions. Our goal is to explore cryptic diversity and to find possible morphological differences congruent with the genetic data, in pseudoscorpion populations belonging to *Globochthonius* and *Neobisium* genera inhabiting different caves in three different karst massifs (Odishi plain; Sataplia-tskaltubo karst massif; Zemo Imereti plateau) of Georgia.

Our preliminary analysis based on molecular data suggests a high degree of cryptic diversity within the cavernicolous members of the studied groups in case of samples collected from different karst massifs.

# NEXT WORK IN PROGRES ON STYGOBIOTIC GASTROPODA FROM GEORGIA FURTHER CONFIRMING THE SUBTERRANEAN BIODIVERSITY HOTSPOT STATUS OF CAUCASUS

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In the series of our recent papers (Grego et al. 2020, Chertoprud et al. 2020, 2021, 2023) the Caucasus was recognized as a remarkable hotspot of stygobiotic and crenobiotic Mollusca diversity after revealing large number of new, range-restricted species and genera within the subfamily Sadlerianinae sensu Szarowska, 2006 (Hydrobiidae) and in crenobiotic to stygophile representatives of subfamily Belgrandiellinae Radoman, 1983. However, our work continues on obligate stygobiotic representatives of the subfamily Belgrandiellinae which also show remarkable radiation of genera and species in the region and the preliminary phylogenetic results suggesting rather complicated convergent evolution of both present subfamilies of Hydrobiidae, resulting in even higher diversity than hitherto supposed.

# HYPOXIA AND SONIC HEDGEHOG DRIVE THE EVOLUTION OF HEART ASYMMETRY IN CAVEFISH

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The Mexican tetra *Astyanax mexicanus* is a single species with surface-dwelling and cave-dwelling morphs. Cavefish have evolved many trait differences relative to surface fish, and one of these is a change in heart asymmetry. As in all vertebrates, surface fish develop a heart on the left side of the midline. In contrast, cavefish develop a heart either on the right or the left side. Heart asymmetry and many other cave fish traits are controlled by Sonic Hedgehog (Shh) signaling. We have studied the environmental conditions responsible for the evolution of heart asymmetry in cavefish. Using surface fish as a proxy for the cavefish ancestor, heart development was studied in surface fish embryos exposed to one of the four challenging conditions of the cave habitat: (1) the absence of light, (2) low water conductivity, (3) low water temperature, or (4) low dissolved oxygen (hypoxia). Darkness, low water conductivity, or water temperatures within the range tolerated by surface fish did not affect heart asymmetry: hearts developed on the left side, similar to controls. In striking contrast, exposure to 20% hypoxia, about the same oxygen concentration as measured in cave water, resulted in significant levels of heart development on the right side, as seen in the natural cavefish phenotype. Subsequent studies showed that cavefish are constitutively hypoxic, even under normoxic laboratory conditions, and that hypoxia affects heart asymmetry by elevating Shh signaling. The results suggest that hypoxia and Shh may have driven the evolution of heart asymmetry in *Astyanax* cavefish.

# REVIEW OF THE CAVE-DWELLING SPIDERS OF THE CAUCASUS

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In general, the spider fauna of the Caucasus is quite rich, comprises over 1100 species and still has a great potential for new scientific discoveries. This region is also recognized as one of the world's major subterranean biodiversity hotspots. In most cases, spiders are among the most common inhabitants of terrestrial caves around the world. But despite these facts, this region remains very poorly investigated in terms of subterranean spider fauna, leaving the spiders a relatively unstudied group. The present state of our knowledge about the cave-dwelling spiders in the Caucasus is still poor and fragmentary and little is known about its distributions in the region. The present review is based on the critical analysis of all literature records and represent the first attempt to summarize the fauna of the cave-dwelling spiders of the Caucasus. So far, a total of 13 species only from 6 families are recorded from subterranean systems. Except one, all analyzed species are recognized as troglophiles, some of them common and widespread in terrestrial habitats as well, and others seem to be facultative inhabitants of underground systems. *Iberina ljevuschkini* Pichka, 1965 is considered as only true troglobitic species and its current dubious taxonomic status (species inquirenda) is discussed.

## DIVERSITY AND DISTRIBUTION OF PALPIGRADI (ARACHNIDA) IN GEORGIA

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Palpigradi is a group of miniature and so often neglected arachnids with a dominant distribution in tropical soils. In northern temperate region the palpigrades can be found almost exclusively in caves. From Europe there are numerous cave records of this group. *Eukoenenia vargovitshi* Christian, 2014 was the first species described from Georgia, based on a single male individual collected in Kveda Shakurani (=Nizhnyaya Shakuranskaya) Cave, Western Caucasus. R. Vargovitsh made a substantial effort in collecting cave arthropods in deep pits of the Western Caucasus in the period 2006–2019. He collected altogether 34 specimens of Palpigradi and this material is recently taxonomically processed based on thorough study of morphological characters. In 2022 the first Palpigradi specimens (both males) were collected in central Georgia (Letsurtsume Cave) by A. Faille, S. Barjadze and E. Maghradze, representing an undescribed species new to science. This discovery was followed soon by record of another male specimen by E. Maghradze in Garakha Cave situated in the same karst region than in previous cave. Taxonomic status of this specimen remains to be taxonomically verified.

The explorations of Palpigradi in Georgia are still at the beginning and the further effort could bring substantial discoveries of the new taxa in karst caves of the country, thus contributing to list of troglobiotic taxa of the Caucasus that has been recognized as a hotspot of subterranean biodiversity.

# PRACTICAL GUIDE FOR BUILDING THE ASTYANAX AND CAVE INVERTEBRATE FACILITY

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Cave species are emerging eco-evo-devo model organisms. The best known is the Mexican cavefish *Astyanax mexicanus*, as it is easily maintained under laboratory conditions and regularly produces large numbers of embryos. Among invertebrates, there are many species that have great potential for answering various research questions, but their use as model organisms is hampered by a lack of maintenance and breeding protocols. Recently, we have established an animal facility at RBI in Zagreb where we keep cave-dwelling and related surface species. We keep colonies of *A. mexicanus*, various crustaceans, snails and planarians. Here we present our experiences to provide guidelines for researchers undertaking similar efforts. We discuss general care practices such as aquarium and terrarium design, housing, water treatment and conditioning, feeding and breeding approaches, decontamination of equipment and tools, and quarantine. We present our fish facility in more detail, as it is a customized modular system consisting of 4 independent racks with automation processes controlled by modular logic modules from Siemens LOGO. The automation controls water treatment with reverse osmosis water, a programmed refill system, a daily water changes of 10% for each rack, temperature control, pH and conductivity control, and UV lamp regime. The alarm system sends warnings in case of malfunction of the main parts of the system or if the water parameters are out of the standard range. The system with automated processes significantly reduces the time spent by staff on maintenance and ensures stable conditions for the fish.



# **INOTRECHUS DOLZHANSKY & LJOVUSCHKIN, 1989, AN ENDEMIC GENUS OF CAVE TRECHINI FROM GEORGIA (COLEOPTERA: CARABIDAE)**

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Georgian cave fauna is still poorly investigated, because of the remote access of karstic areas of Georgia and the lack of specialists focusing on subterranean Invertebrates. Culver et al. (2006) suggested the karstic regions of Georgia as a potential hotspot of subterranean biodiversity. Nineteen species of troglobiont beetles are reported from the Georgian caves belonging to the following families: Carabidae, the richest family with 18 species, and Staphylinidae represented by 1 species only.

Eighteen Georgian caves host troglobitic ground beetle species belonging to seven genera: *Inotrechus* Dolzhansky & Ljovuschkin, 1989, *Taniatrechus* Belousov & Dolzhanskij, 1994 (Endemics of Georgia), *Jeannelius* Kurnakov, 1959, *Meganophthalmus* Kurnakov, 1959 and *Troglocimmerites* Ljovuschkin, 1970 (endemics of Caucasus), *Duvalius* Delarouzée, 1859, *Cimmerites* Jeannel, 1928, all belonging to Trechini, a tribe especially rich in species in the Western Palearctic.

The genus *Inotrechus* Dolzhansky & Ljovuschkin, 1989 is characterized by well-defined troglomorphic features and is represented by only two species yet: *I.injaevae* Dolzhanskij et Ljovuschkin, 1989 described from Sakishore Cave, Racha region and *I.kurnakovi* Dolzhanskij et Ljovuschkin, 1989 described from Prometheus Cave, Imereti region. No further records of the species have been reported to date.

Here we present the preliminary results of a revision of the genus based on a morphological and molecular study and more than 50 specimens sampled in 11 caves of Georgia, belonging to 4 karst massifs of Western Georgia. In addition to new localities for the two known species, a new species was discovered in the frame of the project.

The position of *Inotrechus* among Trechini is discussed, and an updated map of the distribution of the genus is provided.

# GENERAL OVERVIEW OF CAVE-DWELLING BATS IN GEORGIA

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Some species of bats use caves, mines, or other underground shelters/roosts for hibernation or as maternity roosts. Bats have been a study subject in Georgia since the beginning of the 20<sup>th</sup> century. Nevertheless, the complete data is still unavailable as only 15% out of 2000 underground sites have been observed, and their investigation is still an ongoing process. Of 30 species of bats in Georgia eight are cave-dwelling, two of them (*Miniopterus schreibersii*, *Rhinolophus mehelyi*) are listed as Vulnerable and one (*Rhinolophus euryale*) as Near-Threatened by IUCN, and two (*Rhinolophus euryale*, *R. mehelyi*) of them are also listed on the Georgian Red List. Additionally, numerous other species have been observed to utilize caves during different seasons.

Cave-dwelling bats' species diversity has also been studied by DNA barcoding, conducted in recent years by our team and within the framework of the Rustaveli National Science Foundation project "DNA-barcoding database of small mammals and fauna composition determination" and Caucasus Barcoding Project (CaBOL). Most of the species taxonomy has been validated and one species - *Myotis tschuliensis* has been found for the first time in Georgia. This *Myotis* species tends to use underground sites for swarming and hibernating.

For future studies, we will evaluate and clarify species diversity, population dynamics, potential threats, and the degrees of anthropogenic pressure in the caves of Georgia. We will reassess the most significant sites with the most susceptible bat communities and plan the best approach to the identified problem.

## IRANIAN REGIONS OF THE CAUCASUS - THE NEED TO STUDY UNDERGROUND ECOSYSTEMS

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The Caucasus is a region between the Black Sea and the Caspian Sea. The southernmost borders of the Caucasus include parts of the north and northwest of Iran. This area includes the provinces of Ardabil, Gilan, the west of Mazandaran province and the northern parts of the two provinces of West and East Azerbaijan. Alborz mountain range covers most of this area and stretches from northwest to east of Iran. Mountainous areas of the country are generally rich in karst landscapes with many caves formed by the chemical dissolution of the limestones. For example, more than karst 60 caves have been reported from Ardabil province. These subterranean ecosystems consist of aquatic and terrestrial environments, which provide suitable conditions for highly specialized organisms that are restricted to these environments. Arthropods are among the most important inhabitants of these ecosystems in terms of diversity and abundance of subterranean fauna. Macro- and micro- arthropods, such as spiders, mites, centipedes, millipedes, springtails and beetles are among the main primary consumers and play an important role in subterranean food webs maintaining their ecosystem functioning. Study of the cave fauna is an important condition for reliable protection of these valuable and vulnerable subterranean ecosystems. However, subterranean ecosystems of the Caucasian region in Iran remain unknown and should be considered in the future studies.

# FIRST PHYLOGENETIC INSIGHTS INTO SOME SUBTERRANEAN PSELAPHINAE BEETLES (COLEOPTERA: STAPHYLINIDAE) OF CAUCASUS

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The Caucasus region includes numerous representatives of Amauropini and Bythinini, the two Pselaphinae tribes with the highest number of cavernicolous Pselaphinae species. Newly obtained molecular data reveal subterranean speciation processes within the two tribes and highlight some first taxonomic outcomes. Existing morphological species groups are compared to phylogenetic clusters and their taxonomic significance is discussed. Within the Amauropine genus *Bergrothia* and the Bythinine genus *Bryaxis* four cases of putative new taxa are shown, while one case of an enigmatic synonymy is being questioned. All of these taxa and their habitat are illustrated. The study contributes to the evidence that Caucasus represents a speciation hotspot for Pselaphinae beetles.

# REVIEW OF THE GENUS *NEMASPELA* ŠILHAVÝ (OPILIONES: NEMASTOMATIDAE) IN THE CAUCASUS

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Among the rich opilionid fauna of the Caucasus region, the subfamily Nemastomatinae of family Nemastomatidae plays an important role. All *Nemaspela* species are exclusively troglobitic. Twelve *Nemaspela* species display a rather disjunct distributional pattern: nine species are known from the Greater Caucasus Mountains, two species - in the Balkan Peninsula and a single species - in the southern part of the Crimean Peninsula, Ukraine. Nine Caucasian taxa are: *N. abchastica* (Ljovuschkin & Starobogatov, 1963), *N. birsteini* Ljovuschkin, 1972, *N. femorecurvata* Martens, 2006, *N. gagrica* Tchemeris, 2013, *N. kotia* Martens, Magradze & Barjadze, 2021, *N. kovali* Chemeris, 2009, *N. melouri* Martens, Magradze & Barjadze, 2021, *N. prometheus* Martens, Magradze & Barjadze, 2021 and *N. sokolovi* (Ljovuschkin & Starobogatov, 1963). Morphology and relationships of *Nemaspela* species within the genus and with hypothetical epigeal ancestors are discussed. A key to the Caucasian *Nemaspela* species and its distributional map is provided.

# TWO NEW SPECIES OF *DEUTERAPHORURA* ABSOLON (COLLEMBOLA: ONYCHIURIDAE) FROM THE GEORGIAN CAVES

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*Deuteraphorura* specimens collected in eleven caves of Georgian were analyzed morphologically and molecularly based on COI gene's barcode region. Two molecular delimitation methods (ASAP, PTP) revealed existence of four species, however, only two of them were distinguished morphologically. Both new species - *D. kozmani* from Imereti region and *D. colchisi* from Imereti and Samegrelo regions belong to a group with pseudocellus on the first thoracic tergum. High molecular diversity within *colchisi* point to recent speciation of *Deuteraphorura* in Georgian caves and highlights the potential of the region as hotspot of subterranean biodiversity.

# A RAPID AND ACCURATE MINION-BASED WORKFLOW FOR TRACKING SPECIES BIODIVERSITY IN THE FIELD AND CAVES

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Genetic markers (DNA barcodes) are often used to support and confirm species identification. Barcode sequences can be generated in the field using portable systems based on the Oxford Nanopore Technologies (ONT) MinION sequencer. However, to achieve a broader application, current proof-of-principle workflows for on-site barcoding analysis must be standardized to ensure a reliable and robust performance under suboptimal field conditions without increasing costs. We demonstrated the implementation of a new on-site workflow for DNA extraction, PCR-based barcoding, and the generation of consensus sequences. The portable laboratory features inexpensive instruments that can be carried as hand luggage and uses standard molecular biology protocols and reagents that tolerate adverse environmental conditions. Further instrumental and protocol adjustments have been implemented and demonstrated for field conditions where electricity is not available, e.g. in a cave. Barcodes are sequenced using MinION technology and analyzed with ONTrack, an original de novo assembly pipeline that requires as few as 1000 reads per sample. The ONTrack pipeline has a user-friendly interface and returns consensus sequences in minutes. The remarkable accuracy and low computational demand of the ONTrack pipeline, together with the inexpensive equipment and simple protocols, make the proposed workflow particularly suitable for tracking species under field conditions, even when extreme, such as in a cave.

## TERRESTRIAL ISOPODS (CRUSTACEA: ONISCIDEA) FROM GEORGIAN CAVES

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At present, 20 species and subspecies of terrestrial isopods are known from 31 caves in Georgia. Five of the species in the genera *Ligidium* (Ligidiidae), *Buddelundiella* (Buddelundiellidae) and *Cylisticus* (Cylisticiidae) are troglaphiles, while all the others in the genera *Caucasoligidium* (Ligidiidae), *Caucasonethes* and *Trichoniscus* (Trichoniscidae Trichoniscinae), *Colchidoniscus* and *Mingrelloniscus* (Trichoniscidae Haplophthalminae), and *Borutzkyella* (Buddelundiellidae) show troglomorphic traits and are considered to be troglobites. In recent years, during new investigations in Georgian caves a large number of Oniscidea has been collected, including a new genus and species of Trichoniscidae Haplophthalminae, a new species of *Colchidoniscus*, and a new species of *Haplophthalmus* (Trichoniscidae Haplophthalminae), a genus not yet recorded from Georgia. All the troglobite taxa are discussed and illustrated and an identification key is provided.



# CAUCASIAN CENTIPEDES (MYRIAPODA: CHILOPODA) WITH SPECIAL FOCUS ON CAVE FAUNA

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The Caucasus has always attracted the attention of biologists, as have its caves and their inhabitants.

The first publications mentioning centipedes from the Caucasus date back to the 19th century (Eichwald, Kessler, Sselivanoff), further papers appeared at the beginning of the 20th century (Lignau, Verhoeff, Muralewitz), mainly taxonomic and faunistic publications. Research from the Soviet era was more systematic and focused on specific areas or topics, as is the case today.

The latest review of cave centipedes lists 11 taxa from the Caucasus, ten troglophilous and a single troglobitic species. This work provides new data on centipedes from Georgian caves. Another troglobitic centipede from Georgian cave was described last year.

**THREE NEW SPECIES CAVE-DWELLING  
PSEUDOSCORPIONS FROM THE GENUS  
*EPHIPPIOCHTHONIUS* BAIER, 1930 (ARACHNIDA:  
PSEUDOSCORPIONES: CHTHONIIDAE) FROM  
ABKHAZIA, GEORGIA (WEST CAUCASUS), WITH  
TAXONOMIC COMMENTS ON THIS GENUS**

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Three new hypogean *Ephippiochthonius* species from karst caves of Abkhazia, Georgia (West Caucasus) are described, including detailed diagnosis and illustrations. These are the first cave-dwelling species of this genus described for the Caucasus. The taxonomic significance of the position is discussed intermediate setae *ih3* and *ih4* on antiaxial side of chelal hand for the genus *Ephippiochthonius*. Based on this and other features, the heterogeneity of the *E. tetrachelatus*-group is discussed. Based on the presence of seta *ph3*, new combinations (comb. nov.) are established for a number of species from Balkans and Anatolia previously assigned to the genus *Ephippiochthonius* as *Occidenchthonius*.

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## NOTES

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