A gall-inducing infection of *Lepista* spp. in Norfolk by *Mycosymbioces mycenophila* - first record for Britain

Anne Edwards*, Tony Leech** & Ian Senior***

In November 2019, one of us (IS) found a group of brown agarics in Earlham Cemetery, Norwich with prominent swellings on their caps (Fig. 1). A few days later, AE, together with Tracy Money and Andy Gardiner, found similar specimens at Ashwellthorpe Lower Wood 13 km to the south-west (Fig. 2). In the latter case the agaric was clearly Field Blewit, *Lepista saeva* but the affected agarics in Norwich lacked any blue colour although they did appear to be a *Lepista* sp. (see below). The Norwich material is referred to below as Collection A and the Ashwellthorpe material as Collection B.

Our first thought was that the agarics were infected with a species of *Syzygospora*, a genus of jelly fungi known to cause 'tumours' on the caps of agarics. *S. tumefaciens* is a rare species recorded from a handful of sites in Britain and *S. mycetophila* is a very similar North American species with possible occurrence in Europe. However, whilst *Syzygospora* spp. cause fleshy growths on the cap, the Norfolk specimens showed, at least in the early stages, pronounced swellings from beneath the cuticle. Moreover, both of the above *Syzygospora* spp. are known only as parasites of *Gymnopus dryophilus* and *Rhodocollybia butyracea*.

Fig. 1. *Lepista ovispora* attacked by the anamorph of *Mycosymbioces mycenophila*. Collection: A. Earlham Cemetery, Norwich. Photograph © Ian Senior.

Microscopic examination of parasite

This description is based on cap cuticle from the surface of the swellings on Collection A. Material from Collection B was not available.

Unbranched conidiophores (probably phialides) to ca. 30 µm long projected from the cap surface. These were slightly tapered from a width of 1.5 µm near the base. Many bore a single conidiospore held at an angle (Fig. 3) suggesting that others had been shed; occasionally a pair of conidiospores were seen. Similar structures were seen protruding sparsely from the gill edge.

The conidiospores were quite variable. At first it was thought that more than one form was present but subsequently a range of intermediates was seen (Figs. 3 & 4). The smallest spores, often those still attached, were torpedo-shaped 5.5-8.0 x 1.5-2.0 µm.

Apparently more mature spores were broader and often slightly longer (to 11.0 x 3.0 µm). Some of these were more cylindrical but retained a tapered end. In one preparation, several of the larger spores were septate and bore processes from one end suggesting germination.

*anne.edwards@jic.ac.uk
**tonyleech3@gmail.com
***ian.senior@it.ox.ac.uk

Fig. 2. *Lepista saeva* attacked by *M. mycenophila*. Collection: B. Ashwellthorpe Lower Wood, Norfolk. Photograph © Andy Gardiner.

Identity of parasite

The failure to find basidiospores finally eliminated *Syzygospora* but a further suggestion was that the swellings were due to infection by *Lecanicillium fungicola* (*Verticillium fungicola*) which causes 'dry bubble' disease in cultivated mushrooms. However, the absence of verticillate branching of the conidiophores made this less likely.

And there the puzzle would have remained, as many do, but for the fact that AE is a molecular biologist working on ash die-back genetics and was able to determine the base sequence of the ITS region from Collection B (GenBank SUB7670150 Seq1 MT671360). To our considerable surprise, she obtained a sequence 99% similar with that published for *Mycosymbioces mycenophila* (GenBank KF030236) and later obtained exactly the same sequence from Collection A (GenBank SUB7670150 Seq2 MT671361). The surprise arose from the fact that this species is a recently described stipitate ascomycete apparently parasitising the base of a *Mycena haematopus* which was found by Jonathan Frank in the Cascade mountains of central Oregon. He described a new genus for the species which he described as follows (Frank, 2014):

Ascocarp: 2–4 cm tall. Purple brown, growing from base of basidiocarps in the genus *Mycena*. **Stipe** elliptical to pinched in cross-section, glabrous 2-4 x 1-2 mm across (Fig. 5). **Hymenium** convex cap-like 1.5-2 x 2.5 mm, appressed to upper stipe. **Asci** aciculate 35–50 x 3–6 µm. Spores ellipsoid, hyaline 5–6 x 2–3 µm.

The type collection is illustrated in Fig. 5, and in Fig. 6 where it can be seen that the cap is covered with small 'pimples' which resemble the protruding perithecia in some members of the *Hypocreales*. In particular, there is a close similarity in general form between this and the truffleclub *Elaphocordyceps capitata*. However, the resemblance of the Norfolk material to *E. capitata* [GenBank JN943317] is only 68.4% over 547 base pairs, so they are not closely related.

The Norfolk specimens in no way resemble this description but the sequences also match two reported from a culture of *Sarocladium mycophilum* (GenBank HG965024; GenBank MH862348). There is a 100% match for a sequence of 526 bases between these and that of *M. mycenophila*. The genus *Sarocladium* was described by Gams and Hawksworth (1975) to accommodate what was previously known as *Acrocylindrium oryzae*, a pathogen of rice. Helfer (1991) used this genus as a repository for his newly described species *S. mycophilum* (found on *Cortinarius casimiri* (= *C. subsertipes*)).

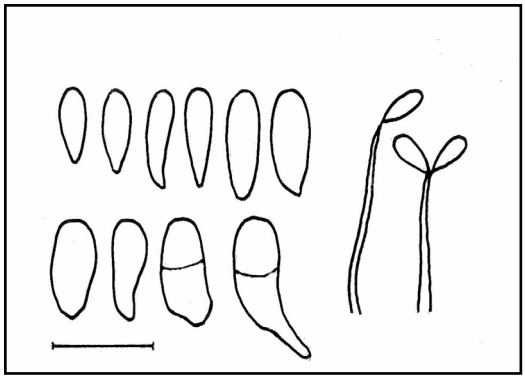

Fig. 3. Conidiospores and conidiophores of *M. mycenophila*. Scale bar 10 µm.

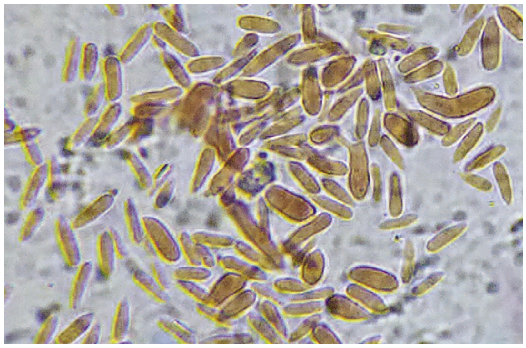

Fig. 4. Conidiospores and conidiophores of *M. mycenophila*.
Photomicrograph x 1000 © Tony Leech.

Fig. 5. *M. mycenophila* at the base of *Mycena haematopus*. Oregon, U.S.A. Photograph © Jonathan Frank.

Fig. 6. *M. mycenophila* cap showing what appear to be protruding perithecia. Oregon, U.S.A. Photograph © Jonathan Frank.

S. mycophilum is the only member of the genus known to be a fungal parasite; most other species are parasites of plants but several have been isolated from human tissues and it is possible that they may cause disease under some circumstances (Perdomo *et al*., 2010). Although the full taxonomic position of *Sarocladium* has yet to be established, it resides in the *Hypocreales* – intriguingly consistent with the macroscopic appearance of the ascomycete fruiting body described above. A taxonomic study by Giraldo *et al*. (2015), however, showed that *S. mycophilum* is phylogenetically distant from the type of *Sarocladium*.

The Oregon *M. mycenophila* is the teleomorph of this mycoparasite as it is the only collection bearing this DNA sequence in which asci have been observed. None were found in the Norfolk specimens which were of the anamorph. Although the name *S. mycophilum* predates that of *M. mycenophila*, exclusion of the former from the genus *Sarocladium* means that the name *Mycosymbioces mycenophila* should take precedence. We are tempted to offer the English name of 'Norfolk Bubble Fungus' but, in the way of these things, it could soon be found more widely.

An unidentified truffle collected in South America has recently been found with a secondary symbiont that genetically matches the above (Jonathan Frank, pers. comm.). This may be further evidence of a relationship with the genus *Elaphocordyceps*.

The possibility that the infection was due to *Lecanicillium fungicola* was finally excluded by comparison with a published sequence for this fungus (GenBank MK106661) for which there was only 67.5% concordance with the Norfolk material over 547 base pairs.

Fig. 7. In some cases, the distortions caused by infection totally obscured the form of the host. Fruitbodies attacked in this way resisted rotting for several months. Collection: A. Earlham Cemetery, Norwich. Photograph © Ian Senior.

Description of affected agarics

Infection is first apparent from a cluster of swellings on the upper surface of the cap (Figs. 1 & 2) which are covered with a whitish bloom. These swellings soon increase in size and fuse to form a cerebriform mass which darkens and may envelope the whole fruiting body (Fig. 7).

Identity of Norwich agarics

The affected fungi of Collection A formed a cluster growing in grass in a semi-wild part of the cemetery. Several species of tree, including poplars, were growing nearby.

Cap: Rusty tawny (British Fungus Flora colour chart); 85-130 mm diameter; shallowly funnel-shaped when mature.

Gills: Fulvous (BFF colour chart); crowded; slightly decurrent.

Stipe: Vinaceous buff (BFF colour chart) with dark fibrils; 75 x 15mm

Spores: White; 6.5-8.5 x 4.0-5.0 µm; ellipsoid; verrucose.

Cystidia: Not seen.

The genus *Lepista* was suggested from the warty spores and moderately crowded gills but no trace of blue colour was apparent on any part of any of the fruiting bodies. Using *Funga Nordica* this led us to either *L. irina* or *L. ovispora*. In the absence of a sweet smell, and because of the caespitose grouping and *Clitocybe*-like form at least when mature (Fig. 8), *L. ovispora* was considered the more likely although the gills were only slightly decurrent, if at all. The 'pale pinkish' spore deposit may have been missed; it is less likely that the 'pink to salmon' tint of *L. irina* spores would have been.

In apparent contradiction to the above, AE was able to determine the DNA sequence for the ITS region of the host agaric and found it was 99.7% identical to a sequence published for *L. irina* (GenBank MH856287) and 99.5% identical with one published for *L. sordida* (GenBank LT716070). However, in the absence of a taxonomic study and due to the lack of published sequences of this region for *L. ovispora*, we are unable to draw firm conclusions.

DNA extraction, amplification and sequencing methods

DNA was extracted using the CTAB method of Lee et al. (1988). DNA amplification was carried out with universal fungal ITS primers (Forward 5' TCCGTAGGTGAACCTGCGG 3' and Reverse 5' TCCTCCGCTTATTGATATGC 3'). PCR products were gel-purified and Sanger sequenced by Genewiz (www.genewiz.com). Sequence comparisons were made using Geneious Prime 2019 2.3.

Acknowledgements

We are very grateful to Jonathan Frank (Dept. of Biology, Southern Oregon University) for providing information on his Oregon specimen, allowing us to use Figs. 5 & 6 and commenting on this manuscript. We thank Alick Henrici for encouraging us in our searches for information, advising on taxonomic matters and commenting on the manuscript. We thank Brian Spooner for examining the material.

Fig. 8. An unaffected agaric near to the infected specimens, provisionally identified as *Lepista ovispora*. Earlham Cemetery, Norwich. Photograph © Ian Senior.

References

Frank, J.L. (2014). *Mycosymbioces mycenophila*. *Index Fungorum* 134: 1.

Gams, W. & Hawksworth, D.L. (1975). The identity of *Acrocylindrium oryzae* Sawada and a similar fungus causing sheath-rot of rice. *Kavaka* 3: 57–61.

Giraldo, A., Gene, J., Sutton, D.A., Madrid, H., de Hoog, G.S., Cano, J., Decock, C., Crous, P.W. & Guarro, J. (2015). Phylogeny of *Sarocladium* (*Hypocreales*). *Persoonia* 34: 10–24.

Helfer, W. (1991). Pilze auf Pilzfruchtkörpern. Untersuchungen zur Ökologie, Systematik und Chemie. *Libri Botanici* 1: 1–157.

Lee, S.B., Milgroom, M.G. and Taylor, J.W. (1988). A rapid, high yield mini-prep method for isolation of total genomic DNA from fungi. *Fungal Genet. News* 35: 23–24.

Perdomo, H., Sutton, D.A., Garcia, D., Fothergill, A.W., Cano, J., Gene, J., Summerbell, R.C., Rinaldi, M.G., & Guarro, J. (2010). Spectrum of clinically relevant *Acremonium* species in the United States. *Journal of Clinical Microbiology* 49 (1): 243–256.