△ Figure 1. *Frullania dilatata.* *All photographs George Greiff*

Studying bryophilous fungi on *Frullania dilatata*

George Greiff describes the commoner fungal parasites of this familiar liverwort

In a previous article (Greiff, 2019), I illustrated the diversity of bryophilous fungi, briefly discussed their biology, and argued that bryologists are best suited to study these organisms. In response, bryologists from across Britain and Ireland have kindly sent me specimens and together we have amassed a collection of bryophilous fungi, including over 200 vouchers of around 90 different taxa (which will be donated to Kew in the future). Several of these collections are nationally significant, and a small number represent species new to science.

The aim of this article is to outline how to begin studying bryophilous fungi, using as examples four common parasites of the common leafy liverwort *Frullania dilatata*. I will discuss how fungi may be spotted in the field, what constitutes a useful voucher specimen, and how species can be examined microscopically.

F. dilatata is host to more than ten species of parasitic ascomycete microfungi, half of which are yet to be found outside central Europe. The examination of *F. dilatata* from across the British Isles offers not only an introduction to common bryophilous fungi, but may also enable the discovery of new species both to these islands and to science.

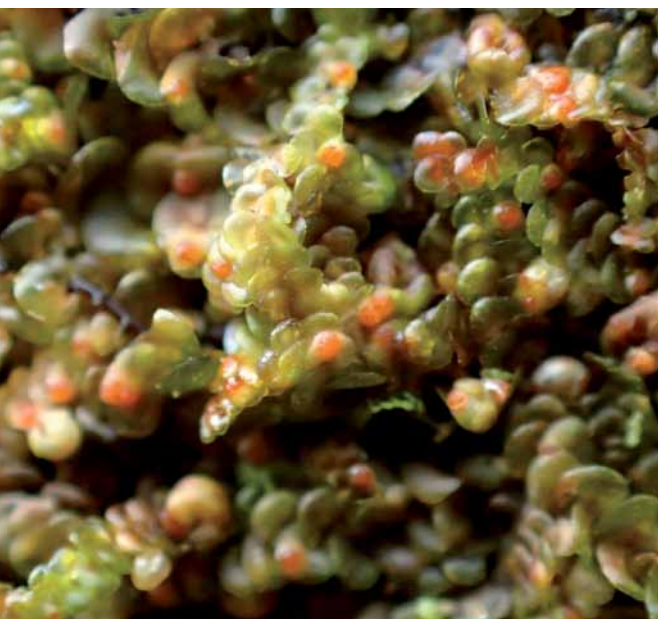
Spotting bryophilous fungi on *Frullania dilatata*

F. dilatata (Fig. 1) is a medium-sized, dorsiventrally flattened leafy liverwort that frequently grows as an epiphyte, closely appressed to the substrate. The colour of the plant can vary from green to reddish-black depending on its age and growth conditions, and the species is easily identified when the large, warty perianths (containing archegonia and, later, developing sporophytes) are present.

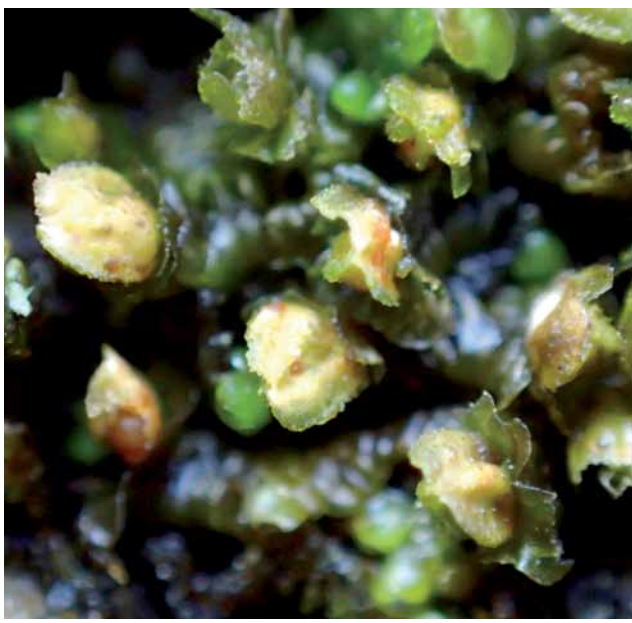
Bryocentria brongniartii (Fig. 2) is specific to *F. dilatata* and is fairly easy to spot and preliminarily identify in the field. It is named in honour of the 19th-century French botanist Adolphe-Theodore Brongniart. Although the fungus does not cause significant damage to the host, infections are visible as small, smooth, bright orange spots perforating through the leaf lobes of the plant. These spots frequently occur in large numbers and are easily seen with a ×15 hand lens (those including lights are best) and can just about be noticed as minute flashes of orange with the naked eye. Beware, however, the filamentous orange alga *Trentepohlia*, which frequently grows on bryophytes and superficially resembles an orange fungal parasite.

Periantria (formerly *Calonectria*) *frullaniae* (Fig. 3) is somewhat less conspicuous than *B. brongniartii*. It also forms brightly coloured, smooth, orange spots on infected hosts. The main difference between the two is their host 'microniche'. While *B. brongniartii* mainly infects the vegetative parts of the thallus, especially the leaves, *Periantria frullaniae* is specific to the perianths of the host. According to Döbbeler (2021), it infects perianths with fertilised archegonia and hijacks resources from the transfer

▽ Figure 2. Orange fruitbodies of *Bryocentria brongniartii* on the dorsal surface of host plants.



▽ Figure 3. *Periantria frullaniae* turning the perianths white. Orange fruitbodies are growing within the infected perianths.



cells, which would normally fuel sporophyte development by transporting nutrients from the gametophyte to the sporophyte. *Periantria frullaniae* is not always easy to detect using a hand lens, and I have found that in excess of ×20 magnification is sometimes required to appreciate the full extent of infected perianths in a sample. Nonetheless, dense populations of sexually reproducing *F. dilatata* frequently display infected perianths, which can be partially bleached white from the fungus. This bleaching, in some cases, betrays the presence of the fungus, even to the naked eye.

Pithyella chalaudii (formerly *P. frullaniae*; Fig. 4) was named after another Frenchman, botanist and bryologist Germain Chalaud (Priou, 2019). Unlike the two species mentioned thus far, *Pithyella chalaudii* forms cup-shaped fruitbodies, usually less than 0.5 mm in diameter, rather than roughly spherical ones. *Pithyella chalaudii* is also a potent necrotroph – the fungus kills host tissues and frequently creates conspicuous dead, greyish-brown rings within host patches. The flesh-coloured fruitbodies of the fungus blend in with the dead host plants, but they are often found around the borders between dead and healthy host tissue, visible under a hand lens in the field. When searching for *Pithyella chalaudii*, bryologists should be wary of two lichen imposters, *Normandina pulchella* and *Coenogonium luteum* (*Dimerella lutea*). Both of these have cup-shaped fruitbodies but they are larger than those of any bryophyte parasite considered here, frequently exceeding 1 mm diameter.

Bryonectria callicarpa (Fig. 5) is, in my experience, impossible to observe or identify in the field. Infected plants look healthy, though

▽ Figure 4. Cup-shaped fruitbodies of *Pithyella chalaudii* on dead host tissue.

▽ Figure 5. Yellowish fruitbodies of *Bryonectria callicarpa*, shown by arrows.

may occasionally be overgrown by algae – the only suggestion that they could be parasitised. *B. callicarpa* occupies a rather unusual microniche: it grows on the ventral (underside) surface of host plants, forming inconspicuous, yellowish, smooth spherical fruitbodies around the underleaves, lobules and rhizoids of infected plants. How the fungus disperses remains a mystery, although I have noted that large mats of *F. dilatata* tend to flake off trees, exposing the ventral surfaces of the plants and possibly allowing fungal spores to be shot through the air. Spotting *B. callicarpa* requires very careful examination of infected material under a dissecting microscope, and I have only ever found the fungus in areas where the host was growing profusely on ash and sycamore trees.

Collecting bryophilous fungi – how much or how little?

Most of the time, bryologists will only detect fungi on their samples once they are under the microscope. Frustratingly, these inadvertent collections are often interesting and exciting, but do not contain enough fungal material to constitute useful voucher specimens. For the tiny fungi considered above, a relatively small amount of host material can yield dozens of fungal fruiting bodies. For example, approximately 3 cm^2 of *F. dilatata* can contain sufficient *B. brongniartii* for a useful museum specimen, with enough orange fruitbodies for re-examination by future researchers, and also for DNA sequencing projects.

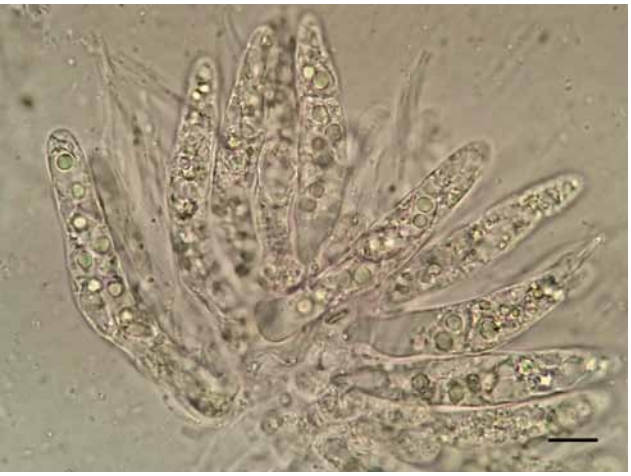
Other fungi, such as *P. chalaudii*, can come up in groups and many fruitbodies can be collected on small pieces of infected host material. In my opinion, 15 to 20 fungal fruitbodies should be the minimum amount collected for a useful voucher. If a fungus has been seen many times (e.g. *B. brongniartii*), fewer fruitbodies can be collected to check the identity of the fungus microscopically but not retained in a voucher. If a small amount of fungal material is inadvertently stumbled upon in a bryophyte sample, one can always return to the site to make a targeted collection from several host plant patches.

Overall, the amount of fungus collected should depend on the objectives of the collection. Early on, I would advise that more is better, especially since we have barely scratched the surface of bryophilous fungal biodiversity in Britain and Ireland and many collections could be valuable. That said, we should always follow sustainable bryophyte collection practices and not risk extirpating host plants. Excessive collecting is discouraged, and with experience *B. brongniartii*, *P. frullaniae* and *P. chalaudii* can be identified on sight in the field (though occasional microscopic examination of the fungal ascospores is recommended).

Examining bryophilous fungi microscopically

The microscopic examination of bryophilous fungi is one of the main challenges faced by bryologists, as fungal material is quite different to bryological material under the microscope. The first step to working on these fungi is to understand what we are looking at, and for, when using high power (×40 or more) compound microscopes.

The four species listed above, as well as most known bryophilous fungi, are ascomycetes (the cup-fungus phylum). This means that their sexual fruitbodies – the orange spots of *Bryocentria brongniartii* and *Periantria frullaniae*, for example – contain asci (Fig. 6). Asci are sac-like structures in which the spores of the fungus develop, and the asci of most ascomycetes contain eight ascospores (Fig. 7). These spores come in a huge variety of shapes, colours, sizes and surface textures, and the examination of



△ Figure 6. Asci of *Bryonectria callicarpa* at ×1000 magnification, containing maturing ascospores. The four mature spores per ascus found at maturity are not yet visible. Bar = 10µm.

△ Figure 7. Liberated spores of *Periantria frullaniae*, which are long and cylindrical with swollen ends. Bar = 10µm.

mature spores is often necessary to confidently identify a fungal sample. This is not so different from the examination of the spores of bryophytes such as *Fossombronia* and *Ephemerum*.

A key aspect to studying bryophilous fungi under the microscope is finding them on a sample. Gently wash away debris from a sample under a tap, or by soaking the material in a dish, before placing it on a piece of dry kitchen roll to remove excess water. The preparation can then be screened under a dissecting microscope. A pair of fine forceps and a dissection needle can be used to remove fungal fruitbodies from the host and transfer them to a small drop of water on a microscope slide. For small fungi like *B. brongniartii*, I would examine three fruitbodies in a single drop of water, but others are far smaller and more might be required to see mature spores. It is best not to include host tissues in initial dissections as these will interfere with the extraction of the fungal spores. However, sometimes the behaviour of the fungal mycelium inside host cells can be helpful for identification purposes.

Following the example of *Bryocentria brongniartii*, once the three fungal fruitbodies are on the slide, gently lower a cover slip over it, as one would over a preparation of moss leaves. Because the fungal fruitbodies are round, and the asci and spores are inside, the next step is to gently tap the cover slip (e.g. with the back of a pencil, glass rod or finger nail). This will rupture the delicate fungal fruitbodies and allow their contents to spread across the slide, revealing the asci and spores. I usually tap between 5 and 30 times depending on the size and fragility of the fungal sample, being careful not to crack the cover slip.

Next, examine the preparation under a compound microscope. Although mostly unnecessary for bryology, a ×100 oil immersion lens (×1000 total magnification) is essential for the serious study of bryophilous fungi. A standard ×40 objective lens (×400 total) can suffice for checking most species, however. High magnification allows distinctive spore

properties to be accurately noted and quantified. For example, the spores of *P. chalaudii* are tiny (3.0–3.5 µm in diameter) and possess warts on their surface that are impossible to make out at lower magnifications. The globular shape of the spores can easily be seen at ×400, in most cases confirming the identity of the fungus.

Stains such as iodine and lactophenol cotton blue are often used by mycologists to enhance the visualisation of certain microscopic fungal characteristics, but the use of these reagents is not strictly necessary for the identification of the fungi described here.

Concluding observations

Frullania dilatata is a hotspot for host-specific bryophilous fungi, and it is hoped that bryologists will try their hands at finding some of the fungi mentioned in this article. Döbbeler (2006) provides detailed descriptions of the species listed here, as well as others I have not discussed. For example, *Octosporella erythrosigma* is superficially similar to *Bryocentria brongniartii* in that its fruitbodies form orange spots on host tissues. However, *O. erythrosigma* seems to be rather rare in the British Isles and differs from *B. brongniartii* in that it has pale hairs on its fruitbodies rather than the smooth surface of the latter species.

Leafy liverworts as a group appear to be excellent hosts for bryophilous fungi. For example, *Plagiochila asplenioides* is host to at least 13 fungi (Marsh *et al.*, 2010). Several species have been reported on *Radula complanata* in mainland Europe (Döbbeler, 1978), but intriguingly host mats I have examined in England, Scotland and Wales have thus far been devoid of fungi. Conversely, the temperate rainforests of western Britain and Ireland appear to house several bryophilous fungi that are currently unknown from mainland Europe.

Overall, studying bryophilous fungi allows us to look at common bryophytes with new eyes and to closely examine bryophyte morphology and ecology. Although these fungi can be tricky to work with, half the job in their identification is to identify the host bryophyte, a relatively easy task for bryologists compared to traditional mycologists.

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