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# CONTENTS



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# Lichen fungi and their relatives

**Life on Earth was single-celled for a long time. Several important milestones mark the path to where we are now, and symbiosis—the "living together" and toolbox-sharing of two organisms—features prominently in this lineup. Scientists are now convinced that the switch to cells with nuclei—eukaryotes—happened around 2.5 billion years ago, when an early unicellular organism in a group called Archaea enveloped, but did not digest, a bacterial cell.**

This new cell—nicknamed by scientists LECA, for Last Eukaryotic Common Ancestor—is thought to have acquired a powerful advantage over its predecessors. Not only could it carry on with its archaean predator machinery, it could tap into the bacterial baggage it had incorporated to generate adenosine triphosphate, or ATP, a major source of energy for the cell. The twinning of these two toolboxes propelled LECA into places where no archaean had gone before. Passed from generation to generation, the captured bacterium would eventually evolve into what we call mitochondria.

→ *Ourasphaira giraldae*, an enigmatic Late Mesoproterozoic to Early Neoproterozoic fossil considered the oldest fungus-like fossil known to date, from around one billion years ago.

Something similar happened again around a billion years later. Some of the eukaryote descendants of LECA took up another group of nifty bacteria that had been on the scene for a while: cyanobacteria. Cyanobacteria had previously evolved the ability to fix carbon through photosynthesis, giving them the luxury of not having to eat other organisms to make a living. The green descendants of this event are now called plants.

### THE RISE OF PROTOLICHENS?

So how did we get from there to lichens? Fungi (and animals) are descended from one of the eukaryotic lines that did not go green. Some of the earliest fungi have been estimated to have arisen around one billion years ago, at the end of the Mesoproterozoic era, probably in shallow marine environments when much more of the Earth's surface was covered in water than it is today. The Mesoproterozoic saw some important advances in the evolution of life, including sexuality and multicellularity. At the same time, fungi had to contend with challenges such as low oxygen levels.



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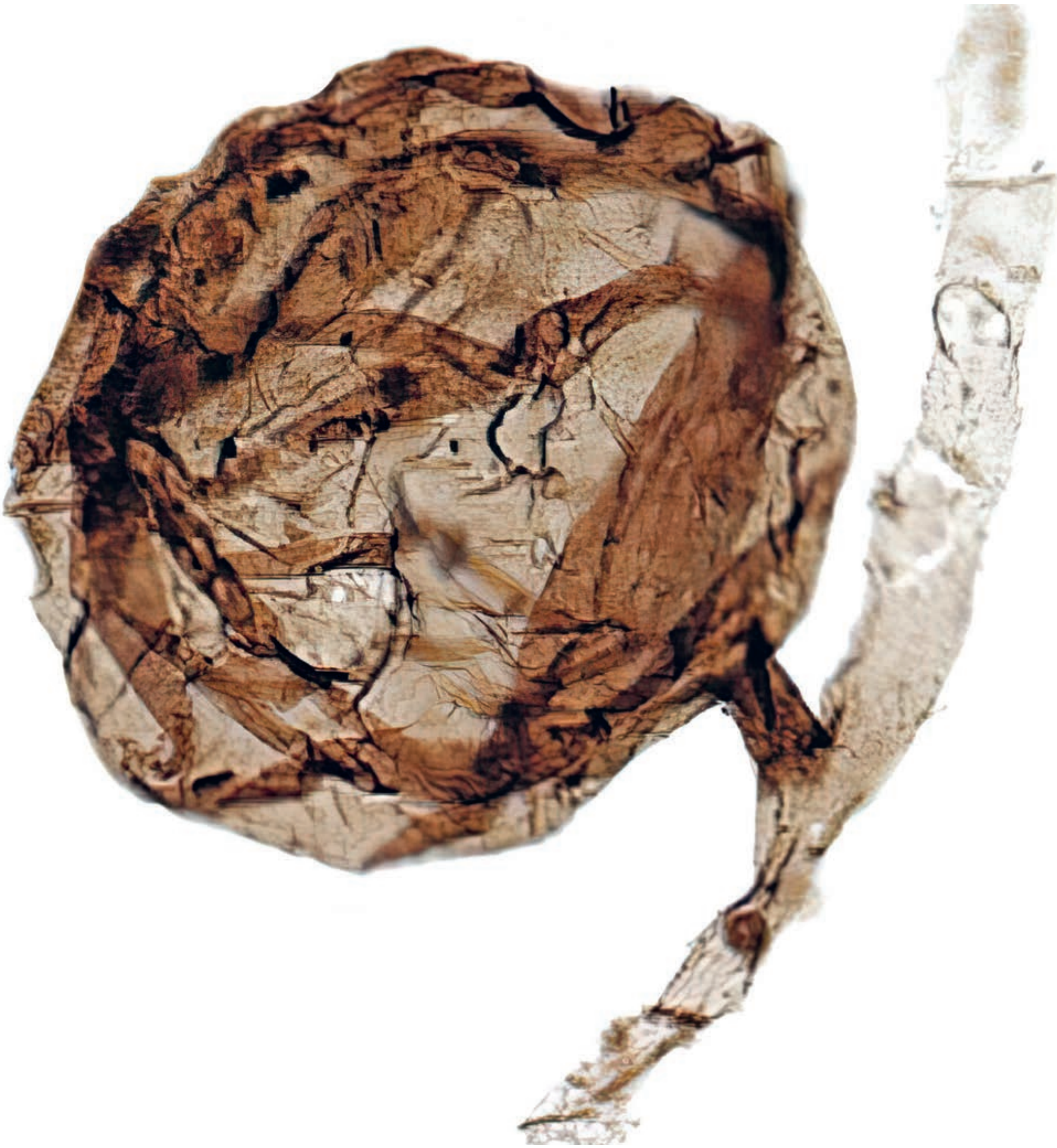



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Keeping tabs on our timeline, Mesoproterozoic fungi would have been in the aqueous company of both a well-established veteran community of cyanobacteria that had already been around for a respectable billion years, as well as some upstart plant prototypes in the form of early green algae that were themselves probably not more than a few cells in size. What kind of lifestyle these early fungi led, and how the terrestrial fungi we know today got started, is the matter of much debate. One intriguing hypothesis is that they began appearing on ancient seashores as lichens—the so-called "protolichen hypothesis." Under this scenario, the majority of modern fungi we are familiar with, from yeasts to mushrooms, would be the descendants of fungi that got their first leg up, so to speak, in a lichen-themed amphibious landing vessel.

As intriguing as this idea is, we do not have a lot of data to back it up. Fossils of fungi and lichens do not preserve well and are exceedingly rare, especially this far back in time. Even with the most sophisticated DNA-based reconstruction techniques, it is difficult to be certain about evolutionary events that far back

↖ *Mycosphaerella punctiformis* is a saprotrophic microfungus occurring on dead or dying leaves, especially of oaks, but is also found in healthy leaves. It is closely related to *Cystocoleus ebeneus*, a fungus involved in tiny, filamentous lichens. The genus *Trichoglossum* (here *T. hirsutum*) likely forms mycorrhizae with plants, but is part of a clade containing the likes of the Candleflame Lichen (*Candelaria concolor*), certain pin lichens, and certain cyanolichens.

↗ The mushrooms *Hydnum repandum* and *Arrhenia spathulata*, the first mycorrhiza-forming, the second associated with mosses, are close to the lichen-forming genera *Multiclavula* and *Dictyonema.*



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in the past. The uncertainty is compounded by the fact that for both fungi and algae, a billion years might be long enough for them to transition from one lifestyle to another and back again, perhaps multiple times. What we know from DNA sequence data is that fungi do indeed go back around a billion years. Recently, the oldest fungus-like fossils have been reported from right where we suspected they would have first evolved: the Late Mesoproterozoic.

## A LONG FLIRT WITH SYMBIOSIS?

Many fungi are associated with terrestrial plants, but it is thought that plant life conquered the land "only" about 500 million years ago. So what happened in the 500 million years between the appearance of the earliest fungi and the appearance of the first land plants? Were fungi and algae already "tinkering" with a relationship during this long period? And if so, in what way and with what outcomes?

Pondering how fungi began to get involved in making lichens goes hand in hand with the question of what kind of lifestyle the predecessors of those fungi had. While the earliest origins of fungi are too far back to reconstruct these lifestyle transitions, a few of the origins of lichens are recent enough that DNA is starting to yield some clues. By reconstructing the genomes of recent common ancestors, scientists have found that lichen fungi in the early phases of a switch to symbiosis have genomes similar to those of saprotrophs—fungi that feed on dead material, such as decaying plants, or algae, for that matter. Some lineages of lichen fungi are indeed still closely related to saprotrophs, or even switch back and forth between a saprotrophic and a lichen lifestyle. Only a few such transitions have been studied using genome data, and more surprises may be in store.



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# The life of a fungal symbiont

**As humans, we tend to relate to other animals in proportion to how closely related they are to us. Other mammals? Those adorable eyes! They care for their babies! The empathy rapidly breaks down when it comes to more distantly related animals such as fish or insects, and it is safe to say it is gone altogether when we consider animals' next of kin, the fungi. To begin life, go through middle age, and eventually die as a fungus is all but impossible for us to imagine. But it doesn't mean we can't try.**



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Animals and fungi are siblings in the tree of life, and both derive their nutrition from eating things—they are heterotrophs. When, early in evolution, the first fungi and animals went their separate ways, animals, even single-celled ones, maintained their ability to engulf their food, a subset of the heterotroph lifestyle known as phagotrophy. Fungi, by contrast, secrete degradative enzymes to break down their food *outside* of their cells and then vacuum it up through the cell membrane. This, in turn, is called osmotrophy, and it fundamentally defines what it means to be a fungus.

## MAT MEETS MAT, A LOVE STORY

Fungi begin life as a spore. They sporulate, producing a sporeling. They can proceed to forming hyphae, which make up a mycelium. Their sex is determined by a specific gene sequence they inherit at an address on their genome known as the mating type locus. Most lichen fungi, about 99 percent, belong to the large group known as sac fungi, or ascomycetes, and these typically possess two different sexes, or mating types. Fungal biologists refer to these rather unglamorously as MAT1 and MAT2. Curiously, some fungi, including many basidiomycetes (the group that includes mushrooms, bracket fungi, and their relatives), have more than two sexes, and some have thousands.

So far, so binary, at least for the sac fungi. But even with only two sexes, to get the job of reproduction done, different fungi take wildly different paths. In the scheme that may seem most familiar to us humans, a fungus may begin life with a single MAT sequence—a sex determinant—and then needs to find a different individual of the same species with the opposite mating type. How a fungus finds a mate is not trivial, as fungi don't bellow out mating calls and travel to find partners. But if the search goes well, they happily conjugate, their nuclei fuse, and a fruiting body, such as an apothecium, is formed. This approach to mating is called heterothallism.

That's all well and good for some fungi, but it is not always such a straightforward MAT-meets-MAT love story, and here is where it gets harder to relate to. Oddly perhaps for us, the important thing for fungi, including those involved in lichens, is that the two MAT DNA sequences may both be there somewhere *in the cell*. So some fungi carry both MAT types on the same genome from a spore onwards, and can form sexual fruiting structures without any second fungal individual at all. This is called homothallism. One of the downsides of homothallism is the lack of genetic diversity, and much effort has been invested in studying the long-term effects of homothallism in fungi related to those in lichens.

## A PHOTOBIONT IN THE BEDROOM

For the many fungal species that are heterothallic, the two mating types need each other to form sexual spores. Since fungi do not have the mobility of animals, getting to each other is a bit of an engineering problem. The solution that has evolved is that mycelia form tiny asexual buds called conidia that are dispersed by wind, rainwater, or the leg of a passing beetle, and some small percentage of these will reach a mycelium of the compatible mating type. Here, they might get lucky and stick to a trichogyne, a hypha sent up by the opposite mating type (see page 53).

And then it's Valentine's Day—but only if the fungi are in a lichen. Remarkably, lichen fungi never reproduce sexually unless a photobiont—an alga or cyanobacterium—is present. Nobody yet understands what exactly the fungus receives to initiate this process, but it must be something that helps get the job done. And this leads us to the next set of players.

← Most lichen fungi studied so far are heterothallic, such as the New Zealand and Tasmanian endemic *Knightiella splachnirima*.



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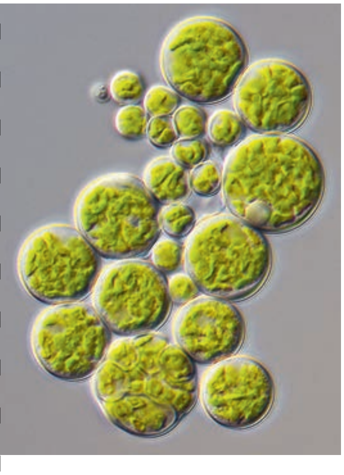
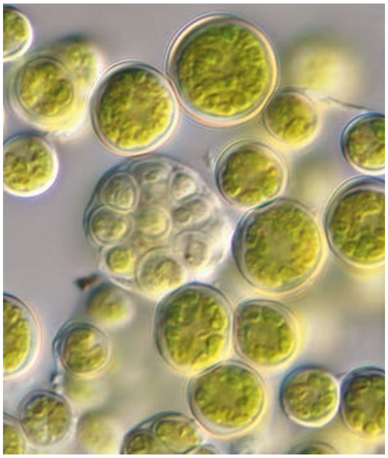
# Lichen algae

**As we saw earlier, the path to recognizing that green, photosynthesizing cells inside a lichen are a separate organism from the fungus in which they are embedded was a long and tortuous one. But that only led to more discoveries. It turns out that these green microorganisms have complex lives of their own.**





## PHOTOBIONTS

Not all of the algae identified as such by scientists in the 1860s were in fact algae. Cell traits and later DNA data revealed that alga-like organisms arose multiple times in the tree of life, and many are not even closely related to each other. Nonetheless, the name "algae" continues to be used for all such organisms that are not bacteria, and "cyanobacteria" for those that are. Lichenologists refer to the photosynthesizing partners, regardless of evolutionary origin, as photobionts.

Of the 20,000+ lichen symbioses that scientists have named, the large majority involve algae. Algae, for their part, are no better off than fungi when it comes to human relatability.

↖← *Trebouxia* (*T. jamesii*) and *Symbiochloris* (*S. symbiontica*) are common green algal lichen symbionts, the latter found in lung lichens and their relatives, such as the neotropical *Yoshimuriella peltigera* (bottom).

→ The parasitic green alga *Cephaleuros virescens* (top) attacks leaves of several trees. The lichenized fungal genus *Strigula* (bottom) associates exclusively with this photobiont, and often causes wounds in the leaves (or growing around them).



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Depending on your perspective, the word "alga" may conjure images of nori, kelp, or toxic blooms, but the algae that occur in lichens are none of the above. They are single-celled, or simple multicellular thread-like organisms. When they occur as free-living organisms, we perceive them as powdery green coatings or minutely hairy orange fuzz on tree bark and rock, if we notice them at all. Like fungi, these algae interact with their surroundings by secreting and taking up biomolecules through their cell walls. Where fungi and animals are heterotrophs, algae are almost always autotrophs—deriving their food from sunlight, water, and carbon dioxide.

## SECRET CRAVINGS

But are they *only* autotrophs? It has long been known that some algae, including those found in lichens, grow as well or better in total darkness as in the light, and for prolonged periods of time. This is something that cannot, for the most part, be said of plants. It suggests that, while these algae can live autotrophically, making their own food from water and $CO_2$, they have a secret back-up plan. Indeed, it is now understood that algae, in addition to performing photosynthesis, can use other sources of carbohydrates for food much as fungi do, and when they do, they are at least partially heterotrophs, just like fungi.

Organisms that switch in such a way between different nutrient modes are called mixotrophs. One curious example is the algal genus *Cephaleuros*, which behaves more like a fungus than an alga, living parasitically on plants, including important fruit trees such as citrus. *Cephaleuros* is also the photobiont of the lichenized fungus *Strigula*.



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## ALGAL REPRODUCTION

Algal sex, like fungal sex, is a cryptic business, and it is not often they are caught *in flagrante*. In fact, algae are thought to rarely reproduce sexually when in lichens, so much so that until 30 years ago lichen textbooks stated matter-of-factly that they were incapable of sex. (Notable exceptions are the aforementioned *Cephaleuros* and the genus *Phycopeltis*, which often produce sporangia in healthy lichens found on leaves in the tropics.) Since then, many lichen algae have been cultured *in vitro* in labs.

In contrast to the fungus, algae almost never reproduce sexually when inside a lichen, and to say these single-celled organisms are difficult to observe in nature when outside of a lichen is an understatement. Nonetheless, they can sometimes be observed reproducing sexually in a petri dish. When a lichen algal cell is feeling frisky, it begins to internally differentiate into specialized structures called zoospores that bear flagella at one end. These are basically little motile whips that can be wiggled to move the cell around, most effectively in water. Algae, like fungi,

↖↗ *Trentepohlia* algae (left and middle left, with close-up middle right) are the only eukaryotic lichen photobionts readily visible when they occur outside lichens. Lichens containing these algae—such as *Thelotrema lepadinum*, the Bark Barnacle Lichen (right)—often have specific habitat requirements.



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come in different mating types, and with luck one of these flagellum-whipping zoospores might meet another of the opposite mating type. They fuse, lose their flagella, and grow into a new algal cell.

## LICHEN ALGAE OUT AND ABOUT

Lichen algae grow well without having fungi around, and most are thought to also live outside of lichens in nature. But where do they live when they are not in lichens? The Austrian phycologist Elisabeth Tschermak-Woess set out to find free-living lichen algae over 45 years ago, and found them on rock and on the bark of various tree species. Lichen algae are generally "aeroterrestrial" algae, meaning that they occur outside water, and exposed to the air, in terrestrial ecosystems. But even so, finding free-living algae (aside from the flashy orange ones—page 62) is a tricky business, and not something that your local iNaturalist community can help with on Saturday-morning outings with hand lenses. Detection of single-celled algae requires careful swabbing and culturing, and then constant babysitting to pick out algal cell colonies and prevent them from being overgrown by microbial contamination.

In recent years, researchers have resorted to sequencing characteristic DNA signatures from the environment to get a handle on where these elusive microbes occur when they are not in lichens. We still do not have a definitive answer, but some of the most intriguing results show that lichen algae most definitely are floating about in the air we breathe. In fact, both Japanese and French researchers have shown that lichen algae are among the most common microbes in snow and rain—suggesting that precipitation droplets may well even nucleate around these microbial specks.



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# Cyanobacteria: metabolic powerhouses

**For most of the history of modern biology, cyanobacteria were not universally recognized to be bacteria. Instead, they were referred to as "blue-green algae," and in some languages they continue to carry this name.**

The slow migration of cyanobacteria in the classification system from the plant kingdom to bacteria started with an 1853 proposal that they were more closely related to non-blue-green bacteria than to algae. This idea was only fully accepted with the application of biochemical techniques and a formalized definition of bacteria. It was not until 1962 that the Canadian microbiologist Roger Stanier proposed a new name, "cyanobacteria," and this was not fully accepted in bacteriology textbooks until 1974. Old habits die hard.

Early scientists can be forgiven for classifying cyanobacteria as algae, and indeed just like the mitochondria discussed at the beginning of this chapter, the chloroplasts that are the photosynthetic hubs of algal cells are now generally accepted to descend from an ancient cyanobacterial symbiont. That being said, algae and cyanobacteria could hardly come from more distant branches of the tree of life.

Cyanobacteria are true bacteria, and their cellular organization is completely unlike that of algae. They also do not reproduce sexually, instead reproducing by specialized vegetative outgrowths called hormogonia. In contrast to green algal symbionts, most cyanobacteria found in lichens are filamentous, even if in the lichen thallus this organization may be modified to resemble single-celled or colonial organisms.

→ *Nostoc* (A), *Rhizonema* (B), and *Stigonema* (C) are the most common cyanobacterial lichen photobionts. *Stigonema* is found as the primary photobiont in *Spilonema* lichens (here *S. paradoxum*, D).

## FIXING NITROGEN

Cyanobacteria serve many functions in ecosystems on land and in the sea, but most can do one thing outstandingly well: fix atmospheric nitrogen. All of life needs nitrogen as a building block of basic molecules like amino acids, and in many places on Earth it is scarce. Cyanobacteria possess a special enzymatic pathway to convert nitrogen gas out of the air into molecules such as ammonium that are more chemically reactive, and thus more accessible to other forms of life (page 200). The importance of this process is so central to life on Earth that cyanobacteria are credited with playing a role in the early colonization of land around 2.6 billion years ago.

The nitrogen-fixing abilities of cyanobacteria make them attractive partners in symbiosis. Many organisms, from cicadas to plants, have evolved close partnerships with cyanobacteria. One of the groups of cyanobacteria most commonly involved in lichens is the genus *Nostoc*. *Nostoc* species usually form chains when living in lichens, and are typically embedded in a gel of their own making.

A common misunderstanding about *Nostoc* in lichens is that they are always involved in nitrogen fixation. The cells of cyanobacterial filaments essentially divide labor, with special enlarged cells called heterocysts engaged in nitrogen fixation but not photosynthesis, and smaller cells engaged in photosynthesis but not nitrogen fixation. The proportion of heterocysts in a *Nostoc* photobiont can be used as a proxy to estimate how much nitrogen fixation the cyanobacterium does in the lichen, if indeed it is fixing nitrogen at all.



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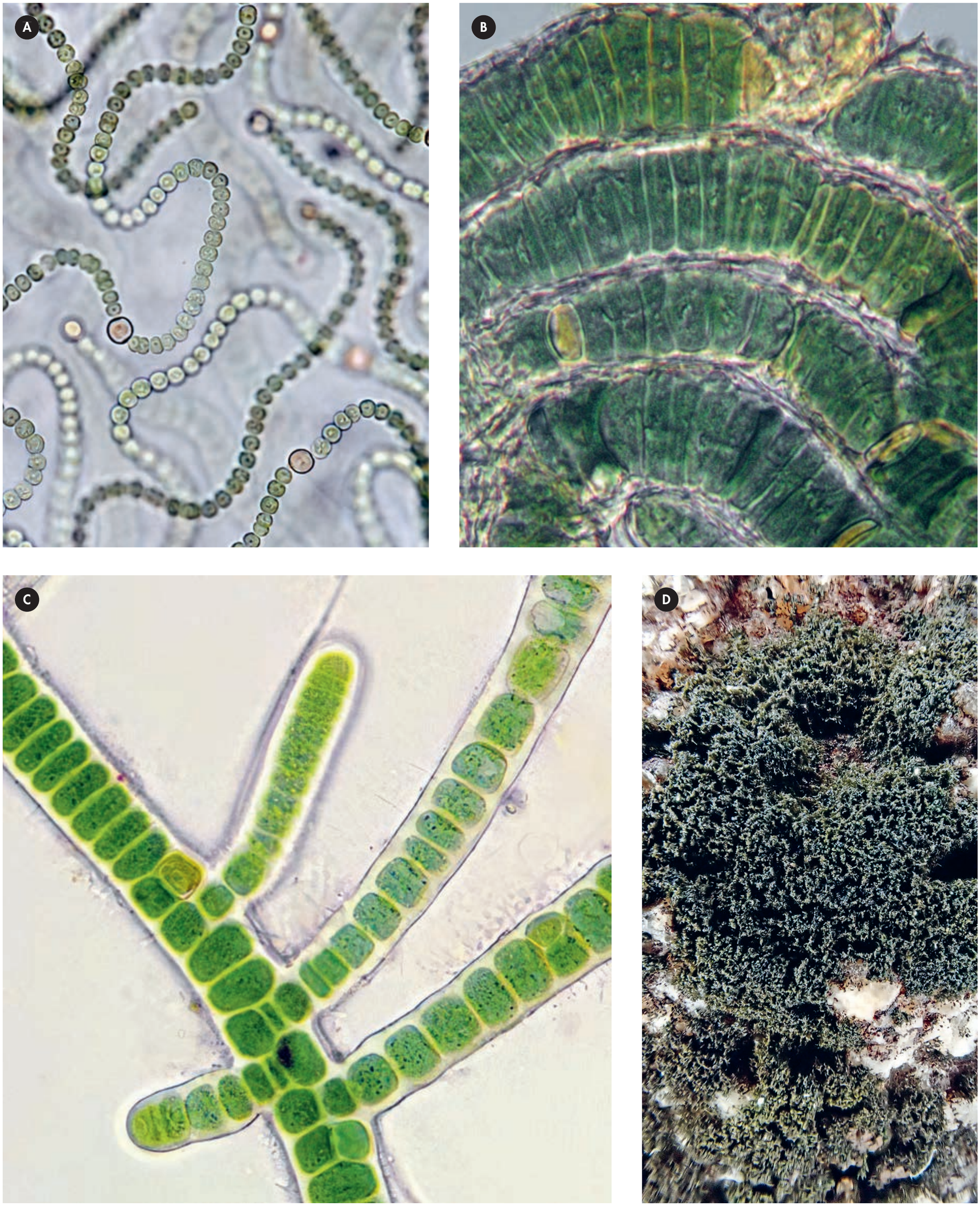



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# A lichen life: from cradle to grave

**Understanding the biology of individual symbionts is key to piecing together the puzzle of lichen symbiosis. Their individual needs must be met. But when they join in making a lichen, many things become possible that neither could accomplish alone.**

↓ Lichen fungi reproducing like fungi. The crust-lichen-forming *Astrothelium megaspermum* disperses its sexually produced spores, which then need to find a new *Trentepohlia* alga to establish another lichen.

So far we have discussed the life cycles of the symbionts, but we haven't especially focused on the lichen itself. How does the lichen, which does not resemble any of its component parts, come to be? One useful metaphor, proposed by the Canadian microbiologist Ford Doolittle, is that of a song sung by a choir with a variable cast of voices. The song remains largely the same—though not exactly—even if individual singers are swapped out, and the song itself can evolve. The rotating door of fungal and algal (and other) symbiont composition makes new lichen songs possible, which can be advantageous to exploring new ecological possibilities. But lichen symbioses have also evolved a way to temporarily lock down the existing singer combination and take some of the luck out of the equation.

## MAINTAINING THE LICHEN SYMBIOSIS

Many lichens forgo fungal sexual reproduction and produce just-add-water go-packs that have everything needed to make a new lichen at a fraction of the cost of sexual reproduction. One of the most common versions of go-pack is called the soredium (plural: soredia), in which a few algal or cyanobacterial cells are enmeshed by a small number of fungal hyphae (see also page 136). Soredia can also carry other symbionts, such as yeasts or bacteria. The success rate of lichen formation from soredia is very high in lab experiments, which suggests that it is in nature as well.

A variation on the soredium theme is the isidium (plural: isidia), in which the package is enclosed in a secreted layer of polysaccharides and thus appears to have a hard shell (see page 136).



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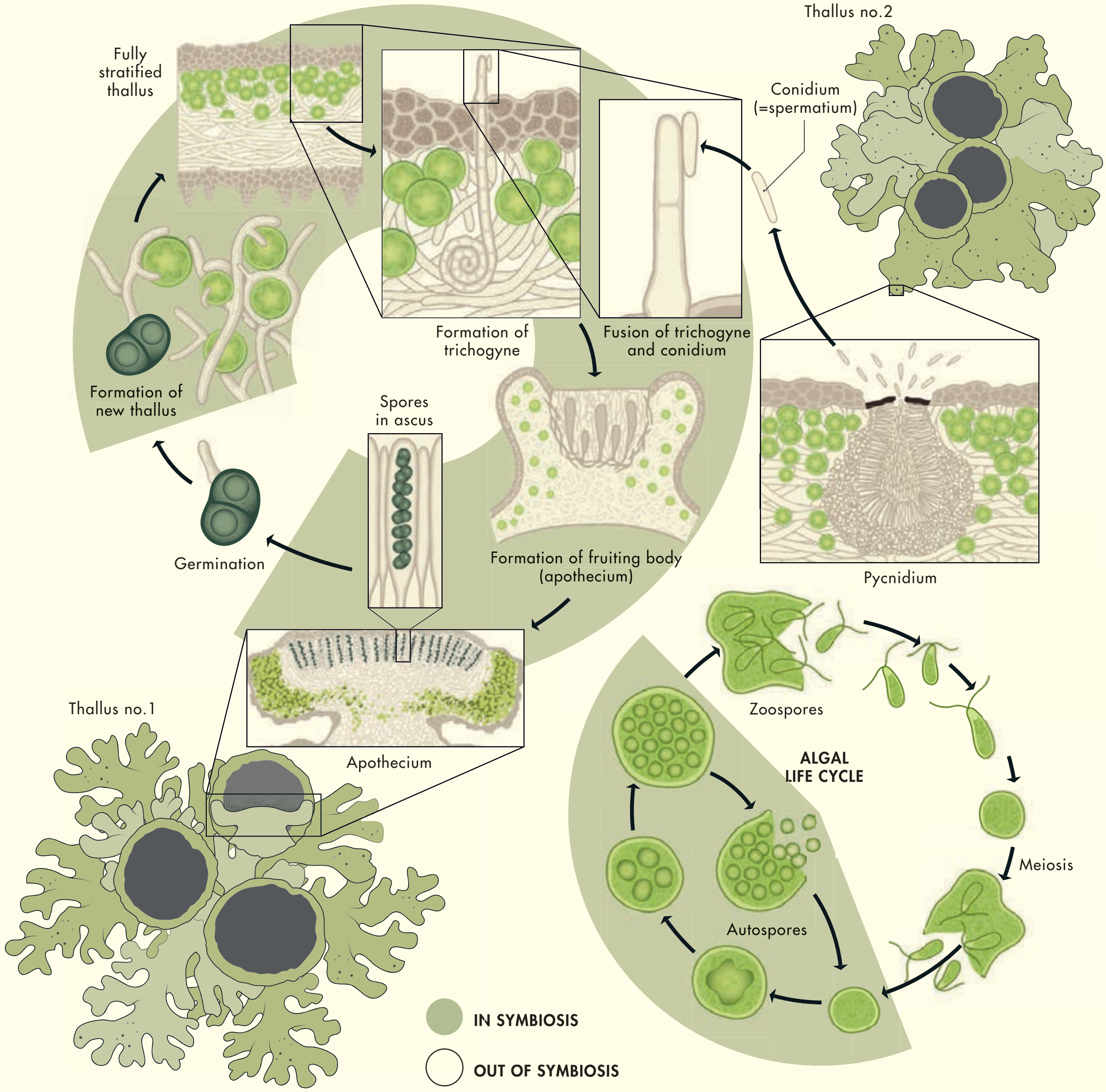


Isidia likely carry even more microbial cargo and probably retain water better than soredia, but have the apparent disadvantage that they are quite heavy as propagules go. Numerous lichens threatened with severe decline or extinction reproduce with isidia. This might be because they cannot readily disperse to new habitats—even if nearby habitat is suitable, it cannot be reached.

Soredia and isidia make it possible not only for fungus and photobiont to co-disperse, but also lichens carry other symbionts such as bacteria and secondary fungi that hitch a ride on thallus propagules; these may play important, if still poorly understood, roles in lichen establishment.

**Sexual life cycles of common lichen symbionts**

The fungus (scheme top left) of a sexual *Physcia stellaris* lichen starts life as an ascospore that germinates outside of symbiosis and, once it has found a compatible alga, forms a new thallus. Sexual reproduction initiates when a microscopic trichogyne from this fungus is fertilized by a conidium from a thallus of a compatible mating type. A *Trebouxia* alga (bottom right) meanwhile reproduces sexually outside of symbiosis, but can also reproduce asexually while in symbiosis, using autospores.



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# Lichen bacteriobionts

**In the early 1920s, an Italian microbiologist by the name of Maria Cengia Sambo was culturing cyanobacteria from jelly lichens when she encountered growths of much smaller bacteria on her culture plates. Another key player in lichens entered the picture, but it would be decades before the discovery would begin to be widely appreciated.**

Cengia Sambo concluded that the bacteria she cultured could fix nitrogen (which indeed many bacteria other than cyanobacteria can do). She was clearly excited by the discovery, and convinced they were part and parcel of the lichen: "*In simbiosi con l'alga stessa e col fungo*," she wrote, "in symbiosis with the alga itself and with the fungus." She was not the only one excited by the discovery. Soviet researchers, in particular, followed up with numerous studies from the 1930s through to the 1980s, trying to nail down more evidence of the role of these "other" bacteria in lichens.

## UBIQUITOUS BACTERIA

We now know that almost all lichens carry these bacteria. As with us humans, bacterial cells can sometimes outnumber all the other cells put together. In lichens, the most common bacteria worldwide come from just four small groups, treated in the bacterial classification system as families. The most common single line of bacteria of these is called *Lichenihabitans*, because, well, it inhabits lichens.

Despite Cengia Sambo's start a century ago, it's still early days in the science of lichen bacteria. It appears likely that many of the pioneering studies did not, in fact, tap into the bacteria we now know to be ubiquitous in lichens, and these, in turn, have rarely been extracted in culture. We only know they exist in so many lichens because when we grind up lichens and extract their DNA, the DNA of the four bacterial families is very often present. While we still don't have experimental evidence on what they do, their DNA has been helpful in this department too, giving many hints.

← A pioneer in lichen microbiology, Maria Cengia Sambo (1888–1939) isolated numerous bacteria from lichens and hypothesized about their function in the symbiosis.



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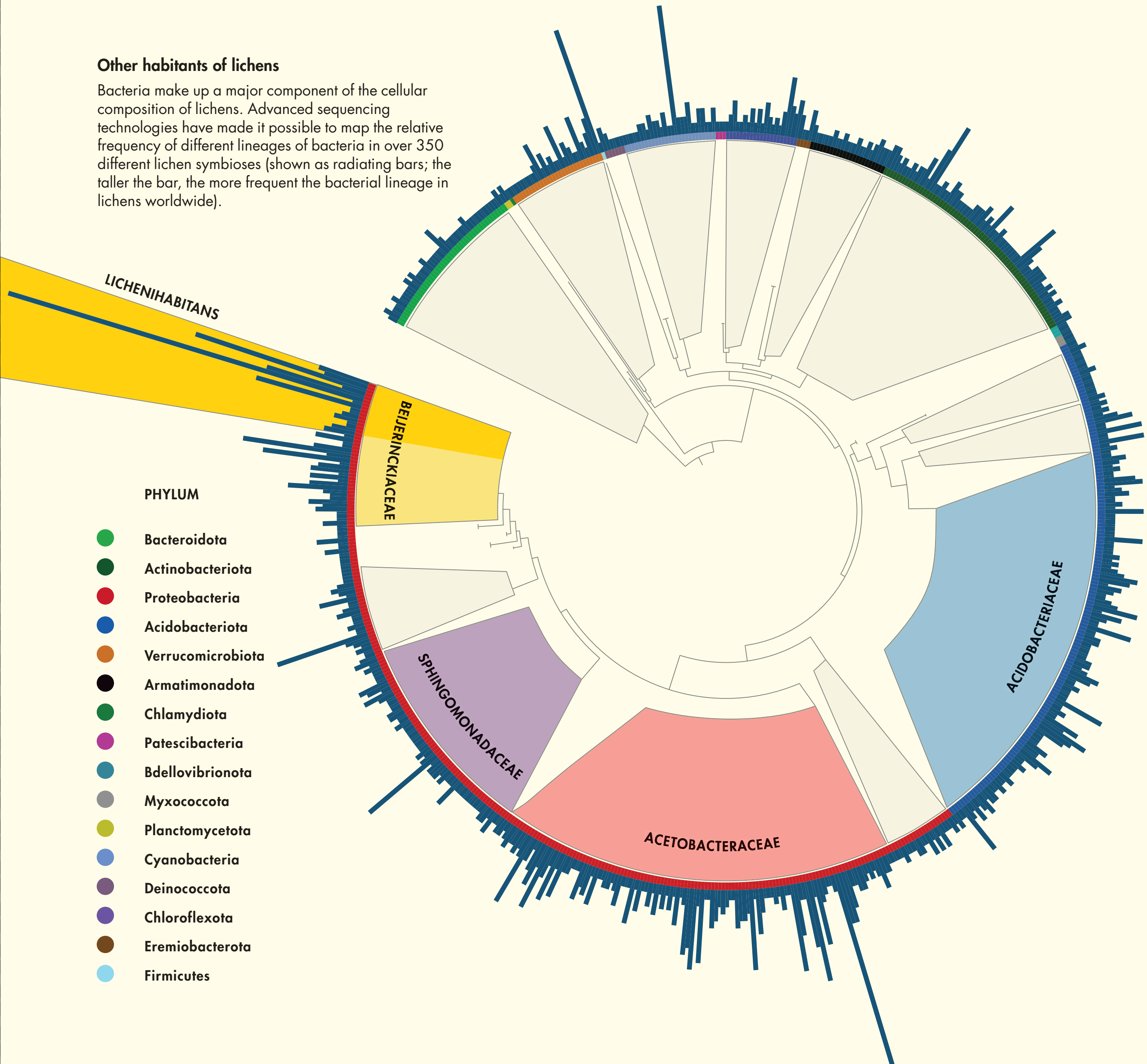
**Other habitants of lichens**

Bacteria make up a major component of the cellular composition of lichens. Advanced sequencing technologies have made it possible to map the relative frequency of different lineages of bacteria in over 350 different lichen symbioses (shown as radiating bars; the taller the bar, the more frequent the bacterial lineage in lichens worldwide).




From the genes the DNA codes for, we now know that the most common lichen bacteria do not, after all, have the machinery for fixing nitrogen; they appear to live off free carbohydrates in the lichen, and they produce vitamins the fungus and alga need. Also, perhaps most oddly of all, they photosynthesize, but not in the way plants do. Instead of generating oxygen with sugar byproducts, they generate high-energy molecules for the bacterium to power other cellular processes.

These closely lichen-associated bacteria have been dubbed "bacteriobionts" by Gabriele Berg and Martin Grube, two of the leading researchers in the field.

One of the most debated topics in lichen science today is whether bacteriobionts are necessary to form a fully developed lichen. Resynthesizing a lichen in the lab is a complicated business with many caveats (page 22), and as of now, we do not know the answer to this question.



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# More than one fungus?

**Lichens may have enjoyed a long time in the scientific limelight as two-partner tangos of fungus and alga, but keen-eyed observers have long known that other fungi often produce fruiting bodies on the surface of a lichen thallus. Some familiarity with the lichen at hand provides telltale evidence that these fungal fruiting structures are not from the main lichen fungal symbiont. What are they doing there?**

With few exceptions, early lichenologists considered these additional fungi to be parasites, or, as they have increasingly come to be known in recent years, lichenicolous fungi. Hunting for, collecting, and identifying lichenicolous fungi is a lichenology subculture of its own, with its own literature, websites, and social media groups. But what does "lichenicolous" really mean, and what kinds of lives do these fungi lead?

## FRIEND OR FOE?

"Lichenicolous" roughly translates to "living in lichens," which helps to establish their address, but tells us little about how these fungi get along with the lichen symbionts. Until very recently, the fruiting bodies of the fungus have typically been the only part that is visible. Only a few fungi, which kill lichen thalli, have visible, spreading mycelia or engender obviously unhealthy symptoms in their hosts. Many others appear to emerge from healthy thalli or apothecia of the lichens themselves, not actually damaging the lichen. What is problematic about this, from the standpoint of interpreting the lifestyle of these fungi, is that the full extent of their mycelium is not easy to ascertain. How much of the lichen do they inhabit?

Studies on the lichenicolous fungi of leaf-dwelling lichens, with their thin thalli, brought to light a notable correlation between the apparent biology of these fungi and their phylogenetic relationships. When growing in a lichen, lichenicolous fungi principally have one of two means of association: either with the lichen fungus or with its photobiont. It turned out that lichenicolous fungi visibly associating with the photobiont of their host generally provoked little or no damage to the host lichen, suggesting that they might be acting as commensals—that elusive category of symbiosis where a partner takes resources, does no harm, but also gives nothing obvious in return. These fungi are often relatives of the lichen fungi that provide their host thalli. On the other hand, lichenicolous fungi associating with the lichen fungus may cause visible and often severe damage to the host lichen, and most of these fungi come from pathogen lineages only distantly related to lichen fungi, such as the genus *Fusarium*.

→ More than 2,000 species of fungi are known to associate with or attack lichens. This small selection features *Biatoropsis usnearum* on *Usnea* lichens (A), *Illosporiopsis christiansenii* on diverse lichens (B), *Pronectria robergei* on *Peltigera* (C), and *Tremella xanthomendozae* on *Xanthomendoza* (D).



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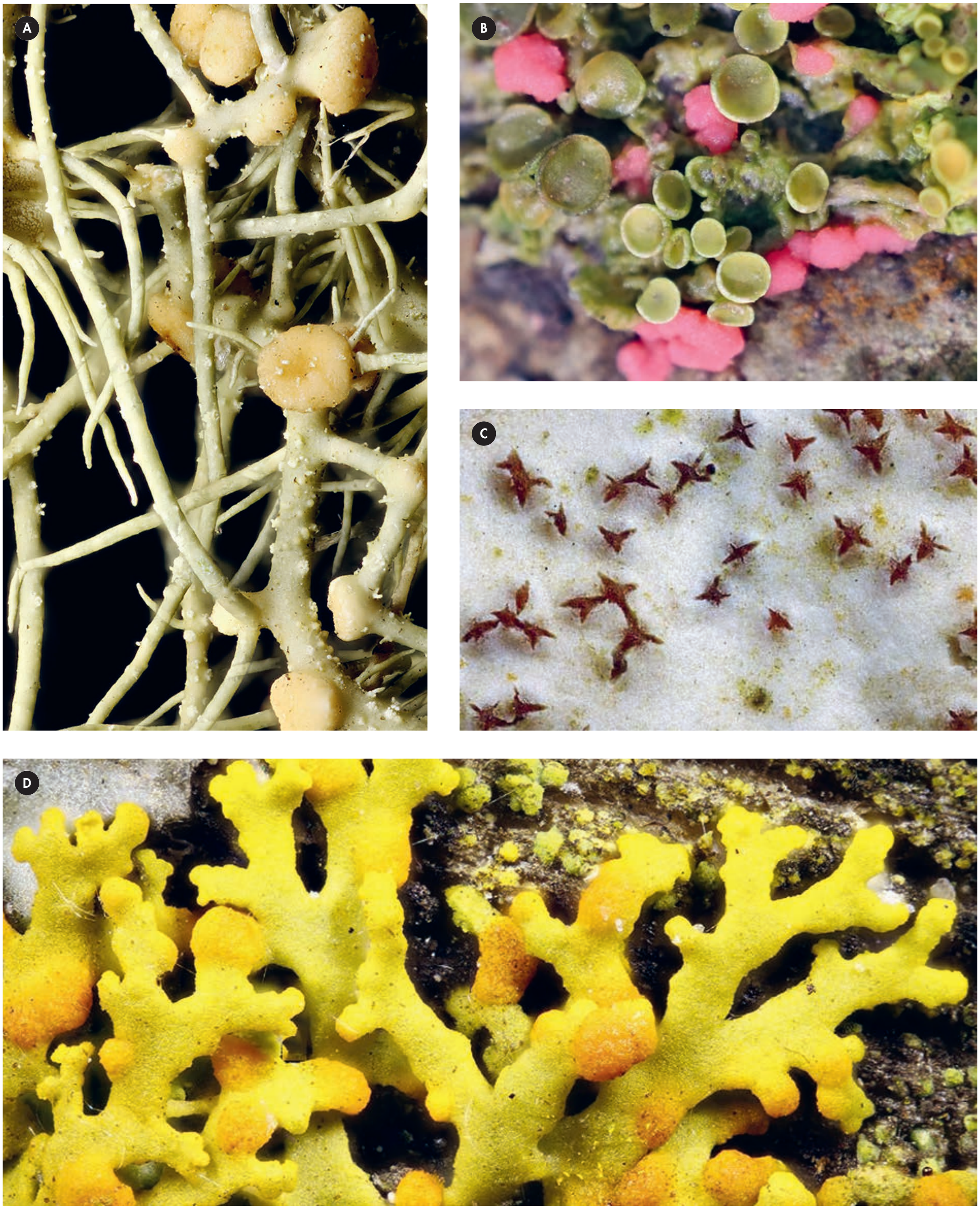




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## LICHEN YEASTS

The application to lichens of laser microscopy techniques more usually encountered in medical research has made it possible to "turn the lights on" inside a lichen and see who is who in the fungal matrix and where they are hanging out. The first applications of this research have revealed additional fungi in the yeast form—single, tiny cells that reproduce by budding—in the outer layer of perfectly healthy-looking lichens. Using DNA signatures, researchers have been able to determine that these yeasts were exactly the same species as some of the surface fruiting bodies named by the intrepid "parasite" collectors of yore. In fact, in several lichen symbioses that have been carefully surveyed to date, they appear to always be there. A prominent scientific journal proclaimed lichens to be "two fungi, not one." But does this hold up?

Lichen yeasts are only beginning to be studied in detail. They are found in one way or another in as many as two-thirds of known lichens, but certainly not all. Even so, evidence is mounting that in specific lichen symbioses—colloquially called "lichen species"—they are an intrinsic part of the lichen wherever it occurs, anywhere in the world. By this measure, they are an easy fit for the term "symbiont," which in the trade is used for any organism that constantly "lives together" with another, irrespective of any benefit or harm to either. But is there benefit or harm? It is currently not possible to selectively eliminate yeasts and leave the other symbionts untouched, so we cannot tell how a lichen might react if we did. Yeasts have also yet to be cultured alone. Attempts at divining the biochemical give and take of yeasts and the "main" fungi based on their genomes have yielded promising leads, but no smoking guns. Lichen yeasts appear to be involved in triggering the production of secondary metabolites such as lichen acids, and they have the enzymes for producing water-absorbing polymers. One can think of many ways in which these traits could be useful to the symbiosis that carries them, and we won't bet against them being necessary and intrinsic components of lichens. But it may be a while before the bets are decided.

→ The yellowish color of the lichen that has been called *Bryoria tortuosa* (above) is caused by the toxic substance vulpinic acid. Its abundance correlates with that of basidiomycete yeasts from the genus *Cyphobasidium*, visualized using fluorescent microscopy in a section of one of the lichen's hairlike filaments (below). The yeasts, which are unicellular, appear in green; the hyphal ascomycete fungus that gives the lichen structure is shown in blue; and the algae are rendered in red.



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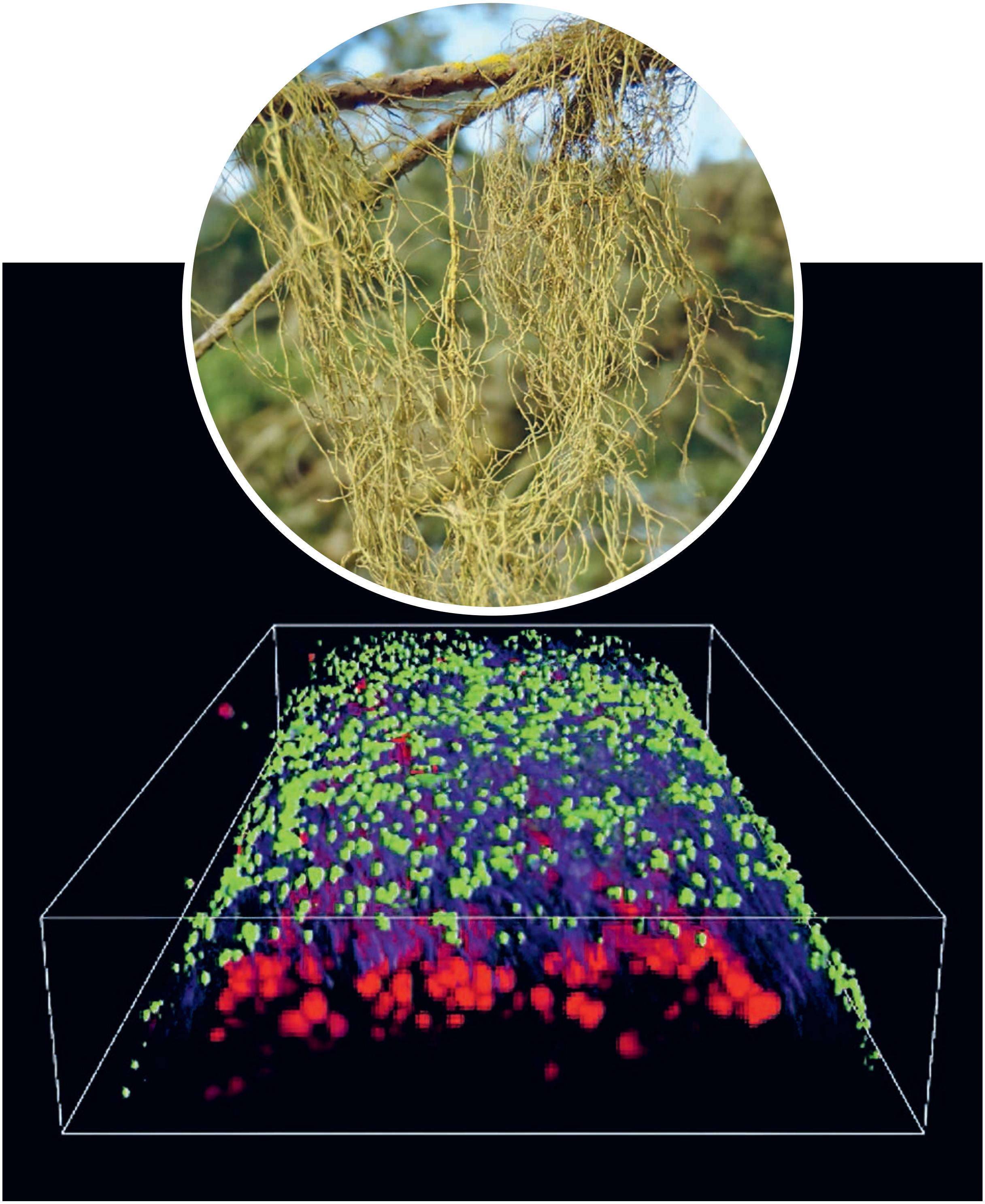



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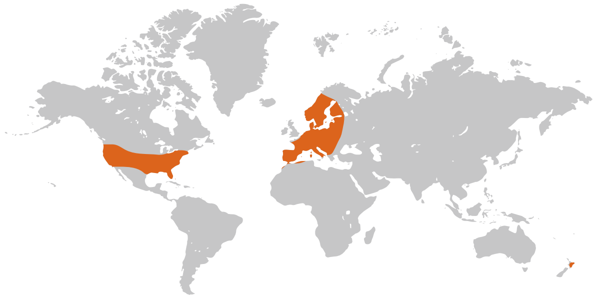

TREBOUXIA LYNNAE

# Lynn Margulis's Trebouxia

A photobiont of large and common lichens

| | |
|---|---|
| SCIENTIFIC NAME | *Trebouxia lynnae* Barreno |
| PHYLUM, FAMILY | Trebouxiophyceae, Trebouxiaceae |
| GROWTH FORM | Single cells that reproduce quickly to form a green mass, but not usually visible in nature |
| SPECIES IN GENUS | 27 named, but many are not yet named |
| HABITAT | Extremely common in terrestrial environments |
| NOTABLE FEATURES | One of the most common lichen photobionts |

**Best not try to carry out a survey of lichen photobionts armed with a hand lens and cell-phone camera. Most are single-celled, and identifiable only on the basis of internal cell structures and DNA sequences. As a result, the classification of photobionts has lagged behind that of the fungi involved in lichens, and even some of the commonest species are still being given scientific names.**

Break open the thallus of a lichen and squint really hard, and you might make out an extremely thin green line sandwiched between the upper and lower fungal layers. It's the world's thinnest burrito, a fungal tortilla with algal filling. Better yet, use a standard light microscope or a scanning electron microscope (page 21), and you will get a sharper view of round cells wrapped in fungal nets or suspended at the ends of what look like fungal suction cups. More often than not, these cells are members of the genus *Trebouxia*, one of the most common groups of photobionts worldwide.

A decision to study *Trebouxia* is a conscious decision to become a microbiologist. The methods are much the same as those one might use to culture bacteria from a mouth swab in a hospital, and analyzing the resulting growth requires patience and a well-stocked lab. The classification of *Trebouxia* is a technical business, and many species have yet to be given formal scientific names. One of the more common species in large foliose and fruticose lichens was named as recently as 2022 by a team led by the Spanish researcher Eva Barreno. She named the species for Lynn Margulis, one of the leading figures of symbiosis research and the person who discovered that mitochondria are descendants of intracellular bacterial symbionts.

→ *Trebouxia lynnae.*



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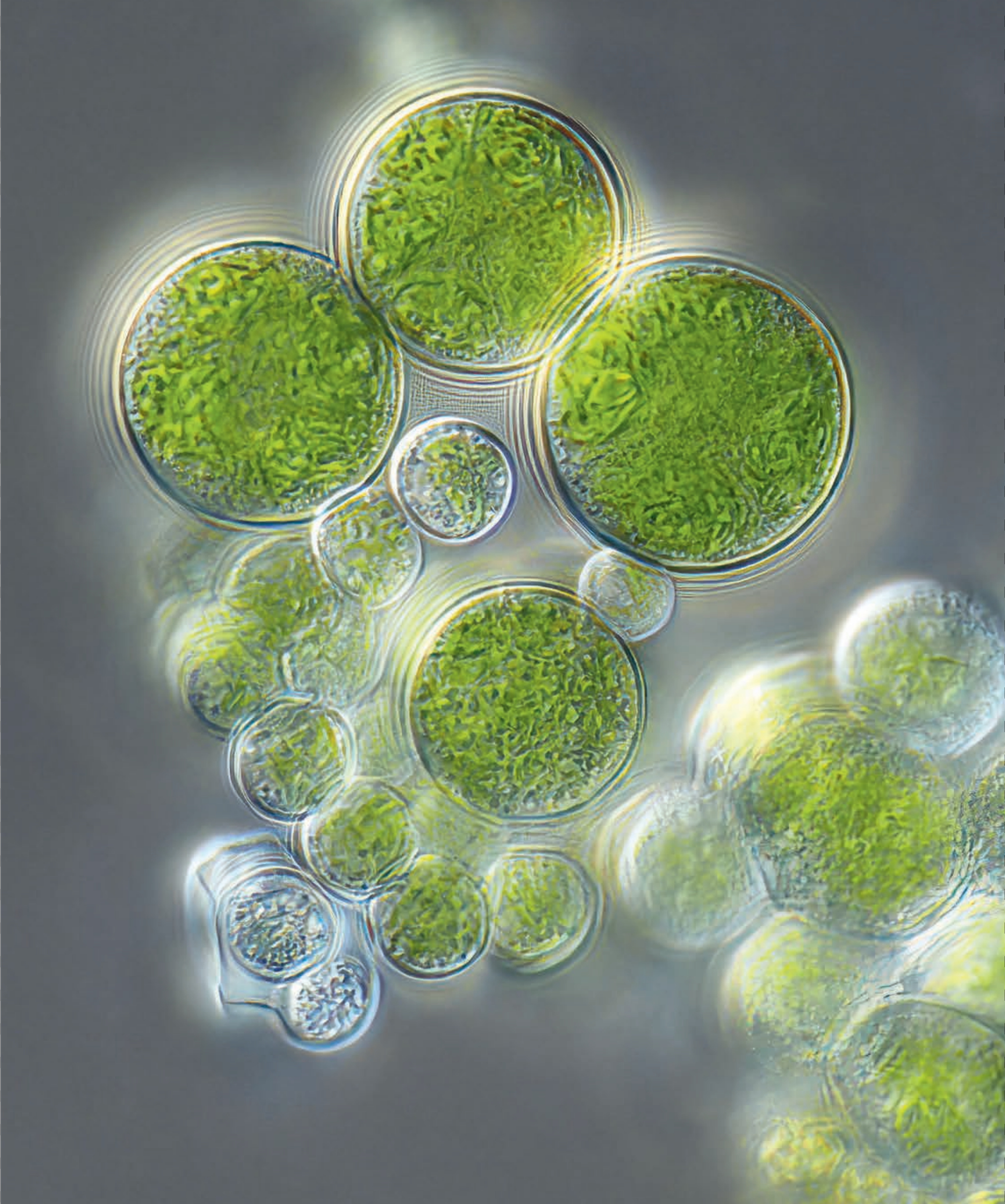

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TRENTEPOHLIA AUREA

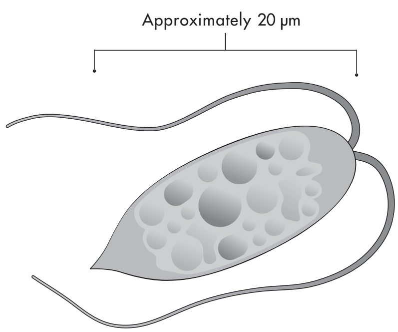
# Golden Trentepohlia

## Orange is the new green

| | |
|---|---|
| SCIENTIFIC NAME | *Trentepohlia aurea* (L.) C. Martius |
| PHYLUM, FAMILY | Ulvophyceae, Trentepohliaceae |
| GROWTH FORM | Single to multiple chain-forming cells with one or more large carotenoid bodies, giving the alga a carrot-orange color |
| SPECIES IN GENUS | 54 |
| HABITAT | Common in foggy coastal regions on trees and rocks, and inland near the ground on tree trunks |
| NOTABLE FEATURES | A common lichen photobiont |

**Like the other major algal genus we profile here, *Trebouxia* (page 60), *Trentepohlia* is a genus of "aeroterrestrial" algae—in other words, algae that live on land, exposed to air. But in many ways, that is where the similarities end.**

Unlike *Trebouxia*, *Trentepohlia* algae are capable of complex multicellularity. Together with a few close relatives, they are also unique among algae in possessing phragmoplasts, which students of plant anatomy might recall are cellular structures involved in laying down the beginnings of new cells.




**Mobile**
The life cycle of *Trentepohlia aurea* includes flagellate spores. These are only known from free-living algae and not from the algae when in symbiosis.

Furthermore, despite being formally classified as green algae, they are usually orange in appearance on account of large carotenoid pigment bodies contained in every cell.

*Trentepohlia* algae are important symbiotic partners of fungi in lichens in several different distinct biomes. They feature prominently in tropical lichens that grow in deep undergrowth of rainforests, but also appear in high-humidity coastal environments from the Mediterranean to Baja California. Despite this decidedly beach-holiday ecology, species of *Trentepohlia* can also pop up as symbionts in some of the most inhospitable places on Earth, at the bases of trees close to the Arctic treeline. And just in case you thought you had the ecology of the genus narrowed down to humid environments, they also occur in lichens (and indeed also as free-living algae) on the sparse vegetation of the Atacama Desert in Chile, one of the driest places on Earth.

→ *Trentepohlia aurea*, on rock in the mountains of Colombia.



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**MICROTHELIOPSIS ULEANA**

# Ule's Leaf-Dwelling Brownspot

Photobiont going rogue

| | |
|---|---|
| SCIENTIFIC NAME | *Microtheliopsis uleana* Müll. Arg. |
| PHYLUM, FAMILY | Ascomycota, Microtheliopsidaceae |
| GROWTH FORM | Tiny crust lichen forming round, brownish patches |
| SPECIES IN GENUS | 4 |
| HABITAT | Strictly on living leaves of tropical rainforest plants |
| NOTABLE FEATURES | The photobiont always produces reproductive structures in this lichen |

**Given that lichens are known as slow-growing organisms, their occurrence on a short-lived substrate such as leaves is surprising. Leaf-dwelling lichens have solved this conundrum not by growing faster but by reaching maturity at extremely small sizes. One such lichen is *Microtheliopsis uleana*, happily reproducing with spores made in dark perithecia. And the photobiont joins in the reproductive frenzy.**

The phyllosphere is a world of its own. It is a miniature ecosystem made up of organisms inhabiting the surface of living plant leaves. Given that the trees in temperate forests are typically leafless for several winter months, the phyllosphere is best developed in tropical rainforests. It consists of lichens and other surface fungi, liverworts and mosses, algae and cyanobacteria (and other bacteria), and invertebrates.

A common element of the phyllosphere is Ule's Leaf-dwelling Brownspot. It is found in rainforests on all continents. Its characteristic feature is the small, round, brown patches that develop spore-bearing, dark perithecia, often arranged in a concentric pattern. It also has what look like tiny hairs. These are actually the sporangia of the photobiont, an alga of the genus *Phycopeltis*, closely related to the aerial alga *Trentepohlia*. *Microtheliopsis* is the only genus of lichen fungi in which the photobiont produces such conspicuous reproductive structures with regularity. Why the alga reproduces in this manner in the presence of this particular fungus is unknown, but it suggests that they have a rather unusual relationship. Live and let live?

→ *Microtheliopsis uleana* growing on a leaf in a Costa Rican rainforest. The tiny hair-like structures are the sporangia of the associated alga.



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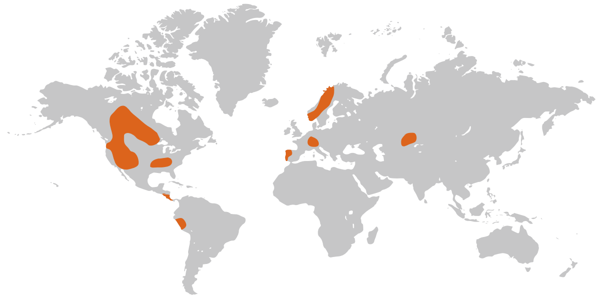

LICHENIHABITANS SPP.

# Lichenihabitans bacterium

Obligate lichen dweller

| | |
|---|---|
| SCIENTIFIC NAME | *Lichenihabitans* spp. |
| PHYLUM, FAMILY | Alphaproteobacteria, Beijerinckiaceae |
| GROWTH FORM | Bacterial colonies, in lichens incorporated in and on the cortex |
| SPECIES IN GENUS | 1, plus many unnamed species |
| HABITAT | Originally cultured from *Psoroma antarcticum* lichens in Antarctica |
| NOTABLE FEATURES | Represents a wider group of bacteria found worldwide on lichens |

**Most people, lichenologists included, do not think of bacterial cultures when they think of lichens. They were not one of the symbionts originally discovered in lichens, and until recently it was not even known that they were a constant part of lichen life. But like us humans, lichens have distinctive bacterial assemblages. The role they play in the lives of lichens is still being studied.**

The culturing of bacteria from lichens began in the 1920s (page 54). As with exploration of the human gut microbiome, however, it was not until recently that technologies have been developed to survey the vast bacterial world associated with lichens using DNA sequencing technology. It turns out that many of the bacterial strains that have been isolated from lichens are one-off occurrences that may be picked up from the environment, but a few seem to have a strong association with lichens and are more or less always there.

Although it is early days in this line of research, this appears to be the case with the bacterial genus *Lichenihabitans*. The genus was given a name in 2019 by a Korean research group led by Yung-Mi Lee, based on a strain isolated from the lichen *Psoroma antarcticum*. Subsequent screening of DNA data from lichens has shown that close relatives, probably also members of the genus *Lichenihabitans*, are found in over 90 percent of lichens sampled from different parts of the world. What *Lichenihabitans* does in lichen symbiosis is not known with certainty. Genomes from closely related strains have been predicted to be able to synthesize vitamin $B_{12}$, which photobiont algae need to make the amino acid methionine.

→ A species of *Lichenihabitans* in culture at the University of Alberta.



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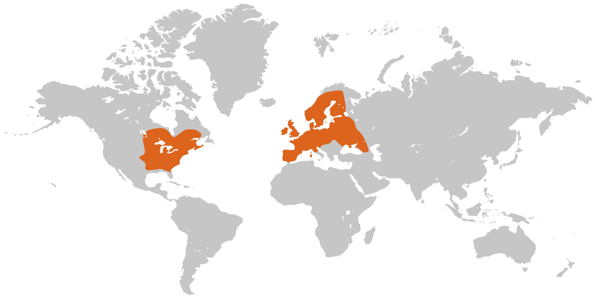

MARCHANDIOMYCES CORALLINUS

# Marchand's Coral Fungus

## Team player and killing machine

| | |
|---|---|
| SCIENTIFIC NAME | *Marchandiomyces corallinus* (Roberge) Diederich & D. Hawksw. |
| PHYLUM, FAMILY | Basidiomycota, Corticiaceae |
| GROWTH FORM | Lichen-attacking fungus producing pink-red fruiting bodies |
| SPECIES IN GENUS | 4 |
| HABITAT | On a diversity of host lichens, but often on Parmeliaceae |
| NOTABLE FEATURES | Teaming up with another lichenicolous fungus to overcome host defense |

**We have seen that lichens are not just the stereotypical symbiosis between fungi and algae or cyanobacteria. Rather, lichens are miniature ecosystems involving and supporting a range of organisms. These also include a diversity of other lichen-inhabiting (lichenicolous) fungi. Not surprising, given that parasitism is one of the major fungal lifestyles.**

Some of the lichenicolous fungi seem to live in peaceful coexistence with the main lichen fungus, taking advantage of the photobiont as commensals, but others fiercely attack the host.

The pink-red reproductive structures (bulbils) of *Marchandiomyces corallinus* are a beautiful sight. But make no mistake: this basidiomycete fungus kills. And it does so on a broad range of lichen hosts.

Studies led by lichen ecologist James Lawrey have shown that lichens do have chemical defenses against this fungus, one of these being lecanoric acid, another gyrophoric acid, the same lichen substances employed in dye production (page 250). These apparently "multitasking" compounds inhibit the growth of the attacking fungus and its cell-wall-degrading enzymes.

But then there is another fungus, the ascomycete *Fusarium*, which is able to degrade these lichen acids, overcoming the host defense. Once the *Fusarium* attacks a lichen, the *Marchandiomyces* (and other lichenicolous fungi) may come in and take advantage of the weakened host. Notably, *Fusarium* is the asexual morph of a species of *Nectria*, some of which are used as biological control agents. A striking example of all the things lichens can teach us.

→ The pink-red pustules (bulbils) of the lichen-attacking basidiomycete fungus *Marchandiomyces corallinus* make for a striking color splash. For the host lichen, here a species involving a fungus from the Parmeliaceae, *Parmelia sulcata*, the attack usually ends in death, at least for portions of the thallus.



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