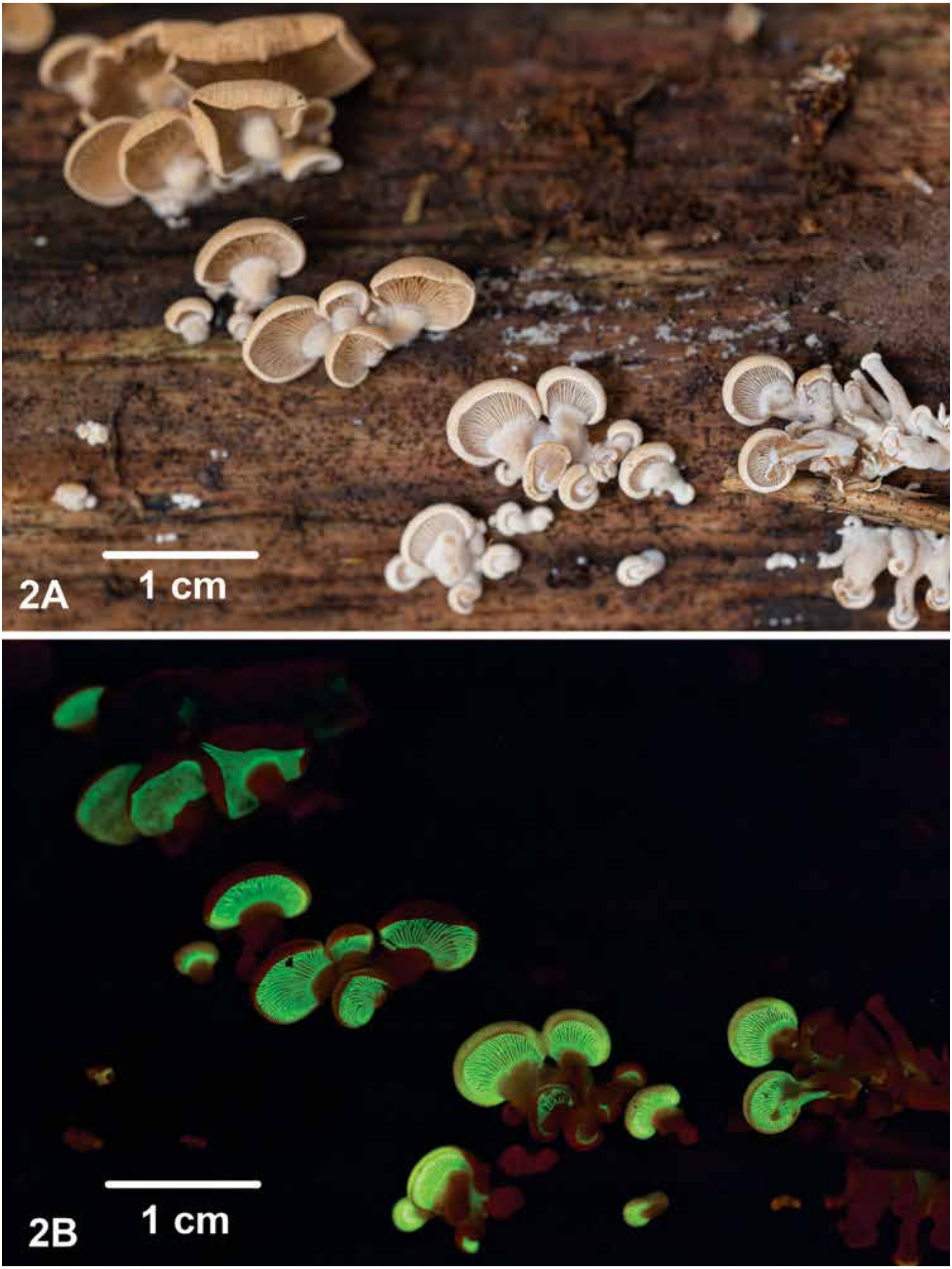
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Fluorescence and Bioluminescence in Mushrooms at Foray Newfoundland & Labrador

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What a Foray!

One of the highlights of last year's foray in Newfoundland and Labrador (FNL) for some of us (quite apart from the wonderful company, excellent food, and diverse fungi) was the exploration of **fluorescence and bioluminescence** in some of the mushrooms that had been collected, and challenging our veteran FNL photographer, Roger Smith, to capture this. And he did!

Foray faculty member Jan Thornhill was first struck by a beautifully fluorescent collection of *Gymnopilus picreus* (Figure 1; an otherwise unremarkable, small reddish-brown mushroom on wood), but it later turned out that quite a few collections of *Gymnopilus* and *Cortinarius* also fluoresced under Michael Burzynski's UV flashlight.

About bioluminescent mushrooms

The bioluminescent mushrooms were *Panellus stipticus*, a species that is common in Newfoundland and Labrador as well as most of North America, Eurasia, Oceania, and Australasia – but only collections from northeastern North America are bioluminescent (Buller, 1924).

This species was found many times during the Foray, on dead branches and trunks of alder, birch, or other hardwoods, and is readily recognized in the daylight by its tan, shelf-shaped fruiting bodies, 0.5–2 cm broad, with very crowded gills radiating from an arc that sharply separates stem from hymenium (Figure 2A). Only very fresh collections are bioluminescent, so when I came upon a really nice collection one day during the faculty foray at Grenville Heights, I wrapped it carefully in waxed paper and brought it back to Max Simms Camp to see if we were lucky. It took some doing to find a truly dark room at the camp, but when I did and allowed my eyes to adjust to the darkness, I convinced myself that my mushrooms were glowing. Some others were not convinced, but we got Roger to tackle the project of providing photographic proof, which you see in Figure 2B. Try it yourself – find a nice collection and impress your friends or use it to pry your kids away from a computer screen to see a glow-in-the-dark mushroom!

Fungi: Fluorescence … and Bioluminescence … Explained!

Let's start with those big words: **fluorescence** is the emission of visible light by a chemical compound that has been "excited" by absorbing light of an activating wavelength: typically, we shine an ultraviolet lamp on a mineral, a lichen, or a mushroom, and it responds by emitting a different wavelength of light, which might be greenish-white, pale blue, orange, lemon yellow or pink (Fig. 1). Fluorescence in lichens has been the subject of a nicely illustrated article in FUNGI (Lücking et al. 2014).

Bioluminescence is the biological production of visible light, as in fireflies, some jellyfish, and yes, some mushrooms (Fig. 2). The greenish-white light that we can see is produced during the enzymatic oxidation of a compound known as luciferin[1] in living cells, whether fireflies, bacteria, jellyfish, or mushroom fungi.

Fluorescence in mushrooms has been used to help detect and recognize poisonous members of the genus *Cortinarius* that contain the deadly toxin orellanine (Laatsch and Matthies, 1991; Keller-Dillitz et al., 1985), but as Jan and others who explored our tables of mushrooms at FNL can tell you, other species of *Cortinarius* (many in the subgenus *Dermocybe*), *Gymnopilus*, *Russula*, *Hypholoma*, and *Tricholoma* are also brightly fluorescent (Wright, 1990). The fluorescent compounds are often unidentified, but in *Leucopaxillus albissimus*, the fluorescent compound was found to be a highly bioactive compound called 2-aminoquinoline (Pfister, 1988). Fluorescence and fluorescent compounds in mushrooms are areas ripe for study, but also provide great entertainment in the woods or at the foray tables!

Bioluminescence in fungi has been known for a long time and was commented on by both Aristotle and Pliny the Elder (Harvey, 1957). Early studies were by Canadian mycologist A.H.R. Buller (1924; 1934), and by E.C. Wassink (1948) and E.J.H. Corner (1950; 1954). However, bioluminescence has been found in only a very small number of fungi – perhaps 100 of the over 100,000 described species of fungi (Oliveira et al., 2012; Wikipedia, 2022). The known bioluminescent fungi are in four lineages of mushrooms

1 The literal translation of luciferin is "light-bearing chemical." The term is a generic one for the light-emitting compounds in bioluminescent organisms, somewhat like referring to your bicycle, a Tesla, and an 18-wheeler as "vehicles" – see Figure 3.

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PUBLISHER'S NOTES: Although many wild mushrooms are quite palatable, some are deadly poisonous. It is advisable to avoid eating any wild organisms, including fungi, unless absolutely certain of identification. And although some mushroom species are edible for many people, those same species may cause allergic reactions or illness in others. When in doubt, throw it out. FUNGI wants to ensure that all readers are long-term subscribers. It is a good idea to have any wild mushroom checked by an expert before eating them. It should be understood that the Publisher and all Editors are not responsible for any consequences of ingesting wild mushrooms. Furthermore, the Publisher and all Editors are not engaged, herein, in the rendering of any medical advice or services. All readers should verify all information and data before administering any drug, therapy, or treatment discussed herein. Neither the Editors nor the Publisher accept any responsibility for the accuracy of the information or consequences from the use or misuse of the information contained herein. Unauthorized reproduction of published content of FUNGI is strictly forbidden, and permission for reproduction must be obtained by application in writing to the Publisher.

▶ *Figure 3. Different "luciferins" across kingdoms of life: A) firefly luciferin; B) the luciferin of dinoflagellates including* Noctiluca*; and C) the fungal luciferin 3-hydroxy hispidin. (A by Yikrazuul - Own work, Public Domain, https://commons.wikimedia.org/w/index.php?curid=6953255, B by Yikrazuul - Own work, Public Domain, https://commons.wikimedia.org/w/index.php?curid=10896498; and C by Wrfrancis - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=63895867).*

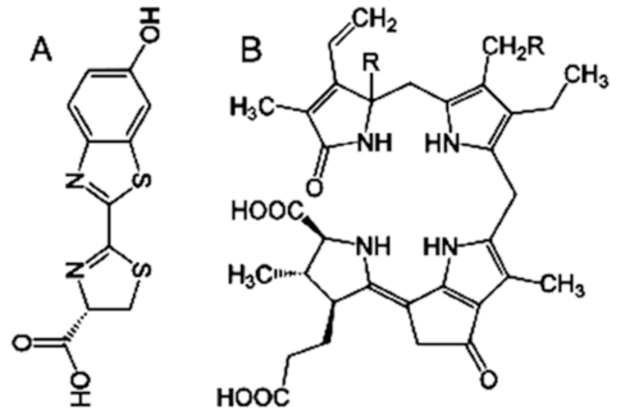

Figure 3.

(Agaricales, Basidiomycota) (Oliveira et al., 2012) and in *Xylaria hypoxylon* of the Ascomycota (Xylariales) (Wikipedia, 2022). In some mushroom fungi, it is the fruiting bodies that glow (or only their gills, as in our *Panellus stipicus*, Fig. 2), while in others such as the honey mushrooms (genus *Armillaria*) the living mycelium glows, but not the fruiting bodies (at least in North America). Chips of glowing wood colonized by living *Armillaria* mycelium have been used to light pathways in Scandinavia (Buller, 1924). *Panellus stipticus* is a relative of *Mycena*, of which over 60 species are known to be bioluminescent, particularly in the tropics (Oliveira et a., 2012).

Bioluminescent mushrooms use a chemical pathway which is different from that found in other organisms

As it turns out, all known bioluminescent mushrooms use a common chemical pathway and substrate (Oliveira et a., 2012), which is different from that found in fireflies or jellyfish. That is, the "luciferin" of fungi is not the same as the luciferin of fireflies, but is a compound called 3-hydroxyhispidin (Purtov et al., 2015) (Figure 3). The precursor compound hispidin is found in many non-luminescing mushrooms (and plants), so the ability to bioluminesce is restricted by the presence of a soluble NADPH-dependent hispidin-3-hydroxylase enzyme and the second enzyme, luciferase. These enzymes can be isolated from bioluminescent fungi by extraction with cold water, whereas a hot water extraction degrades the enzymes but gives a better yield of the substrate 3-hydroxyhispidin or its precursor hispidin. Knowing this, Purtov et al. (2015) mixed cold water extracts of the tropical bioluminescent mushroom *Neonothopanus nambi* with hot water extracts of non-luminescent fungi such as *Pholiota squarrosa* or luminescent ones such as *Panellus stipicus*, *Mycena citricolor*, and *Armillaria borealis*, and visible light was produced by all.

Why bioluminesce?

"Why?" you might ask. What is the purpose of constantly producing visible light from your gills that is visible (to us, at least) only when it is nearly completely dark? The same question could be asked about fluorescence in fungi – why should the gills and spores of some mushrooms, and the stems or caps of others, emit visible yellowish-green light when excited by ultraviolet light?

Again, the phenomenon probably occurs all day, while the mushrooms are being bombarded by sunlight containing UV wavelengths, but we just can't see the fluorescence emitted except when it is dark. Does fluorescent emission of light protect spores from damage by UV light? Maybe. However, this explanation doesn't help provide a rationale for bioluminescence, especially for bioluminescence of *Armillaria* mycelium within rotting wood, where no sunlight is penetrating. Perhaps it is just nature's way of keeping up the "Oh wow!" factor in mycology.

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Notes on the photographs

As the official photographer of Foray NL since 2004, my main task has been to photograph each specimen after it has been identified by the experts, before passing it on to the database team for entry into the database. In order to do this efficiently, I use a large light tent illuminated on each side by LED panels to give soft, even light which minimizes shadows and shows details of each specimen to best advantage. I place the specimens on a plain grey background which minimizes distraction and allows the specimens to stand out clearly. A small B&W centimetre scale to indicate specimen size completes the setup, which is then photographed using a Canon 70D digital SLR camera with a 17-50mm f/2.8 Tamron zoom lens, mounted on a copy stand. While this setup is fine for documenting the huge numbers of specimens of many different sizes that come through (1,215 specimens during Foray NL 2022), it is not so good for capturing the occasional specimen of particular interest to someone at the Foray. These are the occasions that cause me to perk up and realize that a challenge may be forthcoming!

Such was the case when Jan Thornhill found a small brown mushroom on one of the tables (*Gymnopilus picreus*, identified by Renée Lebeuf) whose gills glowed a brilliant yellow-green when she shone a UV flashlight (Convoy S2+ UV 365nm, provided by Michael Burzynski) on its underside. Jan and Helen Spencer then asked me if I could photograph the phenomenon, so I immediately accepted the challenge. I changed lenses to a macro lens (Canon EF-S 60mm f/ 2.8) so that I could focus more closely on the small mushroom. Helen then held the UV flashlight and I asked her to shine it on the underside at different angles to best show the fluorescence, which was in fact so bright I didn't have to dim my lights to allow it to show up. Figures 1A and 1B show the mushroom as it appeared under visible light only, vs under visible light plus UV illumination. The edges of the gills appeared to show the strongest fluorescence.

The next challenge came from Greg Thorn, who had collected a beautiful specimen of *Panellus stipticus* on a dead branch. Knowing that this species is one of several dozen that are bioluminescent, Greg found a dark room and, after allowing his eyes to adjust, was convinced he could see a faint green glow from the underside of the mushrooms. He then brought the specimen to me, and, as it was after 11:45 pm (a typical time for the hard-working Foray Faculty, who almost never sleep), we were able turn off the lights and make the room almost completely dark. In the same way that astronomers use long exposures to allow the faint light from distant galaxies and nebulae to accumulate on the camera film or sensor, thus revealing intricate details invisible to the human eye, I knew I had to use a long exposure to enhance the faint glow from the mushrooms. Modern digital cameras also have another tool available, that of very high ISO settings, (what we used to call film speed), which greatly increase the sensitivity of the digital sensor to incoming light.

I had with me one of Canon's new mirrorless cameras, the R5. While probably overkill for this particular assignment, the Canon R5 does have the ability to shoot at very high ISO settings. I chose ISO 6,400 and used my Canon RF 85mm f/2 macro lens at f/6.3. This is a medium f-stop allowing some depth of field to keep as many of the mushrooms in focus as possible, while not greatly extending the exposure time. For the setup in visible light, I first arranged the branch so that the maximum number of fresh mushrooms (said by Greg to produce the most bioluminescence) were visible and lying in a plane roughly parallel to the camera back, which minimized the depth of field necessary to get them all in focus. I set the camera on Aperture Priority auto exposure, set the lens on manual focus, focused on the most prominent mushrooms, then took the shot. (See Fig 2A for the result). Leaving the camera settings and focus the same, I asked one of the people crowded around the table to turn off the lights and we all watched expectantly as I fired the shutter. After a 30-second exposure (the default maximum time that Canon cameras give on Auto settings), the rear screen on the camera lit up with blazing green mushrooms, much to our delight. See Fig 2B for the final result, which has been enhanced somewhat using photo editing software to exaggerate the brightness of the luminescence. It would not appear that bright to the human eye. Note that just as Greg had predicted, the largest (and oldest) two mushrooms in the upper left corner no longer show luminescence.

–*Roger Smith, October 2022*