



# PROJECT INTERIM REPORT

## 1<sup>st</sup> ERANet-LAC Joint Call

**ELAC2014/DCC-0178**

**Project acronym:** CAVICE

**Project title:** Cave ice microbiom: metabolic diversity and activity in response to climate dynamics and anthropogenic pollution

**Project Duration:** from 06.01.2016 to 31.12.2018

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## SUMMARY

The overall interest of the project is to characterize the diversity and metabolism of total and active microcosm from cave ice, and evaluate past, present and future consequences of climate driven ice retreat and human impact on biogeochemical and ecological processes by studying temporal and spatial changes in ecosystems present in ice and glacier caves from different geographical locations, and to establish a strategic framework for multidisciplinary research in ice ecosystems, including investigation of the applicative potential of ice microcosm in nanotechnology development. The goals of the project are i) the first characterization of the functional diversity and activity of a cave ice microbiome using metagenomic and metatranscriptomic Next Generation Sequencing, ii) to assess the impact of climate dynamics and anthropogenic pollution on microbial communities preserved in this particular glacier environment, and iii) to identify novel cold adapted metal nanoparticle producing strains for bionanotechnologies.

The project started in 2016 and accomplished the main objectives and activities emphasized for this period. Ice samples were collected from the Europe and South American locations, including the glacier caves from Argentina (Viedma Glacier) and Chile (Grey Glacier), and the from the ice caves from Norway (Svarthammar), Romania (Scărișoara) and Slovakia (Dobšinská and Demänovska) with the participation of all the consortium teams. Physicochemical and geochemical analyses were carried out on Scărișoara ice of up to 2000 years old. Recent climate analysis of Scărișoara was initiated and carried out by long-term monitoring of water isotopic composition outside and inside the cave. Samples from the ice block of *Svarthammar* were collected for stable isotope analyses (432) and for  $^{14}\text{C}$  dating (14). Microbial DNA was extracted from the ice samples collected from all locations for determining the bacterial diversity; for Svarthammar ice samples, this step will be carried out in January 2017 with the participation of a Master student from the Romanian IBB partner in Norway UoB laboratory. Illumina sequencing of 16S rDNA was performed for samples collected from Grey Glacier dominated by *Firmicutes* and *Bacteroidetes*, while 454 pyrosequencing of five ice samples of Scarisoara showed the presence of *Proteobacteria*, *Firmicutes* and *Actinobacteria* in ice of up to 900 years old, a high representation of *Bacteroidetes* and *Cyanobacteria* in recent ice exposed to light, and the presence of *Archaea* in 400 and 900 years old ice. Shotgun metagenomics sequencing of 7 ice samples of up to 2000 years old from Scarisoara cave is in progress. Bacterial cultures and isolated colonies were obtained from Scarisoara ice cave and Grey Glacier ice samples and their identification based on 16S rDNA is currently underway.

Two CAVICE kick-off meetings were organized in Argentina and Slovenia, respectively, to ensure the participation of all the European and Latin American consortium partners for discussing a series of scientific and management collaborative aspects. CAVICE results were presented in three conferences (4 oral presentations and 4 posters), with the participation of members of 4 of the consortium partners.

This project is expected to provide pioneering knowledge on cave ice microcosm reflected in a better understanding of how the ice microbiomes mirrors the past and actual climate changes and the anthropogenic activities. We appreciate that this study will contribute to the knowledge for predicting the dynamics and evolution of the ice microbiomes in relation with the climatic patterns and anthropogenic pollution. Identification of pollution biomarkers would ease the monitoring activities, being of great help for the caves administrations in their effort to better preserve these unique sites. The novel cold adapted microorganisms able to synthesize metal nanoparticles that we attempt to identify are of broad applicative potential in nanotechnologies and medicine. This interdisciplinary project brings together specialists from various fields of Life Sciences, thus ensuring the improvement of the scientific level of the participating teams, and favoring further joint applications. Moreover, the project contributes to the formation of top-rank young scientists through an excellent training of the participant Master students and PhD students.

## RESULTS AND OUTPUTS 2016 - SYNTHETIC REPORT

<b>WP</b>	<b>Technical Milestone 2016</b>	<b>Accomplished</b>	<b>Observations</b>
WP1	Sampling of ice sediments from the Romanian, Norway, Argentina and Chile ice caves and glacier caves (Month 4)	Yes	Ice samples were obtained for all considered location and 2 supplemental Slovakian ice caves
WP2	DNA extraction from ice samples (Month 4)	Yes, partial	UoB (Norway) - due to sampling field trip organization in October 2016 this step will be performed in January 2017 in collaboration with IBB (Romania)
WP2	16S rRNA PCR amplification and sequencing (Month 7)	Yes, partial	UoB (Norway) - due to sampling field trip organization in October 2016 this step will be performed in January 2017 in collaboration with IBB (Romania)
WP3	Climate reconstruction of Scărișoara Ice Cave (Month 12)	Yes	IBB (Romania) Recent climate monitoring in the Romanian ice cave
WP4	Chemical analyses of Scărișoara Ice Cave (Month 12)	Yes	IBB (Romania) Physicochemical analyses of ice samples up to 2000 years old from Scărișoara Ice Cave  ERIS (Romania) Chemical analyses of Scărișoara cave ice samples
	Consortium networking	Yes	2 management workshops (kick off meetings) were organized with participation of all partners representatives
	Results dissemination	Yes	Participation of IBB, ERIS, UA and UoB partners to 2 international conferences (3 posters and 3 oral presentation) and 1 national conferences (1 poster and 1 oral presentation)

## 1. SAMPLING FIELD TRIPS

Ice samples were collected from all the emphasized locations, including the two ice caves from Romania and Norway, and the two glacier caves from Argentina and Chile. Additional ice and water samples were obtained from adjacent locations and from 2 Slovakian ice caves.

### 1.1 Viedma Glacier, Argentina, 26 February-1 March 2016

Participants: Argentina (Dr. Maria Farias), Chile (Dr. Paris Lavin), Romania – Partner IBB (Dr. Cristina Purcarea, Dr. Aurel Persoiu, PhD student Corina Itcus), and – Partner ERIS (Dr. Alexandra Hillebrand-Voiculescu).

Ice samples were collected from Viedma Glacier Cave at exposed and remote locations, and from the glacier border that forms an open cave for determining the microbial diversity and for geochemical analyses.



### 1.2 Grey Glacier, Chile – 11-12 May, 2016

Participants: Chile - (Paris Lavin, Pablo Aran, Esteban Severino, Patricio Munoz)

Samples were taken of the Grey glacier, located inside the National Park Torres del Paine, XII Region of Magallanes and Antártica Chilena. Ice samples were collected from the cave formed in Grey Glacier corresponding to a more compacted ice (blue colored ice), from the superficial part and the inner zone of the glacier ice, and iceberg samples were collected from the Grey Lake near Glacier Grey. The water samples (5 liters) were collected from 4 different locations of the Grey lake.



### 1.3 Svarthamar Ice Cave, Norway – 3-9 October 2016.

Participants: Norway UoB team (Dr. Lise Ovreas, Dr. Stein-Erik Laurizen, Sverre Aksdal, PhD student Christos Pennos and Hilde Rief Armo) and Romania – IBB team (Dr. Aurel Persoiu, Dr. Cristina Purcarea, Drd. Corina Itcus).

Ice samples of up to 700 years old were collected from 7 locations of Svarthamar Ice Cave, Norway for microbiological analyses, 14 ice samples for  $^{14}\text{C}$  dating and 432 ice samples for isotope analyses.



#### 1.4 Dobšinská and Demänovska Ice Caves, Slovakia, 7-14 November 2016.

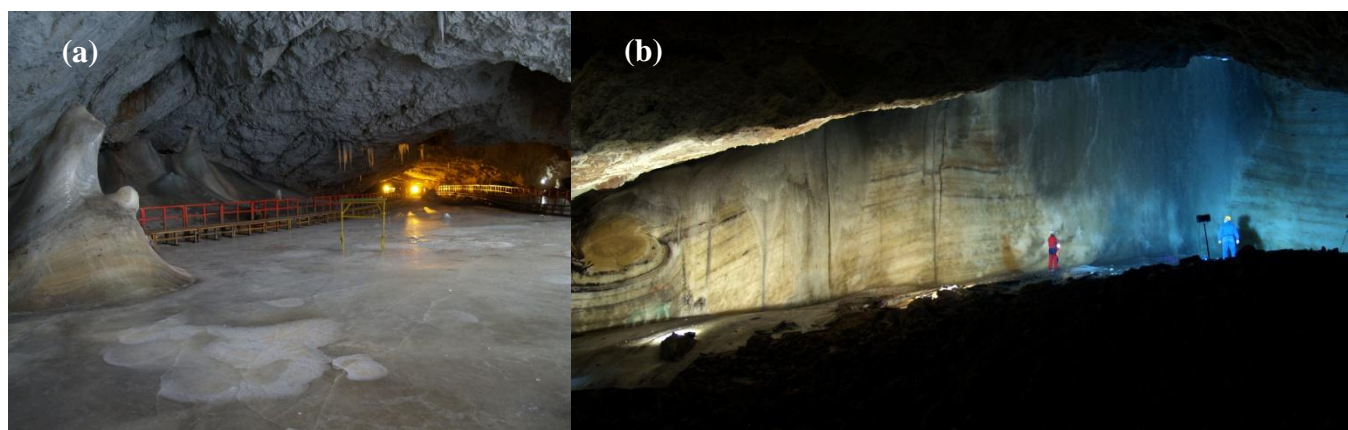
Participants: RO-ERIS (Dr. Alexandra Hillebrand-Voiculescu) - initiated new collaborations with Dr. Višňovská Zuzana and Dr. Michal Rendoš, Slovak Caves Administration, and collected ice samples from both caves.



#### 1.5 Sampling locations in Scărișoara Ice Cave, Romania

In order to perform the metagenomic analysis of microbial community from the ice block of Scărișoara Ice Cave we used seven ice samples of different age and sediment content collected from the perennial ice block from the Great Hall (a) and Little Reserve (b) areas of the cave under aseptic conditions:

- 1-S and 1-L - Recent ice (1 year old) collected from the ice block surface in the Great Hall
- 400-O – 400 years old ice collected from Little Reserve ice wall
- 900-O and 900-I : 900 years old ice collected from Little Reserve ice wall from organic-rich and clear ice layers
- 1500-I: 1500 years old ice – collected by vertical drilling from the Great Hall
- 2000-I: 2000 years old ice - collected by vertical drilling from the Great Hall



## 2. CAVICE MANAGEMENT WORKSHOPS

**2.1 The first CAVICE management workshop** was held on February 25, 2016, at El Chalten, Argentina, with the consortium participants from four out of five partners (IBB, ERIS, UA si CONICET), where was discussed the project scientific strategy.

**2.2 The second CAVICE workshop was held on 20 May, 2016, in Postojna, Slovenia,** with the occasion of 7<sup>th</sup> International Workshop on Ice Caves, with the participation of the Norway team members Dr. Lise Ovreas and Dr. Stein-Erik Laurizen and the Romania IBB members Dr. Cristina Purcarea, Dr. Aurel Persoiu and Drd. Corina Iteus, for discussing the Project collaboration strategy

and the organization of the field trip for ice sampling collection at Svarthamar Ice Cave, Norway, in October 2016.

### 3. CONFERENCES

The results obtained were presented in 2 international conferences (3 posters and 3 oral presentation) and a national conferences (1 poster and 1 oral presentation), with participation of the IBB, ERIS, UA and UoB partners of the CAVICE consortium.

**7<sup>th</sup> International Workshop on Ice Caves (IWIC VII)**, Pastojna, Slovenia, 16-22 Mai 2016

Participants: Romania - IBB (Cristina Purcarea, Aurel Persoiu, Corina Itcus); Norway – UoB (Lise Ovreas, Stein-Erik Lauritzen) - 1 oral presentation and 3 posters

- Persoiu A. *Stable isotopes in cave ice: tracking changes in moisture source in Eastern Europe*. Oral presentation
- Lauritzen SE, Engelién J, Bastaad L, Holmlund P. *Dynamics of the Svarthamar ice cave, Fauske, North Norway*. Oral presentation
- Lauritzen SE. *Speleogenesis of the Svarthamar ice cave, Fauske, North Norway*. Oral presentation
- Itcus C, Pascu MD, Hillebrand-Voiculescu A, Persoiu A, Brad T, Purcarea C. *Cultured bacterial diversity in Scărișoara ice cave*. Poster
- Itcus C, Pascu MD, Hillebrand-Voiculescu A, Brad T, Persoiu A, Onac BP, Purcarea C. *Distribution of bacteria and archaea in the ice block of Scărișoara cave (Romania)*. Poster
- Lauritzen SE, Øvreås L. *Cave ice microbiom along a Chronosequence in Svarhamar Ice Cave, Norway*. Poster

**XXXVIII Congreso Chileno de Microbiología-SOMICH**, Valdivia, Chile, 22-25 November

Participants: Chile - UA (Cristina Dorador, Pablo Aran, Esteban Severino, Patricio Munoz) – 1 poster

- Munoz P, Aran P, Severino E, Dorador C. *Diversidad microbiana psicrófila del Glaciar Grey, Parque Nacional Torres del Paine, Chile*. Poster

**56th Annual Scientific Session of the Institute of Biology**, Bucharest, Romania, 9 December 2016

Participants: RO-IBB (Cristina Purcarea, Ioan Ardelean, Victoria I. Paun) – 1 oral presentation

- Itcus C, Pascu MD, Hillebrand-Voiculescu A, Persoiu A, Brad T, Ardelean I, Purcarea C. *Microbial communities from Scărișoara Ice Cave*. Oral presentation

### 4. 16S rRNA GENE SEQUENCING AND METAGENOMICS

**RO-IBB**: Melted ice samples from **Scărișoara Ice Cave** at 4°C were concentrated on 0.22 μm Millipore filters and the genomic DNA was extracted using Qiagen Blood and Tissue kit and an adapted protocol with an initial lysis step by incubation with mutanolysin. DNA extraction was performed in triplicate.

The bacterial and archaeal diversity of ice samples up to 900 years old from Scărișoara Ice Cave, Romania, was determined by 454 pyrosequencing of 16S rDNA, indicating the presence of *Proteobacteria*, *Firmicutes* and *Actinobacteria* in all analyzed strata, with the dominance of *Bacteroidetes* and *Cyanobacteria* in recent ice samples, while *Archaea* was present only in 400 and 900 years old ice.

Shotgun metagenomics of 7 ice samples (1-S/L, 400-O, 900-O/I, 1500 and 2000) from Scărișoara Ice Cave, Romania, was performed by Génome Québec, Montréal, Canada, by Illumina HiSeq. The Shotgun DNA library was prepared for Illumina sequencing using NEB Ultra II and the Sequencing is currently under way using Illumina HiSeq 2500 PE 125bp sequencing lane.

Chile-UA: After filtration on 0.22 µm polyethersulfone filters, total DNA was extracted from the Grey Glacier samples using MOBIO Power Biofilm kit. Illumina MiSeq of 16S rRNA gene sequencing of ice samples from Grey Glacier indicated the occurrence of *Firmicutes* and *Bacteroidetes* as major bacterial phyla.

## **5. RECENT CLIMATE MONITORING IN SCĂRIȘOARA AND SVARTHAMMAR ICE CAVES**

RO-IBB: We have installed climate monitoring equipment in *Scărișoara Ice Cave*, Romania, and are continuously monitoring the isotopic composition of water both outside (precipitation) and inside (drip water) the cave.

In October 2016, UoB (Norway) and RO-IBB teams collected 432 samples for stable isotope analyses from *Svarthammar Ice Cave*, Norway, as well as 14 samples for <sup>14</sup>C dating. The radiocarbon dating will provide us with an age-depth model that will be used in combination with the results of oxygen and hydrogen stable isotope analyses in order to reconstruct the climate variability over the past ~1000 years.

## **6. GEOCHEMICAL ANALYSES**

RO-IBB: Physicochemical analysis - The conductivity and pH of Scărișoara Ice Cave samples 1-S/L, 400-O, 900-O/I, 1500-I and 2000-I were measured immediately after ice melting at 4°C. The pH values ranged around neutral for 1-S, 400-O, 1500 and 2000 ice samples, with slightly basic values for 1-L and 900 years old ice (pH 7.87-7.92). Meanwhile, the conductivity decreased exponentially with the ice age.

RO-ERIS: Chemical analyses of the samples from Scărișoara Ice Cave were measured by RO-ERIS partner, showing high organic carbon content and concentrations of Mn, Fe, Cu and Zn in 400 years old ice and the presence of Ag in recent ice samples 1-S and 1-L and in 400-O. This latter observation favors the further investigation of Ag nanoparticle-synthesizing bacteria from this ice cave (WP5). High nitrate and sulfate concentrations were found in recent ice located in touristic areas.

## **7. CULTIVATION**

Ice samples from all glaciers and ice caves locations were inoculated in various growth media (R2B, ABM, TSB, LB, MB, Sabouraud) at 4°C and 15°C to isolate cultured psychrotolerant/psychrophilic bacterial strains by the IBB, UA, CONICET and UoB partners. Identification of isolated by 16S rRNA gene sequencing is currently performed (UA, RO-IBB).

The project implied the signing of a Consortium Agreement between all partners. For the early stage researchers training, 3 of the consortium partners hired 1 Master student (IBB) and 6 PhD students (2 IBB, 1 ERIS, 3UA).