ROMANIAN ACADEMY
INSTITUTE OF BIOLOGY BUCHAREST

Ph.D. THESIS
SUMMARY

Microorganisms Diversity in Perennial Ice from Scărişoara Ice Cave

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CONTENTS

INTRODUCTION

The general idea of this doctoral thesis was its approach by means of molecular biology. The target of the study was represented by the microbial diversity contained in the Scărișoara ice block associated with the chemistry and the age of the ice strata.

The microbial communities represent an excellent study model of molecular diversity (precise delimitation, homogeneity, stable conditions imposed by temperature and chemical composition of water), low temperature, which subsequent research will be able to harness.

Ice embedded microcosm represents a vast source of new species and an alternative for past climate reconstitution. Among glacial environments, ice caves represent one of the scarcely investigated frozen habitats.

In this thesis, in order to describe the microcosm embedded in the perennial ice from karst ecosystems in relation with past climatic changes, we considered both preservation and functional role of microbial species in the cave.

Due to the increasing interest regarding psychrophilic microorganisms because of their great biotechnological potential, this investigation of the microbial diversity from the perennial ice block from Scărişoara Ice Cave represents the first study of the biodiversity of prokaryotic and eukaryotic microorganisms from caves ice deposits in correlation with the age and geo-climatic characteristics of the ice.

Cryospheric deposits such as ice sheets of the glaciers contain the best paleoclimatic record, providing relevant data for the understanding of climatic variations and evaluation of the anthropic impact on the environment.

The less studied members of the cryosphere are ice caves which are formed by perennial accumulations of frozen water (Perșoiu & Onac, 2012), and represents the smallest group of the global cryosphere.

Ice caves are formed by the water that reaches to the solid phase in one part of a natural cave such as lava tubes or limestone caves, that registers a temperature under 0°C constantly over the year and it is composed of important amounts of perennial ice, being thermally insulated from the outside temperatures (Barck, 1913; Wigley & Brown, 1976).

Studying different icy environments led to a better understanding of the role and adaptation mechanism of the microbial diversity with respect to these types of extreme habitats.

At international level, numerous studies of the ice containing areas were conducted and the discovery of life in particularly cold and isolated environments has altered the notion of livable areas of the Earth. Thus, at the end of the 1990s, life forms were discovered at about 3590 m glacier depths (Karl, et al., 1999; Priscu, et al., 1999) and in subglacial deposits (Sharp, et al., 1999). Microorganisms have also been encountered in permafrost (Rivkina, et al., 2000) and in marine ice (Junge, et al., 2004).

Fundamental in defining the ecological role that microorganisms have in frozen systems is knowing their metabolic activity at temperatures below 0°C. Latent cells from frozen systems are consuming carbon-C, nitrogen-N, phosphorus-P and other vital and unreplaceable nutrients as opposed to metabolic cells that participate actively in biogeochemical phases. Determination of metabolic activity under the sub-zero temperature of the water raises methodological problems. Numerous protocols have been established in an attempt to calculate the level of the microbial processes at these extreme temperatures (Mikucki, et al., 2011).

Even if the diversity regarding microorganisms present in underground ice blocks is less studied, many more other frozen environments were investigated, as for example Arctic and Antarctic ice, polar ice sheets and glaciers, permafrost, highland glacier forefields etc. These environments found inside of the caves, were targeted for paleoclimatic research for the information resulted from geochemical proxies (Racoviță & Șerban, 1990; Yonge & MacDonald, 1999; Citterio, et al., 2004; Kern, et al., 2004; Luetscher, 2005; Maggi, et al., 2011). Ice caves from different altitude areas, where the mixture of underground cavities morphology and their climatic conditions permit the conservation of ice were linked to azonal icy climatic conditions (Perșoiu & Onac, 2012). Partial research comprise isolation of prokaryotes and eukaryotes (Margesin, et al., 2004; Vincent, 2007; Namsaraev, et al., 2010; Uetake, et al., 2010) from this kind of environment.

As far as we know, a microbial chronological distribution was not reported on ice containing alpine caves.

Recent investigation of Hawaiian lava tubes (Teehera, et al., 2017) highlighted the presence of bacterial and archaeal various communities in silica and calcite (Zak, et al., 2008) deposits and ice pond water. The ice bacterial community was dominated by Proteobacteria phylum (39%), similar to the recent ice deposits of Scărișoara Ice Cave, besides Bacteroidetes (18%), Verrucomicrobia (8%) and candidate division OD1 (7%), also spread in the 1-S, 1-L and 400-O alpine cave ice samples. Meanwhile, the mineral samples of Hawaiian volcanic caves had a high relative abundance (86%) of Actinobacteria, similar to 900-years old ice layers of the alpine ice block, and Euryarchaeota OTUs were also found in the volcanic ice cave, but very scarcely represented (<<1%) unlike the Medieval Warm Period-formed ice layers of Scărișoara Cave. However, in spite of some commonly found taxa in these two types of caves, a wider diversity and unique bacterial OTUs and particular microbial distribution were characterizing the prokaryotic structure from the perennial ice block of Scărișoara Ice Cave.

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The field work for prevailing samples that were analyzed in this thesis was performed in Scărișoara Ice Cave which is located in the Western Romanian Carpathians (Bihor Mountains). Scărișoara Cave is geographically situated at 46°29′23″ North latitude and 22°48′35″ East longitude at an altitude of 1165 meters above the sea level and is mainly formed on limestone. The ice block hosted by the cave was dated up to more than 13,500 years old and it is one of the largest, with a volume of approximatively 100,000 m^3 (Holmlund, et al., 2005; Perşoiu & Pazdur, 2011). Many researches and studies on Scărișoara Ice block were conducted by several authors in order to understand and describe the climatic factors and processes related to glaciology (Racoviță, 1927; Șerban, et al., 1948; Rusu, et al., 1970; Racoviță & Viehmann, 1987; Racoviță, 1994; Racoviță & Onac, 2000).

In recent years, due to the interaction between ice mass and air temperature, Scărișoara Ice Cave became a major reference point for the climate reconstruction and environmental changes (Onac, et al., 2007; Feurdean, et al., 2011; Perșoiu, et al., 2011; Perșoiu & Pazdur, 2011). Scărișoara' s perennial ice block was formed through water freezing as opposed to other glacier caves (Perșoiu, et al., 2011). During the hot season (from spring till autumn), the outside water infiltrates the cave area (Great Hall) leading to the formation, on the surface of the ice block, of a ~20 cm deep lake which in the cold season, takes a solid form resulting in an ice layer of similar thickness that contains sediments brought by water in the summer period. This repetitive process leads to the formation of ice layers with a various organic and inorganic sediment content (Perşoiu, et al., 2011; Perşoiu & Pazdur, 2011). In 1949, E. Pop identified in the limestone of Scărișoara Cave chemolithotrophic organisms as nitrifying bacteria (Pop, 1949).

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This thesis, constituting a basic research study, represents the first characterization of the microbiome from perennial ice accumulated in caves, encompassing total and cultured bacterial community and cultured fungal community structure from different locations of Scărișoara cave ice block, up to 2000 years old ice strata.

Scărișoara Ice Cave (Romania) contains the world's largest cave ice block that was dated up to more than 10,500 years old (dated with ^{14}C), formed in bedded limestones of Upper Jurassic age. Specific permafrost conditions of the cave environment during ice layers formation were correlated with climate patterns. In this context, our main interest was to identify the microbial biodiversity in ice deposits from Scărişoara Cave, and to determine a chronological distribution of ice microorganisms and a corresponding climate-based biomarker. This study encompasses phylogenetic and physiological analysis of uncultivated and cultivated bacteria and fungi, and uncultured archaea isolated from ice layers of different ages and organic content.

Being a less studied environment, the research regarding the microbial biodiversity from alpine ice caves brings a big impact in the context of climate change through species conservation in ice that can be defined as climate biomarkers.

While the ice cave microbiome is almost undocumented, this investigation representing the first study of microorganisms in the Scărișoara Ice Cave is relevant to fundamental research by determining biodiversity of a very little-known ecosystem type through linking the diversity of microbial communities with the age of ice layers in the cave, exposure to light / darkness, organic ice content.

The research regarding the identification of microbial diversity embedded in ice has an increasing interest because of the huge applicative biotechnological potential of the psychrophilic microorganisms as biocatalysts in low-temperature processes.

Novel bacterial species were isolated from the perennial ice of this cave in view of identifying psychrophilic and psychrotolerant microorganisms with enhanced properties useful in bionanotechonologies.

Objectives

1. *Study of the microbial density, viability, diversity, and chronological distribution of microbial communities from cave ice sediments*
2. *Phylogenetic and physiological characterization of uncultured and cultured bacteria and fungi isolated from different ice layers up to 2000 years*
3. *Selection and characterization of new species adapted to extreme environments with applicative potential in biotechnology*
4. *Identification of microbial markers for climate variations during ice formation in this ice cave*

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The present paper is structured in two parts divided in five chapters and contains 39 figures and 17 tables.

The first part presents the current state of the art, including general information on the cryosphere and ice caves as part of cryospheric deposits; ice microbiome; psychrophilic microorganisms; site description; research importance; materials and methods.

The second part presents the original contributions, constituted by the results obtained in the present study and discussions on microbial diversity from the Scărișoara Ice Block. The presented paper ends with a chapter of general conclusions and further research directions preceded by a list of papers published on the subject of the PhD thesis. The bibliography comprises a number of 172 papers and books cited in the text.

The techniques used regarding the research on microbial diversity from Scărișoara ice block comprises a series of methods as follows: microbiological (liquid/solid cultivation), molecular (DNA extraction, PCR amplification, DGGE, Sanger sequencing, pyrosequencing 454, instrumental (flow cytometry, scanning electron microscopy), chemical (pH, EC, TDS, carbon/nitrogen content, salinity, nutrients), bioinformatics (statistics and phylogeny).

Ice samples belonging to different ages were extracted from C^{14}-dated ice layers of perennial ice block from Scărișoara Cave (Perşoiu & Pazdur, 2011).

Seven different samples of 1, 400, 900, 1500- and 2000-years old ice were prelevated from different locations of the ice block. Two 1-year-old ice samples were collected using a vertical drilling procedure from the Great Hall area, one (sample **1-S**) from a sun exposed region of the block and the other (sample **1-L**), located in the center of the cave, from a poorly lit area.

The 399 cal. yrs BP ice (sample **400-O**), and 887 cal. yrs BP ice (samples **900-O/I**) were extracted from the ice wall situated in the Little Reservation area using a horizontal auger, after removing approximatively twenty centimeters of the ice wall surface.

The 1500- and 2000-years old samples were collected also by vertical drilling. For each location the sampling procedure was conducted under aseptic conditions and the ice samples were extracted in triplicate (Hillebrand-Voiculescu, et al., 2014).

In order to associate the microbial diversity with the chemical and physicochemical properties of the samples extracted from ice, the mass of carbon and nitrogen, along with measurements of the pH, electrical conductivity EC, and total dissolved solids TDS parameters of ice samples were determined. The results show that the parameters of the melted ice samples alternate with both the age of the sample and also with the organic content of the sediment.

Pyrosequencing analysis showed a common microbial core comprising 161 distinct OTUs (33.7% of prokaryotic taxa), while 218 OTUs (45.6% of taxa) were shared between different ice layers. Among these, the largest phylotypes number (41) was shared between 400 and 900 years old ice, while very few common prokaryotic OTUs were found in both recent ice 1-L (1-6), or 1-S (1-2), and older ice samples 400-O, 900-O and 900-I, respectively.

The phylogenetic distribution of bacterial taxa in the five cave ice deposits showed strong phyla dissimilarities across the ice block. The identified taxa belonged to 30 phyla, with a major incidence of Proteobacteria (33.9%), Actinobacteria (25.6%) and Firmicutes (17.2%). The highest relative content of Proteobacteria OTUs was found in the clear ice deposits 1-L (54%) and 900-I (42%). Actinobacteria constituted the major group of the 900-O (up to 54%) and 900-I (39%) ice strata, while both 1-S and 400-O samples had a lower (11%) representation of this phylum. Firmicutes OTUs were mainly observed in 400-O ice (up to 37% of total taxa). Six other phyla showed a substantial relative abundance (>1%) across the cave ice block.

Crenothrix, Cryobacterium and Polaromonas genera have been highlighted in all ice samples (with a higher percentage of species belonging to Polaromonas genus in 900-I ice sample), due to the content of psychrophilic species. Among the cold-adapted species, OTUs belonging to Chryseobacterium genus were registered in all the ice samples as psychrotolerant microorganisms.

Archaeal 16S rRNA OTUs belonging to 7 phylotypes were identified in 400- and 900-years old ice strata.

The relative abundance of taxa belonging to archaeal phyla Crenarchaeota, Thaumarchaeaota and Euryarchaeaota was highly variable in the three ice layers. Crenarchaeota was the dominant phylum of the 400-O (62%) ice layer, while its relative content was rather low (2+5%) in both 900-O and 900-I ice samples. OTUs belonging to Euryarchaeaota were mostly found in 900-O (52%) and 900-I (95%), while only 13% contributed to the 400-O archaeal community. Thaumarchaeaota was present in organic-rich ice strata, with the highest representation in 900-O (45%), and 400-O (25%) samples.

The geochemistry of the 1-S, 1-L, 400-O, 900-O and 900-I cave ice samples was previously determined (Ițcuș, et al., 2016), revealing neutral to slight alkaline pH (7.45-8.03) and decreasing electrical conductivity (EC) values (124.2-15 µS cm^{-1}), total organic carbon content (TOC) decreasing with the age and sediment content of the ice in the 33.38-3.03 mg L-1 interval, and total nitrogen (TN) content of 0.53-2.15 mg L^{-1}.

The analysis of the microbial phyla composition from all investigated ice deposits in relation with the ice geochemical parameters, explained 88% of the data dissimilarity of the ice samples regarding the distribution of bacterial and archaeal phyla that appeared to be explained by the geochemistry substrate of the ice layers.

Thus, the pH had large positive loadings, while EC and TOC had negative loadings with about equal contribution to sample partition. 400-O ice sample showed a higher score being well separated from the other samples based on the TN ice content, with a strong correlation of Chloroflexi, Firmicutes and unassigned phyla distribution. The relative content of Cyanobacteria and Proteobacteria from recent ice 1-S and 1-L appeared to be influenced based on EC. The content of Actinobacteria and Chlamydia in old ice strata 900-O and 900-I appeared to be strongly dependent on the pH of the ice substrate.

The microbial distribution in the perennial cave ice layers revealed a heterogenous bacterial and archaeal communities across the ice block. A total of 435 distinct OTUs belonging to these prokaryote kingdoms were found in recent ice strata from the ice block surface, 400-years old ice, formed during the Little Ice Age (LIA) period, and 900-years old ice, corresponding to Medieval Warm Period (MWP) (Perşoiu, et al., 2011; Perşoiu, et al., 2017). Thus, the major bacterial phyla from this icy habitat formed during recent (1-year-old ice), LIA and MWP period corresponded to Proteobacteria, Firmicutes and Actinobacteria, respectively.

Specific bacterial and archaeal OTUs were found in 400 (cold and dry LIA period) and 900 years old (warm and wet MWP period) cave ice constituting putative biomarkers for climate characteristics during ice formation. Among the 26 unique phylotypes inhabiting the LIA-formed ice, OTUs belonging to genus Methanobacterium (Euryarchaeota) revealed the presence of anaerobic hydrogenotrophic methanogens (Whitman, et al., 2014). OTUs belonging to the Miscellaneous Crenarchaeotic Group (MCG), a predominant archaeal group in anoxic environments, mainly in deep marine habitats (Fry, et al., 2008), with possible significant role in the global biogeochemical cycles, were highly present (40-69%) in 400-O.

The current data on Scărișoara Ice Cave microbiome underscored a highly diverse bacterial community throughout the cave ice block, and the occurrence of various archaeal taxa in old ice layers formed during 400- and 900-years BP, revealing large dissimilarities in the ice-embedded microbial profiles varying with the age, sediment content and light exposure of the ice. Both heterotrophic and autotrophic prokaryotes were identified in the recent and old ice deposits, with the putative presence of various anaerobic microbial groups. In addition, climate variations recorded during ice layers formation within LIA and MWP time-intervals appeared to have an impact on both major bacterial and archaeal phyla, with distinct microorganisms identified up to genera level that might serve as putative climate proxies.

Our study revealed the existence of culturable bacteria in all analyzed samples from Scărișoara ice block up to 2000 years old ice, similar to other extreme habitats (Skidmore, et al., 2000; Lee, et al., 2011; Bell E., 2012).

The analysis of 18S rRNA gene sequences evidentiated 23 OTUs that corresponds to cultivated fungi at 4°C (16 OTUs) or 15°C (13 OTUs) from all ice samples. Among these, 21 OTUs were specific for distinct ice samples while 7 OTUs appear between different layers of ice. Resulted sequences from the ice samples were registered with a high percentage similarity and coverage with various homologous OTUs belonging to extreme environments (e.g. SM. 1-L. T1-15 KY614581 with *Thelebolus* sp. GU004225 originated from Antarctic soil with an identity coefficient of 98% on a 96% coverage.

In the DGGE patterns from both 4°C and 15°C cultivated samples, the psychrophilic yeast *Mrakia stokesii* and *Mrakia gelida* appears to be common to all five ice layers being the most predominant OTU (14). In the samples from recent ice and 400 years old, *Teberdinia hygrophila* (a cryophilic yeast) was found with an identity of 98% being commune with Murmansk region White Sea from Russia and with alpine soil from Caucasus Mountains (Sogonov, et al., 2005).

Fungal DNA genes, belonging to 5 phyla where examined through a phylogenetic analysis.

The fungal diversity from ice it is described scarcely in extreme environments as the Poles: North Pole (Butinar, et al., 2011) and South Pole (Connell & Staudigel, 2013). Culturable fungi were discovered in old Antarctic ice sheets (Taylor, et al., 1997; Christner, et al., 2003). Particular types of cave ice fungi were identified in Scărișoara with a major amount of OTUs might originate from the outside and imprisoned within the ice strata in the presence of organic sediments accumulated during summer period (Kern & Perșoiu, 2013; Perșoiu, 2018).

Identified fungi from Scărişoara Ice Cave can offer data's regarding the climate change. Due to the global warming, microorganisms living in the ice could be released (Kern & Perșoiu, 2013). Studies on the adaptation and role of the fungal content of ice caves could bring valuable information for research and cryopreservation that eventually could lead to bionanotechnological applications (Margesin & Feller, 2010).

In this research, 19 OTUs isolated from Scărișoara ice block shared a small percentage of identity and coverage with other relatives from GenBank database, being registered as novel strains.

CONCLUSIONS

The present study is relevant to fundamental research, being the first microbiological study on the microbial community embedded in Scărișoara Ice Block.

In this initial research regarding the microbial diversity trapped in the ice block from Scărișoara Cave (Romania), it was identified the occurrence of viable microorganisms (Bacteria and Eukarya) in all ice strata.

Differences between the growth patterns of microbial communities from each ice sample (1-S, 1-L, 400-O, 900-O, 900-I, 1500-I and 2000-I) were given by the sunlight exposure, the age of the ice and the organic content.

The chemical composition of recent and old ice strata embedding these microorganisms presented important shifts in their levels as a role of the organic content and the age of the ice layers. This assumption is sustained by a decreased bacterial growth observed for the older ice samples (900-O/I) as compared to 400 years old organic sample which suggests a lower density of cultivable strains.

Even though the samples are different by age, there is also a clear variance in terms of the influence of the outside climate during the ice strata deposition from which old organic samples (400-O, 900-O) and clear ice (900-I) were collected. Samples 900-O/I originate from the ice deposition throughout Medieval Warm Period (MWP), a time interval with warm and dried summers pointed by the expansion of *Fagus sylvatica* forests (Vesterdal, et al., 2008; Feurdean, et al., 2011).

As a dissimilarity, the 400-O ice sample it is related to a layer deposed in the period named Little Ice Age (LIA). In that time, forests constituted from *Picea abies*, a marker for acidic soils, predominated the landscape around the site sampling area, indicating a wet and cold period (Cankar, et al., 2006; Feurdean, et al., 2011).

The climatic conditions that persisted during the Little Ice Age over the Medieval Warm Period led to recurrent inflow of great volumes of water in the cave, carrying substantial amounts of organic matter and nutrients enhancing the increase of the microbial diversity.

This thesis, that reports on the existence of cultivable and uncultivable microorganisms in up to 2000-years old ice from Scărișoara Ice Cave (Romania), and dependence on age, temperature and organic substrate of the microbial communities from this icy habitat, is the first survey of an ice cave.

While, to date, this particular type of ecological niche received a very limited attention, the present study made the first step in unraveling the diversity of microbiota thriving in underground ice deposits from this cave in correlation with past climatic changes.

The sequences reported in this study were assigned in GenBank DNA database under the following accession numbers:

- 16S rRNA gene raw 454 pyrosequencing reads NCBI Sequence Read Archive - BioProject accession number PRJNA263762, corresponding to BioSamples accession numbers SAMN03140079 (1-S), SAMN03140080 (1-L), SAMN03140081 (400-O), SAMN03140082 (900-O), and SAMN 0314 0083 (900-I)
- 16S rRNA DGGE fragments (cultured bacteria) - KF85203-KF853221, KJ454416-KJ454425, and KP219085-KP219133
- 18S rRNA DGGE fragments - KY614706-KY614719 (uncultured fungi) and KY614552- KY614602 (cultured fungi)

Finally, the methods used in this study converge to interdependent results that highlight the same microbial composition, dependent on age and organic substrate, which correlates with climatic periods and can lead to the creation of a common pattern.

Further research directions

A deeper analysis of the microbial diversity and distribution in different cave ice strata could lead to identification of climate microbial proxies.

Screening of the metabolic diversity and active microbial fraction from this icy habitat will contribute to understand the role of cave ice microbiome to environmental habitat.

Comparison of cave ice bacterial community with that of Alpine, Arctic and Antarctic glaciers will contribute to understand the particularities of ice-contained microbiome formation, activity and resilience in perennial cave ice as compared to exposed glacier ice.

Another future direction is the selection, isolation and characterization of new microbial species adapted to extreme low temperatures environments that constitute premises for developing novel strains and biomolecules for bionanotechnological applications.

The identified patterns can be further used as starting points in developing models for exobiological studies of ice-containing planets.

LIST OF PUBLICATIONS

The results obtained from the scientific approach of this doctoral dissertation were exploited by the following papers and conference participations:

A. Published articles

1. Brad, T.#, **Iţcuş, C**.#, Pascu, M.D., Perşoiu, A., Hillebrand-Voiculescu, A., Iancu, L., Purcărea, C. (2018). *Fungi in perennial ice from Scărișoara Ice Cave (Romania)*. Nature Scientific Reports, 8(1), 1-9. (IF 4.122)
2. **Iţcuş, C.**, Pascu, M.D., Brad, T., Perşoiu, A., Purcărea, C. (2016). *Diversity of cultured bacteria from the perennial ice block of Scarisoara Ice Cave, Romania*. International Journal of Speleology, 45(1), 89-100, (IF 2.057)
3. Hillebrand-Voiculescu, A.#, **Iţcuş, C**.#, Ardelean I., Rusu, A., Perşoiu, A., Brad, T., Popa, E., Onac, B.P., Purcărea, C. (2014). *Searching for cold-adapted microorganisms in the underground glacier of Scarisoara Ice Cave, Romania*. Acta Carsologica, Volume 43, 319-329. (IF 0.792)
4. Hillebrand-Voiculescu, A.#, Rusu, A.#, **Iţcuş, C**., Perşoiu, A., Brad, T., Pascu, M.D., Ardelean, I., Onac, B.P., Purcărea, C. (2013). *Bacterial 16S-rRNA gene clone library from recent ice stalagmites of Scărișoara cave*. Rom. J. Biochem Volume 50, 109-118. BDI

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B. Articles submitted for publication

1. **Iţcuş, C**.#, Pascu, M.D.#, Perşoiu, A., Brad, T., Iancu, L., Lavin, P., Purcărea, C. (2018). *Bacterial and archaeal community structures in perennial cave ice*. Submitted to Nature Scientific Reports
2. Mondini, A., Donhauser, J., **Iţcuş, C.**, Marin, C., Perşoiu, A., Frey, B., Purcărea, C. (2018). *High-throughput sequencing of fungal communities across the perennial ice block of Scărișoara Ice Cave*. Submitted to Annals of Glaciology

C. International Conferences

1. Cristina Purcarea, **Corina Itcus**, Constantin Marin, Soon Gyu Hong, Victoria I. Paun, Aurel Persoiu, Paris Lavin, Traian Brad, Alexandra Hillebrand-Voiculescu, Denisa Pascu, Cristian Coman, Iris Tusa, Manuela E. Sidoroff, 2018, ”*Ice Microbiome: From Antarctic Glaciers to Alpine Ice Caves*” POLAR 2018 - XXXV SCAR Biennial Meetings - Open Science Conference, Davos, Elveția, 15 – 26 Iunie
2. Victoria Ioana Paun, **Corina Itcus**, Constantin Marin, Aurel Persoiu, Paris Lavin, Alexandra Hillebrand-Voiculescu, Antonio Mondini, Carmen Badaluta, Cristina Dorador, Cristina Purcare, 2018, *Chronosequence of Active Bacterial Community*

from an Alpine Ice Cave, POLAR 2018 - XXXV SCAR Biennial Meetings - Open Science Conference, Davos, Elveția, 15 – 26 Iunie

3. Purcărea, C., **Iţcuş, C.,** Păun, V.I., Marin, C., Perşoiu, A., Brad, T., Bădăluță, C.A. Icaza, G., Dorador, C., Lavin, P., Donhauser, J., Frey, B. (2018). *Searching for microbial biomarkers for past climate changes in Scarisoara ice cave*, 8th International Workshop on Ice Caves IWIC-VIII, Potes, Picos de Europa National Parc, Spain, June 11-16.
4. Purcărea, C.**, Ițcuș, C.**, Păun, V.I., Marin, C., Perșoiu, A., Hillebrand-Voiculescu, A., Brad, T., Bădăluță, C., Lavin, P. (2018) *Cave ice microbiome.* International Symposium on Cryosphere and Byosphere, Kyoto, Japan, March 14-19.
5. **Ițcuș, C.**, Pascu, M.D., Paun, V.I., Perșoiu, A., Hillebrand-Voiculescu, A., Brad, T., Purcărea, C. (2017). *Reconstruction of Scarisoara ice cave microbiome based on 16S rRNA gene sequencing and shotgun metagenomics*, 7th International Conference on Polar and Alpine Microbiology (PAM), Nuuk, Greenland, September 8 -12.
6. **Ițcuș, C.**, Pascu, M.D., Hillebrand-Voiculescu, A., Brad, T., Perșoiu, A., Onac, B.P., Purcărea, C. (2016). *Cultured bacterial diversity in Scarisoara Ice Cave*, 7th International Workshop on Ice Caves, Postojna, Slovenia, May 16 - 22.
7. **Ițcuș, C.**, Pascu, M.D., Hillebrand-Voiculescu, A., Brad, T., Perșoiu, A., Onac, B.P., Purcărea, C. (2016). *Distribution of Bacteria and Archaea in the ice block of Scarisoara Cave, Romania*, 7th International Workshop on Ice Caves, Postojna, Slovenia, May 16 - 22.
8. **Ițcuș, C.**, Pascu, M.D., Hillebrand-Voiculescu, A., Brad, T., Perșoiu, A., Purcărea, C. (2015). *Diversity of cultured ice cave microcosm.* 6th International Conference on Polar and Alpine Microbiology (PAM), České Budějovice, Czech Republic, September 6-10.
9. **Ițcuș, C.**, Pascu, M.D., Hillebrand-Voiculescu, A., Brad, T., Perșoiu, A., Onac, B.P., Purcărea, C. (2015). *Prokariotic community structure across the ice block of Scarisoara Cave determined by 454 pyrosequencing.* 6th International Conference on Polar and Alpine Microbiology (PAM), České Budějovice, Czech Republic, September 6 - 10.
10. Pascu, D.M., **Iţcuş, C.**, Ardelean, I., Cirnu, M., Hillebrand-Voiculescu, A., Brad, T., Perşoiu, A., Purcărea C. (2015). *Diversity of phototrophic bacteria in Scarisoara Ice Cave.* 6th International Conference on Polar and Alpine Microbiology (PAM), České Budějovice, Czech Republic, September 6-10.
11. **Iţcuş, C.,** Pascu, M.D., Hillebrand-Voiculescu, A., Brad, T., Perşoiu, A., Purcărea, C. (2015). *Bacterial diversity in perennial ice deposits from Scarisoara Ice Cave.* International Symposium of the "Young Researchers in BioSciences", Cluj-Napoca, Romania, July 22-26.

12. Pascu, D., **Iţcuş, C**., Cirnu, M., Hillebrand-Voiculescu, A., Brad, T., Perșoiu, A., Ardelean, I., Purcărea, C. (2015). *Molecular and microbiological study of phototrophs from Scarisoara Ice Cave.* International Symposium of the "Young Researchers in BioSciences", Cluj-Napoca, Romania, July 22-26.
13. **Iţcuş, C.,** APECS World Summit (2015). "*The Future of Polar Research*" Sofia, Bulgaria, June 6-8.
14. **Iţcuş, C**, Pascu, M.D., Hillebrand-Voiculescu, A., Brad, T.., Perșoiu, A., Ardelean, I., Purcărea, C. (2014). "*Diversity of cultured and uncultured ice cave microbiota*", 10th International Congress on Extremophiles, Sankt Petersburg, Rusia, September 6-11.
15. **Iţcuş, C.**, Pascu, D.M., Hillebrand-Voiculescu, A., Brad, T., Perșoiu, A., Purcărea C. (2014). *Molecular analysis of bacterial diversity from Scarisoara Ice Cave.* 1st International Symposium of the "Young Researchers in BioScience", Cluj-Napoca, Romania, July 23-27.
16. Pascu, D.M., **Iţcuş, C.**, Ardelean I., Cirnu M., Hillebrand-Voiculescu, A., Brad, T., Perșoiu, A., Purcărea C. (2014). *Metabolic activity and diversity of phototrophs from Scarisoara Ice Cave.* 1st International Symposium of the "Young Researchers in BioScience", Cluj-Napoca, Romania, July 23-27.
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22. Hillebrand-Voiculescu, A., **Iţcuş, C.**, Rusu, A., Ardelean, I., Pascu, D., Perșoiu, A., Brad, T., Popa, E., Onac, B.P., Purcărea, C. (2012). *Microbial Diversity in the Subterranean Ice Deposits of Scărişoara Cave.* A New Approach of the Academic

Research in Biology, IBB Annual Conference, Bucuresti, Romania, December 11-12.

23. **Iţcuş, C.,** Hillebrand-Voiculescu, A., Pascu, D., Perșoiu, A., Brad, T., Purcărea, C. (2012). *Cell growth and DGGE analysis of microorganisms from Scarisoara Cave ice sediments.* A New Approach of the Academic Research in Biology, IBB Annual Conference, Bucuresti, Romania, December 11-12.
24. Hillebrand-Voiculescu, A., **Iţcuş, C.**, Rusu, A., Perșoiu, A., Brad, T., Popa, E., Onac, P. B., Purcărea, C. (2012). *Microbial Biodiversity in ice sediments from Scărişoara Ice Cave (ROMANIA)*, The 5th International Workshop on Ice Caves, Barzio, Valsassina, Grigna, Italia, September 16-23.

D. National Conferences

1. Iancu, L., Păun, I., **Iţcuş, C.**, Mondini, A., Purcărea, C. (2017). *Microbial diversity through 16S rRNA Illumina sequencing and shotgun*, Perspectives of bioinformatics in Romania, Biology and Geology Faculty, Babeș-Bolyai University, Cluj-Napoca, Romania, November 24.

2. **Iţcuş, C.**, Pascu, M.D., Hillebrand-Voiculescu, A., Brad, T., Perşoiu, A., Brad, T., Ardelean, I., Purcărea, C. (2016) *Comunităţi microbiene din Peştera Gheţarul Scărişoara*, 56th Annual Scientific Session, Institute of Biology of the Romania Academy, Bucharest, Romania, December 10.

3. **Iţcuş, C.**, Hillebrand-Voiculescu, A. Pascu, D., Perşoiu, A., Brad, T., Purcărea, C., (2013). *Microorganisms diversity in Scărişoara Ice Cave.* 53rd Annual Scientific Session, Institute of Biology of the Romania Academy, Bucharest, Romania, December 10.

4. Pascu, D., Ardelean, I., **Iţcuş, C.**, Hillebrand-Voiculescu, A., Perşoiu, A., Brad, T., Purcărea, C. (2013). *Highlighting the phototroph microorganisms in perrenial ice from Scărişoara Ice Cave.* 53rd Annual Scientific Session, Institute of Biology of the Romania Academy, Bucharest, Romania, December 10.

5. **Iţcuş, C.**, Hillebrand-Voiculescu, A., Pascu, D. M., Perşoiu, A., Brad, T., Purcărea C. (2013) *Identifications of microorganisms from Scărişoara Ice Cave - cell growth and DGGE analysis*. National Symposium "Cryosphere 2013" Piatra Neamţ, Romania, February 21-24.

E. Awards

1. ICGEB grant – ICGEB Course „*Fluorescence Microscopy*", Trieste, Italy, 24.10.2017 – 26.10.2017 (~500 euro)
2. Training fellowship - International course „ *Karst Landscape, Geopark, Natural Heritage, Environmental Geology Mapping and Data Mining.*", Nanning, China, 20.09.2015 - 5.10.2015 (Scholarship 2500 euro)

3. Training fellowship - International course „*Karst Hydrogeological Investigation, Dynamic Monitoring and Application in River Basin*", Guilin, China, 17.11.2013 - 30.11.2013 Certified: Excellency certificate (UNESCO prize and Scholarship 2500 euro)
4. Poster prize **- Iţcuş, C.**, Hillebrand-Voiculescu, A. Pascu, D., Perșoiu, A., Brad, T., Purcărea, C., (2013). *Microorganisms diversity in Scărişoara Ice Cave*. 53rd Annual Scientific Session, Institute of Biology of the Romania Academy, Bucharest, Romania, December 10 (100 RON)
5. Training scholarship - COST Action: ES1103 „*Bioinformatics for microbial community analysis*" University of Liverpool, Centre for Genomic Research, Liverpool, UK, 11.12.2012 - 14.12.2012 (1000 euro)

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