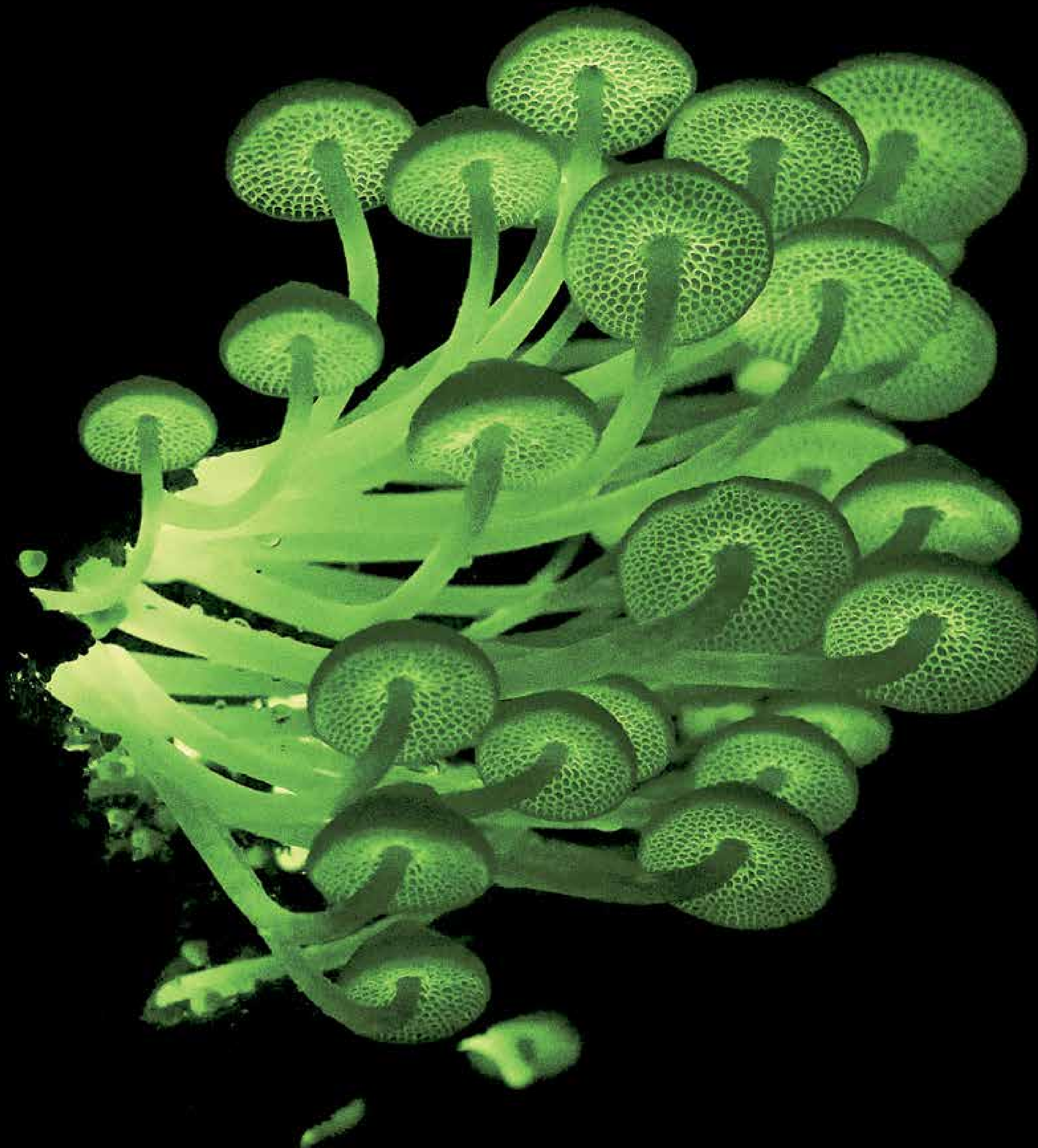

Bioluminescence in fungi is usually found in the fruiting bodies as seen here in Mycena *(*Filoboletus*)* manipularis. *Photo by Taylor Lockwood.*

Gigantic bioluminescent fungal networks of the Amazon

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Deep within the Amazon rainforest there is an extraordinary wonder of the fungal world that has gone unnoticed. It is a massive mycelial network living within the lower leaf litter that can be seen at night as a gigantic bioluminescent layer covering the forest floor. This expansive organism blankets huge areas but remains hidden until it is aroused to produce a vigorous glow. Many people have seen or heard about mushrooms or rotting wood that glow in the dark but in the Ecuadorean Amazon this bioluminescent fungus outperforms all other examples with its wide-ranging luminescence that sets the forest aglow.

The first time I saw bioluminescent fungi was when I was working as an instructor at a Massachusetts Audubon summer camp during my college years. One evening per session, the young campers had a night camping with night hikes to experience the sights and sounds of the forest. On a cloudy and completely dark night while hiking, the campers became apprehensive seeing sticks on the trail that had broken and were giving off an eerie glow. I had told them they may encounter nocturnal birds and other animals but had not mentioned they may come across glowing wood. Walking through a completely dark forest had already made the kids a bit uneasy and tense. When they saw the weird, unexpected glow, they started to become really scared. This was the first time I had seen "foxfire," which is a term used by Mark Twain and many others to describe a "false fire" type of light emitted from wood decayed by some fungi. I quickly tried to explain the phenomenon before panic set in. Having mentioned Mark Twain and the adventures of Huckleberry Finn and Tom Sawyer where they found foxfire, the camper's fear of a ghostly source for the light diminished rapidly and they had great fun finding more glowing sticks and waving them around. Since that first encounter, I have had many more adventures with bioluminescent fungi. Usually, I find fungi known to be bioluminescent and bring the fruiting bodies or decaying wood inside to view them at night. This is a lot easier and safer than tramping through a dark forest in the middle of the night looking for them.

Fungi such as *Armillaria* species (the honey mushroom), produce white mycelial fans under the bark that are bioluminescent and others such as *Panellus* in temperate forests and *Neonothopanus* and *Mycena* in the tropics produce fruiting bodies that glow in the dark. If it is a very dark night and your eyes have adjusted to night vision, the blueish-green glow can be significant.

Bioluminescence occurs in some insects, bacteria and marine animals as well as in fungi. There are estimated to be over 100 species of fungi that are bioluminescent in forests throughout the world. All of these are in the Basidiomycota and all appear to cause a white rot type of wood decay. Sixty-eight bioluminescent species are *Mycena*. This is a large percentage of the known bioluminescent species. However, there are more than 500 species of *Mycena* so not all have fruiting bodies that glow in the dark.

The mechanism of visible light emission from fungi appears to be a two-step process. As described in a recent paper in the *Proceedings of the National Academy of Science* by Ke et al., the compound hispidin is hydroxylated by an enzyme to luciferin. Oxygen is then added by the enzyme called luciferase, producing a high energy intermediate which emits light during decomposition. The amount of light emitted during this chemical process is low and the wavelength of fungal bioluminescence ranges between 520 and 530 nm.

A neurophysiologist researcher and friend at the University of Minnesota, Steven Hoffman, briefly explained this process of "dark adaptation": "The light sensitive portion of the eye, the retina, is a thin sheet that lies at the back of the eye. In the majority of humans, it is composed of three types of color receptive "cone cells," which through a mixture of their favorite wavelengths of

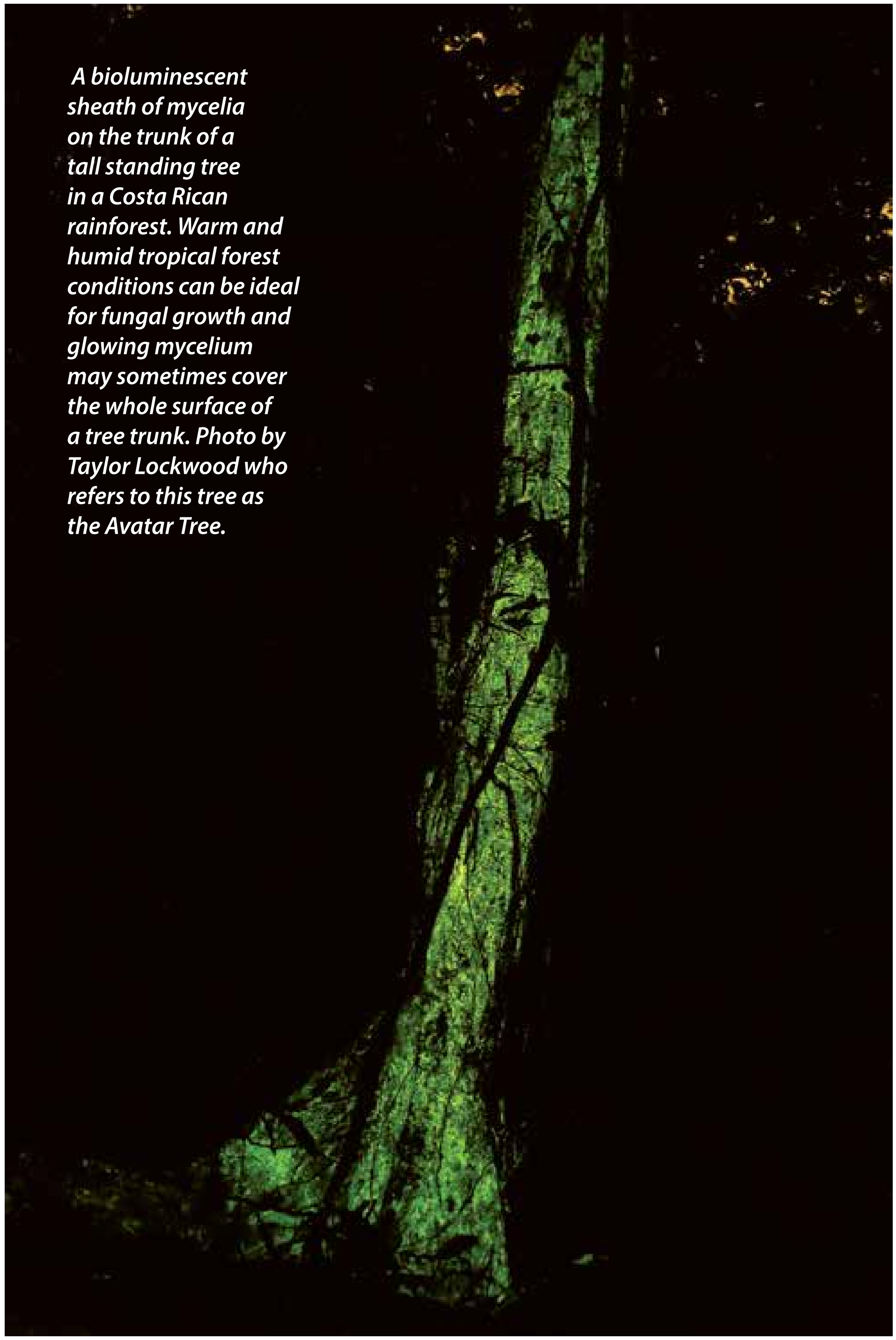

A standing dead tree in the Amazon rainforest at Yasuni National Park with bioluminescent mycelia. The fungus colonized the lower trunk of the decaying tree. Photo by Josh Kielsmeier-Cook.

color, allow us to perceive the different hues that make up the "visible light" portion of electromagnetic spectrum. These cells have a high density near the fovea, or center of gaze, of the eye. Another type of cell found in the retina are rods, which are more sensitive in dark environments and able to respond to just a single light photon. These cells have a higher density at the periphery of the retina. This is why when stargazing you can often detect a dimly lit star in the periphery, or out of the corner of your eye, yet as soon as to shift your gaze to focus on the star it disappears. Due to the range of intensities of light humans (and many other organisms) have evolved with, these cell types have an adaptive capability to vary their photosensitivity. However, due to the biophysics of this, processing time (roughly 20 minutes) and *very* dark conditions (which remove other light "noise" sources) are needed to fully appreciate the beauty of the relatively low energy photon emission from the fungi."

During forest pathology field classes, students often encounter small conifers that have been killed by *Armillaria* root rot. These can be pulled from the ground, taken inside to view and the outer bark removed to expose the white mycelial fans that are bioluminescent. Often, some students do not see the mycelium glowing and this is usually due to not having the room dark enough. Also, the mycelium must be fresh; trees killed by *Armillaria* that are old or dried out do not glow. Other factors such as the type of substrate, pH, and temperature can also influence bioluminescence. If you isolate *Armillaria* from an attacked tree into pure culture in agar-based medium, the mycelia often do not glow, but if you grow the fungus on a culture media that has breadcrumbs in it (just breadcrumbs, agar, and water), it glows brightly. Apparently, the various vitamins and other nutrients in breadcrumbs provide all the requirements that are needed. You can also culture it on sterilized wood and get good bioluminescence.

One amazing encounter I had with fungal bioluminescence occurred when teaching a field mycology class with Professor Maria Ordóñez at the Pontificia Universidad Católica del Ecuador, Yasuni Research Station in the biggest National Park of Ecuador. The research station is deep within the Yasuni Biosphere Reserve, a UNESCO World Heritage Site in Ecuador with 2.7 million hectares of continuous virgin tropical forests. It is one of the richest biodiversity hotspots in the world and our research at the site has shown that it is also extraordinarily biodiverse in fungi. Students in the class had lectures and field collection forays followed by lab work to identify morphological and microscopic characteristics to prepare specimens for accession in the Fungarium at the University in Quito. While at the station, students had heard from others doing research at night on bats and poison dart frogs, that sometimes bioluminescent fungi could be found. They were extremely interested in seeing this and eager to go out for a night foray. The biodiversity of Yasuni National Park includes a diverse population of very poisonous snakes, spiders, hornets, biting ants, and of course larger animals like jaguars. The terrain is a jungle with steep slopes in some areas and the heavy rain can turn the ground into very slippery mud. On a daytime foray, you must be exceedingly careful and with so many potential hazards, I was reluctant to take a large group of students into the jungle at night. However, these students were persistent and had little fear. After discussing the potential hazards and developing a plan to be extra cautious, a night foray was set. We traveled single file though the dark on an established trail to look for glowing mushrooms. We slowly walked for almost an hour with no success. We walked for another 20 minutes and still nothing. Discussions about whether we should turn around and go back took place as students stirred around and a decision to go on for just another 10 minutes was made. We did this, saw nothing, and very disappointedly turned around to walk back. As we returned and got to the site we had stopped at earlier, where 20 students had paced and kicked the leaves, we were awestruck to see the entire forest floor glowing. The bioluminescence here was not from mushroom fruiting bodies but from the network of fungal mycelium that was growing under layers of leaves. Wherever the bottom leaves of the leaf litter were turned over, there was bioluminescence and the longer we stayed at the site, the stronger the glow became. We had not seen the bioluminescence when we first passed through the site. It was only after some time had passed and we returned to the site that the glowing of fungal colonized leaves was evident. They kicked more leaves and 30 minutes later there was bioluminescence everywhere. The entire forest floor around us glowed!

After this awe-inspiring experience, we wanted to know the identity of this fungus and how well distributed it was throughout the forest. We also wanted to understand why there was a lag time between disturbing the leaf litter and the surge in bioluminescent activity. To get answers, graduate students, Cristina Toapanta Alban, Josh Kielsmeier-Cook, Nick Rajtar, and I, as well as researchers Maria Ordóñez and Charlie Barnes, explored the Amazon forest at night during several expeditions as we worked on different projects (more on Amazon fungi: FUNGI 15[1], Winter 2022; and https://doi.org/10.1186/s40529-023-00403-x). We were able to culture

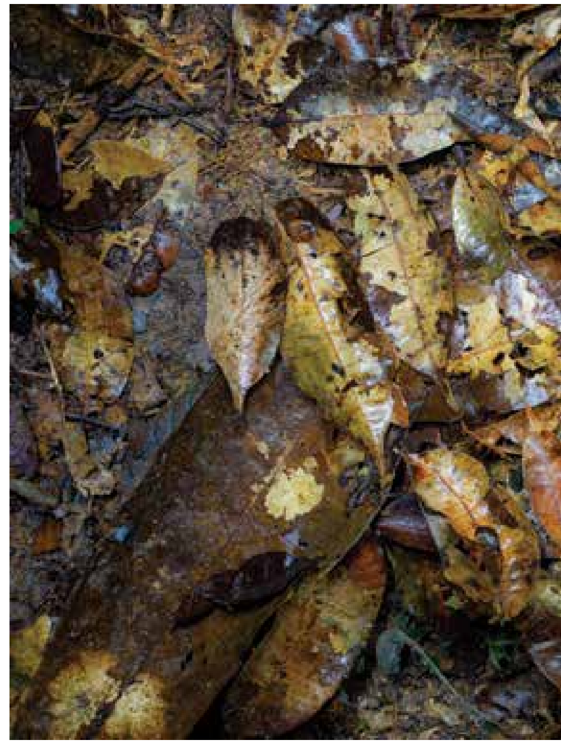


A bioluminescent sheath of mycelia on the trunk of a tall standing tree in a Costa Rican rainforest. Warm and humid tropical forest conditions can be ideal for fungal growth and glowing mycelium may sometimes cover the whole surface of a tree trunk. Photo by Taylor Lockwood who refers to this tree as the Avatar Tree.

Another tropical fungus with bioluminescent fruiting bodies is* Neonothopanis gardneri. *Photo by Taylor Lockwood.

The leaf litter along a trail at Yasuni National Park at night illuminated with a headlamp. To be able to see the bioluminescence, complete darkness is needed and flashlights need to be off for about 20 minutes so your eyes adjust to night vision.

the fungus and sequence several gene regions which indicated this fungus is an undescribed species of *Mycena*. Just about every upland site we checked around the research station had the same bioluminescent fungal mycelia under the leaf litter. However, during the dry season when it has not rained for weeks, there is barely any glow, but when the leaf litter is wet and the mycelium is robust, the glow can be intense. Some areas of bioluminescence were very large networks many meters in size and others were smaller more localized zones



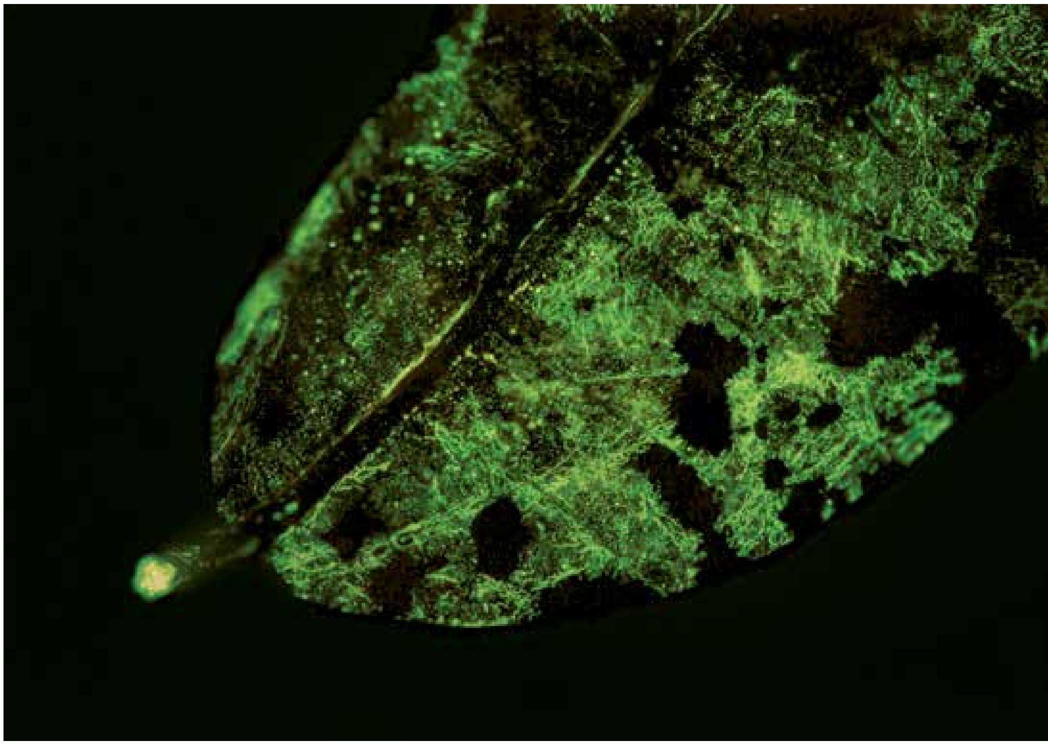
A section of leaf litter that had been disturbed showing leaves, sticks and a faint white covering that is the fungal mycelia growing over the organic matter. The photo was taken at night with a camera flash providing light. Photo courtesy of Tim Cockerill, Falmouth University.

of fungal colonization. Just how big some of these mycelial colonies may be needs additional investigation by brave individuals who want to tramp through thick jungle conditions and potentially dangerous encounters during the darkest of nights.

Having obtained a pure culture of the fungus and getting all the appropriate permits from the National Park and other Ecuadorian officials, I was able to study the new *Mycena* species in the laboratory. It is a saprotrophic fungus and can grow on leaves, wood, or other substrates. I have grown it on nutrient agar, sterile grain in large jars as well as on leaves, wood shavings, and grain in mushroom grow bags. The fungus produces a constant low level of bioluminescence day and night, but it is only seen in a completely dark room after your eyes have adjusted to night vision. A low level of bioluminescence is usual; however, this changes dramatically with some agitation. Even a small pinch on one side of the grow bag initiates increased bioluminescence throughout the bag. The intensity of the glow also

increases with time. The process of accelerated bioluminescence starts a few minutes after some physical stimulus and increases to peak production about 3 hours later. It then diminishes and returns to its original low level after a few more hours. A signal of some sort initiated by physical stimulus triggers and transfers an enhanced bioluminescence response throughout the mycelium. One small area of the mycelial mass gets agitated and over a relatively short time the entire colony glows brightly.

Induced bioluminescence has been reported previously. In Merlin Sheldrake's book, *Entangled Life*, he discusses how one part of a mycelial network may communicate with more distant parts and reports observations made by mycologist Steven Olsson. Professor Olsson grew *Panellus stipticus* in culture and found that if one part of the mycelium was wounded with a pipette tip, the wounded area immediately lit up immensely and after 10 minutes, the light had spread throughout the petri dish. He concluded that some sort of rapid communication system had to be operating within the mycelial network. In the large bags of *Mycena* mycelia that I grew in the lab, a similar transfer took place from one area of the mycelium that was disrupted to mycelia throughout the entire bag. The same type of reaction seems to be also taking place in the Amazon rainforest, but the transfer occurs over a much larger area as mycelial networks within the leaf litter get disrupted and exposed. When this happens, all the underlying mycelial mass reacts with an acceleration of bioluminescence that becomes



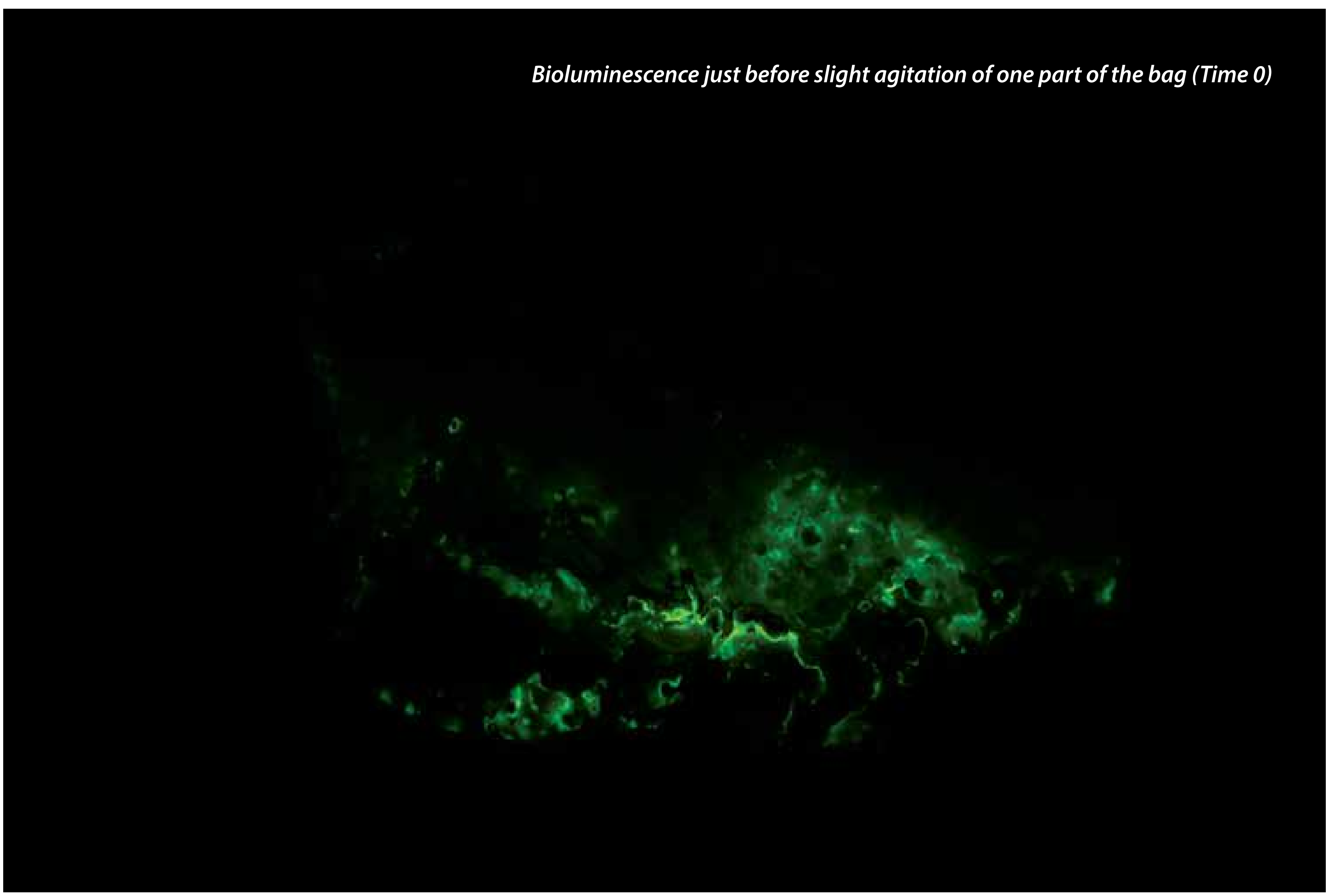
TOP RIGHT: The same area as seen in the light photo to the left showing the bioluminescent mycelia covering leaves and sticks. This image shows only a small zone of a huge area that was glowing. Photo courtesy of Tim Cockerill, Falmouth University.

BOTTOM RIGHT: A glowing leaf from the disturbed forest floor with bioluminescence inside the petiole and covering the surface of the leaf. Photo courtesy of Tim Cockerill, Falmouth University.

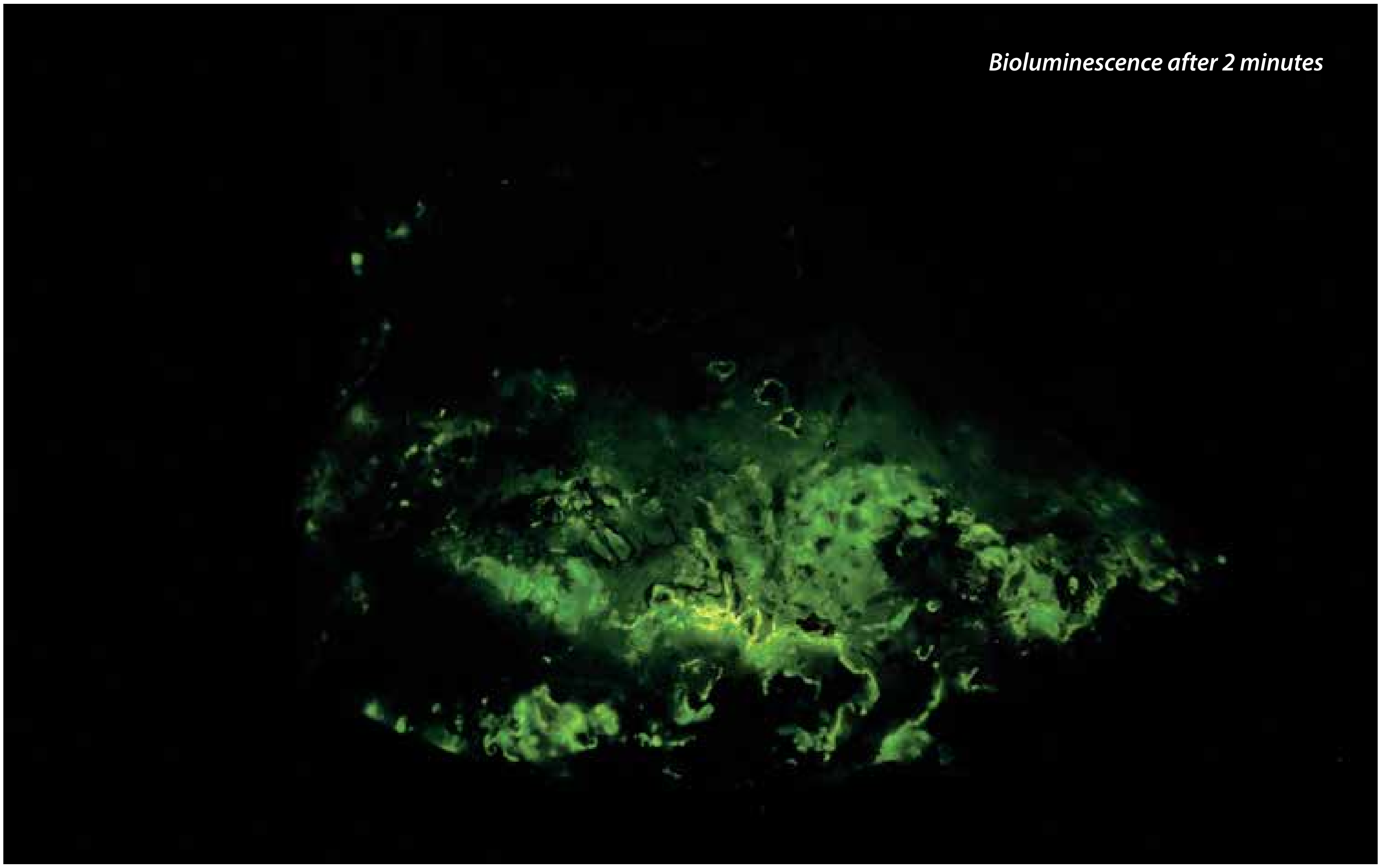
widespread over the forest floor.

Why some fungi produce bioluminescence is up for debate. Some have indicated that the light produced by bioluminescent fruiting bodies may attract insects to aid in spore dispersal while others suggest the light may repel possible fungivores. The bioluminescence in *Mycena* could have some purpose to ward off predation or it could just be a byproduct of increased metabolic functioning after disturbance. One thing is for certain, there is a chemical or other stimulus that can move throughout the mycelium very quickly. This sleeping giant of a fungus, long unnoticed in the dark forest of the Amazon, is now seen in a new light as a bioluminescent wonder of the fungal kingdom. In this truly incredible world we live in, what other bioluminescent fungi are waiting to be discovered?

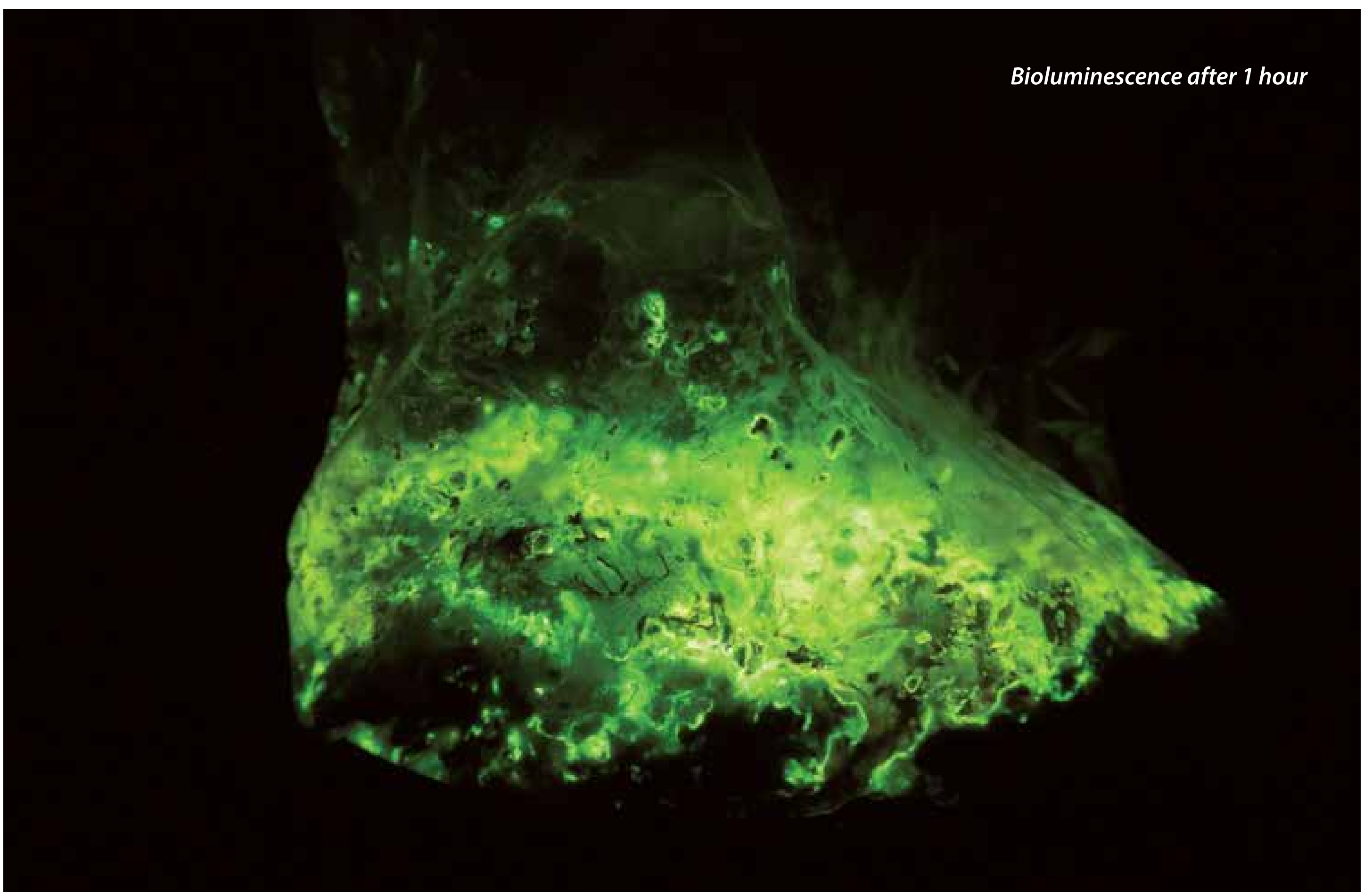
A photo time series showing mycelia of the Mycena *species cultured from leaves at Yasuni National Park and grown on a sterile substrate in the laboratory. Photos (above through page 34) showing the colonized bag illuminated with light, before slight agitation of one part of the bag, and after 2 minutes, 1 hour and 3 hours. The bioluminescence progressively expanded throughout the bag and became stronger over time. The glow reached a peak at about 1 hour and then gradually faded (glow after 3 hours on page 34–35).*



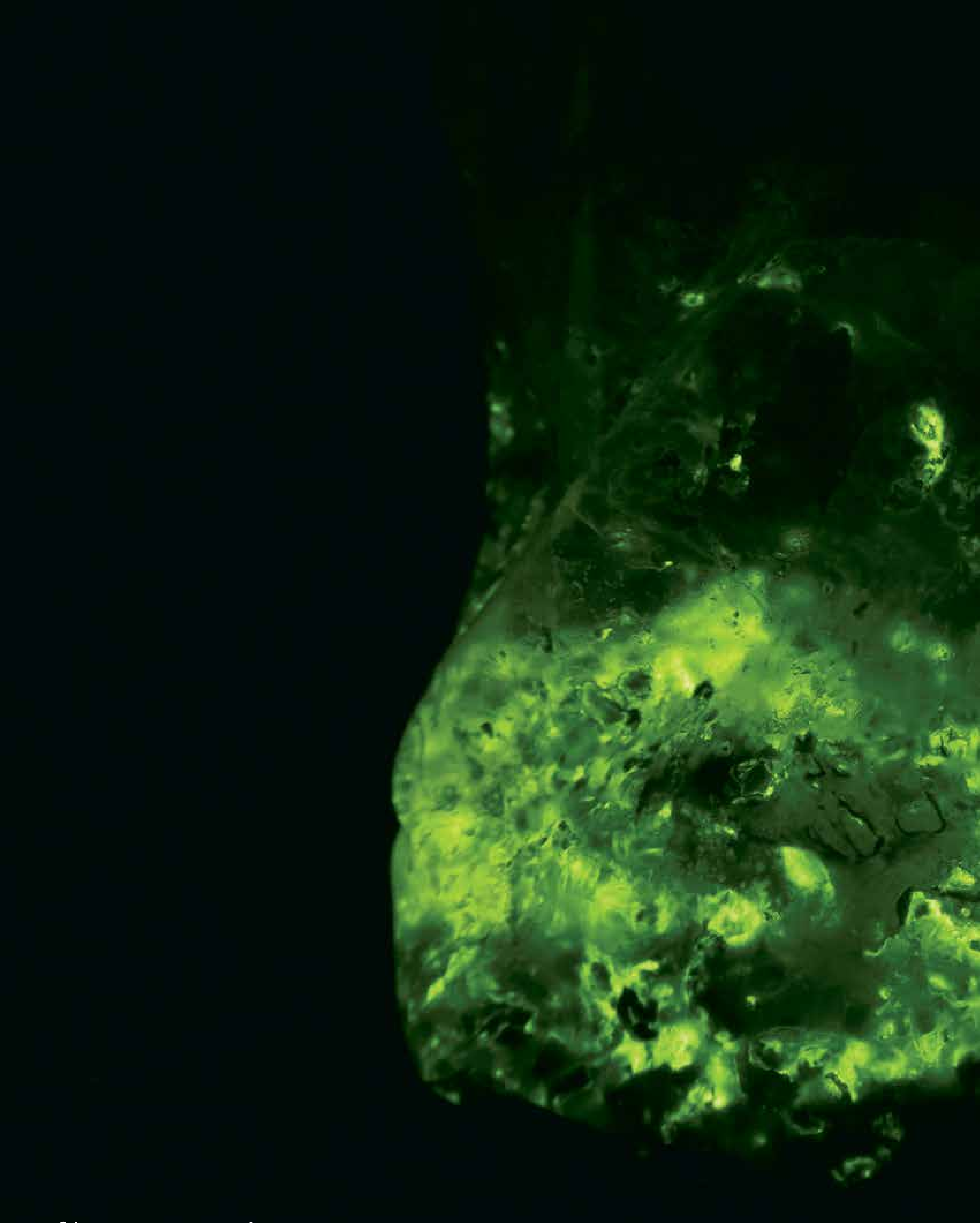
Bioluminescence just before slight agitation of one part of the bag (Time 0)



Bioluminescence after 2 minutes



Bioluminescence after 1 hour



Bioluminescence after 3 hours