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Field Mycology

Field Mycology is a quarterly magazine, published by the British Mycological Society. It provides articles about fungi of interest to the field mycologist, covering all aspects of identification, conservation, recording and collection, for all levels of expertise.

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Back cover: *Amanita muscaria*, a watercolour pencil drawing © Roclyn Thomas-Owen.

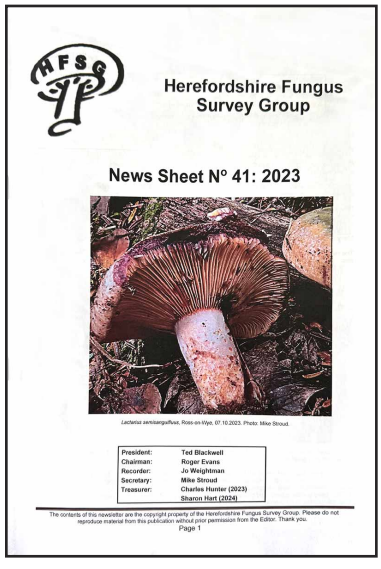
EDITORIAL

We are fortunate in the UK to have numerous local fungus groups which people can join to learn about the many differing aspects of mycology from collecting for study, microscopy, DNA sequencing, to cultivation, foraging for food, photography and many others.

The British Mycological Society's website lists 44 such groups distributed across the whole country, so there are very few places in Britain where you are not within reach of a local group (https://www.britmycolsoc.org.uk/field_mycology/recording-network/groups). This means that it is now easier than ever to find like-minded people to help you learn and encourage you in your studies. You will usually find both expert mycologists as well as beginners and all stages between so you should not feel intimidated about joining.

Most groups run local fungal forays to enable you to find and see species in the wild and most also publish newsletters to keep members up to date with activities and interesting finds made throughout the year.

As an example, one such group who send me their newsletter is the Herefordshire Fungus Group. They are one year older than this magazine, having just had their 25th anniversary meeting in August of last year. They produce an excellent newsletter in full colour recording their many activities and highlighting the more interesting species that they come across.


HFSG

Herefordshire Fungus Survey Group

News Sheet N° 41: 2023

Lactarius zonarioaurifluus, Ross-on-Wye, 07.10.2023. Photo: Mike Stroud.

President:	Ted Blackwell
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Their newletter is typical of many such produced by local groups around the country and serves to illustrate the sterling work of studying, recording and teaching mycology undertaken by the many people who so generously give of their time to run these groups.

So, if you know of anyone just starting out in mycology or who perhaps is already an enthusiast but not a member, then do encourage them to seek out and contact their local group.

This spring and early summer have been among the wettest on record here in rural Norfolk where I now live (and of course elsewhere in the country also) and the rain shows no sign of abating; but I am writing this in late July and the fungi here have yet to appear in any numbers.

Looking at the various online fungal groups however, distributed around the country, I see that other, more northerly parts seem to be in full flush already. It will be interesting to see what sort of mushroom season we get this year, particularly with the mycorrhizal species. I would assume that the trees are happy with the damp weather so surely their mycorrhizal partners should be so too? I wait with bated breath for the first big flush of the season, especially *Russula* species which myself and my colleague Mario Tortelli are working hard on at the moment—fingers crossed!

Postscript

Two days after writing the above on July 17th I have just found my first *Russula* of the season: *R. versicolor*, a strict birch associate, along with a number of other smaller fungi, mostly *Marasmius* and *Mycena*, so the season here has now got underway—and of course it is still raining!

Fungal Portrait: 99

Tricholoma arvernense

Geoffrey Kibby

Fig. 1. *Tricholoma arvernense,* a robust species associated with *Pinus.* Boat of Garten, Cairngorms National Park, Scotland. Photograph © Geoffrey Kibby.

Amongst the greenish yellow to copper coloured species, *Tricholoma arvernense* Bon is a moderately uncommon member of the genus found in Scottish conifer woods, mainly under *Pinus*, more rarely with *Abies* (Fig. 1). Apart from Scotland there is one record on FRDBI from Northern Ireland.

It is typically rather stout with honey-yellow to greenish yellow or copper caps which have fine, innate, radiating darker fibrils and streaks. The gills are broad, fairly crowded and white to pale greyish, often with somewhat yellowish, serrate edges. The stem is cylindric to clavate, white, fibrous and sometimes tinted pinkish at the bse. The flesh is firm and has a strongly farinaceous odour when cut, while the taste is farinaceous with a slight rancid overtone. Spores are rather small, broadly ellipsoid 4.0–6.7 x 3.3–5.2 µm. Clamp connections are frequent in its tissues. Despite some morphological similarities to some other species, according to Christensen & Heilmann-Clausen (2013) it occupies a rather isolated position phylogenetically amongst other European species and they place it in its own clade.

Most likely to be confused is the deciduous forest species *T. sejunctum* (Fig. 2) which associates with *Quercus*, *Fagus*, *Corylus* and *Carpinus*, often on clay or calcareous soils. It is when you are in mixed coniferous and deciduous forests that confusion may arise. It tends to have more distinctly greenish yellow colours and larger spores 5.2–8.0 x 3.7–6.4 µm. Also clamp connections are absent.

Fig. 2. *Tricholoma sejunctum*, a deciduous forest lookalike of *T. arvernense*. Photograph © Geoffrey Kibby.

Fig. 3. *Tricholoma viridilutescens*, in moss under *Picea* in Scotland. Photo © Jo Weightman.

Another coniferous species with greenish-yellow colours which could be confused with either of the above species is *T. viridilutescens* (Fig. 3) which tends to have dark olivaceous black fibrils, especially at the cap centre, often becoming entirely greyish to brownish black with age and with quite large spores 5.3–8.7 x 4.4–7.3 µm. It associates with *Picea* and *Abies* and in Britain it is probably confined to Scotland. Records from England with deciduous trees must be treated as doubtful, possibly just darker than normal *T. sejunctum*.

Reference

Christensen, M. & Heilmann-Clausen, J. (2013). The genus *Tricholoma*. *Fungi of Northern Europe* Vol. 4. Svampetryk.

Hydropus inopinatus - a new species described from the UK

Penny Cullington*

Background

In August 2021 a mysterious small mycenoid mushroom was found by Barry Webb in Buckinghamshire. It appeared on a piece of damp well-rotted *Pinus* which he'd collected from Burnham Beeches. For about a year its identity remained a mystery — even after amplification and sequencing by Eric Janke and Aberystwyth University respectively — until further collections were made from two different Hampshire sites, by Mike Harrison and Eric Janke. All three collections turned out to have matching sequences, placing the species clearly in the genus *Hydropus* s.s. although they did not cluster with any other currently available sequence. This was reported in FM 24(1) - *Hunting for the identity of a Hydropus species found in Buckinghamshire and Hampshire,* Cullington *et al.* (2023) - comprising the full story, species description and photos, phylogenetic tree with GenBank accession nos.

The delay before publishing

Though we were already of the opinion that the species was likely to be genuinely new, there were two obstacles preventing formal publication. Firstly, alongside our natural instinct to err on the side of caution, there was a basic practical issue. Any newly described species must have a designated type collection to be kept in a fungarium as voucher for future reference and study. This we did not have! All existing material from our first three collections had been used up for sequencing or microscopic study. This is a tiny mushroom with cap only a few mm across when mature. Furthermore when each collection was made the possible significance of its identity was not realised therefore minimal material had been collected.

Secondly, further research was needed to ensure that the species had not already been described in some paper unbeknownst to us. We'd already received advice from *Hydropus* expert Jerry Cooper in New Zealand supporting Eric's placement of the species within the genus, now considerably reduced (see our previous article for further explanation). Cooper's feelings were that our species was likely to have a northern European distribution rather than worldwide, that as such it was one of very few European species within *Hydropus* s.s., all of which are rare, and he encouraged us to publish. As belt and braces, Eric now contacted Vladimír Antonín, working on the genus in Europe and best placed to advise us if we had a genuinely undescribed species. Back came the news that he had no knowledge of anything matching having being reported, thus in effect giving us the go ahead. What we lacked now were further collections to provide a designated type and to broaden our experience of the species in order to fill out the description.

More recent collections

In October 2022 — a few months after our two Hampshire collections but in fact prior to our FM article—unbeknownst to us Mario Tortelli found what turned out to be this same species in Mereworth Woods, Kent (Fig. 1). Struggling to determine it even to genus, he sent a sample to Alvalab (in Spain) for sequencing, receiving the result that it matched nothing known. Realising how similar in appearance Mario's collection was to our recently published photos in FM, a check was then made by Geoffrey Kibby comparing Mario's Alvalab sequence to ours now in GenBank. Bingo! It proved to be a 99.74% match. Interestingly, however, this find was not from conifer wood but from a well rotted *Castanea* stump. No material or notes from this collection survive, just Mario's convincing photo and the sequence remain.

In 2023, in January, August and October, Mike Harrison made four further sightings, all from well-rotted conifer wood but at different locations within Morgaston Wood, The Vyne, the Hampshire site where he'd made his original collection the previous year. One of his August collections was then successfully sequenced by Eric, providing a further exact match (this now the fourth entered into GenBank as *Hydropus* sp.) but unfortunately we had just the record and

the sequence - no remaining material, notes or photo.

In July 2023 Barry Webb noticed the species appearing once again, fruiting quite prolifically in Burnham Beeches on two different rotting *Pinus* stumps, one being the stump from which the wood for his original collection came, the other from a totally different area. He made a further collection from Penn Wood several miles away, though this time on well-rotted *Picea*. Barry took me to both sites and together we endeavoured to make viable collections as best we could though mature perfect specimens were hard to come by.

These tiny insignificant mushrooms are clearly irresistible to the local slug population!

Fig. 1. *Hydropus inopinatus*, Mereworth Woods, Kent, 12 October 2022. Photograph © Mario Tortelli.

Several times Barry deliberately camouflaged miniscule immature clusters with litter in the hope that they'd be further developed in a day or so, only to find no sign of them on his return - just the telltale trail of slime with some rather satiated gastropods nearby! Though we could detect no smell from the species it was clearly emanating some alluring signal. Finding perfect collectable specimens proved a challenge: besides being tiny, when large enough to pick they tend to have a coating of adhering woody debris (possibly due to the slug slime?), also when dried they shrivel to such an extent that there's virtually nothing usable left to work with. We did eventually manage to make a reasonable collection from the original Burnham Beeches spot, providing us with a sporeprint and morphological notes in the hope that this would be our designated type collection (Fig. 2).

A snag!

Though we were confident that this latest Burnham Beeches collection was a perfect match for our species, we needed a matching DNA barcode to prove it beyond doubt. Frustratingly over the ensuing months Eric, despite his best efforts, made numerous unsuccessful attempts to obtain a useable amplification, trying every method he could think of. Whether this was due to slug / fungal / other contamination or inefficient drying technique we have no idea – these things happen! In desperation I decided to deplete our valuable collection further by sending him another cap, and failing that we had the spore print as a last resort though I was reluctant to lose that. However, in June this year I received the

Fig. 2. *Hydropus inopinatus*, holotype K-M001442993, Burnham Beeches, Buckinghamshire, 5 July 2023. Photograph © Penny Cullington.

encouraging news that he'd succeeded with the latest sample and that the results were back from Aberystwyth University with a 100% match to Barry's original collection. Thus we were now in a position to go ahead with publication.

Taxonomy

***Hydropus inopinatus* P. Cullington, M. Harrison, E. Janke, & B. Webb, sp. nov.**
Registration Identifier IF901514
Fig. 2

Etymology: *inopinatus* means unexpected and reflects the authors' surprise when sequencing revealed the species as belonging to *Hydropus* s.s.

Holotype: UNITED KINGDOM, England. S. Buckinghamshire: Burnham Beeches, in mixed woodland on a very rotten soggy *Pinus* stump near a stream. Alt. ca. 54 m.; 51°33'26.61"N, 0°37'14.34"W; 05 July 2023, B. Webb & P. Cullington HFRG_PC230705_1; Holotype accessioned in K as K-M001442993, GenBank accessions PP982811 & PP992745.

Diagnosis: A small mycenoid greyish white mushroom with a smooth partly translucent pileus, decurrent lamellae, a finely pruinose stipe, 2-spored basidia, spores amyloid, smooth, amygdaliform, 8–9 x 5–6 µm, cheilocystidia clavate to subutriform, pleurocystidia not seen, caulocystidia as cheilocystidia. Substrate damp very rotten stumps, both coniferous and deciduous.

Description

Habit mycenoid. **Pileus** 1–5(6) mm diam, at first inrolled convex, as it expands margin deflexed and undate, eventually ± applanate with sunken centre and margin ± reflexed in places, thin-fleshed, outer half translucently striate, inner half smooth, surface at first finely pruinose, this less apparent with age, dull greyish white at first, becoming white in outer half, retaining grey tinge in inner half. **Lamellae** (sub)decurrent, at first arcuate then horizontal, medium to widely spaced with blunt uneven edges, L = 14–18 interspersed with lamellulae variable in length, white. **Stipe** central, to 8(10) x 1–2 mm, ± cylindrical with slightly swollen clavate base at first, either straight or curved dependent on the angle of emergence from the substrate, white, entirely finely pruinose, base strigose with fine mycelial strands attaching to substrate; stipe when cut exuding colourless fluid. **Odour** and **taste** not observed.

Basidiospores 8–9(10) x 5–6 µm, smooth, amyloid, ellipsoid to subphaseoliform. **Basidia** 78–110 x 18 µm, mainly 2-spored, some 1-spored, some 4-spored, with oil droplets within, sterigmata 15-18 µm long. **Cheilocystidia** 26–40 x 10–12 µm, clavate to flexuose cylindrical or utriform, some with pedicel, forming a palisade, finger-like protuberances seen in some specimens. **Pleurocystidia** not seen. **Pileipellis** a cutis of short cylindrical cells with rounded ends. **Caulocystidia** similar to cheilocystidia, clustered in bundles throughout the stem length. **Clamps** not observed.

Substrate and habitat

Growing gregariously, loosely clustered on damp very well rotted stumps or wood having fallen from stumps having partly disintegrated and in contact with damp soil. Collections known so far mainly on *Pinus*, one on *Picea*, one on *Castanea*, from Buckinghamshire, Hampshire and Kent.

UNITE Species Hypothesis (1.5% threshold)

SH0972688.10FU

Additional specimens examined

Buckinghamshire: Burnham Beeches, 31 Aug. 2021, B. Webb HFRG_BW210831_1, GenBank accession (ITS + LSU) OQ133570, no material remains (Fig. 1 in Cullington *et al.*, 2023). *Ibid.*, 2 Jul. 2023, B. Webb & P. Cullington K-M001443237. *Ibid.*, 7 Jul. 2023, B. Webb & P. Cullington K-M001443238. *Ibid.*, 21 Jul. 2023, B. Webb & P. Cullington K-M001443236. *Ibid.*: Penn Wood 15 Jul. 2023, B. Webb & P. Cullington K-M001443239.

Hampshire: The Vyne, Morgaston Wood, 22 Sept. 2022, M. Harrison HFRG_MH220922_1 (in GenBank as HFRG_MK220922), GenBank accession OQ133585, no material remains. *Ibid.*, 12 Aug. 2023, M. Harrison Hampshire Fungus Recording Group Fungarium HFRG_MH230812_1, GenBank accession OR896139. *Ibid.*: Waggoners Wells, 18 Sept. 2022, E. Janke HFRG_EJ220918_2, GenBank accession OQ133584, no material remains (microcharacters shown in Figs. 3–5 in Cullington *et al.*, 2023).

Kent: Mereworth Woods, 12 Oct. 2022, M.

Tortelli SFSG_MT221012_1, GenBank accession PP982809, no material remains (Fig. 1).

General comments

It seems remarkable that this small yet quite distinctive species should not have been described until now. Bearing in mind that one of its notable characteristics appears to be its habit of fruiting in troops (as is evident from our photos), another that – at the Burnham Beeches location at least – it has fruited repeatedly in the same spot over several years [see also postscript below] it seems unlikely that it is genuinely rare. A third characteristic of particular note – one which we should possibly have picked up on earlier to point us to genus – is the watery translucent fluid contained within the stem, this after all the feature to which the genus name refers. However, *Hydropus* is by no means unique amongst mycenoid genera in having this feature though it may also have been a contributory factor in the placing of some species – now moved elsewhere – within this genus prior to the DNA era (See FM 24(1) for further discussion re this). The genus is large, though of the 179 species listed to date in Species Fungorum the vast majority are tropical, very few are European, and the four accepted as British are amongst those now placed in different but closely related genera, leaving our new species as the sole UK representative within *Hydropus* s.s. – its barcode placing it near to the generic type, *H. fuliginarius*, an American species. (See phylogenetic tree Fig. 4).

It is hoped that following this publication more collections will materialise to give us further information about range and host substrate. All records so far are from southern England, collected mostly in August but also in October with one surprisingly in January. It would appear that as long as the wood substrate is damp, really well rotted and disintegrating this species is not that choosy about the host tree, having been recorded on both conifer and deciduous woods. It remains to be seen if this is borne out in future.

A final general observation regarding records of new species

One problem we as field mycologists are experiencing more and more is that in the last few years the growing number of new species both to science and to the UK are not yet covered in any available generalist keys. Keeping abreast of new developments and discoveries has become no easy task. If not prepared to spend considerable time searching through the numerous papers (not necessarily in English) in which such species are described, one has little or no chance of taking them into account as part of ones identification process. Not everyone has the facility or finance to have collections sequenced, consequently it is likely that many interesting and potentially valuable collections go either mis- or unidentified.

In an ideal world the routine practice of keeping photos, micrographs, notes, dried samples of all such collections is the way to go in the hope that the day of easily available cheap barcoding is not that far away. How many of us have the time and dedication to do this? Life's too short…

Fig. 3. *H. inopinatus*, Burnham Beeches, Buckinghamshire, July 2024. Photograph © Barry Webb FRPS.

Acknowledgements

Thanks are due to many people who've made this publication possible: to the co-authors of our new species, Barry Webb, Mike Harrison and Eric Janke, also to Mario Tortelli, for finding and making collections; to the British Mycological Society and City of London Corporation (owners of Burnham Beeches) for providing some funding for sequencing; to Aberystwyth University and Alvalab for sequencing; to Lee Davies for curation at RBG Kew; to Paul Kirk for registering the species in Index Fungorum; to Geoffrey Kibby (who suggested the epithet) and Martyn Ainsworth for valuable help and advice. Finally I'd like to add particular thanks to Eric Janke for his dedication, skill and persistence in preparing the extraction and amplification of the samples ready for sequencing, to say nothing of the analysis, collation and subsequent taxonomic and phylogenetic interpretation of the results.

Postscript

Having finalised this article (in early July) I received the news from Barry Webb that the recent persistent rains have triggered the reappearance of this enigmatic little mushroom. At Burnham Beeches it's coming up not only on the original *Pinus* stump but also on its neighbour not far away, still only 3–4mm tall and as yet undamaged by slugs (Fig. 3).

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Reference

Cullington, P., Harrison, M., Janke, E., (2023). Hunting for the identity of a *Hydropus* species found in Buckinghamshire and Hampshire. *Field Mycol.* 24(1): 5-9.

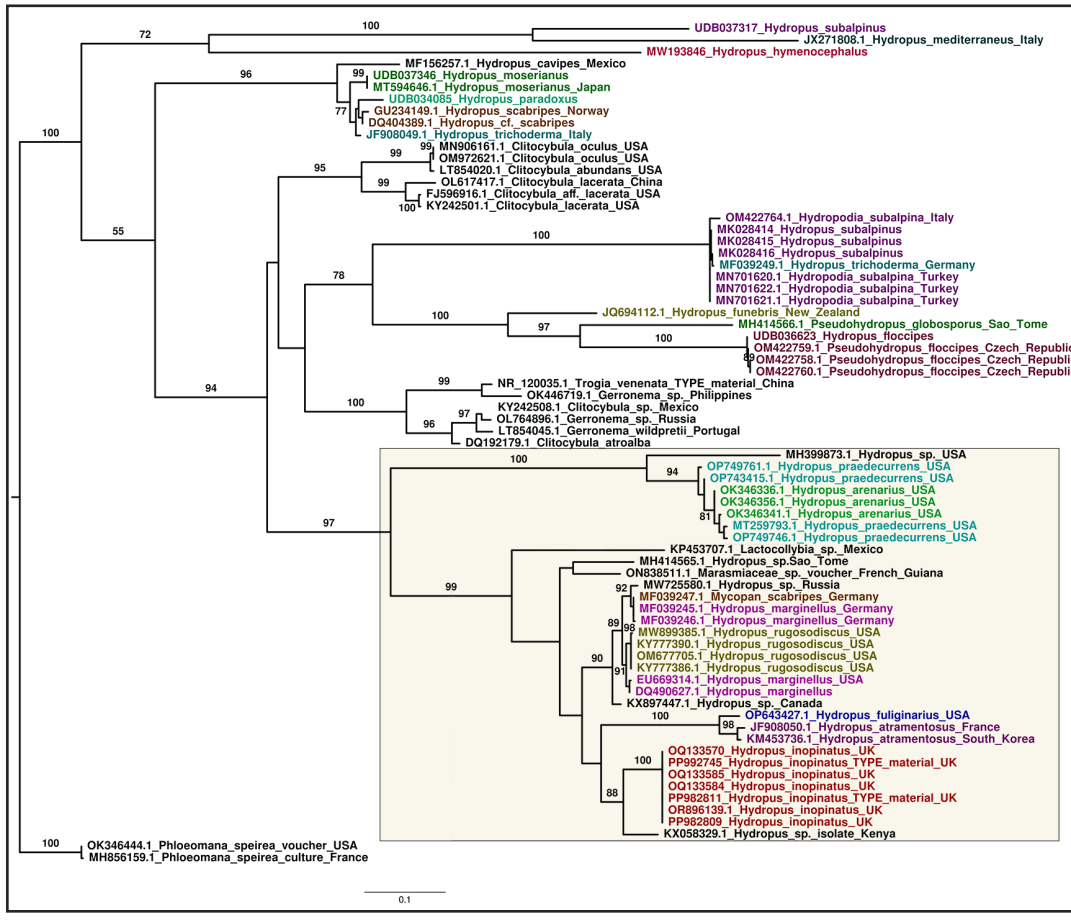

Fig. 4. Phylogenetic tree of the ITS region showing position of the seven UK collections of *H. inopinatus* alongside *H. fuliginarius* within the *Hydropus* sensu stricto clade (shown in the coloured box) together with other *Hydropus* sensu lato species listed in GenBank and UNITE, which are highlighted.

Take your auger out with you!

Caroline Hobart*

August 2015 was an exciting time. During a visit with my father to Wales and out on a remote mountain road, I stopped to look at the spruce plantations up near Rhyd. I collected and subsequently recorded the first UK collection of *Chamonixia caespitosa* (Hobart 2016). Nine years later the world has moved on. It is now a common research practice to put an auger in the soil, draw out the contents and take it to the lab for sequencing using HTS (High Throughput Sequencing): eDNA is here (environmental DNA) and it tells us all we need to know, or does it?

In January 2024 I was contacted by Dr Andy Taylor from the James Hutton Institute. Andy, a molecular ecologist and old friend, had been extracting soil samples for eDNA in spruce plantations at Creagan near Oban. He had, in amongst the huge numbers of sequences, a result that matched a sequence of *Chamonixia caespitosa*. Surprised that I had already found this rarely reported species in Wales, he asked if I had a photo for a press release. I was able to provide a rather poor, blurred image that I hadn't used in my FM article and I began thinking about eDNA as well as the *Chamonixia* find.

Firstly the *Chamonixia*

Following my 2016 article I had thought more about the rarity of the fungus. In 2018 after a further site visit and noticing that vast tracts of the surrounding woodland were being felled, I contacted a number of people in Wales to see if this fungus could be red-listed. The responses were helpful, but not encouraging. It was apparently very difficult to prioritise fungi associated with native trees, but near impossible for fungi in non-native forests. *Chamonixia* could be fairly common in these Welsh woodlands, but there were relatively few people recording hypogeous fungi, so it might not be possible to determine its true rarity or distribution, therefore a case to preserve it might be difficult. It was suggested that I attempt to assess it for the IUCN global Red List.

Recently, in an email discussion with Andy, I suggested to him that it was probable that it also grew elsewhere; I was delighted to read in the newspaper that he had had more luck than me. With DNA tools that I had no access to, and probably more 'clout', he has been able to persuade Forestry and Land Scotland to support a project to properly study soil samples in other Scottish plantations. This should eventually give us a better idea of its rarity and distribution. All this was reported in the Guardian newspaper on the 12th of April, 2024.

So finally eDNA

It is becoming ever more common for soil samples to be taken in grasslands, forests and other land and water masses to monitor biodiversity.

Fig. 1. *Chamonixia caespitosa* showing the intense blue staining of the surface and at right the internal pale gleba. Found in a *Picea* plantation, near Rhyd, Wales, 2015. Both photos first published in FM17(2): 60 in 2016, © Caroline Hobart.

Technological advances have been made in the last 15 years and as a result sequencing costs have come down. The last eDNA costing I obtained in 2022 was about £300.00 per sample for looking at fungi. For those interested in an excellent overview of methodology, process, advantages and pitfalls of this technique see Deiner *et al* (2017). A further example of an eDNA study, this time in grassland, monitoring for fungal diversity and abundance in the White Peak, a limestone plateau in the Peak District, can be found in Griffith *et al (*2022). More recently, despite government funding squeezes, Natural England have been sampling oak woodlands using eDNA in the south west of England.

In summary eDNA using HTS will, in combination with traditional survey methods, no doubt become the next tool in the armoury of the amateur mycologist/citizen scientist. Take your auger out with you!

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References

Carrell, S. (2024). Rare truffle find in Scottish spruce forest sends fungi experts on alien species hunt, *Chamonixia caespitosa* found during rewilding project in west Highlands while removing non-native Sitka spruce. *The Guardian* Fri 12 Apr 12.00 BST Last modified on Sat 13 Apr 2024 05.07 BST

Deiner, K., *et al*. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*, 26(21): 5872–5895. https://doi.org/10.1111/mec.14350

Griffith, G. W., Detheridge A. P. & Bye, R. (2022). *Use of eDNA analysis of soil samples to evaluate the fungal conservation value of grassland areas in the South West Peak: lessons for woodland creation proposals.* IBERS, Aberystwyth University.

Hobart C. A. (2016) *Chamonixia caespitosa*: first record for Britain of a rarely found fungus. *Field Mycology* 17 (2). 60–63. https:// doi:10.1016/j.fl dmyc.2016.04.009

Fig. 2. *C. caespitosa* found in the Jura, France, Sept. 2012. Photograph by Gerhard Koller, ex Wikimedia Commons.

Drawing and painting fungi - different techniques

Geoffrey Kibby*

Even in this age of digital photography, and when everyone can capture images on a smart phone, traditional painting techniques still have a place in natural history illustration. In this article I will explore three techniques available to those who wish to produce an image of the fungi that they find.

Many people will say that photographs of organisms are all that one needs for identification but most naturalists know that paintings and other illustrative techniques can capture or illustrate in a way that might otherwise be difficult in a photo. A painting can enhance, correct or otherwise 'pull out' features you wish to emphasise. This is quite apart from the aesthetic pleasure and appreciation of the artist's skill that a fine illustration can evoke. Added to this is the fact that—in my experience—illustrating a fungus is one of the best ways to learn about and remember it. The intense concentration and examination required to capture the fine nuances of a particular mushroom helps to firmly fix its characteristics in one's mind.

I have been making natural history illustrations since I was first able to handle a pencil or brush; first of dinosaurs (still one of my passions) then of insects and flowers and finally of mushrooms. I discovered the latter when I was 12 or 13 and have been hooked ever since. I quickly realised that they were incredibly diverse and that their amazing colours, shapes and textures lent themselves perfectly to illustration.

Originally I used watercolour paints but soon discovered coloured pencils and found that they suited my particular style better and have used them ever since, at least until I ventured into digital illustration (but more on that later). What I want to show here, both with my own illustrations and those of other mycologists who have kindly allowed me to reproduce their artwork, is how different techniques can produce totally different but equally valid ways of capturing the essence of a fungus.

Watercolour painting

Although I used watercolours for some years they were never my personal forté, I was happier with more opaque media such as gouache or acrylic and latterly coloured pencils. I always envied (and still do) those who can achieve the classic transparent, deceptively simple effects and clean colours so characteristic of good watercolourists. My heroine of this technique has always been Beatrix Potter, many of whose fungus paintings are available in various books. I first saw her paintings in the now long out of print Wayside and Woodland book of mushrooms, and have been entranced by her work ever since. I have been privileged to see many of her beautiful originals which are kept in the Ambleside Library.

Many modern mycologists continue in this tradition and I am indebted to David Mitchel for allowing me to reproduce here two of his lovely paintings: firstly *Mitrula paludosa* (Fig. 1) and secondly *Gliophorus psittacinus* (Fig. 2). They are good examples of capturing the essence of a fungus with clean washes of colour and often minimal detail. David is soon to produce a book of his work which I greatly look forward to and I am sure it will be reviewed here once it becomes available.

Advantages of watercolour can be the speed of applying colour, the almost infinite hues obtainable by careful mixing along with the general brightness and transparency of the pigments. Difficulties include mastering the technique, avoiding muddy colours through over-mixing and the classical technique of leaving the paper unpainted where you want pure white highlights.

To get the best results it is worth investing in both good quality paints and particularly good brushes. Cheap, poor quality brushes will only lead to frustration and annoyance; get good quality sable brushes if possible and your budget allows. Windsor & Newton are an old and highly regarded company and manufacturer of both paints and brushes but there are other companies also. Your local art suppliers should be able to give you advice on what to choose.

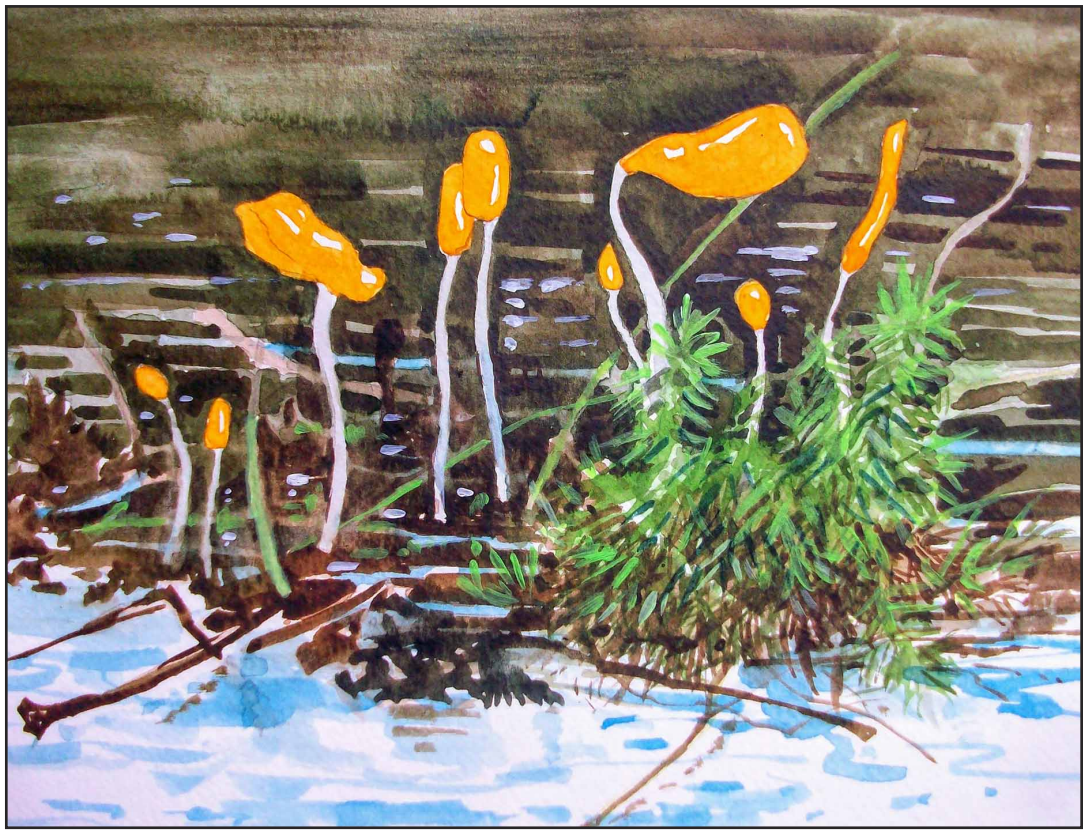

Fig. 1. *Mitrula paludosa* captured in a watercolour painting © David Mitchel.

Fig. 2. *Gliophorus psittacinus*, a watercolour © David Mitchel.

Colour pencils

When I talk of colour pencils I am referring to water-soluble colour pencils. As their name suggests these pencils are soluble if wetted and are not as waxy as traditional colour pencils. Although you can use water to dissolve the pigment and produce a wash I rarely do so, preferring to restrict this function to areas where I want a spot of more intense colour. Most of the time I will blend the pencil colours together dry and directly on the paper.

There are a number of manufacturers; I have tried a number of them and in my experience you get what you pay for. Popular brands include Caran d'Ache (my favourite), Faber-Castell, Derwent and several others. I would recommend going to a reputable art supply store and seeing what they stock. I find the very cheap brands available in high street stationers, etc are generally disappointing in terms of light fastness (i.e. not fading quickly when exposed to long periods of light), how easily they blend and purity of colour. The better known manufacturers will produce a very large range of colours. For

example, Caran d'Ache provide up to 100 colours in some of their ranges. But be warned, they can be very expensive! You can get all the colours you need with much smaller sets and by blending colours together. There are many excellent books and videos available on using water soluble pencils; ideal if you have not used them before.

Some brands although highly respected have caused me problems. For example I have tried pencils by Derwent several times as they have a very wide range of hues but every time when using the palest shades, creams, pale pinks, etc the sudden inclusion of a speck of some deeper colour within the pale colour is very annoying and difficult to remove. Perhaps I have just been unlucky, others may have had no problems at all.

There are also choices to be made in how hard or soft the pencils are, several manufacturers offer softer pencils for easier blending and colouring of large areas. Mostly I use the harder pencils as they allow for the finer detail so often required when drawing fungi.

Colour pencils lend themselves to a loose, less blended style of drawing (a style I don't seem able to achieve) and some examples are given here (Figs 3 & 4 and the back cover), all produced by an excellent young artist, Roclyn Thomas-Owen, aged 11, who is well on his way to becoming an accomplished mycologist and illustrator. His illustration of *Amanita muscaria* on the back cover is a good example of how colour pencils can capture the textures and colours of fungi.

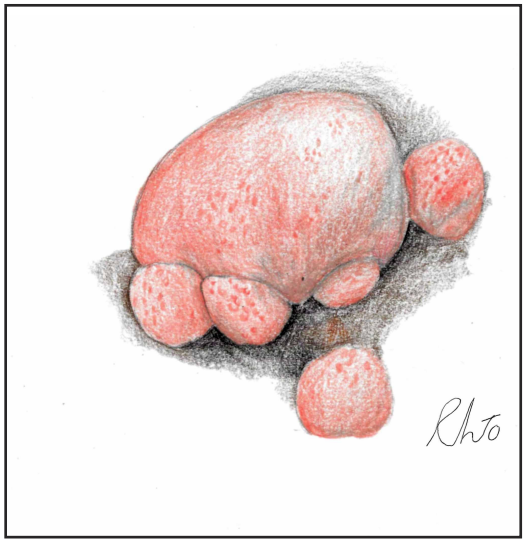

Fig. 3. *Lycogala epidendrum*, a colour pencil drawing © Roclyn Thomas-Owen.

Fig. 4. *Sarcoscypha coccinea* a colour pencil drawing © Roclyn Thomas-Owen.

My own style of pencil drawing, developed over many years is of a more highly blended, detailed approach, examples of which can be seen in Figs 5 & 6. The most intensely coloured areas in my pictures will have been made by wetting the tip of the pencil to gain maximum deposition

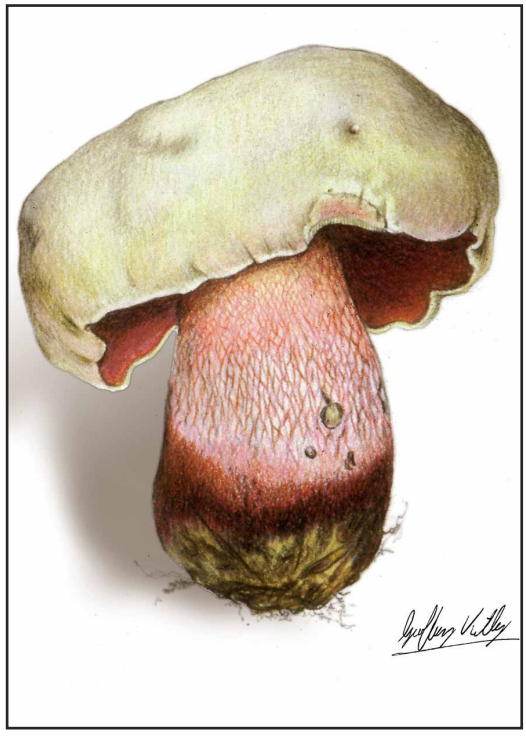

Fig. 5. *Rubroboletus legaliae*, a colour pencil drawing © Geoffrey Kibby.

of pigment on the paper. Paler areas with more varied colours present are made by light pressure of the dry pencil and gently overlaying layers of different colours to get the required hue. Tightly rolled paper blending stumps, available in most art stores, may be used to help smooth out areas or some companies produce special blending pencils which contain the pencil medium or solvent in concentrated form and help to mix colours together.

Disadvantages of colour pencils are that producing a picture, particularly of the highly detailed sort, is generally a slow process, and the medium is unforgiving of mistakes. It is not always easy to correct errors, pencil erasers are not entirely satisfactory at removing colour pencil, especially if you were using firm pressure when drawing, thus pushing the pigment deep into the paper surface. On the other hand, because pencil drawing is usually rather slow and methodical, mistakes are perhaps fewer than in watercolour painting, at least in my personal experience.

Digital drawing/painting

With the advent of computer monitors and graphic tablets that allow drawing directly on the screen a new era of illustration has begun.

I have experience with two manufacturers, Wacom, who produce both graphic monitors and graphic tablets and Apple, famous for their iPad series of tablets; there are of course several other manufacturers available but I have no experience of their products.

I use a large, 23 inch Wacom monitor to do all the layout of both this magazine and my books and this allows me to both draw and edit directly on the screen with the digital 'pencil' that comes with the monitor. It is extremely high resolution and accurate in colour and forms an essential part of my daily work flow. All my recent artwork however is produced on an Apple iPad Pro with a 13 in screen and the Apple pencil. For those who have my 4-volume set of field guides, volume one was produced with colour pencil drawings while the drawings in Vols 2–4 were made entirely on the iPad. The software used to produce illustra-

Fig. 6. *Leratiomyces squamosus* var. *thraustus*, a colour pencil drawing © Geoffrey Kibby.

tions is very important and having tried and purchased a great many I always return to Procreate (https://procreate.com). It is inexpensive (£12.99 at time of writing) and is slick, easy to learn and very fast and responsive to the Apple pencil. It comes with lots of digital brushes and pencils built into the software which mimic the textures and structure of real world brushes and paints.

The advantages of digital art for me are speed and the ability to easily correct, alter and modify drawings, very important when producing hundreds of paintings for a book. Also the ability to use layers allows for techniques and possibilities not available to traditional media. If you imagine sheets of transparent glass stacked on each other, with each sheet able to be painted on and able to interact with the sheet below in different ways you will have some idea of the power of layers. Also, because you can set both the size of the drawing and the resolution of the digital 'paper' it is possible to produce large and highly detailed images. Finally you can ask Procreate to record and play back every brush stroke in a short movie, ideal for teaching and demonstrations.

Fig. 7 shows part of a digital painting of *Gomphidius glutinosus* drawn from life and showing the level of detail, shading, etc possible.

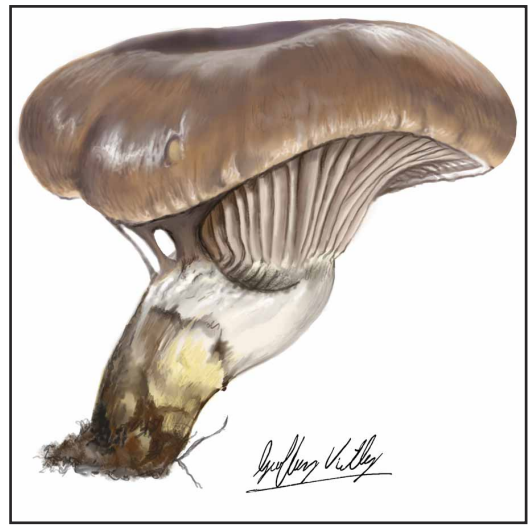

Fig. 7. *Gomphidius glutinosus* a digital illustration drawn on an iPad with Procreate software © Geoffrey Kibby.

Artificial intelligence (AI)

Finally, I cannot finish without mentioning this most recent development, loved by some, hated by many. Whether AI will, as many believe, lead to the destruction of humans in the not too distant future, as in the movie Terminator, is open to debate, but that it is revolutionising the ability of anyone to produce pictures is undeniable.

I have avoided using the words 'create' and 'art' in this context. as in the view of many no art or creation by a person is present, pictures being produced solely by written prompts to the computer, I will leave that debate for others!

Fig. 8 shows an example of a picture produced by AI software in the latest pre-production beta of Adobe Photoshop with the following simple prompt: "Three *Russula emetica* in moss and needle litter in a dense pine forest". Photoshop took 11 seconds to produce this result. A little bit Walt Disney in style and not very accurate, but not bad. AI still has some trouble with fungi, probably because it has yet to acquire a large database of images as references to work from. Images produced of more everyday items such as cars, furniture, people, etc are much more impressive.

But is this art? I leave you to decide.

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Fig. 8. A digital image conjured up by AI using a simple text prompt in Photoshop.

Inocybe griseovelata - with or without caulocystidia?

Andy Overall*

A short note/update on *Inocybe griseovelata*, featured in my recent article on Bedfont Lakes. Field Mycology Vol. 25 (2): 42–43.

Following the publication of the above article, a discussion took place via email between Penny Cullington, Eric Janke, Charles Aron and myself, regarding the presence or absence of caulocystidia in various collections of this species. Eric Janke pointed out that he has sequenced this species five times across 2021, 2022 and 2023 making it his most popular *Inocybe* subject with sequenced collections.

Inocybe griseovelata is a difficult species to key out. It's not mentioned in *Funga Nordica* as it was absent from the Nordic area when the key was written. It is included in the Outen & Cullington key (2015), as a species without caulocystidia but with a note stating it can occasionally have them. Stangl (1989) implies they are absent and Kuyper (1986) states they are absent or rarely found at the extreme stem apex.

On the website www.Inocybe.org hosted by Ditte Bandini, the caulocystidia of *I. griseovelata* are described by Horst Glowinski as not being true cystidia ("Kaulozystiden: keine echten Kaulozystiden"). Furthermore, a description by Bandini *et al*. (2021) of the lectotype specimen states "Caulocystidia not studied (to preserve the material") which left the presence of caulocystidia open to debate. As Eric points out, this lectotype collection is the one we have all been following. However, an incorrect accession number which was corrected in Bandini *et al*. (2023), lectotypified again using a different specimen. They recognised that this supersession meant that their sequenced epitype lost its standing, but did not do anything about re-designating an epitype because they had "not had the opportunity to study the lectotype'. Note that Bandini *et al*. (2021) say on p. 1085 that "*I. griseovelata* also has long and slender caulocystidia".

Where does that leave us? We can only report what we find and if that evidence was at odds with the type, are we looking at a different species or is it just that this species can be with or without caulocystidia? Most probably the latter. Which then begs the question, how important are caulocystidia in *Inocybe* taxonomy?

I didn't mention caulocystidia in my aforementioned article, so I decided to look at the dried material; no easy task. Following Penny's advice of leaving a small section from the upper stem in ammonia for 10 mins or so, I managed to remove a small, thin strip for observation in Congo Red. Bingo! I found caulocystidia. (See Figs 1 & 2). Further investigation revealed caulocystidia in both the mid and lower sections of the stipe.

A 2012 collection from Netham Park, Bristol, by the late Justin Smith, identified by Ellen Larsson and examined by Penny Cullington, was also found to have caulocystidia (Fig. 3). A further collection by Jesper Launder on 16/10/22 examined by Penny had caulocystidia in the upper third of the stem.

Charles Aron had also found caulocystidia close to the stem base in a collection from Aberlleiniog, near Beaumaris, NW Wales on 28/09/2021. (Fig. 4).

However, in some of Eric Janke's collections it was difficult to observe 'true caulocystidia', often closely resembling the hymenial cystidia. Closer inspection however of one of the collections did eventually show distinct caulocystidia to be clearly visible (Fig. 5).

From these examples it seems the evidence is quite overwhelming for caulocystidia to be quite often present on the stem of *I. griseovelata*, be it upper, mid, lower or throughout the entire length of the stipe, despite historic accounts stating otherwise. Therefore, we must surmise that the presence of caulocystidia may not be consistent.

On a separate but related note, this species may not be as rare as I indicated in my previous article and is most probably overlooked and misidentified due to lack of up-to-date information and keys. This is borne out by the provisional names given to some of the collections cited above, such as, *I.* cf. *flocculosa*, *I.* cf. *phaeoleuca*, *I. bruuneoatra* and *I.* cf. *pseudodestricta*. There

are currently five sequence-verified collections.

The problems surrounding *Inocybe griseovelata* are covered more extensively in Penny Cullington's article in *Field Mycology* 14 (1) p.18.

My thanks to Penny, Charles and Eric for their invaluable input.

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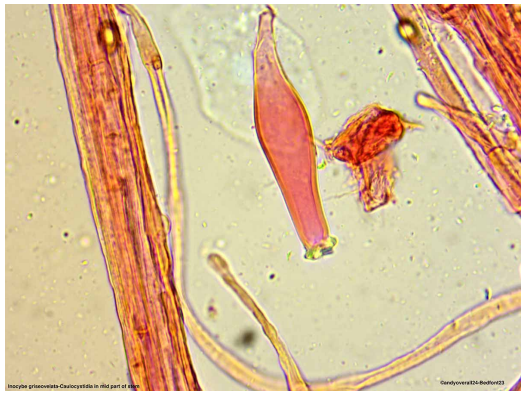

Fig.1. *I. griseovelata,* caulocystidium in central section of stem. Photo © Andy Overall.

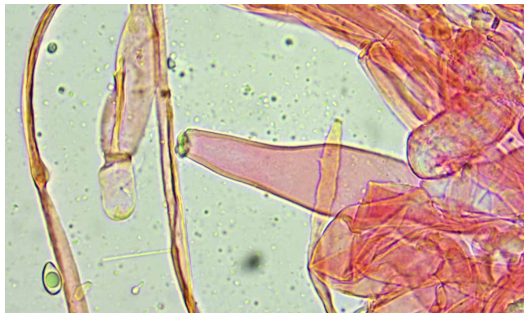

Fig. 2. *I. griseovelata*, caulocystidia on upper stem. Photo © Andy Overall.

Fig. 3. *I. griseovelata,* Netham Park, 19.07.12 caulocystidia from top 3rd of the stem. Photo © Penny Cullington

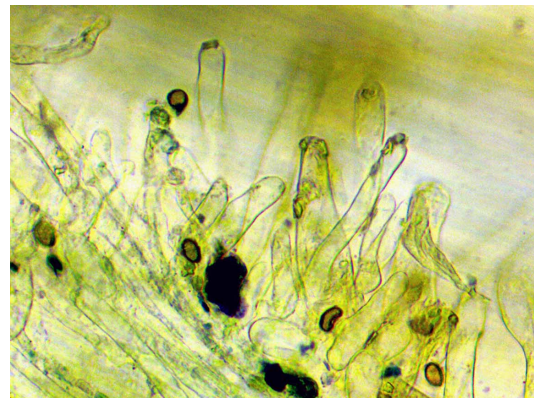

Fig. 4. *I. griseovelata,* caulocystidia 1 cm from stem base, Aberlleiniog, 28.9.21. Photo © Charles Aron.

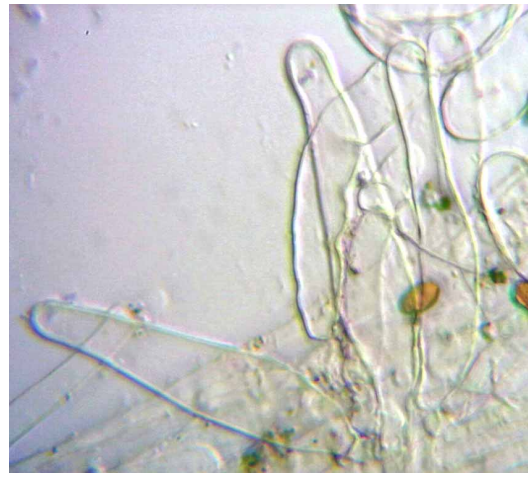

Fig. 5. *I. griseovelata*, caulocystidia from mid-stem. Photo © Eric Janke.

References

Bandini, D. *Inocybe* website www.inocybe.org

Bandini, D. *et al.* (2021). A fresh outlook on the smooth-spored species of *Inocybe*: type studies and 18 new species. *Mycological Progress* 20:1019–1114).

Bandini, D. *et al.* (2023). Noch mehr Risspilze (4): Vierzehn neue Arten der Familie *Inocybaceae*. *Mycologia Bavarica* 23: 1–50.

Kuyper, T.W. (1986). A Revision of the Genus *Inocybe* in Europe. *Persoonia* Suppl. 3:1–247.

Outen, A. R. & Cullington, P. (2015). *Keys to British species of Inocybe*. Edition 3.1. Publ. by the authors.

Stangl, J. (1989). *The Genus Inocybe in Bavaria* English Edition (2011) from www.summerfieldbooks.com

Mallocybe batrachiorum (*Inocybaceae*) a new species found in wet habitats

Charles E. Aron*, Ditte Bandini** & Bernd Oertel***

Abstract. *Mallocybe batrachiorum*, found in moist to wet habitats in Germany, the Netherlands and the United Kingdom is proposed as new to science. The species is described in detail with photos and illustrations of macro- and micromorphology.

Key words: *Inocybaceae*, ectomycorrhizal, ITS, LSU.

INTRODUCTION

The species described here belongs to the new genus *Mallocybe*, recently elevated from subgenus within the *Inocybaceae* (Matheny *et al*., 2020). *Mallocybe* species are often characterized by a tomentose to lanose or scaly pileal surface, a more or less flattened pileus, and usually broadly adnate lamellae. They tend to be rather squat, with a short stipe and a unicolorous ochraceous to reddish-brown pileus. Microscopically, in contrast to species of *Inocybe* s. str., which possess metuloid cheilo- and pleurocystidia, mostly with apical crystals, *Mallocybe* species have thin-walled, often catenate, cheilocystidia while pleurocystidia are absent. Spores are smooth with an obtuse apex and basidia are necropigmented (Bandini *et al.,* 2022a).

Mallocybe species are ectomycorrhizal partners of broad-leaved and coniferous trees, also some shrubs, including *Cistus* in the Mediterranean (Daskalopoulos *et al*., 2021) and occasionally herbaceous plants. They often occur in open habitats such as coastal dunes and slacks, (see e.g., Bon, 1984; Courtecuisse, 1986; Watling & Rotheroe, 1989; Noordeloos, 2020) and in these habitats they frequently associate with *Salix* alongside *Hebeloma*, *Cortinarius* as well as other species of *Inocybaceae* (CEA, personal observations). They also occur as pioneers in quarries and ruderal sites (Bandini *et al*., 2022a). *Mallocybe* species are also widely distributed in Arctic-Alpine regions, associated with dwarf *Salix*, *Betula* and *Dryas* and the herbaceous plant, *Bistorta* (Cripps *et al*., 2010). Exploration of the Swiss and French Alps (Favre, 1955; Kühner, 1988) resulted in the discovery of many new species of *Inocybaceae*, including *Mallocybe* species. In similar habitats they are also known from the Rocky Mountains of North America (Cripps *et al*., 2010) and from Arctic regions such as Greenland (Borgen *et al*., 2006) and Svalbard (Huhtinen, 1987; Gulden & Torkelsen, 1996). They are widely distributed over the globe but over much of the world the genus has remained largely unexplored, however, there are recent discoveries of novel taxa from Africa (Aignon *et al*., 2021), China (Mao *et al*., 2022), and Pakistan (Saba & Khalid, 2020; Saba *et al*., 2023; Naseer *et al*., 2024).

With their rather uniform appearance *Mallocybe* species are a challenge to identify and phylogenetic analysis is more difficult than with other genera of *Inocybaceae* (Bandini *et al*., 2022a). The genus is relatively poorly known in the British Isles, for example, with only eight species listed on the Fungal Records Database for Great Britain and Ireland (FRDBI) while on the Isle of Anglesey alone at least half a dozen species are known to occur (CEA, personal observations), so the true figure for the British Isles as a whole is likely to be somewhat higher. Index Fungorum lists 84 species of *Mallocybe*.

Following Bandini *et al*., 2021, species are only described as new if "*they differ from existing species by the combination of at least three independent characters that are constant among representatives of the new species, and the representatives of the new species are monophyletic in phylogenetic ITS (ITS+LSU) analyses. If constant ecological differences between new species and existing species could be recognized, they were also considered meaningful, but we did not describe species based on ecological or ITS differences, without morphological differentiation*".

MATERIALS AND METHODS

Material was collected on forays from Germany, the Netherlands and the United Kingdom. From fresh material smell, pileus colour, shape and surface texture were noted along with stipe

colour and surface texture, colour and density of lamellae and nature of the lamellar edge. Colour of exsiccata was also recorded as well as habitat and surrounding trees.

Photos of fresh collections were taken in the field and in a studio setting using a Ricoh WG-50 digital camera with a 5x zoom lens and a Panasonic Lumix GH2 with a Leica DG Macro-Elmarit 1:2.8/45-mm lens. Colour codes are taken from Munsell (2009, as 'Mu'); terminology follows Vellinga (1988) and Kuyper (1986). Fungarium acronyms are according to Holmgren *et al*. (1990).

Microscopic structures, including cheilocystidia, caulocystidia, spores and basidia were examined from fresh or dried material in water and 3% KOH. Photographs of microscopic elements were made using a Zeiss AxioCam ERc5s and an LCMOS videocam. Measurements were made using Zeiss Axiovision version 4.8 and Toupview software. 120 spores and 45 basidia (not including sterigmata) and cystidia were measured from 3 collections of the new species. All measurements are given as length by width. Q values of microscopic elements were calculated (length divided by width). The microplate was drawn by D. Bandini. TK25 and SH refer to German and UK mapping grids, 1:25,000.

The DNA of specimens was extracted and the ITS and LSU regions sequenced by Alvalab (Oviedo, Spain, http://alvalab.es/). Sequences were assembled and edited using Geneious (version 6.1.2, Biomatters Ltd., Auckland, New Zealand). Forward and reverse sequences were merged, edited if necessary, and a consensus sequence was generated for every sample. Sequences were submitted to GenBank. FASTA sequences were studied in MEGA 11 (https://megasoftware.net/; Tamura *et al*., 2021). The comparative evaluation of the ITS sequences to record the adjacent species of the new *Mallocybe* species were made with BLAST analysis (Altschul *et al*., 1990). If necessary, the sequences were trimmed to the exact length of ITS or LSU. In GenBank (https://www.ncbi.nlm.nih.gov/genbank/) the setting "Optimize for somewhat similar sequences, blastn" and in UNITE (https://unite.ut.ee/) the massBLASTer was used. Collections of unpublished private ITS or LSU sequences were used for comparison using the GenBank tool "Align two or more sequences", the so-called "Local BLAST" method, using only ITS sequences of full length or LSU sequences as references. This avoids the frequent BLAST errors which occur when comparing with reference sequences that are too short.

RESULTS AND DISCUSSION

Following morphological and molecular analysis we hereby introduce *Mallocybe batrachiorum* as a species new to science based on its distinct morphology and phylogeny. The new species belongs to the genus *Mallocybe* and it is genetically well-delimited from other species of the *Delecta* clade of this genus (Matheny *et al*. 2023).

Taxonomy

***Mallocybe batrachiorum* Aron, Bandini & B. Oertel sp. nov.**

Registration Identifier: Mycobank MB 853975.

Fig. 1.

Etymology: The epithet 'batrachiorum' refers to *Batrachia*, a superorder of amphibians, as the species grows in wet places favoured by them.

Holotype UNITED KINGDOM, Wales, Anglesey, VC52, Penrhoslligwy, Mynydd Bodafon, SH48308553, alt. 28 m, on boggy ground with *Alnus glutinosa*, *Betula pubescens* and *Picea sitchensis*, 22 Aug. 2021, leg. C.E. Aron CEA4539, (holotype K-M001442629; isotype priv. fung. DB, DB22-8-21-Aron). GenBank ITS & LSU: PP789108 (from isotype).

Diagnosis

Mallocybe batrachiorum is a small to medium-sized, relatively slender species with brownish to ochraceous, smooth to finely tomentose cap, a fibrous stipe with a yellowish base, especially when young, smooth spores measuring 7.9–11.1 (av. 9.3) x 4.7–6.4 µm (av. 5.4 µm), thin-walled, rather inconspicuous, catenate cheilocystidia and hyphoid, segmented caulocystidia confined to the extreme stipe apex. Found on moist to wet, acid ground with *Alnus glutinosa*, *Betula pubescens*, *Picea abies*, *Picea sitchensis*, *Salix caprea* and *Salix repens*. This combination of characters, as well as the ITS sequence data distinguishes *M. batrachiorum* from all other species in the genus *Mallocybe*. The closest known genetically related species is *M. himalayana*.

Description

Pileus 15–40 mm wide, campanulate or domed-

convex when young, becoming convex, conico-convex, then broadly convex or expanded, usually without, rarely when young with a low, broad umbo. Margin at first strongly incurved, later decurved to straight or even uplifted when old. With age depressed, sometimes with a slight umbo, no remnants of a velipellis observed. Colour at first brownish, often with a paler margin, later usually ochraceous in different nuances, sometimes with an orange tinge at the centre, sometimes up to foxy- or rusty- ochraceous or foxy to rusty with a reddish tinge, especially towards the centre (Mu 10YR 6/6-6/8; 7.5YR 4/8, 5/6–5/8, 6/8; 5YR 5/8, 6/6–6/8, 5/6–5/8; 2.5YR 2/8, 4/10, 5/12). Surface smooth to finely tomentose, at the centre pubescent to minutely squamulose, with age somewhat excoriate towards the margin. Young basidiomata cortinate at first, later with remnants of a pale ochraceous cortina.

Lamellae moderately crowded to rather crowded (c. 35–60), broadly adnate, sometimes with decurrent tooth, edge even, to wavy in mature specimens; at first (pale) cream with a greyish hue, later (pale) ochraceous with or without a greyish to brownish hue; with age reddish brown, snuff-brown or umber; edge concolorous or pale.

Stipe 28–55 × 2–4 mm, straight, or curved, especially towards the base; when young covered with a fine, pale straw-coloured tomentum, later longitudinally striate or glabrous, ochraceous-brownish to brownish with or without a greyish hue or brownish with a reddish hue; at the base mostly faintly to rather intensely yellowish; pruinose only at the apex.

Context at first (watery) pale whitish in the pileus, later up to greyish-brownish; pale ochraceous in the stipe, at the base pale to bright yellowish when young; stipe hollow with age.

Smell indistinct to slightly sweetish, slightly spermatic when cut.

Colour of exsiccata in pileus yellow-brown to brown with reddish hue (Mu 7.5R 4/8, 5YR 2/6, 5/6–5/8), lamellae and stipe concolorous or a little lighter in colour, no darkening or blackening on drying.

Spores 7.9–11.1µm (av. 9.3 µm, SD 0.7µm) x 4.7–6.4 µm (av. 5.5 µm, SD 0.4 µm); Q = 1.5–2.2 (av. 1.7, SD 0.1) (n = 120 of 3 coll.), larger in the holotype (av. 10.1 x 5.6 µm); smooth, (sub)amygdaloid, (sub)ellipsoid, without or with only faint suprahilar depression, apex obtuse.

Basidia (27–36 µm x 7–9µm) generally 4-spored, rarely also 2-spored.

Lamellae edges sterile, with cheilocystidia, or partly fertile, intermixed with basidia.

Cheilocystidia 16–40 µm (av. 24 µm, SD 5 µm) x 6–14 µm (av. 10 µm, SD 2 µm); Q = 1.7–3.4 (av. 2.6, SD 0.5) (n = 45 of 3 coll.) (sub)cylindrical to subclavate, sometimes somewhat deformed, often catenate (1-4 segments) with the final element always being the longest one.

Caulocystidia only near the apex of the stipe, consisting of narrow, segmented hyphoid elements up to about 50µm long.

Pileipellis constituted by an epicutis made up of parallel hyphae 9–25 µm wide, with coarsely encrusting yellowish pigment and sometimes with pale yellow parietal pigment.

Ecology and distribution

Mallocybe bactrachiorum is, thus far, only known from single sites from Germany, the Netherlands, and the United Kingdom from close to sea level to 860 m ASL. From soil and environmental samples in UNITE it appears that this species is also present in Estonia and Belgium. All the collections were from wet ground on acid soils, in woods of *Alnus glutinosa*, *Betula pubescens*, *Picea abies*, *P. sitchensis*, *Salix caprea* and *S. repens,* thus it may associate with a wide range of trees, including both broad-leaved and conifers. Climate ranges from very oceanic/cool temperate on Anglesey (UK) to more continental in Germany. Given the widespread distribution, climatic amplitude, and range of associated trees this species might be expected to occur more commonly but could easily be overlooked in its boggy habitats.

In the German and Welsh localities the species was closely associated with *Sphagnum* and other mosses in very boggy, acid habitats. In contrast the Netherlands collection was found on more or less pure sand but again in a very moist, acidic location. At the Welsh site it has been found fruiting from August to October but has been absent most years and was not detected when the area was thoroughly searched for macrofungi in 2014.

The Welsh collections, including the holotype, were made in an area of carr woodland within a spruce plantation. Other fungi recorded from the site include *Cortinarius bibulus*, *Hebeloma fusisporum*, *Naucoria* species, *Lactarius lilacinus* and

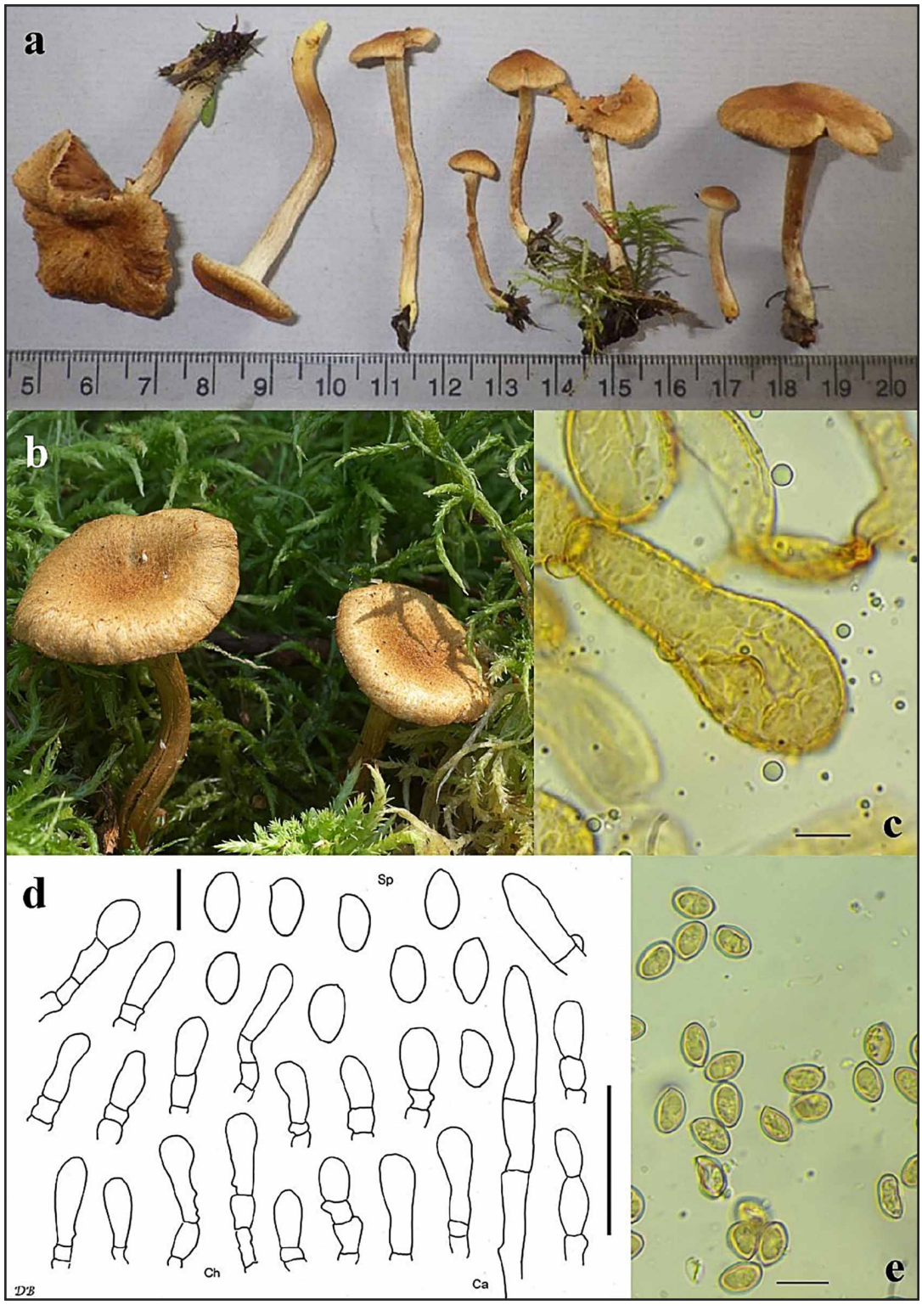

Fig. 1. *Mallocybe batrachiorum*. **a**-coll. CEA4539 (holotype K-M001442629). **b**-coll. DB 20-7-22-5. **c**-encrusted hyphae of pileipellis, CEA4539, scale bar: 10 µm. **d**-microscopic characters (CEA4539), Ca-caulocystidia, Ch-cheilocystidia, Sp-spores, scale bar spores: 10 µm, scale bar cystidia: 20 µm. **e**-spores (CEA4539), scale bar: 10 µm. Photographs © C.E. Aron (a, c, e), D. Bandini (b); drawing D. Bandini.

Lactarius tabidus. The surrounding spruce plantation has proven very rich in mycorrhizal fungi, especially *Cortinariaceae*, including the novel taxon, *Thaxterogaster monaensis* Liimatainen, Wang & Niskanen (see also Liimatainen *et al*., 2022 and Aron, 2023). Other species of *Inocybaceae* were generally scarce and included *Inocybe napipes* and *I. stellatospora*, on the more acidic areas of the forest, with *I. ochroalba* and *I. sindonia* on the more base rich soils.

The species is also known from Belgium through an environmental sequence in GenBank (MH815167). Additionally, there are 20 soil sequences from Estonia in UNITE.

Other collections studied

GERMANY, Bayern, Ostallgäu, Roßhaupten, Senkele, TK25 8330/1, alt. 860m ASL, amidst *Sphagnum* in very wet boggy location with Picea abies, 20 Jul 2022, leg. D. Bandini, det. D. Bandini & B. Oertel, (SMNS-STU-F-0901843, DB20-7-22-5, GenBank ITS & LSU: PP761176). NETHERLANDS, Friesland, Ameland, Hollum, with *Salix caprea* and *Salix repens*, 19 Sep 2011, leg. & det. D. Bandini (SMNS-STU-F-0901842, DB19-9-11-13, Genbank ITS: PP761171); United Kingdom, Wales, Anglesey, Mynydd Bodafon, Penrhoslligwy, SH48308553, alt. 28m ASL, on boggy ground with *Alnus glutinosa*, *Betula pubescens* and *Picea sitchensis*, 20 Oct 2016, leg. C.E. Aron, det. D. Bandini (CEA3938, UDB0754135).

Comments

One of the most striking features of our new species is the yellow stipe base, especially marked in young specimens. Microscopically, this species is confusing as it appears to lack cheilocystidia, however, cheilocystidia are present but difficult to find and easily confused with basidioles. Indeed, this phenomenon also prompted Ludwig (2017) to describe *Inocybe* (*Mallocybe*) *acystidiata*. However, close examination of the type material by D. Bandini revealed that cheilocystidia were indeed present.

Similar species based on sequence data

Mallocybe himalayana Y.G. Fan, R. Khurshid & A. Naseer, a recently described and genetically quite similar Asian species has similar coloration to *M. batrachiorum* but the pileus is scalier and the stipe more heavily fibrous and lacking a yellowish base. Microscopically, it has shorter cystidia and the elements of the epicutis are less inflated. It appears to associate with conifers and there is no mention of a preference with moist habitats in the type description (Naseer *et al*. 2024). It exhibits the highest similarity regarding its ITS sequence with a match of 97% (with a difference of 9 base pairs and 11 gaps).

Mallocybe siciliana (Brugal., Consiglio & M. Marchetti) Brugal., Consiglio & M. Marchetti has a match of 96% and is rather similar in habit and colour and occupies moist habitats but usually on more basic soils. However, it has a (sub)squarrose to (sub)squamulose pileal surface with copious veil remnants and lacks a yellow stipe base. Microscopically, it has wider, balloon-shaped cystidia (Brugaletta *et al*., 2017).

Also close are *M. delecta* (P. Karst.) Matheny & Esteve-Rav. and *M. nuptialis* Bandini, B. Oertel & U. Eberh., both with a match of 95%.

Other morphologically similar species

Mallocybe agardhii (N. Lund) Matheny & Esteve-Rav. has a pronounced fibrous ring and copious whitish velipellis. Also, it grows on basic, often sandy soils, confined to *Salix* (Lund, 1845).

Mallocybe leucoloma (Kühner) Matheny & Esteve-Rav. (Kühner, 1988) and *M. subtomentosa* (Peck) Matheny & Kudzma, also differ in having a whitish velipellis (Peck, 1897). *M. leucoloma* has longer spores and develops a (sub)lanose pileal surface with age, while *M. subtomentosa* has shorter spores and grows with conifers, usually in drier locations.

M. cotoneovelata (E. Ludw.) Matheny & Esteve-Rav. also occurs with conifers, on drier, often sandy soils and has smaller spores on average (Ludwig, 2017). Also, *M. cotoneovelata* tends to have more robust basidiomata than *M. batrachiorum*.

M. arthrocystis Kühner has a more conical, vividly coloured, mostly entirely pubescent, pileus (Kühner, 1988; Cripps *et al*., 2010). *Mallocybe hebelomoides* Matheny & Esteve-Rav. differs, apart from the alpine habitat, in having a rather speckled, ochre-brown pileus which is less smooth with age (Kühner, 1988).

M. plebeia Bandini, B. Oertel & U. Eberh. differs in its wider, sometimes almost truncate spores and growth on drier, basic soils. This taxon does show some yellowing on the stipe but

does not have a yellow stipe base (Bandini *et al*, 2022b).

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We extend our sincere thanks to Rubab Khurshid from the Fungal Biology and Systematics Laboratory at the Institute of Botany, University of the Punjab, Lahore, Pakistan, for providing us with the ITS sequences of the holotypes of *Mallocybe himalayana* and *M. kashmirana*. Ursula Eberhardt is thanked for supplying us with sequence data for material deposited at the STU collection.

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References

Aignon, H.L., Naseer, A., Matheny, B.P., Yorou, N.S, & Ryberg, M. (2021). *Mallocybe africana* (*Inocybaceae*, *Fungi*), the first species of *Mallocybe* described from Africa. *Phytotaxa* Vol. 478(1): 49–60. https//doi.org/10.11646/phytotaxa.478.1.3

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. (1990). Basic local alignment search. *Journal of Molecular Biology* 215: 403–410. doi: 10.1016/S0022-2836(05)80360-2

Aron, C.E. (2023). *Thaxterogaster monaensis* recently described from Anglesey. *Field Mycology* 25(2): 59–60.

Bandini, D., Bandini, G., Bensch, K., Rodríguez Campo, F.J., Oertel, B., Valade, F. & Eberhardt, U. (2023). Three new smooth-spored species of *Inocybe*, two new epitypes, and updates on earlier epitypifications. *Integrative Systematics* 6 (2): 1–37. https://doi.org/10.18476/2023.787646

Bandini, D., Oertel, B. & Eberhardt, U. (2021). A fresh outlook on the smooth-spored species of *Inocybe*: type studies and 18 new species. *Mycological Progress* 20: 1019–1114. https://doi.org/10.1007/s11557-021-01712-w

Bandini, D., Oertel, B., Eberhardt, U. (2022a). Noch mehr Risspilze (3): Einundzwanzig neue Arten der Familie Inocybaceae. *Mycologia Bavarica* 22: 31–138. https://doi.org/10.1007/s11557-021-01712-w

Bandini, D., Oertel, B. & Eberhardt, U. (2022b). More smooth spored species of *Inocybe* (*Agaricales*, *Basidiomycota*): type studies and 12 new species from Europe. *Persoonia* 48: 91–149. https://doi.org/10.3767/persoonia.2022.48.03.

Bandini, D., Oertel, B., Eberhardt, U. (2024). Even more fibre-caps (5): Eleven new species of the family *Inocybaceae* and epitypification of *Pseudosperma rimosum*. *Mycologia Bavarica* 24: 1–52.

Bon, M. (1984). Macromycètes de la zone maritime picarde (8ème supplément). Les inocybes sabulicoles. *Documents Mycologiques* 14: 11–17.

Borgen, T., Elborne, E., & Knudsen, H. (2006). A checklist of the Greenland Basidiomycetes. Greenland Bioscience 56: 37–59 (*Arctic and Alpine Mycology* 6).

Brugaletta, E., Consiglio, G., Marchetti, M. (2017). *Inocybe siciliana*, una nuova specie del Sottogenere *Mallocybe*. *Riv. Micol.* 60(3): 195–209.

Courtecuisse, R. (1986). Transect mycologique dunaire sur la Cote d'Opale (France) II: Les groupements de l'hygrosère. *Documents Mycologiques* 17(66):1–70.

Cripps, C.L., Larsson, E., Horak, E. (2010). Subgenus *Mallocybe* (*Inocybe*) in the Rocky Mountain alpine zone with molecular reference to European Arctic-alpine material. *North American Fungi* 5 (5): 97–126. DOI:10.2509/naf2010.005.0057

Daskalopoulos, V., Polemis, E., Fryssouli, V. *et al*. (2021). *Mallocybe heimii* ectomycorrhizae with *Cistus creticus* and *Pinus halepensis* in Mediterranean littoral sand dunes — assessment of phylogenetic relationships to *M. arenaria* and *M. agardhii*. *Mycorrhiza* 31: 497–510. DOI: 10.1007/s00572-021-01038-1

Favre, J. (1955). Les champignons supérieurs de la zone alpine du Parc National Suisse. *Ergebnisse der wissenschaftlichen Untersuchungen des Schweizerrischen Nationalparks* 5: 1–212.

Gulden, G. and A-E. Torkelsen (1996). Part 3. Fungi I. *Basidiomycota*: *Agaricales*, *Gasteromycetales*, *Aphyllophorales*, *Exobasidiales*, *Dacrymycetales* and *Tremellales*. Pp. 173-206. In: Elvebakk, A. and P. Prestrud, (eds.): A catalogue of Svalbard plants, fungi, algae and cyano-bacteria. *Norsk Polarinstitutt Skrifter* 198.

Holmgren, P.K., Holmgren, N.H., Barnett, L.C., (1990). *Index Herbariorum*, 8th edn. Botanic Garden, New York.

Huhtinen, S. (1987). New Svalbard Fungi. Pp. 123-151. In: Laursen, G.A., J.F. Ammirati and S.A. Redhead (eds), *Arctic-alpine Mycology* II, Plenum Press, New York. Pp. 364.
Kühner, R. (1988). Diagnoses de Quelques Nouveaux Inocybes récoltés en Zone Alpine de la Vanoise (Alpes Françaises). *Documents Mycologiques* 19(74):17–22.
Kuyper, T.W., (1986). A revision of the genus *Inocybe* in Europe I Subgenus *Inosperma* and the smooth-spored species of subgenus *Inocybe*. *Persoonia* Suppl: 3.
Liimatainen, Wang, Savage, Niskanen & Kytövuori. (2022). *Index Fungorum* 524: 2.
Ludwig, E. (2017). *Cortinariaceae* I: *Cortinarius* (part I), *Galerina*, *Hebeloma*, *Hebelomina*, *Inocybe*, *Phaeogalera*. *Pilzkompendium* 4: 143–152.
Lund, N., (1845). *Conspectus Hymenocetum Holmiam Crescentium* (Christianiae): 40.
Mao, N., Xu, Y.-Y., Lv, J.-C., Fan, Li, (2022). New Species of *Mallocybe* and *Pseudosperma* from North China. *Journal of Fungi* 8(3): 256. https://doi.org/10.3390/jof8030256
Matheny, P.B., Hobbs, A.M., Esteve-Raventós F. (2020). Genera of Inocybaceae: new skin for the old ceremony. *Mycologia* 112(1): 83–120.
doi: 10.1080/00275514.2019.1668906
Matheny, P.B., *et al.* (2023). A phylogeny for North American *Mallocybe* (*Inocybaceae*) and taxonomic revision of eastern North American taxa. *Fungal Systematics and Evolution* 12: 153–201.
doi: 10.3114/fuse.2023.12.09.
Munsell, O. (2009). *Soil Color Charts*. X-Rite Grand Rapids MI.
Naseer, A., Khurshid, R., Fan, Y.-G., Khalid, A.N. (2024), Two new species of *Mallocybe* from Himalayan forests of Asia. *Phytotaxa* 640(3): 255–264;
doi: 10.11646/phytotaxa.640.3.3
Noordeloos, M.E., (2020). *Paddenstoelen* III. KNNV Uitgeverij.
Peck (1897). Report of the State Botanist 1894. *Ann. Rep. NY St. Mus*. 48: 109.
Saba, M., Khalid, A.N. (2020). *Mallocybe velutina* (*Agaricales*, *Inocybaceae*), a new species from Pakistan. *Mycoscience*, 61(6): 348–352.
https://doi.org/10.1016/j.myc.2020.06.006
Saba, M., Khalid, A.N., Sarwar, S, (2023). New species of *Mallocybe* (*Agaricales*, *Inocybaceae*) from Pakistan, based on morphological and molecular evidence. *Mycokeys* 99: 171–186.
https://doi.org/10.3897/mycokeys.99.86844
Tamura, K., Stecher, G., Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* 38(7): 3022–3027.
doi: 10.1093/molbev/msab120.
Vellinga, E.C. (1988) Glossary. In: Bas, C., Kuyper, T.W., Noordeloos, M.E., Vellinga, E.C. (eds), *Flora Agaricina Neerlandica* Vol. 1. A.A. Balkema, Rotterdam.
Watling, R., Rotheroe, M. (1989). Macrofungi of sand dunes. *Proceedings of the Royal Society of Edinburgh Section B Biological Sciences* 96: 111–126.
doi: https://doi.org/10.1017/S0269727000010885

Psathyrella kellermanii: a little known and under-recorded species

Sandra Bell*, Mike Harrison** & Eric Janke***

On 17th August 2023, SB found some rather striking mushrooms with what looked like a heavy coating of icing sugar growing in an outdoor lettuce pot; she was intrigued to find out what they were. Having some mycological knowledge, she was careful to collect one of the sporophores and make a spore-print which was black suggesting some form of *Psathyrella*.

As the said pot was in Aldermaston, she contacted MH, who runs the Thames Valley Fungus Group. He was far from sure what SB had found, with *P. hirta* being the nearest macroscopic match but this was listed as a dung species. MH also contacted the Hampshire Fungus Recording Group but they had no better suggestions. The pot was subsequently transferred to MH and finally to EJ, in whose greenhouse it produced another tuft of mushrooms at the end of August.

Microscopic examination showed slightly capitate cheilocystidia, some with more than one "head", so not matching *P. hirta*, which has lageniform-fusiform and small clavate cheilocystidia.

As has now become normal in these circumstances, EJ tried to amplify the ITS barcode region and obtained a decent sequence. Searching the GenBank database revealed two matching sequences under the name of *Psathyrella kellermanii* (KC992920 from Belgium and MK053807 from Germany).

Our searches have revealed little information about this species other than its original description by Peck in 1906 (as *Galera kellermanii*) from a single collection from a greenhouse in Columbus Ohio owned by a Dr. W. A. Kellerman after whom the species was named. It was transferred into *Psathyrella* in 1959 by Singer, who comments that it "is very rare in the United States and obviously introduced with plant material".

In addition to the original collection in 1906, GBIF contains one record from Germany in 2015, two (of the same collection?) from France in 2010, three (again, from the same location) from Belgium in 1996 and one from Cincinnati in Ohio in 1933. None of these records specify whether the collections were from outside or under cover. Clearly it is a rare or very under-recorded species.

The macroscopic and microscopic features are quite distinctive and even without the benefit of molecular analysis, it is clear from the description that we are looking at the same species – once one knows what to compare it against. The absence of the species from the literature must surely have exacerbated any under-recording and we hope that this article will go some way to remedy the situation.

Description

Sporocarps 10–30 mm grey-brown with granular appearance, at least when young.
Stem 30–50 x 1.5–3 mm, white, pruinose over whole length.
Habitat on compost in a plant-pot.
Smell indistinct – "mushroomy".

Microscopy

Granular material on pileus composed of a mixture of hyphae and pyriform-globose 20–45 µm in diameter.
Cheilocystidia: utriform-capitate, some with multiple "heads" 35–55 x 10–15 µm.
Caulocystidia: similar but slightly less capitate. 35–45 x 10–20 µm.
Spores: 8–10 x 4.5–6 µm with 1 µm germ-pore.

Discussion

Given the apparent scarcity of the species, one might wonder how it turned up in a plant pot in Aldermaston. SB has tried to investigate the provenance of the compost in the pot in which the mycelium seemed to have been growing and that used to cultivate the transplanted lettuce seedling. The bulk in the pot was a multipurpose mix comprising composted conifer bark from UK forestry crops with a little composted green waste and a small proportion of coir. The bark and green waste are both of UK origin but it is not possible to trace the ingredients to their original

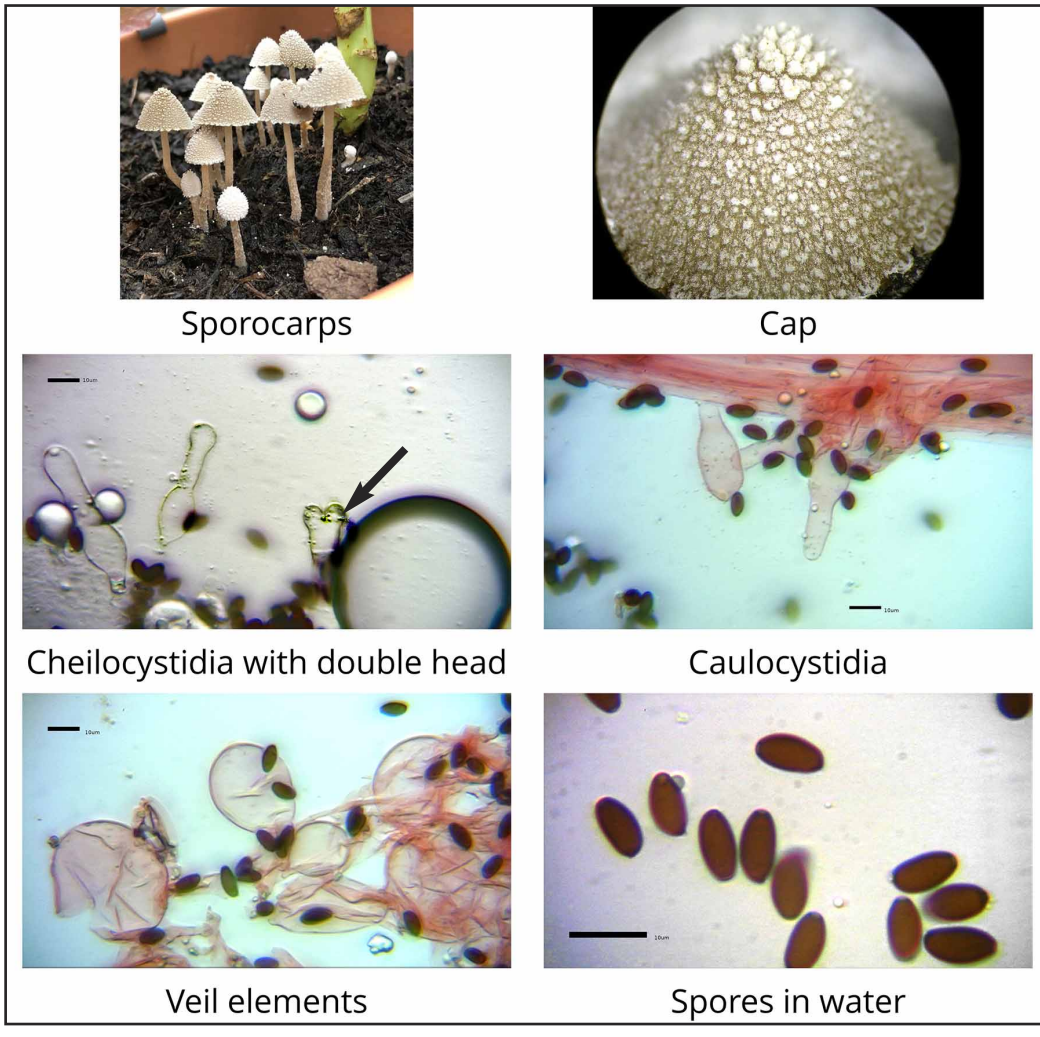

Fig 1. Photographs showing sporocarps, cheilocystidia, caulocystidia and veil elements at x400 and spores in water at x1000. Photos © Eric Janke.

locations. The seedlings were raised in a 60% peat and 40% coir and wood fibre mix but the manufacturers have been reluctant to provide further details. Neither the suppliers of the bulk of the compost or of the seedlings recognised photographs of the final sporocarps. This leads us to conclude that the mycelium was either present in one of the original components of the composts or that stray spores from an unknown source entered the process at some point.

EJ has recorded a number of unusual species from his greenhouse, all growing in DIY compost, so it is possible that the latter is a more likely conclusion and should encourage readers to look for specimens in their own plant pots over the summer.

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References

Peck C. H. (1906). A new species of *Galera*. *J. Mycol*. 12(4): 148–149.
Singer R. (1959). New and Interesting Species of Basidiomycetes VI, *Mycologia* 51(3): 392 .

A puzzling, perhaps undescribed *Conocybe* from a plant pot

Graham Mattock*

It's amazing what you can find at your local garden centre. During such a visit in Winchester, Hampshire in February 2024 I noticed a cluster of small brown mushrooms fruiting from the compost in a pot of Rosemary. The strongly sulcate, hygrophanous caps ranged in colour from light ochre to greyish beige; the slightly lined, mottled stems were also variously coloured with no sign of a ring or ring zone at the apex, having a slight white bulb at the base. A red-brown spore deposit had elliptical, smooth spores averaging 11.75 x 7.25 µm, with a small germ pore. Cap cuticle was cellular, all confirming my suspicions that this was a *Conocybe* (Fig. 1).

Using Anton Hausknecht's *Conocybe* key in *Funga Nordica* (Knudsen & Vesterholt, 2012) my specimen with a mixture of hair-like and lecythiform caulocystidia at the stem apex placed it in sect. *Pilosellae*. Continuing with the key I reached *C. fuscimarginata* (Murrill) Singer, a species described from Florida and whose description fitted well with my collection.

Fig. 1. A *Conocybe* species found in a plant pot in Winchester, February 2024. Photo © Graham Mattock.

As there are only 12 records for *C. fuscimarginata* on the FRDBI database I asked Eric Janke if he would DNA sequence my collection. A good clean sequence was obtained but was only a closest match of 95.7% to a sequence labelled *C. fuscimarginata* on GenBank i.e. not close enough. Using all the *C. fuscimarginata* sequences on GenBank Eric produced the accompanying phylogenetic tree (Fig. 2). There appear to be three obvious clades containing sequences labelled as *C. fuscimarginata*: one adjacent to my collection (which is labelled EWJ240322-06 in red), containing Swedish, Canadian and German sequences. A second but distinct group containing sequences from Venice and China, and a third which is nowhere close, containing sequences from Stuttgart and Venice. The Swedish sequence is perhaps significant because this is referenced in a paper co-authored by Hausknecht (Toth, 2013). However, none of the sequences on GenBank are from the holotype.

So unless a sequence from the holotype is forthcoming my collection remains as *Conocybe* cf. *fuscimarginata*. Sequencing can throw up as many questions as it answers…

Acknowledgements

I thank Eric Janke for sequencing my collection and for providing the phylogenetic tree.

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References

Knudsen, H. Vesterholt, J. Eds. (2012). *Funga Nordica*. Nordsvamp.

Toth, A., Hausknecht, A., Krisai-Greilhuber, I., Papp, T., Vagvolgyi, C. & Nagy, L. (2013). *Iteratively Refined Guide Trees Help Improving Alignment and Phylogenetic Inference in the Mushroom Family Bolbitiaceae*. PLOS ONE, Open Access. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0056143

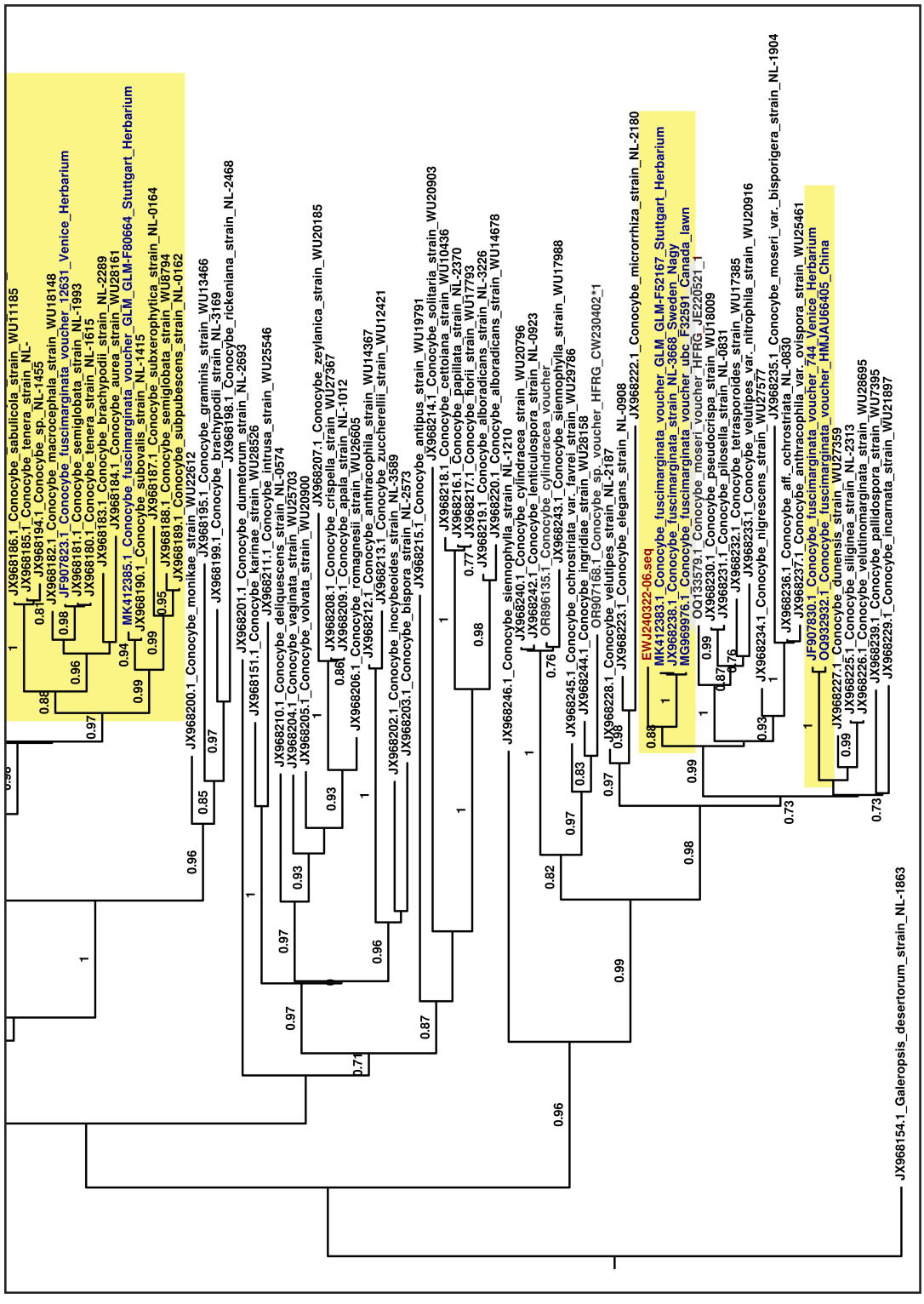

Fig. 2. Part of a phylogenetic tree produced in GenBank showing three clades which each contain sequences labelled as *C. fuscimarginata*. The Hampshire collection is in red. Phylo tree produced by Eric Janke.

Ophidiomyces ophidiicola in Britain, the cause of ophidiomycosis (snake fungal disease)

Steven J.R. Allain* & Tony Leech**

If it is the case that plant pathogenic fungi are under-recorded on FRDBI (Henrici, 2021), animal pathogenic fungi are likely to be even less well recorded. This is because unambiguous identifications can rarely be made from observing the lesions present, and the techniques available for identification are not generally available to the field mycologist. However, skin infections caused by the keratinophilic fungus *Ophidiomyces ophidiicola*, which has been recently shown to occur in Britain, are visible on the sloughed skins of Barred Grass Snakes (*Natrix helvetica**) and so could come to the attention of field mycologists.

Ophidiomyces ophidiicola was first described (as *Chrysosporium ophiodiicola*) by Rajeev *et al.* (2009) from a captive Black Rat Snake (*Elaphe obsoleta obsoleta*) that had been taken from the wild in Georgia, USA. It was shown to be distinct from other *Chrysosporium* anamorphs that cause scale infections in a wide variety of reptiles by its sequence in the ITS region and the morphology of its conidia. *Chrysosporium ophiodiicola* was reported to produce conidia measuring 4–6.5 (9) x 2–3 µm at the termini of hyphae and on lateral branches, and arthroconidia measuring 7.5–10 x 2–2.5 µm from fragmenting hyphae.

The genus *Ophidiomyces* was erected to accommodate this fungus in 2013 when DNA sequencing showed it to be genetically distinct from members of the genus *Chrysosporium*. The teleomorph is not known. The fungus belongs to the order *Onygenales*, members of which are able to decompose keratin, the major protein component of skin, hair and feathers.

Ophidiomycosis, or snake fungal disease (SFD), for which *O. ophidiicola* is the primary causative agent, was first recognised in 2006 in Timber Rattlesnakes (*Crotalus horridus)* in New Hampshire, USA. The disease has now been recorded in dozens of snake species in both North America and Europe (Burbrink *et al.*, 2017; Blanvillain *et al.*, 2024). In a retrospective examination of pathology material collected from captive snakes at the Smithsonian National Zoological Park, Washington DC, USA, the fungus was found in seven species of snake with the earliest record from 1983 (Anderson *et al.*, 2021). The first British record of the disease in which association with *O. ophidiicola* was confirmed was in 2015 from a Grass Snake found in eastern England (ARC Factsheet), although the earliest case of the disease known so far dates back to 2010 thanks to the use of a slough archive (Franklinos *et al.*, 2017). The locations of these occurrences have not been published. So far there have been a limited number of cases from both wild and captive snakes recorded in Asia (Sun *et al.*, 2022; Takami *et al.*, 2021), as well as in Africa and South America. This means that we currently lack the data to determine whether *O. ophidiicola* is native to Great Britain or whether it has been introduced. So far, no species of lizards have been found to be infected with *O. ophidiicola*.

Clinical signs of ophidiomycosis and the severity of disease vary from species to species. The most common signs, seen on the ventral surface of the snake, include: flaking and crusting of the scales, displaced or discoloured scales, more frequent moulting and the swelling of infected tissues (Figs. 1 & 2). The appearance of clinical signs may be affected by co-infections with other fungi or bacteria. Lesions can be seen on sloughed skins. In some snakes, the effect of the fungus appears superficial and seems to cause no serious issues. Indeed, it is possible for *O. ophidiicola* to be detected by PCR on a snake without

[*Footnote: Recent taxonomic revision has resulted in the native British grass snake formerly known as the Common Grass Snake (*Natrix natrix helvetica*) becoming the Barred Grass Snake (*N. helvetica*). For more information, see Kindler *et al.* (2017)]

Fig. 1. Ventral scales of a Barred Grass Snake showing clinical signs of ophidiomycosis including changes in colour, crusting and scale margin erosion. For more details see Allain *et al*. (2024). Photo: Steven Allain.

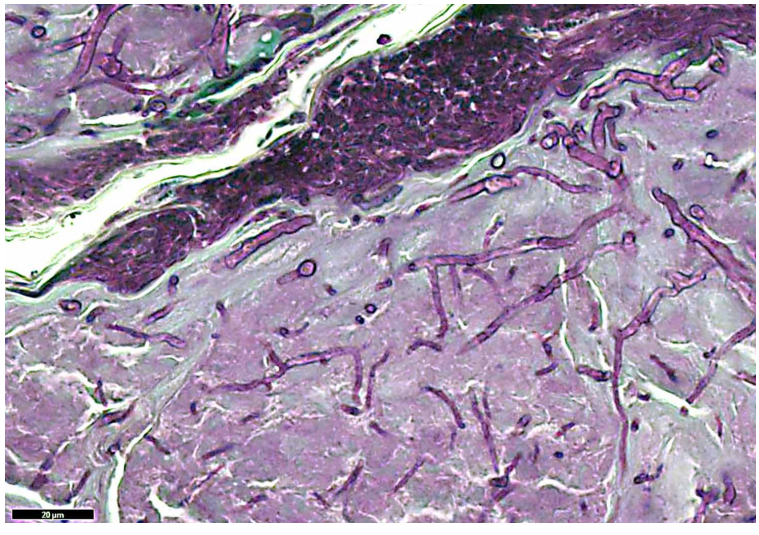

Fig. 2. Photomicrograph showing numerous branching fungal hyphae of *Ophidiomyces ophidiicola* associated with an area of epidermal thickening and necrosis. Periodic acid-Schiff stain; magnification 600x. Reproduced with permission from the Zoological Society of London.

observable symptoms – perhaps as a consequence of subclinical infection. Conversely, the disease may progress to infect internal organs, and in some snake populations in North America mortality is significant (Lind *et al*., 2018). Effects on behaviour, particularly a tendency for snakes to spend more time in the open thus increasing the incidence of hypothermia and predation, may contribute to mortality.

Between 2010 and 2016, 33 carcasses and 302 moulted skins of three species of snake were collected in Britain and examined for skin lesions and the presence of *O. ophidiicola* DNA (Franklinos *et al*., 2017). Skin lesions were observed in nearly one quarter of the samples, and, of these, about half of the Grass Snake samples showed the presence of *O. ophidiicola* DNA. No such DNA was detected on any of the seven Smooth Snake (*Coronella austriaca*) samples with lesions. One Adder (*Vipera berus*), without skin lesions, was PCR positive. This study was the first demonstration of *O. ophidiicola* in wild European snakes and also showed that the presence of this fungus could not be assumed from the appearance of the lesions alone.

From 2019 to 2021, one of us (SJRA) studied the large population of *N. helvetica* at Watermill Broad, Cranwich, in south-west Norfolk (TL7795), where almost 30% of individual snakes identified had skin lesions consistent with ophidiomycosis (Allain *et al*., 2024). The majority of

these were positive for *O. ophidiicola* DNA. As well as using PCR to determine the presence of *O. ophidiicola*, histology was carried out to detect the fungal hyphae and arthroconidia associated with the fungus in some *N. helvetica* carcasses and skin sloughs.

It is assumed that transmission of ophidiomycosis is by spores acquired from the environment or by direct contact between snakes. This clearly has implications for workers handling snakes from different sites, and strict biosecurity protocols should be used when moving between sites where populations of snakes are known. This does not just affect those of us working with wild reptiles, but everyone who utilises the countryside to observe the natural world, as we could be inadvertently spreading the spores around to unsuspecting populations of our vulnerable reptiles.

Acknowledgements

We would like to thank past and present Trustees of Watermill Broad Nature Reserve, Cranwich, Norfolk, for granting us access to study the population of *N. helvetica* present at the site. Additionally, SJRA would like to thank his supervisory team and collaborators for the support throughout his doctoral studies as described above.

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References

Allain, S.J.R., *et al*. (2024) Characterisation, prevalence and severity of skin lesions caused by ophidiomycosis in a population of wild snakes. *Scientific Reports* 14, 5162. https://doi.org/10.1038/s41598-024-55354-5

Amphibian and Reptile Conservation. *Snake Fungal Disease* (SFD). arc-trust.org/snake-fungal-disease.

Anderson, K.B., *et al*. (2021). Retrospective review of ophidiomycosis (*Ophidiomyces ophiodiicola*) at the Smithsonian's National Zoological Park (1983-2017). *Journal of Zoo and Wildlife Medicine* 52(3): 997–1002.

Blanvillain, G., *et al*. (2024). Contribution of host species and pathogen clade to snake fungal disease hotspots in Europe. *Communications Biology* 7(1): 440.

Burbrink, F.T., Lorch, J.M. & Lips, K.R. (2017). Host susceptibility to snake fungal disease is highly dispersed across phylogenetic and functional trait space. *Science Advances* 3(12): e1701387.

Franklinos, L.H.V., *et al*. (2017). Emerging fungal pathogen *Ophidiomyces ophiodiicola* in wild European snakes. *Scientific Reports* 7: 3844. https://doi.org/10.1038/s41598-017-03352-1

Henrici. A. (2021). Notes and records. *Field Mycology* 22(4): 141–142.

Kindler, C., *et al*. (2017). Hybridization patterns in two contact zones of grass snakes reveal a new Central European snake species. *Scientific Reports* 7: 7378.

Lind, C.M., McCoy, C.M. & Farrell, T.M. (2018). Tracking outcomes of snake fungal disease in free-ranging pygmy rattlesnakes (*Sistrurus miliarius*). *Journal of Wildlife Diseases* 54(2): 352–356.

Rajeev, S., *et al*. (2009). Isolation and characterization of a new fungal species, *Chrysosporium ophiodiicola*, from a mycotic granuloma of a Black Rat Snake (*Elaphe obsoleta obsoleta*). *Journal of Clinical Microbiology* 47(4): 1264–1268.

Sun, P.L., *et al*. (2022). Infection with *Nannizziopsis guarroi* and *Ophidiomyces ophiodiicola* in reptiles in Taiwan. *Transboundary and Emerging Diseases* 69(2): 764–775.

Takami, Y., *et al*. (2021). First report of ophidiomycosis in Asia caused by *Ophidiomyces ophiodiicola* in captive snakes in Japan. *Journal of Veterinary Medical Science* 83(8): 1234–1239.

Notes and Records

Alick Henrici*

In the last issue I discussed the troubles that mycological taxonomists are having with the concept of a family in these days of DNA-based taxonomy. This despite it being a major level in the taxonomic hierarchy instituted by Linnaeus and followed today in all branches of the natural world. I took my examples from the widely differing concepts of *Tricholomataceae* in current literature. I now turn to *Clitocybaceae*, as this family has recently been the subject of a major rumpus among agaric taxonomists.

What's gone wrong with *Clitocybaceae*?

In Kibby (2020) *Clitocybaceae* is described as "invalidly published but widely used". As luck would have it valid publication followed in November of that same year. The concept of such a family had been around ever since 1876, but then only described in French as Clitocybacées and thus unrecognised by the ICN (International Code of Nomenclature) until 2020.

There is no need to specify a type genus when describing a new family. It is implicit in the family name. The type genus of *Clitocybaceae* is thus *Clitocybe*. But the type species of a genus must be defined. That of *Clitocybe* is *C. nebularis*, and that's where things have been going wrong. An important paper (Zheng-Mi *et al.*, 2023), reports extensive DNA studies (much more than just ITS) across a wide selection of species from the family, leading to a much fuller understanding of its content and structure. They found that 18 of the *Clitocybe* species studied all contained muscarine, reinforcing the general feeling that most *Clitocybe* species are poisonous. These 18 appear clearly congeneric, whereas *C. nebularis* differs widely in its DNA and contains no muscarine. Two other previously undescribed Chinese species were the only ones to group with *C. nebularis*. Thus these three appeared to be the only known species that truly belonged in *Clitocybe*.

The position was comparable to the time when the type of *Coprinus*, *C. comatus*, was found to be unrelated to almost all other *Coprinus* species. All the many other species were moved to other new genera (*Coprinopsis*, *Coprinellus*, *Parasola*). Zheng-Mi *et al.* didn't actually mention this precedent but initiated similar large scale generic rearrangement. They found that the bulk of their *Clitocybe* species were sufficiently similar to *Collybia cirrhata* (type of *Collybia*) to be combined there rather than needing a new genus. So their 18 *Clitocybe* species, including several well known in Europe, were duly combined in *Collybia*.

This drastic solution promptly provoked much adverse on-line comment. What about the rest of the 500 or so currently described *Clitocybe* species world wide? More to the point, the *Coprinus* precedent didn't apply in this case. When Persoon erected *Coprinus* this was the only species he mentioned. Nothing else could replace it. It had to stay as *Coprinus*; all the others had to be something else. By contrast *Clitocybe* started life described by Fries as a section of his all-inclusive *Agaricus*. Several species were placed there but no type was designated. ICN has complex rules about how the type of a genus is determined in such historic circumstances. Nowadays a genus won't be valid unless a type species is specified.

Choosing a type for *Clitocybe*

At one time the type of a genus tended to be the first species listed. This sometimes turned out to be an unfortunate choice. The rules were changed: the type became the one advocated by whoever first chose on taxonomic grounds rather than by a mechanical rule. In this case it was two Americans: Clements & Shear and they proposed *C. gibba* (now the type of *Infundibulicybe*). But they were operating under an American code (which according to ICN must now be discounted). *C. nebularis*, proposed by Donk, was adopted, although considered very unsatisfactory by Singer (1986) in two long abstruse paragraphs. He thought it was very atypical, rightly as it turns out, and insisted it had to be *C. gibba*. A further American, Bigelow, also objected to *C. nebularis* and proposed *C. clavipes* (now the type of *Ampulloclitocybe*). For the time being *C. nebularis* wins. Both the other proposed types are now in other families, not merely other genera.

What is now needed is yet another type species for *Clitocybe*, chosen from among the central poisonous (muscarine containing) species, thus avoiding the proposed wholesale upheaval. *C. nebularis* needs to be ousted. This can be done, but only through publication of a formal proposal in the journal *Taxon* and subsequent approval of this proposal at the next International Botanical Congress. Such a proposal has now been published (Zheng-Mi & Yang, 2024) proposing that *C. phyllophila* should become the new conserved type species (Fig. 1).

This proposal, somewhat surprisingly, was published only around four months after the original paper and by two of the same authors. However they acknowledge generous support given by John McNeill, who was on the editorial committee of the 2017 version of the ICN, in putting it together. Possibly they had been unaware that such a procedure was possible. Alternatively they may have been prompted to think again merely by the volume of objections to their original approach. Assuming approval, a new generic name will then need to be chosen to house *C. nebularis* and its two Chinese relatives.

Some notes on the British *Clitocybaceae*

This is a small family in terms of numbers of genera, known in Britain from around two dozen *Clitocybe* species, 10 *Lepista* (of which one sometimes placed in *Paralepista*), 1 *Singerocybe* (formerly *Clitocybe*), 1 *Pseudocollybia* and 3 *Collybia* species. These last 4 are the remnants of the formerly large genus *Collybia*, most of which were reported in 2010 to be remote from the type species *C. tuberosa* and thus moved to *Gymnopus* or *Rhodocollybia*.

These notes derive from the tree and associated muscarine data in Zheng-Mi *et al.* (2023).

- *C. nebularis* is basal to the British *Clitocybaceae*, i.e. the earliest to diverge from the rest of the family.
- The former *C. phaeophthalma*, now in *Singerocybe*, is another outlier in the family, fairly remote both from *C. nebularis* and from the remaining *Clitocybe* species. These two both lack muscarine. The only other British *Clitocybe* shown to be similarly lacking is *C. odora*. This has rather similar DNA to the rest of *Clitocybe*, but possibly deserves a genus of its own.

Fig. 1. *Clitocybe phyllophila*, recently proposed to be the conserved type species of the genus *Clitocybe*, replacing *C. nebularis*. Photograph Perivale Wood, Ealing, London © Geoffrey Kibby.

- *Dendrocollybia* (muscarine status unrecorded) is a third outlier in the genus, whereas the two *Collybia* species sampled (*C. tuberosa* and *C. cirrhata*) unexpectedly turn out to belong in the heart of *Clitocybe* and to likewise contain muscarine. Hence the proposal to recombine most of *Clitocybe* in *Collybia*. In the *Taxon* paper this is reversed and *Collybia tuberosa* (type species) is combined in *Clitocybe*. Both genera were initially described in the same publication in 1857, so either way is permissible, but preserving *Clitocybe* makes much better sense if it can be achieved!
- The rest of the family can be thought of as two large genera, *Lepista* and *Clitocybe*. *Lepista* appears rather too diverse to remain a single genus and several segregates have been proposed, but with *C. nebularis* removed *Clitocybe* appears fairly homogeneous.

Another hot topic: Treatment of FDTs

'What are FDTs?' you ask. They are the so called 'Fungal Dark Taxa', i.e the now very numerous fungi known only through 'metabarcoding', also known as high throughput sequencing (e.g. of soil samples). Their sequences show them to match no published species that has been sequenced, sometimes indeed no published genus, but as no physical structures have been seen to allow description and preservation of voucher material as required by the code, no name can be validly published. Some such sequences have been found repeatedly in economically important contexts within particular research projects. There is an obvious need for a central registration system to coordinate such findings alongside (or possibly within) the current ICN for fungal taxa. But all sorts of questions arise. For instance, what sequence lengths of what portions of DNA should be considered worthy of registration? What other details should be supplied concerning the source of the sequence?

We can look forward to a time in the not too distant future when whole genome sequences become the norm. But the whole genome is that of an individual, not of a species. It is too much, but for instance ITS alone is too little. What bits of the genome should be insisted on before an FDT is registered? There has been a recent 3-page overview of the FDT problem (Zhou, 2024). But I find its proposals for a path to an agreed system within fifteen years highly optimistic. They remind me too strongly of all the current commitments to have the world's climate under control by 2050.

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References

Kibby, G. (2020). *Mushrooms and Toadstools of Britain & Europe* Vol.2. Privately printed. fieldmycol@yahoo.co.uk

Singer, R. (1986). *The Agaricales in Modern Taxonomy* (4th Edn.). Koeltz Scientific Books.

Zheng-Mi, H. & Yang, Z.L. (2024). (3014) Proposal to conserve the name *Clitocybe* (*Basidiomycota*) with a conserved type. *Taxon* 73(2): 635–636.

Zheng-Mi, H., Zuo-Hong, C., Bau, T., Geng-Shen, W. & Yang, Z.L. (2023). Systematic arrangement within the family *Clitocybaceae* (*Tricholomatineae*, *Agaricales*): phylogenetic and phylogenomic evidence, morphological data and muscarine-producing innovation. *Fungal Diversity* 123: 1–47.

Zhou, L-W. (2024). The strategy for naming fungal 'dark taxa'may involve a transition period and genomics. *Fungal Biology Reviews* 48, 100358: 1–3.

Book review

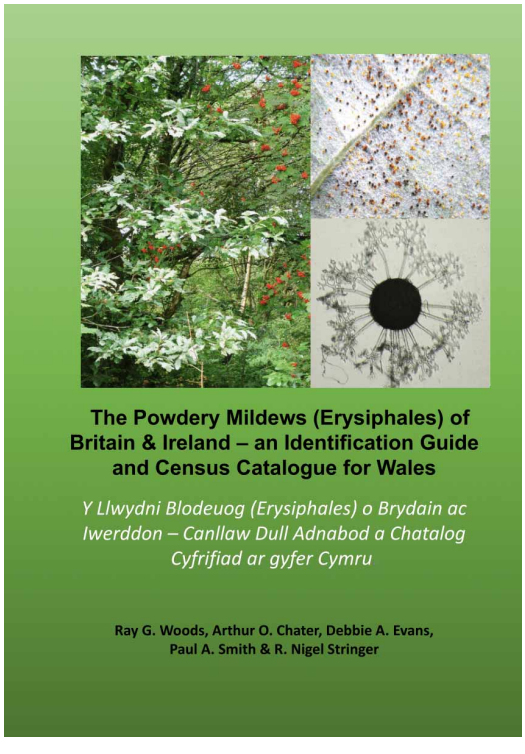
The Powdery Mildews (Erysiphales) of Britain & Ireland – an Identification Guide and Census Catalogue for Wales.

Woods, R.G., Chater, A.O., Evans, D.E., Smith, P.A. & Stringer, N.

Aberystwyth: A.O. Chater. (2024).
ISBN 978-0-9565750-7-4. Pp116.

Price: £10 + £3.50 p&p.
Also available online for free download at: https://www.aber.ac.uk/waxcaps/downloads

This is the latest in the series of accounts of Welsh plant pathogens, all of which have been produced in a similar format, and published by Arthur Chater. This one is a fully revised and much extended version of the *Census Catalogue of Welsh powdery mildews* which was published just five years ago (Chater & Woods 2019). That 5-year period has seen considerable progress in the taxonomy of these fungi and their host ranges, based particularly on molecular studies, and, with some new British records, has quite significantly extended the species lists for Britain and, of course, for Wales. The 2019 edition recognised 122 species for Wales and 166 for the British Isles, and was itself a clear indication of the progress made with these fungi during the previous few decades. Dennis (1986), for example, had just 65 species for Britain. Hence, the need for a revised edition is evident – there are now 150 species recorded from Wales, and around 200 for Britain. This proliferation of recorded species seems likely to continue and the present edition is again presented as a 'work in progress'.



As the title indicates, this is a greatly extended work, including now not just the Welsh but all the British species of *Erysiphales* and their host associations, though excluding hosts which are entirely greenhouse or indoor plants, and with their country distributions noted. It is a welcome contribution, presented as an aid to the identification of species and with the hope of encouraging wider study of these fungi which are important ecologically as well as commercially in agriculture and horticulture.

Following a general introduction to these fungi and background information, the book includes short sections covering conservation, identification and examination. These describe and illustrate with clear coloured images the distinction from the downy mildews (*Peronosporales*) and the white moulds (*Ramularia* and allies) which are also plant pathogens and could, at least by beginners, be confused with powdery mildews. The main characters of powdery mildews are described, including the mycelium, conidia, and appressoria, as well as the chasmothecia (the tiny, globose, ascus-bearing fruitbodies), and the attractive, ornate appendages which they commonly bear.

The main part of the book, over 100 pages, presents tables of the mildews, their hosts and distribution. The first of these tables is a list of host genera and their associated mildews, usefully including extra-limital species from Western Europe which may well occur in Britain, those known from Britain being distinguished in bold. Where more than one species of mildew is known from a given host, brief distinguishing characters are given which should allow reliable identification in most cases. Then follows the Census Catalogue of the powdery mildews of

Britain and Ireland, an alphabetical list of all recorded host species, their associated mildews with their country distribution and, for species known from Wales, the vice-counties from which they have been reported. The inclusion of all British host species is something which has not been readily available since the earlier comprehensive listing of the British species by Ing (1990-91). The final table is an alphabetical list of mildews with their host species and, again, their country distribution.

The book concludes with a glossary, and list of References. A brief but interesting discussion of the history in Britain of the powdery mildews of oaks (illustrated on the front cover) and of vines appears on the inside of the back cover. A minor typographical error has crept in here, the recently-recorded oak mildew *Erysiphe hypophylla* being accidently given as *E. hypodytes*. It is one of the very few errors which have been noted, another being *Golovinomyces ambrosiae*, the mildew of *Helianthus* and *Rudbeckia*, accidentally given as its synonym *G. latispora* in the listing under *Helianthus*.

The inclusion of extra-limital species from nearby mainland Europe is, according to the authors, partly due to the lack of a reliable checklist of British species. However, this is not quite the case, as a recent checklist was published by Ing (2021) and has been apparently overlooked. It is useful in including synonymy, is helpful for general references and comparison with earlier records not given in the present work, distributions which include also the Channel Islands and Isle of Man, and basic frequency. A quick comparison of this checklist with the current work shows a very close agreement, though already there have been a few changes. For example, recent revisionary study of *Blumeria* (Liu *et al.* 2021), grass-infecting mildews for which, hitherto, a single species has been recognised, has distinguished a further five species with different host ranges.

This is an authoritative work and the most comprehensive account of the British *Erysiphales* currently available. It will be an essential reference for anyone with an interest in plant pathogens, or fungal recording and identification. As noted, though, it is not the end of the story; continuing taxonomic revisions are likely to distinguish additional taxa, and further species are likely to turn up. In addition, further host plants are likely to be recognised. Indeed, various other hosts have been reported here but are currently unpublished and require confirmation. The mildews recorded from greenhouse and indoor plants, which were included by Ing (1990-91) but excluded here, are also in need of revision.

As with other titles in the series, this is a well-produced publication, A4 in size and ring bound allowing for easy opening of the pages. It has, as usual, a protective, clear plastic covering over an attractive green card cover with coloured photos of oak mildew, mildew on *Fraxinus*, and the ornate chasmothecium of *E. hypophylla*. It is excellent value as hard copy and, as an added bonus once again, is also available online for free download.

Brian Spooner

References

Chater, A.O. & Woods, R.G. (2019). *The Powdery Mildews (Erysiphales) of Wales. An Identification Guide and Census Catalogue*. Aberystwyth: Chater.

Dennis, R.W.G. (1986.) *Fungi of the Hebrides*. Kew: Royal Botanic Gardens.

Ing, B. (1990 – 1991). An introduction to British Powdery Mildews 1–7. *Mycologist* 4: 46, 48, 88–90; 125–128, 172 – 177; 5: 24–27, 61–67, 156–157.

Ing, B. (2021). New Checklist of British *Erysiphaceae*. *Field Mycol.* 22(4): 123–129. (195 spp)

Liu, M. *et al.* (2021). Taxonomic revision of *Blumeria* based on multi-gene DNA sequences, host preferences and morphology. *Mycoscience* 62: 143–165.

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