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Forum

Safeguarding Earth's biodiversity by creating a lunar biorepository

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Abstract

Earth's biodiversity is increasingly threatened and at risk. We propose a passive lunar biorepository for long-term storage of prioritized taxa of live cryopreserved samples to safeguard Earth's biodiversity and to support future space exploration and planet terraforming. Our initial focus will be on cryopreserving animal skin samples with fibroblast cells. An exemplar system has been developed using cryopreserved fish fins from the Starry Goby, *Asterropteryx semipunctata*. Samples will be expanded into fibroblast cells, recryopreserved, and then tested in an Earth-based laboratory for robust packaging and sensitivity to radiation. Two key factors for this biorepository are the needs to reduce damage from radiation and to maintain the samples near –196° Celsius. Certain lunar sites near the poles may meet these criteria. If possible, further testing would occur on the International Space Station prior to storage on the Moon. To secure a positive shared future, this is an open call to participate in this decades-long program.

Keywords: cryopreservation, extinction, ethics, fibroblast cells, fishes, the Moon

Biodiversity on Earth is increasingly threatened and at risk (IPCC 2007; https://www.ipcc.ch/site/assets/uploads/2018/02/ar4_syr_full_report.pdf). Even under the most optimistic models of global climate change, a staggering proportion of Earth's biota will go extinct (Dirzo et al. 2022). Because of myriad anthropogenic drivers, a high proportion of species and ecosystems face destabilization and extinction threats that are accelerating faster than our ability to save these species in their natural environment (Sala et al. 2000, Dirzo et al. 2022). There is an urgent need to envision innovative strategies to conserve Earth's biodiversity to protect ecosystems of the future.

Cryopreservation technologies provide one such innovative strategy (Angeles and Catap 2023) whereby cells can remain frozen but alive for hundreds of years. With increasing success, collections of cryopreserved materials can be thawed to recover DNA, intact cells, and even whole functional organisms (Daly et al. 2018, Powell-Palm et al. 2023). Many institutions globally maintain cryopreserved biological collections, especially those concerned with human health, but fewer biorepositories hold live wildlife samples in a frozen state. Nevertheless, all these biorepositories require intensive human management, electrical power, and an ongoing supply of liquid nitrogen, which makes them susceptible to unpredictable natural and geopolitical disasters. Today, many frozen collections are stored in urban centers, making them even more susceptible to destabilization threats (Johnson and Owens 2023).

In the face of potential catastrophic ecosystem loss, such as coral reefs from climate-related warming, we propose the creation of a lunar biorepository to maintain samples in a cryopreserved state with little human intervention. In 4.5% of the Moon's southern pole, seasonal temperature variation is stable year-round at or below –196° Celsius (°C; Williams et al. 2019). Such a biorepository would safeguard biodiversity and act as a hedge against its loss occurring because of natural disasters, climate change, overpopulation, resource depletion, wars, socioeconomic threats, and other causes on Earth. Initially, this would be a vault for live, cryopreserved samples of the most at-risk animals on Earth to safeguard our biodiversity and to support future space exploration, as well as planet terraforming taxa, with other organisms and plants to be added in the future. Our goal is to cryopreserve most animal species on Earth. In addition to safeguarding Earth's biodiversity, a lunar biorepository would advance our fundamental understanding of how cells behave in space and would also preserve animal, plant, and microbial samples that may be essential to human ex-

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ploration of the solar system or galaxy. The biorepository could store biomaterials for food, filtration, microbial breakdown, and ecosystems engineering.

Long-term storage of living cells from animal species requires temperatures at –196°C or below to suspend all biological activity. There is nowhere on Earth cold enough to store animal samples without human intervention. But there are places on the Moon that reach –196°C (including some that remain constantly below –225°C; Clery 2023), which is cold enough for stable storage. This is especially true at the lunar poles, where deep craters are permanently shadowed. Lunar lava tubes at midlatitudes may also achieve temperatures necessary for biopreservation. The Arctic Svalbard Global Seed Vault, in Norway (https://academic.oup.com/bioscience/article/58/3/190/230676), is a passive biorepository that maintains seeds at –18°C because of the natural surrounding temperature of the permafrost. Changing climatic conditions threaten the stability of the Svalbard Seed Vault, but on the Moon there is no atmosphere and, therefore, no threat of climate change.

Human activity on the Moon in the decades to come may increase dramatically (Clery 2023) and establishing and maintaining a long-term backup of life from Earth is of critical scientific value. Ideally an international agreement on a shared lunar biorepository would provide an effective long-term solution to protect life.

Recently, a lunar ark has been proposed to save endangered species in a subterranean storage structure on the Moon (Diaz Flores et al. 2021). It would depend on solar energy for power and to keep it cold, making it susceptible to failure because of energy loss. Furthermore, the proposal includes an engineering design without consideration of the biological aspects of the biorepository other than it would hold cryopreserved seeds, spores, and gametocytes. In contrast, we focus on the biological aspects of a lunar biorepository with a passive design that does not depend on generating power to maintain ultralow temperature because it would be near the poles. We detail ideas of the cryobiology, the type of cells needed, engineering for the cells to keep them safe over time, and governance of the repository. Moreover, we have begun to collect cells for the repository using an exemplar.

Ours is a transdisciplinary approach involving ecologists, biologists, cryobiologists, systematists, geneticists, geologists, engineers, and expertise in law and bioethics to develop social, technical, and logistical solutions, as well as governance considerations that might make this endeavor a reality. Rapid progress toward this goal might be facilitated by harmonizing these active and passive approaches for a lunar repository.

Methodology and roadmap

We propose that the lunar biorepository initially incorporate a range of animal taxa that are endangered on Earth and have the potential to or would be required to rebuild human-friendly sustainable ecosystems during space flight, on another planet, or back on Earth. Because animal life depends most basically on plants, to support the development of *de novo* ecosystems the biorepository would need to expand beyond animal taxa in the future. The first groups that might be placed in the biorepository are summarized in Box 1.

For animals, fibroblast cells from skin samples, and potentially some invertebrate larvae, can be uniformly collected and cryopreserved. A few biorepositories around the world are banking fibroblast cells from humans (Hutton et al. 2021) as well as a variety of wildlife species for conservation

Box 1. Critical groups for possible initial inclusion in the lunar biorepository.

Ecological engineers
Pollinators
Extreme environment fauna
Primary producers
Temperate to cold water fishes
Threatened and endangered animals
Organisms important in space exploration
Wild relatives of domesticated organisms
Species of cultural importance

(Elyasi Gorji et al. 2021, Alexsandra Fernandes et al. 2023). Cryopreserved fibroblast skin cell lines have been established for the African Savanna Elephant from materials conserved at the San Diego Zoo (Jansen van Vuuren et al. 2023). Under culture conditions, these cells form flattened disks only 3 to 6 nanometers in height and have the amazing capacity to become pluripotent (LeBleu and Neilson 2020). Cells from more than 15 wild mammalian species have been reprogrammed into pluripotent stem cells that have characteristics of embryonic cells (Takahashi and Yamanaka 2006, 2016, Swegen et al. 2023). Understanding the molecular processes that underly this reprogramming is an active area of research, making fibroblasts a key choice for this future-looking program. Moreover, cryopreserved cardiac stem cells have recently been sent from Earth to the International Space Station (ISS; Rampoldi et al. 2021, 2022). Consequently, the mechanisms for robust packaging that can withstand liquid nitrogen temperatures, microgravity, and vacuums are being addressed for cryopreserved samples in space. Although, with emerging technology, it may be possible to resurrect organisms from their genomic sequence alone, current organismal cloning is not successful without additional cellular components.

The Starry Goby system

We chose *Asterropteryx semipunctata* Rüppell, 1830, the Starry Goby, as an exemplar species for cryopreservation for several reasons. It is a marine reef fish that, like many reef-associated fishes, lives closely with corals with which they have a mutually beneficial relationship. Considerations for mutualistic and other ecological relationships must be factored into decision-making for inclusion in the lunar biorepository. Selection of the Starry Goby as an exemplar species emphasizes the need to include the corals that are critical to its well-being. The phylogenetic position of a species is another important consideration for inclusion in the lunar biorepository (Pellens and Grandcolas 2016). The Starry Goby is a member of the family Gobiidae, which, with over 2000 species, is one of the largest families of bony fishes. It is abundant, is not threatened with extinction (Larson 2019), and is in aquaculture for the aquarium trade. As a bony fish, it is an ideal subject on which to test cryopreservation and packaging protocols. Preliminary studies on the cryopreservation of fish fins and the extraction and cryopreservation of their fibroblasts (Mauger et al. 2006, Moritz and Labbe 2008) have been successful, and the Starry Goby has already been used as an exemplar to cryopreserve testicular stem cells with success (Hagedorn et al. 2018, Bouwmeester et al. 2022). It is easy to collect both males and females and to dissect skin samples that may be cryopreserved and used to expand fibroblast cells to be packaged for radiation testing.

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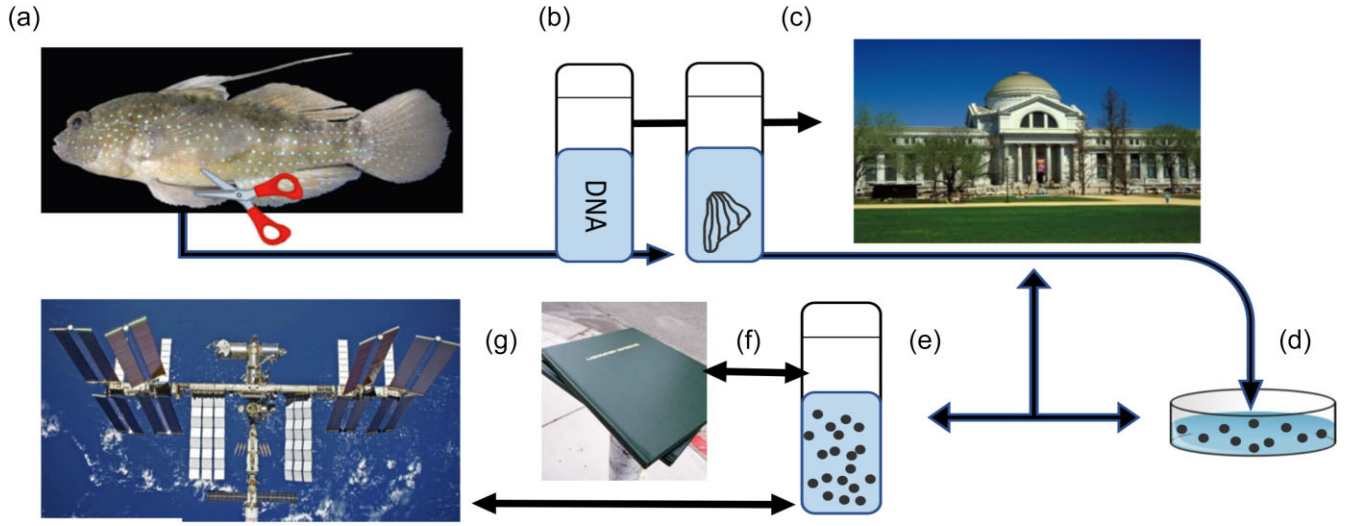

Figure 1. The proposed flow diagram to create cryopreserved cells and test them in space. (a) Sampling of pelvic fins from the Starry Goby. (b) Fins and DNA samples can be stored in a biorepository. Fins can be placed dry in a cryovial, with a sterile damp sponge and with cells expanded into fibroblasts or cryopreserved and held in a biorepository. (c) An Earth biorepository, such as the Smithsonian National Museum of Natural History, where cryopreserved samples can be held for decades or potentially longer prior to launching into space. (d) Fibroblasts from fins expanded in the lab. (e) Fibroblast cells cryopreserved. (f) Cryopreserved cells and cryopackaging tested on Earth for robustness under space conditions. (g) Space-ready cryopreserved samples are sent to the International Space Station for testing and then returned to Earth for analysis of viability and changes to DNA.

As a first step, we are developing an exemplar system to test and establish the metrics and protocols for sample collection. We collected 10 specimens of the Starry Goby in Kane'ohe Bay, Hawai'i, in 2023 (figure 1). Fish were processed as voucher samples at the Smithsonian's National Museum of Natural History; each specimen's size and sex were recorded, and a tissue sample and high-resolution images were taken using standard methods (Hagedorn et al. 2018). The two pelvic fins from each specimen were sampled, cryopreserved (Moritz and Labbe 2008), and stored for subsequent expansion into fibroblast cells. Our vision is that these fibroblasts would be distributed into a variety of space-hardy cryopackaging and tested under space-like conditions on Earth. Candidate packaging for the cells would be tested next on the ISS.

We plan to leverage the continental-scale sampling that is currently underway at the US National Science Foundation's National Ecological Observatory Network (NEON). NEON collects approximately 100,000 samples annually from 81 freshwater and terrestrial field sites using standardized protocols. These samples include vouchered and cryo- or ethanol-preserved tissues from a diversity of taxa, including mammals, insects and other arthropods, fishes, algae, and plants. Through NEON's Assignable Assets program (SanClements and Mabee 2021), we can start sample collections and process them to generate fibroblast cells. One of the most difficult and yet to be determined parts of this process is the technological roadmap for these cells and how we safely get them into space.

We must identify robust packaging to hold the cells in space and conduct the first trials on the ISS. We do not how know long the samples will need to be tested for sensitivity to radiation and microgravity on Earth or on the ISS. The effects of microgravity are observed largely in changes to human physiology, specifically to cytoskeletal disorders (Bradbury et al. 2020). Because the cells are cryopreserved at –196°C, there will be little to no active physiology once all water in the sample is completely frozen (Pegg 2007), thereby limiting all metabolic processes. Therefore, the cryopreserved cells may escape the effects of the one-sixth gravity of Earth on the Moon. We know that the cryopreserved cells might receive about 3 days of solar and cosmic radiation in transit each way to and from the Earth (if the samples were brought back to Earth to use). We plan to ship the cryopreserved cells in containers that reduce radiation but have not yet identified that packaging. Moreover, there could be delays moving packages to their final storage location on the Moon. Therefore, we may have to plan for several months of radiation exposure. Once the samples are surrounded by about 1 meter of regolith, much of the cosmic and solar radiation is mitigated (Akisheva and Gourinat 2021).

Ice recrystallization is a concern in a lunar biorepository, as it is for an Earth biorepository (Chang and Zhao 2021). This is especially so if there are temperature fluctuations in transit but less so in areas of the Moon that are permanently shadowed and have a uniform daily and seasonal temperature around –196°C. It is also important to note that both the freezing and thawing steps will be performed on Earth and will not be subject to microgravity conditions. A lunar biorepository would not necessarily face more issues with ice crystallization in samples than do other long-term biorepositories. As the team's expertise grows, we will invite international partners, especially those engaged with the International Union for Conservation of Nature. It is important to note that although we propose to start the program by cryopreservation of skin fibroblasts for several critical species to show feasibility, we envision expansion to other cell types, including gametocytes (i.e., oocytes and sperm).

Challenges and potential solutions

A lunar biorepository holds great promise for securing Earth's biodiversity and supporting human exploration and terraforming of other planets. The challenges and benefits of a Moon-based versus Earth-based biorepositories are summarized in table 1.

The first challenge is that we must produce robust packaging for our samples that can withstand the rigors of space over long time periods.

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Table 1. Comparison of attributes of an active Earth-based versus passive Moon-based biorepository for cryopreserved material.

	Active Earth-based repository	Passive Moon-based repository
Cost to establish	1	5
Cost to maintain	5	2
Governance to establish	2	5
Accessibility of samples	1	5
Concerns about physiological issues of radiation and microgravity	1	5
Standard packaging needed for samples	1	5
Flexibility of locations	2, power outages	5
Energy to maintain temperature at –196°C	5	1
Susceptibility to natural disasters (e.g., hurricanes, climate change)	5	2
Susceptible to social instability (e.g., wars, power outages)	5	1

Note: Ranked from 1 (low, easy) to 5 (high, difficult).

The second challenge is degradation of exposed samples by radiation. Countermeasures to radiation include using antioxidant cocktails in concert with protease inhibitors during the cryopreservation of the samples to reduce radiation-induced oxidative stress and death (Kennedy 2014). In addition, providing physical barriers, such as water, lead, cement, regolith, and newly developed materials, can passively or actively block radiation (Naito et al. 2020, Warden and Bayazitoglu 2020). This may be remediated by using proven radiation-hardened materials used for satellites (Messenger 2020).

A third challenge is temperature. Although certain areas of the surface of the Moon may reach 100°C during the lunar day (equivalent to approximately 14 Earth days in length), craters at the north and south poles, known as *permanently shadowed regions* (PSR), or deep lava tubes at midlatitudes may have a temperature close to –196°C, ideal for long-term storage of biomaterials without significant human intervention. The logistics of transporting biomaterials into these areas at liquid nitrogen temperatures is challenging but tractable, assuming soon-to-be-launched rovers and astronauts can deploy these types of experiments.

A fourth challenge is that the PSRs are thought to contain substantial amounts of ice. Not only is the origin of this ice of scholarly interest to planetary scientists, but it may be an important *in situ* resource for future human missions, providing drinking water, fuel, and oxygen for space explorers. Therefore, PSRs may be highly restricted and managed. Nevertheless, that this ice exists on the Moon indicates that storing frozen or vitrified hydrated samples in similar areas should be possible without sublimation. The NASA Artemis Program plans to eventually sample ice from a PSR and transport it in a frozen state back to Earth. This will involve building pathways to get into and out of these deep polar regions and transporting intact low-temperature material back to Earth. These ongoing efforts will help frame the challenges and solutions for our future work.

A fifth challenge is the long-term effect of microgravity on cells. Microgravity testing was performed on cryopreserved cardiac cells for only months on Earth. When these cryopreserved cells were launched into space and cultured on the ISS, they lived and expanded (Rampoldi et al. 2021, Rampoldi et al. 2022). Still, the long-term effect of microgravity on cryopreserved samples is not known.

Governance

Planning and operating a lunar biorepository will require careful consideration of ownership and long-term governance issues. The Svalbard Global Seed Vault is a public entity established and funded by the Norwegian government and is overseen by an international advisory panel "representing depositing gene banks and stakeholders" (www.seedvault.no/about/purpose-operations-and-organisation). For the lunar biorepository, we recommend a governance process that mirrors Svalbard's: the establishment of a collaborative planning process involving key stakeholders who will include public and private funders, scientific partners, countries, others providing samples, and public representatives. A lunar biorepository to secure the future of Earth's biodiversity should be a public entity, with mechanisms for cooperative oversight. Many questions will require stakeholder input, such as what type of operating procedures might ensure that the preserved samples remain within the public domain from the individual country of origin (or their designee) and how the lunar biorepository might be protected within a treaty governing the site or region in which it is located. A feasibility study for a safety backup system for the Svalbard Global Seed Vault addressed these and other issues (Acker et al. 2017). We are confident that, with wide global stakeholder input and careful considerations of the challenges facing cryopreserved collections, we can ensure a future that will meet the needs of Earth and planetary exploration for this proposed biorepository on the Moon.

Conclusions and next steps

This is a decades-long program. Realizing a lunar biorepository will require collaboration by a broad array of nations, cultural groups, agencies, and international stakeholders to develop acceptable sample holding, governance, and long-term plans. Protecting Earth's life must be a top priority in the rush on the Moon sites for industries and many types of science (Clery 2023). Our near-term next steps include expanding our partnership base, especially to include laboratories and agencies who work in space research; extracting and cryopreserving fibroblast cells from the cryopreserved fins of fishes and testing their packaging under space-like conditions on Earth; securing support for testing in the ISS; and creating sample and banking methodologies for partners collecting on Earth.

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Data availability

No new data were generated or analysed in support of this research.

Author contributions

Mary Hagedorn (Conceptualization, Data curation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing), Lynne R. Parenti (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Supervision, Writing – original draft, Writing – review & editing), Robert A. Craddock (Conceptualization, Writing – original draft, Writing – review & editing), Pierre Comizzoli (Conceptualization, Writing – original draft, Writing – review & editing), Paula Mabee (Conceptualization, Writing – original draft, Writing – review & editing), Bonnie Meinke (Conceptualization, Writing – original draft, Writing – review & editing), Susan M. Wolf (Conceptualization, Writing – original draft, Writing – review & editing), John Bischof (Conceptualization, Writing – original draft, Writing – review & editing), Rebecca D. Sandlin (Conceptualization, Writing – original draft, Writing – review & editing), Shannon N. Tessier (Conceptualization, Writing – original draft, Writing – review & editing), and Mehmet Toner (Conceptualization, Writing – original draft, Writing – review & editing)

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