Fungal Microscopy

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Why use a microscope for mushroom ID?

Many mushrooms can be accurately identified to species by sight, however taxonomically significant features are often not visible to the naked eye.

For genera such as Inocybe, Conocybe or Galerina, a microscope is usually necessary to determine the species.

Microscopy is very helpful for determining the genus of mystery mushrooms.


CEMENTO
CRUZAZUL
SLIDES


KOH
Columbia

Types of microscopes

Stereo microscopes are lower magnification microscopes, and the sample is often lit from above.

Compound microscopes are higher power microscopes, and the sample is sandwiched between a microscope slide and a cover slip, and lit from below.

Stereo microscopes are good for preparing slides for viewing in a compound scope.

Stereo Microscope

Compound Microscope

Inexpensive Biological Microscope

The least expensive microscope that works well for Mycology is the Amscope B-120ms. It costs about $200. Another good inexpensive scope is a Swift 380.

You should also get a stage micrometer to calibrate your measurements, and

a camera to take photos.

Better microscopes

If you have more to spend on a scope, you can get better optics.

Plan optics flatten the image.

Plan achromatic both flatten the image and correct for chromatic aberration.

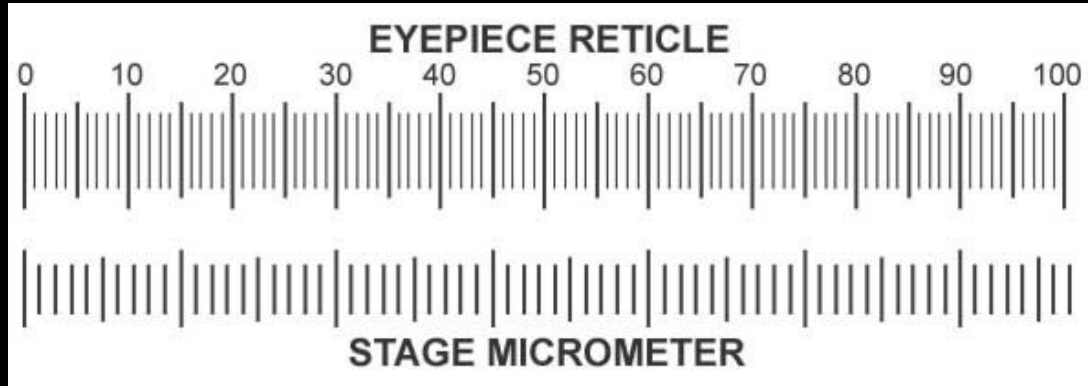
Plan apochromatic objectives are best.

Illumination

* Brightfield is standard
* Darkfield makes the background black
* Rheinberg illumination changes the background and/or subject color
* Phase contrast makes clear objects stand out, but adds optical aberrations
* Differential interference contrast is the best, but is expensive.

Stage Micrometer

To make accurate measurements, a stage micrometer is used for calibration.
Measurements can be done with an eyepiece reticle, but digital measurements are better.



Digital Measurements

Several programs are available to help you measure objects in your micrographs.

The best one for mycology is Piximetre, however it only works on Windows. ImageJ is another, and works on Linux, Mac OSX and Windows.

Using a stage micrometer, you can find out how many pixels per micrometer your images have, and make very accurate measurements quickly.

Measuring spores with Piximetre

- Quickly and accurately measure spores and other microscopic features
- Free download from http://ach.log.free.fr/Piximetre
- Calibrate with a stage micrometer
- Automatically calculates spore ranges, mean and Q values
- Add scale bars

10.6 [11.4 ; 12.4] 13.2 x 5.8 [6.3 ; 6.8] 7.2 micrometers

Q = 1.6 [1.7 ; 1.9] 2.1 ; N = 7 ; C = 95%

Me = 11.9 x 6.5 micrometers ; Qe = 1.8

12.13 6.60

10.72 5.99

11.65 7.10

11.60 6.48

12.21 6.68

12.47 6.30

12.72 6.41

Cell phones as cameras

* Cell phones work well, and often provide better images than microscope cameras.

* Autofocus changes size of the image.

* Distance from the ocular changes size of the image

* The phone needs to be held at the right distance and angle – a spacer helps. Cell phone holders are also useful.

Rehydrating dried material

Mushrooms can be examined either fresh or dried. Mushrooms that are hundreds of years old still usually have their microscopic features intact.

Dried mushrooms should be rehydrated either with a drop of 70% isopropanol, followed by water, or a drop of 5 – 10% potassium hydroxide.

Microscopy Chemcals

* A drop of water is used to mount the sample
* Or you can use KOH, which often causes taxonomically significant color changes
* If you add a little dish soap to the water, that will help with the air bubbles
* Congo red and Methylene Blue are common stains. You can also use diluted food coloring.
* Melzers reagent contains iodine and chloral hydrate.

Taking Photographs

You can get a digital eyepiece, which is a USB camera.

You can get an adapter for DSLR cameras.

Slide Preparation

The most important thing is to get the smallest amount of material you possibly can. This is extra important if you are using the higher magnification levels. .5 mm is far too thick. It helps a lot to use a dissecting scope, but is not required.

A cross section of a mushroom cap shows several microscopic features in the same mount. It is helpful to cut on a slight angle, so one side is impossibly thin and the other side is too thick. Then cut off the thick side.

Spores

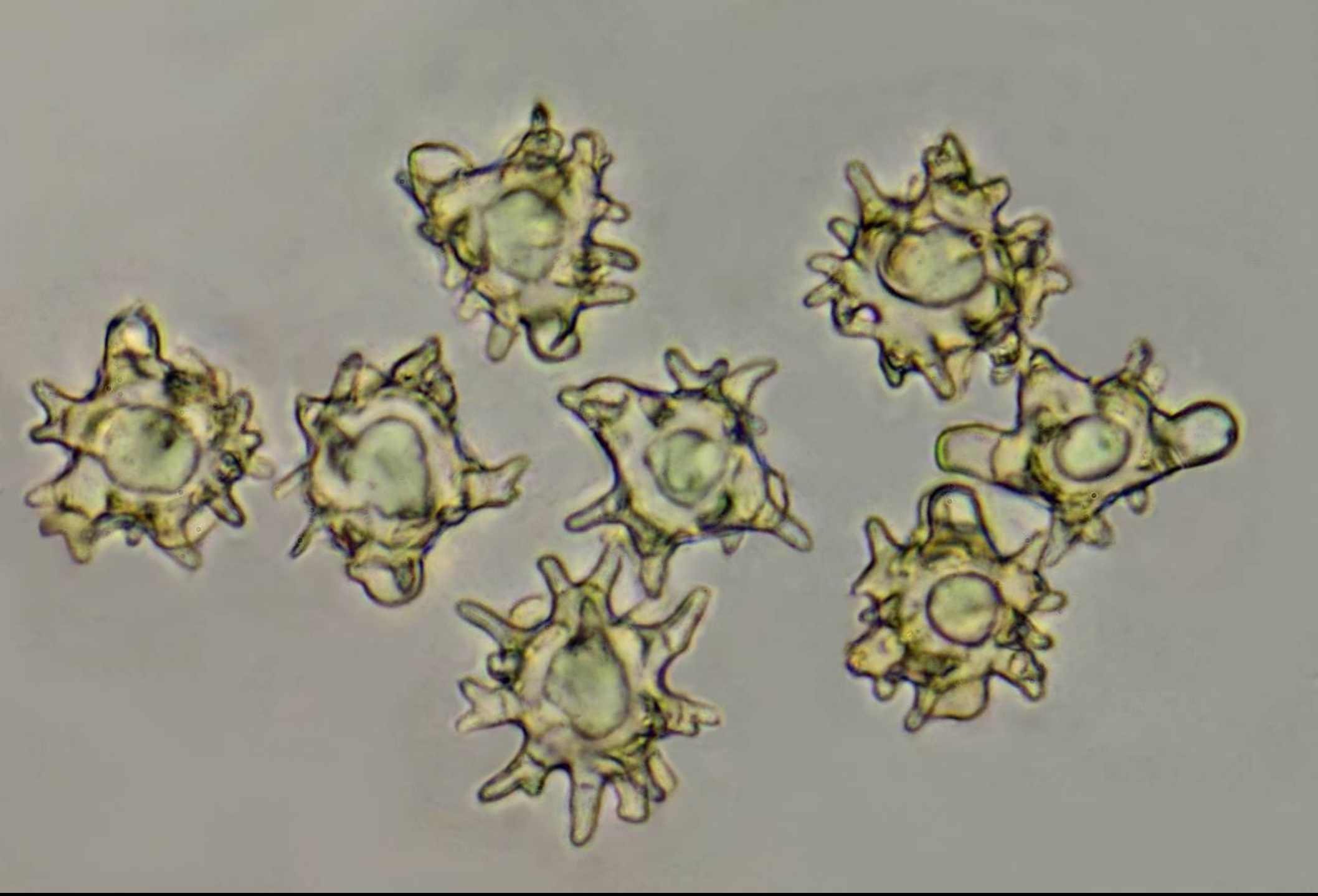
- Produced on basidia
- Usually measure between 3 and 80 micrometers.
- Best viewed from a spore print
- Use highest magnification objective, preferably oil
- Press down hard on the cover slip to force all the spores into the same focal plane


Puccinia evadens

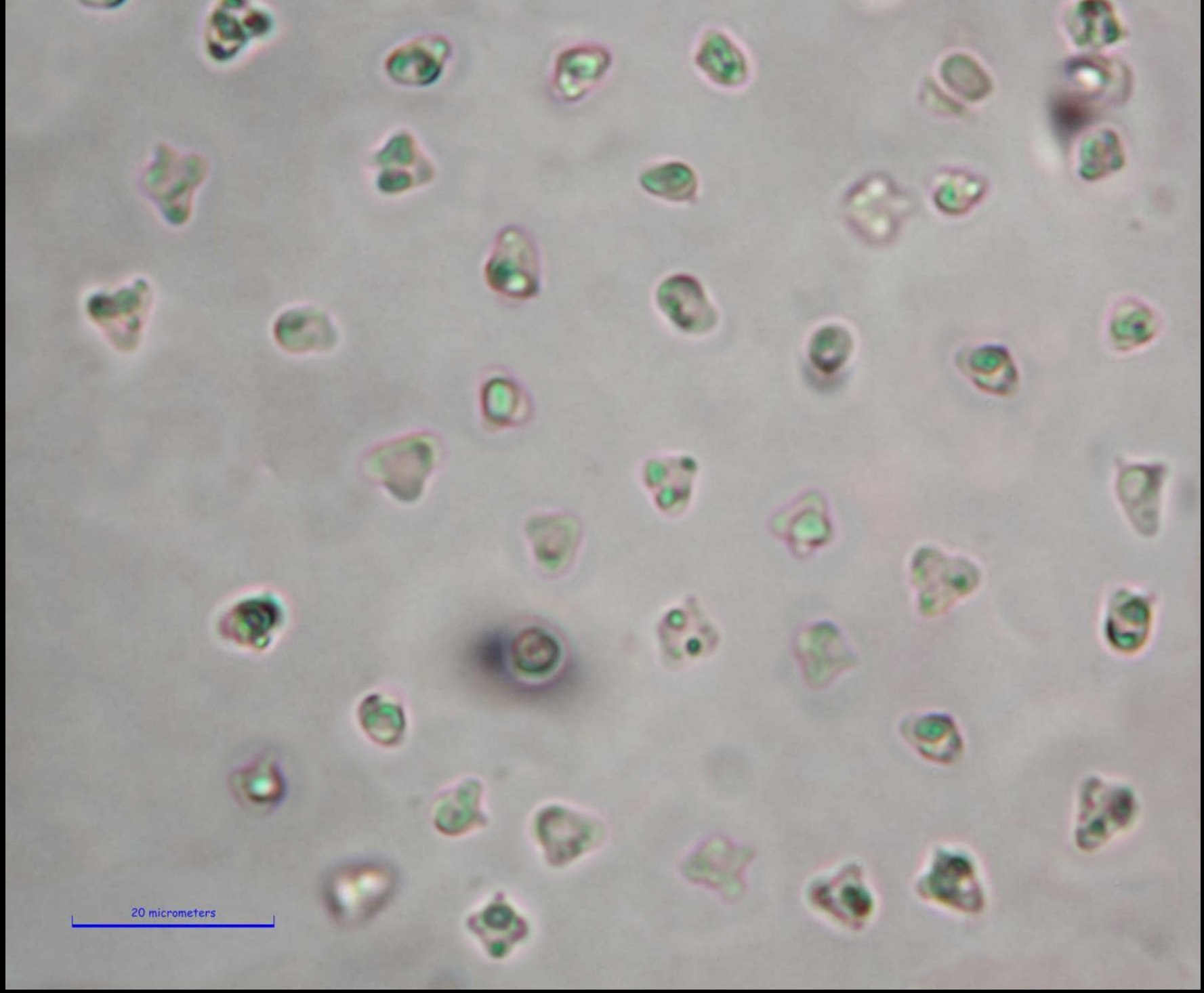
Psilocybe baeocystis

Tuber oregonense spores

Asterophora lycoperdoides spores

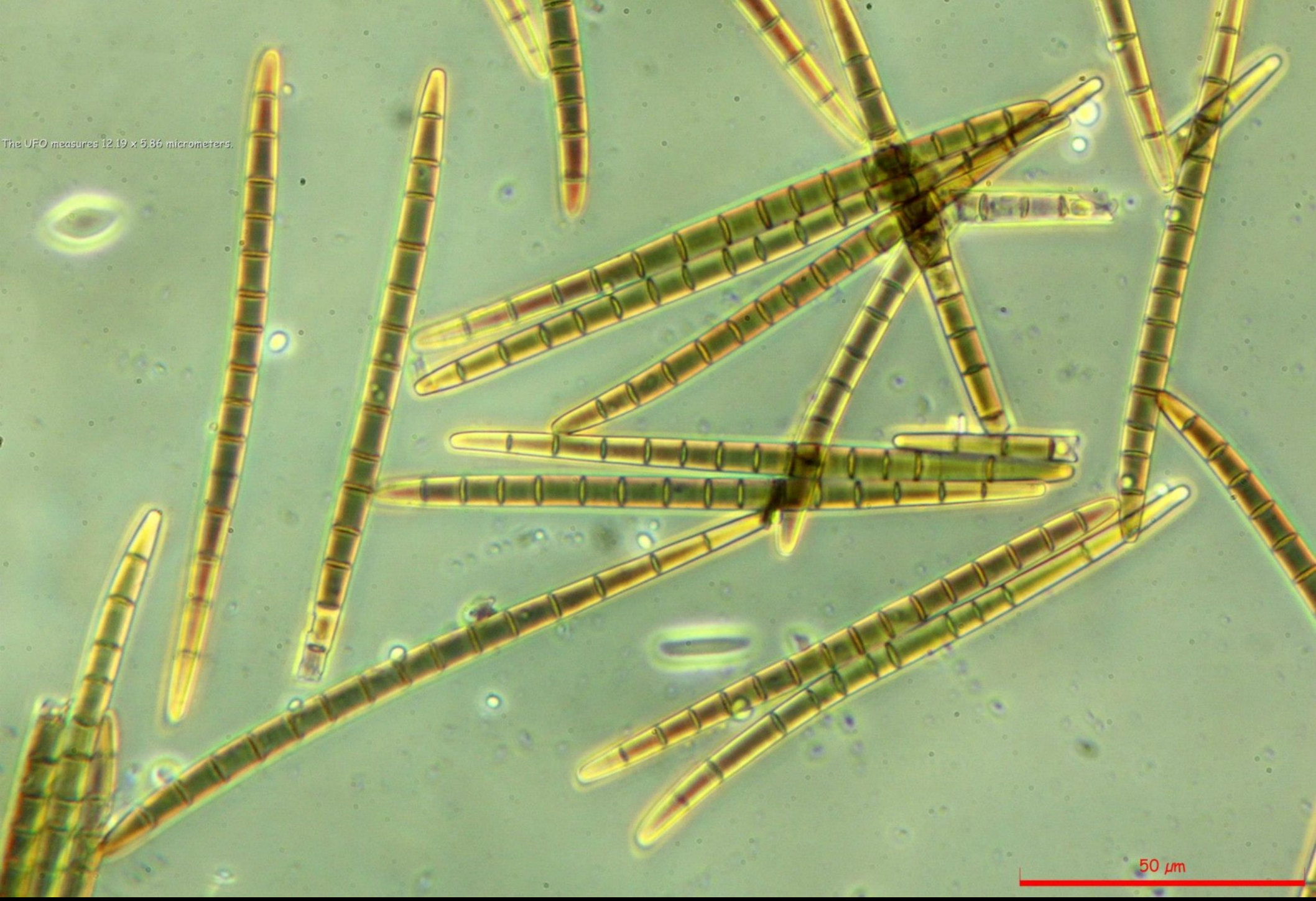


Ten images stacked with CombineZP

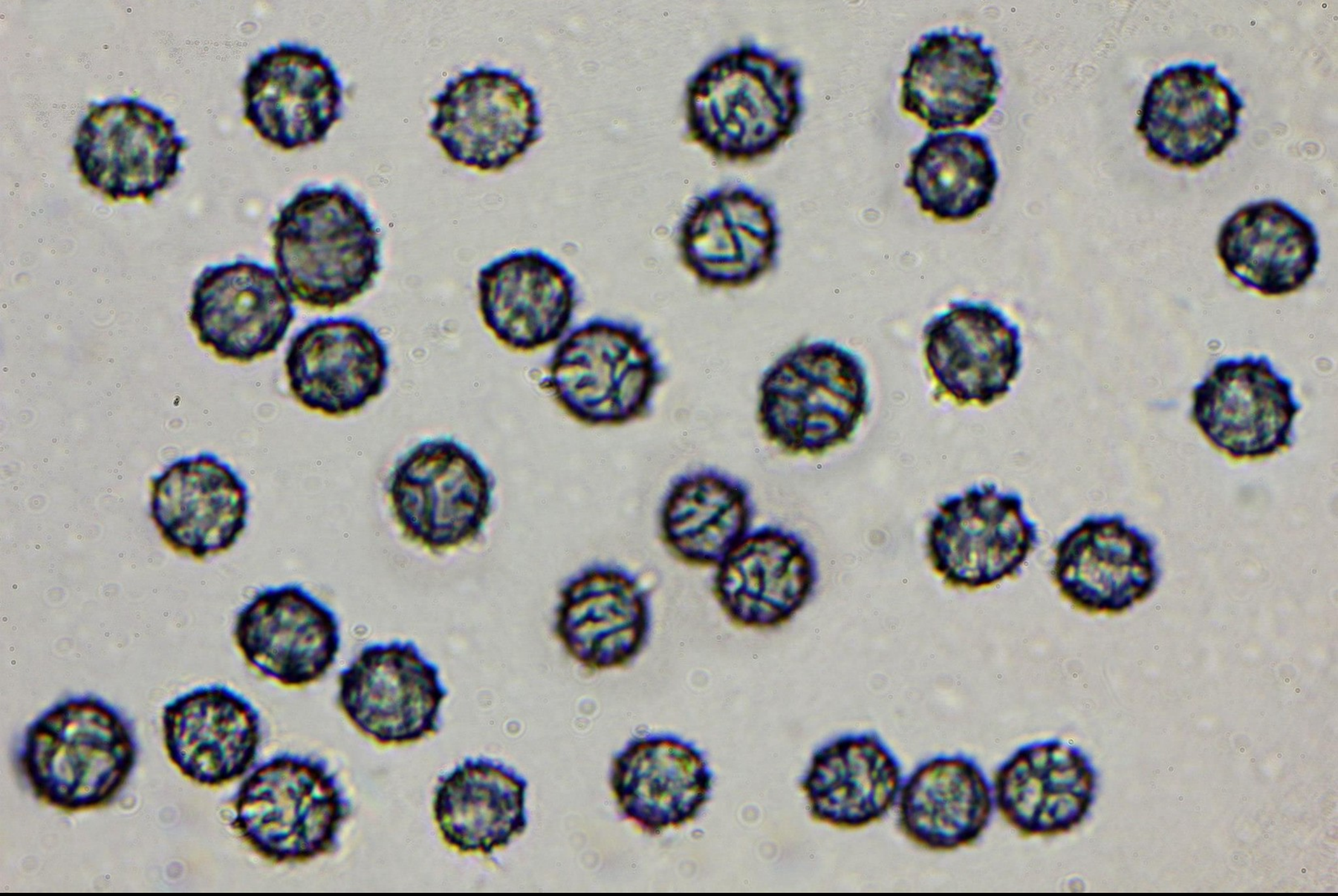


Tricholosporum tropicale spores

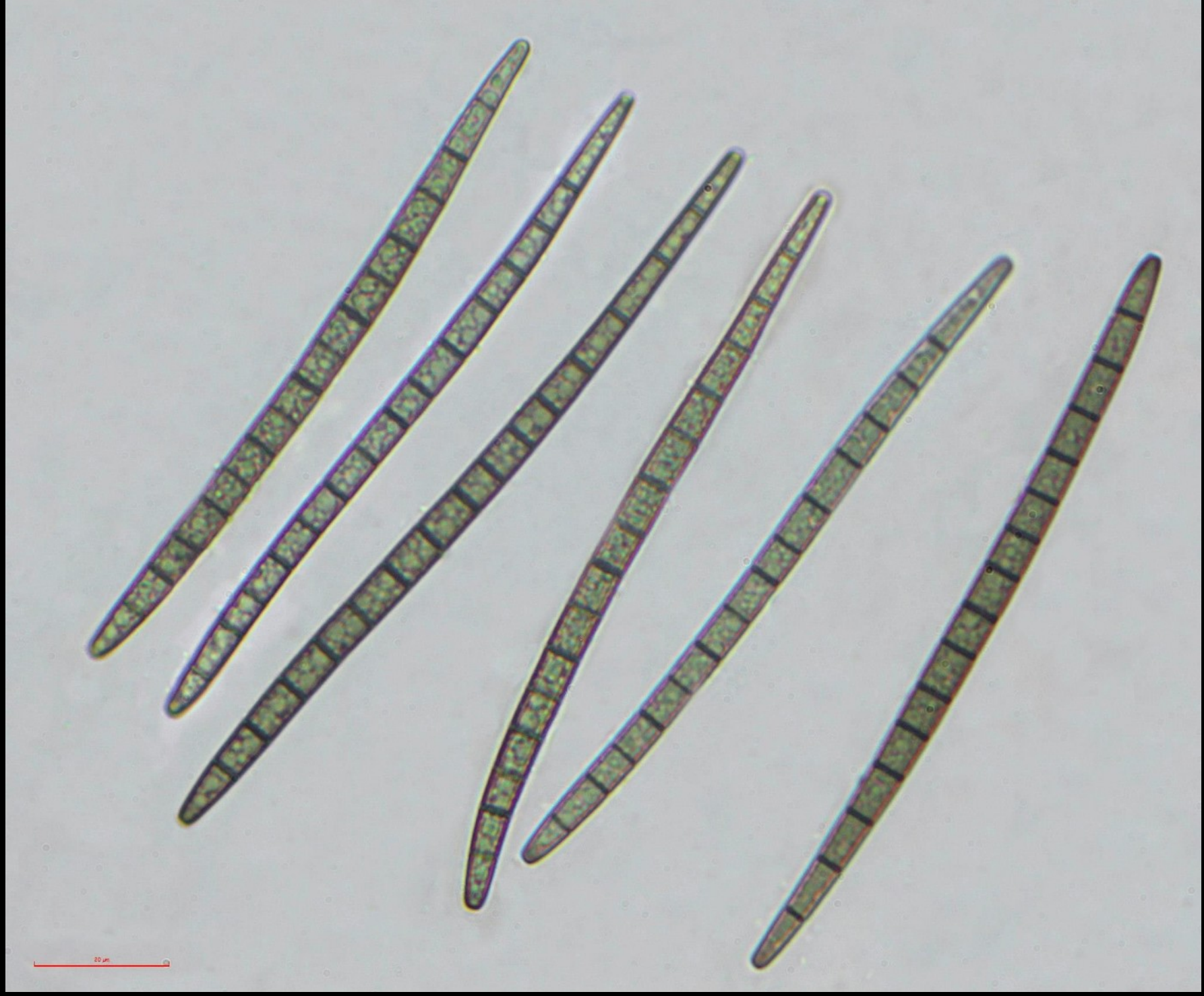
Amanita sect. Lepidella amyloid spores



Trichoglossum hirsutum spores



Lactarius rubidus amyloid spores

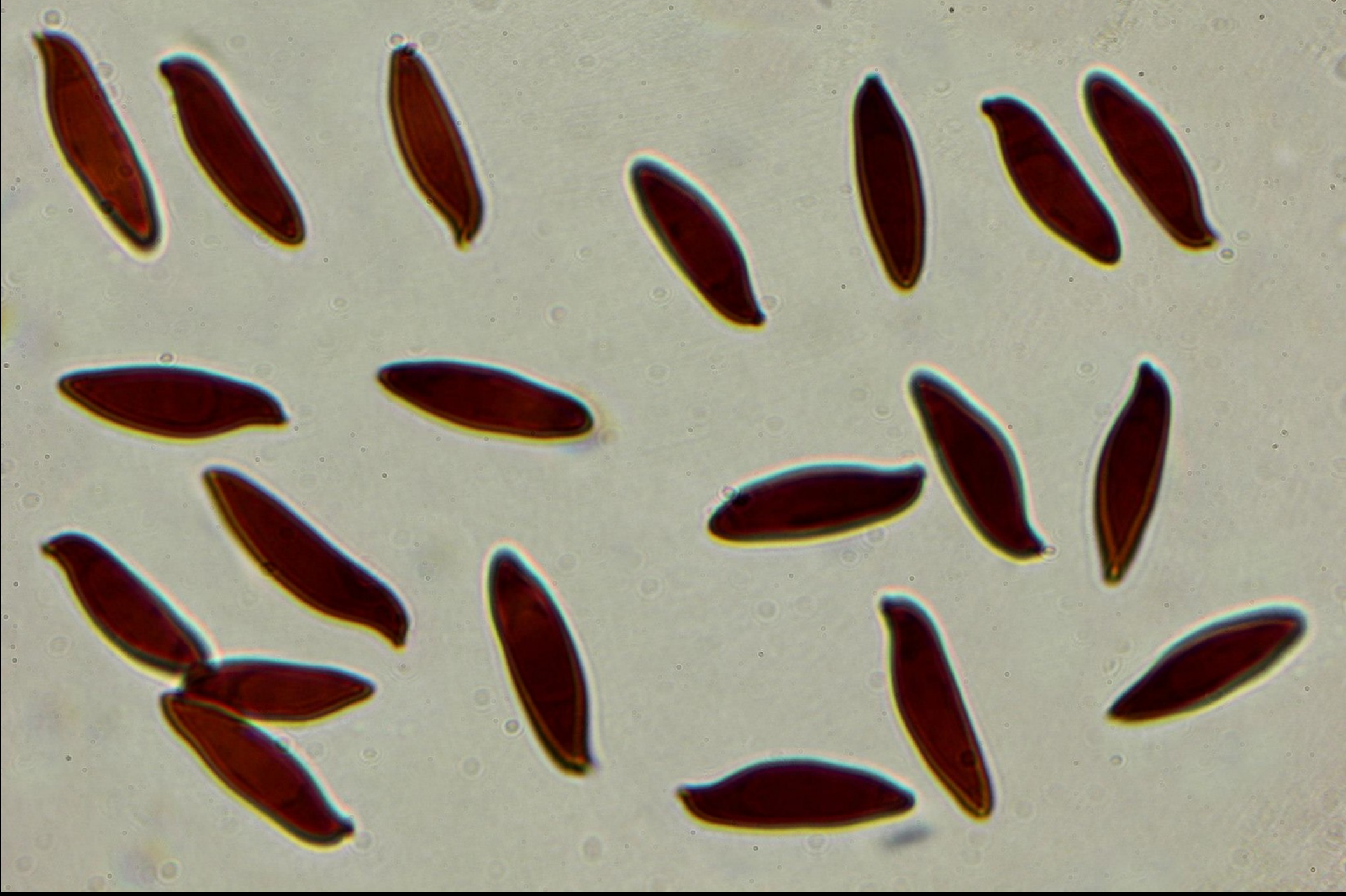


Trichoglossum hirsutum spores

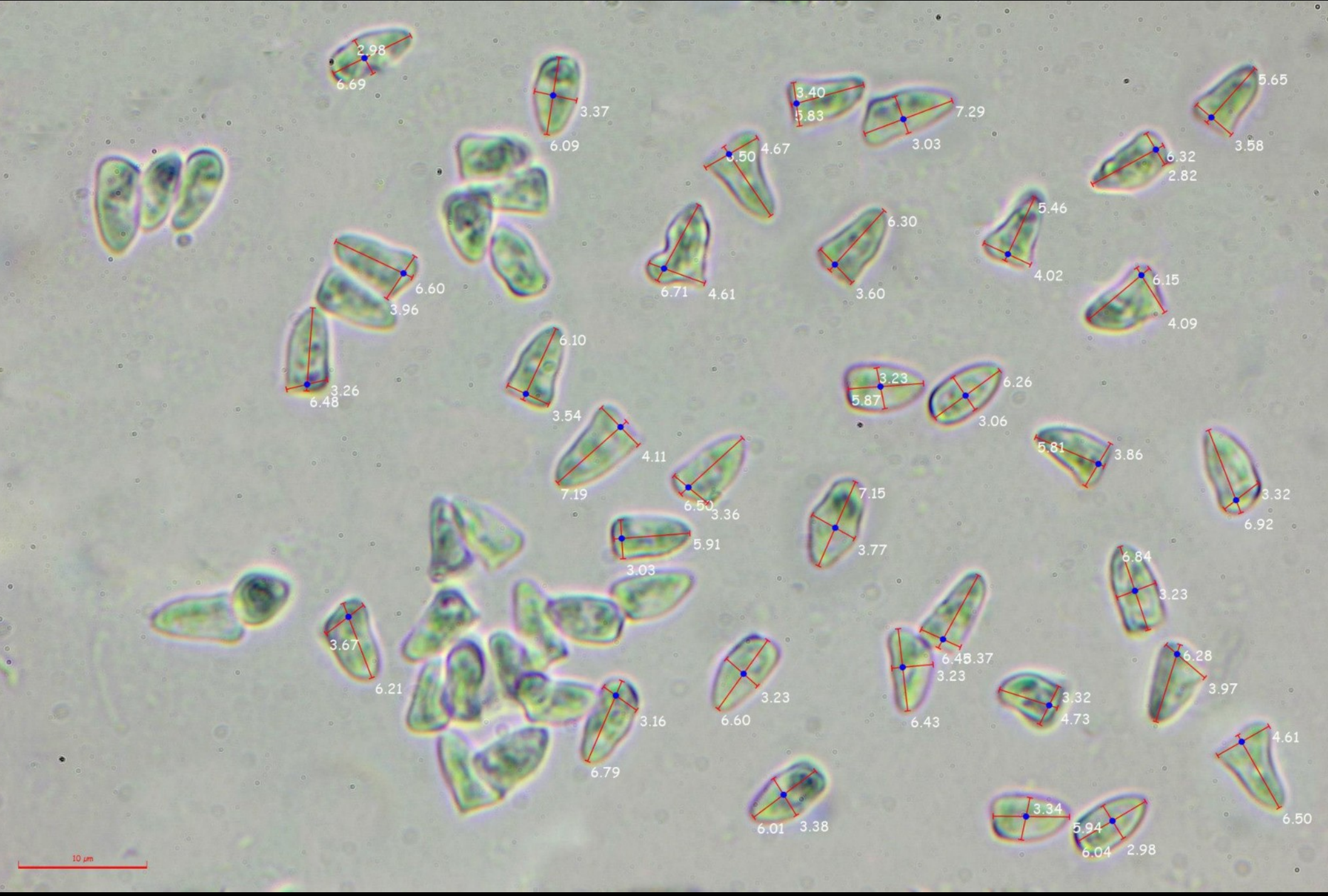
Psilocybe subaeruginosa spores

Psilocybe subaeruginosa spores

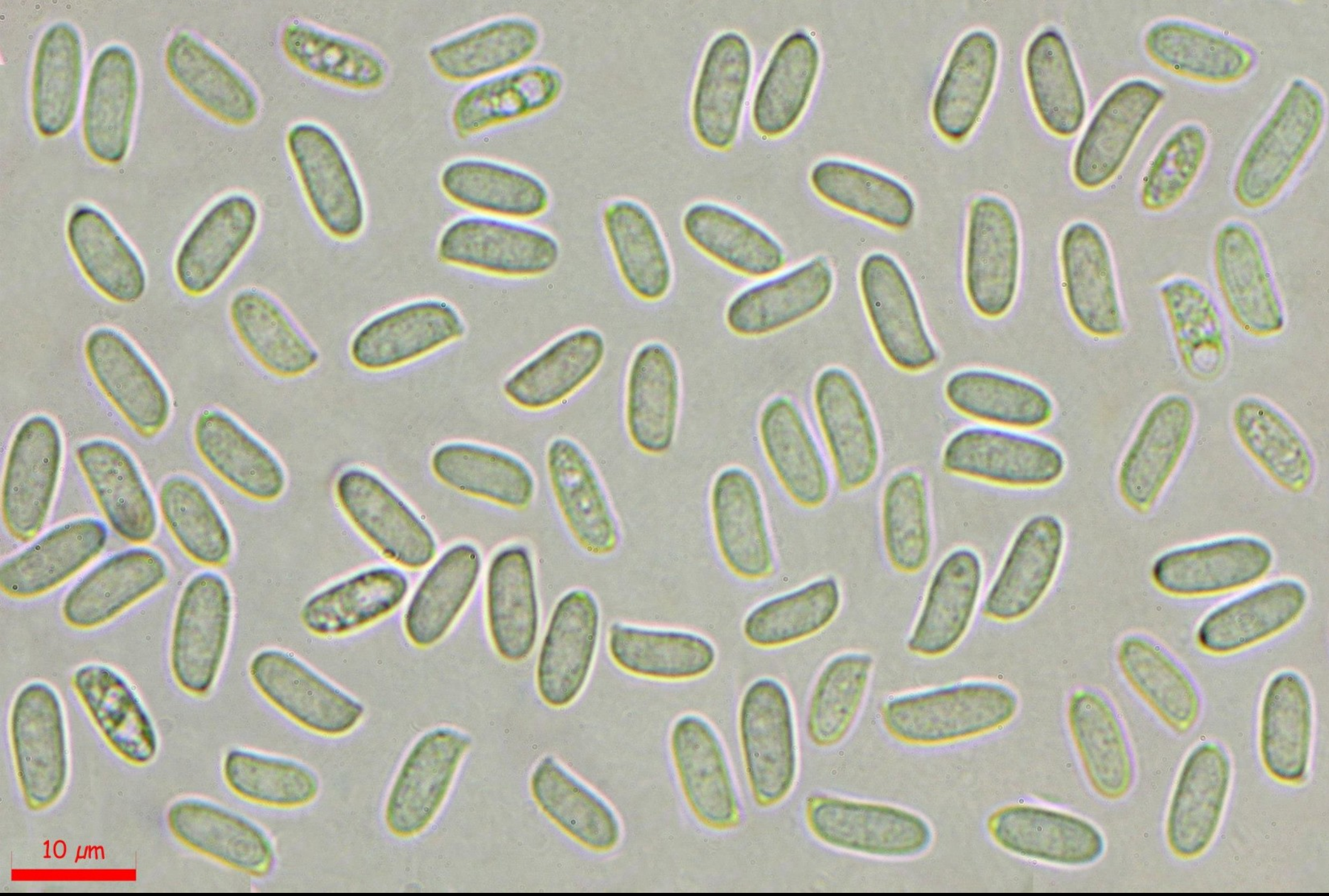
Psilocybe cubensis spores



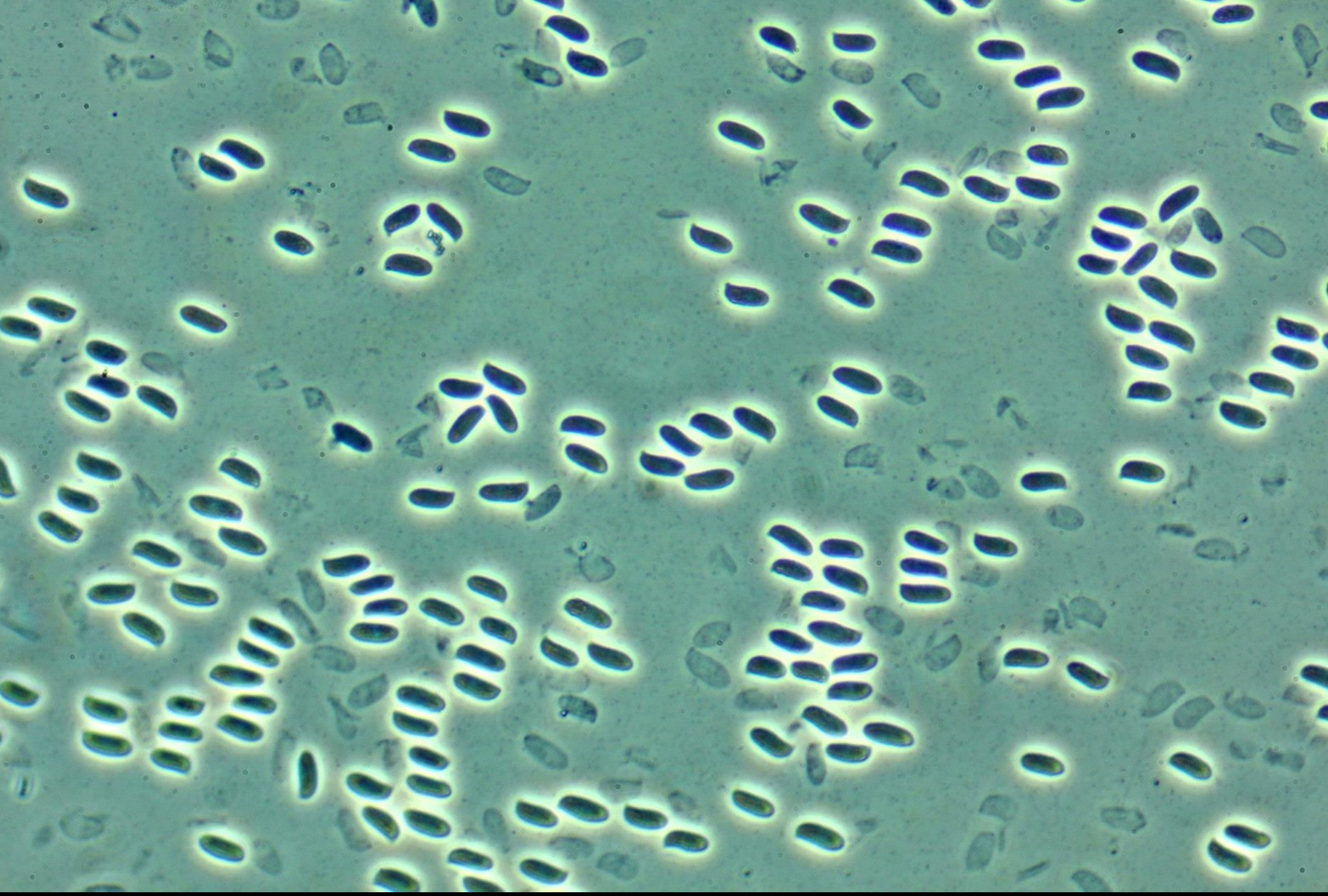
Lepipta sphenicispora dextrinoid spores



Lepiota castaneidisca

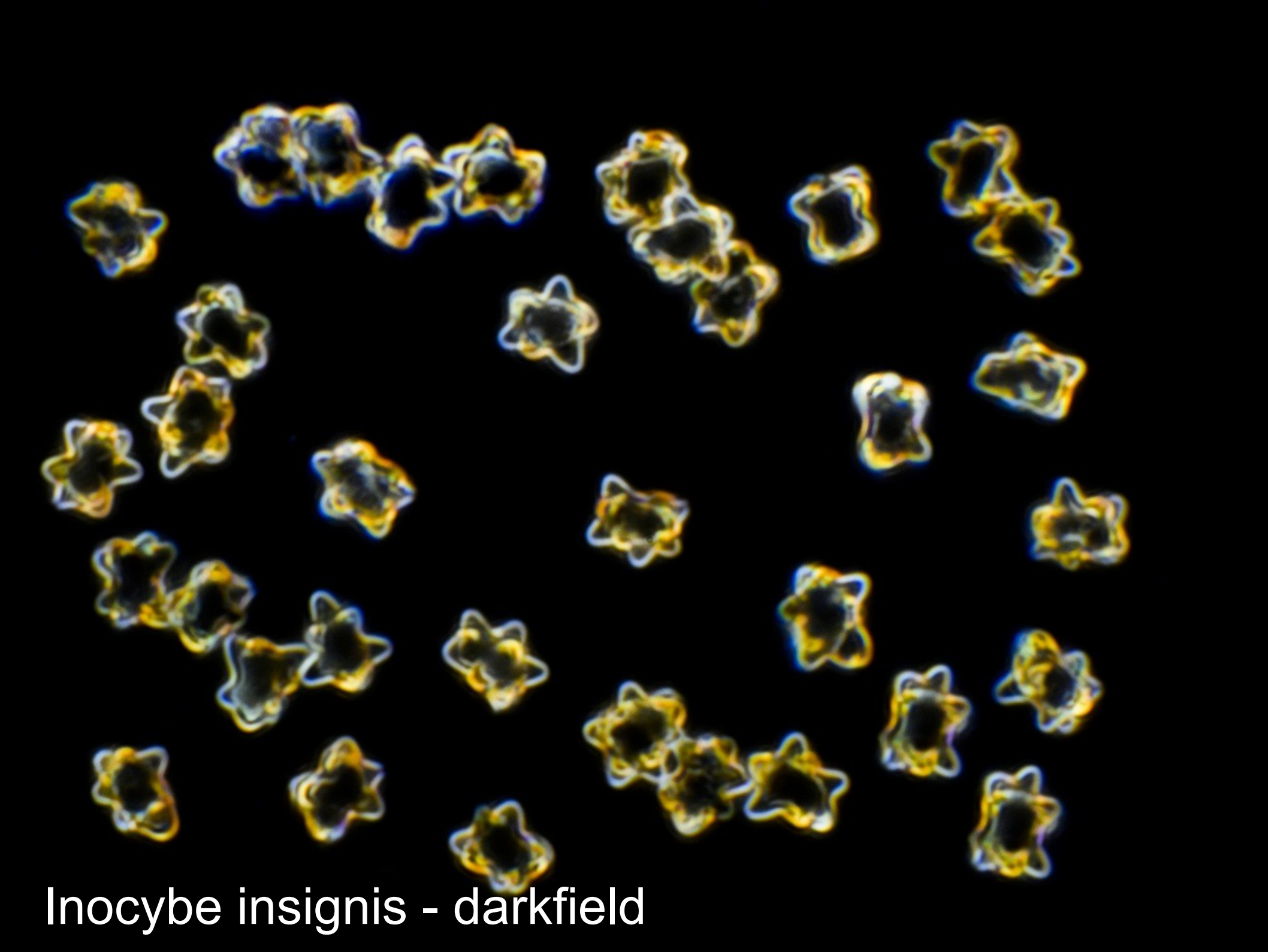


Pleurotus ostreatus

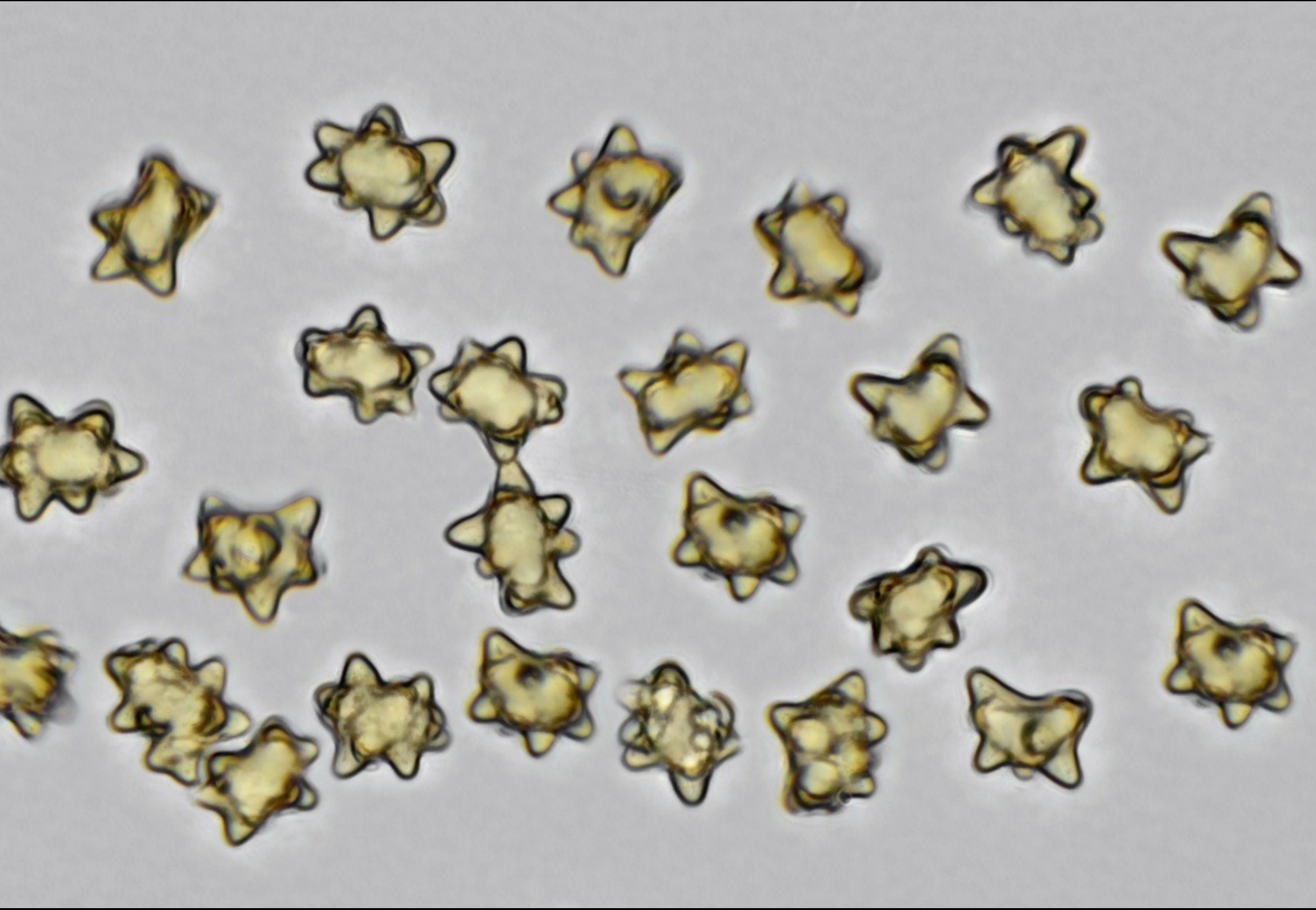


Pleurotus ostreatus

Panaeolus foenisecii



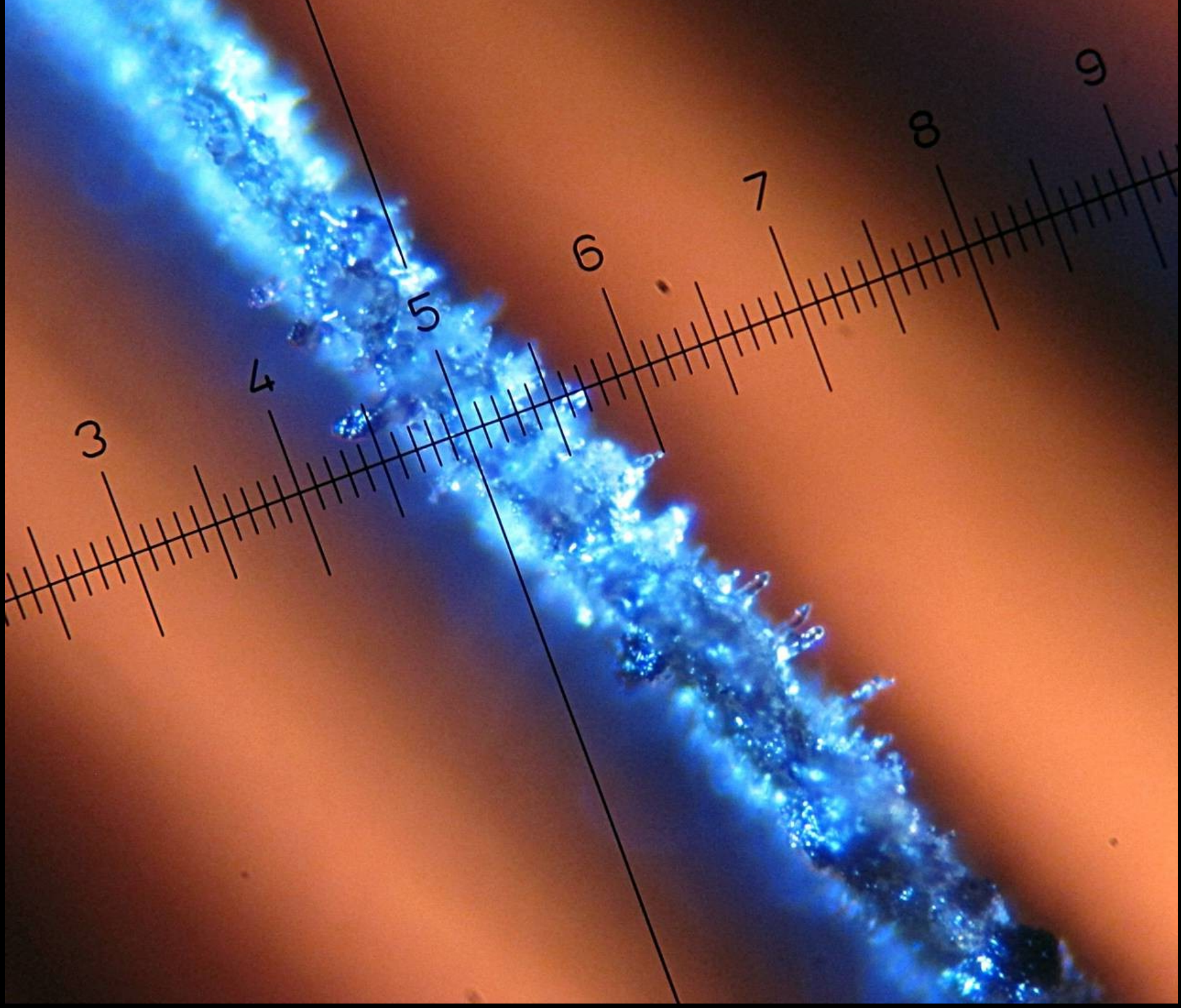
Inocybe insignis - darkfield



Inocybe insignis - brightfield

Cheilocystidia

- Cheilocystidia are the cystidia that are on the edges of the gills.
- Taxonomically significant.
- Best viewed with a crush mount so you can see the whole cell.
- Usually measure between 10 and 50 micrometers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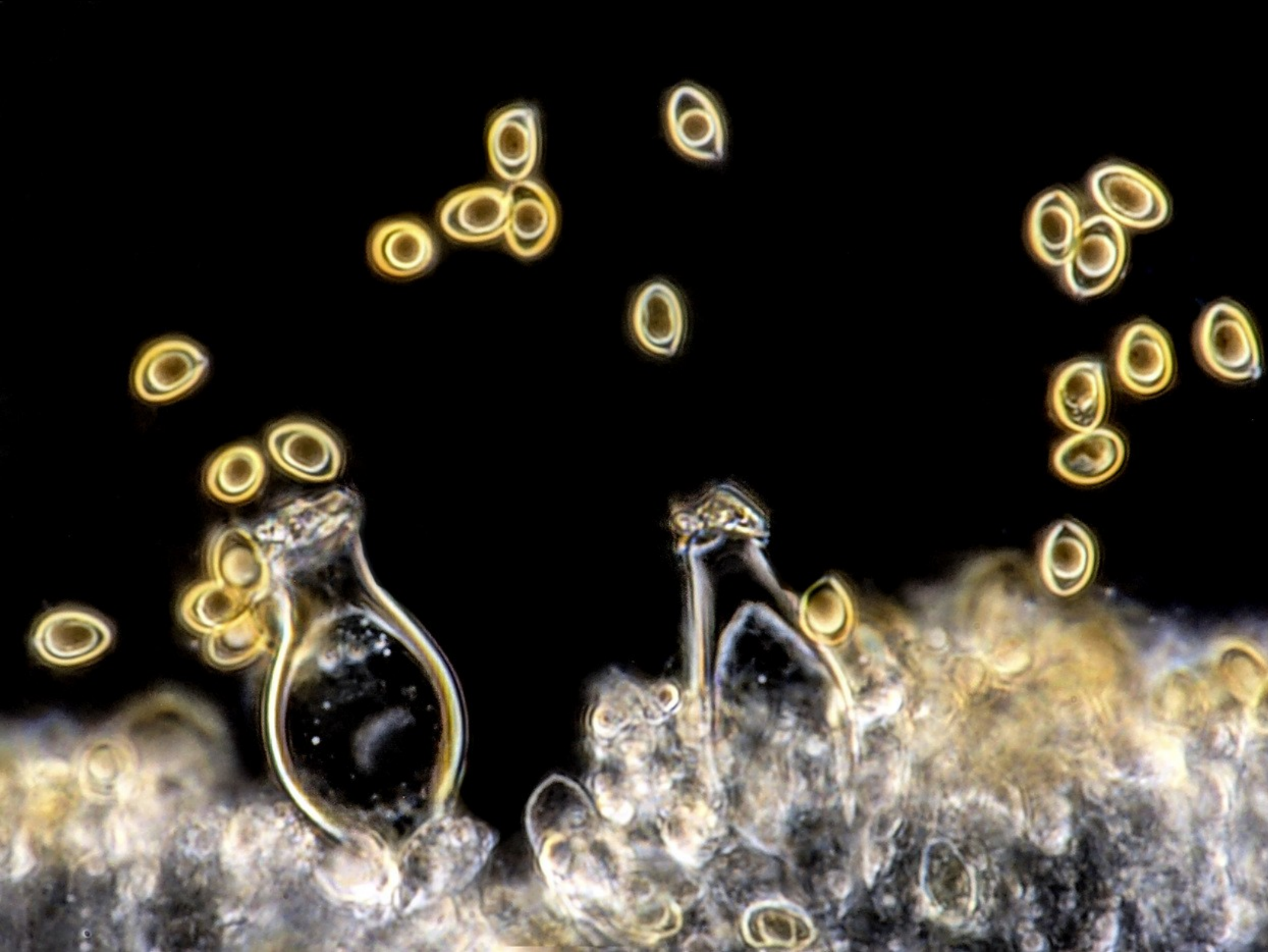
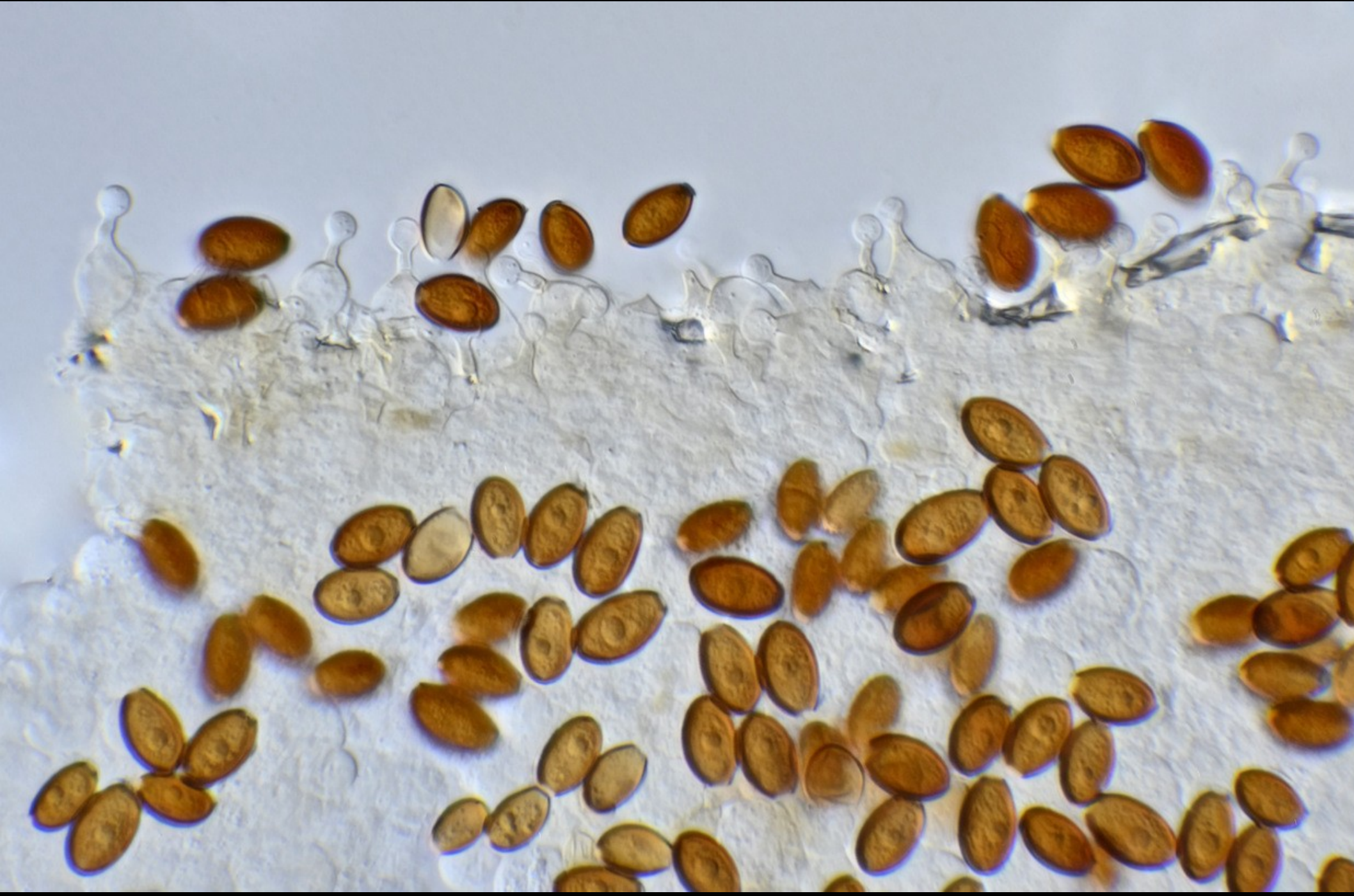
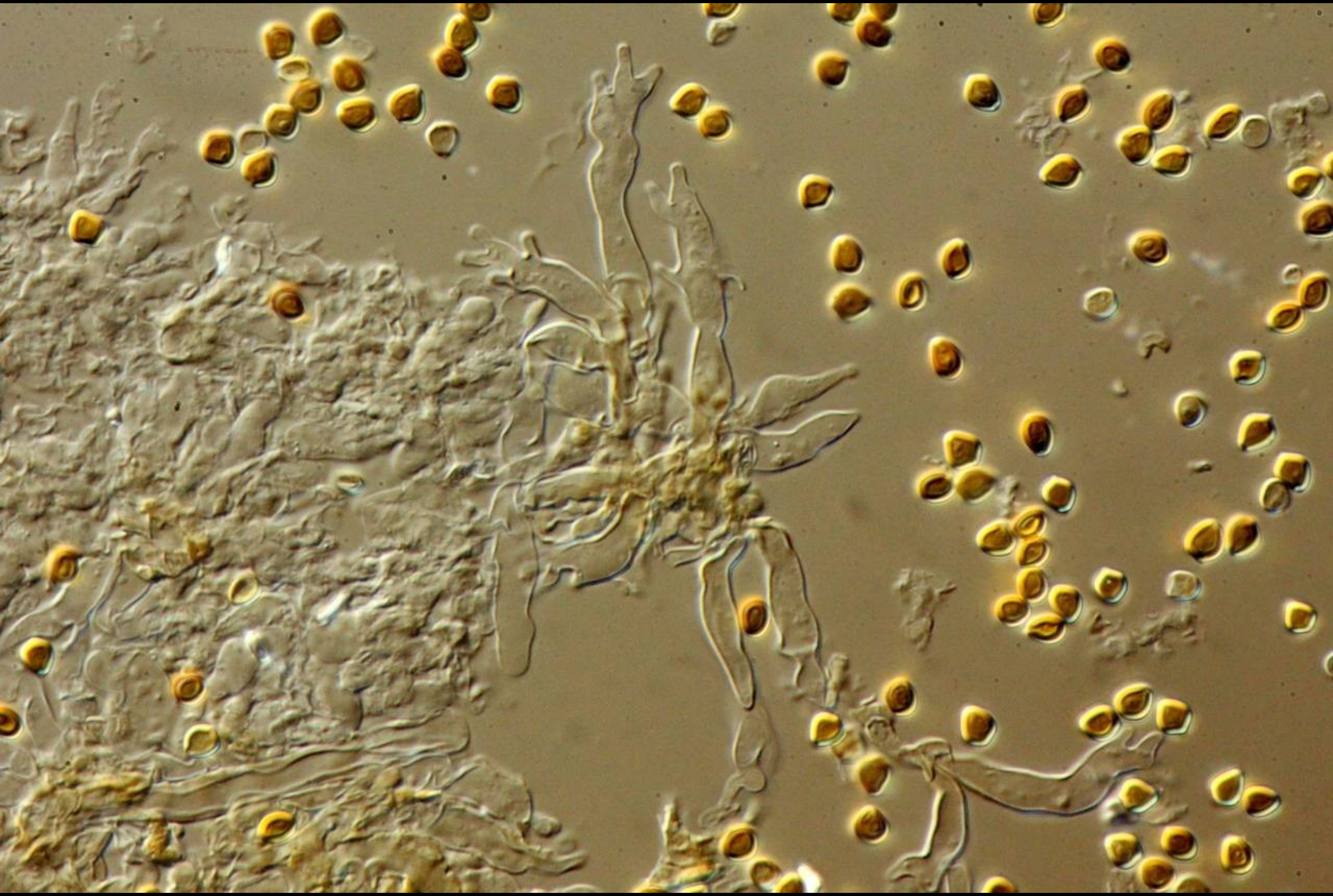




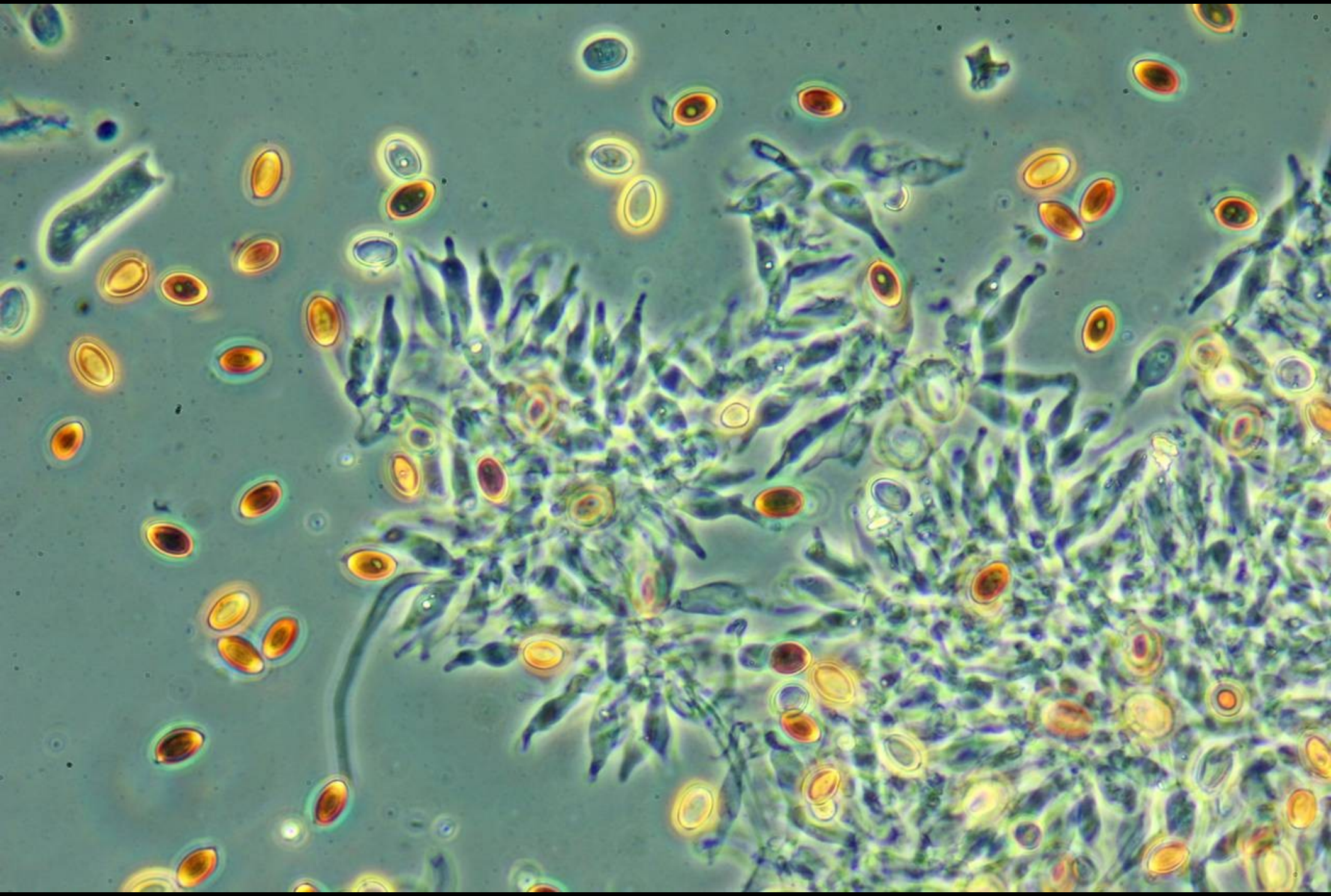
Conocybe aurea gill edge with cheilocystidia

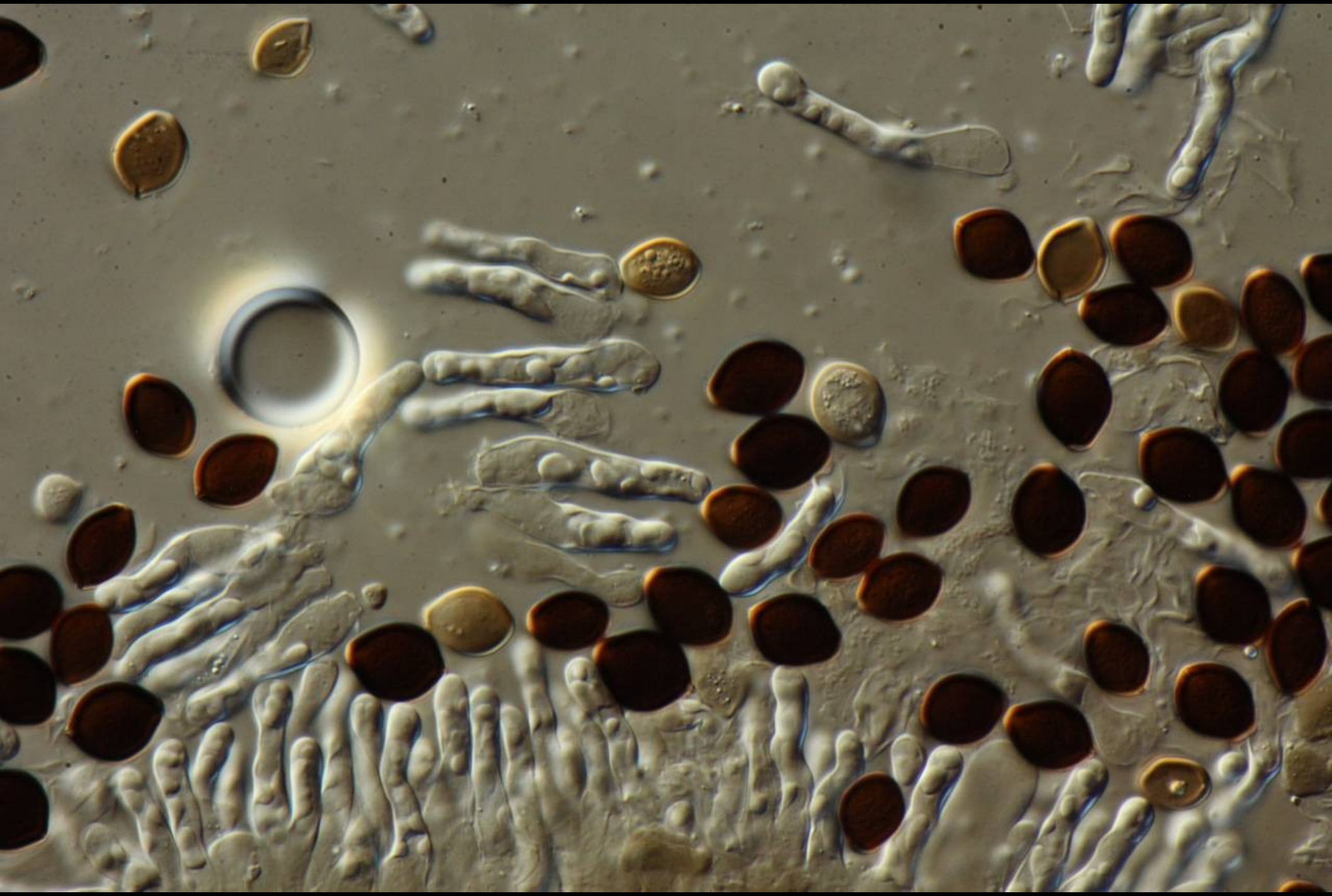
Psilocybe subaeruginosa cheilocystidia

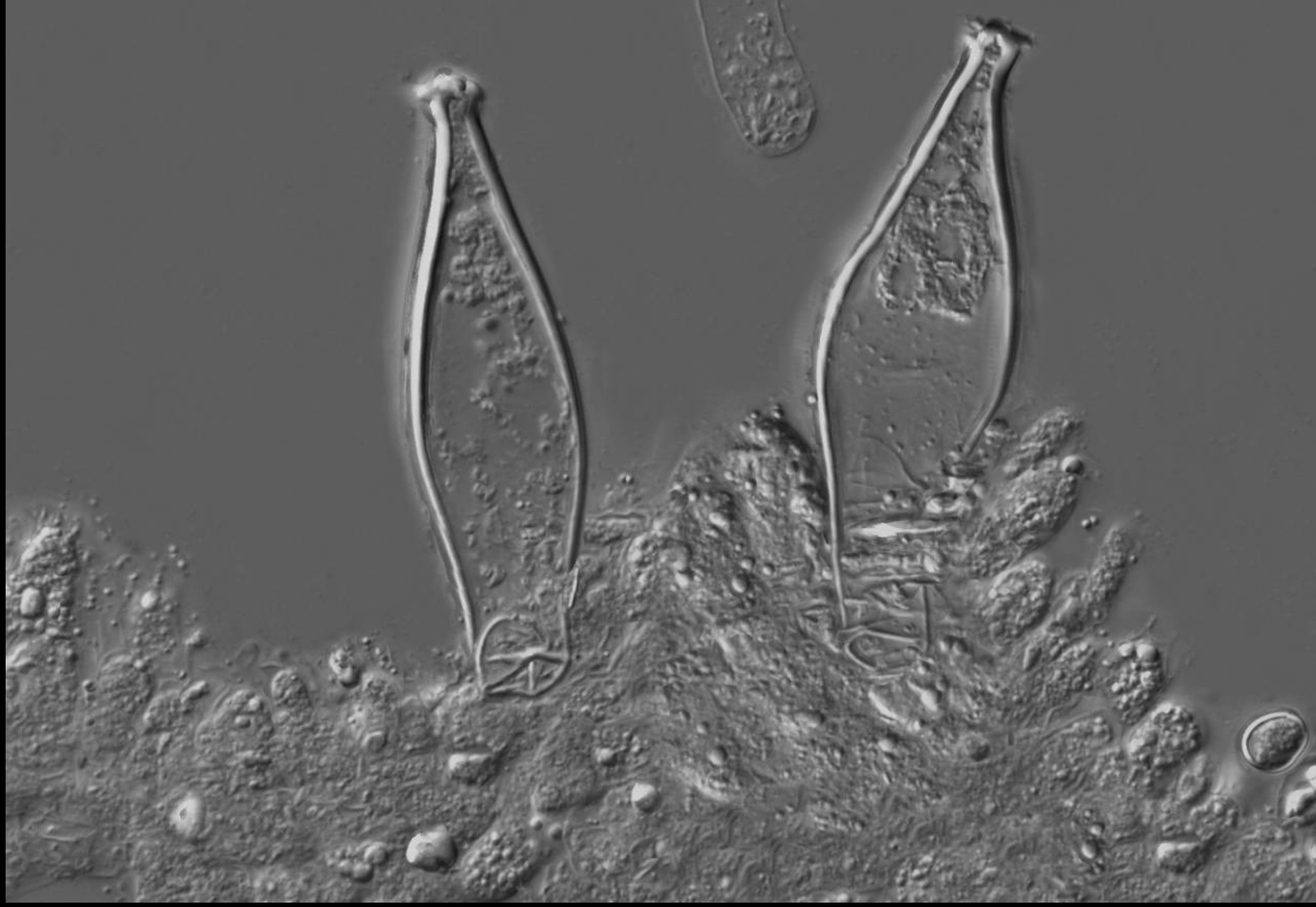


Psilocybe yungensis cheilocystidia

Psilocybe quebecensis cheilocystidia







Pluteus cervinus group cheilocystidia

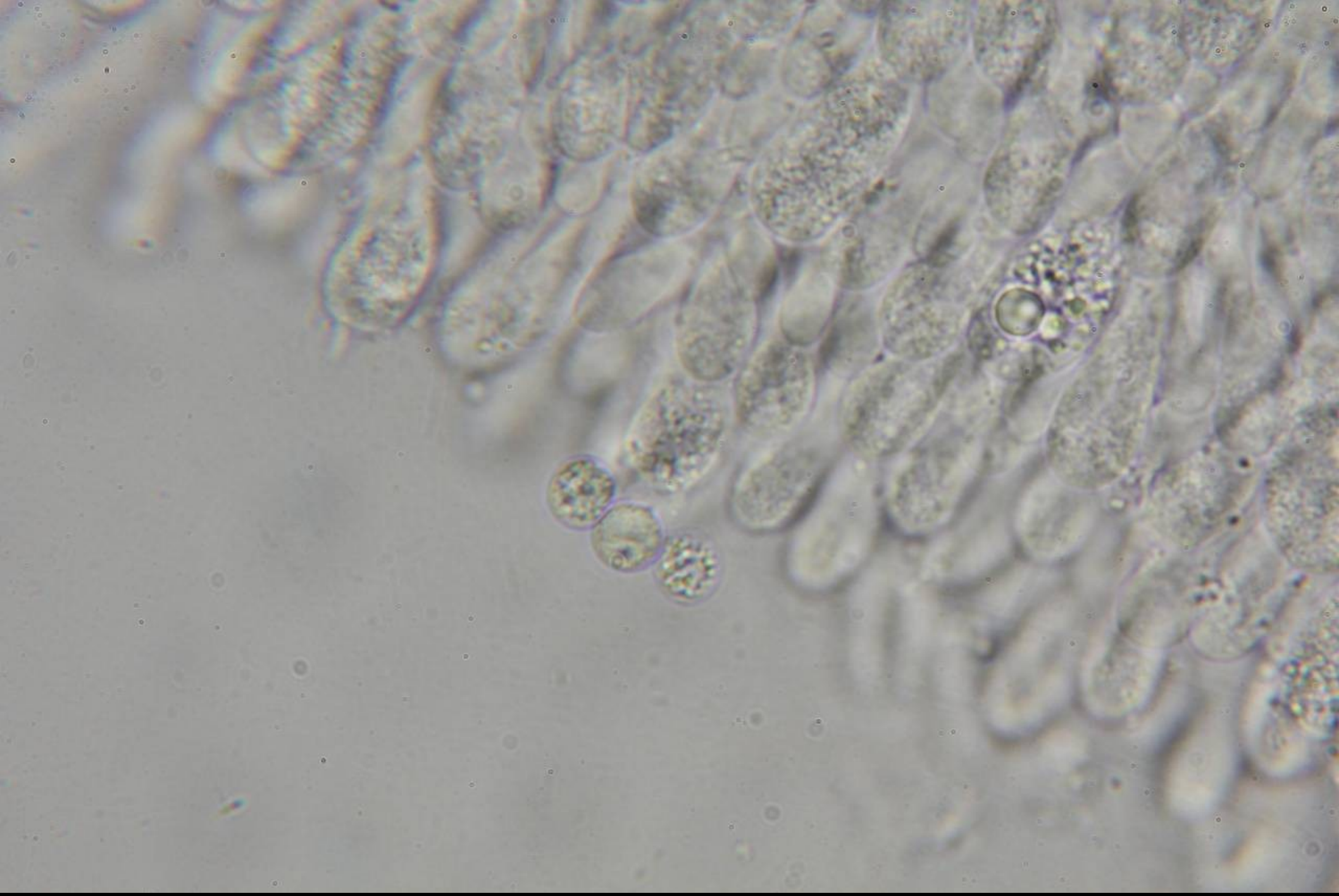
Pluteus cervinus group cheilocystidia

Basidium

- The cells that form the spores
- They have spines on the end called sterigmata where the spores grow
- Usually 4 spored, but sometimes they have 2 or other numbers
- Pural form is basidia
- Immature ones are basidioles
- Often have granular contents which can

distinguish them from basidia and basidioles

Copelandia bispora basidia

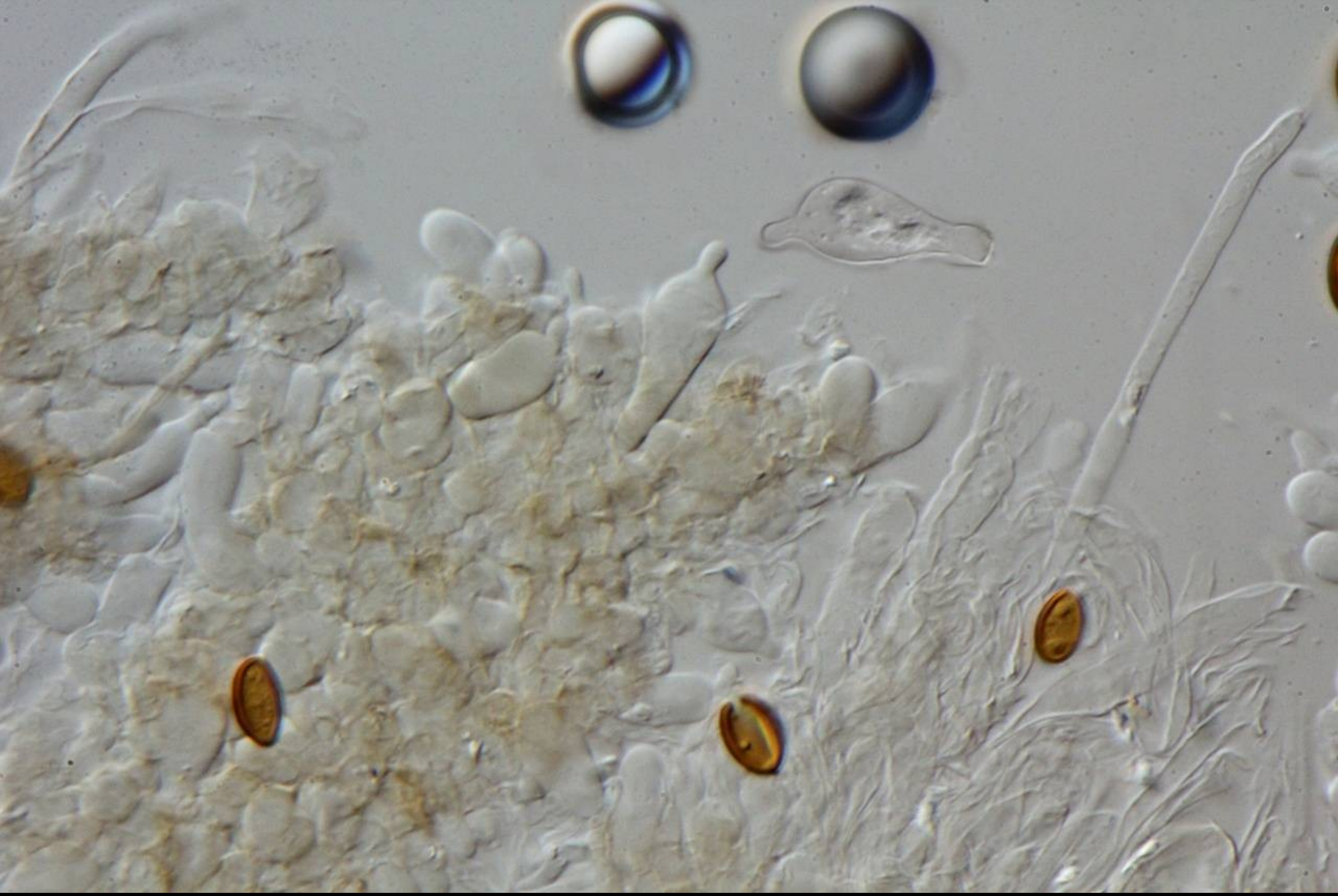


Clitocybe sp. basidium with spores attached

Psilocybe pelliculosa basidia, basidioles and spores

Pleuocystidia

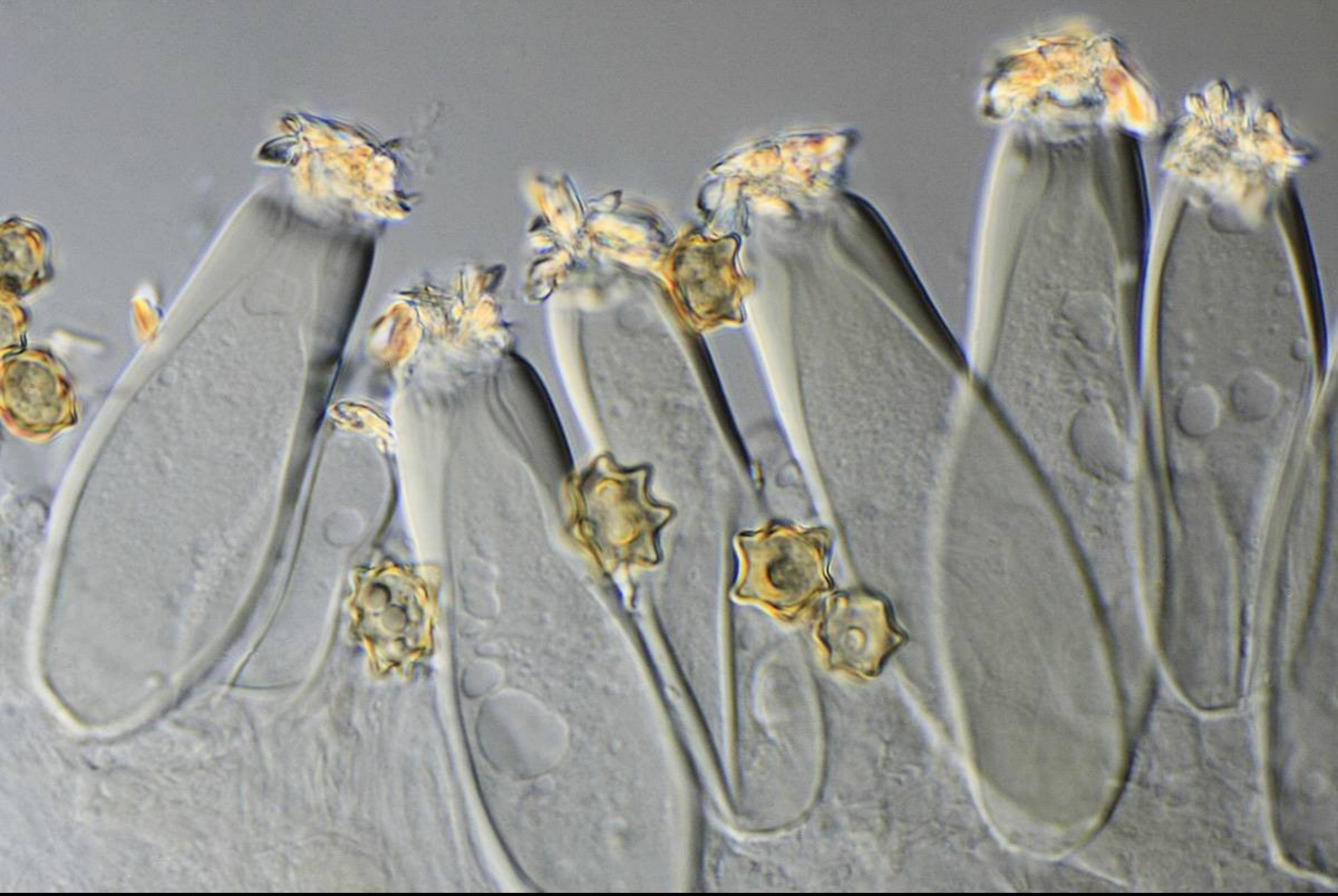
- Cells on the gill faces
- Taxonomically significant.
- Best viewed with a crush mount.
- Usually measure between 10 and 40 micrometers



Psilocybe subaeruginosa pleurocystidia

Coprinopsis atramentaria pleurocystidia

Copelandia bispora pleurocystidia



Inocybe sp. pleurocystidia

Photo by Linas Kudzma

Caulocystidia

* Cystidia found on the stem
* Most abundant near the apex
* It’s best to avoid picking up mushrooms by the stem to keep the caulocystidia from collapsing


Conocybe aurea caulocystidia

Pseudocystidia

* Unlike pleurocystidia, they are equally likely to occur on the gill face or gill edge.

* They are grey in color

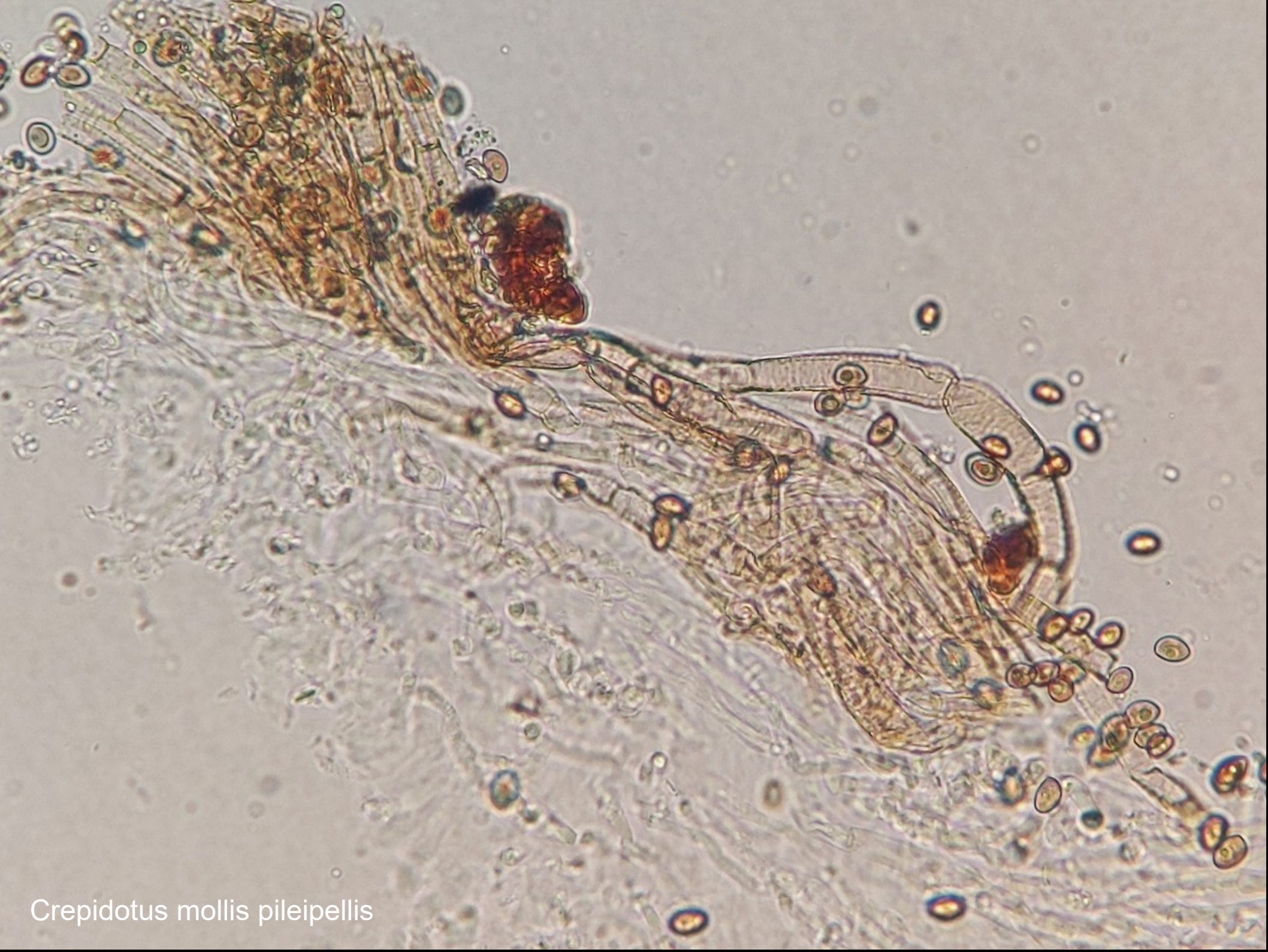
* They arise from either the lamellar trama or the subhymenium.

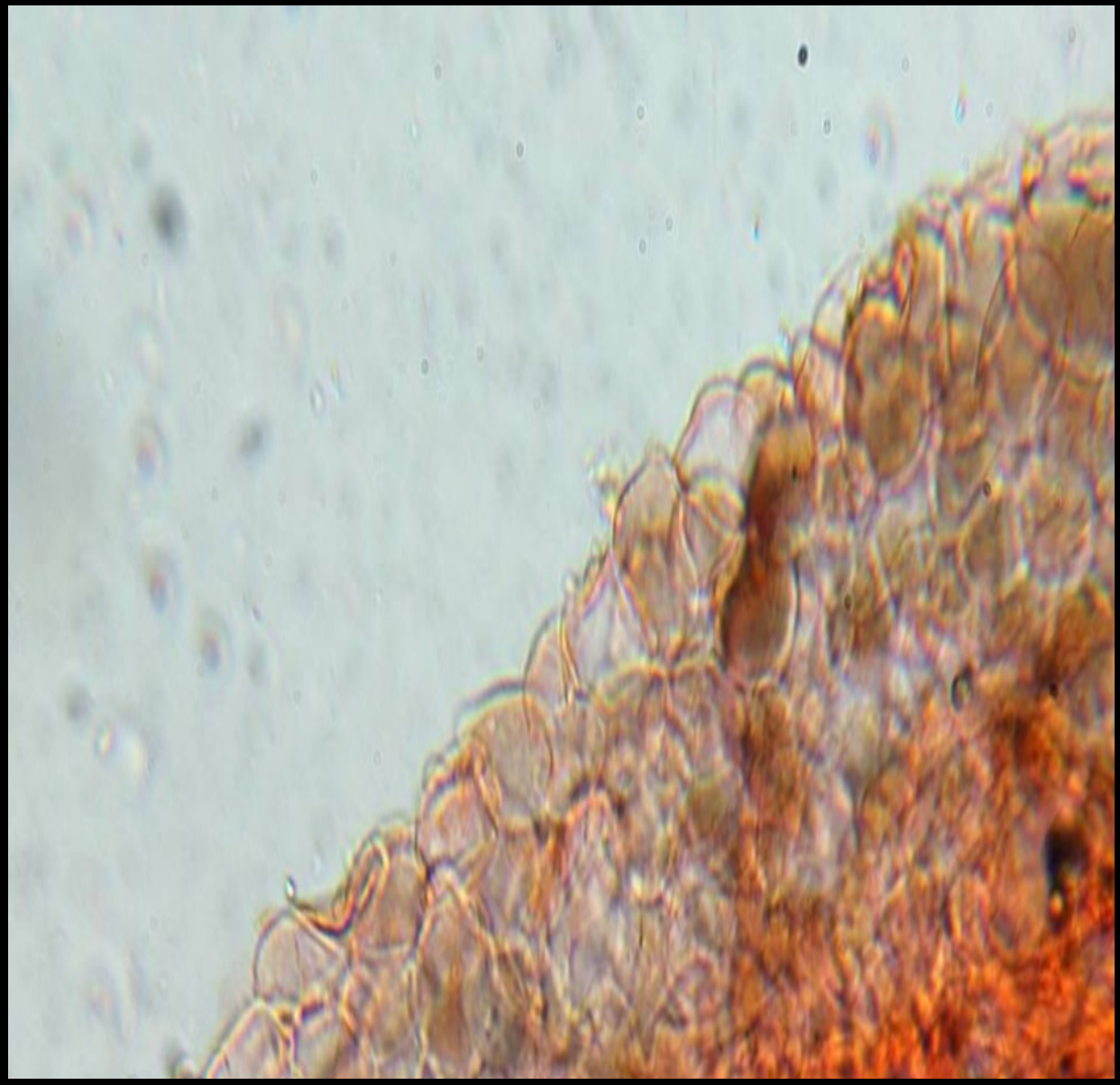
Psilocybe zapotecorum pseudocystidia

Pileipellis

- The layer of cells on the top of the cap
- Usually viewed in cross section, however it is also useful to view a perpendicular (scalp) section
- Sections must be extremely thin to view it clearly (a veces, no siempre. Si queries ver pileocystidia, si.)

Coltricia pileipellis


Crepidotus mollis pileipellis



Pluteus chrysophlebius pileipellis

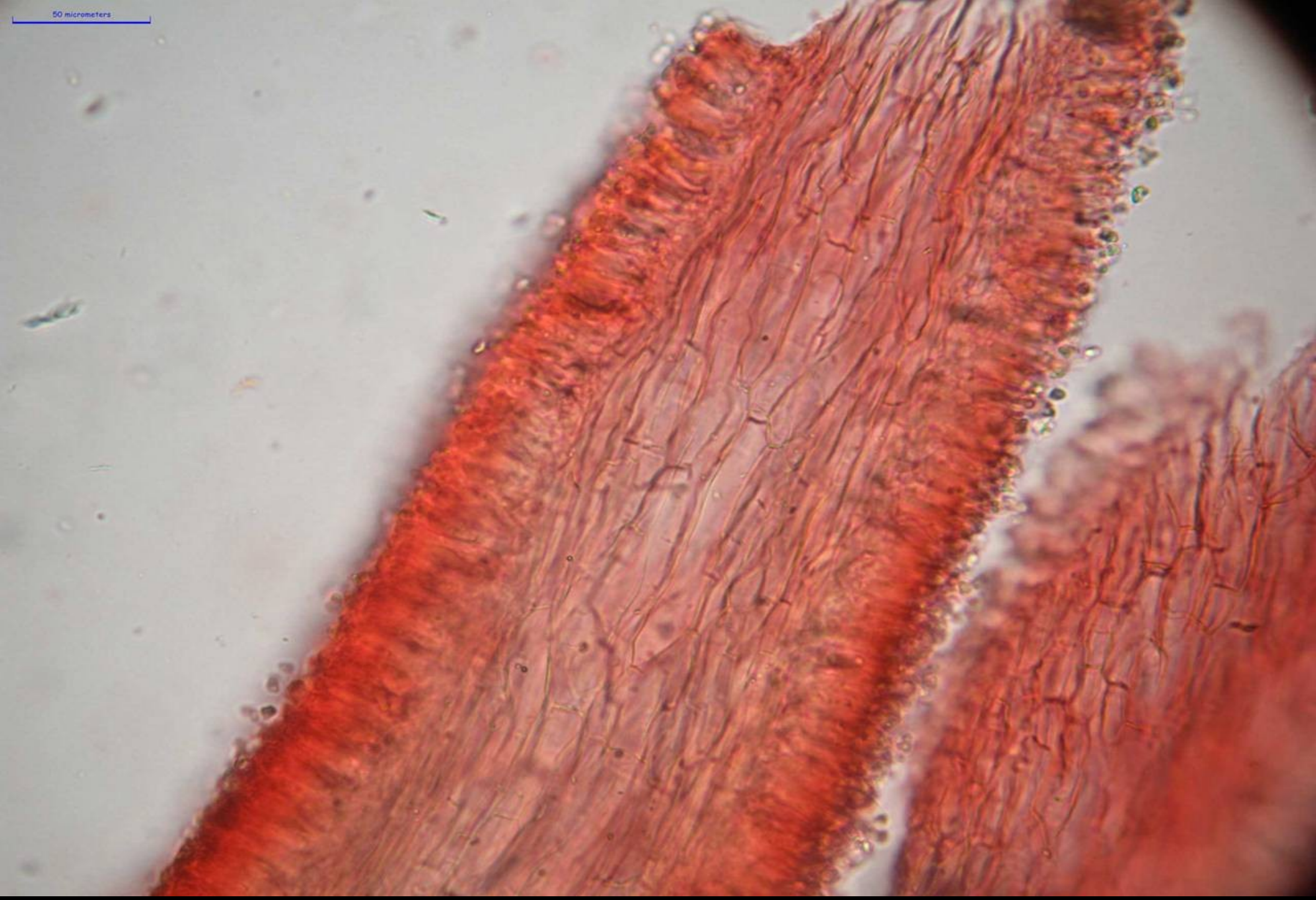
Gymnopilus sp. pileipellis

Lamellar trama

- The cells inside the middle of the gill
- Best viewed in a very thin gill or pileus cross section

Psilocybe pseudoaztecorum lamellar trama

Photo by Alonso Cortés-Pérez

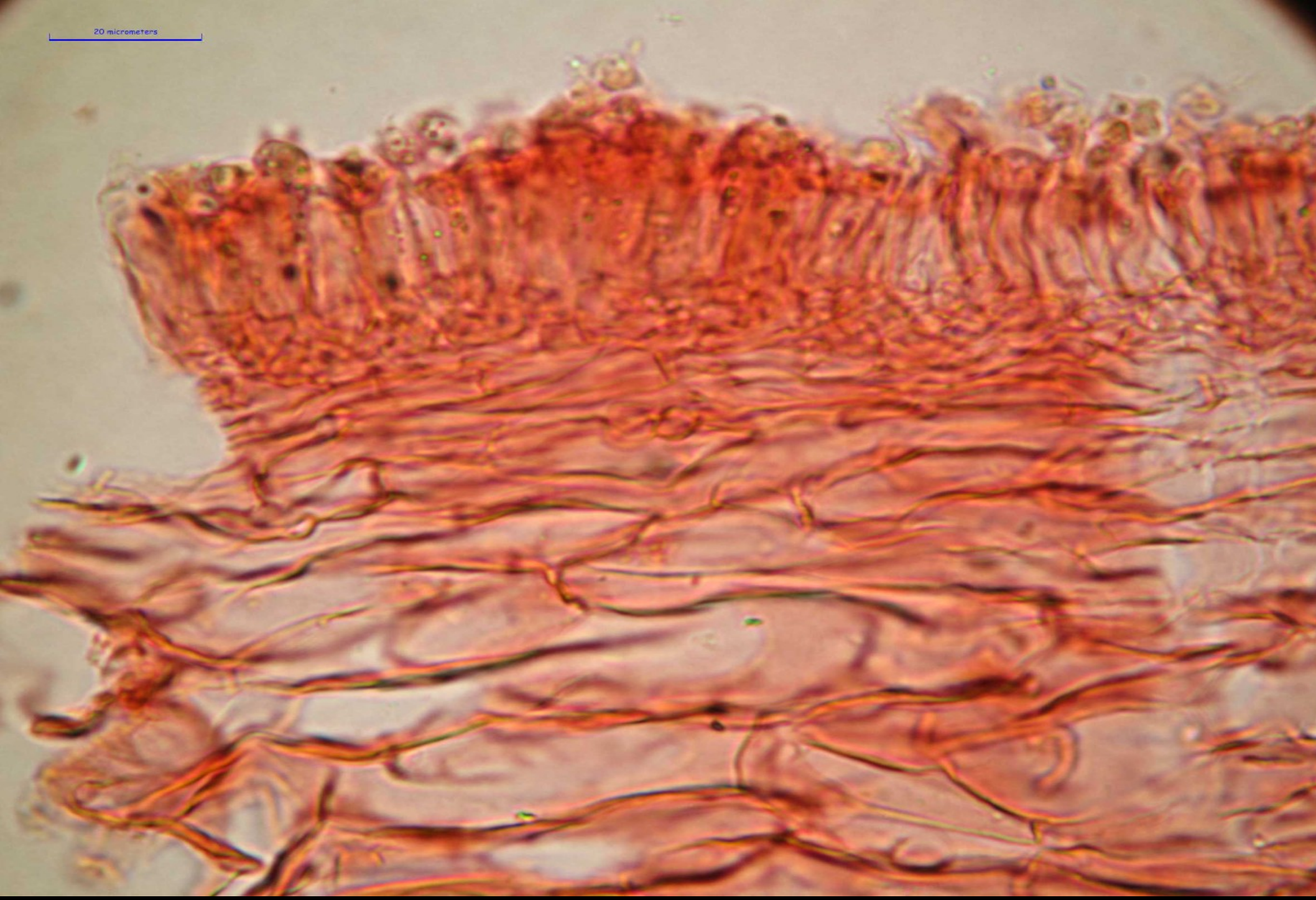


Tricholosporum tropicale lamellar trama

Amanita sect. Caeeareae lamellar trama

Subhymenium

- The cells between the lamellar trama and the row of basidia
- View by making about 20 extremely thin gill cross sections with a dissecting scope and staining with congo red



Amanita sect. Caeeareae subhymenium

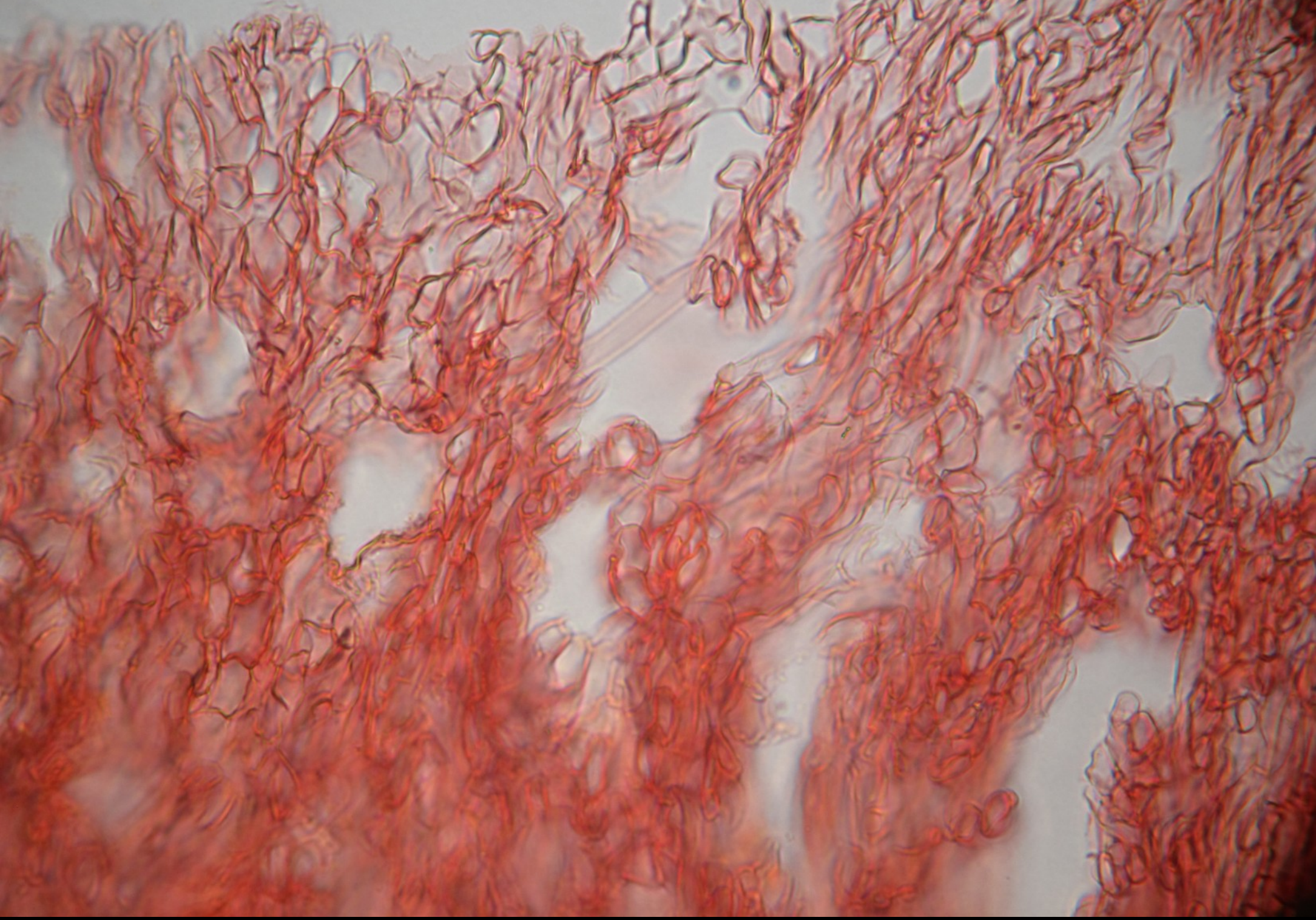
Pileocystidia

- Cystidia on the top of the cap
- Visible in crush mounts or extremely thin cross sections of the pileipellis

Tricholosporum tropicale pileocystidia

Pileus trama

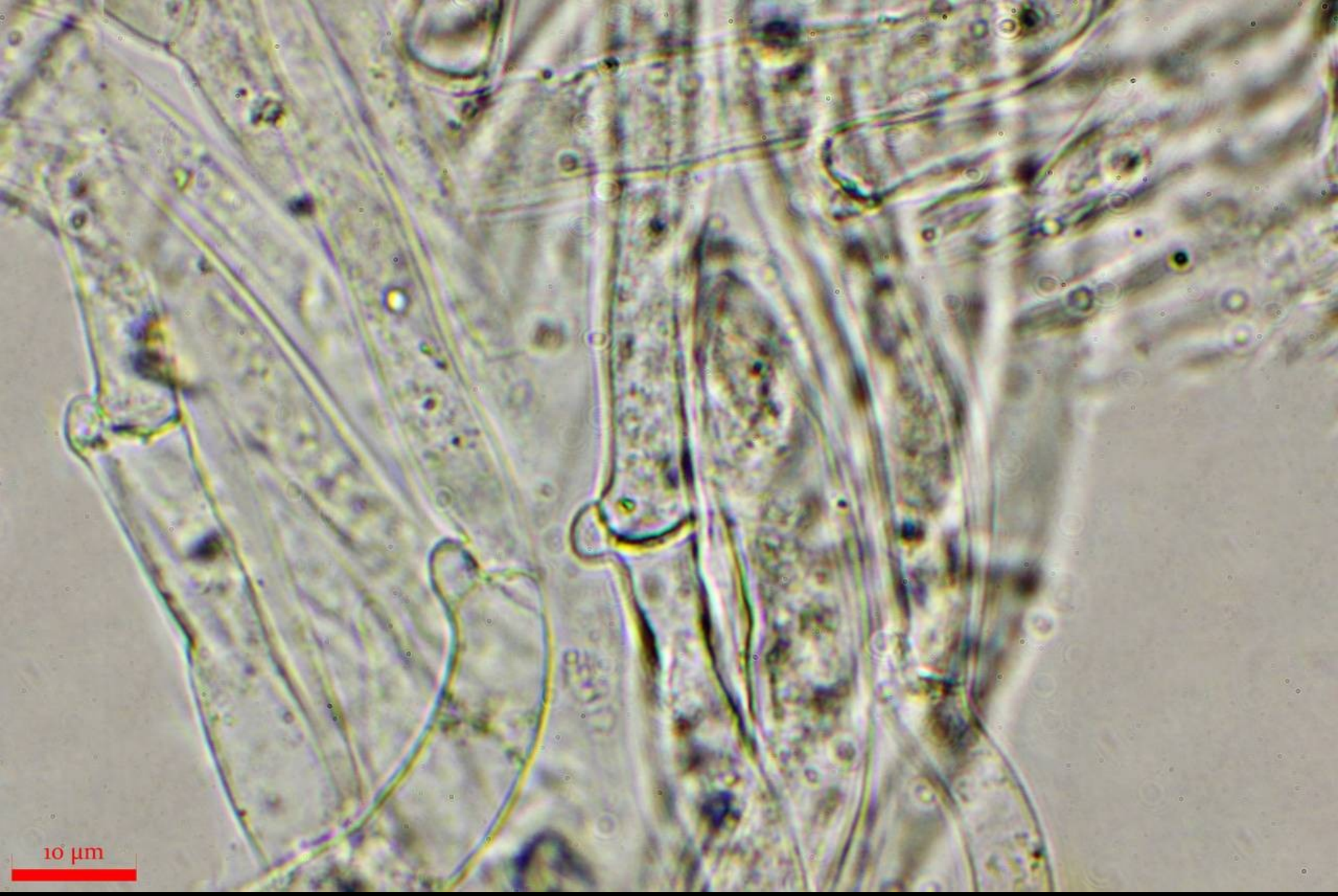
- The cells inside the pileus
- View in extremely thin cross section



Tricholosporum tropicale pileus trama

Clamp connections

- Bumps at hyphae junctions
- Creates genetic variation among hyphae by creating more nuclea via mitotic division
- View with crush mounts



Pluteus cervinus group clamp connections

Resources

https://www.facebook.com/groups/FungalMicroscopy

http://images.mushroomobserver.org/Fungal%20Microscopy.pdf

Amateur Microscopy FB group:
https://www.facebook.com/groups/2545390180608 54

How to Identify Mushrooms to Genus III: Microscopic features

https://files.shroomery.org/attachments/21366346-Optimized%20How%20To%20Identify%20Mushrooms%20To%20Genus%20III%20Microscopic%20Features%20-%20OCR.pdf

Questions?

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