



n February of 1977, an oceanographic expedition studying hydrothermal vents at the bottom of the Pacific Ocean made a discovery that changed biology forever. Two kilometers deep, near a volcanic zone northeast of the Galápagos Islands, explorers onboard the deep-ocean submersible Alvin found four dense agglomerations of clams, mussels, crabs, anemones, and other creatures. Some of them were living among an alien-looking variety of tubular worms that vaguely resembled giant albino tulips. In awe, the researchers — probably inspired by the stark contrast with the barren seafloor beyond the vents - named the fourth, tubewormbedecked spot the "Garden of Eden."

At the time it was a mystery how this lush ecosystem could survive at such depth. Biologists had assumed that sunlight was the energy source that powered all life on Earth, effectively constraining the habitable zone to a thin layer on the planet's surface. Within that layer, photosynthetic organisms capture solar photons and use the energy to split water molecules. They then combine the hydrogen with carbon from the atmosphere to form sugars, releasing oxygen as a byproduct. In that scheme, all the other organisms, no matter how high up in the food chain, depended on the yield of these primary producers.

But these giant tubeworms survive thanks to their ability to host chemosynthetic bacteria inside themselves. Like photosynthetic organisms on the surface, these bacteria also produce their own sugars, but instead of using sunlight there is none 2 kilometers deep in the ocean — they obtain energy from chemical reactions. In this case they do it by oxidizing hydrogen sulfide present in the warm waters. Later research revealed that other types of chemosynthetic bacteria live freely inside and around the vents, occupying the bottom of the food chain in these isolated ecosystems.

This discovery showed that life is much more versatile and resilient than previously thought, opening the scope of where to look — and what to look for — when searching for life. It has led to the realization that, in our quest to find signs of life on Mars, maybe we've been looking in the wrong place.

## From Underwater to Underground

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It didn't take long for scientists to realize that one of the places they should be looking for chemosynthetic life forms was under their own feet. At first they piggybacked on com-

> mercial drilling and mining operations, then later on they explored caves and conducted their own drilling campaigns. By the early 1990s, researchers had collected enough evidence to show that Earth's crust is populated by a variety of microbes sustained by chemosynthetic organisms.

This deep biosphere, as it's called, extends from a few meters below the surface to several kilometers down, depending on the local conditions. It's mainly populated by bacteria and other single-celled organisms called archaea, although recent research has also found fungal species and even animals, such

as nematode worms and tiny multicellular creatures called rotifers. In some places, where the conditions allow it, these communities can live in the pores and cracks of rocks up to 10 kilometers deep.

An expansion of scientific drilling projects in the last two decades has confirmed the findings and extended the range of subsurface environments where microorganisms can live, from the ancient continental crust to the younger and more



▲ HYDROTHERMAL TULIPS Giant tubeworms (Riftia pachyptila) live among anemones and mussels at a deep-sea vent on the Galápagos Rift. This is one of the largest concentrations of Riftia found so far.



▲ NEMATODES Members of the species Monhystrella parvella inhabit a stalactite 1.4 km underground in the Beatrix gold mine in South Africa. Each nematode is a couple hundred microns long.

dynamic oceanic crust. This implies a huge diversity of living conditions, for the most part under high temperature and pressure, but also in the near-freezing environments under the polar ice sheets or basking in the heat of radioactive minerals.

Although life is pervasive underground, it's more austere than on the surface. The lack of sunlight and oxygen limits the energy supply. Subsurface organisms have slower metabolisms and are much less abundant than their surface counterparts. While one gram of surface soil can host more than 10 billion microbes, one gram of oceanic crust may contain only 10,000 cells, and continental crust one-tenth of that.

However, the volume of the deep biosphere is huge when compared to the surface world. Subsurface inhabitants therefore make up an important fraction of all life on Earth. "We've estimated that subsurface microbial life is about 10<sup>30</sup> cells," says Tullis Onstott (Princeton University), a pioneer in the field who has been involved in subsurface-life research since the 1990s. "That's more than [the] stars in the visible universe and as many cells as in the surface world." Onstott's team estimates that subsurface life could represent around one-tenth of Earth's total biomass.

In order to subsist, chemosynthetic organisms — or *chemolithotrophs*, as they are called when they are able to extract energy from inorganic compounds — need to pair substances that can act as electron receivers and electron donors. When an electron jumps from the donor to the receiver, there is a small energy release these microbes can exploit. Luckily for them, many geological processes can provide this kind of chemical pair.

One example is the decay of naturally occurring radioactive elements within the rocks, such as uranium, thorium, or potassium. As these disintegrate, they emit high-energy particles that can break water molecules. This process, called *radiolysis*, releases huge quantities of hydrogen and reactive oxygen. Hydrogen is like a super food for microorganisms: It's so eager to donate electrons that even poor receivers such as sulfates can oxidize it, making it the ideal microbial fuel for the deep underground.

Another source of free hydrogen is *serpentinization*, a process in which iron-rich minerals react with water, filling the environment with leftover hydrogen that microbes can use. In some cases serpentinization can also produce hydrocarbons such as methane, another favorite meal for many microorganisms. Not only can they grab its hydrogen for food, they can also use it as a source of carbon.

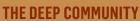
Recent analyses have shown that underground dwellers are genetically diverse. There are even microbe species that are unique to the subsurface and cannot survive on the surface, raising important questions. Is subsurface life merely a result of what trickles down from the surface? How long have these species been evolving in the darkness? Could life have originated underground and then colonized the surface?

Both surface and subsurface life follow the same DNA blueprint, suggesting that life had a single origin, but it's not clear how or where it initially appeared. "There is much we don't understand about the origin of life on this planet," says Barbara Sherwood Lollar (University of Toronto, Canada), a geologist specialized in characterizing underground water reservoirs. "Certainly I don't think we understand yet the kind of environment where life first arose."



▲ ANCIENT WATERS Members of Barbara Sherwood Lollar's team take samples of water in rock fissures deep in Beatrix Mine in South Africa. The team has found evidence of life in this mine and others.

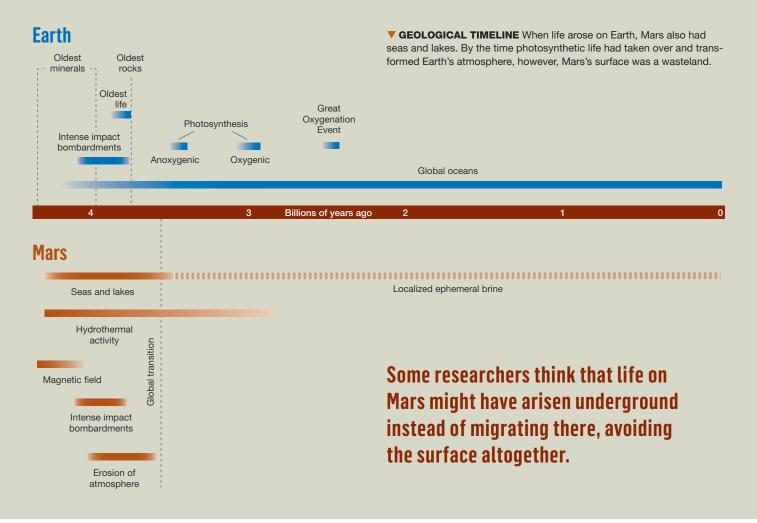
In 2013, Sherwood Lollar and her team discovered the oldest underground water ever found in Earth's crust, a thin network of veins 1.5 billion years old hidden 2.4 kilometers deep inside a mine in northern Ontario, Canada. This mineral-rich water contains the electron donors and receivers that organisms need to survive, showing that these habitable environments can be preserved over long time scales, something that could also happen on Mars. Sherwood Lollar and her collaborators have found signs of microbial life in the water, but although they are sure the life isn't a modern arrival, there is no way to say how long it's been there. It could have been isolated from the upside world for at least hundreds of millions of years.



According to a 10-year study by the international Deep Carbon Observatory team, 70% of Earth's bacteria live underground.







But on other worlds, could pockets of underground life have originated independently from the surface?

"It's a question that is very important but we cannot address yet, whether or not life can originate in a subsurface environment," Onstott says. "When you think of Europa or Enceladus or any of the icy satellites and planets that exist out there that have subsurface oceans but never had a surface ocean, if life can originate in the subsurface then there's a chance that life exists there."

Even if humans cannot explore the oceans of Enceladus yet, there is a place within our reach that has potential for underground life: Mars.

## **Next Stop: The Red Planet**

Although the Martian surface is currently inhospitable to life, various lines of evidence indicate that until about 3½ billion years ago, the Red Planet had surface water and an atmosphere (*S&T*: July 2018, p. 14). If life had time to appear on the surface during the billion or so years of clement conditions, then it might have also colonized the Martian underground, where conditions would have remained stable long after the surface became hostile. These life forms could have left fossils or other signs of their presence. Some scientists

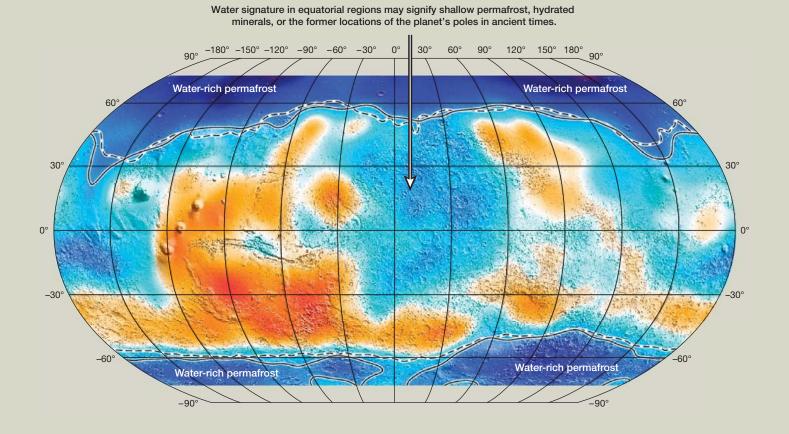
even think that this life could persist underground today.

Recent studies suggest that the same geological processes that provide energy for subsurface microorganisms on Earth — serpentinization and radiolysis — occurred on Mars. NASA's Mars Odyssey spacecraft has found an abundance of the radioactive elements thorium, potassium, and uranium in the modern Martian crust. In eons past, these elements could have produced a global habitable subsurface several kilometers thick, thanks to radiolytically generated hydrogen. This could have provided enough chemical energy to support microbes for hundreds of millions of years, as long as there was enough water to split.

"There is no reason why you couldn't take the same organisms we find three kilometers down in South Africa and just teleport them to the subsurface of Mars, they would do just fine," says Onstott. "Deep below the surface [life] could be quite pervasive and quite active."

On the other hand, Mars might not have given life the chance to evolve on its surface. By current estimates, life appeared on Earth sometime prior to 3.7 billion years ago, roughly the same time that Mars's outer core stopped churning and the planet lost its magnetic field, exposing its atmosphere to the gusty solar wind. Photosynthetic life appeared on

▼ UNDERGROUND WATER This map from the ExoMars Trace Gas Orbiter shows hydrogen's distribution (bluer colors mean more hydrogen) in the uppermost meter of Mars's surface. Hydrogen might indicate water, water absorbed into the surface, or minerals formed in water.



Earth soon after, and by the time it became widespread enough to dramatically boost our atmosphere's oxygen content, Mars's surface had been a frozen and hyperarid desert bombarded by high-energy radiation for more than a billion years.

For this reason, some researchers think that life on Mars might have arisen underground instead of migrating there, avoiding the surface altogether. In their view, searching for evidence of life on the Martian surface is a biased approach fueled by what we see on Earth. Here, photosynthesizing surface life is extremely abundant, favored as it is by a protective atmosphere and magnetic field, a moderate climate, and easy access to water. One way or the other, scientists increasingly think that Mars research needs to shift its focus from the surface to the underground. Even if life got a foothold above ground, the harsh surface conditions might have wiped out any organic remains or other revealing signs, thwarting any life-searching missions based on surface features.

"Anything related to life, extinct or extant, leads us to the subsurface," says Vlada Stamenković (NASA Jet Propulsion Laboratory), an ardent advocate for subsurface Martian exploration. "It's clear that if we really want to understand if there ever was or is life on Mars, then we really have to dig into the subsurface and explore what's beneath."

## **Looking for Subsurface Life**

Finding out if there is or has ever been underground life on Mars is not a straightforward proposition. First of all, we don't really know the physical properties of what lies below the surface. Things like temperature and water availability remain big unknowns. We only know there is a frozen layer close to the surface and a core that is probably still warm, making it likely that a temperate zone exists somewhere in between.

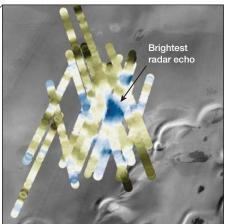
"These are reasonable conjectures, but we won't really know until we study Martian geophysics," says Ricardo Amils (Center for Astrobiology, Spain), who has worked extensively in characterizing southern Spain's Rio Tinto region, considered one of the best Martian analogs we have on Earth. Still, he's confident scientists need to look beneath the surface to fully assess the Red Planet's habitability. "If there is life on Mars it has to be in the subsurface, there is no doubt about that."

That's why NASA's Insight lander is an important first step towards the exploration of the Martian underground. The probe landed on Mars on November 26, 2018, carrying a seismometer, a thermal probe, and radio antennas, which together will reveal key aspects of Martian geophysics, such as the size and physical properties of the planet's core, mantle, and crust, as well as details about its inner heat flow.

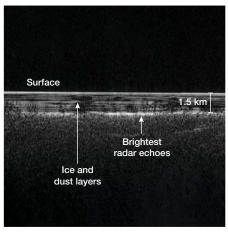
## Mars South Polar Region



Mars Express Radar Footprints



Radar Image of Subsurface



▲ SUBSURFACE LAKE? Multiple passes by the European Mars Express orbiter reveal a highly reflective layer about 1.5 km below layers of ice and dust near Mars's south pole. Scientists suspect the 20-km-wide "anomaly" (blue triangle in radar footprints, center image) is a brine patch or lake. The righthand panel shows an example radar profile of the region.

Another open question is the availability of liquid water below the surface. Orbiters have found that a frozen layer of soil and water ice called permafrost is common in the polar regions and covers large swaths of the rest of the planet, including equatorial areas. But little is known about what lies below. In July 2018, scientists using the MARSIS radar instrument onboard the European Space Agency's orbiter Mars Express announced they'd detected hints of liquid water 1.5 kilometers deep below the southern polar cap. While the finding remains controversial, researchers think that these are probably brines, bodies of water-soaked salt.

The detection would support the idea of an underground where high pressure and milder temperatures can make liquid water available. Although water's presence wouldn't mean life is present, it certainly would make things easier. "Life doesn't need a lot of water," says Amils. "Until now it was said that if there isn't liquid water there can't be life. Well, there is water."

Obviously, the most direct way to solve these questions is drilling, but current and future planned missions have limited digging capabilities. The Curiosity rover can grind just a few centimeters into the rock, and the upcoming European ExoMars 2020 rover will be able to drill up to 2 meters down. Insight, meanwhile, spent months stalled at a fraction of its 3-meter goal — still nowhere near where a deep biosphere might have existed.

"Mars exploration has been focusing so much on the surface that there has been very little investment in real subsurface exploration," says Stamenković, who is leading a concept design for a solar-powered Martian drill called Ares Subsurface Great Access and Research Drill (ASGARD), able to reach depths of at least 1 kilometer. Stamenković was recently part of a workshop hosted by the Keck Institute for Space Studies, where scientists and industry representatives discussed the future of underground Martian exploration. "We've realized that, actually, drilling technology has a lot to offer, there has

been just little investment so far," he says. "As with many things on this planet, it's not the technology. It's the funding that is limiting."

However, Stamenković's optimism clashes with Onstott's experience chasing deep life. Even on Earth, he warns, drilling through frozen rock is an energy-intensive process that is prone to equipment failures and unexpected engineering challenges, from frozen pipes to broken drill bits.

For the time being, researchers will have to exploit other opportunities to study the Martian subsurface until drilling technologies become available. That means relying on indirect measurements and looking for certain features that are far less exciting than those uncovered by drilling kilometer-deep holes. Instead, researchers could look for exposed crustal rocks with unusual metal or carbonate accumulations, *biotextures* in rocks caused by interactions with microbes, or the buildup of organic molecules in fractures or fluid inclusions in rocks.

While the arguments for going deep in Martian exploration are sound and the possible outcomes fascinating, it seems that we will have to put our curiosity on hold. Even if indirect methods can reveal hints at what lies below the surface, only specialized instruments and drilling will provide definitive answers. Based on his experience, Onstott thinks that humans will not reach the Martian underground until after humans establish a base on Mars and need to access water below the surface for a permanent colony. "That's an important question for them," Onstott says. "When they access the water, will there be Martian organisms in it?"

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Read the story of the 1977 hydrothermal vents discovery at https://is.gd/1977ventsdisc.