Microbe Hunter

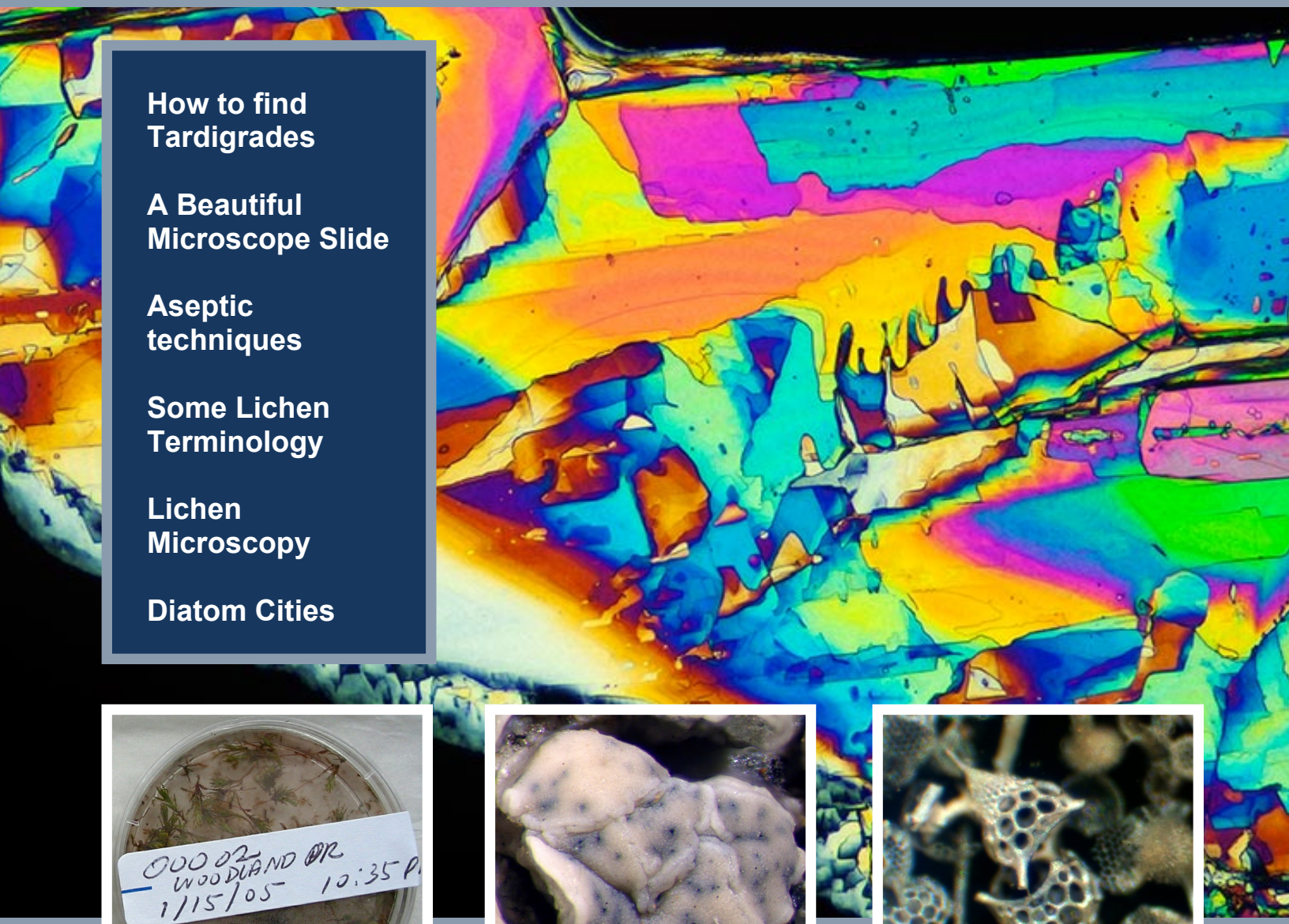
Microscopy Magazine

ISSN 2220-4962 (Print)
ISSN 2220-4970 (Online)

Volume 1, Number 10
October 2011

The Magazine for the Enthusiast Microscopist

http://www.microbehunter.com



Tardigrade isolation

Lichen Microscopy

Radiolaria

ABOUT

Microbehunter Microscopy Magazine
The magazine for the enthusiast microscopist
MicrobeHunter Magazine is a non-commercial project.

Volume 1, Number 10, October 2011

ISSN 2220-4962 (Print)
ISSN 2220-4970 (Online)

Download: Microbehunter Microscopy Magazine can be downloaded at: http://www.microbehunter.com

Print version: The printed version can be ordered at: http://microbehunter.magcloud.com

Publisher and editor:
Oliver Kim, Ziegeleistr. 10-3, A-4490 St.Florian, Austria
Email: editor@microbehunter.com
Web: http://www.microbehunter.com
Tel.: +43 680 2115051

Text and image contributions by:
Leonardo Balbi
Rodney Brightwell
Brudersohn (cc)
Debivort (cc)
Charles E. Guevara
Mike Guwak
Paul Hebert (cc)
Oliver Kim
J. Marqua (cc)
Mike Shaw
Anthony W. Thomas

Editorial: Article and image submissions are welcome and should be sent to: editor@microbehunter.com.
For submission guidelines, consult the website at:
http://www.microbehunter.com/submission

Front Cover:
Large image: Anthony Thomas
Left image: Mike Shaw
Middle: Mike Guwak
Right: Leonardo Balbi

ANNOUNCEMENT

Visit the Forum!

It is now possible to discuss the individual articles of the magazine. Every issue has a separate sub-forum for discussion.
www.microbehunter.com/forum

Facebook

Do you have any microscopy links to share?
Do it here on facebook:
www.facebook.com/microbehunter

CONTRIBUTE!

Write for Microbehunter!

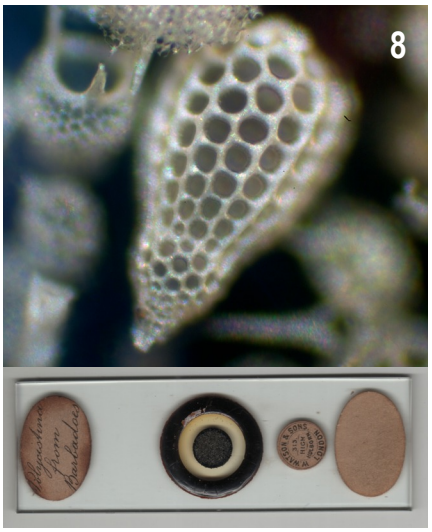
Please contribute both articles and pictures. Share your experiences, problems and microscopic adventures. If you are a researcher using microscopes, tell the readers what your research is about. Please contribute, even if you consider yourself inexperienced. If you are a struggling beginner, tell us something about the problems that you encountered. If you are an active enthusiast microscopist then share your projects, experiences and observations. Are you a teacher or lecturer? Share your microscopic experiences from school or university. This magazine is made by an enthusiast microscopist for other enthusiasts. Let's work together to make this project a successful one.
Please send all contributions to:
editor@microbehunter.com

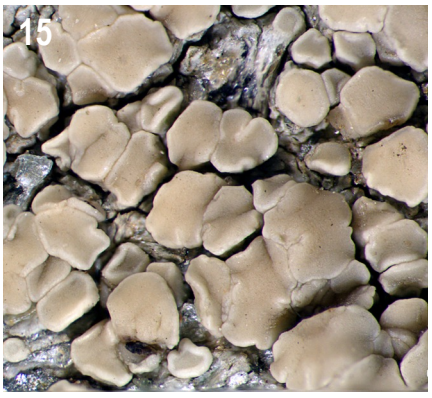
You must own the copyright of the contributions and you retain the copyright of all submitted articles and pictures. While we are not able to pay you for your efforts, we will, of course, give you full credit for your contributions.

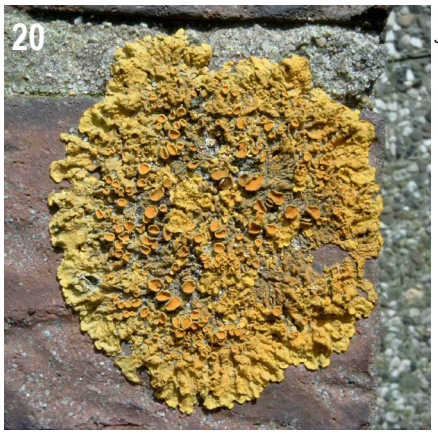
Guest Bloggers! Yes, guest blogging is also a possibility. Write microscopy-related blog posts, send them to me and I will publish them on the web site. Naturally, I'll put a link to your blog. Condition: it must be original content and you must be the copyright holder of the text (obviously). When submitting articles, please indicate if you want to have them published on the blog or in the magazine (or both).

Before submitting anything, please read the submissions page on the website: www.microbehunter.com/submissions.

CONTENTS







Answer to the puzzle (back cover):Chicken feather (*Gallus domesticus*)

How to find Tardigrades

Tardigrades, or water bears, are cute little creatures that can easily be observed with a stereo microscope.

Mike Shaw

Tardigrades (figure 1) are microscopic creatures that are a maximum of one millimeter in size, but usually are found to be about half that size. These are little creatures that live in moist lichen, moss, or leaf litter.

They are harmless, and cute. Yes cute. They are often referred to as "Water Bears" because they look like little bears with six puffy legs, and they have claws that look like those a grizzly bear would have. In fact, they have two eyes, a nose and a mouth, kind of like a bear. So how do you find them, how long do they live, what climates do they live in? Those are some often asked questions.

They can live a hundred years, withstand extreme heat and cold, and you find them everywhere. They protect themselves by going into a type of hibernation called cryptobiosis. They roll up into a dried little ball, and just stay dormant, with no sign of life whatsoever. Scientists have rehydrated them from a piece of moss in a museum collection that was a hundred years old. They've been frozen and defrosted, put under pressure, subjected to very high temperatures, and zapped with X-Rays. They come out alive and well. Just add water.

The easiest place to start is to find some moist lichen, that the yellow fuzzy stuff on a tree in your yard. Lichen grows on the shady side of the tree (figures 2-4).

Scrape some lichen into a paper bag, an ordinary lunch bag, or perhaps an envelope. Don't use plastic bags, because the moisture in there will allow mold to grow, and looking at mold is a different science project!

The best lichen to collect is not that hard crusty kind, shown in the bottom picture. Sometimes the lichen is greenish yellow, the softer, the better. See the picture in the bottom middle. This lichen is fairly good. It is greenish yellow and soft.

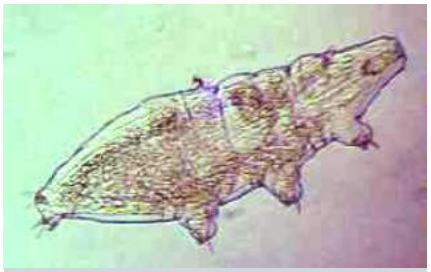


Figure 1: Tardigrade from West Orange, NJ

Begin by finding a good tree with soft fluff that grows on the dark side of the tree. Besides trees, you can also find tardigrades in moss. Go for the soft young moss. In figure 5, you can see moss in all of its stages of growth, and even some lichen growing with it. Tardigrades like to hide in fluff, so that's

Figures 2-4: Yellow lichen on tree, fluffier lichen on tree, hard crusty lichen.

Figure 5: Moss in all of its stages.

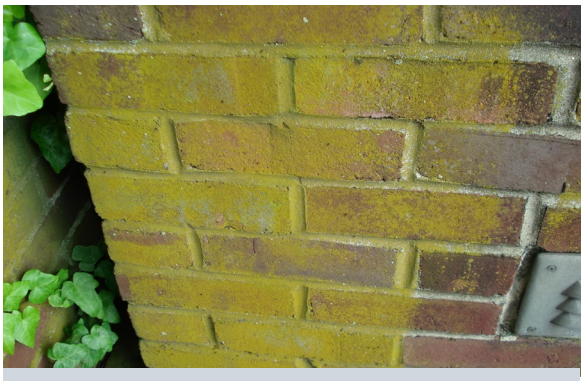


Figure 6: Lichen on brick.

what to look for. Simply pull up some clumps of moss and put it in a paper bag.

Again, use paper bags, not plastic bags, when collecting moss and lichen, because that allows air to dry out what you have collected. You do not want mold to grow on your moss or lichen, and that's what will happen in a plastic bag that is air tight. So always use paper bags, the kind you get to pack lunch sandwiches in.

You might even find some lichen on a rock or a brick wall, as shown in figure 6. It's fine. Tardigrades are sure to be in this lichen.

Next, you will need a clear plastic petri dish, or something like it. You could use a clear plastic container, like the lid from a food container, the top of a little plastic box for cosmetics, a piece of cut of blister packaging that hangs on the rack in the store.

Most small products are blister packed (figure 12), that is packaged in a hard plastic shell backed by the color cardboard product description. Cut out a piece of plastic that will hold about a half inch of water, like a little bowl or water for a hamster. The "dish," as we will call it, does not have to be round.

Sprinkle some lichen into it, just a tiny pinch. Remember, this is a microscopic journey, so a pinch is a ton of material under the microscope. Then add some bottled water, again, just about half in inch. Set aside, and let the lichen soak over night. Ideally, you could use plastic petri dishes, as you can cover these to prevent dust and mold spores from getting into the water. In the picture here, you can see that I have

Figure 7: Looking for tardigrades through a compound microscope

Figure 8: Looking for tardigrades through an inexpensive dissecting microscope

set up two petri dishes (figure 11), one with moss and one with lichen. When you add the water and let it sit, it is called a "suspension."

You will need a microscope. A dissecting microscope (Figure 8) is best, but there are some very good amateur microscopes available at reasonable prices. Sometimes called a binocular stereo scope, it will open up a whole new world to you. Here is the one I use, which I purchased second hand for under $100.00 on E-Bay. This is a very high quality Bausch & Lomb dissecting microscope, however you don't need anything so fancy.

Nowadays, you get fairly good optical quality for your money spent. You can get one from a few places on line such as

http://www.scientificsonline.com/ similar to the one shown in figure 8.

Figure 9 (top left): Petri Dish with Fiber Optic Light

Fighre 10 (top right): Dissecting Microscope with fiber optic illuminator and cable on left. Tardigrade suspension on the base.

Figure 11 (bottom): Petri Dish Suspensions of Lichen (left) and Moss (right)

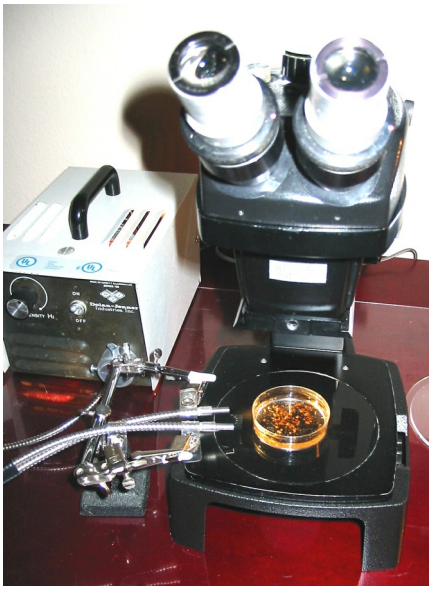


Figure 12: Blister pack.

The nice thing is that you do not need high power to see and observe tardigrades.

Your lowest magnification will be perfect, as only 15 to 30 times magnification is all you need to find a tardigrade. Also, check out what I have in my Microscope Store.

In fact, higher powers make it more difficult to search through the jungle of lichen you have created. When ready to examine the specimen under the microscope you need to place a piece of black paper under your clear dish or container. Place that on the microscope stage. In figure 9, you see a petri dish with light from the side. Somehow, you will have to mount or set up a narrow beam of light, from a flashlight or even a high intensity desk lamp. Shoot the beam across the bottom of specimen dish.

In other words, you do not illuminate the lichen from underneath, like you would if you were examining a glass microscope slide. No. You will have to illuminate the lichen suspension from the side. A horizontal beam makes any tardigrades or other creatures now appearing in your specimen dish glow white, and they will stand out clearly.

Now you have to focus the microscope, at lowest power, on the debris that rests on the bottom of the dish. If you try to use a higher power objective lens, you will wind up with it under water. You do not want that to happen, or you will ruin the optics. So, use the lowest power objective, and that will give you what's called a good working distance, or separation from the surface of the water.

Once you have focused on the bottom of the dish, and the light is streaming sideways across brightly illuminating the lichen, you can methodically examine the specimen. You are hunting for bears. These are slow moving creatures, which is why they have been named tardi-grades, or slow-walkers. What does a tardigrade look like?

If you Google search, you will see many examples. Your Google search would probably lead you to my website with loads of pictures showing the various types of tardigrades found in my New Jersey Tardigrade Survey: www.tardigrade.us

Looking in your petri dish, don't be discouraged if you don't find any at first. Sometimes, they can take two or three days to emerge from their hibernation. Sometimes you have to look in another sample of lichen or in a sample of moss or lichen. Eventually, you will find one.

There is a saying that the first tardigrade you ever find is the hardest to find.

Happy Hunting! Mike Shaw. ■

Daphnia or *Simocephalus*?

I would like to thank Josh Grosse for pointing out a possible misidentification of a water flea in the September 2011 issue. He wrote: "I'm writing in regard to a possible misidentification in the September 2011 issue of MicrobeHunter. Here you have some excellent photos of a water flea under the label *Daphnia*. However these usually have a tail spine, except in the first moult, and a lower forehead. Based on this, I think this is more likely some other relative, probably *Simocephalus*."

Suspected *Simocephalus*: there is no tail spine.

***Daphnia pulex*: notice the tail spine.**
Image credit: cc-by-sa Paul Hebert

A Beautiful Microscope Slide

Victorian microscope slides are not only nice to look at from outside. Some of the specimens that they carry are at least as interesting to observe. Here we have a look at some Radiolaria.

Leonardo Balbi

The paths we walk in this life are very curious. I have always been fascinated by science and related subjects, with a very special predilection for biological sciences, so that when I had to choose a university course, I chose biology.

While going trough the various paths of life, I came to not work in the area of my training, but moved to the technology and informatics. I can't complain. My current work allows me to enjoy biology, and my passion, microscopy, as an engaging hobby.

Acquiring equipment and materials to fuel this passion, I got from one e-bay dealer one antique slide, mounted for epi-illumination view. I do not know exactly who made this slide, but it contains the label from W. Watson & Sons, London (figure 1), undated, but probably made at the end of the nineteenth century or early twentieth centuries. (If someone with more knowledge about this type of material can send me more information, or even indicate some literature, all feedback is welcome.)

This slide caught my attention because it was mounted with radiolaria, which may be the most awesome group of organisms to me. At this point, a casual reader might wonder: "but, what are radiolaria?"

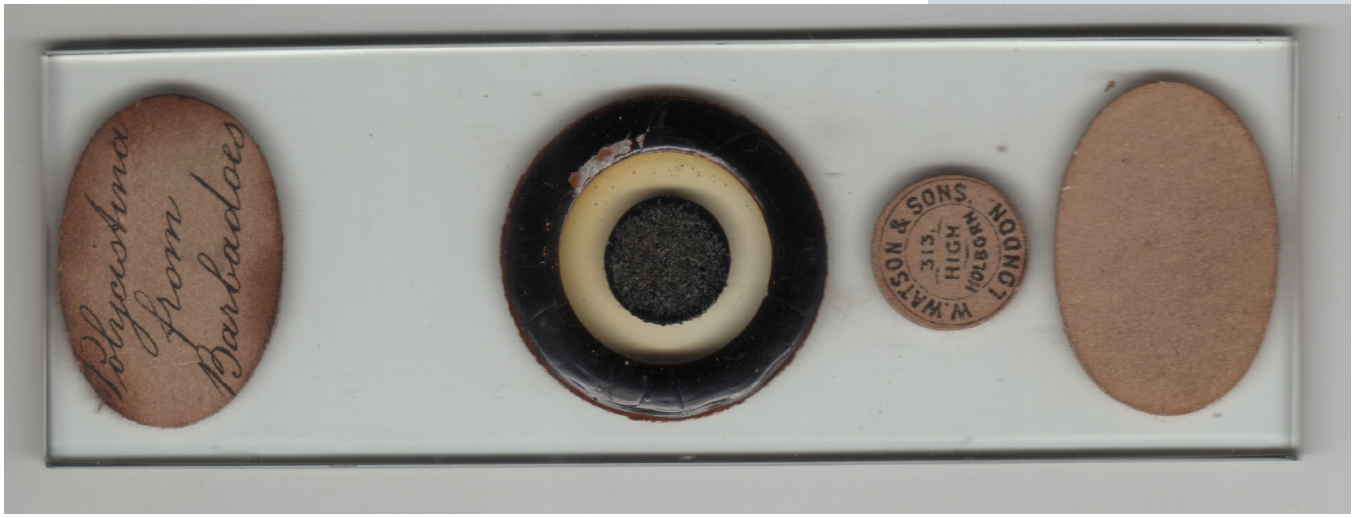
Radiolarians are single-celled micro-organisms, exclusively marine, planktonic, solitary or colonial. Belonging to the class *Actinopoda*, whose members differ from other protists by the presence of rigid pseudopods and a membrane that surrounds the endoplasm (capsular membrane) separating it from the ectoplasm. They are known in the fossil record through his endoskeleton, composed originally by hydrated silica - opal.

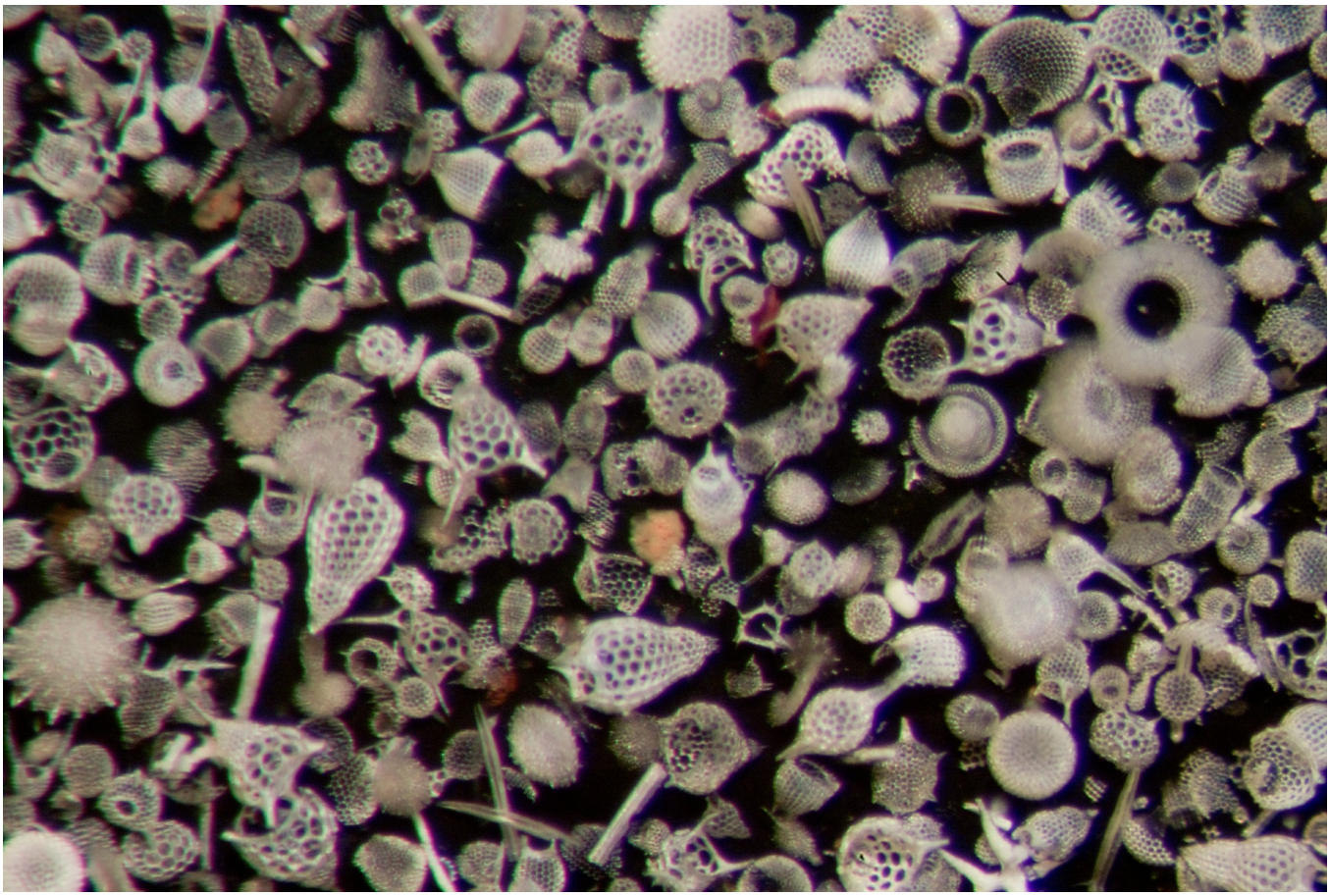
Again, thinking of my inquisitive reader, he now asks: "Okay. I know what they are. But what is so special about them?" - Well - I say - they say a picture is worth a thousand words. On the following pages are some images of this slide, and the beauty of radiolarians on it.

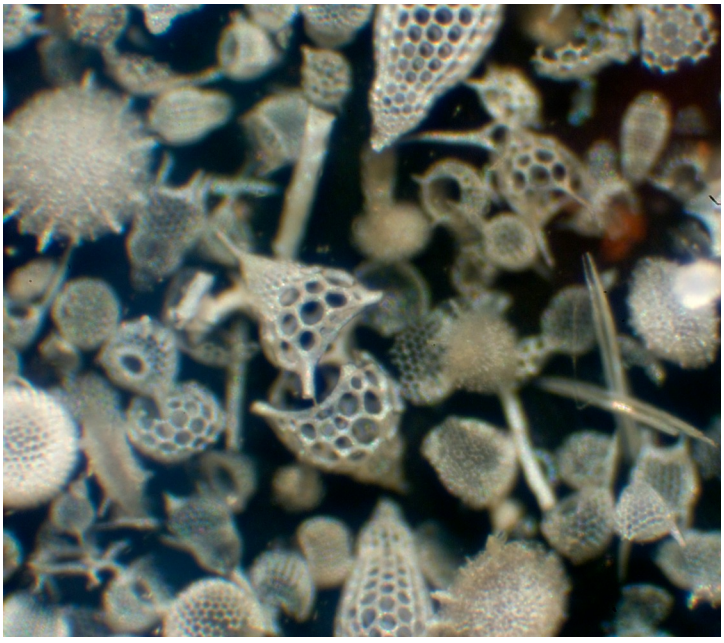
■

Figure 1: Label from W. Watson & Sons

Figure 2: General view of slide describe in text (Epson CX4500 scanner).







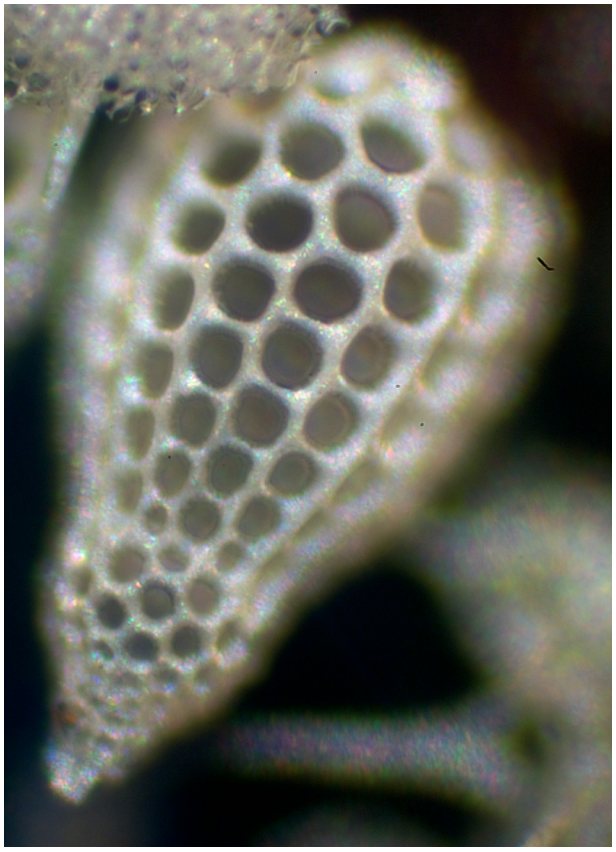


Figure 3: Some specimens on slide (Zeiss SM XX).

Figure 4: Another view of slide (Ortholux I - 5x)

Figure 5: Detailed view of a Nasselarian.

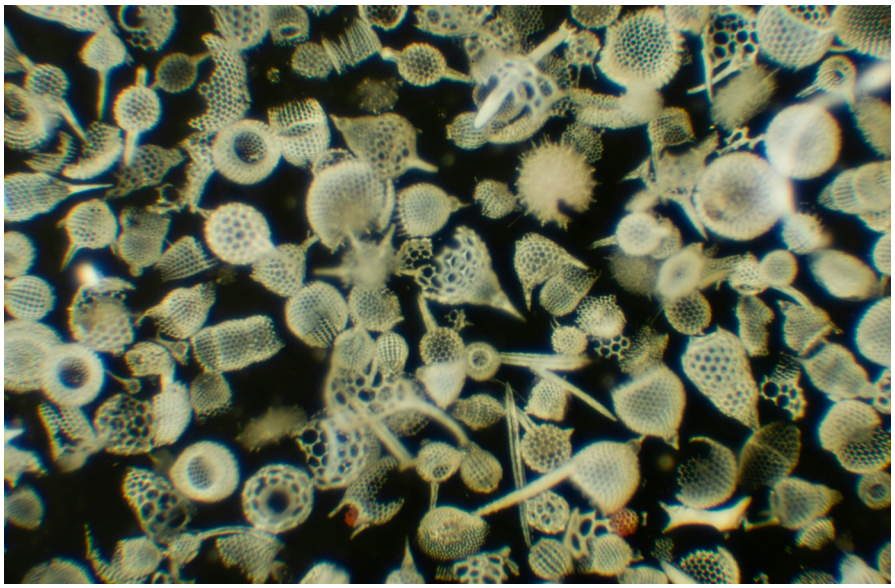


Figure 6: Another field of view (Ortholux I - 5x)

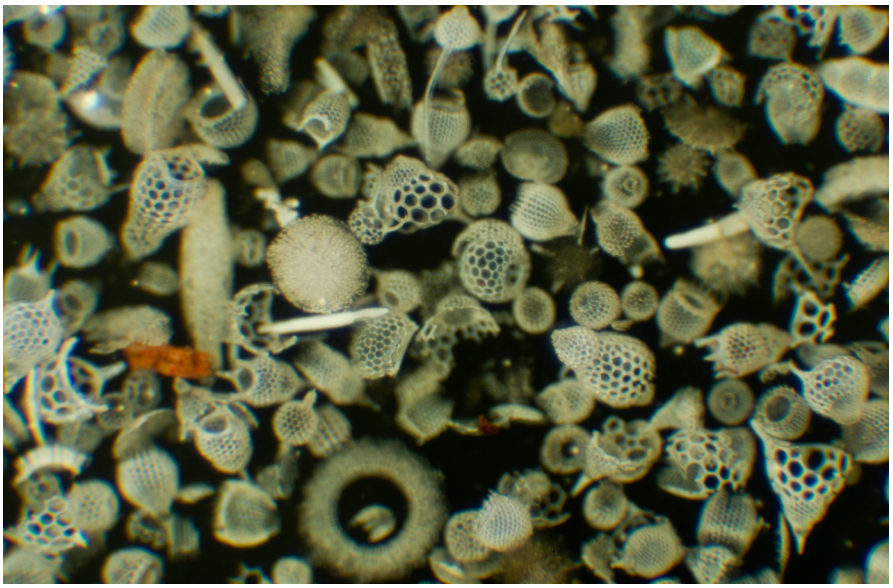


Figure 7: Conical forms (*Nassellaria*) and Spherical forms (*Spumellaria*)

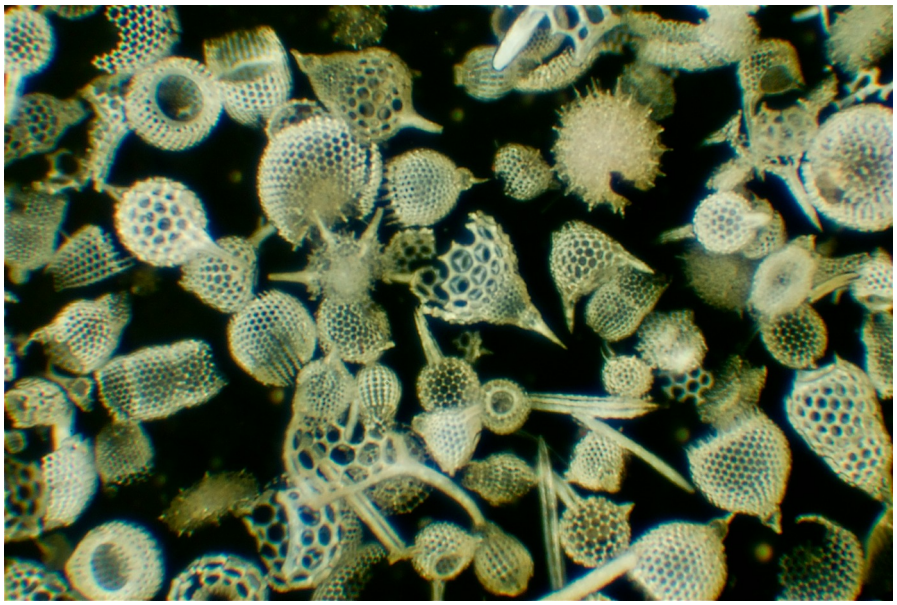


Figure 8: All specimens on this slides are fossil forms from Brabados radiolarite (a rock formed mainly by radiolarians)

Introductory Microscopy Projects for Children and Students

Are you searching for simple microscopy projects for classrooms or children?

Oliver Kim

Here is a list of microscopy ideas that could be conducted with students and children:

Observing dust samples: Students should collect house-dust and bring it to class to be observed under the stereo or compound microscope. Careful, some people may be allergic to dust!

Observing sand and soil samples: Students should collect sand and soil samples to be observed under the stereo microscope. The different color and texture of the sand grains can be observed.

Observing textile fibers: Observing various fibers obtained from clothing (cotton, polyester, nylon etc.). Different colors and textures become visible under the microscope.

Which printer is the best? Students bring in print-outs of different pictures on different types of paper. The printing resolution can be observed under the stereo microscope.

Observing water life: A large jar is filled with pond water and a little soil. Algae and other organisms will (hopefully) develop over the course of a few weeks. Do not let the water rot!

Fungi from cheese (figures 1 and 2): Camembert, Brie, Blue Cheese etc. contain edible molds (not hazardous) and can be used. This is much safer than rotting food and observing the molds. Alternatively one can also look at non-filamentous fungi, such as yeast. Blue cheese is inoculated with *Penicilium*.

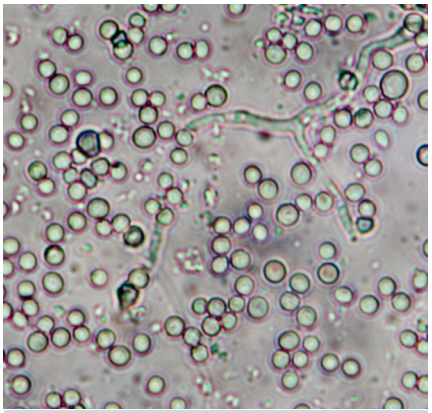
Vegetables and fruits: The teacher cuts the tomatoes and mushrooms in various ways, they can be observed under the stereo microscope. Do not eat the food afterward, you never know what chemicals were left behind on the microscope by previous classes…..

Hair samples: Each student donates one hair and then they have to match them with the hair left behind on the "crime site". This is a playful approach into forensics and gives the observation some purpose. Maybe a competition between different groups is also a nice idea. The teacher may have to prepare a set of permanent slides with some hair samples.

Coins: Coins collect many scratches (and dirt) over the years. How can the scratches be quantified? Is it possible to predict the age of a coin by looking at the number of scratches? The year is imprinted in the coin.

Observing human cheek cells: This is a classic, really. Using a cotton swab, some epithelium cells from the inside of the mouth are collected and transferred to a microscopic slide.

There are also a few things that one better does not observe in a classroom setting. Safety, after all, should play a high priority. This includes spoiled food material and any microogranisms obtained from the human body or animals. Blood samples or other body fluids may be sources for infection. Polluted water is also problematic for obvious reasons. Water from polluted rivers, lakes may contain toxic substances and harmful microorganisms. ■



Figures 1 and 2: Blue cheese. Scratch some of the cells off the cheese and make a wet mount.

Safety Issues for Microscopists

Safety first! Do you know which microorganisms can be found in your sample?

Oliver Kim

Much has already been said and written about the precautions that one should take when dealing with organic solvents, fixatives, and stains, which are needed for preparing microscopic specimens. Organic solvents (such as xylene) can be inhaled and many volatiles pass easily through the mucous membranes into the blood. Certain fixatives will react with substances in the cells, where they may denature proteins and cause a wide range of other chemical modifications. Stains can be a particular problem, especially if these interact with the DNA of the organisms, as used for making nuclei visible. In this case the stains may be cancer-causing. As a matter of fact, some more traditional substances used in microscopy, such as Hoyer's mounting medium, contain ingredients that are addictive and are a controlled substance and are therefore not freely available.

Much less has been written about the precautions that one should take when dealing with the organisms themselves. It is now my objective to address some precautionary measures when dealing with organisms that are to be microscoped.

Amateur microscopy certainly can not be considered a high-risk hobby, especially when one looks at ready-made permanent slides. Here the organisms in question safely killed and embedded in mounting medium. The issue starts to look a little different when one engages in collecting, concentrating and possibly even growing microorganisms for microscopic observation. Safety issues like this are not only relevant to amateur microscopists, but also for teachers who want to conduct basic microbiological and microscopic work in a school laboratory. In this case the organisms are alive and depending on the type of organism, they may pose a possible health hazard.

It it not, and can not be the intention of this article to give a detailed overview of the aseptic procedures used in a microbiology laboratory. I am not going to address the growing of bacterial colonies on agar petri dishes or the the making of a nutrient broth for the enrichment of bacteria. I am also not going to address the proper use of an inoculation loop and a Bunsen burner for sterile colony transfer. These laboratory methods are, in my opinion, too specific for the majority of amateur microscopists and require a properly equipped lab and appropriate training. Such issues can also not be covered in the little space available. The growth of (unknown) bacteria on agar plates or liquid culture medium also poses a potential health hazard, as the bacterial densities can be extremely high, and I generally would be cautious when working with nutrient media. There are also legal issues associated with these methods, as the legislation of some countries only permit the enrichment and growth of bacteria for certified laboratories. As a matter of fact, the growth of unknown bacteria isolated form the environment even requires the application of aspetic methods of an elevated biohazard level. Readers who are interested in these methods should consult introductory microbiology books, which cover these aspects in detail.

Rather, I would like to place a focus on the methods that are relevant for microscopists. In particular, I would like to address the making of a hay infusion, the observation of pond water as well as the observation of molds and other fungi.

Aseptic technique

The term aseptic technique refers to a medical or laboratory procedure that is performed under sterile conditions. The aseptic technique fulfils several objectives. First, the technique should protect the sample under investigation from contamination. This is of particular importance when culturing microorganisms, as fast-growing contaminants may possibly grow faster than the microorganism that one is interested in. The sample may thus quickly become overgrown by unwanted microorganisms.

While still working in a microbiology lab, I was told that a student working towards his diploma thesis accidentally sub-cultured a contaminant for several months. All of the experimental tests were performed on this contaminant and at the end of the thesis work the obtained data of several months was considered worthless. A quick check of the microorganism under the microscope would have quickly revealed the mix-up. For those of you who were wondering: Luckily I was not the unfortunate student.

Second, the aseptic technique should protect oneself from infection by potentially pathogenic microorganisms. The procedure therefore includes measures that prevent the inhalation of microorganism containing aerosols as well as the prevention of skin contact and ingestion.

Last, the environment and other people should be protected as well. Proper disposal of petri dishes and microorganism-containing sample materials is therefore necessary and often also required by law.

Risk Assessment

The dangers of contacting an infection depend on several aspects:

Immune status of the person: The weaker the immune system of the person, the higher the chance of contacting an infection. For this reason, only handle unknown bacteria if you are healthy and have no immune system problems.

Infectivity of the organism: Some pathogens can be infective at a low concentration, others require a higher concentration. Keep the concentration of the microorganisms low.

Density of the organism: The higher the density, the higher the chance that a critical level of the microorganism is reached to cause infection. Just as above, keep the density of the microorganisms low and only grow them if it is not possible to observe them from natural samples.

Mode of transmission: Different pathogens prefer a different method of transmission. Certain pathogens, for example, are transmitted over the air, others over water and still others over food. Others require insect vectors for transmission.

Most microorganisms are harmless, but one never knows what substances they are producing when grown at a higher concentration. Certain *Cyanobacteria*, for example, are known to cause eye irritations or allergies.

Hay infusion issues

A hay infusion is a culture medium which is commonly used to grow protists, such as the well-known *Paramecium*, for microscopic observation. Hay infusions have been popular since the beginning days of microscopy and are still a popular way of obtaining protozoa for educational uses in schools and universities.

There are two ways in which a hay infusion can be made. A handful of hay is boiled with water to extract nutrients, which serve as a food source for the microorganisms. The obtained culture medium must then be inoculated with the microorganisms that one wants to enrich. Pond water containing ciliates, for example, can be used. Alternatively one can try to enrich the microorganisms that can be naturally found on the hay. In this case the hay-water mixture is not boiled, but simply left standing for 24-48 hours. A thin iridescent biofilm will start to form on the water surface. This film is teeming with bacteria. In the presence of ciliates, the number of bacteria may decrease over time, and a progression of different organisms can be observed.

One should be aware that unknown (and therefore potentially pathogenic) microorganisms may also start to grow in the hay infusion. The boiling process does not necessarily kill all of the microorganisms present on the hay. It is not uncommon to find heat-resistant spores of *Bacillus* and *Clostridium* on the hay. After the cooling of the infusion, these spores may start to germinate giving rise to live, possibly pathogenic, bacteria. The fact is, that you simply do not know what you are growing and appropriate safety precautions should be taken.

It is not possible to determine the pathogenicity of bacteria by microscopic observation. A range of biochemical and genetic tests are necessary. The enthusiast microscopist should therefore treat such a hay infusion with utmost care. Do not ingest the hay

Figures 1 and 2: Two kinds of biofilms. In both cases unidentified microorganisms are present. I would assume that the left biofilm (bottom side of a shower stopper) is potentially more dangerous (possible accumulation of bacteria that were washed off from the human skin). The water with the green algae (right) was never in contact with humans and is therefore possibly safer, but one never knows.

infusion, avoid skin contact (especially if there are open wounds), do not inhale the aerosols and prevent spills. Generally avoid contact of the liquid with mucous membranes, including the eye. Also make sure that the hay is clean and has not been in contact with excrements of animals. You may otherwise enrich bacteria from the animal's digestive system. If a spillage or skin contact has occurred, then use 70% ethanol for disinfection (mix 7 parts of alcohol with 3 parts of water). A higher concentration of alcohol may actually have a lower disinfection efficiency.

Do not simply flush the hay infusion down the toilet. This may cause aerosol formation. Add chlorine bleach to the infusion and allow the substance to work for a few hours. Some people may be concerned that the bleach will then also find its way into the waste water, which is not very environmentally friendly. I would agree, but have no solution to this issue. Be aware that the addition of 70% ethanol to the infusion will dramatically lower the concentration of the alcohol. You can add concentrated alcohol to the infusion but this is a cost issue (and the glass jar may not be large enough).

Pond water safety

Even pond water may be the source of some unexpected surprises. I recently introduced mosquito larvae into my household this way. The mosquitoes caused me quite some irritation at night. Be aware that keeping a jar of standing water may even be illegal in countries with Malaria, which can be spread by certain mosquitos.

Other issues relate to the water quality of the pond water, may or may not be very high. Decomposing animals close to the sampling site can give rise to microorganisms that one does not want to have in the household.

Ponds which are clean enough for swimming should not be problematic, there are rare cases, where people did get infected by certain protozoa, however. Water samples from ponds which are rich in (potentially irritating) *Cyanobacteria* or eutrophicated should be handled with more care. Some ponds may be close to agricultural areas and there is the possibility for manure to run into the ponds.

Molds

Molds can be easily grown by treating an appropriate substrate (such as bread) with a soil-water suspension. Fungi will start to grow and release spores into the air. These spores may not only contaminate other types of food in the household, but may also be responsible for allergic reactions when inhaled. Many types of mold also produce potent toxins, which are capable of causing severe health problems.

Prevent the spreading of spores by keeping the container with the mold closed and avoid air currents which may distribute the spores.

If you want to investigate molds for educational purposes, then I would suggest that you try to first use edible molds, as can be found on foods, such as cheeses.

General Advice

Here is some general advice when handling samples that contain microorganisms.

Open wounds: Do not handle microorganism-containing media if you have open wounds or cuts in your skin. Intact skin can be considered as a very effective physical barrier against infection and open wounds can be problematic.

Disinfection: Disinfect hands and surfaces with 70% ethanol. More concentrated ethanol may actually work less efficiently in killing microorganisms.

Disposal: Autoclave the used culture medium at 120°C for 30 minutes. This should also be able to kill spores. If you do not have an autoclave available, then cover the petri-dishes or culture medium with chlorine bleach. Allow sufficient time for these substances to work. When you add bleach, be aware that this is a corrosive substance when concentrated. Eye and skin contact must really be avoided. Also be aware that liquid bleach becomes more diluted when you add it to liquid culture medium, losing its efficiency.

Avoid aerosolization: Some microorganisms spread over air. Avoid spillage of the culture medium and carefully add the disinfectant to the medium before disposal, avoiding splattering of the liquid.

Keep bacterial counts low: Make sure that the sample (such as a hay infusion) contains many ciliates that consume the bacteria. Keep the level of nutrients low to avoid too many bacteria from forming and ensure that the medium has sufficient oxygen supply for the ciliates to grow.

Do not use polluted water: Dirty and polluted water can contain contains many bacteria and a lower count of the more interesting ciliates. If the water sample was isolated from a stream that came in contact with household waste water, then it may be possible that pathogenic enterobacteria are present.

Do not decompose food: Some teachers like to decompose food to demonstrate the spoiling process to children. Be aware that *Clostridium perfringens* may be found on spoiled meat or poultry. This bacterium can cause food-borne illnesses. Personally, I would not use microorganisms from spoiled food for educational microscopy. I would resort to much safer and easily available bacteria and fungi. These can be isolated from fresh cheese, or example.

Do not culture bacteria obtained from humans: In particular, do not inoculate growth medium with bacteria from the skin. You may be growing *Staphylococcus*, otherwise.

Keep petri dishes closed: This minimizes the risk of accidentally touching the agar surface, which may be covered by bacterial colonies. Generally speking, I do not recommend the growth of unknown bacteria in petri dishes by people who do not have basic microbiological training in aseptic technique. The bacterial concentrations are simply too high to be safe.

What is the take-home message? A good portion of common sense and basic hygienics will greatly reduce the possibility of you catching an infection and will hopefully keep you healthy. ■

Some Lichen Terminology

An understanding of mycological terminology is necessary to fully understand the anatomy of fungi and lichens.

Oliver Kim

Give this one a try: *The thallus of a lichen is composed of hyphae, which may be paraplectenchymatic. The lichen forms either perithecia or apothecia, depending on the species. These reproductive structures contain a hymenium carrying the asci and the sterile paraphyses. The epithecium of the perithecia or apothecia give a lichen its characteristic color, unless it is hyaline. The asci produce spores, which may also be hyaline and septate. Lichens may also form soredia for reproduction.*

Did you have problems understanding this text? Here is a list of terms that may help you to shed light into the mystery. These terms may also help you understand the article on the following pages.

Lichens: These are fungi that live in a symbiotic relationship with a photosynthetic organism (algae or cyanobacteria).

Hyphae: These are long, filamentous structures found in many fungi. They collectively form the mycelium of the fungus.

Fruiting bodies: These are the organs of a fungus which form the spores. In colloquial English, the fruiting bodies are known as the "mushroom" of a fungus. The fruiting bodies contain the ascii, which form the spores.

Thallus: This is the vegetative (non-sexual) part of a lichen. It is composed of the hyphae of the lichen.

Apothecia: These are fruiting bodies which are (in contrast to the perithecia) open and cup-shaped (Figures 2, 3).

Perithecia: This is another type of fruiting body of a fungus. The perithecia are closed and possess a small hole through which the spores are released (Figure 1).

Epithecium: This is the thin (often pigmented) outermost layer of a fruiting body.

Hymenium: This is the tissue layer of a fruiting body which produces the asci (these produce the spores).

Ascus, asci: These are specialized cells of a fungus. They form the spores (typically 8).

Hyaline: A structure that is hyaline allows light to pass through. It is transparent.

Spores: these are the reproductive cells of a fungus. They are formed inside the ascii. The spores germinate to form a new fungus

Paraphyses: These are long sterile cells that are located in the perithecia (the fruiting body). They do not form spores. The asci are interspersed between the paraphyses.

Figure 3: The cup-shaped apothecia can easily be seen on this lichen.
Image credit: cc-by-sa by Brudersohn

Soredia: these are structures of lichens used for asexual reproduction. They are composed of fungal hyphens which are in close association with their photosynthetic partner (green algae or *Cyanobacteria*).

Paraplectenchymatic: This is an adjective describing the shape of the cells composing the hyphae. Paraplectenchymatic hyphae are short celled and the direction of the hyphae is not apparent. The hyphae therefore have a much more "cellular" appearance and are not as stretched

Septate: The spores of some lichens are not individual, but are joined to each other. They are then separated by a thin septum. These spores are referred to as being septate. ■

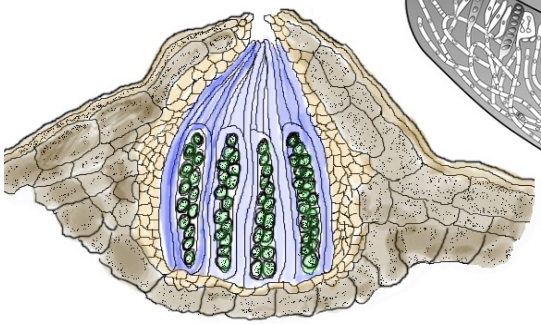


Figure 1 (left): Closed perithecia with a small opening at the top to release the spores.
Image credit: cc-by-sa by J. Marqua

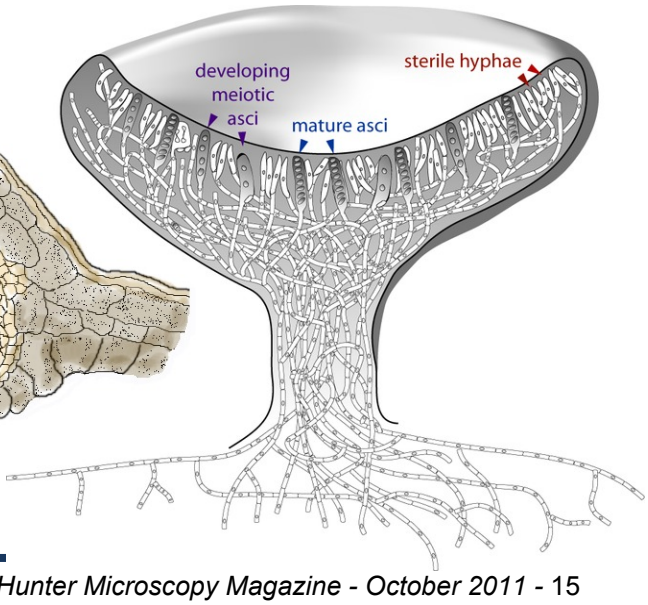


Figure 2 (right): The cup-shaped apothecia. The sterile hyphae are the paraphyses. The asci form the spores
Image credit: cc-by-sa by Debivort

Microscopic observations of Lichens

Lichens are fungi that live symbiotically with a photosynthetic partner. Who would have guessed that lichens can can be parasitically infected by other fungi as well?

Mike Guwak

In this photo essay, I present the lichen *Lecanora polytropa* and its parasitic partner *Cercidospora epipolytropa*. Let's start out with a short characterization of an uninfected *Lecanora polytropa* lichen.

Lecanora polytropa (Hoffm.) Rabenh.

Thallus: Grey-white, without soredia.

Apothecium: Ocher colored and 1-1,5 mm in size. The juvenile stage is characterized by a clearly brighter thallus border. The borders of more mature stages are pushed downwards and are almost not visible.

Epithecium: The epithecium has a brown pigmentation, which can be dissolved in KOH.

Spores: The spores are hyaline, there are 8 spores per ascus and they are single celled. The spores measure 9-14 x 5-6,5 µm (Figure 5).

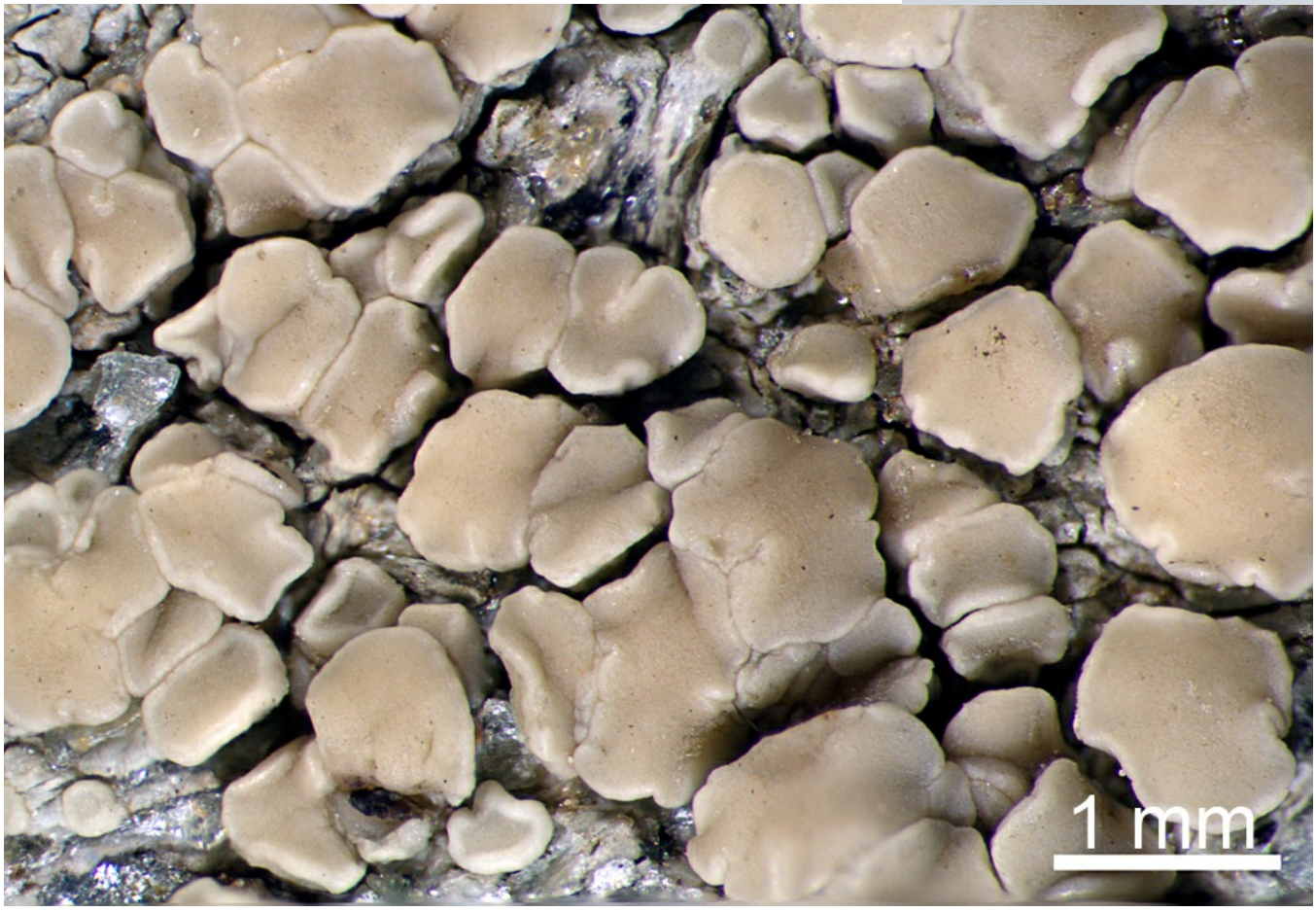
Chemical composition: Thallus K-, C-, KC+ slightly yellow, P-. The letters are abbreviations for different chemical reactions performed on the lichen. A minus sign denotes a negative reaction without color change. K: KOH reaction, C: Calcium hypochlorite, P: para-Phenylendiamin reaction.

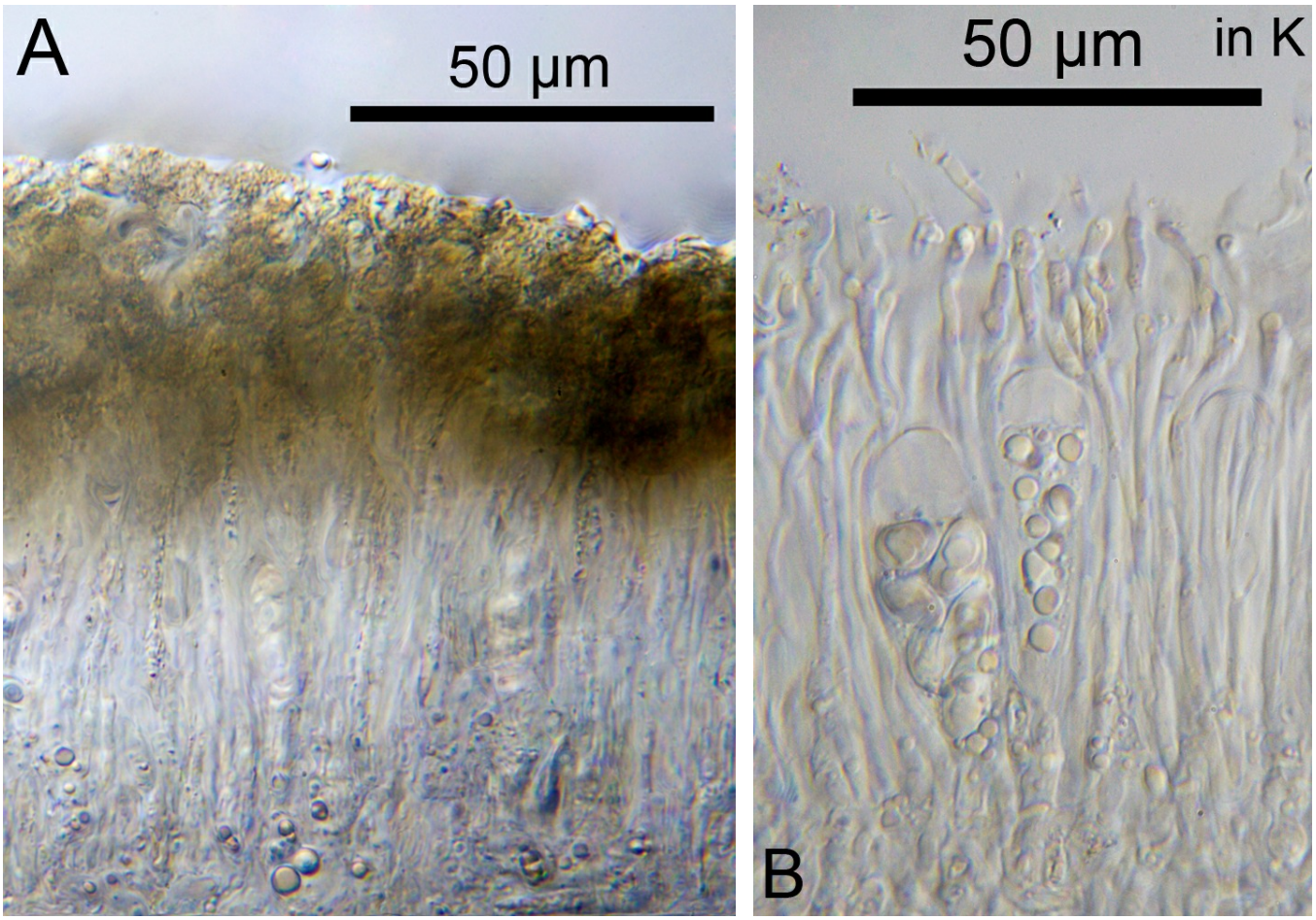
Are you interested in more pictures of lichens? Check our Web site at:

www.flechtenmikroskopie.de

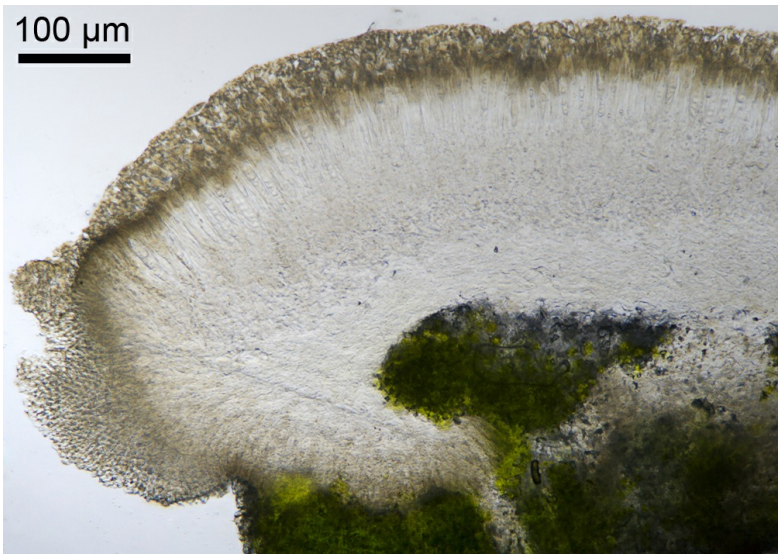
This site is by Mike Guwak and Dr. Ralf Wagner. Here you can find a variety of different lichens that we collected over time. We keep on adding more lichens.

Figure 1 (bottom): Overview of an uninfected *Lecanora polytropa*.





Figures 2 and 3: The brown pigments of the epithecium (left) dissolve in the presence of KOH (right). This is a relevant criterion for identifying the lichen.



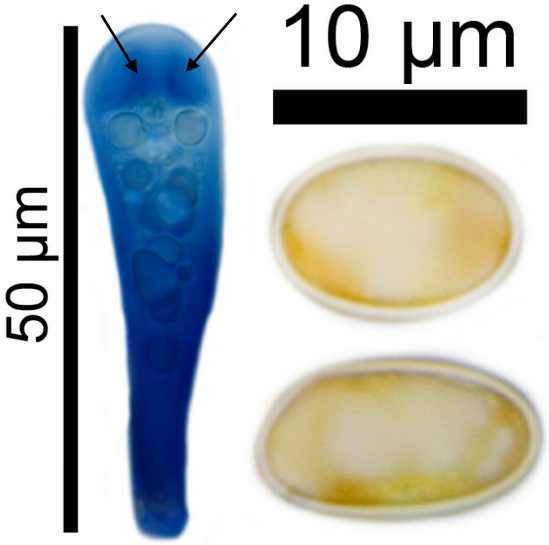
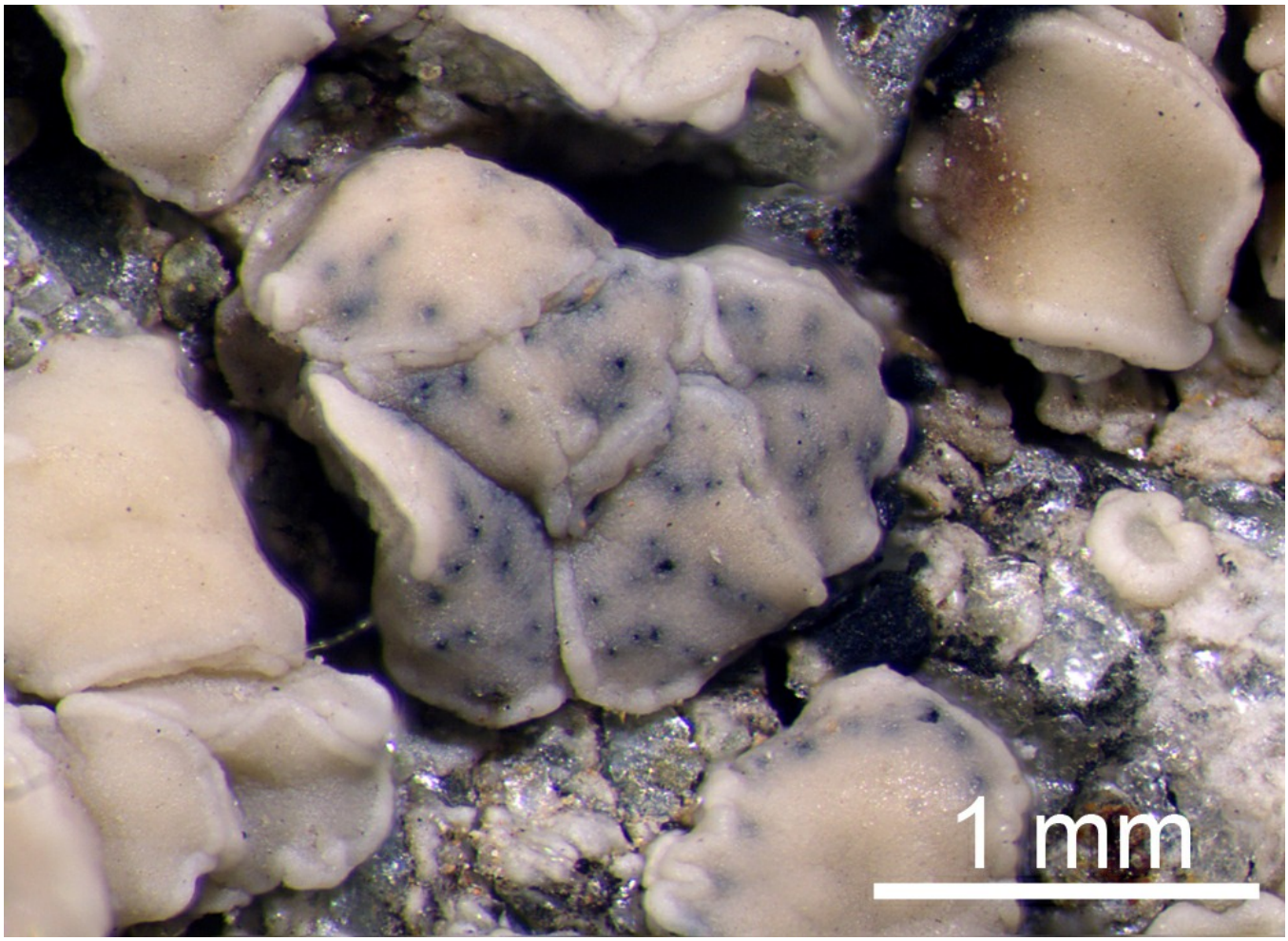


Figure 4 (left): Cross section through the apothecium.
Figure 5 (center): An ascus. The arrows point to the apex of the ascus.
Figure 6 (right): The spores measure about 10µm across and are transparent.



Cercidospora epipolytropa (Mudd.) Arnold

Cercidospora epipolytropa is a fungus which is able to infect the lichen *Lecanora polytropa*. The infection can be clearly seen in the form of blue-green pigmented spots on the host. The following description refers to the parasitic fungus, and not to the host lichen.

Perithecia: 0,08-0,13 mm wide, embedded in the hymenium of the host *Lecanora polytropa*

Wall of perithecia: The lower half contains interwoven, more or less parallel almost hyaline hyphae, which can only be vaguely differentiated from the host tissue.

Pigmentation: The wall in the upper area towards the mouth is blue-green to blackish pigmented, and becoming paraplectenchymatic. Pigment becoming more intensely blue-green with addition of KOH.

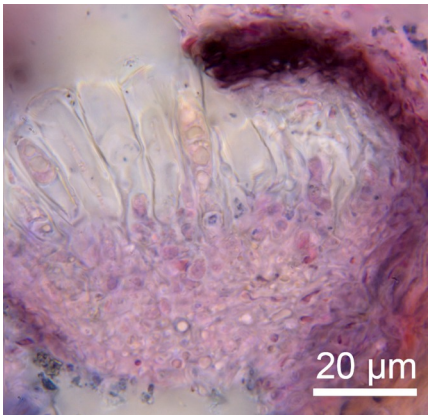
Spores: 13-23 x 4-8 µm, 1- septated, 8 spores per ascus, boat-shaped.

Comments: *C. epipolytropa* is best identified by ist spore size and its occurence on the host *Lecanora polytropa agg.* ■

Figure 1 (top): The lichen *L. polytropa* infected by C. epipolytropa (the blue-green spots).

Figure 2 (center): The parahyses. These are sterile cells in the fruiting body.

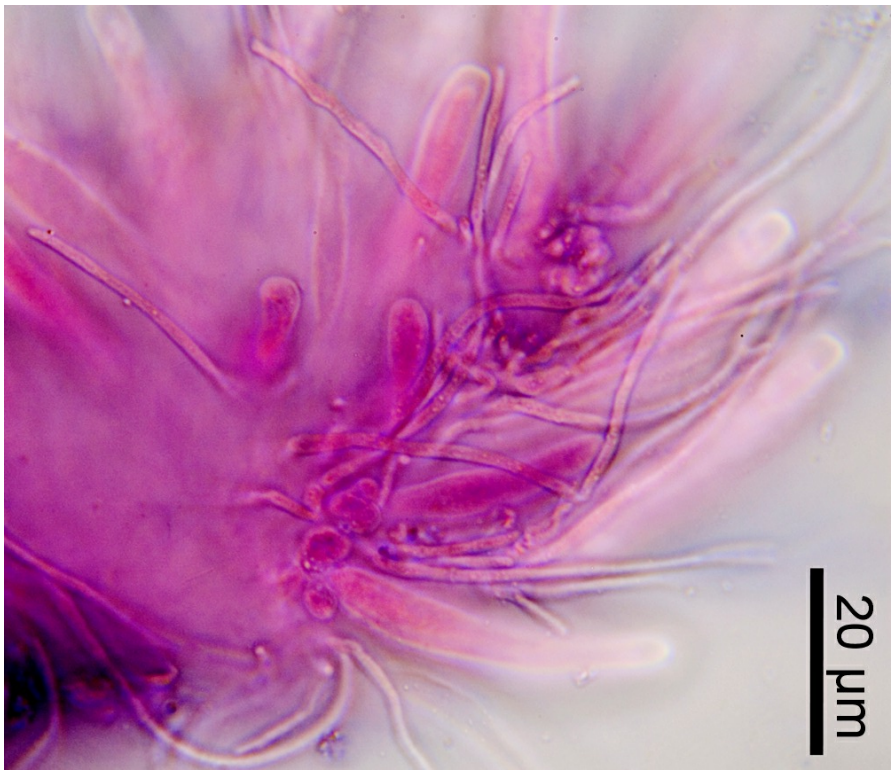
Figure 3 (bottom): Two spores are associated with each other and separated by a single septum ("1-septate").



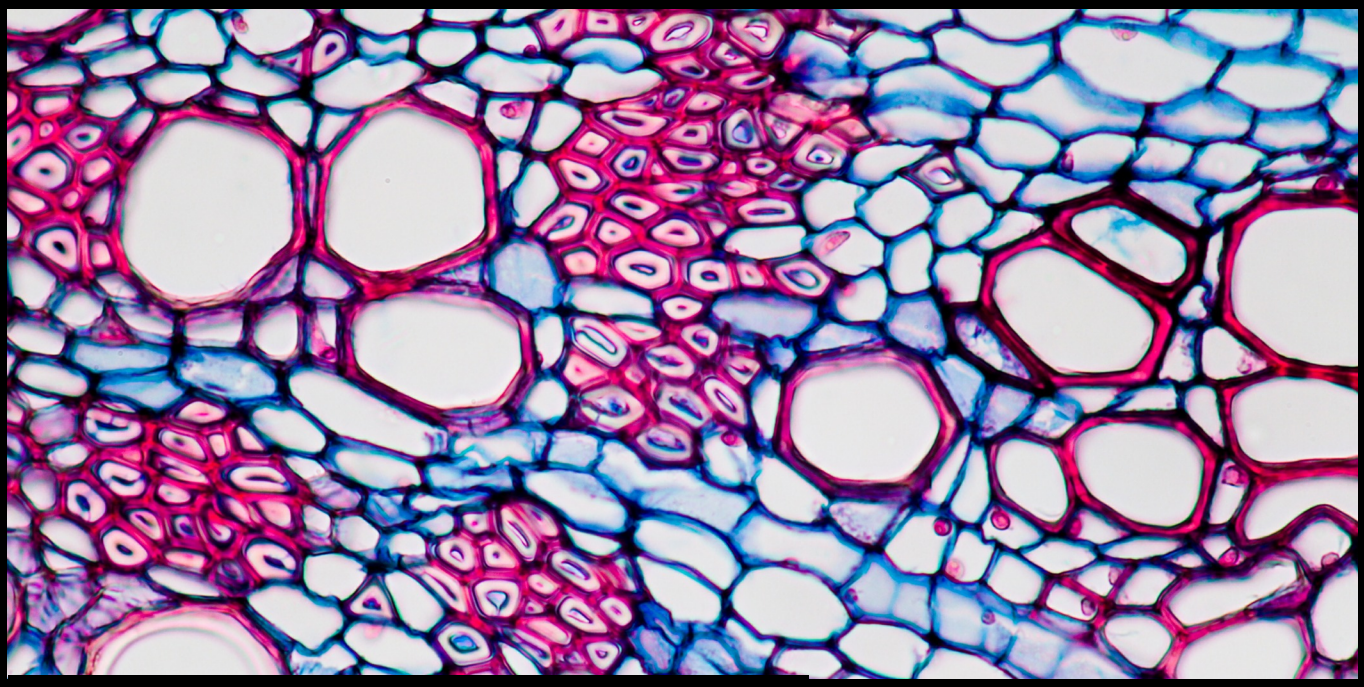
Figures 4-6 (top left, top right and left): The three images show cross sections through *C. epipolytropa*. In the absence of pigmentation towards the base of the fungus, it is nearly impossible to distinguish the fungal tissue from the one of the host lichen. The pigmentation increases towards the opening at the top.

Figure 7 (bottom left): a single ascus containing 8 spores.

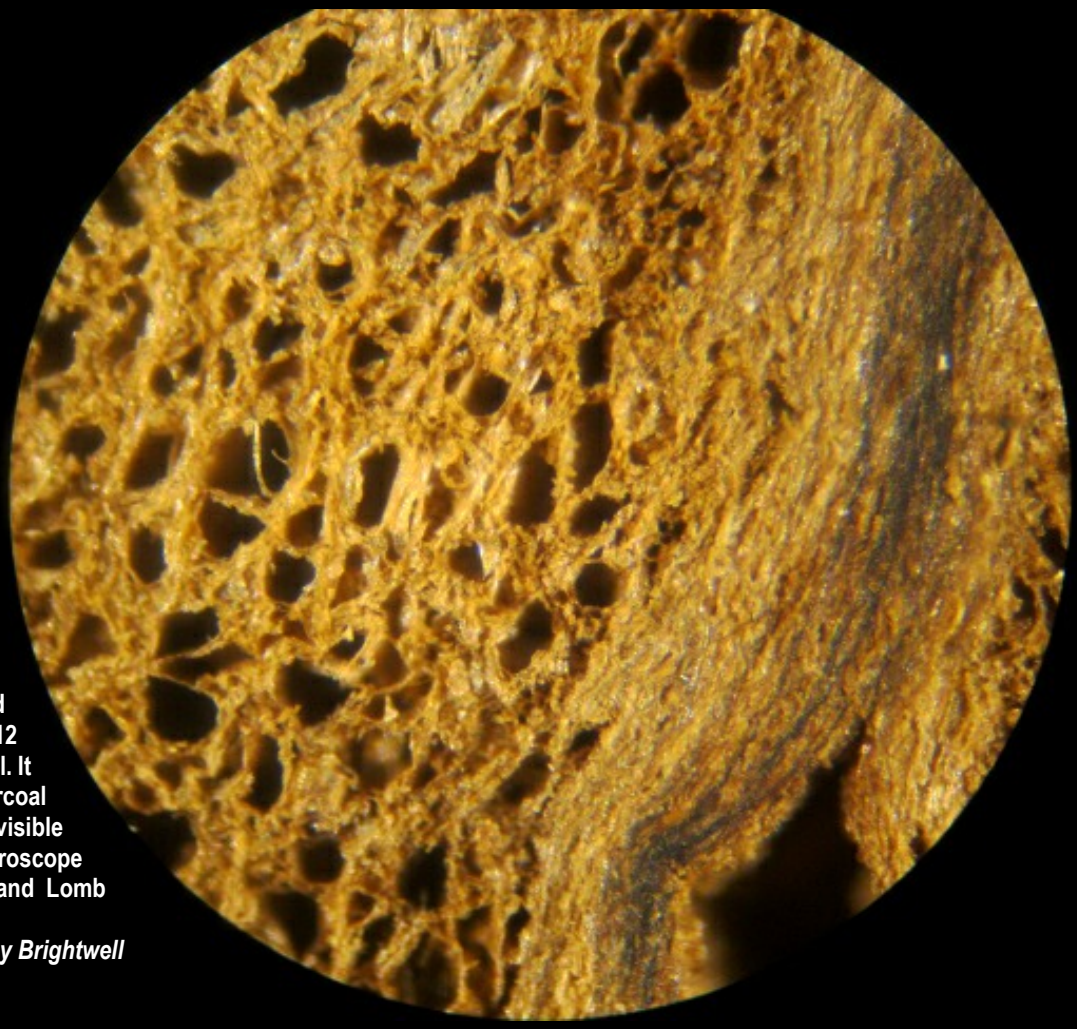
Figure 8 (bottom right): The images shows paraphyses, which are long sterile cells in the spore-forming layer of the fungus.



Send images to: editor@microbehunter.com

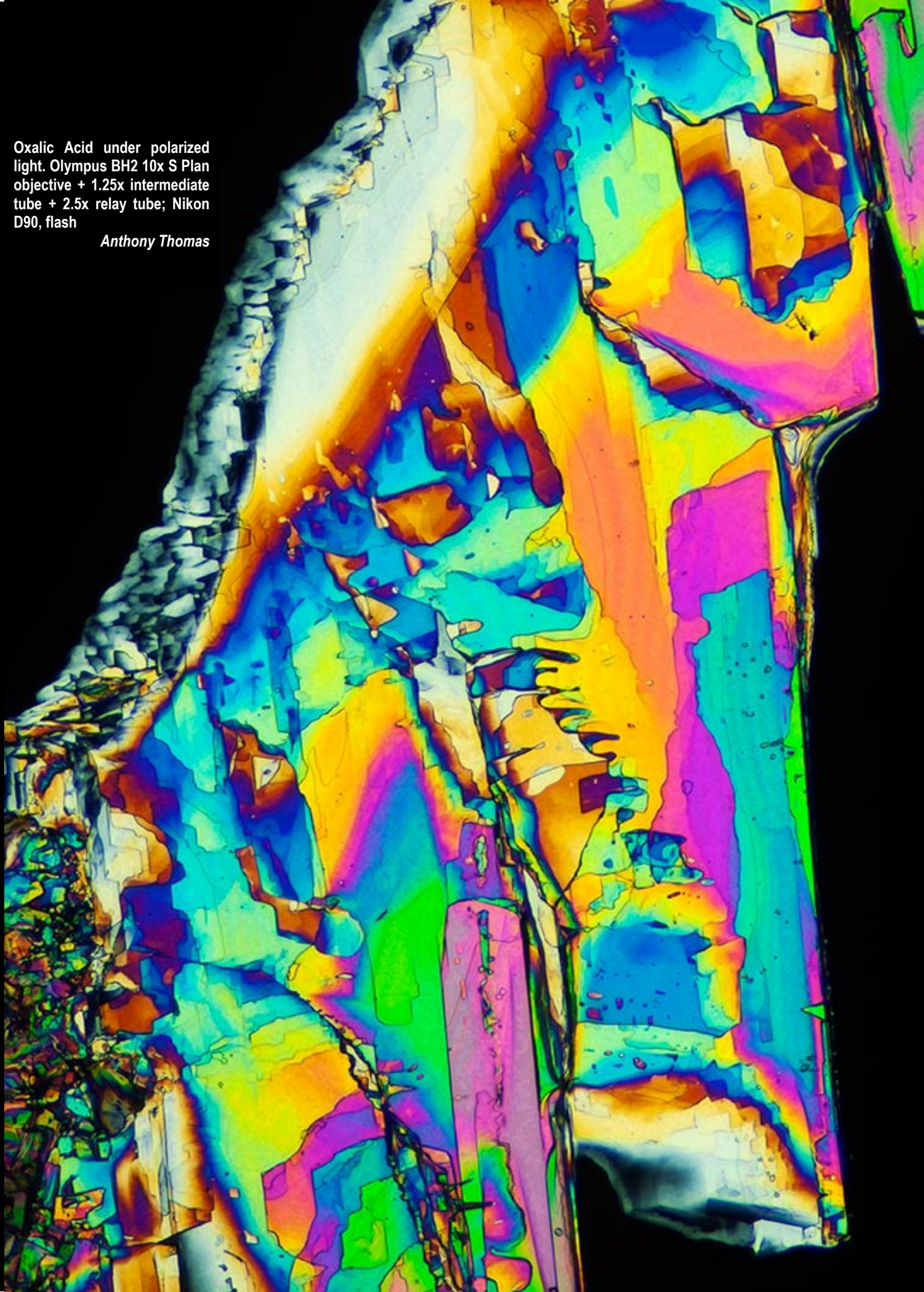


Cross section through the root of a *Lupinus* plant. *Oliver Kim*



Wood slice section. This old wood was dug up out of our pond and was aprox. 10-12 feet below ground level. It apears to have charcoal traces in it which are visible lower right. The microscope used was a Bausch and Lomb Dynazoom at 120x.

Rodney Brightwell



Oxalic Acid under polarized light. Olympus BH2 10x S Plan objective + 1.25x intermediate tube + 2.5x relay tube; Nikon D90, flash

Anthony Thomas

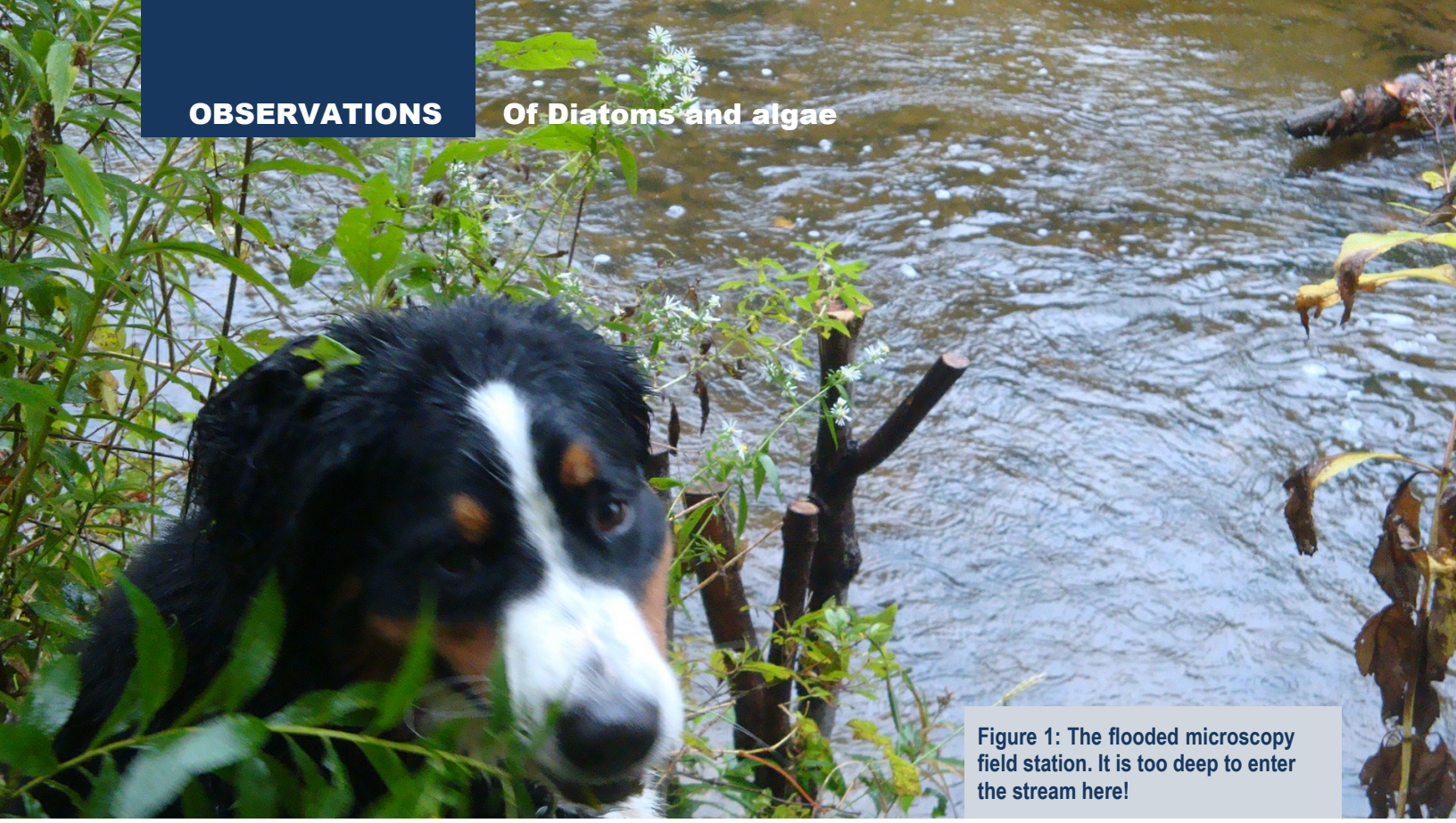


Figure 1: The flooded microscopy field station. It is too deep to enter the stream here!

Diatom Cities

If your freshwater field microscopy agenda "turns sour" - then it's time to make some lemonade of the microscopy hike!

Charles E. Guevara

The radical stream flooding rapidly increased in depth, and also its torrential strength. This, naturally, always occurred when I had the free time to hike to my field microscopy station (read the article in the August 2011 issue about the field station). The torrent prevented me from reaching my field station platform, and I could not get to my field location with the purple sulfur bacterial blooms.

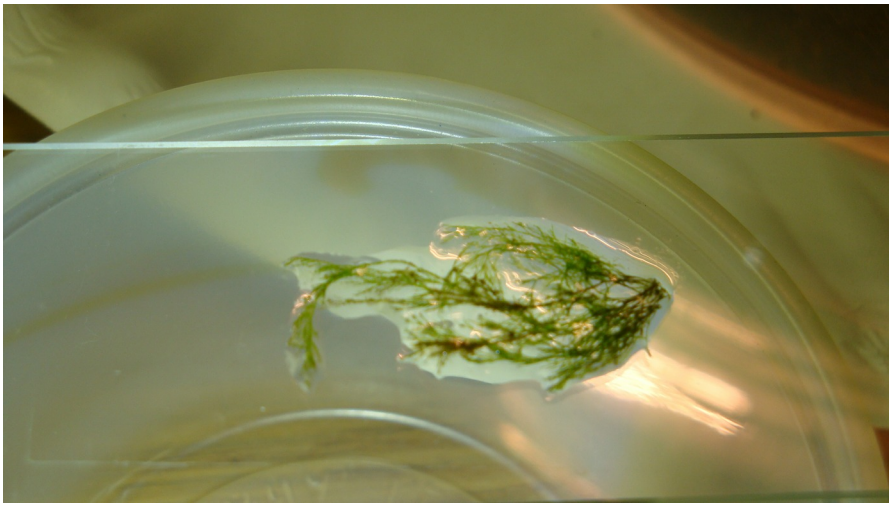
I could not even find my clever cover-slip collection gadgets, or my strong magnets in a glass vial. I could only find the temperature gauges, which I spiked into the bacterial bloom locations in the stream, towards the bottom of the shore.

Ahh, but a 1964 text in my cluttered study read: "The Algae". This text tantalized me with a wonderful section (Chapter 14, "Freshwater Ecology", section "Epiphytes", pp. 371-374). This chapter contained graphs, charts and plots of variables but also a 'pearl' to pursue on my flooded-out microscopy hike. One page 374 it read: "Another interesting feature is the frequent association of *Gomphonema* with the basal cells of *Oedogonium*, but so far there is no evidence to suggest whether this is a casual relationship or not". Now here was field microscopy hike objective for my puppy and me (after I hiked back home to return my nifty field microscope kit). I mustered up my stream

Figure 2: The intact field-microscopy station (Read the August 2011 issue) for an explanation.

Figure 3 and 4 (top, middle): A stream rock at my work bench, full of epiphytes.

Figure 5 (bottom): The wet-mount preparation. Macroscopically you can see where the epiphytes cluster! Yes... yes!!



hike equipment and my non-microscope-assistant-puppy, and the usual plastic pail with a specimen jar.

I suppose, on an allegorical level, I searched for 'Diatom Cities', but the field microscopy imbued by Dr. Chapman's published thoughts was significant, and motivated me to revisit a seasonal epiphytic assemblage of stream organisms.

Please do not enter a raging stream, but rather do enjoy with me this encounter of algae in harsh currents - algae attached to a rock about 7 cm beneath the churning stream surface.

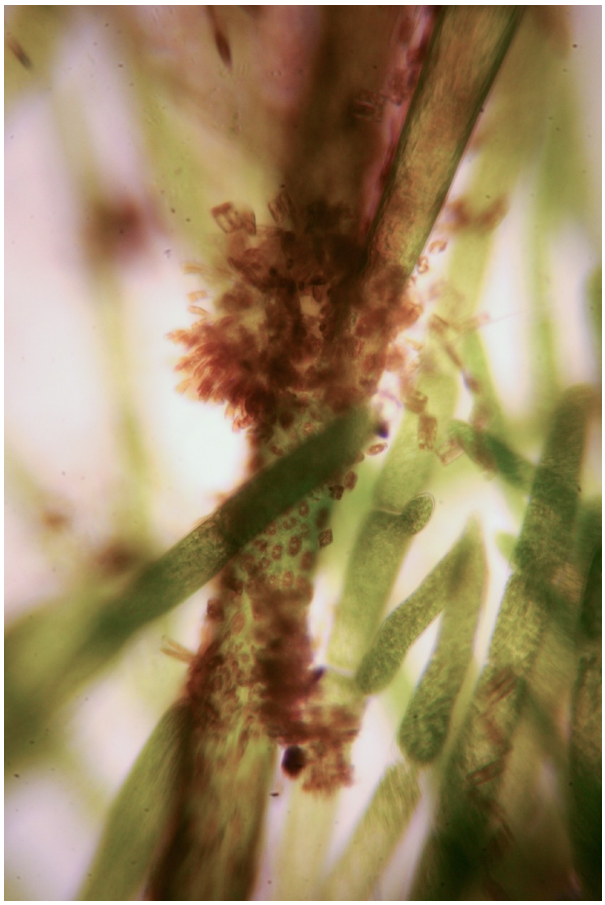
Text sources:

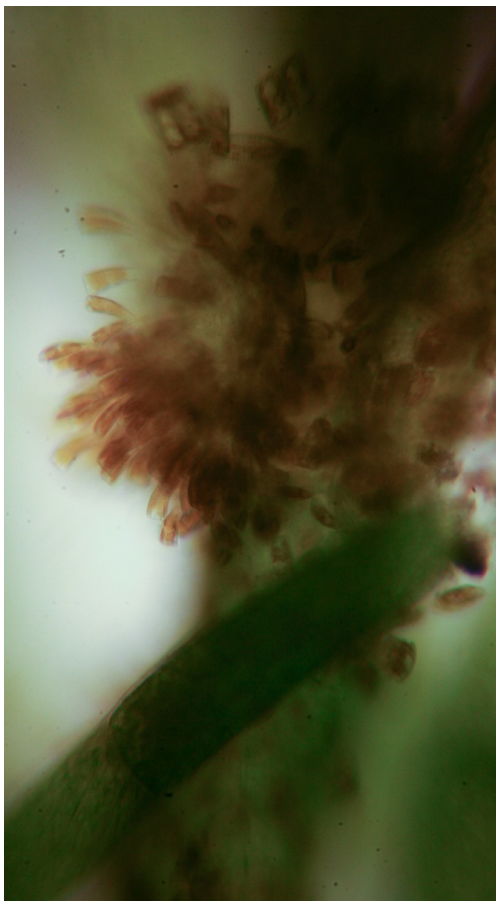
"The Algae", V.J.Chapman, Macmillan & Co LTD/ St Martin's Press, 1964
a wonderful text! Narratives as if you are attending Dr.Chapmans lectures!

"How to Know the Fresh-Water Algae", G.W.Prescott,WM.C.Brown Co.,1954
So useful over the years, thank you, Dr.Prescott!

"Diatoms of North America", William C. Vinyard, Mad River Press, 1979

This was very helpful on an area I know little about, yet constantly encounter. The section on pages 77-79 titled "Derivations of Generic Names" is brilliant. For example, it states that the genus name *Cocconeis* originates from Greek, with "cocco" being a berry and "neis" beig feeble. *Gomphonema* comes from the Greek "gompho" (wedge-shaped) and "nema" (a thread). *Diatomella* is made of the words *Diatoma* and the Latin dimuitive "ella". The "di" in *Diatoma* comes from the Greek word for "through", while "tom" means to cut. So "diatoms", are Greek rooted as: "cut through"?!! ■

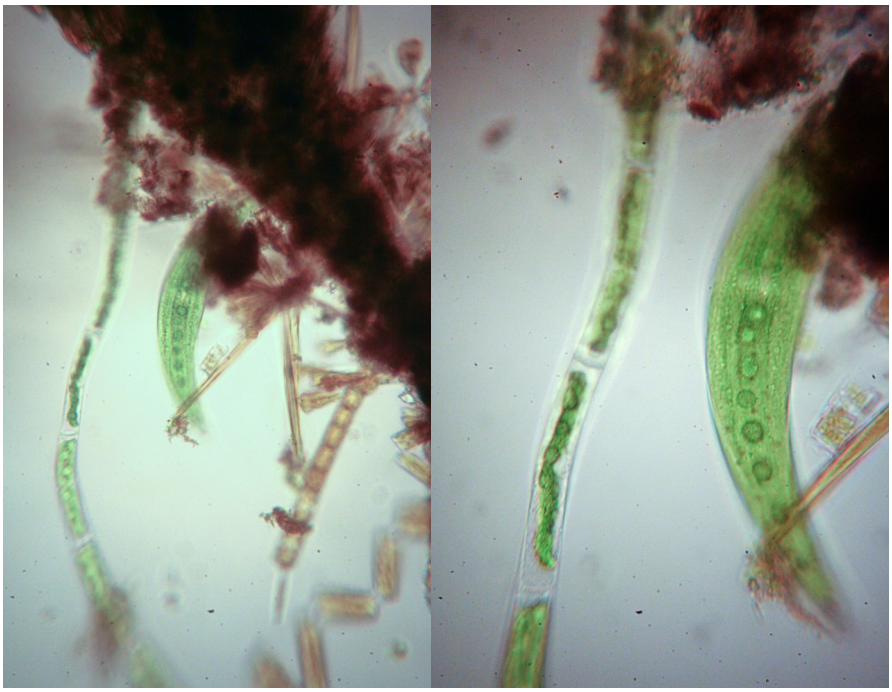




Figures 7, 8 (top): Diatom cities!

Figure 9 (far left): There are different dominant species in different areas of the algal thallus substrate. This becomes evident in low-power observations.

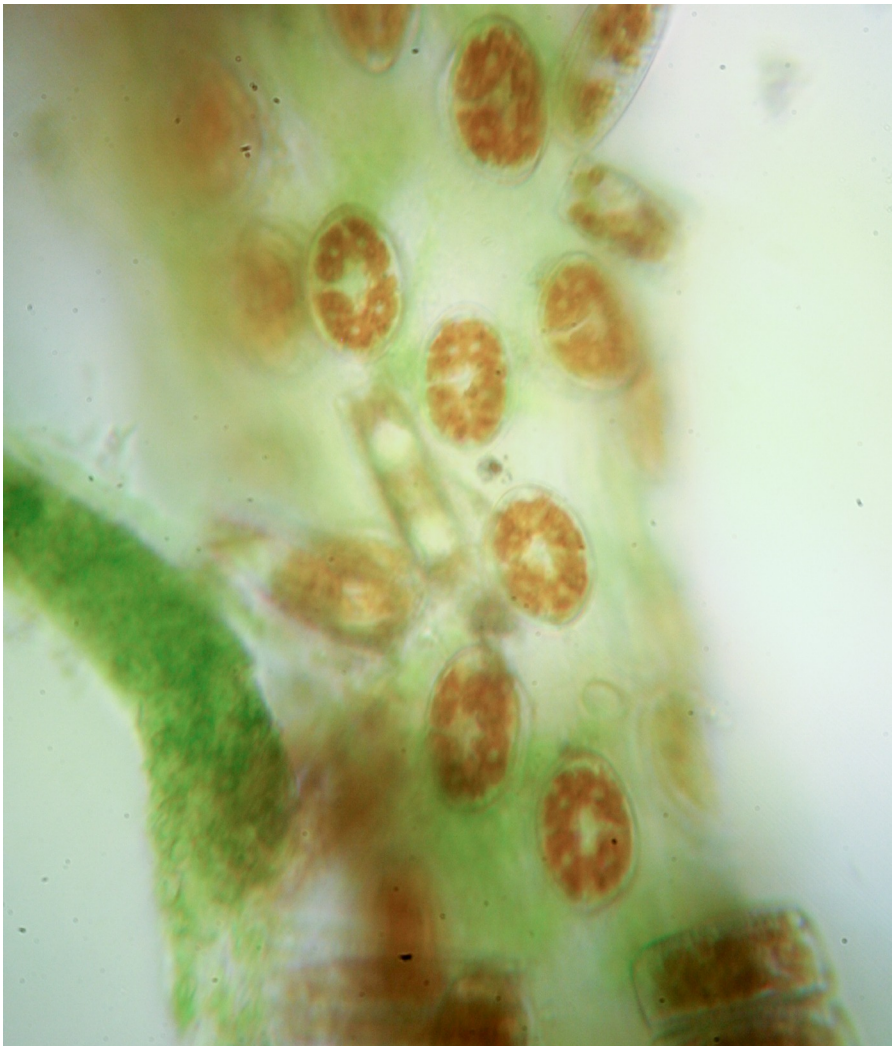
Figure 10 (left): *Gomphonema* species in a bloom of epiphyte assemblages.

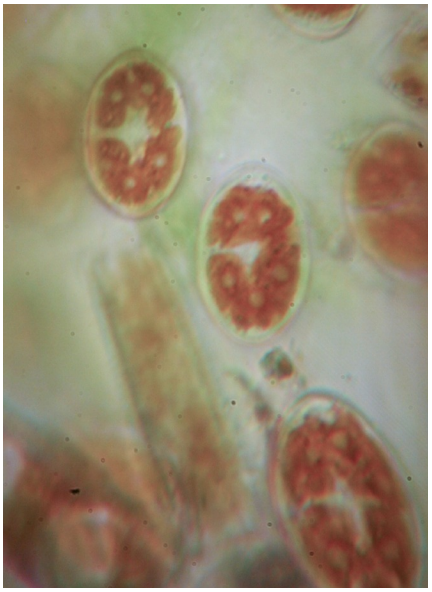


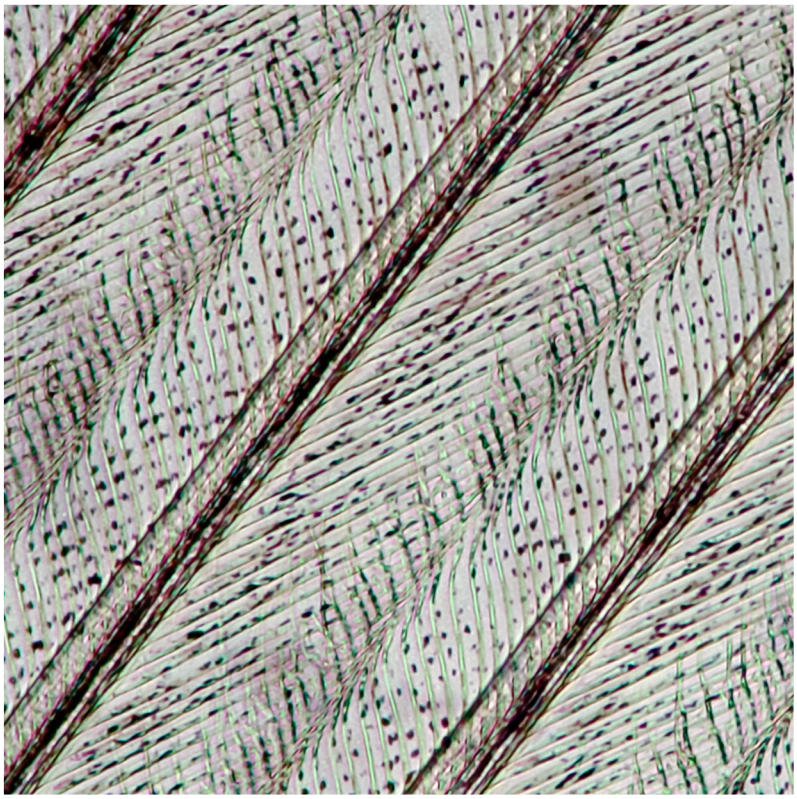
Figures 11 and 12 (left) a Diatom City with a desmid (*Cosmarian*).

Figure 12 (bottom left, right): Cocconeis species area of epiphyte dominance on this algae substrate

Figure 13 (bottom right): Just Like this Chironomid midge larvae, I have traversed a few Diatom Cities. I am now with many questions, I deeply enjoyed this field microscopy hike. Thank you all, whom also enjoyed this field microscopy hike!







What's this? Answer on page 3.