Growing Rogue

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Izzie: Welcome to Morel Dilemma, an exploration of why some mushrooms are so highly sought, some are so heavily cultivated, and some are so very dangerous. So far on this podcast, we have talked extensively about the fungi that live where we live - fungi that grow in the woods, on trees and rocks, in the roots of plants, and even inside of animals, including ourselves. Terrestrial fungi, the fungi that grow on land, have been my main focus so far because they're the ones we interact with most often. But what if I told you there was another world of fungi? They shore don't surface very often in the news. They don't really make waves. But they're there, and they're vital. They are the water fungi.

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Izzie: You are probably familiar with water molds if you ever have to clean a bathroom. Black and brown slime gathers around the faucet if you wait too long to 409 the counter. You also know about water molds from when your cream cheese goes bad. Water molds are the aquatic fungi responsible for all that grossness, and they are present in just about every body of water on earth.

Some aquatic fungi are the asexual, or non-fruiting, versions of terrestrial fungi like the ones that make mushrooms. However, the two major types of water fungi live exclusively in water. The chytridiomycetes, also known as chytrids, are thought to be the oldest "true" fungi, related to the original lineage of fungi that developed in ancient oceans. The oldest known chytrid fossils date back more than 600 million years.

The second type of water fungi are the oomycetes, also known as "egg fungi" or "downy mildews". Their history is more difficult to determine. A recently discovered piece of fossilized amber from the Cretaceous period, 65.5 million years ago, may contain an oomycete. They aren't a modern invention, at any rate.

Like terrestrial fungi, most aquatic fungi are either saprobic, digesting dead material, or parasitic, stealing the nutrients of another organism to survive. *Un*like most terrestrial fungi, water molds are notoriously specific. Chytrids are super happy on fir and pine pollen, for example; some oomycetes exclusively eat protein-rich foods like dead flies, while others prefer starchy foods like potatoes and fruit. In order to gather and identify new molds, mycologists can do something called mold prospecting, where they put something unusual into a body of water and see what molds grow on it. For instance, they could put a tropical fruit like a kiwi into a pond in the Himalayas. The idea is to bait an organism that usually can't get enough of its preferred food source to be visible in normal culturing methods. The more substrates a scientist baits with, the higher diversity of saprobic water molds they can expect to find. Parasitic oomycetes can feed on each other, on algae, plants, fish eggs or scales, and on amphibians - basically anything that lives in the water succumbs to oomycetes at some point, while it's alive or after it's died.

[Musical tone]

Izzie: Now, although they are called "water molds", these organisms are not universally considered to be fungi. There are a few distinctions that blur the line.

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Izzie: First, the so-called true fungi have cell walls that contain chitin, a structural sugar unique to fungi and insects. While chytrid cell walls contain chitin, oomycete cell walls are made of cellulose and glycan instead. Second, the true fungi grow with haploid hyphae; however, oomycetes grow with diploid cells throughout life, as humans do. Finally, the true fungi are immobile, with spores that rely on the wind to take them where they need to go, but chytrid spores have flagella, tiny, cellular tails that let them move through water like sperm. Because of these distinctions, many biologists consider oomycetes and chytrids not to be fungi, and classify them in various other kingdoms.

Listen: All of taxonomy is in a constant state of refinement and editing, especially when DNA evidence conflicts with morphological evidence. Scientific classification largely depends on who you're asking. When I worked at the fungal herbarium at the New York Botanical Garden, a third of my job was just filing specimens with historical names in sections with the current names. Once I went to the herbarium director about several labels listing species that weren't in the herbarium, or any of the online databases. The director looked at the labels and said something like, "Oh, these are from Susan. Yes, she's been working on defining a new genus for about ten years now. Just put them in as she wrote them."

All this is to say that while oomycetes and chytrids might have some structural differences from other fungi, they grow the way fungi grow, eat the things fungi eat, and don't fit cleanly into any other categories. For that, I call them fungi, and they get to be on this podcast.

[Music ends]

Izzie: Chytrids don't generally interact with freshwater and terrestrial habitats enough to garner human attention. Oomycetes, on the other hand, are prevalent in soils, and adept at parasitizing terrestrial plants. As a result, they are very important to human agriculture, especially the downy mildews. These are parasitic oomycetes that cover plants in stiff spore-releasing stalks called sporangia that make the plant look furry, or downy, from a distance. The water mold cuts off the leaves from the rest of the plant, starving it while stealing its sugars and releasing spores that spread and germinate rapidly in water. Chilly, damp environments like those of northern Europe are so conducive to oomycete growth that time from initial introduction of the mold to complete destruction of a field crop takes only a couple of days.

In 1846, oomycetes ravaged Irish potato crops in the greatest downy mildew disaster of human history. The potato plant is native to North America, as is the oomycete *Phytophthora infestans*. However, while the potato was moved across the pond hundreds of years ago and cloned repeatedly, becoming a staple food crop all over Europe, the blight stayed put until 1846. It probably came over on a ship from the United States, and when it arrived, it found an entire country full of genetically identical, susceptible plants. Almost every potato plant in Ireland was destroyed in the space of one week. This devastation resulted in the Irish Potato Famine, during which one million people died and another 1.5 million emigrated out of Ireland.

The disaster of *Phytophthora infestans* is a tragic cautionary tale against clonal agriculture, which unfortunately is still the dominant agricultural method in use. Maybe that's why oomycetes still regularly threaten avocado, pineapples, grapes, lettuce, corn, and cabbage crops, to name a few. However, as we saw in Episode 10, other fungi partner with terrestrial plants, providing soil nutrients in exchange for sugar and helping the plants defend against disease and pathogens. It's important to remember that fungi perform a variety of functions in our soil-based world, which they have developed over just 425 million years. Likewise, they perform a wide range of jobs in the ocean - where they've had much longer to find a niche.

[Musical tone]

Izzie: Like plants, fungi first evolved in the ocean and then made the jump to land. Plants and fungi already had a solid partnership before they colonized land together. Of course, not all the fungi and plants moved to terrestrial habitats, and there is still an amazing diversity of both in waters that never touch agricultural soil.

As on land, fungi in aquatic habitats are major decomposers of plant material. Perhaps their biggest contribution to the ocean is be their ability to degrade lignocellulose, a combination of cellulose sugar and a polymer called lignin that gives woody plants their structure. Lignin is one of the toughest materials in the world to digest, and the fungal claim to fame is the rapid rate at which they can break it down. An enormous mass of wood is regularly flushed into the sea; for example, in 2009, Typhoon Morakot dumped 18 and a half billion pounds of wood into the ocean. Through the efforts of our friends the fungi, wood is a significant source of energy in aquatic ecosystems.

To be fair, fungi aren't the only organisms that can digest wood - they're just the fastest and the most widespread. Some marine bacteria can digest lignin, but not as quickly as fungi. Some mollusks can do it, and contribute considerably to wood breakdown on the deep sea floor, but they can't handle the low oxygen concentrations in intertidal zones or ocean sediment. In those zones especially, fungi are responsible for major calorie turnover. As on land, though, lignin digestion is just one job that fungi undertake. Aquatic fungi can also digest animal remains, including corals and mollusk shells; live symbiotically, in submerged lichens or in the roots of marsh grasses; cause diseases in marsh trees, fish, mollusks, crustaceans, and octopi; and even parasitize algae. So who's doing all these jobs?

[Musical tone]

Izzie: There are two types of marine, or ocean-living, fungi. Obligate marine fungi are "obligated" to live and reproduce in the ocean or in estuarine habitats like salt marshes, intertidal zones along the coast, and mangrove forests. Facultatively marine fungi, on the other hand, are those that generally live in freshwater or even terrestrial environments, but are able to grow, and sometimes reproduce, in saltier conditions.

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Izzie: Aquatic fungi aren't a single taxonomic group, and a genus that includes freshwater or terrestrial fungi may also contain marine species. Generally speaking, the aquatic mycobiome reflects water temperature and food source more than geography or genus. That can make identifying aquatic fungi difficult, even with DNA sequencing. Facultative species are particularly tricky; a terrestrial species found under the ocean's surface may live there, sure, but it may have just washed in from a different habitat and managed not to die yet. It's easier to get numbers for obligate marine species because, well, you know they didn't just fall into the water by mistake.

In 1979, a famous paper by Kohlmeyer and Kohlmeyer estimated that there were only 500 marine fungal species. Today, there are about 450 known obligate marine fungi, more than half of which were discovered after that paper's publication. In 1991, mycologist Dr. Hawksworth said he, quote, "conservatively" estimated 1.5 million fungal species on Earth overall. Ten years later, he wrote that that estimate was far too low, and calculated that the number of known species expands as much as 20 to 49 percent per decade that we search. This massive increase in species identification comes from the advent of new technologies, and from more extensive research into poorly studied areas, like - drumroll, please - freshwater and marine habitats. In 2007, mycologist Dr. Raghukumar calculated that, far from the 1979 estimate, there could actually be more than 14,000 marine fungal species.

[Music ends]

Izzie: Despite the progress being made on identifying and quantifying obligate marine fungi, there are a surprising number of missing evolutionary links between the ancestral chytrids and modern marine fungi. Most known obligate marine fungi apparently didn't evolve in the ocean, but came back to aquatic life after being terrestrial for a while. For example, most marine wood-rotting fungi are descended from wood rotters that live on land. So are the fungi that occupy and digest dead corals. It's possible that, for some reason, modern culturing methods don't work on the fungi that evolved from the same lineage as the chytrids but never left the oceans. Their absence is a major gap in our understanding of marine fungal evolution.

Between low species numbers and this major missing link, it might seem like mycologists aren't devoting nearly enough attention to aquatic habitats - but that's not quite fair.

[Musical tone]

Izzie: First of all, the ocean is 70% of the earth's surface, possibly representing 90% of Earth's living space. That incredible mass of water contains a wide range of habitats, ranging in temperature from extremely cold polar waters to boiling-hot hydrothermal vents, covering depths of several thousand meters and pressures that would flatten a person. Open ocean, sand flats, seagrass meadows, and coral reefs are just a few of the marine biomes. It's estimated that humans have only explored five percent of the ocean floor, to say nothing of the open water. Where to even begin?

[Musical tone]

Izzie: Another factor holding marine mycologists back is that, as we saw in the lichen episode in season 1, if you don't already know a particular group of fungi is present, it can be maddeningly hard to find. And the lichen study was using DNA-based, culture-independent techniques. While those studies can be useful, many marine fungi are only known because the DNA sequences of their spores showed up in the water. That means scientists can't know if they actually grow in the oceans, or what they eat. Culturing microorganisms can be a safeguard against accidentally adding a non-marine organism to the marine species list. But as with terrestrial species, the majority of marine species cannot be cultured with current technology. A study on one order of marine chytrids found that only 0.06 to 3.65% of the species can be cultured.

[Musical tone]

Izzie: In an effort to capture some more of the diversity, scientists sometimes perform mold prospecting in marine waters. In the ocean, prospecting is often limited to very nutritious bait, like pine pollen or brine shrimp. This seems like a great idea until you remember that too much nitrogen fertilizer can kill plants - overload an organism, even with something it usually likes, and it will die. Therefore, highly nutritious baiting will only reveal organisms that rely on a high-nutrient diet, and the others will remain uncounted. The full diversity of marine fungi remains elusive.

[Musical tone]

Izzie: Finally, remember from the orchid episode that it can be super hard to identify fungal species if they don't fruit and sporulate? Well, most aquatic fungi are unicellular. The weblike growth of hyphae on dry land is a great adaptation to dry land, when a fungus is anchored and that's how it spreads around looking for nutrients. But in the water, where a large web would be constantly buffeted and broken by currents, and offer no real advantage in terms of locating nutrients, being single celled is a much more efficient route. Even the fungi that develop multicellular phases, or fungi with fruiting terrestrial forms, only engage in asexual, mold-like growth and reproduction underwater. They reproduce by budding, not by sporulating. Fungal fruits, so useful for identification, are absent.

[Musical tone]

Izzie: I know I haven't fooled you. While my love of fungi is boundless, there's a special place in my heart for mushrooms, and I could never live with myself if I delivered an episode to you, my rapt audience, that didn't have mushrooms in. So I know that you know that there have to be aquatic fungi that fruit. Well, yes there are.

Some ascomycete fungi fruit *from* the water, or at least, from very wet substrates. Basically, the hyphal network is growing in this waterlogged wood or soppy soil - it's growing like a terrestrial fungus, but underwater. When ready, these fungi send up stipes above the water's surface that sprout mushroom caps. *Vibrissea truncorum* is a cute example, growing on wood submerged in cold running water. Mushrooms that have wet feet, so to speak, have been found growing from submerged wood in North America, Europe, Japan, Thailand, and Costa Rica. Sometimes the areas that host these mushrooms flood, submerging the caps temporarily, but the spores are only released in dry conditions. So while they are adorable, and pictures of them are very artsy indeed, these are *not* underwater mushrooms. I'm afraid no fungi actually grow their fruit underwa -

Just kidding! We live in a beautiful, incredible world where crazy things happen all the time, so of course there are fungi that fruit, and even sporulate, under water.

In 1995, Dr. Desjardin and colleagues described the habitat and appearance of *Gloiocephala aquatica*, a saprobic agaric that also has the honor of being the world's first known underwater mushroom.

Gloiocephala aquatica was collected from the banks of several Argentinian lakes, where California bulrush grass grows densely and dies at the onset of winter each year. Ice quickly forms on the surface of the lake, and the water dips almost to freezing: 35 degrees Fahrenheit or 1.6 Celsius. But that doesn't deter the *Gloiocephala* mycelia from breaking down the bulrush stalks under the water line, or from sending out their tiny fruits when it's time to reproduce. The mushrooms are adorable, little rubbery plates coming off the stipe, looking like little ping-pong paddles just over half a millimeter in diameter.

Desjardin and the other researchers explored the area around the lakes throughout the year, but never found any of the little fruits above the ice. So this is definitely not a terrestrial mushroom that got caught in a flood; these fruits fully develop underwater. Unfortunately, the spores don't seem to have gotten the memo.

[Music begins]

Izzie: Instead of spores that passively drift from the cap, many terrestrial fungi have something called ballistospores. These are just what they sound like: spores that rocket away from the mushrooms as though shot from a cannon. First, the mushroom develops the spore in a way that it can collect a droplet of water. The surface which the spore grew from and is still attached to also collects a drop of water. Eventually, the droplets become large enough to touch, and - trust me on this - the change in surface tension as those droplets merge blasts the spore away from the cap with an acceleration force of 25,000 g's, ten thousand times the pressure astronauts feel when they leave the Earth's atmosphere! Instead of going into orbit, though, the spores quickly slow down - the initial blast is usually just enough to fire them onto a gentle breeze that takes them off on their way.

[Music ends]

Izzie: This is all very impressive, and it works terrifically as long as you're in an environment where you can gather two individual water droplets. But as soon as you submerge the assembly in water, the whole idea of ballistospores sort of fizzles out. When *Vibrissea truncorum* and other wet-footed fungi get flooded, they can still often sporulate because as the water rises around them, air is trapped under their caps. Their ballistospores can be shot into the bubble - which contains air, after all - and move around when the water level decreases and the bubble pops. That wouldn't work for *Gloiocephala aquatica*, because the ping-pong paddles grow entirely under the water and are never exposed to air. Instead, Desjardin's team thinks that the spores are not forcibly discharged in this species, but instead swept away by water currents. Once in the water, the spores might spread via wind-induced eddies in the water, or stick to aquatic animals or birds.

The lack of related underwater fungi and the presence of the obsolete ballistospore setup imply that like many aquatic fungi, *Gloiocephala aquatica* descends from a terrestrial fungus. Why might this mushroom have moved underwater? Well, while the water is certainly frigid, frozen-over lakes never actually freeze. The ice acts as an insulator, keeping the water warm enough for very cold-tolerant life to continue - this is also why fish can survive freezing winters. The steady environment might have been a happy accident for a particularly cold-tolerant strain of *Gloiocephala*, and however many generations later, we end up with - triumphant music - the world's first underwater mushroom.

And hey - if a half-millimeter ping-pong paddle doesn't feel mushroomy enough for you... I have good news.

[Music begins]

Nicole: Hi, Izzie, my name is Nicole and I am calling from beautiful New York, New York. And I got a bone to pick with you about those underwater fungi. They do not exist. They can't! There's a big logic gap there. Water giveth and taketh away life from fungi, so that doesn't work. You'd be keen to know that those aren't underwater fungi; I think they're probably aliens from another planet. That is all.

[Music ends]

Izzie: After the Desjardin paper in 1995, scientists found eleven more basidiomycetes that live and sporulate underwater. However, it wasn't until 2005 that your new favorite fungus, well, "surfaced". My friends, this here is a real mushroom. Tall, thin stipe; umbrella-shaped cap; and honest-to-goodness gills underneath - it's the whole nine yards.

As with many amazing discoveries throughout history, this one took a long time to be recognized. The paper announcing a new, underwater species called *Psathyrella aquatica* came five years after its initial discovery, and the way was paved with disbelief. I sat down with Doctor Darlene Southworth, one of the authors of that paper, to hear about her experience describing the world's first underwater cap-and-gill mushroom.

Here's how she begins the story: Hydrologist Robert Coffan was on picnic with his family in Oregon. He waded into the water a bit, looked down, and saw something strange.

Darlene: He was picnicking on the 4th of July along the Rogue River, and he's a consulting hydrologist. And so they dabble around in the water, that's what hydrologists do, and he noticed this.

Izzie: What Robert noticed was an out-of-place mushroom, sprouting in the middle of a river, with its cap way underwater. And he's no dummy about mushrooms; in fact, he's an amateur mycologist.

Darlene: You know, he hunts morels.

Izzie: So he knew that it was very weird to see this upright mushroom where it could not have simply fallen, apparently growing in this place that never gets dry. And it was standing up in a strong current - that region of the Rogue River puts out about 2900 gallons per second in July - which make the chances of its being there by accident even slimmer. A quick look around the river established that there was nothing similar growing nearby on land. Robert knew he had found something exceptional, so he snapped a photo and started contacting mycologists when he got home.

Izzie: He didn't have much luck.

Darlene: He contacted a couple of mycologists in Oregon, but they sort of shined him off. One said, "Well if you see that again call me." So what do you say next? And then the other one said, "Well I've never heard of that." And again, so – what do you say after that?

Izzie: The mycologists' reactions were disappointing, and surprising to me. Usually in mycology, amateurs are welcomed and taken pretty seriously.

Darlene: Amateurs are definitely respected. So I don't quite know … maybe they were too far away. One was in Corvallis, one was in Eugene, and he's here in Medford. So they – they just don't make the connection.

Izzie: But their indifference couldn't stop Robert. He knew he was looking at something very important, and actually made collections of the mushroom. Fast forward two years to 2007, when Robert meets Dr. Southworth.

Darlene: I had a poster up on the wall about some other fungi, so he contacted me, and […] instead of saying I've never heard of that, I said, "Okay, show me."

Izzie: So Robert sent along his picture to the person who finally believed him. It's a very striking picture, too - you see a mushroom really just growing straight out of the sand into the middle of the water.

Darlene: And then I said, "Well, take me to the site." And he did. And thereby hangs the tale. You know, in terms of figuring out how long it took us to determine that it grew underwater, that was like fifteen minutes. "Oh, I see, these mushrooms are growing underwater. Got it."

Izzie: For its unusual lifestyle and its original location, Robert referred to his find affectionately as the "Rogue mushroom," but that wasn't going to get the mushroom officially recognized.

Darlene: For a while, Robert thought you could publish something that said "I found a mushroom growing underwater" without knowing what it was. And I said, "Well, good luck with that! I can't do that."

Izzie: Dr. Southworth and her lead researcher, Jonathan Frank, began working to identify the mushroom from keys - basically a branching table that leads you to a genus identification based on traits of the mushroom like cap and spores. The spores of the Rogue mushroom look very much like the spores of Coprinus mushrooms, which include the inky cap or shaggy mane mushrooms that you'll recall from the foraging episode. However, the underwater fruit wasn't liquefying itself the way Coprinus mushrooms do.

Darlene: I found that it was the genus one over from Coprinus, and that was Psathyrella. So that settled that right away. So that was not difficult. It was getting to the species that was hard.

Izzie: Now, Psathyrella is a genus with 414 known species in North America that are notoriously tricky to tell apart. Large structures, like mushrooms, might be morphologically similar between species that are related only distantly. Meanwhile, small structures like spores might vary within a single species. Some strains can be considered the same species even if their DNA is very different. Jonathan took on the daunting task of poring over spore s and samples in the microscope. When no morphological matches were forthcoming, he turned his attention to the genetic code.

By comparing DNA isolated from eight of the Rogue River mushrooms, Jonathan confirmed that they all matched - so, they were members of the same species. The next step was to figure out just what species that is.

When biologists are trying to identify something new with DNA methods, they commonly compare their own samples to the sequences stored in online libraries. GenBank is one such library, where scientists from all over the world can access massive stores of data they didn't have to work on personally. Unfortunately, the utility of the database depends on scientists actually uploading their own data. And in 2007, there weren't many Psathyrella species in GenBank for Jonathan to compare with the underwater mushrooms.

The numbers increased for the first time when Dr Southworth performed some very intense networking, reconnecting with a classmate from her days at Berkeley, who was now a professor with a graduate student working on Psathyrella. The second increase came a year later, when Jonathan, sifting through GenBank yet again, came upon a veritable treasure trove.

Darlene: Jonathan said, "There's 75 more species in GenBank!" I said, "Whoa. Who's that?" And it's a person from Vienna who I had met at a meeting, and a few keystrokes later I had his paper. So we acquired this knowledge through this kind of network of mycologists.

Izzie: As they compared the underwater mushroom to this increasing number of other Psathyrella species, it became more and more apparent that the Rogue mushroom represented a new species. And it wasn't a one-time mutation or fluke - these mushrooms really are growing in the middle of the river, year after year.

Darlene: I've been back every year since 2007 to just say hi, verify that they actually persist there. We know exactly where the site is, so we just go there and we look up and down this reach of the Rogue River, and sometimes we find it on the other side of the river, up a little further, down a little further but generally we know where the site is.

Izzie: They've never found it in any other stretch of the river, but there it remains: each year, a couple of these mushrooms pop up in the middle of the current. They may fruit on waterlogged wood, from gravel or even straight from the sand of the riverbed, but they're all the same species. In 2009 Jonathan, Robert, and Dr Southworth's team submitted a paper proposing that the species be designated *Psathyrella aquatica*. But instead of triumphant music, the paper was accompanied by yet more of the doubts that plagued Robert's initial sighting.

Darlene: Was it difficult to get others to accept that the mushroom was new? Yes. Or that it grows underwater, yes. Nobody wanted to believe that. Somebody told his students that someone must have kicked it underwater.

Izzie: The distrust might be more forgivable if Psathyrella was a genus known exclusively from very dry areas, but actually, many *Psathyrella* fungi have been found on damp or wet soil. In fact, three of the six species closest to *Psathyrella aquatica* have been collected in damp habitats like that. That means that, like *Vibrissea truncorum*, their hyphae are growing underwater.

Darlene: So somehow they took the step further and get underwater...

Izzie: If you're a fungus with wet hyphae living in an area that gets flooded a lot, you might eventually develop some adaptations that let you live entirely underwater. In the case of *Psathyrella aquatica,* the major adaptation permitting an underwater lifestyle was probably just a bit of extra wax in the cells.

Darlene: By being just a little bit waxier, they may be able to not get yucky with water. You know, if you take ... *Agaricus* from the Safeway, and you soak them in water, they get icky? Well somehow these are just waxy enough that they don't get soggy and gushy. So that's a relatively small adaptation, and if they're really related to things that are wet-footed, who don't mind having their hyphae underwater, then it's not that big a deal.

Izzie: And remember, while it has a lot of stark contrasts to a dry habitat, life underwater has great advantages.

Darlene: It's so stable! And it doesn't have to rain, and it doesn't get too hot or too cold, so their season is longer. We've seen them this year in like mid-June, we've seen them as late as October. Can you find morels for that length of time in the same area? No!

Izzie: Moving into such a cozy habitat also sort of helps in the debate about whether *Psathyrella aquatica* is a new species. In biology there are a lot of definitions for what it means to be a species, but the one I find most often is that members of a species can reproduce only with each other. For various reasons - whether an issue of mechanics or genetics - an organism can't reproduce with any organism outside its species. This logic is cyclic and flawed, but generally accepted. That means that if a group of organisms reproduce with each other, and nobody else, for long enough, they might become unable to reproduce with anyone else - they'd be a new species. When a species branches off this way, it's called *speciation by reproductive isolation*.

If a variety of mushrooms evolved spores that could develop underwater, but the next-closest species didn't, then the spores under the water would be isolated from the spores on land. As we know, two spores have to meet up to make a mushroom, but in this case the underwater spores would only meet up with other underwater spores. This is a good argument for why the Rogue mushroom didn't match any terrestrial Psathyrellas, and would support the idea that it's a new species.

So, speaking of spore dispersal in water, how does *Psathyrella aquatica* manage it? With ballistospores, of course! These ballistospores do still need air to discharge, and unlike *Vibrissea*, the Rogue mushroom is never exposed to air; however, somehow, this mushroom that grows submerged in half a meter of water has air bubbles down there. You can see them clinging to the gills in that first picture from 2005.

Darlene: So what we think, based on that bubble – and it took us a long time to think this through – we think that they're released into this gas bubble. So they aren't released free into the water.

Izzie: Alright, the spores get fired out of the cap and into the bubble, where they touch the water and immediately go into the flow of the river to get spread downstream, right? Actually, no. Just like the mushroom itself, the spores are waxy, which means they repel water.

Darlene: It wasn't something we sought or expected. You know, we're just sort of working here. We just watch. And if you stir the spores in water, they don't disperse.

Izzie: So instead of having spores mix in with the current as they fall, you actually get spores gathering on the underside of these air bubbles under the cap. When you gently lift the mushroom out of the water, the bubbles stay intact for a few seconds. When they burst, the spores fall heavily into a dense spore print. So as with *Gloiocephala aquatica*, it seems the spores haven't adapted to a wet habitat as well as the fruits and the hyphae have. But there's an advantage to not sporulating directly into moving water.

Gloiocephala aquatica, fruiting in lakes, can rely on gentle, wind-stirred eddies to move spores somewhere favorable. By contrast, the Rogue mushroom is getting pounded with a current pouring 2900 gallons of water each second in a particular direction. If the Rogue mushroom's spores mixed with the water more easily, the fungi would only ever be able to spread *down*stream. Waxy as they are, the Rogue spores can instead hang out in their bubbles until some disturbance pops them, and then hang onto the nearest surface that is also waxy - for example, water insects, fish, mosses, or algae. Some of those - the insects and fish, mostly - could offer spores a chance at moving upstream. The spores could even cling to waterlogged wood and leaf litter floating in the river, providing them with a food source wherever they settle.

Sticking on insect larvae and getting eaten by fish would have the same effect, since spores pass through the gut undigested and would end up in nutrient-rich fish poop.

Of course, it's almost impossible to track the actual movement of the spores, because they are so small and numerous. They could also attach to a substrate, get jostled off of it, and reattach to something else. At this point, it's all speculation.

Darlene: Whenever we give a talk, by the end of the talk everybody's hallucinating, trying to make up an excuse. So the insect larvae get eaten by fish, who get eaten by great blue herons, who fly upstream, and so on. We can make up a really great story. We have no data to support this, except that the spores are not really wettable.

Izzie: It's possible that the spores aren't responsible, or solely responsible, for *Psathyrella aquatica* reproduction. The fungus might spread asexually via the hyphae under the stream bed.

Darlene: Yeah, one big colony underwater, it's possible. Our DNA methods don't let us answer that question.

Izzie: The fungus most likely uses a combination of these techniques, but the hyphal method seems like it's the most successful - at least, the Rogue mushroom team has never been able to find any specimens far enough from the original to suggest successful spore dispersal.

Darlene: We have looked upstream in tributaries and we've looked downstream, and we can't find them. We can't find the fruiting bodies.

Izzie: And despite all the searching, in the 11 years since Robert first found the Rogue Mushroom, no other gilled mushroom has ever "surfaced" underwater.

Darlene: Why don't other fungi do this? I have no idea, I haven't talked to them. But… there could be others. You know, we aren't looking. No one is looking underwater.

Izzie: Because of how little mycological attention aquatic habitats receive, Dr Southworth has been calling on amateurs and professionals alike to keep their eyes on the water for more mushrooms. This has generated several false reports - generally people reporting mushrooms like *Vibrissea truncorum* that got submerged in floods.

Darlene: They were not *Psathyrellas,* they were not fruiting underwater; they were just very soggy.

Izzie: They also got one maybe-report from a scuba diver. The guy called Dr Southworth, very excited about the mushroom, but when they went back to look at the site, he couldn't locate the specimen.

Darlene: Do I believe him? I believe him. But if I can't find it I can't go, there's nothing to be done.

Izzie: Still, there's one recent report that has Dr Southworth very excited. A few months before our conversation, she had received an email from someone near Lake Payette, in Idaho.

Darlene: It was the best picture anyone had ever sent that said "I could be Psathyrella." At that point, it was like late October, early November, I almost got in my car and drove to Boise, but it's a long way.

Izzie: Instead, she asked him to send the specimen in. Morphological analysis of both the mushroom and the spores match the Rogue River samples exactly, and the DNA comparisons are in the works.

[Music begins]

Izzie: What's beautiful is that this report brings the story full circle.

Darlene: He was not a mycologist, he had been up at a family gathering and they had stopped at this lake on the way back home towards Boise, and he saw these and ...his wife said why don't you take a picture and send it to that email address? And he did!

Izzie: Two amateur mycologists on family outings 11 years apart, finding something unusual in the water. Maybe this time people will take it seriously!

[Music ends]

Izzie: I hope this episode has opened your eyes to the marvelous world of underwater fungi, and the difficulty in exploring aquatic habitats and describing aquatic species. Jonathan and Dr. Southworth's team is still on the lookout for any underwater mushroom you see.

Darlene: I read my email every day to see if anybody's found any. Look anywhere underwater. We don't know if they occur in lakes, look in streams; wherever you are, look closely. If you're going by in a raft, you probably won't notice them ... But if you just happen to be standing in a little water and you look down, there it might be!

Izzie: If you find anything, snap a pic and send it to Dr. Southworth via her email, southworth@sou.edu.

Robert also wants me to remind everyone that the Rogue mushroom is just one example of the unique biodiversity found in the beautiful 3.3 million-acre Rogue Basin in Southern Oregon. It's a marvelous place to explore whether you enjoy rafting the rivers, hiking the beautiful woods, learning about the rich geology or the 8500 years of human habitation. And, of course, the mushroom diversity goes without saying.

[Music begins]

Izzie: Morel Dilemma is written and produced by me, Izzie Gall. Our theme song is "Fungi Among I", composed and performed by John Bradley, with additional music by Mihai

Sorohan. You can find more of Mihai's music at mihaisorohan.bandcamp.com. Special thanks this episode to Nicole for doing the intermission.

I also want to thank Dr. Southworth for giving me the inside scoop about the work involved in convincing the world that your underwater mushroom is real. Dr. Southworth, Jonathan, and Robert: thank you for your commitment to underserved and underwater fungi!

Morel Dilemma is on Patreon, where you can receive cool rewards for donating to the podcast, and donations start at just $1 a month. There's some fun stuff related to this episode going there soon, so be sure to check it out. You can also post a review on iTunes, or wherever you listen. Next episode combines fungi, dinosaurs, and spaaaaaace! Call 347-416-6735 if that tickles your interest or your imagination, and leave a mushroomy message to have your voice in the next episode. However you show it, thank you for your support! Mycelia later!

[Music ends]

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