From *Fungi Kingdom News*, Summer 2018

Using the Microscope to Help get that I.D.

II. Hyphal Structures

By Sue Lancelle

Onward with our microscopy adventure! In the last newsletter issue, we provided an introduction to the microscopy of fungal spores. In this column, we will discuss microscopy of the basic gill structures in members of the gilled fungi (Agaricales). Once you learn about these structures, you can extend your study to the surface of the cap and the stipe, and the arrangement of the hyphae in these parts of the mushroom. You can also apply these same techniques to study members of the Boletales, keeping in mind that you are typically dealing with pores rather than gills. In future columns, we will try to cover some of the structures found in other basidiomycetes and ascomycetes.

The structures we are interested in are hyphae – the filaments that make up the fruiting body of the fungus – or developmental modifications of hyphae leading to specialized cells. Gills are typically lined with cells that are either basidia, the spore producing cells; basidioles, which are immature basidia that are not producing spores; or cystidia, cells of unknown function that are typically larger and shaped differently than basidia and do not produce spores. The important diagnostic features of these cells are how many spores the basidia produce, and the presence, shapes, sizes and locations of the cystidia. The presence or absence of specialized hyphal structures called clamp connections is another characteristic that is used to help identify fungi.

In order to understand where these structures are, you must think about the three-dimensional arrangement of the mushroom cap and gills, and then visualize how we can look at the structures in two dimensions on a slide. This will usually involve making a very thin section through part of the cap and gills in order to get a cross-section through the gills. In that way, you can look at the face of the gills and the gill edge.

There are different ways to accomplish this. One way is that described by Largent et al. *(How to Identify Mushrooms to Genus III*), and illustrated in Figure 1. The first step is to cut a small wedge in the mushroom cap, starting at the center if possible, large enough to include several gills (Figure 1A, B). Using a very sharp single-edged razor blade, cut the cap rim (on your wedge) off to expose the gills (Figure 1C). Pinch the wedge between your thumb and index finger to form a horseshoe shape (the tighter you can hold this tissue, the easier the sectioning will be) (Figure 1D,E). Then, slicing carefully toward your thumb, remove one or more very thin sections. Using a dissecting needle or something similar, transfer the sections to a drop of slightly soapy water, 5% KOH, or a stain solution (more on stains later) (Figure 1E).

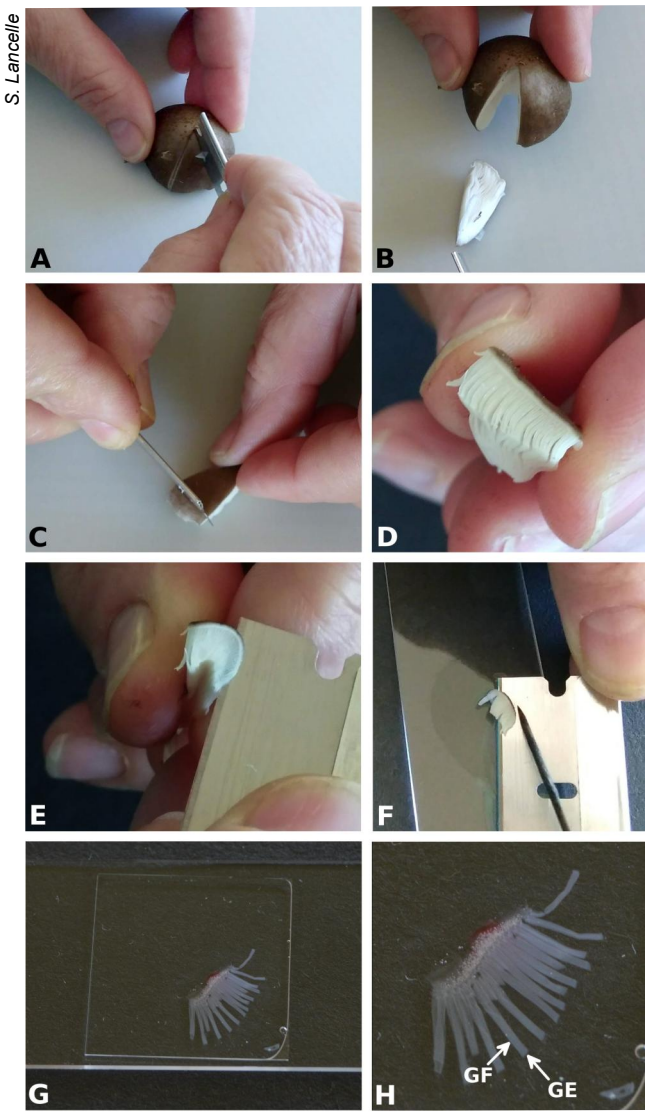

Figure 1. Obtaining a section. A and B. Cut a wedge from the mushroom cap. C. Slice off the cap edge, revealing the gills (D). E. Squeeze the piece of cap into a horseshoe shape and slice a very thin section, gently bumping up against your thumb to finish the cut. F. Transfer the section to a couple drops of solution (water, KOH or dye). G. Cover the section with a cover glass and add solution to eliminate air bubbles. Gently tap down on the cover glass to squash a bit if desired. H. Orientation of the gills, showing the location of the gill face (GF) and gill edge (GE). Note that this section is thicker than ideal, but is used for illustrative purposes. Even thick sections such as these reveal important information!

If the thought of sectioning against your thumb gives you nightmares of trips to the ER for stitches, it really isn't that dangerous if you work slowly. If you would still rather not try this method, you can always make a longitudinal slice in a piece of carrot and slide the wedge of cap tissue into it. Then squeeze the carrot together and cut some thin sections, right through the carrot as well. You can gently tease away any pieces of carrot as you place the sections on the slide.

Cover the tissue with a cover glass and add more liquid if necessary so that you have no air spaces under the cover glass (Fig 1G). If the section is somewhat thick, you may need to gently tap on the cover glass with the blunt end of the dissecting needle or something similar to squash the tissue a bit.

For purposes of illustration, the section in Figure 1 is much thicker than is ideal, but even with a thick section, you can glean a lot of good information. The key is to keep track of the orientation of the tissue, so you know where the gill edges and faces are (Figure 1H). It is also important to realize that very small or very large caps, as well as very mature tissue, is much more difficult to section. Do not get discouraged, because even less than perfect sections can be very useful.

Another type of very useful preparation is to cut a small piece of the whole gill that includes the edge (cut a shape like a trapezoid that will let you keep track of the gill edge), place it in liquid on the slide, and gently squash it a bit under a cover glass. This will let you double-check for the presence of cystidia on the gill edges, since it is sometimes difficult to get the gill edge on a gill cross-section. If there is a variety of cystidial shapes on the gill edge, using a whole mount will also make it easier to see that.

Just a note on terminology: Just like describing fungal spores, there are so many terms used in describing the various hyphal structures that it can be overwhelming. It really helps to have some kind of glossary with illustrations at hand to help you sort it out. The aforementioned book *How to Identify Mushrooms to Genus III: Microscopic Features* by David Largent, David Johnson and Roy Watling is an excellent source; other excellent books that are not currently in print but are available on the used market include *How to Know the Gilled Mushrooms* by Alexander Smith, Helen Smith and Nancy Weber and *A Glossary of Mycology* by Walter Snell and Esther Dick.

Generating Contrast

If you view a fresh section of fresh tissue in a standard light microscope, it can be difficult to see much of anything. This is because cells are made mostly of water, and water doesn't inherently generate contrast. Expensive research microscopes may have specialized optics that can generate contrast in the image you see (phase contrast or Nomarski DIC, for instance), but most people will need to generate contrast another way. One way of doing this is to shut down the diaphragm in the condenser until you can see the tissue. Figures 2 and 3 show the difference between leaving the condenser diaphragm open as it normally would be for light microscopy, and shutting it down a bit to get some contrast. Be aware that doing this reduces the resolution (or detail) that can ultimately be generated, but you do need to see the tissue, so we need to accept that trade-off.

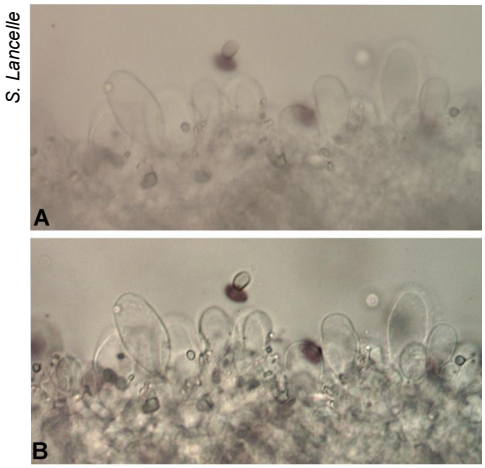

Figure 2. Generating contrast. A. Image obtained from a normally aligned light microscope. B. Same are as in A, with the condenser diaphragm closed down a bit to generate contrast.

Another way to see the tissue is to use a colored stain. Mycologists use many different stains, some for very specific uses, but good general purpose stains include Congo Red and Cotton Blue. Place tissue sections directly in the stain, let it sit for a few minutes, then draw off the stain with a tissue or small bit of paper towel and replace with water or KOH. Sometimes even after using a stain, it helps to close the condenser diaphragm down a bit (Figure 3).

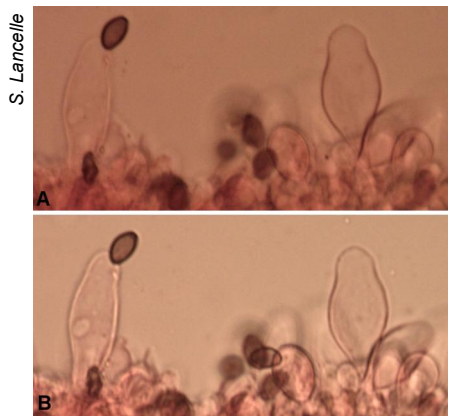

Figure 3. Use of dye to generate contrast. Tissue has been stained with Congo Red (A). In (B), the condenser diaphragm has been closed down a bit to add even more contrast.

Basidia and Basidioles

Basidia are the spore-producing cells and are found all over the gills. In almost all cases, the basidia are club-shaped, so the shape itself is not usually a defining characteristic. Spores are attached to little prongs, termed sterigmata (sterigma in the singular) at the apex of the basidia (Figure 4A). Basidioles look like basidia, but do not have sterigmata. Most often there are four sterigmata per basidium, although you may have to look at the basidium on an angle or focus up and down a bit to see all of them (Figure 4B, C). Some species have two-spored basidia (Figure 4D, E), sometimes there is a mixture of two- and four-spored basidia, and sometimes the number of spores per basidium changes as the cap matures! Thus you must be careful in describing this feature and do a lot of careful observation to be certain. Be aware that you typically see spores in various stages of development (Figure 4B-E); this is why, when you are looking at spores, it is best to measure ones that have fallen on a spore print if possible, to make sure you are measuring them in a mature state.

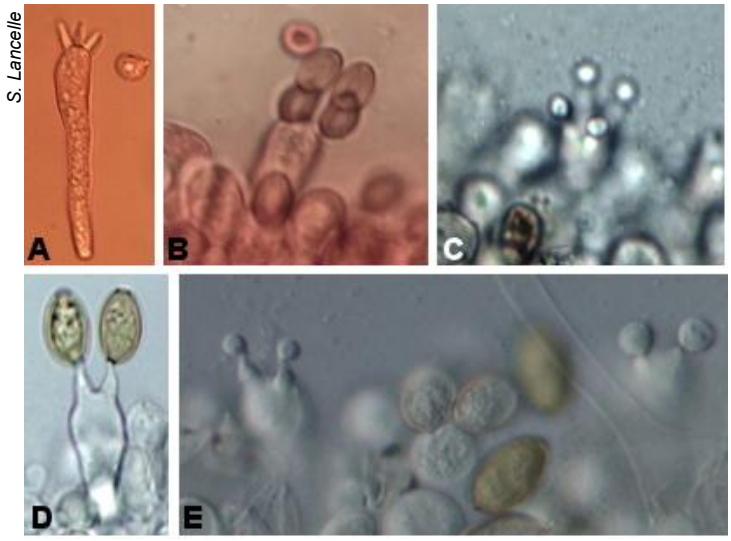

Figure 4. Basidia. A. Typical club-shaped basidium with four sterigmata. B. Four nearly mature spores on a basidium. Sometimes it takes careful focusing up and down to see all four. C. Four very young spores, observed with the basidium at an angle. D, E. Two-sterigmate basidia from *Conocybe macrospora*.

Sometimes basidia and basidioles contain granules that take up certain dyes, and this can be a diagnostic characteristic. Figure 5 shows the "cyanophilic granules" that stain with Cotton Blue in the basidia of *Lyophyllum decastes*.

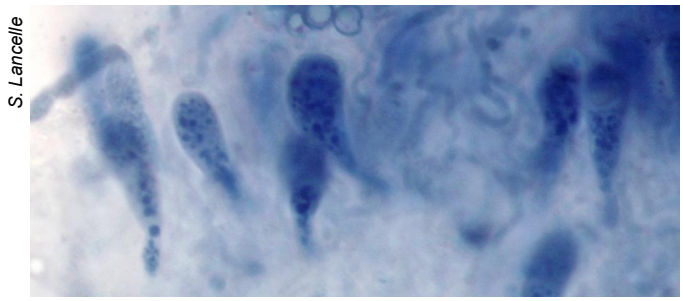

Figure 5. Cyanophilic granules. Basidia from *Lyophyllum decastes* stained with Cotton Blue, showing the dark granules inside.

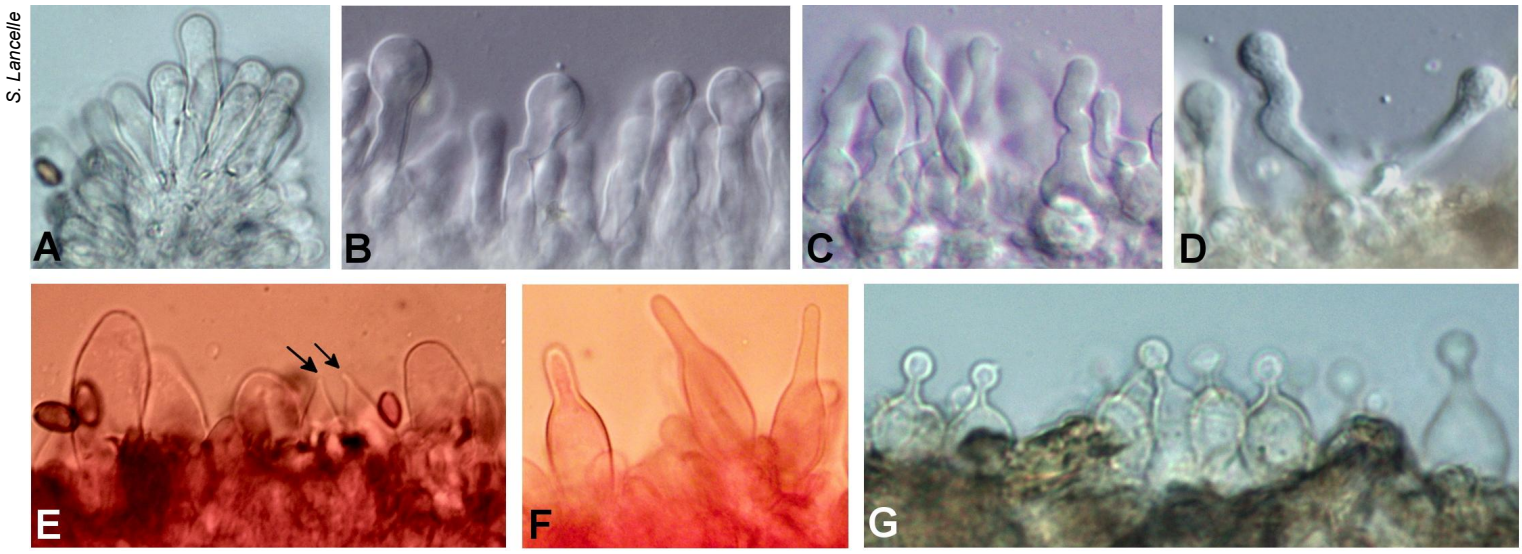

Figure 6. Variety of cheilocystidia shapes. Cheilocystidia are found on the gill edges. A. Cylindrical, *Psathyrella candolleana*. B. Capitate (with a bulbous apex), from an unknown species. C. Strangulate, from a coprinoid species. D. Cylindrical to strangulate with a capitate apex, *Flammulaster erinaceellus*. E. Two shapes, widely cylindrical and mucronate-rostrate (with a pointed or beaked apex, arrows), *Stropharia rugosannulata*. F. Ventricose (widest in the middle), *Agrocybe arvalis*. G. Lecythiform, typical of some *Conocybe*.

Cystidia

The key to distinguishing cystidia from basidia or basidioles is that cystidia are typically larger and/or longer, or else a very different shape. Cystidia found on the gill edges are generally called cheilocystidia, with other variations of that term describing variations on a basic thin-walled cystidium (leptocystidium). Figs. 6 and 8A show a variety of cheilocystidia shapes, imaged in various ways.

Cystidia found on the faces of the gills are generally called pleurocystidia. They may be the same shape as the cheilocystidia, or they may be an entirely different

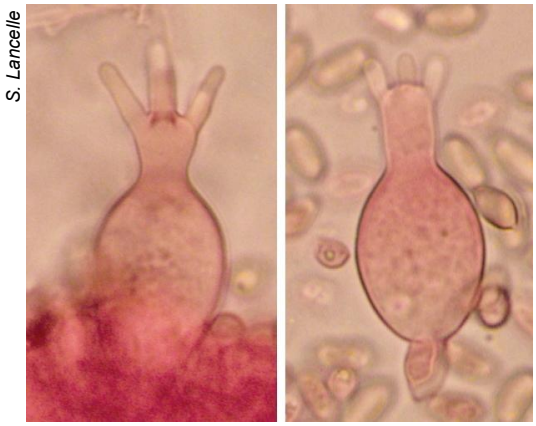

Figure 7. Pleurocystidia of *Agrocybe arvalis*. Ventricose with a digitate (with finger-like projections) apex.

shape – compare Figure 6F with Figure 7, both from *Agrocybe arvalis.* The distinctive "horned" pleurocystidia of *Pluteus cervinus* (Figure 8A) are very different from the variety of shapes displayed by the cheilocystidia of the same species (Figure 8B).

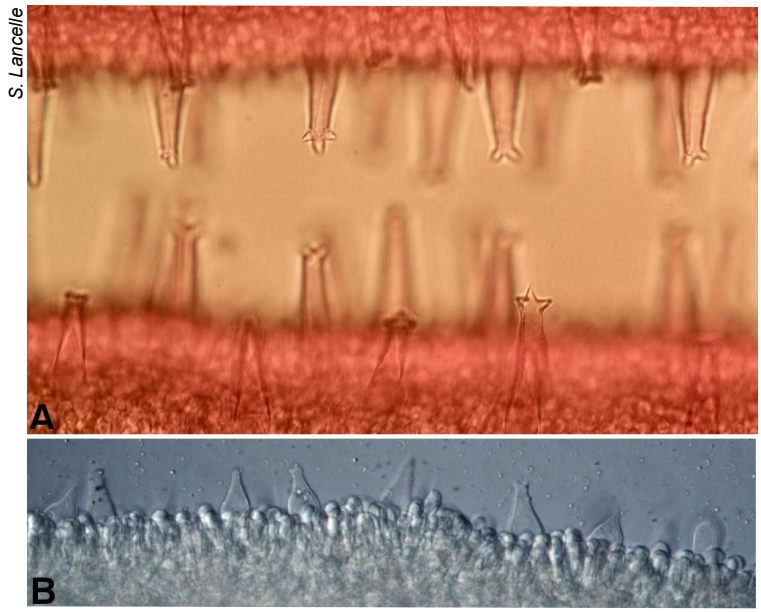

Figure 8. Cystidia of *Pluteus cervinus.* A. The characteristic pleurocystidia are thick-walled and contain a number of "horns" at the apex. B. Variety of shapes of cheilocystidia, seen here with a whole mount of a piece of a gill.

Cystidia can develop crystalline inclusions inside the cells (Figure 9); these are referred to as chrysocystidia, and are typical of the genera *Stropharia* and

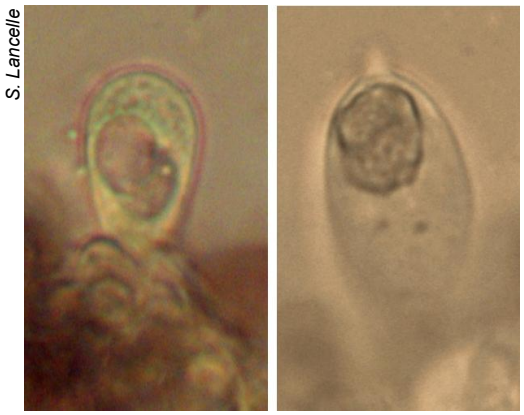

Figure 9. Pleurocystidia from *Protostropharia alcis.* These contain large crystalline inclusions. The descriptive term then becomes "pleurochrysocystidia."

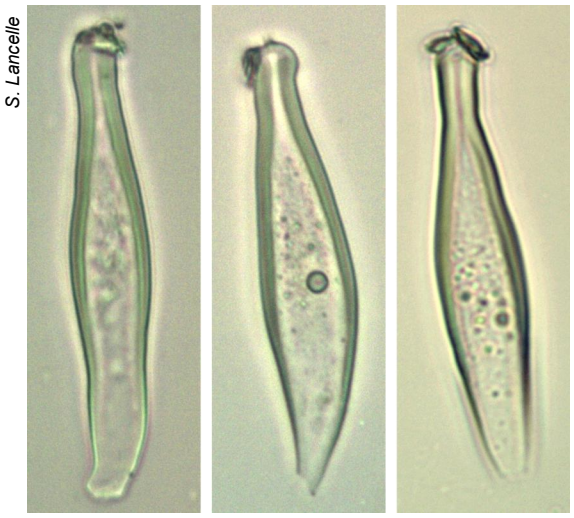

Figure 10. Pleurocystidia from *Inocybe* sp. These are typical of some species in the genus, with encrustations at the apex and thick walls (lamprocystidia).

Protostropharia. The characteristic pleurocystidia of some *Inocybe* develop encrustations on the outside of the cell at the apex (Figure 10). Thick-walled cystidia such as these are termed lamprocystidia.

Be aware that characteristic cystidia can also be found on the surface of the cap (pileocystidia) and the stipe (caulocystidia), and sometimes it helps to look carefully for those as well.

It can be very difficult to measure cystidia, because of the need to see the entire cell. After careful observation of your slide preparation, you can try gently tapping or pressing on the cover glass to gently squash the tissue. This sometimes releases the cells so that you can get a clearer view.

Clamp Connections

Another diagnostic microscopic characteristic that you

often see mentioned in formal descriptions of fungi is the presence or absence of clamp connections. These are swellings that may occur at the point of the hyphal septae, or crosswalls, and can be found at the bases of basidia, basidioles, cystidia, or along any of the other hyphae. They function in distribution of cell nuclei during hyphal elongation. Figure 11A shows what clamp connections look like when you are able to tease out the hyphae from the rest of the tissue. More commonly, you are looking for them in a mass of tissue, as shown in Figure 11B. You have to look carefully but after a while you can pick them out if the tissue isn't too thick.

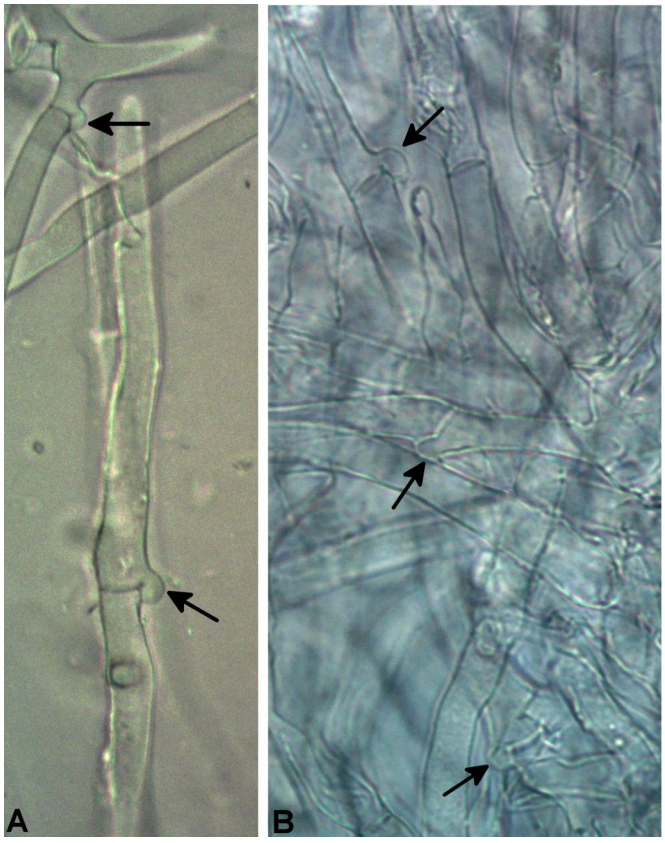

Figure 11. Clamp connections in *Infundibulicybe squamulosa*. A. The clamp connections are swollen areas that are seen at the locations of septae, or crosswalls, in some species. They are easy to see when the hyphae can be teased away from the rest of the tissue, but are often seen in a mass of tissue (B), where you have to look more carefully for them.

This has been just a brief overview of some of the important microscopic features of mushroom gills. Delving into the micromorphology of fungi is a challenging but worthwhile endeavor if you want to take your ID skills to the next level. Warning: it can become a very consuming pastime!

My heartfelt thanks to Dr. Roy Halling for his generous and patient advice and guidance during my quest to learn how to study the micromorphology of fungi.