Fun with micro-fungi

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Introduction

If asked to draw fungi, most people would draw toadstools, quite possibly Fly Agarics with their well-known red caps and white spots. The typical stem and cap shape of toadstools is the most prevalent image in the minds of most British citizens. But myriad shapes of fungi exist. The 'macro'-fungi that we see in the countryside, including toadstools, and also the bracket fungi found on trees, are the province of most field mycologists. Around the UK, groups of enthusiasts regularly 'foray' in woodlands, meadows and other habitats, recording the distribution of these larger fungi. In other parts of the world 'foraying' for fungi means finding food to eat. But the British have never been particularly cosmopolitan in their taste for fungi, preferring to eat only an extremely limited range of species and only confident to buy these from green-grocers or supermarkets.

The smaller or 'micro'-fungi tend to be the study subject of professionals in the realms of plant and animal diseases, food production, and the production of chemicals such as enzymes for detergents. But there is a huge amount of fun to be gained by interested amateurs with microscopes who wish to explore the world of micro-fungi. Of course, it takes more than a dissecting microscope and a compound microscope to really enjoy looking at micro-fungi. There are tools and skills which make the task easier, and good, accessible and readable literature is a huge boon.

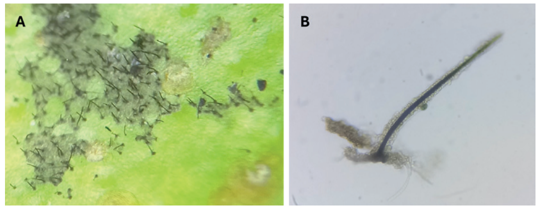


Fig. 1. *Dennisiella babingtonii.* A: on the surface of a *Rhododendron* leaf. B: a single seta showing the transparent cells surrounding a ventral dark core.

Micro-fungi come in a huge variety of shapes and forms. Some can be at the limit of what can be seen by the naked eye while others don't even exhibit their existence until under a compound microscope. The evergreen leaf in Fig. 1A shows a close-up of a black crust on a *Rhododendron* leaf: the kind of 'muck' that would probably be wiped off by fastidious gardeners. This is *Dennisiella babingtonii.* The small hair-like structures are known as 'setae'. They have an amazing and distinctive look when mounted in water and examined at high magnification. In Fig. 1B you can see the central dark core and the transparent cells that surround the core.

Three groups of micro-fungi can be particularly enjoyable for microscopists: plant pathogens, lichenicolous fungi and aquatic hyphomycetes. The first two groups now have some very approachable modern literature, while the aquatic hyphomycetes can be more of a struggle but are so intriguing that you'll want to take a look.

Plant pathogens

Pathogens are organisms that gain their nutrition from living hosts – often harming

Fig. 2. Plant pathogenic fungi. A: *Alternaria thunbergiae* causing leaf spots on *Thunbergia alata* (Black-eyed Susan). B: *Erysiphe berberidis var berberidis* causing powdery mildew on *Mahonia aquilifolium*. C: *Puccinia umbilici* causing rust on *Umbilicus rupestris* (Navelwort).

them, or even killing them, in the process. Gardeners will be familiar with powdery mildews, downy mildews (actually oomycetes rather than fungi!) and rusts. Fungi can also form 'leaf spots', dead areas of a leaf where the fungus often continues to develop and may form tiny, black spore-producing bodies; and galls, in which the fungus has disrupted the cell replication process to encourage extra growth in which the fungus can develop (Fig. 2). For most people these are not welcome forms of biodiversity in the garden but for someone with microscopes they are a source of beauty and study material.

Fig. 3. The front cover of Termorshuizen & Swertz (2011) *Roesten van Nederland*.

Rusts

What makes some groups of plant pathogens extremely easy to study (and record if you're interested in biological recording) is good, modern literature. The rust fungi have been written up by Termorshuizen & Swertz [1] in the volume illustrated in Fig. 3. This really useful book has keys arranged by plant host (so if the rust being studied is on red campion, *Silene dioica*, then the key will separate the two rust fungi that occur on this plant). Each rust is described with text in both Dutch and English and has colour photographs.

Rusts can have up to five different spore types and often spread their life cycle over two totally unrelated host plants (Fig. 4). But many have reduced the complexity of their life cycles by losing some of the spore types, often in conjunction with becoming confined to one host species. So that's five different spore types to look for and five different spore-producing structures!

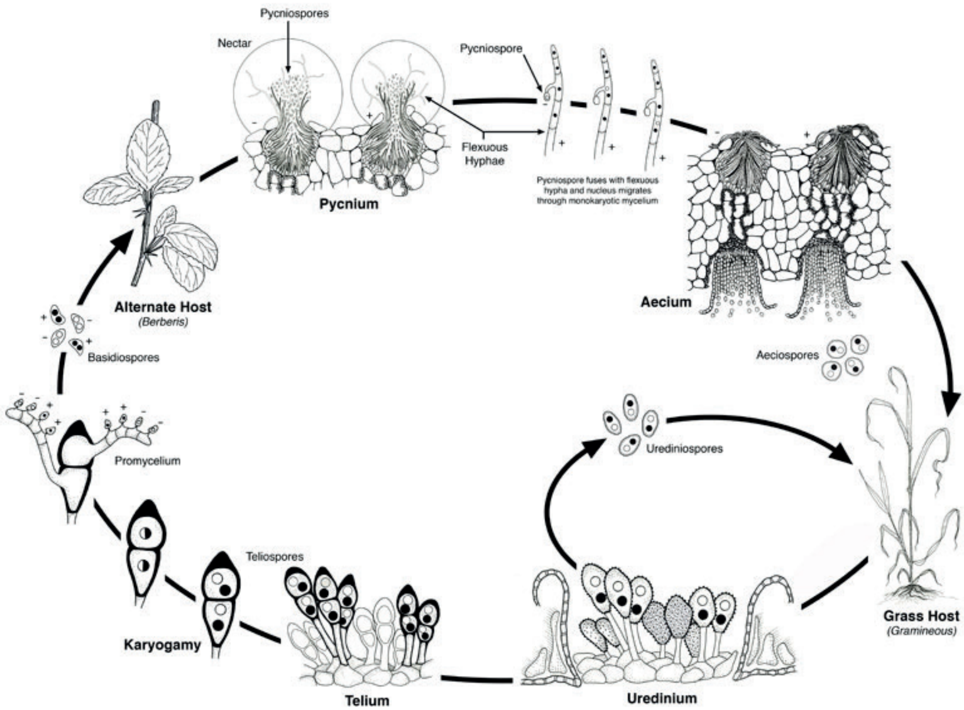


Fig. 4. Life cycle of *Puccinia graminis*. © Agricultural Research Service, US Department of Agriculture.

For the UK, a Welsh group of plant pathogen enthusiasts published a *Rust Fungus Red Data List and Census Catalogue for Wales* [2]. This describes the most threatened rust fungi in detail with excellent colour photos but also has a list of all rust fungi known in Wales, a list that is very similar to the list for the whole of the UK. Later publications from the group cover smuts (which produce huge numbers of spores from various parts of infected plants), powdery mildews, downy mildews and white moulds (Fig. 5).

Mildews and moulds

The powdery mildews, downy mildews and white moulds, can be collectively described as 'white, fluffy stuff' (Fig. 6). At this stage they are all in anamorph form producing simple spores by 'budding' at the tip of specialised hyphae known as 'conidiophores'. All produce their conidiophores on the surface of the plant that they are infecting. As a general rule, powdery mildews produce their spores on the top surfaces of leaves while the downy mildews and white moulds produce theirs underneath. But there are always exceptions to man-made rules. They're not always white, either. They can be pale shades of cream, grey and even lilac. However, it's very easy to check which of the three groups is present on a leaf or stem and to use the plant host to quickly make an identification with the help of those Welsh guides. It's also a very cheap group of organisms to explore. The Welsh guides are all available as free pdfs and, if you already have a hand lens or dissecting microscope and a compound microscope, the rest of the equipment needed is inexpensive.

Fig. 5. Guides to Welsh plant pathogenic fungi. A: Woods et al. (2015) Rust Fungus Red Data List and Census Catalogue for Wales. B: Woods et al. (2018) Smut and Allied Fungi of Wales; A Guide, Red Data List and Census Catalogue. C: Woods et al. (2024) The Powdery Mildews (*Erysiphales*) of Britain & Ireland, an Identification Guide and Census Catalogue for Wales. D: Chater et al. (2020) Downy Mildews (*Peronosporaeae*) and White Blister-rusts (*Albuginaceae*) of Wales. E: Chater et al. (2021) White Moulds, *Ramularia* and *Phacellium* Anamorphs, in Wales and Britain: A Guide and Welsh Census Catalogue.

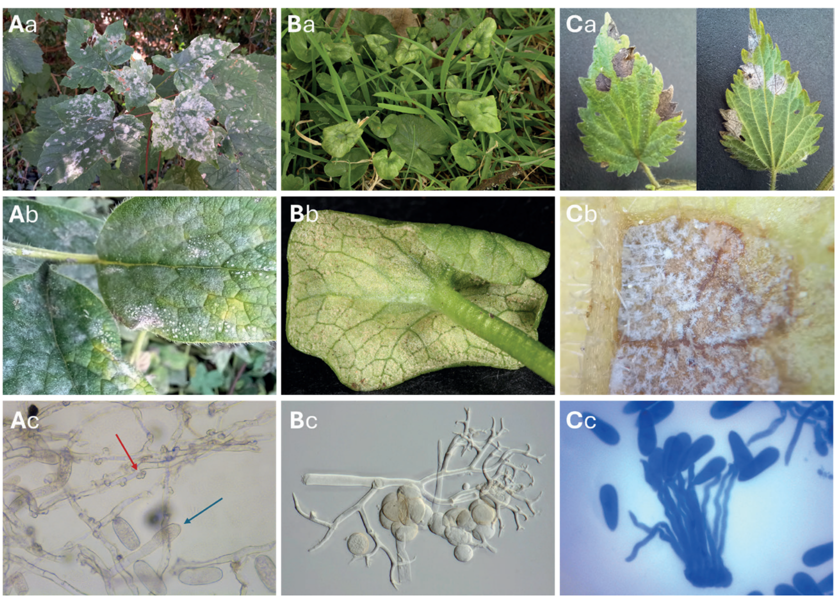


Fig. 6. 'White fluffy stuff' at varying magnifications. A: Powdery mildews. a: *Phyllactinia marissalii* on Sycamore. b *Golovinomyces asperifoliorum* on Comfrey. c. *Erysiphe galegae* on Goat's Rue. (red arrow = appressoria, blue arrow = single conidium on a conidiophore). B: a-c Downy mildew, *Peronospora ficariae* on Lesser Celandine at increasing magnifications © Malcolm Storey, www.bioimages.org.uk. C: White moulds. a. *Ramularia urticae* on top and bottom of Common Nettle leaf. b. *Ramularia digitalis* on back of Foxglove leaf. c. *Ramularia rubella* on Broad-leaved Dock, stained with Analine blue.

The first step is to examine an unhealthy-looking leaf for sporulating material. If there isn't enough fungal material visible to be seen with the naked eye, a hand-lens or a dissecting microscope may reveal some. If not, placing the plant material into a moist atmosphere will usually produce some sporulation. An 'incubation chamber' is easily made with some wet tissue inside an air-tight, plastic box. Using boxes upside down and placing the wet tissue with the plant material on top of it *on the inside of the lid of the box* makes it easy to lift the bottom of the box away and slide the lid and specimen under a dissecting microscope for later examination. Chinese take-away type boxes are ideal. Their semi-transparent plastic allows light through which may keep leaves photosynthesising for a short while. An example of the lid of an incubation chamber in position under a stereo microscope is shown in Fig. 7.

Once some 'white fluffy stuff' – the conidiophores and the spores developing on them – are present, a 'Sellotape strip' slide can be made to look at the fungus. It is possible to scrape up some of the fungus with the tip of a scalpel blade or a mounted needle, but this often pushes lots of material into a tight clump that is difficult to interpret when mounted on a slide. Using Sellotape is more likely to keep the material spread out and arranged in a more similar way to its arrangement on the plant parts. A 'Sellotape strip' slide begins as a normal microscope slide with a small drop of water on it. A piece of Sellotape about the same size as a cover slip is then cut and held in one corner to

Fig. 7. Arrangement for viewing specimens on the lid of an incubation chamber.

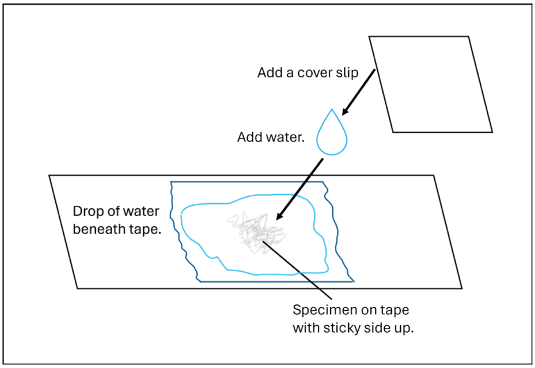


Fig. 8. Instructions for making a 'Sellotape strip' slide.

prevent fingerprints appearing on the glue. This piece of tape is then pressed onto the fungal material and peeled away, bringing the fungal material with it. The tape should then be placed *sticky-side-up* onto the drop of water on the slide. It likes to stay attached to the finger holding it of course, but it can usually be persuaded to be held onto the slide by the surface tension of the water droplet. A drop of water is then added to the fungal material and a cover slip is carefully dropped on top (Fig. 8).

Powdery mildews

Powdery mildews have feeding hyphae that run across the surface of the plant material (Fig. 6 Ac). These may have appressoria – swellings that push into the plant cells to absorb nutrition. The conidiophores grow at right-angles from the feeding hyphae and form spores at their tips, either singularly or in chains. The spores tend to be broadly elliptic or barrel-shaped. They are relatively large and easy to see, despite being colourless and having thin walls. Sometimes they have tiny, totally straight, objects in them that catch the light and glint as the focus is taken slightly in and out. These are fibrosin bodies and are good identification features as they only occur in some genera and are never found in others.

Downy mildews

Downy mildews feed inside the plant and only their conidiophores occur on the surface (Fig. 6 Bb, Bc). These are dichotomously branched structures that split into two and then into two again and sometimes again. The resulting structure looks similar to a deer's antler. One single spore-like body is produced at the tip of each branch. In fact, these spore-like bodies 'germinate' later to release a large swarm of swimming zoospores but this is rarely seen on a slide. The branching structure of the conidiophores is enough to identify material as belonging to a downy mildew.

White moulds

White moulds similarly feed inside the plant and only their conidiophores occur on the surface. Unlike downy mildews they are unable to push their conidiophores through the surface of living plant material, so they appear in clusters, 'caespituli', from stomata (Fig. 6 C). The conidiophores are usually unbranched but often produce their spores either singly or in chains from various points on the conidiophore. Wherever a spore is produced a dark scar remains on both the conidiophore and on the spore. Spores produced in chains therefore have a dark scar on both ends. The colourless spores vary in shape between species, from nearly spherical to cylindrical and can have transverse septa. Even within a single species, and on one specimen, spores can be reasonably variable, especially those that

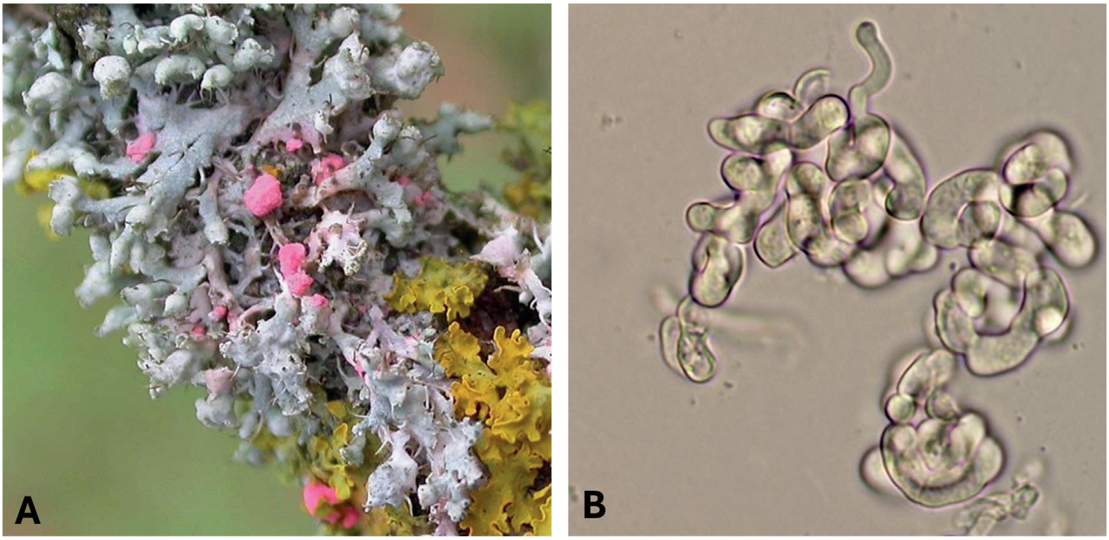


Fig. 9. *Illosporiopsis christiansenii* on the lichen *Physcia adscendens*. A: Pink spore masses. © Mark Powell. B: Coiled spores. © Lukas Large.

are cylindrical. They might show different lengths (but less variation in width) and different numbers of septa.

Identification to species level

Once the type of fungus is identified, the appropriate Welsh guide, lists the species present in the UK on each host and often helps with the separation of species when more than one occurs on the same host. Literature for other groups of plant pathogens is rather limited and most is out-of-date. So, the fungal groups covered by the Welsh series of guides are definitely the places to start.

Lichenicolous fungi

Lichens are composite organisms where a fungus has evolved to feed on the photosynthetic products of an internal partner. Usually, these partners are algae, but they can be cyanobacteria. Other fungi have evolved to feed on the lichen partnership. These are called lichenicolous fungi. Just as with plant pathogens, many lichenicolous fungi are host specific, so knowing the lichen can help you identify the infecting fungus.

The majority of lichenised fungi are ascomycetes – fungi which produce their sexual spores in sacs known as asci. And many of the fungi that feed on lichens, the lichenicolous fungi, are also ascomycetes. This means that both the host lichen and the lichenicolous fungi are often capable of producing the same kinds of fungal structures: pycnidia, apothecia and perithecia. It can therefore be difficult for someone without a good knowledge of what a lichen should look like to be able to spot some of the lichenicolous fungi that infect them. In fact, in the past, mistakes have been made when describing lichens because structures of invading fungi have been descried as belonging to the lichen! But, for those who don't know lichens well, there are some brightly coloured lichenicolous fungi to look out for and one common lichen host

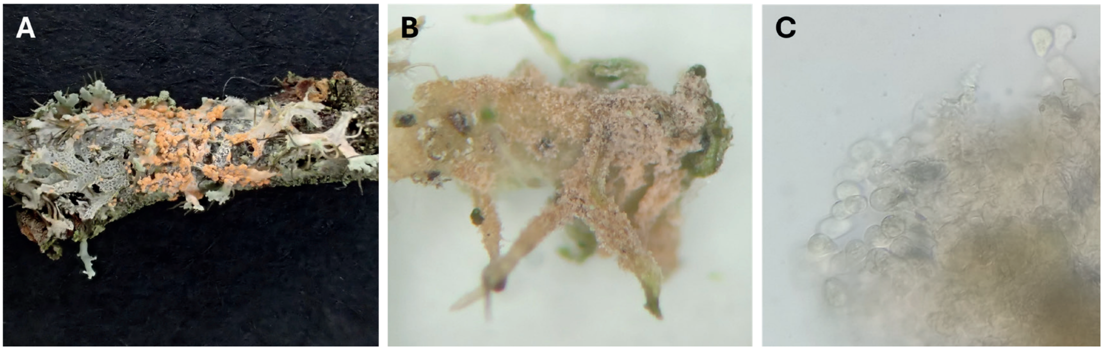


Fig. 10. *Erythricium aurantiacum* on the lichen *Physcia tenella*. A: Bulbils on the decomposing lichen thallus. B: A fertile surface covered with basidia found underneath the decomposing lichen thallus. C: Basidia and basidiospores.

that can easily be explored.

Illsoporiiopsis christiansenii (Fig. 9) produces bright pink blobs of asexual spores that resemble the colour of 1960's 'shocking pink' lipstick, although the blobs become a deeper rose pink as they age. The fungus is most common on two small, bushy, grey lichens belonging to the genus *Physcia*. These two lichens enjoy extra nitrogen in their environment so have become very common near well-used roads, chicken sheds, other sites of animal husbandry, and fields that are regularly spread with liquid manure. They are often found with a bright yellow lichen known as *Xanthoria parietina*. It is possible for *I. christiansenii* to spread onto *X. parietina*. Checking that you have found *I. christiansenii* is easy. A small pink spore mass can be transferred to a microscope slide and mounted in water. At high magnification, a tangle of coiled, colourless spores will be seen. There are no similar spores produced by any other lichenicolous fungus in the UK.

On the same two grey lichens, a salmon-coloured lichenicolous fungus can also be found. In fact, in recent years this has become more common than *I. christiansenii*. *Erythricium aurantiacum* (Fig. 10) is a basidiomycete. That is, it forms its sexual spores on the outside of enlarged cells on the surface of the fungus. Each spore develops on the end of a tiny prong called a 'sterigma'. *E. aurantiacum* most frequently reproduces by vegetative means through the production of 'bulbils' and this is what is usually noticed on a lichen – tiny, salmon-coloured balls of fungal material which can be near-spherical, ellipsoid or irregular in shape. The bulbils often cluster together so that they are noticeable with the naked eye. The lichen surface beneath them is often stained a similar colour and this is where the basidia can be found. The best place to look for basidia is underneath the stained lichen where the air stays humid for long periods. A thin section taken into the lichen at this point may show basidia with their stigmata and spores. If this lichenicolous fungus is growing in a very sunlit place, it can be a bright, almost fluorescent, orange which suggests that the orange pigment can act as a sunscreen to protect the fungal cells from damage.

Marchandiomyces corallinus (Fig. 11) is also pink. This is found on a large number of

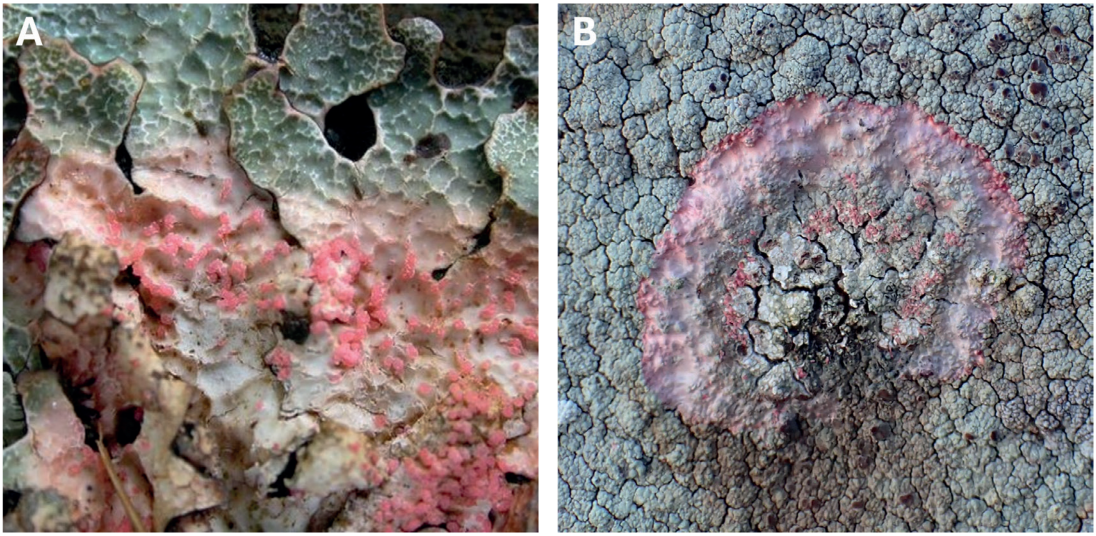


Fig. 11. *Marchandiomyces corallinus*. A: Bulbils on the foliose lichen *Parmelia sulcata*. © Mark Powell. B: On the crustose lichen *Ophioparma ventosa*. © Chris Cant.

different lichens. It's another basidiomycete but it has never been seen with basidia. It appears to reproduce only through the distribution of bulbils: bright rose pink, usually elongated, bulbils, that can look as if they've been squeezed out like toothpaste. Description sheets for these lichenicolous fungi and for some others can be found on the British Lichen Society (BLS) website [3]. The BLS also provide a guide to the lichenicolous fungi that can be found on the bright yellow *Xanthoria parietina* (Fig. 12) a leafy lichen which is extremely common in nitrogen-rich environments.

There are records to date of twenty-nine lichenicolous fungi growing on *X. parietina* in the UK. Many of these are described in the BLS guide which can be downloaded from their web site [3]. The guide has a key to the species and is a good introduction to this group of fungi.

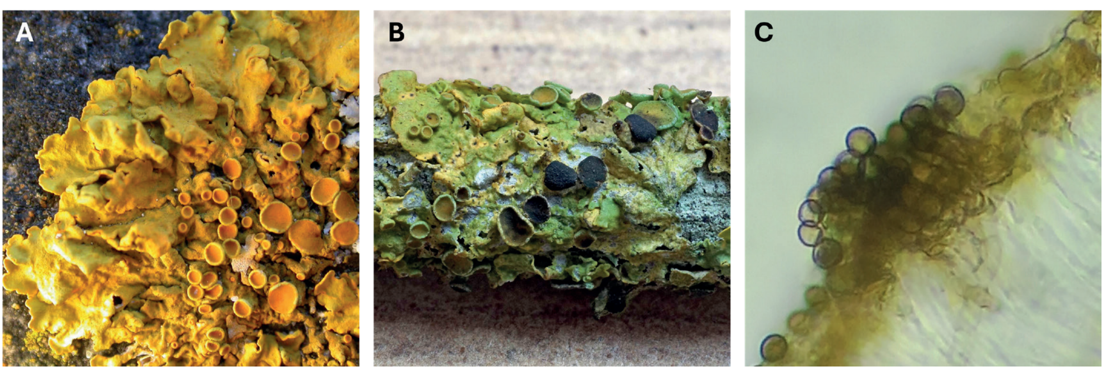
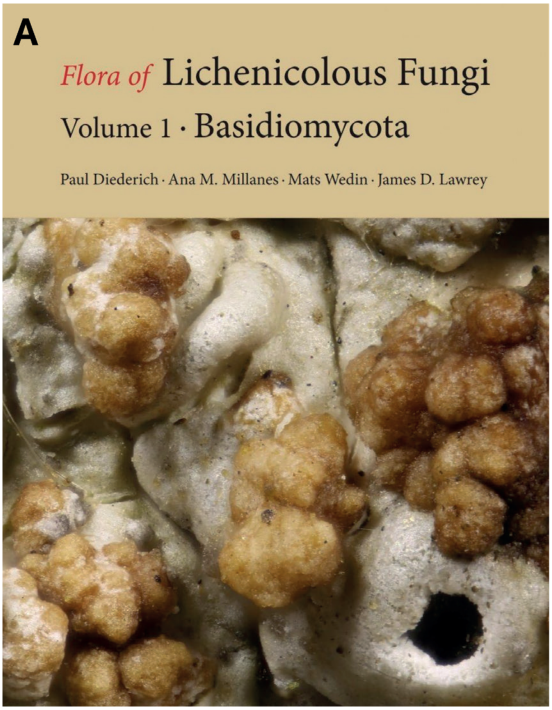


Fig. 12. *Xanthoria parietina* and one of its lichenicolous fungi. A: Healthy *Xanthoria parietina* © Mark Powell. B: Black fruiting bodies (apothecia) infected by Xanthoria smut, *Xanthoriicola physciae* © Mark Powell. C: Spores of *Xanthoriicola physciae* on the yellow surface of a *Xanthoria* fruiting body.



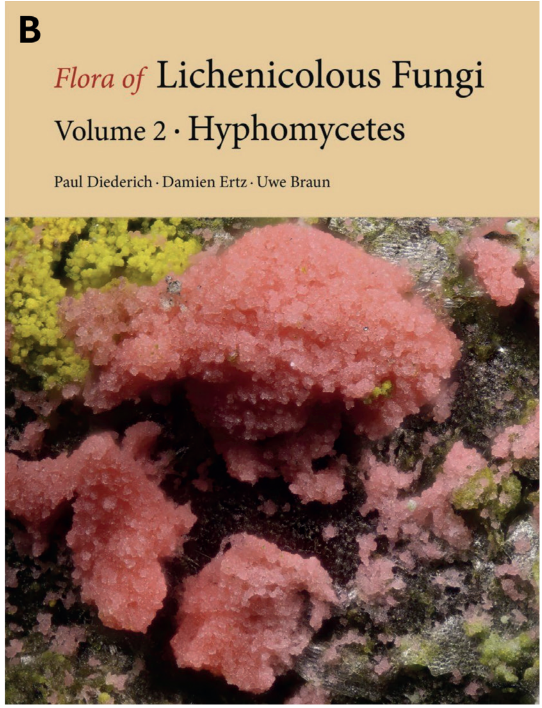


Fig. 13. Front covers of Volumes 1 and 2 of the *Flora of Lichenicolous Fungi*. A: Basidiomycetes. B: Hyphomycetes.

Lichenicolous fungi can also produce galls – unusual extra growths on their host lichen. Many of the fungi that do this are basidiomycetes. Or lichenicolous fungi can be 'hyphomycetes', producing visible mycelium and/or conidiophores and spores, over the surface of lichens. These two groups, basidiomycete lichenicolous fungi and hyphomycete lichenicolous fungi, have been recently written up in the first two volumes of an ambitious *Flora of Lichenicolous Fungi* written by an international group of mycologists led by Paul Diederich, covering lichenicolous fungi around the entire globe [4,5]. The volumes are available in hardback or as free pdf downloads from the Musee Nationale d'Histoire Naturelle in Luxembourg [6]. Fig. 13 shows the front covers of the two Floras.

Aquatic hyphomycetes

If you get a thrill out of admiring amazing shapes under the microscope, the spores of aquatic hyphomycetes are well worth searching for. These are released into flowing water by tiny fungi growing on dead plant material that has fallen into rivers and streams, even tiny dykes that have some flow. Without these fungi our waterways would be choked with dead vegetation. As with all other hyphomycetes, these fungi are growing in an asexual form and produce their spores on the tips, and sometimes on the sides of, special hyphae called conidiophores that stick out of the decaying plant material. When fully developed the spores are released into the water.

Most of this ecological group of fungi prefer dead leaves rather than more woody material. Their released spores need to travel

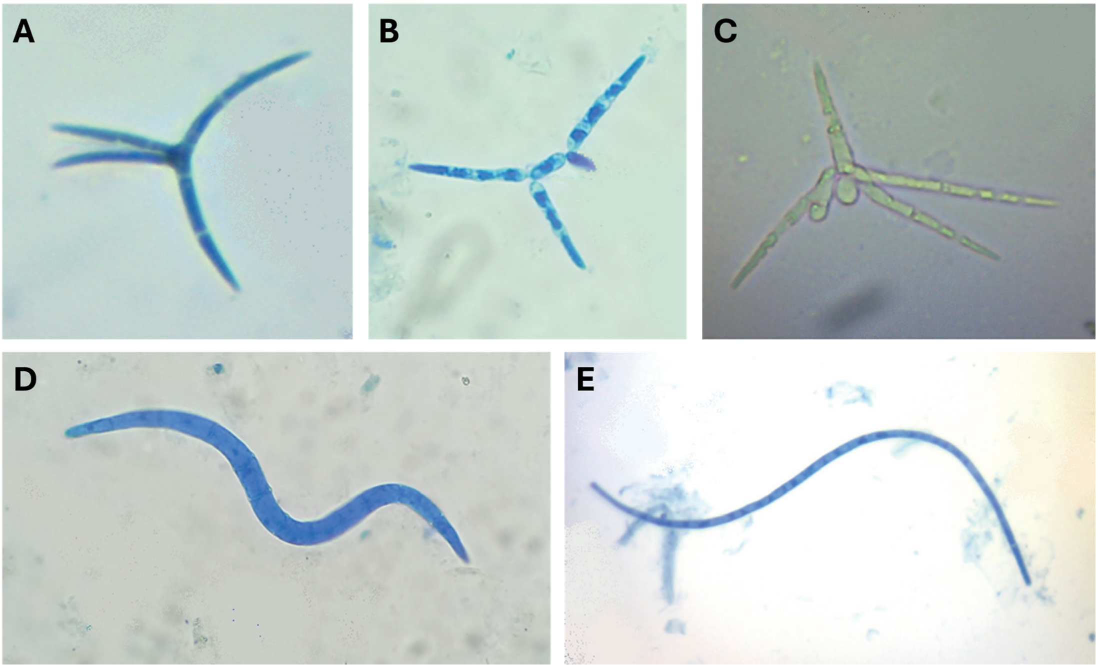


Fig. 14. Aquatic hyphomycete spores. A, B, D and E are dyed with aniline blue. A-C show tetraradiate spores with four 'arms'. A: *Alatospora acuminata*, one of the UK's smallest and most common aquatic hyphomycetes. B: *Articulospora tetracladia*. C: *Tetracladium marchalianum*. D-E show sinuous spores which curve in two dimensions. D: *Anguillospora crassa*. E: *Aquillospora rosea*.

in the water until they encounter another suitable leaf to grow into and feed on. How do these spores stay afloat? How do they ensure that they travel well? How do they fasten onto a leaf rather than being washed past? The answers seem to be in their design, particularly in their shape. Each fungal species has come up with a slight variation on some simple 'rules of thumb', so often only an examination of a spore's shape is needed to provide an identification of the species. Two basic designs are most prevalent: four-armed spores; and sinuous spores i.e. spores that are long and narrow and twist in two-dimensions (Fig.14).

Aquatic hyphomycetes were first studied by C. Terence Ingold in the 1940s. Because of this, the group is often known as Ingoldian fungi. It is still possible to buy Ingold's *Guide to Aquatic Hyphomycetes* (1975) [7] from the Freshwater Biological Association website. Although many more species have been described since this was written, it's still a good starting point and cheap at only £10. Luckily for passing microscopists, aquatic hyphomycete spores tend to get trapped in the menisci of bubbles in river foam, so this is the easiest way to collect them: find some river foam and scoop it up into a jar (Fig. 15). Scooping the foam up into some type of sieve can be useful as any unwanted water runs away.

Aquatic hyphomycete spores germinate as soon as they touch a solid surface. This is one of the design mechanisms that helps them to colonise fresh plant

Fig. 15. The author collecting river foam.

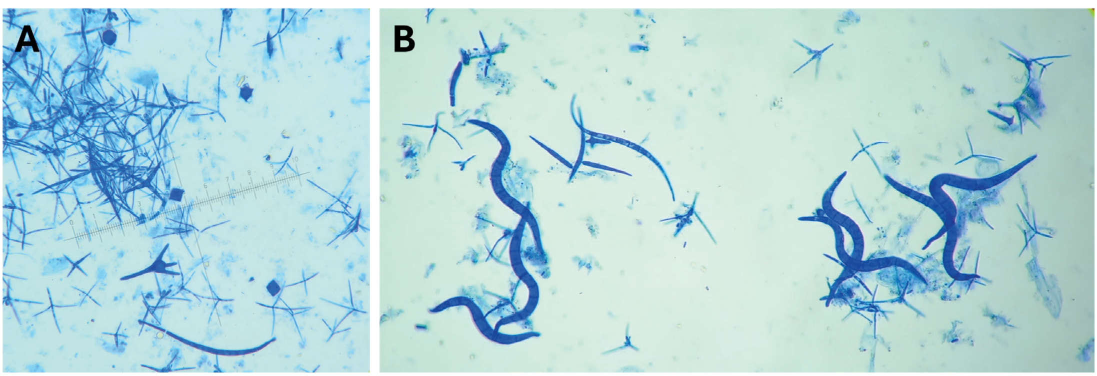


Fig. 16. Two river foam samples, both dyed with aniline blue, photographed under a x20 objective. A: River Aeron in Aberaeron below a weir, November 2024. B: Small stream in woodland in Parc Natur Penglais in Aberystwyth, November 2024.

material – as soon as they touch something, germination starts. Their shapes mean that often more than one part of a spore comes into contact with a surface. Spores will germinate simultaneously from each touching point, increasing the likelihood that the spore will stay attached and the fungus can grow into the plant material. Unfortunately for both the fungi and for collectors, the spores don't appear to be able to distinguish between different types of surfaces so spores will be wasted germinating on useless surfaces such as river stones and the inside of glass jars or plastic containers! For this reason, spores are usually killed as soon as they are collected. The usual chemical to use for this is formal acetic alcohol. Dyes often help to visualise the spores and to break down the foam. Depending on the solvent in the dye, these may also kill the spores and prevent germination. However, if collections are looked at quickly, spores will still be recognisable even if the spores have not been killed. A tiny drop of fluid resulting from the breakdown of the foam, can be placed on a microscope slide and covered with a cover slip. Since the spores are so tiny, it is important to use the minimum drop size that will allow full coverage of the area under the cover slip. If available a x10 or x20 objective lens can be used to scan methodically back and forth over the slide to search for spores (Fig. 16). As well as Ingold's book, a useful key to aquatic hyphomycetes is available via the ASCOfrance website [8]. This key was developed for use in a British Mycological Society workshop held in Sheffield in 1989.

The sinuous spores are more difficult to identify from shape alone as there are fewer character differences between them and the size ranges often overlap. Because of this the conidiophores and the way that the spores develop can be important but these characters can only be seen if the fungus is growing on a leaf or in culture such as in a petri dish. Even if many of the long, sinuous spores can't be named, there is still a lot of fun to be had with spores that have a more distinctive shape.

In 2015 Chris Yeates sampled some foam from a stream in Yorkshire. As well as many other species, he found spores of *Collembolispora barbata* (Fig. 17). This fungus has very distinctive spores that can't be easily confused with anything else. Chris's sample was only the second collection of this fungus in the world. It had been discovered in a stream in Portugal in 2001 and was formally described in 2003. Since then, the species has been seen twice more in the UK, once in Bristol in 2021 and once in a different Yorkshire location in 2024. You can read about Chris's Yorkshire foam sample on the ascomycete.org web site [9].

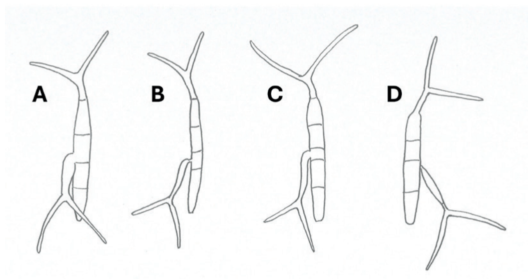


Fig. 17. *Collembolispora barbata* spores. A: North-west Portugal. Described in 2003. B: Mid-West Yorkshire (vc64), Harrogate, 2024. C: South-west Yorkshire (vc 63), Near Slaithwaite, 2018. D: North Somerset (vc 6), Bristol, 2021.

In conclusion

Why not have a look for some of these three groups of fungi and enjoy another part of the rich diversity of the microscopic world?

References

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3. Description sheets for a range of lichenicolous fungi can be found on the British Lichen Society website at britishlichensociety.org.uk/identification/lichenicolous-fungi/species

4. Diederich P., Millanes, A.M., Wedin M., and Lawrey, J.D. 2022 *Flora of Lichenicolous Fungi, Vol. 1.* Musée National d'Histoire Naturelle, Luxembourg 351pp.

5. Diederich, P., Ertz, D. and Braun, U. 2024 *Flora of Lichenicolous Fungi, Vol. 2* Musée National d'Histoire Naturelle, Luxembourg 544 pp

6. Musée National d'Histoire Naturelle (National Museum of Natural History), Luxembourg. www.mnhn.lu/science/flora-of-lichenicolous-fungi/?lang=en

7. Ingold, C.T. 1975 *Guide to Aquatic Hyphomycetes FBA, Cumbria, England* 96pp. *ISBN: 900386223*

8. *ASCOfrance* is a French membership organisation for students of Ascomycetes. Its resources include images and downloadable keys, including Descals et al. (1989) Aquatic Hyphomycetes. www.ascofrance.com/uploadscument/1989DescalsAquaticHyphos-0001.pdf

9. *Acomycete.org* is web portal concerned with fungi belonging to the *Ascomycota.* It includes an electronic journal and digital library. The Chris Yeates article is available at: ascomycete.org/Journal/Article/art-0283

Note: Dr Newbery gave the M.C.Cook lecture on micro-fungi in July 2024.

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