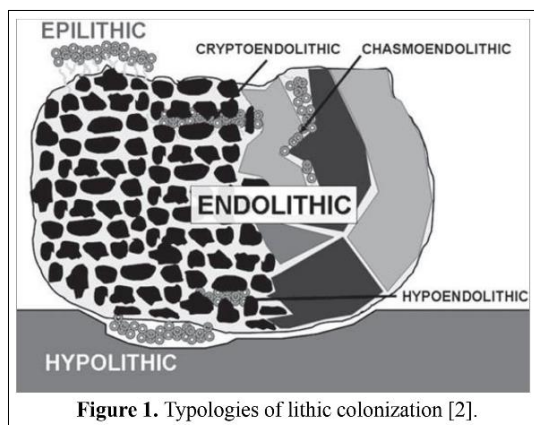


## Metabolic processes and trophic interactions in Antarctic cryptoendolithic communities

**Relevant Categories:** Bacteria/Archaea; Fluorescence activated cell sorting; Fungi; Algae; Metabolomics; Metagenome/metatranscriptome; Other: Microbial Interactions

### Specific Aims

The extreme cold temperature and aridity of Antarctic ecosystems necessitate adaptation to survive under conditions that are at the limit of supporting life. Antarctic cryptoendolithic communities are life-forms that contribute to ecosystem functionality in the hyper-cold and ice-free drylands of the Victoria Land, Continental Antarctica [1]. Under environmental conditions that are normally incompatible with active life, the endolithic habitat provides thermal buffering, physical stability, protection against UV radiation, excessive solar radiation and enables water retention. Microbial communities occupy sandstone pores (Fig. 1) and generate distinct coloured bands running parallel to the rock surface, where different microorganisms are distributed [2].



In an era of rapid Climate Change and expanding desertification, the study of microbial ecosystems from extreme environments will enable development of tools for understanding how microbial interactions have been altered to enable persistence. This includes specifically studying how metabolic processes are adapted to support continued activity under the extreme aridity and oligotrophy. We have gained experience in the last two decades using both in fieldwork and laboratory experimentation on these microbial communities including a metagenomic study of a selection of 20 rocks supported by a JGI CSP project (CSP 503708; PI: L. Selbmann, co-PI: J.E Stajich).

Data from our CSP have generated a deep characterization of microbial diversity in Antarctic endolithic ecosystems; besides, the metabolic processes as carbon and nitrogen fixation and the role of biotic interactions and the spatial organization in its functioning remain still uncovered. In this proposal, our goal is to build an understanding of the metabolic processes and trophic microbial interactions in the rock-inhabiting communities after a proper reanimation (wetting, light, temperature). Applying to the Environmental Molecular Sciences Laboratory (EMSL) technical capabilities (proteomics & metabolomics) and Joint Genome Institute (metagenomics) will provide a global view of the mechanisms and pathways that support adaptations for persistence in extreme cold and aridity. We aim to identify the metabolic pathways and the underlying molecular mechanisms which support life in these harshest conditions on Earth. We also seek to characterize the spatial organization, rock microstructure, pore structure characteristics (e.g. porosity, pore size and connectivity) and water flow properties of the rock matrix to develop spatial phylo-trophic and interactions map, utilizing a combination of microscopic and microanalytical techniques.

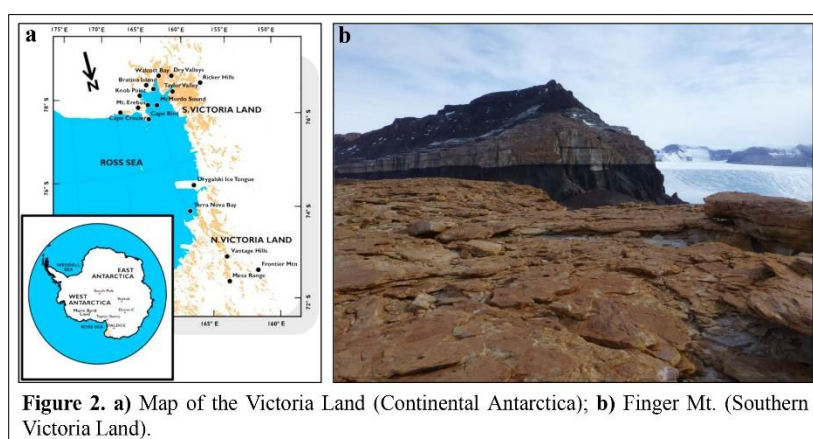
### Mission

Arid and semiarid systems cover more than 40% of the global land surface, and they are among the less explored ecosystems. These environments are characterized by low moisture availability and temperature extremes. The ice-free areas of the Antarctic Deserts are universally considered among the harshest environments on Earth and the Martian counterpart on our planet [3, 4]. Cryptoendolithic communities are almost the only life-forms in these areas and main contributors to environmental/biogeochemical processes metabolizing C, N and other macronutrient cycles. Nevertheless, little is known about metabolites and proteins produced and therefore, the importance of

these communities in regulating nutrient cycling. This project seeks to discover the underlying biology of Antarctic cryptoendoliths as they respond to and modify their environments. Identification and comparisons of enzymes and metabolites that appear enriched will be useful in predicting important pathways that have evolved to support these life-forms on the extreme edge in global drylands, in an era of Climate Change and rapid desertification. The detection of metabolites crucial for carbon or nitrogen fixation will enable identification of the functional classifications and ecosystem roles of microorganisms in these communities. Indeed, in this oligotrophic environment, carbon sequestration is a major challenge [5, 6], mainly performed by algae and cyanobacteria as phototrophs. In addition, a better understanding of how these ecosystems liberate carbon resources through expression of enzymes will help in development of assay to estimate carbon and nitrogen cycling activities. Yet, fungi play fundamental roles in geological processes namely “geomycology” [7], including organic and inorganic transformations and element cycling, rock transformations, bioweathering [8], mycogenic mineral formation and in other processes eventually relevant for environmental biotechnology such as bioremediation. This proposal fits topically within the DOE and BER mission areas focused on the global Carbon Cycle and Biogeochemistry and on understanding ecosystem processes and function, microbial interactions, and the unique biology of arid-land microorganisms. This proposed work leverages and combines the unique expertise and vanguard technologies available at both the EMSL and the JGI, and could only be accomplished in collaboration with these DOE laboratories.

## Background/Introduction

Endolithic microbial ecosystems are found in hot and cold drylands worldwide and in some hyper-arid biomes as the only specialized life-forms able to function. In the Antarctic ice-free areas, mainly in Victoria Land (Continental Antarctica, Fig. 2), the environmental conditions are at the limits for supporting life. There, cryptoendolithic communities are self-sustaining ecosystems thriving in the most adverse conditions known on Earth. These communities are quite stable, due nature of rock substratum where the microorganisms live in strict spatial association with minerals, typically occupying a niche in a few millimetres’ depth below rock surface [2]. The observation of a heterogeneous distribution of microbial communities at various depths in the rock indicates additional microenvironments structure in these endolithic habitats which is likely driven by the specific metabolic processes of microbes [9]. The absence of higher plants and animals in these regions limits these systems to exclusively microbial members, making cryptoendolithic communities as ideal model systems of low-complexity to study how life adapts and evolves under degradation of desert towards the dry limit for life.



**Figure 2.** a) Map of the Victoria Land (Continental Antarctica); b) Finger Mt. (Southern Victoria Land).

Recent studies elucidated the biodiversity, structure and composition of cryptoendolithic communities [10-13], including their spatial organization [14], but has only produced very patchy information about the physiology and stress responses. This hampers our understanding of the mechanisms that enable the metabolic machinery to remain active under

conditions that are lethal for the most organisms.

Using a combination of whole-community metagenomics, proteomics, and metabolomics at the JGI and EMSL on both dormant and reanimated communities, we aim to gain an unprecedented and integrated view of the metabolic processes that underlie community re-activation through identification of enzymes and metabolites and of species which produce them (Aim 1).

In addition, we propose to build a species and metabolite interactions network map, resolving the contributions of different microorganisms, their interaction relationships, and the established among microbial cells and the lithic substrate, combining XCT coupled with hydraulic measurements, SEM-EDX, and FIB/SEM EDX imaging on colonized and uncolonized samples (Aim 2). Finally, we seek to understand how microorganisms, in the dormant and reanimated Antarctic cryptoendolithic communities, interact metabolically and contribute to carbon and nitrogen cycling using NanoSIMS (Aim 3).

Cryptoendolithic microbial ecosystems have unique properties as a model system for studies of microbial ecology in both hot and cold deserts. We expect that data generated in this project would provide critical insights to be also applied to microbial endolithic ecosystems in drylands worldwide.

## Approach

We will study microbial endolithic colonized sandstones, both dormant and reanimated, which exhibit differences in community composition and rock porosity. In particular, two different typologies of sandstone will be analysed to highlight the influence of the rock substratum on the organization and development of the community:

- i) the *Beacon sandstone* is a formation of the Beacon Supergroup, dating back to the Devonian-Triassic (400 to 250 MYA) and characterizes the landscape of the McMurdo Dry Valleys (Southern Victoria Land), constituted mostly of orthoquartzite [15, 16];
- ii) the *sandstones from the Northern Victoria Land* have a more recent origin, dating from the Triassic to the Jurassic (252 to 145 MYA), and are characterized by a higher presence of matrices among quartzite grains.

Both colonized and uncolonized sandstones will be studied to detail the distribution of microorganisms along the rock depth and the influence of microbial colonization in different sandstone typologies on the hydraulic properties and water fluxes. Dry, 48, 96 hrs post-wetting rocks will be incubated in a  $^{13}\text{CO}_2$  and  $^{15}\text{N}_2$  atmosphere and will be analysed according to the techniques described later in detail in this “Approach” section.

Rock samples have been collected along the Victoria Land (Continental Antarctica) during the XXXI (Dec. 2015 - Jan. 2016) and XXXIV (Dec. 2018 - Jan. 2019) Italian Antarctic Expeditions, in the framework of ongoing projects funded by the Italian National Program for Antarctic Researches (PNRA, PI Laura Selbmann). Rocks are stored in the Mycological Section of the Italian Antarctic National Museum (University of Tuscia) at  $-20^\circ\text{C}$ .

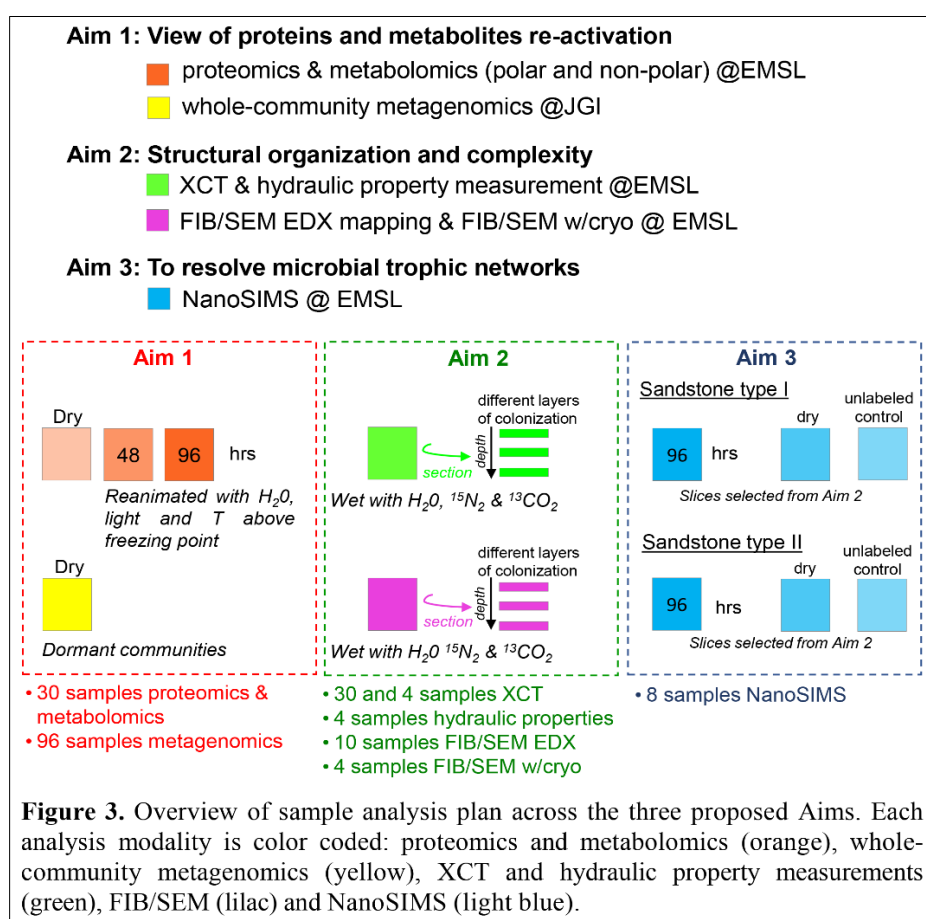
The samples have been collected from locations well characterized for the presence of cryptoendolithic colonization, as for instance:

- i) Linnaeus Terrace, ( $77^\circ35' \text{ S } 161^\circ0.49' \text{ E}$ ) in the McMurdo Dry Valleys is the region from which Antarctic cryptoendolithic communities have been described for the first time [2]. This area is designed as an ASPA (Antarctic Specially Protected Areas) to protect these outstanding environments and the access is possible with special permission only;
- ii) Battleship Promontory, ( $76^\circ54' \text{ S } 160^\circ54' \text{ E}$ ) in the McMurdo Dry Valleys is another region well studied for cryptoendolithic colonization and is designed as an Antarctic Specially Protected Areas (ASMA);
- iii) Pudding Butte, ( $75^\circ51' \text{ S } 159^\circ58' \text{ E}$ ) is a huge sedimentary outcrop in the Northern Victoria Land where cryptoendolithic communities have been largely reported [10, 11];
- iv) Mt New Zealand, ( $74^\circ10' \text{ S } 162^\circ30' \text{ E}$ ) where cryptoendolithic colonization has been reported at very high altitude (3,200 m a.s.l.) [10-12].

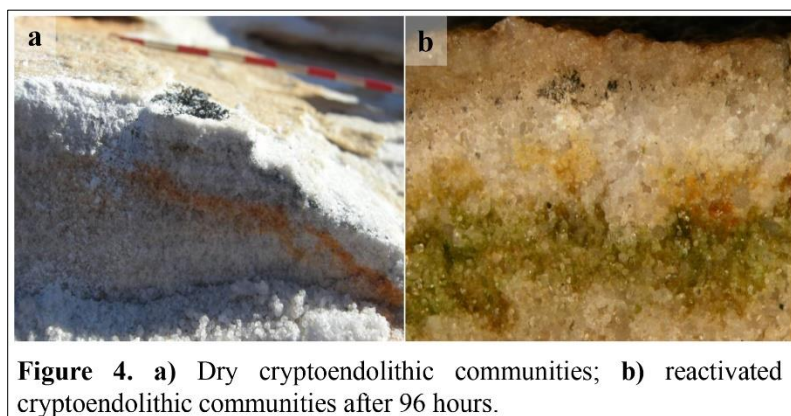
Samples from these and other locations surveyed in the above-mentioned Antarctic Campaigns will be included in this study.

We anticipate that rocks samples will be available prior to the start of the funding period (by the end of the 2020).

Figure 3 summarizes the proposed sampling and analysis plan for the three Aims detailed below.



**Aim 1) View of proteins and metabolites re-activation.** We will compare both community responses under dry conditions, when the community is dormant, and after reanimation by wetting, light and temperature above freezing point. RNA extraction from these specimens was unsuccessful till now, therefore we are not proposing transcriptomic approaches; we will apply metabolomics (polar and non-polar metabolites) and proteomics in collaboration with Mary Lipton (EMSL).



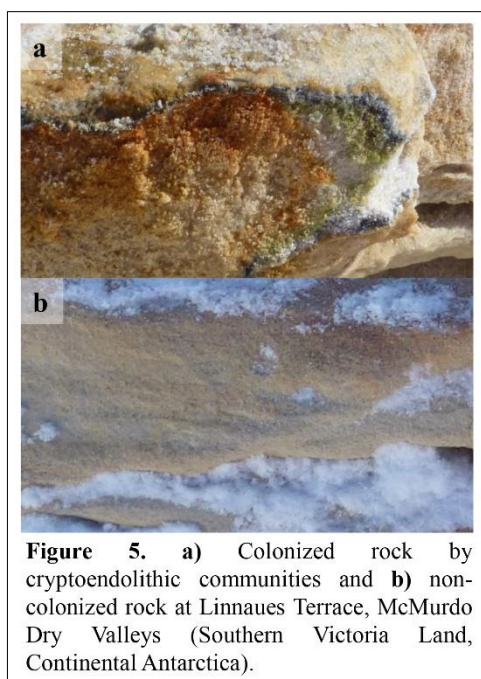
We propose to analyse 30 samples and 3 replicates for each sample. To be able to pair proteomics and metabolomics, a single rock sample will be reanimated, then split in half after the appropriate time-point and processed, accordingly. Dry, 48, 96 hrs post-wetting rocks (Fig. 4) will be examined to determine how gene expression and C/N trophic dynamics unfold after reanimation. At each time point, rocks samples will be submerged in cold ( $-20^{\circ}\text{C}$ ) HPLC grade methanol to quench metabolic activity [17], followed by rapid liquid nitrogen freezing and storage until being shipped out to EMSL/JGI.

Protocols for a successful extraction of metabolites have been already optimized in a preliminary work we performed based on untargeted metabolomics which aimed to explore the stress-response [18]. Metagenomics on 96 samples will be conducted at the JGI, in collaboration with Christa Pennacchio,

to provide phylogenomic resources and integrate data generated at EMSL. Recent shotgun metagenomics experiments have been performed by Selbmann and Stajich labs in collaboration with JGI, in the frame of a JGI-funded project (“Metagenomics Reconstruction of endolithic communities from Victoria Land, Antarctica”, CSP 503708; PI: L. Selbmann, co-PI: J.E Stajich), where 5-20 ng/ul DNA was obtained from Antarctic rocks colonized by cryptoendolithic communities. According to this, we expect diversity on the order of 300-350 and 100-200 for prokaryotes and eukaryotes, respectively [19, 20].

Whole-community metagenomics (JGI), proteomics and metabolomics (EMSL) will produce an integrated view of functional responses, including metabolites of central metabolism and products of photosynthesis/nitrogen-fixation, but also polysaccharides and lipids that might be synthesized in response to reanimation.

**Aim 2) Structural organization and complexity.** We will generate information on microstructure and chemical composition of rock matrix at microscale as well as a detailed picture of the interactions established among microbial components and between them and the lithic substrate. A selection of both colonized and non-colonized samples (Fig. 5) will be analysed by a combination of different



**Figure 5.** a) Colonized rock by cryptoendolithic communities and b) non-colonized rock at Linnaeus Terrace, McMurdo Dry Valleys (Southern Victoria Land, Continental Antarctica).

microscopic and microanalytical techniques.

With Mark Bowden and Tamas Varga (EMSL), we will use X-ray computed tomography (XCT) on a selection of 30 samples to measure porosity, pore size and distribution and connectivity and water flow properties, creating a whole 3D image. From XCT results, we will select at least 4 samples (two different sandstone typologies, comparing colonized and non-colonized samples to highlight the influence of microorganisms on the hydraulic properties) to investigate permeability measurements. We have discussed hydraulic measurements with Mark Bowden and we will further work together to optimize protocols.

In parallel, a detailed study utilizing SEM combined with energy dispersive X-ray spectroscopy (EDX) at MNCN-CSIC, following Wierchos & Ascaso [21], will be performed on 10 colonized representative samples in collaboration with co-PI A. De los Ríos, providing information on the size or morphology of microorganisms and locate individual cells. This study will localize potential microbial interactions in colonized pores from different

depths (coinciding with different coloured layers of colonization) on the samples by SEM. Briefly, rock samples will be fixed with glutaraldehyde and osmium tetroxide, dehydrated in ethanol series and later embedded in LR-White resin, analysed by SEM using back-scattered electrons and then shipped to EMSL for further analysis.

Areas of interest selected after XCT and SEM-EDX will be then analysed with cross sectional FIB/SEM with EDX chemical mapping on samples previously prepared at MNCN-CSIC.

At least 4 unprocessed samples in cryo-mode (cryo-frozen specimens) will be studied at microscale ( $10^2$ 's of nm scale resolution), in collaboration with Daniel Perea and Scott Lea (EMSL).

Our research strategy focuses on analysing endolithic microorganisms without disturbing the microhabitats enabling us to characterize different layers of colonization and the different biotic and abiotic interactions established in the endolithic community.

**Aim 3) To resolve microbial trophic networks.** Some of the selected slices analysed on Aim 2 will be examined by NanoSIMS to get insights on cryptoendolithic microorganisms' metabolism for carbon and nitrogen sequestration. Following Mergelov et al., [22], we will explore the elemental distribution directly at the surface ( $10^2$ 's of nm scale resolution) of biofilm-to-mineral biogeochemical interfaces. Electron multiplier secondary ion collectors will be used. Briefly, a small selection of dry and 96 hrs post-wetting reanimated samples (4), previously analysed in Aim 2 (to localize potential microbial

interactions in colonized pores from different deepness) will be studied by NanoSIMS to produce isotopically-resolved compositional maps, in collaboration with Scott Lea and John Cliff (EMSL). Unlabeled samples will be analysed as controls.

With this approach we seek to get insights on the coordinated trophic responses, individuating taxa involved in reanimation [23].

### **Regulatory compliance**

All the samples that will be analysed in this proposal have been collected in Antarctica, in the frame of projects (PI Laura Selbmann) funded by the Italian National Program for Antarctic Researches (PNRA). The Nagoya Protocol does not apply to the genetic resources from Antarctica, neither Antarctica applies its own regulation or exists an access legislation in place.

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- organisms is one of the pathways for the beginning of soils on Earth. *Scientific reports*, 8(1):1-15, **2018**.
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**Appendix 2 – CVs**  
**PI, Laura Selbmann**

**A. Profession Preparation**

University “La Sapienza”, Rome (I)	Biology	M.Sc,	1993
University of Tuscia, Viterbo, (I)	Biochem Biol Evol	Ph.D.,	2000
University of Tuscia, Viterbo, (I)	Mycology, Evolution	Postdoc,	2000-2006

**B. Appointments**

2015-Present	Associate Professor, Department of Ecological and Biological Sciences (DEB), University of Tuscia, Viterbo, (I),
2006-2015	Senior Researcher, Department of Ecological and Biological Sciences (DEB), University of Tuscia, Viterbo, (I)

**C. Products**

**(i) Publications Most Closely-Related to Proposed**

Coleine C, Albanese D, Onofri S, Tringe S, Pennacchio C, Donati C, Stajich J, **Selbmann L** (2020). Metagenomes in the borderline ecosystems of the Antarctic cryptoendolithic communities. *Microbiology Resource Announcements* 9(10).

Coleine C, Pombubpa N, Zucconi L, Onofri S, Stajich JE, **Selbmann L** (2020). Endolithic fungal species markers for harshest conditions in the McMurdo Dry Valleys, Antarctica. *Life* 10(2):13.

Coleine C, Masonjones S, Sterflinger K, Onofri S, **Selbmann L**, Stajich JE (2020). Peculiar genomic traits in the stress-adapted cryptoendolithic Antarctic fungus *Friedmanniomyces endolithicus*. *Fungal Biology* In Press. DOI 10.1016/j.funbio.2020.01.005

Coleine C, Stajich JE, Pombubpa N, Zucconi L, Onofri S, **Selbmann L** (2020). Sampling strategies to assess microbial diversity of Antarctic cryptoendolithic communities. *Polar Biology* 43:225–235

Coleine C, Stajich JE, Zucconi L, Onofri S, **Selbmann L** (2020). Sun exposure drives Antarctic cryptoendolithic community structure and composition *Polar Biology* In Press. DOI 10.1007/s00300-020-02650-1

Coleine C, Stajich JE, Pombubpa N, Zucconi L, Onofri S, Canini F, **Selbmann L** (2019). Altitude and fungal diversity influence the structure of Antarctic cryptoendolithic Bacteria communities. *Environmental Microbiology Reports* 11(5):718-726.

Albanese, D., Coleine, C., Rota-Stabelli, O., Onofri, S., Tringe, S., Stajich, J. E., **Selbmann, L.**, & Donati, C. (2020). Antarctic cryptoendolithic bacterial lineages of pre-Cambrian origin as proxy for Mars colonization. *bioRxiv*. DOI 10.1101/2020.02.27.967604

**(ii) Five Other Recent Significant Publications**

Wijayawardene, NN, Hyde, KD, Tawfeeq Al-Ani, LK, Tedersoo, L, [...], **Selbmann L**, et al., (2020). Outline of Fungi and fungi-like taxa. *Mycosphere* In press. DOI 10.5943/mycosphere/11/1/8

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**Selbmann L**, Zucconi L, Onofri S, Cecchini C, Isola D, Turchetti B, Buzzini P. (2014). Taxonomic and phenotypic characterization of yeasts isolated from worldwide cold rock-associated habitats. *Fungal Biology* **118**: 61-71.

Egidi E, de Hoog GS, Isola D, Onofri S, Quaedvlieg W, de Vries M, Verkley GJM, Stielow JB, Zucconi L, **Selbmann L** (2014). Phylogeny and taxonomy of meristematic rock-inhabiting black fungi in the dothidemyces based on multi-locus phylogenies *Fungal Diversity* **65**:127-165.

**Selbmann L**, Isola D, Egidi E, Zucconi L, Gueidan C, de Hoog GS, Onofri S (2014). Mountain tips as reservoirs for new rock-fungal entities: *Saxomyces* gen. nov. and four new species from the Alps. *Fungal Diversity* **65**:167-182.

#### **D. Synergistic Activities**

1. Leadership
  - 2009-present responsible of the Mycological section of the Italian Antarctic National Museum(MNA) <http://www.mna.it/genova-ass/genova-micologia-universit%C3%A0-della-tuscia>. I joined three Antarctic Campaigns: 2010/11, PNRA XXVI expedition; sampling activities as component of research unit. Antarctic Campaign 2015/16, PNRA XXXI expedition; PI of the proposal and coordinator of field activity. Antarctic Campaign 2018/19, PNRA XXXIV expedition, PI of the proposal and coordinator of field activity
2. Teaching and Mentoring
  - 2006 – 2019 I have or am currently supervised 3 Postdocs, 8 PhD, 32 Master-students, numerous Diploma students .
  - Since 2016 I teach Botany (BS Biology), 2006-2012 Mycology, 2012 present Mycology and Phylogeny (MSc in Molecular Biology).
3. Community
  - I am Co-Leading STRES project (<http://www.stresblackfungi.org/>) and “Metagenome of Antarctic cryptoendolithic communities for understanding their responses to environmental stress due to altitude and sun exposure” in partnership with Joint Genome Institute of the Department of Energy.
  - I organized the Symposium “Perspectives and Potentialities of the JGI Doe Fungal - Omics Resource” at the European Fungal Genomic Conference, Rome 17-20 February 2020
  - I lead the Project MIUR PNRA16\_00006 (AMUNDSEN), MIUR 2013/AZ.17 of the Italian National Program for Antarctic Researches (PNRA), responsible of the Research Unit of the Antarctic Project PNRA16\_00101 (MIDAS). Guest Editor for the Journal Life, Component of the Editorial Board for the Journal Life, Astrobiology and Plant and Fungal Systematics. Member of the MIRRI-Italy (Microbial Resource Research Infrastructure).
4. Awards
  - 2008 Fellowship NL-TAF-4012 SYNTHESYS (Synthesys of systematic resources, the European Union-funded Integrated Infrastructure);
  - 2005 Fellowship NL-TAF-775 SYNTHESYS

**Co-PI, Jason E. Stajich****A. Profession Preparation**

Duke University, Durham, NC	Computer Science	B.S.,	1999
Duke University, Durham, NC	Genetics & Genomics	Ph.D.,	2006
University of California, Berkeley, CA	Mycology, Evolution	Postdoc,	2006-2009

**B. Appointments**

2017-Present	Professor, Department Microbiology & Plant Pathology, University of California-Riverside (Dept name changed in 2017)
2014-2017	Associate Professor, Department of Plant Pathology & Microbiology, UCR
2009-2014	Assistant Professor, Department of Plant Pathology & Microbiology, UCR
2006-2009	University of California-Berkeley, Postdoctoral Fellow

**C. Products****(i) Publications Most Closely-Related to Proposed**

Kowalski CH, Kerkaert JD, Liu KW, Bond MC, Hartmann R, Nadell CD, **Stajich JE**, Cramer RA. (2019). Fungal biofilm morphology impacts hypoxia fitness and disease progression. *Nature Microbiology* 4:2430–2441.

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Spatafora JW, Chang Y, Benny GL, Lazarus K, Smith ME, Berbee ML, Bonito G, Corradi N, Grigoriev I, Gryganskyi A, James TY, O'Donnell K, Roberson RW, Taylor TN, Uehling J, Vilgalys R, White MM, **Stajich JE**. (2016). A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia* 108(5):1028-1046.

**(ii) Five Other Recent Significant Publications**

Yang C-T, Vidal-Diez de Ulzurrun G, Gonçalves Pedro, Lin H-C, Chang C-W, Huang T-Y, Chen S-A, Lai C-K, Tsai IJ, Schroeder FC, **Stajich JE**, Hsueh Y-P. (2020). Natural variation in the predatory behavior of nematode-trapping fungi facilitates the establishment of a new robust model strain. *Proc National Academy of Sciences* (12) 6762-6770.

Wang Y, Youssef N, Couger MB, Hanafy R, Elshahed MS, **Stajich JE**. (2019). Molecular Dating of the Emergence of Anaerobic Rumen Fungi and the Impact of Laterally Acquired Genes. *mSphere*. 2019; 4(4): e00247-19.

Chang Y, Desirò A, Na H, Sandor L, Lipzen A, Clum A, Barry K, Grigoriev IV, Martin FM, **Stajich JE**, Smith ME, Bonito G, Spatafora JW. (2019). Phylogenomics of Endogonaceae and evolution of mycorrhizas within Mucoromycota. *New Phytol.* 222(1):511-525.

Mondo SJ, [...], James TY, O'Malley MA, **Stajich JE**, Spatafora JW, Visel A, Grigoriev IV. (2017). Widespread adenine N6-methylation of active genes in fungi. *Nature Genetics.* 49:964-968.

Rosenblum EB, James TY, Zamudio KR, Poorten TJ, Ilut D, [...] Medina EM, Restrepo S, Flechas SV, Berger L, Briggs CJ, **Stajich JE**. (2013). Complex history of the amphibian-killing chytrid fungus revealed with genome resequencing data. *Proc Natl Acad Sci U S A.* 110(23):9385-90.

## D. Synergistic Activities

### 1. Leadership

- Director of Microbiology Graduate Program, UC Riverside (2015-Present) where I emphasize recruitment and retention of graduate URMs and women to reflect UCR's makeup as an undergraduate Minority Serving Institution. Chair, UCR Graduate Council (2018-2020).

### 2. Teaching and Mentoring

- Faculty Mentor in UCR's Minority Access to Research Centers <http://marcu.ucr.edu> (2010-Present); Faculty in UCR HHMI SALSA and Honors learning communities and mentor lab for NSF REU Computational Plant Cell Biology, HSI-STEM, UCR-CAMP bridge programs.
- I have or am currently supervised 9 PhD Students (1 URM; 3 female) and 8 Postdoc fellows (2 URM) and one is currently a faculty member. I serve on 45 PhD thesis committees (20 current) and mentored 23 undergrads as researchers in my group (10 URM, 12 female).
- I teach bioinformatics & data programming courses at UCR <https://biodataprogram.github.io/> and through workshops at Marine Biological Lab (Molecular Mycology and Cold Spring Harbor).

### 3. Community

- I am committed to development of community resources for fungal genomics. I Co-Leading 1000 Fungal Genomes project (<http://1000.fungalgenomes.org>) in partnership with Joint Genome Institute of the Department of Energy. I co-initiated FungiDB – a Fungal Genome database (<http://fungidb.org>) and provide an advisor role to the project. I spearhead community via Twitter and online blogs for these projects: @fungalgenomes and @fungidb – <http://fungalgenomes.org/blog>
- I build our scientific community of fungal genetics and biology through my service as co-Vice-Chair (2018) and co-Chair (2020) for Fungal Cellular and Molecular Biology Gordon Conference; Fungal Genetics (2013-2019) & Neurospora (2014-2019) Policy Committees; Mycological Society of America (2018-2021, 2010-12 Councilor), and Scientific Advisory Boards for Wormbase, Ensembl Genomes, FungiDB. I am an Associate Editor at the journals *Mycologia*, *Genetics*, *Genome Biology & Evolution*, *Microbial Resource Announcements* (ASM Press), *Fungal Genetics & Biology*.

### 4. Awards

- 2020 Fellow, American Academy of Microbiology
- 2019-24 CIFAR Fellow. Fungal Kingdom: Threats & Opportunities
- 2015 Kavli Fellow, Kavli Frontiers of Science
- 2014 C. J. Alexopoulos Prize, Mycological Society for America
- 2006–2009 Miller Institute for Basic Research in Science, Postdoctoral Research Fellowship
- 2003–2006 National Science Foundation, Graduate Research Fellowship

## Co-PI, Asunción de los Ríos

### A. Profession Preparation

Complutense University of Madrid	Biology	M.Sc,	1990
Complutense University of Madrid	Biology	Ph.D.,	1997
Karl Franzens University, Graz, Austria	Molecular taxonomy	Postdoc,	1997-2000

### B. Appointments

2010-Present	Staff scientist, Department of Biogeochemistry and Microbial Ecology, National Natural Sciences Museum, CSIC, Madrid, Spain
2006-2010	Staff scientist, Environmental Sciences Centre, CSIC, Madrid, Spain
2002-2006	Ramon y Cajal Research associate, Environmental Sciences Centre, CSIC, Madrid, Spain
2001	Postdoctoral Reincorporation (Fellow), University of Lleida, Lleida, Spain

### C. Products

#### (i) Publications Most Closely-Related to Proposed

**De Los Ríos, A**, Wierzchos, J, Sancho, LG, and Ascaso, C. (2003). Acid microenvironments in microbial biofilms of Antarctic endolithic microecosystems. *Environmental Microbiology* 5(4):231-237.

**De los Ríos, A**, Wierzchos, J, Sancho, LG, & Ascaso, C. (2004). Exploring the physiological state of continental Antarctic endolithic microorganisms by microscopy. *FEMS microbiology ecology* 50(3):143-152.

**De los Ríos, A**, Sancho, LG, Grube, M, Wierzchos, J, & Ascaso, C. (2005). Endolithic growth of two Lecidea lichens in granite from continental Antarctica detected by molecular and microscopy techniques. *New Phytologist* 165(1):181-190.

**De Los Ríos, A**, Grube, M, Sancho, LG, & Ascaso, C. (2007). Ultrastructural and genetic characteristics of endolithic cyanobacterial biofilms colonizing Antarctic granite rocks. *FEMS Microbiology Ecology* 59(2), 386-395.

**De Los Ríos, A**, Wierzchos, J, & Ascaso, C. (2014). The lithic microbial ecosystems of Antarctica's McMurdo Dry Valleys. *Antarctic Science* 26(5):459-477.

Garrido-Benavent, I, Pérez-Ortega, S, Durán, J, Ascaso, C, Pointing, SB, Rodríguez-Cielos, R, ... & **De los Ríos, A**. (2020). Differential Colonization and Succession of Microbial Communities in Rock and Soil Substrates on a Maritime Antarctic Glacier Forefield. *Frontiers in Microbiology* 11:126.

#### (ii) Five Other Recent Significant Publications

Tornos, F, Oggerin, M, **De los Ríos, A**, Rodriguez, N, Amils, R, Sanz, JL, ... & Slack, JF. (2019). Do microbes control the formation of giant copper deposits? *Geology* 47(2):143-146.

Ortiz-Álvarez, R, Fierer, N, **De los Ríos, A**, Casamayor, EO, & Barberán, A. (2018). Consistent changes in the taxonomic structure and functional attributes of bacterial communities during primary succession. *The ISME journal* 12(7):1658-1667.

Garrido-Benavent, I, Pérez-Ortega, S, & **De los Ríos, A.** (2017). From Alaska to Antarctica: species boundaries and genetic diversity of *Prasiola* (Trebouxiophyceae), a foliose chlorophyte associated with the bipolar lichen-forming fungus *Mastodia tessellata*. *Molecular phylogenetics and evolution* 107:117-131.

Sanders, WB, & **De los Ríos, A.** (2017). Parenchymatous cell division characterizes the fungal cortex of some common foliose lichens. *American journal of botany* 104(2):207-217.

Fernández-Martínez, MA., Pérez-Ortega, S, Pointing, SB, Green, TA, Pintado, A, Rozzi, R., ... & **De los Ríos, A.** (2017). Microbial succession dynamics along glacier forefield chronosequences in Tierra del Fuego (Chile). *Polar Biology* 40(10):1939-1957.

## D. Synergistic Activities

### 1. Leadership

- 2007-present: I am responsible of the Molecular ecology laboratory of Microbial Ecology and Geomicrobiology Group from National Natural Sciences Museum.
- I lead the Project titled: Biodiversity, three-dimensional organization, ecosystem functioning and interdependencies in cryptogamic covers of arid and polar regions- CTM2015-64728-C2-2-R funded by Spanish Science Ministry, active until the end of 2020.
- I have participated in 4 Antarctic campaigns: Dry Valleys with Antarctic New Zealand Program at 2009 and Livingston Island with Spanish Antarctic Program at 2014, 2017, 2019.

### 2. Teaching and Mentoring

- 2006 – 2019 I have or am currently supervised 2 Postdocs, 5 PhD, 10 Master-students, numerous Diploma students.

### 3. Community

- President of the Spanish Biodeterioration, Biodegradation and Bioremediation Group (Spanish Microbiology Society) from 2010 until 2018.
- Council member of Spanish Microbiology Society from 2010 until 2018
- Council Member of International Biodeterioration and Biodegradation Society from 2010 until the present
- Organizer of the “Spanish IX Symposium on Polar Studies”, Madrid 5-7 September 2018.
- Member of Scientific-technic panel, Biodiversity subarea (área de Ciencias y tecnologías medioambientales) of Spanish State Research Agency from July 2018.

### 4. Awards

- Extraordinary Doctoral UCM Prize (1999)
- The Grady L. and Barbara D. Webster Structural Botany Award (2018) from Botany Society of America.

## APPENDIX 3 – Resources Requested

## NSLS-II

If you are interested in using the XFM at NSLS-II, please complete the information below.

NSLS-II RESOURCE REQUEST					
Resource	# of Samples	Estimated date of sample shipment to NSLS-II	Units of Requested (specify unit)	Project Team Expertise per Resource	Support Requested
XFM					

## EMSL

For questions about the EMSL resource request form, please contact the appropriate Capability Lead:

<https://www.emsl.pnnl.gov/emslweb/scientific-capabilities>

EMSL RESOURCE REQUEST					
Add lines as needed for # of resources					
Resource	# of Samples	Estimated date of sample shipment to EMSL	Units of Requested (specify unit)	Project Team Expertise per Resource	EMSL Support Requested
Mass Spectrometer: GC MS (for both non-polar and polar metabolites identification)	90	Nov 1, 2020	90	We will reanimate samples and incubate in a $^{13}\text{CO}_2$ and $^{15}\text{N}_2$ atmosphere. Samples will be lyophilized and sent to the EMSL for metabolites (and proteins) Extraction.	Metabolites extraction; running (polar and non-polar metabolite analysis), and analyzing mass spec results.
Mass Spectrometer: Orbitrap	90	Nov 1, 2020	300	We will reanimate samples and incubate in a $^{13}\text{CO}_2$ and $^{15}\text{N}_2$ atmosphere. Samples will be lyophilized and sent to the EMSL for proteins (and metabolites) Extraction.	Protein extraction; running, and analyzing mass spec results.
X-ray Computed Tomography	30	Nov 1, 2020	150	We will supply a selection of colonized and non-colonized rock, focusing on 2 different sandstone typologies.	Analysis and selection of samples to be processed for Physical Property Measurement System.

Physical Property Measurement System	4	Nov 1, 2020	200	Representative samples will be supplied.	Consultation to optimize sample size and protocols. We will be able to send lab representatives to work with the EMSL team.
NanoSIMS	8	March 1, 2021	150	We will reanimate samples and incubate in a $^{13}\text{CO}_2$ and $^{15}\text{N}_2$ atmosphere. Rock samples will be fixed with glutaraldehyde and osmium tetroxide, dehydrated in ethanol series and later embedded in LR-White resin and then shipped to EMSL for further analysis.	Assistance with all aspects of NanoSIMS sample preparation, imaging, and analysis. We will be able to send lab representatives to work with the EMSL team for periods of a week at a time, for several weeks as needed.
FIB/SEM EDX mapping	10	March 1, 2021	120	Rock samples will be fixed with glutaraldehyde and osmium tetroxide, dehydrated in ethanol series and later embedded in LR-White resin and then shipped to EMSL for further analysis.	Analysis and selection of samples to be processed with NanoSIMS.
FIB/SEM w/cryo	4	March 1, 2021	100	Rock samples will be fixed with glutaraldehyde and osmium tetroxide, dehydrated in ethanol series and later embedded in LR-White resin and then shipped to EMSL for further analysis.	Consultation to optimize protocols and analyzing results. We will be able to send lab representatives to work with the EMSL team for periods of a week or for several weeks as needed.

### JGI

For questions about the JGI resource request form, please contact [jgi-jira+pmosupport@lbl.gov](mailto:jgi-jira+pmosupport@lbl.gov).

**JGI RESOURCE REQUEST**



Resource	# of Samples	Estimated date of sample shipment to JGI	Analysis Support Requested beyond JGI's <a href="#">standard pipelines</a>
<b><u>Sequencing resources:</u></b>			
Bacterial/archaeal <i>de novo</i> genomes (single organisms)			
Bacterial/archaeal single cells: <i>samples for FACS sorting</i>			
Bacterial/archaeal single cells: <i>cells to be sequenced</i>			
Bacterial/archaeal RNA-seq (single organism)			
Bacterial/archaeal resequencing			
Fungal <i>de novo</i> genomes			
Fungal RNA-seq (single organism)			
Fungal resequencing			
Algal <i>de novo</i> genomes			
Algal RNA-seq (single organism)			
Algal resequencing			
Plant <i>de novo</i> genomes			
Plant RNA-seq (single organism)			
Plant resequencing			
Metagenome (no iTags)	96	Dec 1, 2020	
Stable Isotope Probing (SIP) metagenomics prep, <i>samples to be fractionated</i> (limit 36 samples)			
Metatranscriptome			
Other sequencing request (methylation, FAIRE, ChIP-seq)			
<b><u>Metabolomics resources:</u></b>			
Polar metabolite analysis (LC/MS) – with/without stable isotope labeling, limit 200 samples			
Non-polar metabolite analysis (LC/MS) – with/without stable isotope labeling, limit 500 samples			
<b><i>Organism detail (add rows if needed)</i></b>			
<b><i>Kingdom</i></b>	<b><i>Genus</i></b>	<b><i>Species</i></b>	<b><i>Genome size (Mb) – for eukaryotes only</i></b>
<b><i>Environmental samples (add rows if needed)</i></b>			
<b>Number of samples</b>	<b>General description of metagenome sample and source</b>		
96	Rocks colonized by cryptoendolithic lichen-dominated communities collected in Antarctica.		


### JGI SYNTHESIS RESOURCE REQUEST

Resource	# of constructs/libraries
Constructs <5 Kb	
Constructs 5-10 Kb	
Constructs >5 Kb	
Combinatorial library	
sgRNA library	
Other	
<b>Total request in Kb (request must be between 50- 500 Kb):</b>	

#### *Biosafety Information (required for all proposals including a synthesis component):*

##### **Are any of the genes or fragments to be synthesized:**

1. Related to the pathogenicity of an organism?\*  Yes  No
2. Known to or has potential to encode any form of infectious agent or viral life-cycle component?\*  Yes  No
3. Known to have any toxicity, or the likelihood that this project might increase toxicity?\*  Yes  No
4. Intended for use in creating a vaccine?\*  Yes  No

##### **Comments (required if you answered Yes to any of the above):**

##### **Biosecurity, Biosafety, Biocontainment, and Environmental screening \***

Describe the Biosecurity, Biosafety, Biocontainment, and Environmental aspects of your proposed research, including both the current aspects and the long term implications of the work (desirable or otherwise). Describe what you will do (and who you will collaborate with) to address any aspects of concern and how you will mitigate any undesirable outcomes. This information will be critically assessed during the JGI's DNA Synthesis [Internal Review process](#), and your research will be delayed if the reviewers request modifications to your proposal due to insufficient consideration or description of these aspects.

##### **Lay Description \***

The JGI reviews all DNA synthesis proposals for Biosecurity, Biosafety, Biocontainment, and Environmental safety. In order to facilitate a better understanding of your proposal for our reviewers, please provide a lay description of your proposal (excluding scientific jargon), so that non-scientific/technical experts can better assess the broader aspects and implications of the work in the context of the research that is proposed.