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## Exploring Space via Astromycology: A Report on the CIFAR Programs *Earth 4D* and *Fungal Kingdom* Inaugural Joint Meeting

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### Abstract

“Fungi on Mars!”: a popular news heading that piques public interest and makes scientists’ blood boil. While such a statement is laden with misinformation and light on evidence, the search for past and present extraterrestrial life is an ongoing scientific effort. Moreover, it is one that is increasingly gaining momentum with the recent collection of martian rock cores from Jezero Crater by NASA’s Perseverance rover. Despite the increasingly sophisticated approaches guiding the search for microbial life on other planets, fungi remain relatively underexplored compared to their bacterial counterparts, highlighting a gap between the astrobiological and fungal research communities. Through a meeting in April 2021, the CIFAR *Earth 4D* and *Fungal Kingdom* research programs worked to bridge this divide by uniting experts in each field. CIFAR is a Canadian-based global research organization that convenes researchers across disciplines to address important questions facing science and humanity. The CIFAR *Earth 4D: Subsurface Science & Exploration* and *Fungal Kingdom: Threats & Opportunities* research programs were launched by CIFAR in July 2019, each made up of approximately two dozen international researchers who are experts in their fields. The *Earth 4D* program, led by co-directors John Mustard (Brown University, USA) and Barbara Sherwood Lollar (University of Toronto, Canada), aims to understand the complex chemical, physical, and biological interactions that occur within and between Earth’s surface and subsurface to explore questions on the evolution of planets and life. The *Fungal Kingdom* program, led by co-directors Leah Cowen (University of Toronto, Canada) and Joseph Heitman (Duke University, USA), seeks to tackle the most pressing threats fungi pose to human health, agriculture, and biodiversity and to harness their extraordinary potential. The programs met to explore areas for synergy within four major themes: (1) the origins of life; (2) the evolution and diversification of life; (3) life in diverse and extreme environments; and (4) extinction: lessons learned and threats. This report covers the research discussed during the meeting across these four themes. Key Words: Fungi—Subsurface—Origin—Evolution—Extinction—Extreme environments. *Astrobiology* 22, 637–640.

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## 1. Fungi and the Origins of Terrestrial Plant Life

FUNGI ARE KEY MEMBERS of terrestrial ecosystems, forming symbiotic relationships with 90% of all land plants (Feijen *et al.*, 2018) and enabling nutrient cycling as Earth's preeminent degraders of organic matter (Willis, 2018). Heather Graham (NASA Goddard Space Flight Center, USA) discussed fungi in the rock record and their role in facilitating terrestrial life on Earth and highlighted that terrestrial fungi pre-date plants in the fossil record, with the earliest fungus detected 635 million years ago and the earliest terrestrial plant over 200 million years later (Krings *et al.*, 2018; Morris *et al.*, 2018; Gan *et al.*, 2021). Graham noted that fossils morphologically consistent with fungi of the phylum Glomeromycota, which is predominantly composed of arbuscular mycorrhizal fungi that form obligate symbiotic relationships with land plants, were detected in the fossil record 40 to 50 million years before the first terrestrial plants, which raises fundamental questions about the physiology and ecology of these presumably non-symbiotic fungi prior to their association with terrestrial plants (Redecker *et al.*, 2000). Graham seeks to gain insight into the lifestyles of these ancient glomeralean fungi using isotopic fractionation and elemental abundance analysis to distinguish saprotrophic from symbiotic fungi based on differences in the isotopic compositions of organic compounds preserved in fungal fossil samples. Graham highlighted *n*-alkanes, a major component of plant leaf structure that also serve to protect against fungal infection, as a biomarker with the potential to provide insight into how fungi have shaped the composition of forests over time. Graham determined that angiosperm tree species, which became more prevalent after the Cretaceous-Tertiary (K-T) extinction, produce high quantities of *n*-alkanes relative to gymnosperms, and postulated that these leafy waxes could have provided angiosperms with a selective advantage given their antifungal properties (Diefendorf *et al.*, 2011; Graham and Freeman, 2014).

## 2. Fungi and the Evolution of Mammals

In addition to impacting the evolution of forest ecosystems, fungi are hypothesized to have selected for the emergence of mammals after the K-T extinction event (Casadevall, 2005, 2012; Casadevall and Damman, 2020), as theorized by Arturo Casadevall (Johns Hopkins University, USA). Casadevall highlighted that reptiles were the dominant megafauna prior to the K-T event, while mammals dominated thereafter despite having a relatively energetically expensive lifestyle (Casadevall, 2005, 2012). Casadevall hypothesized that fungi imposed a selective filter that favored the evolution of mammals, which are highly resistant to fungal infection as a result of their warm body temperature and adaptive immunity (Robert and Casadevall, 2009; Bergman and Casadevall, 2010). In support of this hypothesis, there is evidence of massive fungal proliferation after the K-T event that could have hindered the re-emergence of reptiles, of which fungi are a major pathogen (Vajda and McLoughlin, 2004). Casadevall termed this hypothesis the fungal infection mammalian selection (FIMS) theory (Casadevall and Damman, 2020), which not only provides explanation for the rise of mammals after the K-T event but also possesses predictive power. FIMS predicts that as global temperatures

approach mammalian body temperature with climate warming, there will be an increase in the prevalence of fungal diseases in mammals due to both expansion of the geographic range of currently pathogenic fungal species and selection for species with pathogenic potential that are presently being restricted by the mammalian thermal barrier (Garcia-Solache and Casadevall, 2010). Moreover, human body temperatures have decreased over the past century, further narrowing this thermal barrier (Protsiv *et al.*, 2021), and Casadevall postulated that *Candida auris* could be the first human fungal pathogen to emerge due to thermal adaptation in response to climate change (Casadevall *et al.*, 2019, 2021).

## 3. Fungi in Extreme Environments

Fungi have a remarkable capacity to survive in diverse and extreme environments. The polyphyletic group of melanotic or black fungi inhabit some of the most extreme environments known, including high-radiation environments such as the nuclear reactor at Chernobyl (Casadevall *et al.*, 2017) and the International Space Station (Satoh *et al.*, 2016). While black fungi are among the most polyextreme-tolerant organisms on Earth, there is a dearth of available black fungal genomes. Jason Stajich (University of California, Riverside, USA) aims to address this paucity through his work with the "Shed light in The daRk lineages of the fungal tree of life" (STRES) project by sequencing nearly 100 strains of black fungi as reference genomes (Selbmann *et al.*, 2020). Through sequencing strains from mostly unsampled genera and from different ecologies and lifestyles, the data acquired will establish a database for fungal metagenomics, biology, and evolution, which will further clarify how black fungi adapted to inhabit the extremes (Selbmann *et al.*, 2020). Moreover, Stajich studied microbial communities across hot and cold deserts such as the Mojave Desert in California and the Antarctic Desert, as well as in marine habitats. Through sampling, metabarcoding sequencing, and network analysis of biological crusts and subsurface soils in the Mojave Desert, Stajich identified that cross-domain fungal-bacterial interactions differed greatly between the surface and the subsurface, highlighting important differences between these proximate yet distinct niches (Pombubpa *et al.*, 2020).

The continental and oceanic subsurface are estimated to contain up to 19% and 30% of Earth's total biomass, respectively, yet they remain relatively unexplored (Kallmeyer *et al.*, 2012; Colwell and D'Hondt, 2013; McMahon and Parnell, 2014; Bar-On *et al.*, 2018; Magnabosco *et al.*, 2018). These spaces represent an untapped resource for fungal discovery, with studies reporting one-third to two-thirds of subsurface fungi identified as potentially novel species (Li *et al.*, 2020). Victoria Orphan (California Institute of Technology, USA) discussed fungi in deep ocean and terrestrial biospheres. She noted that molecular-driven discoveries of subsurface fungi have uncovered remarkable diversity, representing many of the major fungal phyla. Orphan highlighted that subsurface habitats often lack oxygen and are low in bioavailable energy and nutrients. While the vast majority of subsurface research to date has focused on archaea and bacteria, fungi are present and are active members of the microbial communities that persist in these remote and harsh

environments (Edgcomb *et al.*, 2011). For example, transcriptomic approaches have revealed differences in expression patterns between surface and deep subsurface fungi, with subsurface fungi expressing a higher level of transcripts associated with survival and complex carbon utilization (Orsi *et al.*, 2013; Pachiadaki *et al.*, 2016). Orphan highlighted that subsurface fungi can also form close interactions with bacteria and archaea, as evidenced by the detection of intimate contact between a fungus and sulfate-reducing bacteria in deep anoxic granite groundwater (Drake *et al.*, 2017, 2021). Interestingly, these researchers hypothesized that fungi were breaking down the necromass of microorganisms in this environment, resulting in the production of hydrogen that in turn fueled the growth of autotrophic sulfate-reducing bacteria, whose activity encouraged pyrite formation within the rock (Drake *et al.*, 2017). Thus, fungi were modifying both biotic and abiotic factors in this subsurface rock environment. Through investigating life in deep-sea methane vents, Orphan in collaboration with colleagues at Caltech identified deep-sea nematodes that were parasitized by microsporidia fungi, thus uncovering the first example of parasitism in a deep-ocean environment (Sapir *et al.*, 2014) and emphasizing the opportunity for novel discovery through collaboration between the two CIFAR programs.

#### 4. Conclusions and Outlook

Despite the remarkable ability of fungi to thrive in extreme environments and their role in shaping and sustaining life on Earth, there remains a dearth of knowledge on the limits of fungal life in the deep subsurface and in space. Both the subsurface and fungi are understudied and underutilized in their potential to expand conceptual models of habitability and inform the study of the origins and evolution of life on Earth and other planets. What is the range of chemistry that can sustain fungal life? What are the physical controls on subsurface architecture that shape fungal communities? What role do subsurface fungi play in the utilization and cycling of nutrients? How might fungi provide valuable insight in the search for extinct and extant life on Mars and other planets? Through sustained collaboration, the CIFAR *Earth 4D* and *Fungal Kingdom* research programs are poised to answer these and other questions and to unlock the untapped potential of fungi to shift terrestrial paradigms on the limits of life.

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#### Abbreviations Used

FIMS = fungal infection mammalian selection

K-T = Cretaceous-Tertiary

STRES = Shed light in The daRK lineagES  
of the fungal tree of life