



4th international meeting

SOS *Proteus*

Conservation of *Proteus* and its habitat facing climate change challenge



Adriatic Speleological Society
Trieste Natural History Museum
Municipality of Trieste
Tular Cave Laboratory

2022
21st - 22nd May

Museo Civico Storia Naturale
via di Tominz 4
Trieste - Italy

Abstract book

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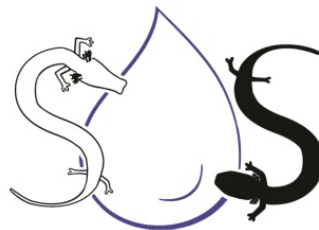
*Upon the 260th anniversary
of the first scientific description of Proteus anguinus [Lacerta caeca]
by Joannes Antonius Scopoli,*

in partnership of
**Speleovivarium Erwin Pichl, Adriatic Speleological Society,
Trieste Natural History Museum, Municipality of Trieste, and Tular Cave Laboratory,**

we are cordially welcoming you at the

**4th International meeting SOS Proteus:
CONSERVATION OF *PROTEUS* AND ITS HABITAT
FACING CLIMATE CHANGE CHALLENGES**

*Research and conservation of Proteus and protection of karst water resources
in climate change perspective*



May 21 – 22, 2022,

at the
**Trieste Natural History Museum,
Trieste, Italy,**

under the patronage of
**European Commission, Italian Ministry for Ecological Transition,
Friuli Venezia Giulia Region, Elettra Sincrotrone Trieste, Italian Speleological Society,
and Regional Speleological Federation FVG**

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SCIENTIFIC PROGRAMME

(LIVE STREAMING @ <https://www.youtube.com/watch?v=kn9XZMfbXzU>)

Saturday, 21st May, 2020

<i>Morning sessions</i>		
9.00 – 9.45	Registration of participants	
9.50 – 10.00	Opening of meeting and welcome speeches	Municipality of Trieste Trieste Natural History Museum Italian Speleological Society Regional Speleological Federation FVG Tular Cave Laboratory
HABITAT ASSESSMENT <i>Chair: Peter Trontelj</i>		
10.00 – 10.20	Hydrogeology of the Classical Karst Region	<u>Luca Zini</u> <i>Department of Mathematics and Geosciences. University of Trieste, IT</i>
10.20 – 10.40	Assessment of environmental risks to groundwater ecosystems for chemical substances	<u>Boris Kolar</u> <i>National Laboratory of Health, Environment and Food, SI</i>
10.40 – 11.00	Characterization and delineation of the Jelševniščica and Otovski Breg catchment areas - actions to identify and mitigate anthropogenic pressure on black and white proteus	Metka Petrič ¹ , Mitja Prelovšek ¹ , Marina Pintar ² , Nina Mali ³ , Rozalija Cvejič ² , Miha Curk ² & Janko Urbanc ³ <i>¹ Research Centre of the Slovenian Academy of Sciences and Arts, Karst Research Institute, SI; ² Department of Agronomy, Biotechnical Faculty, University of Ljubljana, SI; ³ Geological Survey of Slovenia, SI</i>
11.00 – 11.20	Chytrid fungi infection in olm: screening for pathogens in wild populations and olm's susceptibility to infection	<u>Rok Kostanjšek</u> ¹ , Mojca Vek ¹ , Martina Turk ¹ , Ion Gutiérrez-Aguirre ² , Nina Gunde-Cimerman ¹ , Zhimin Li ³ , Elin Verbrugghe ³ , Maja Lukač ⁴ , Frank Pasmans ³ , Ivan Cizelj ⁵ & An Martel ³ <i>¹ Department of Biology, Biotechnical Faculty, University of Ljubljana, SI; ² National Institute of Biology, SI; ³ Wildlife Health Ghent, Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, BE; ⁴ Department of Poultry Diseases With Clinic, Faculty of Veterinary Medicine, University of Zagreb, HR; ⁵ Zagreb Zoo, HR</i>
11.20 – 11.40	Conservation activities managed inside the Riserva Naturale Regionale Laghi Di Doberdò e Pietrarossa, Italy: historical mission, present challenges and future hopes - Pavees Società Cooperativa	<u>Gerardo G. Sarno</u> <i>Regional Nature Reserve of Doberdò and Pietrarossa lakes, IT</i>
11.40 – 12.00	<i>Coffee break</i>	
Keynote session: CLIMATE CHANGE CHALLENGES <i>Chair: Raoul Manenti</i>		
12.00 – 12.30	KEYNOTE LECTURE Climate change or global warming?	Renato R. Colucci <i>Institute of Polar Sciences, National Research Council, IT</i>
12.30 – 12.50	Climatic pollution and underground life: threat or evolution?	Nicola Bressi <i>Trieste Natural History Museum, IT</i>
13.00 – 14.40	<i>Lunch break</i>	

<i>Afternoon sessions</i>		
EVOLUTION OF <i>PROTEUS</i> <i>Chair: Rok Kostanjšek</i>		
14.40 – 15.00	The evolutionary role of the genome size of <i>Proteus</i>	<u>Ettore Olmo</u> , Adriana Canapa, Federica Carducci & Maria A. Biscotti <i>Department of Life and Environmental Sciences, Marche Polytechnic University, IT</i>
15.00 – 15.20	Multiple independent transitions to cave life and high diversity in the olm	<u>Hans Recknagel</u> , Valerija Zakšek & Peter Trontelj <i>SubBio lab, Biotechnical Faculty, University of Ljubljana, SI</i>
CHALLENGES IN SCIENCE OUTREACH <i>Chair: Katarina Koller Šarić</i>		
15.20 – 15.40	A dramatic tale of scientific fraud and political propaganda with <i>Proteus</i> in the main role and a Slovenian biologist as tragic hero, along with some new data	<u>Peter Trontelj</u> , Rok Kostanjšek & Hans Recknagel <i>Department of Biology, Biotechnical Faculty, University of Ljubljana, SI</i>
15.40 – 16.00	From science to clickbait	<u>Gergely Balázs</u> ¹ , Brian Lewarne ² & Gábor Herczeg ¹ ¹ Eötvös Loránd University, HU; ² Devon Karst Research Society, UK
16.00 – 16.20	Black proteus information centre in Jelševnik, Slovenia	<u>Nina Jankovič</u> & Andrej Hudoklin <i>Institute of the Republic of Slovenia for Nature Conservation, SI</i>
16.20 – 16.40	Neotenus dark dwellers Lygophilia	<u>Robertina Šebjanič</u> <i>Independent artist, SI</i>
16.40 – 16.50	<i>Coffee break</i>	
POSTERS <i>Chair: Lucia Mancini</i>		
	Predator recognition is not a major problem in <i>Proteus anguinus</i>	<u>Enrico Lunghi</u> ¹ , Andrea Melotto ² , Olivier Guillaume ³ , Gentile Francesco Ficetola ² & Raoul Manenti ^{2,4} ¹ Ruđer Bošković Institute, HR; ² Department of Environmental Science and Policy, University of Milano, IT; ³ Moulis Subterranean Laboratory, CNRS, FR; ⁴ Laboratory of Subterranean Biology “Enrico Pezzoli”, Parco Regionale del Monte Barro, IT
	New developments in <i>Proteus</i> eDNA quantification in standing and running water	<u>Špela Gorički</u> ¹ , Gregor Aljančič ² , Blaž Kogovšek ³ , Uršula Prosenc Zmrzljak ⁴ & Rok Košir ⁴ ¹ Scriptorium biologorum – Biološka pisarna, SI; ² Institute Tular Cave Laboratory, SI; ³ Research Centre of the Slovenian Academy of Sciences and Arts, Karst Research Institute, SI; ⁴ BIA Separations CRO, Labena d.o.o, SI
	Experience of introducing the olm to the Caves of Oliero, Venetian Prealps, Italy	<u>Marco Lazzarotto</u> <i>Oliero grotte Valbrenta Ivanxteam, IT</i>
	Speleovivarium Erwin Pichl (1990–2022): conservation, disclosure, change	<u>Edgardo Mauri</u> <i>Speleovivarium Erwin Pichl, Adriatic Speleological Society, IT</i>
	Neotenus dark dwellers Lygophilia	<u>Robertina Šebjanič</u> <i>Independent artist, SI</i>
	Monograph “Proteus” (Association Hyla, Zagreb, 2019: 250 p.)	<u>Katarina Koller Šarić</u> ¹ , Dušan Jelić ² , Branko Jalžić ³ & Petra Kovač Konrad ¹ ¹ Association Hyla, HR; ² Croatian Institute for Biodiversity, HR; ³ Croatian Biospeleological Society, HR

Sunday, 22nd May, 2022, World Biodiversity Day

RESEARCH HIGHLIGHTS		
Chair: Nicola Bressi		
9.00 – 9.20	A virtual tour at Elettra: a powerful investigation tool in Trieste	Lucia Mancini ^{1,*} <i>Slovenian National Building and Civil Engineering Institute, SI; *Former affiliation: Elettra-Sincrotrone Trieste S.C.p.A., IT</i>
9.20 – 9.40	3D exploring of <i>Proteus anguinus</i> by X-ray computed microtomography	<u>Markéta Tesařová</u> ¹ , Lucia Mancini ² , Edgardo Mauri ³ , Gregor Aljančič ⁴ , Magdalena Năpăruș Aljančič ^{4,5} , Rok Kostanjšek ⁶ , Lilijana Bizjak Mali ⁶ , Tomáš Zikmund ¹ , Markéta Kaucká ⁷ , Federica Papi ³ , Jana Goyens ⁸ , Anass Bouchnita ⁹ , Andreas Hellander ¹⁰ , Igor Adameyko ¹¹ & Jozef Kaiser ¹ ¹ Central European Institute of Technology, CZ; ² Elettra-Sincrotrone Trieste S.C.p.A., IT; ³ Speleovivarium Erwin Pichl, Adriatic Speleological Society, IT; ⁴ Institute Tular Cave Laboratory, SI; ⁵ Research Centre of the Slovenian Academy of Sciences and Arts: Karst Research Institute, SI ⁶ Department of Biology, Biotechnical Faculty, University of Ljubljana, SI; ⁷ Max Planck Institute for Evolutionary Biology, DE; ⁸ Laboratory of Functional Morphology, University of Antwerp, BE; ⁹ Department of Information Technology, Uppsala University, SE; ¹⁰ Department of Integrative Biology, University of Texas at Austin, TX, US; ¹¹ Medical University of Vienna, AT
9.40 – 10.00	Does age matter? Examining olms from 16-80 year of age.	<u>Susanne Holtze</u> ¹ , Olivier Guillaume ² , Anne Ipsen ³ & Thomas B. Hildebrandt ¹ ¹ Leibniz Institute for Zoo and Wildlife Research, DE; ² Moulis Subterranean Laboratory, National Center for Scientific Research, FR; ³ Gesellschaft für Freilandökologie und Naturschutzplanung mbH, DE
10.00– 10.20	Linking different approaches for sex identification of <i>Proteus anguinus</i>	<u>Tajda Gredar</u> , Lilijana Bizjak Mali & Rok Kostanjšek <i>Department of Biology, Biotechnical Faculty, University of Ljubljana, SI</i>
10.20 – 10.30	<i>Coffee break</i>	
FIELD RESEARCH		
Chair: Gregor Aljančič		
10.30 – 10.50	Behavioral observations of the olm (<i>Proteus anguinus</i>) in a karst spring	<u>Ester Premate</u> , Žiga Fišer, Žan Kuralt, Anja Pekolj, Tjaša Trajbarič, Eva Milavc, Živa Hanc & Rok Kostanjšek <i>Department of Biology, Biotechnical Faculty, University of Ljubljana, SI</i>
10.50 – 11.10	Activity of the olm (<i>Proteus anguinus</i>) in Italian spring habitats, close to A4 highway: conservation implications	<u>Raoul Manenti</u> ¹ , Benedetta Barzaghi ¹ , Edgardo Mauri ² , Marco Restaino ³ , Veronica Zampieri, Bianca Lombardi & Gentile Francesco Ficetola ¹ ¹ Department of Environmental Science and Policy, University of Milano, IT; ² Speleovivarium Erwin Pichl, IT; ³ Adriatic Speleological Society, IT
11.10 – 11.30	Preliminary study in cephalic morphology of <i>Proteus anguinus</i> between individuals found outside caves vs. those found deep in caves	<u>Benedetta Barzaghi</u> ^{1,2} , Gentile Francesco Ficetola ¹ , Edgardo Mauri ^{3,4} , Marco Restaino ³ , Bianca Lombardi ¹ & Raoul Manenti ^{1,2} ¹ Department of Environmental Science and Policy, University of Milano, IT; ² Laboratory of Subterranean Biology “Enrico Pezzoli”, Parco Regionale del Monte Barro, IT; ³ Adriatic Speleological Society, IT; ⁴ Speleovivarium Erwin Pichl, IT
11.30 – 11.40	Images of proteus (video film)	Ciril Mlinar - Cic <i>Water Cycle Institute, SI</i>
11.40 – 11.50	<i>Coffee break</i>	

Round Table Session: e-SCIENCE AND OPEN SCIENCE TO ADDRESS CHALLENGES IN CONSERVATION OF <i>PROTEUS</i> <i>Chair: Magdalena Aljančič</i>		
11.50 – 12.10	Round table keynote Invasive species - a key issue in biodiversity	Alberto Basset ^{1,2} ¹ Department of Biological and Environmental Sciences and Technologies, University of Salento, IT; ² LifeWatch ERIC, IT
12.10 – 12.20	Building virtual laboratories to monitor <i>Proteus</i> and its karst groundwater habitat	Gregor Aljančič ^{1,2} , Magdalena Aljančič ^{1,2,3} , Tanja Milotić ^{4,5} , Octavian Machidon ⁶ , Alina Machidon ⁶ , Jim Casaer ^{4,5} , Tanja Pipan ^{3,2} ¹ Institute Tular Cave Laboratory, SI ² LifeWatch Slovenia, SI ³ Research Centre of the Slovenian Academy of Sciences and Arts, Karst Research Institute, SI ⁴ Research Institute for Nature and Forest, Open Science Lab for Biodiversity, BE ⁵ LifeWatch Belgium, BE; ⁶ University of Ljubljana, Faculty of Computer and Information Science, SI
12.20 – 12.40	Round Table Discussion	

Social and cultural programme

Friday, 20th May

If you are already in Trieste on **Friday evening**, the meeting point is at the Natural History Museum of Trieste (Via Tominz 4, Trieste) at **18.15**.

From there, a bus transfer is organised to take us to the Trieste Karst tourist farm "Milič Zagrski" (Località Sagrado 2, Sgonico, Trieste; <https://www.miliczagrski.com/>), returning back to the Museum before 23.00.

Saturday, 21st May

Saturday evening social dinner at the "Pizzeria Da Pino" at 19.30.

It is a pizzeria, but also a restaurant in the centre of town (www.dapino.it/2020/08/26/da-pino-trieste/). Those who confirm their presence at the secretariat, will receive a coupon with a special discount of 12 € reserved only for the participants of SOS *Proteus*. Then you can order and pay a meal of your choice.

After the dinner, you will have the opportunity to visit the **Speleovivarium Erwin Pichl** (<https://sastrieste.it/index.php/speleovivarium/>).

Sunday, 22nd May

On **Sunday afternoon** (15.30), we invite you to a free guided tour of the Grotta Gigante (Jama v Briščakah) near Sgonico, in the Trieste Karst (www.grottagigante.it/).

Registration required at the registration desk. Participants should organize their own transport to the cave by car or by bus (line 42 from Piazza Oberdan).

ABSTRACTS

Hydrogeology of the Classical Karst Region

Luca Zini

Department of Mathematics and Geosciences, University of Trieste, IT

The Classical Karst Region is plateau of 750 km² located across NE Italy and SW Slovenia. It contains epigean and hypogean karst forms, whose concentration, dimension and type have made this area the worldwide symbol of karst phenomena. Rainfall and surface waters are immediately swallowed by the karstified bedrock, where a network of caves is developed, transferring the waters vertically through the epikarst and vadose zone and collecting them in the aquifer characterized by large horizontal or sub-horizontal conduits transporting the waters to the spring's area. The main part of the aquifer is located in Slovenia, but the whole karst coast and the springs area are located in Italy. The aquifer recharge is represented by: a concentrated allogenic recharge due to Reka River inputs, a diffuse autogenic recharge due to effective precipitations, and a diffuse allogenic recharge due to the Isonzo River contribution. The main spring is the Timavo with an average discharge of about 30 m³/s. Moving west worth, there are several small springs with an average discharge up to 2 m³/s. Along the coastline, several small springs are present. Since 200 years the Reka-Timavo transboundary aquifer, attracts the attention of researchers to satisfy the increasing need of good quality drinking water. Actually, the Classical Karst aquifer is tapped by Slovenian and Italian aqueducts to supply the Slovenian and Italian karst areas and the Trieste town.

Assessment of environmental risks to groundwater ecosystems for chemical substances

Boris Kolar

National Laboratory of Health, Environment and Food, SI

The current EU legislative frameworks for chemical substances (industrial chemicals, biocides, pesticides and feed additives) as well as the Water framework directive (WFD) consider groundwater as a source of drinking water for human consumption. Risk assessment and the protection goals for chemical substances (except assessment of pharmaceuticals in veterinary and human use) are set to protect groundwater as a source for drinking water and not as ecosystems. Based on the evidence of their vulnerability, we propose that risk assessments of GW ecosystems should be a compulsory part of the overall risk assessment of pesticides, biocides and feed additives, as well as the aim of protection of WFD. Ecotoxicological studies are proposed to support that claim.

Characterization and delineation of the Jelševniščica and Otovski Breg catchment areas - actions to identify and mitigate anthropogenic pressure on black and white proteus

Metka Petrič¹, Mitja Prelovšek¹, Marina Pintar², Nina Mali³, Rozalija Cvejič², Miha Curk² & Janko Urbanc³

¹ *Research Centre of the Slovenian Academy of Sciences and Arts, Karst Research Institute, SI*

² *Department of Agronomy, Biotechnical Faculty, University of Ljubljana, SI*

³ *Geological Survey of Slovenia, SI*

In October 2021, targeted research project acronimed HaČloRi started in the western part of the Bela Krajina where black and white proteus are endangered due to anthropogenic

overpressure or pollution threats. The project is focused on the definition of mitigation measures that would effectively assure suitable habitat for living and reproduction of proteus in the catchment area of the Jelševniščica and the Otovski Breg karst springs. To define scientifically supported, relevant and targeted mitigation measures, determination of rate and sources of pollution of underground water with special emphasis on nitrate is studied. Since mitigation measures as well as pollution are spatially dependent, spatial determination of catchment area (and hydrochemical characteristics) of both springs is in progress. Hydrogeological study involves two major predefined as well as nearby springs, which already indicate characteristic differences between low-altitude and high-altitude catchment areas. Definition of catchment area will be based also on studies of sediment ecotoxicology and provenance. Parallel to mentioned activities, presence of classical, modern organic and microbial contaminants is analysed by continuous measurements, temporary and passive sampling. By continuous flow rate and nitrate concentration measurements at springs, as well as later evaluation of nitrogen emission primarily from agriculture and waste water sources, quantitative nitrogen mass balance will be calculated; it will show the share of individual activities in the catchment area to the total nitrogen load defined at karst springs. Background concentration of selected parameters is going to be defined by sampling in nearby caves and in smaller potentially much less polluted springs. Adequate reduction of pollution and threats is expected to be possible primarily in agriculture and by proper treatment and discharge of municipal wastewater. Project activities of three partners will interdisciplinary connect ecological, hydrogeological, agronomical and environmental research.

Chytrid fungi infection in olm: screening for pathogens in wild populations and olm's susceptibility to infection

Rok Kostanjšek¹, Mojca Vek¹, Martina Turk¹, Ion Gutiérrez-Aguirre², Nina Gunde-Cimerman¹, Zhimin Li³, Elin Verbrugge³, Maja Lukac⁴, Frank Pasmans³, Ivan Cizelj⁵ & An Martel³

¹ Department of Biology, Biotechnical Faculty, University of Ljubljana, SI;

² National Institute of Biology, SI

³ Wildlife Health Ghent, Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, BE

⁴ Department of Poultry Diseases with Clinic, Faculty of Veterinary Medicine, University of Zagreb, HR

⁵ Zagreb Zoo, Zagreb, HR

Pathogenic chytrid fungi *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal) represent a significant threat to amphibian diversity, driving severe declines of over 500 amphibian species worldwide. While the globally distributed Bd plays a major role in declines of anuran species, Bsal has proven capable of dramatic declines of populations of European newts and salamanders (Scheele et al., 2019). As an endemic species with a narrow ecological niche, the olm may be highly vulnerable to infections with lethal pathogens, such as Bsal (Spitzen-van der Sluijs et al. 2016).

To elucidate the threat Bsal infection poses to olms, we conducted a survey on the presence of chytrid fungi in natural olm populations and captive specimens in Slovenia. In addition, we analyzed olm's susceptibility to Bsal by exposing animals to the pathogen in a controlled environment and monitoring infection and disease dynamics over a six month period.

Our survey suggests current absence of Bsal in natural populations of olms in Slovenia, and in any of the facilities legally keeping these amphibians in captivity (Kostanjšek et al., 2021). Experimental inoculation of olms resulted in asymptomatic but persistent infection, with limbs as

preferred sites, lack of exponential fungal growth in the olms' epidermis and dampened virulence gene expression in *Bsal* after exposure to olm skin compounds (Li et al., 2020).

Although our results indicate that the olm is one of few western Palearctic urodeles tolerant to *Bsal* infection, this vulnerable species may still act as a subterranean disease reservoir, and costs of subclinical infection may compromise olm fitness in the long term.

References:

Kostanjšek, et al. (2021). *Salamandra*, 57: 162–166.

Li, et al. (2020). *Scientific reports*, 10: 16480.

Scheele, et al. (2019). *Science*, 363: 1459–1463.

Spitzen-van der Sluijs et al. (2016). *Emerging Infectious Diseases*, 22: 1286–1288.

Conservation activities managed inside the Riserva Naturale Regionale Laghi Di Doberdò e Pietrarossa, Italy: historical mission, present challenges and future hopes - Pavees Società Cooperativa

Gerardo G. Sarno

Regional Nature Reserve of Doberdò and Pietrarossa lakes, IT

We present conservation activities for wild life underlying the establishment of the Doberdò and Pietrarossa Lakes Regional Nature Reserve, rediscovering the first, pioneering projects, from which the updated initiatives in response to the equally modern protection challenges have evolved theoretically and technically of the environment. Ideas for work and reflection on which to develop a future of eco-sustainable management of the natural resources of wildlife and more.

CLIMATE CHANGE CHALLENGES

KEYNOTE LECTURE

Climate change or global warming?

Renato R. Colucci

Institute of Polar Sciences, National Research Council, IT

Although people tend to use the terms Climate Change and Global Warming interchangeably, their meaning differs substantially. “Climate change” refers to changes in precipitation, temperature, wind patterns, or other climatic parameters over a long period of time. “Global warming” refers to the rise of global temperatures in the atmosphere producing an increasing amount of changes and feedback in the climate system. So, what are we facing nowadays? Is this caused by the pressing need for mankind to produce energy or represent a natural recursive event?

Climatic pollution and underground life: threat or evolution?

Nicola Bressi

Trieste Natural History Museum, IT

Our climatic pollution is causing the global warming and the so called "climate crisis". Will this crisis affect also the underground ecosystems? Of course yes. We will briefly analyse different way of impact of climatic pollution on cave biodiversity and the possible consequences, with particular regard to management and conservation of the Olms (*Proteus anguinus* complex).

EVOLUTION OF *PROTEUS*

The Evolutionary role of the genome size of *Proteus*

Ettore Olmo, Adriana Canapa, Federica Carducc & Maria A. Biscotti

Department of Life and Environmental Sciences, Marche Polytechnic University, IT

Unveil the genetic mechanisms underlying the adaptation of organisms to extreme environments represents one of the most intriguing challenges of evolutionary biology *Proteus anguinus*, the cave salamander, exhibits extraordinary morphological and physiological adaptations to the subterranean environment. Moreover this species is characterized by a very high genome size (estimated over 40 Gbp) probably depending on a high percentage of transposable elements. Several studies suggest that this hypertrophic genome can affect two adaptive mechanisms that played a key role in urodeles evolution. First, a mechanism would be related to heterochrony, a disturbance of the embryonic development which causes paedomorphosis consisting in the retention of immature characters in adulthood (progenesis) and often a delayed somatic development respect to sexual maturity (neoteny). The second mechanism is related to the cell size increase that leads to a consequent lower metabolic rate due to a decrease of the cell surface/volume ratio. *Proteus anguinus* having large genome size, is characterized by low metabolism, slow development and paedomorphosis which are typical of the so called "frugal evolutionary strategy" and are very advantageous for the adaptation to extreme environments as high altitude, cold mountain water and the subterranean habitat typical of this species.

Multiple independent transitions to cave life and high diversity in the olm

Hans Recknagel, Valerija Zakšek & Peter Trontelj

SubBio lab, Biotechnical Faculty, University of Ljubljana, SI

In comparison to biodiversity on the surface, subterranean biodiversity has largely remained concealed. The olm (*Proteus anguinus*) is one of the most enigmatic extant cave inhabitants, yet until now little was known on its genetic variability and evolutionary history. Olms inhabit subterranean waters throughout the Dinaric karst, with a seemingly uniform phenotypic appearance of troglomorphic traits: an elongate body, snout and limbs, degenerated eyes and loss of pigmentation ('white olm'). Only a single small region in Southeastern Slovenia harbours olms with a phenotype more typical of surface animals: pigmented skin, presence of eyes and a blunt snout with short limbs ('black olm'). Here, we use a combination of intensive sampling with mitochondrial DNA and genome-wide SNP data to investigate the molecular diversity,

evolutionary history and biogeography of olms along the Dinaric karst. We find extraordinary diversity, with nine deeply divergent lineages that separated between 4 – 17 mya, while molecular diversity within lineages was extremely low. These show no signal of recent admixture and very limited amount of historical gene flow. Biogeographically, the contemporaneous distribution of lineages mirrors hydrologically separated subterranean environments, while the historical separation of olm lineages follows micro-tectonic and climatic changes in the area. The reconstructed phylogeny suggests at least four independent transitions to the cave phenotype, or alternatively a single transition and one reversal to the surface phenotype. The deep divergence between and low divergence within olms makes them extremely vulnerable to anthropogenic threats and highlights the importance of protecting subterranean habitats and each lineage separately.

CHALLENGES IN SCIENCE OUTREACH

A dramatic tale of scientific fraud and political propaganda with *Proteus* in the main role and a Slovenian biologist as tragic hero, along with some new data

Peter Trontelj, Rok Kostanjšek & Hans Recknagel

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

History of science remembers the Austrian biologist Paul Kammerer (1880–1926) as one of the most iconic forgers of all times. Working at a private institute called Biologische Versuchsanstalt in Vienna, he tried to induce heritable phenotypic change by altering environmental conditions. His most famous study animals were midwife toads and fire salamanders. Kammerer was celebrated as a scientist during his lifetime but some considered him controversial from the beginning. He was infamously praised by the Stalinist Soviet regime, whose ideology preferred the neo-Lamarckian molding of traits over Darwinian evolution by natural selection. The controversy over Kammerer has remained intense till the present day. Many of his results were debunked as fraudulent, but some see him as an unknowing victim of intrigue and a pioneer of epigenetics.

One of his contemporary critics was his close collaborator, the Slovenian biologist Franc Megušar (1876–1916). After becoming suspicious of Kammerer's work, Megušar tried diligently but vainly to reproduce his results. Because of his openly uttered criticism, Megušar had to leave the institute in 1913. He was never rewarded for his courageous sacrifice for scientific truth. Both protagonists departed tragically—Megušar died on the Eastern front of WWI, Kammerer committed suicide.

However captivating, Kammerer's story is not the main focus of this presentation. It serves as background for the often overlooked but still important role of *Proteus anguinus*, also used in Kammerer's neo-Lamarckian experiments. He claimed to be able to induce a change from live-bearing to egg-laying reproduction by exposing the animals to unnatural conditions of daylight and high water temperature. Knowing that *Proteus* is egg-laying in its normal cold-water environment, one wonders whence the confidence to claim viviparity as reproductive mode for *Proteus* in the first place. This confidence might have come from a detailed and trustworthy report of observed live-bearing at Stična from 1831, known as the Stratil Protocol. For all of us who have wondered about the trustworthiness of this report, a revealing field observation was made in 2021. In this observation from the Stična area, caught *Proteus*, temporarily held in a

small vessel, regurgitated live fire salamander larvae. For any person not familiar with the look of larval *Proteus*, the suddenly appearing little urodelan larva along with an adult olm could be strongly suggestive of a mother giving birth to her young. This recent observation is yet another piece of evidence exposing Kammerer's fraud.

From science to clickbait

Gergely Balázs¹, Brian Lewarne² & Gábor Herczeg¹

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In 2020 we have published an article in the Journal of Zoology entitled Extreme site-fidelity of the olm (*Proteus anguinus*) revealed by a long-term capture-mark-recapture study. *Proteus anguinus* is an iconic subterranean species and as such every now and then related scientific results hit the threshold of the newspapers, internet blogs and television channels. After publishing our results, we got numerous requests from all over the world to give interviews, make comments and provide more information. Naturally the information was often distorted for better selling. Sometimes it only affected the headline but sometimes the content was false as well. Sometimes the well-known science communicators were better in that sense but sometimes they performed worse than a random wide scope internet blog. The research groups and NGOs working on proteus-related topics and protection plans are the spokesmen of the species and in wider sense of all subterranean ecosystems therefore they are responsible for what reaches the public. Yet in some cases, we even did not get a chance to have any influence on what was being published. All in all, we believe that beside the occasional obviously wrong information and misinterpretations, at the end more people are aware of the existence of these wonderful creatures than before. It was a rather turbulent period with good and bad moments, but one thing is for sure: there were lessons to learn and share.

Black proteus information centre in Jelševnik, Slovenia

Nina Jankovič & Andrej Hudoklin

Institute of the Republic of Slovenia for Nature Conservation, SI

The info centre is located in Jelševnik, Bela krajina. It has been founded with the desire to inform people about the importance of nature conservation. The centre opened in the framework of »The Black Olm« project with the purpose of informing the public about the ecological sensitivity of the area populated by this endemic and extremely endangered cave species.

Neotenus dark dwellers | Lygophilia

Robertina Šebjanič

Independent artist, SI

Neotenus dark dwellers | Lygophilia in an artwork and online book; weaves together mythologies and sciences, history and future, fears and desires, continents, cultures, humans and non-humans. Lygophilia folds and unfolds the stories carried by those fascinating creatures that are the Mexican axolotl and the Slovene proteus. From immortality to regenerative medicine - both animals are, as adults, in a state of "eternal youth" (neoteny) showing extraordinary

longevity and regenerative abilities that put them at the centre of ancient myths as well as current cutting-edge scientific researches.

Further reading: video capsule, online publication of the project Neotenus Dark Dwellers -Lygophilia (Robertina Sebjanič, edited by Annick Bureaud; <https://robertina.net/neotenus-dark-dwellers-lygophilia/>, <http://project.memorekall.com//neotenus-dark-dwellers—lygophilia//?w=1> Firefox or Safari web browsers).

POSTERS

Predator recognition is not a major problem in *Proteus anguinus*

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In this study we compared the behaviour of the olm (*Proteus anguinus*) with another aquatic caudate species that has colonised groundwater: the Pyrenean newt (*Calotriton asper*). The latter maintains both surface and subterranean populations, while, only cave adapted populations of the former exist. Both species are apex predators in groundwater habitats, while the Pyrenean newt is a mesopredator in surface waterbodies. Colonising caves may promote the loss of anti-predator response towards surface predators, and an increase prey detection ability. To test these hypotheses, we integrated classical behavioural characterisations with the assessment of lateralisation. Behavioural experiments were performed using laboratory-reared individuals in Moulis (France). We performed 684 trials on 39 Pyrenean newts and eight olms. Under both darkness and light conditions, we tested how exposure to different chemical cues (predatory fish, prey and unknown scent) affected the activity and lateralisation of individuals. Both cave and surface Pyrenean newts responded to predator cues, while olms did not. In Pyrenean newts, predator cues reduced the time spent in movement and time spent in lateralisation associated with hunting. Our results show that predator recognition is maintained in a species that recently colonized subterranean environments, while such behaviour is missing in *P. anguinus*.

New developments in *Proteus* eDNA quantification in standing and running water

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This is a continuation of a crowd-funded eDNA project whereby we sampled water at several sites along the Pivka channel in the Postojna Planina Cave System and tried to determine if the concentration of proteus eDNA in water samples detected by ddPCR correlate with population sizes estimated in the capture-mark-recapture survey conducted almost concurrently by our

colleagues from the Department of Biology of the University of Ljubljana. The results after two analyses carried out in 2017 and 2018 were promising but inconclusive.

In the second phase of our investigations into the dynamics of eDNA release rate by *Proteus*, we turned to the captive animals at the Tular Cave Laboratory. First we sampled water from two large permanent pools housing white proteus to determine how differences in sample volume, filter pore size, and animal density affect observed eDNA concentrations. After preliminary results (additional sampling parallels have yet to be analyzed) the observed eDNA concentrations in standing water most prominently reflected filter pore sizes but did not correlate with animal densities. It was not possible to determine if this was the result of a lower release- or higher decomposition rate in the smaller, more densely populated pool, or simply a methodological artefact. The good news is, the isolated eDNA stored at -80 °C appeared to be very stable and no losses were detected after over a year and half of storage.

Next, we transferred two animals separately to two plastic barrels filled with fresh water, and subjected them to a stream of water simulating water current velocities measured during our field work in different segments of the Pivka channel in the Postojna-Planina Cave System. The most promising finding of the running water simulation was that a single animal in 100 L of water released detectable quantities of eDNA in less than a minute of sampling time. On the down side, the observed eDNA concentrations were very prone to DNA extraction losses. It is therefore imperative that in the future a known quantity of an appropriate DNA standard be added to the samples prior to filtration to monitor filtration and extraction error. Then we will hopefully be able to reliably continue with the development of this indirect method for monitoring proteus population sizes.

Experience of introducing the olm to the caves of Oliero, venetian prealps Italy

Marco Lazzarotto

Oliero grotte Valbrenta Ivanxteam, IT

Speleovivarium Erwin Pichl (1990–2022): conservation, disclosure, change

Edgardo Mauri

Speleovivarium Erwin Pichl, Adriatic Speleological Society, IT

In setting up the Speleovivarium, Erwin Pichl wanted to put at the disposal of people a place where anyone could come closer to the knowledge of life in darkness. The environment is conceived as a museum, combining the enhancement of an anti-aircraft tunnel with the goal of naturalistic scientific dissemination, research and protection of underground wildlife. In 1980, *Proteus anguinus* was first identified in the waters of the Timavo River (Dolce-Pichl), but the high pollution of aquifers and a compromised environmental situation have suggested the idea of ex situ preservation for *Proteus anguinus*. In 1990 the Speleovivarium was opened, with a shared action between the Adriatic Society of Speleology and the Civic Museum of Natural History. The microclimatic conditions of the tunnel were very similar to those of a cave, the easy access to a suitable location for study and dissemination, so much so that in 1995 it was recognised as a small museum by the VGF Region. The work started by Erwin Pichl continues after thirty years with management on a voluntary and free basis.

Actions by Speleovivarium and others have helped to encourage local governments to address the environmental issue in order to develop regulations and protection laws, which combined with dissemination and national and EU policies, made it possible to significantly improve the local environment. Therefore, ex situ conservation must be included in a more complex framework. (zoo regulation) which presuppose continuity of resources and professions that are not consistent with voluntary and free action. However this is less of a problem because we have noted a gradual rise in temperature. In thirty years we have recorded an increase equal to 1.5 °C, became forced to refrigerate some of the aquariums.

In Trieste, the historic site of the birth of speleology and the study of the Karst, we can hope on support, promotion and the continuation of the Speleovivarium mission at public facilities of the Trieste Natural History Museum and the Aquarium, so the public can meet and respect the most extreme forms of life that nature is showing us.

PROTEUS monograph (Association Hyla, Zagreb, 2019: 252 p.)

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The PROTEUS monograph is the most comprehensive publication on the olm (*Proteus anguinus*), printed in Croatian and English languages in 1,000 copies. The book presents all the knowledge about the olm from the middle of the 18th century until today. In addition to the detailed biology of the species, the book also presents the latest research on the olm, which includes environmental DNA methods, ultrasound diagnostics, *ex situ* breeding, etc. Two hundred and fifty-two pages of a book rich in photographs and illustrations will easily draw you into the mysterious world of the subterranean world and slowly reveal its secrets to everyone.

RESEARCH HIGHLIGHTS

A virtual tour at Elettra: a powerful investigation tool in Trieste

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A short virtual tour of Elettra, the Italian synchrotron radiation facility in Basovizza (Trieste), will be presented. The Elettra laboratory is an international research center offering to academic and industrial users the access to a large portfolio of characterization techniques.

Some examples of scientific applications will be presented focusing the attention on non-destructive 3D X-ray imaging techniques.

3D exploring of *Proteus anguinus* by X-ray computed microtomography

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X-ray computed microtomography (microCT) is a non-destructive imaging with a broad range of applications. However, the use of microCT for imaging of soft tissues has been limited by low contrast of non-mineralized tissues. In this study, we used contrast-enhanced microCT for the 3D segmentation of soft tissues in the head of *Proteus anguinus*. We accessed existing collections in Speleovivarium Trieste, Tular Cave Laboratory and University of Ljubljana to avoid collecting specimens from nature. These animals died by natural causes and their bodies were preserved. We applied non-destructive staining by iodine or phosphotungstic acid (PTA), non-destructive imaging by microCT and then returned the specimens back to the collections. 3D atlas of head was created for larval, juvenile and adult specimens, showing the cartilage of the chondrocranium, muscles, the position, shape and size of the brain, eyes, ear labyrinths and olfactory epithelium. Also, we compared proteus with *Ambystoma mexicanum*, also known as axolotl, to make an exemplary comparison between cave- and surface-dwelling paedomorphic salamanders. 3D models based on microCT data may help to understand the nature and origin of the mechanisms behind adaptations to the subterranean environment.

Does age matter? Examining olms from 16–80 year of age

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The olm (*Proteus anguinus anguinus*) is a unique and extremely long-lived neotenus cave-dwelling amphibian. Due to habitat decline and a low reproductive rate, olms are increasingly threatened and captive breeding may become important to ensure future survival of the species. Basic knowledge, especially on their reproductive physiology is scarce. To increase our knowledge on the range of reproductive developmental stages, we examined 35 olms of known ages ranging from 16 to 80 years, including one female one month after oviposition, as well as two of her larvae. The examined animals live in natural cave systems under semi-natural conditions at Hermman's cave in Germany (n=5) and Moulis in France (n=30). Using ultra-high resolution ultrasound at frequencies of up to 70 MHz (Vevo 2100 and 3100, FUJIFILM VisualSonics) enabled us to assess health and reproductive status. The largely monomorphic individuals were unequivocally sexed (9 males, 26 females). Reproductive status was assessed in terms of follicular diameters and location of ova inside the female reproductive tract, and in

terms of testes sizes in males. Follicle diameters ranged between 0.5 and 3.5 mm, testes diameters between 1.7 and 7.0 mm. We further detected various subclinical pathologies, such as liver cysts and gout. There was a strong correlation between inner organ sizes, body length, weight and diameter, while heart rate negatively correlated with body size parameters. Body diameter of females positively correlated with follicle size. Interestingly, within age groups considerable body size variations occurred. Our results will be discussed in the light of our previous ultrasound findings in olms both in captivity at Zagreb Zoo as well as in the wild at Rupečica Cave, Croatia. Ultrasound proved a suitable non-invasive tool for health and reproductive monitoring and for enhancing our understanding of reproductive status and development of olms.

Linking different approaches for sex identification of *Proteus anguinus*

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In *Proteus anguinus*, sex identification is difficult due to the absence of sexual dimorphism and homomorphic sex chromosomes. The aim of our study was to develop a non-destructive and reliable approach for sex identification and gonadal maturity of *P. anguinus*. Our long-term study was based on the systematic analysis of the external morphology of 17 animals, detection of the sex-specific protein vitellogenin, and measurement of sex steroids (estradiol and testosterone) in the blood of the animals. We linked all the above approaches to sex and gonadal maturity and the results were partially evaluated by histological analysis of the gonads. We found that females and males could be identified by the significant increase in estradiol and testosterone concentrations, respectively. Multiple measurements of sex steroids allowed us to estimate the duration of gametogenesis in both sexes. In addition, active oogenesis can be determined by the presence of vitellogenin in the blood and of growing oocytes seen through the unpigmented skin. An unexpected decrease in estradiol concentration and vitellogenin during oogenesis is associated with oocytes degeneration and follicular atresia formation, resulting in a decrease in the size and number of visible oocytes. The increase in testosterone concentration in males coincides with a swollen cloaca, however sex identification by the latter is unreliable. The developed approaches allow sex identification during active gametogenesis and provide an important tool for planned captive breeding of *P. anguinus*.

FIELD RESEARCH

Behavioral observations of the olm (*Proteus anguinus*) in a karst spring

Ester Premate, Žiga Fišer, Žan Kuralt, Anja Pekolj, Tjaša Trajbarič, Eva Milavc, Živa Hanc & Rok Kostanjšek

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In the last decades, a moderate to substantial decline of the olm (*Proteus anguinus*) has been detected at several sites throughout its range and unearthed the urgent need for a conservation management plan to protect this charismatic species. Since then, much effort has been made to develop tools and methods for monitoring its populations, to establish captive-breeding programs, and even an initiative for rescuing washed-out individuals and a protocol for

returning them to their natural habitat have been set up. While these actions are essential for effective conservation, their success relies heavily on our knowledge of the species biology. For example, it has long been recognized that conservation management can benefit from understanding animal behavior and that ignoring the latter can lead to failure of conservation efforts. As the behavior of olms in their natural habitats is poorly known, we studied it in a population whose individuals regularly occur in a natural karst spring at night. For ten consecutive nights in July 2019 we observed olms directly on-site using red headlights and supplemented these observations via IR camera trap recordings for four nights. Direct observations cohered with the recordings regarding the time of the olms' appearance and the surface-subsurface corridors they used to enter or leave the spring. Video-tracking animals on recordings enabled us to quantify the olms' movement activity by estimating total path covered as well as average and maximal speed for several individuals. We constructed simple ethograms and investigated whether olms interacted when multiple individuals co-occurred in the spring. Despite the short-term nature of the study, we obtained valuable data that complement the existing knowledge on olms' behavior in natural habitats. We believe our findings and experience gained will improve the design of future behavioral studies and are a step towards the integration of knowledge on olm's behavior into its conservation plans.

Activity of the olm (*Proteus anguinus*) in Italian spring habitats, close to A4 highway: conservation implications.

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The olm is considered as a classic example of troglobiont organism. However, in the past different observations of individuals of the typical troglomorphic populations have been reported for springs of Venetia Giulia. Since 2020 we started a field monitoring assessing factors allowing springs exploitation by the olm and comparing occurrence and activity patterns in both surface and subterranean habitats. The aim of this work is to point out the non-random active use of surface habitats by the olm, and to identify priorities to preserve the ecotone habitats at the border with groundwater that the species exploits in Venetia Giulia. From June 2020 to March 2021 we performed multiple surveys, both during day and during night to detect olm occurrence in 50 springs and 8 caves of Gorizia and Trieste districts in the surroundings of Doberdò del Lago locality. We also characterised the abiotic features of each spring and we monitored the occurrence of potential fish predators and of some target invertebrate species. We detected the olm at least once in 10 springs, with a maximum of 8 individuals occurring together. Some of the sites where olm's exploitation was particularly frequent occur close to the A4 Italian highway; in one of these sites, we also recorded the occurrence of a larva of 3.56 cm, suggesting the occurrence of breeding. We suggest that epigean habitats and borders with surface may have an overlooked importance for animals adapted to subterranean environments, including the olm. Their conservation is a priority for the study area. Future enlargement of the highway should attentively avoid to alter functionality of surrounding spring habitats; moreover, we underline the importance of favouring environmental diversification of promoting regular management and monitor human fruition of springs mouths in the classic karst area to improve *P. anguinus* conservation.

Preliminary study in cephalic morphology of *Proteus anguinus* between individuals found outside caves vs. those found deep in caves

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The olm (*Proteus anguinus*) is considered the most representative example of all the stygobiont fauna. Nevertheless, it is also reported in some resurgences located in Friuli Venezia Giulia (North-eastern Italy). Like most hypogean animals, the proteus has some very marked adaptations that allow it to exploit the underground environment. The aim of this study is to verify if there are differences in the structural morphology of the head between the olms that are found in caves compared to the individuals found in surface habitats. During 2021, 28 olms, 18 from underground habitats and 10 from external sites, were photographed in standard conditions with a metric reference. For each individual, only the cephalic region has been considered. The pictures were processed thanks to the software TPSdig. For each photo analyzed were placed 2 landmark homologues, matching the emergence of the gills branches, and 48 semi-landmark to define the contour of the head through a continuous curve. The results indicate a significant effect of the environment on the shape of the olm's head, in fact the shape of the head appears more elongated in the front and narrower in the parietal region in the olms coming from the hypogean environment. These findings could therefore suggest a stable attendance of superficial habitats by some individuals. They can also stimulate further research on how caves can be mistakenly considered by many as an "evolutionary dead end" and on how stygobionts can exploit the epigeal environment and maybe adapt to it.

Images of proteus (video film)

Ciril Mlinar - Cic

Water Cycle Institute, SI

It has long been known that olms from different caves differ slightly in their external characteristics. Only recently, however, genetic studies have suggested a possible breakdown into several potential independent species. Geographically separated populations along the entire range could belong to nine different species. Individual populations differ genetically and morphologically. In the decades of field observation, the most peculiarities I noticed in the population from the Bilpa Spring, Slovenia. The first, most noticeable, is their above-average size, as adult animals with estimated length of up to 35 cm in length or even more stand-out from afar. The chest is wider than usual, the head is large, with a long, narrow and angular muzzle, the gills are relatively small. The eyes remain well visible in adult animals, they appear much larger than in all other white populations. The five-minute film *Images of proteus* shows olms from the Bilpa Spring. Is this population also a potential independent species?

Round Table Session

e-SCIENCE AND OPEN SCIENCE TO ADDRESS CHALLENGES IN CONSERVATION OF *PROTEUS*

On May 22, when we celebrate World Biodiversity day, we propose a round table session that includes a short presentation of LifeWatch ERIC research infrastructure (Prof. Alberto Basset) with examples of new studies to proteus within LifeWatch Slovenia node, dedicated to proteus research. A debate with the participants will be in place in order to assess the current needs in proteus research that could be addressed in the future with the help of the LifeWatch ERIC.

Round table keynote

Invasive species - a key issue in biodiversity

Alberto Basset^{1,2}

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Building virtual laboratories to monitor *Proteus* and its karst groundwater habitat

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Here we present a research project that addresses questions about the mechanisms and processes of contaminant transport in aquatic karst systems and their influence on *Proteus* behaviour and ecology. A Slovenian and Belgian team, representing several institutions that are already partners in LifeWatch ERIC and eLTER research infrastructures, plans to establish two virtual laboratories (vLabs) dedicated to the assessment of subterranean biodiversity and its karst habitat: i) ProteusWatch vLab and ii) Karst Groundwater Habitats vLab. The two envisioned vLabs will complement each other with data on proteus behaviour (monitored by infrared video cameras and imaging sonar) and correlate it to the sensed physical and chemical changes in groundwater (e.g., pollution levels, meteo-events/ flooding). The project will exploit the latest advances in machine and deep learning technologies to provide advanced exploratory analysis capabilities of video tracking. The Agouti platform developed and deployed at the LifeWatch Belgium, will be adapted and implemented for both planned vLabs as a part of this project.

SOS Proteus



Abstract book of the 4th International meeting *SOS Proteus*

May 21-22, 2022, Trieste, Italy

Organized in partnership of Speleovivarium Trieste, Speleological Society Adriatic, Natural History Museum Trieste, Municipality of Trieste, and Tular Cave Laboratory

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Digital edition helps to save proteus and groundwater ecosystems!