



Pembrokeshire Fungus Recorder

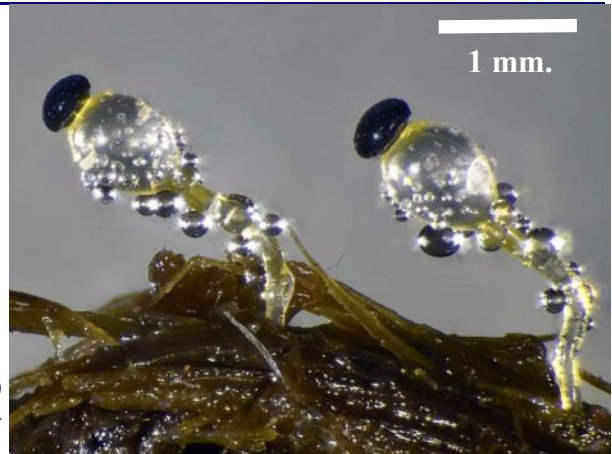
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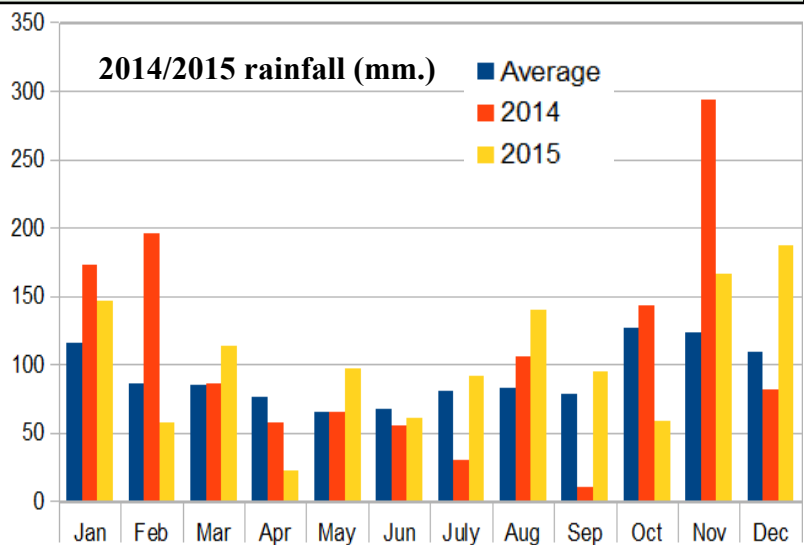
Pilobolus kleinii (dung cannon or hat thrower)
© Mike Crutchley



Introduction

A dry October led into a persistently wet November and December - raising hopes for a productive mycological climax to the year. However, it wasn't to be, and neither grassland sites nor woodlands were spectacular, but as always, there were a few interesting records.

In this issue we concentrate on the organised network events including two on UK Fungus Day together with Adam's report on woodland finds. A full newsletter means that we've kept a few interesting records for the next issue in April.



Weather data courtesy of FSC, Orielton

A special feature in the newsletter is a report on a project which will help us learn more about the application of DNA techniques to the study of fungi. We are using *Hygrocybe conica* (blackening waxcap) as our target species as it is now regarded as a species complex, and is not uncommon in Pembrokeshire. We are especially grateful to the Pembrokeshire Biodiversity Partnership, Gareth Griffith at Aberystwyth University, and the Bentolab team from UCL for their support and encouragement.



David Harries, PFRN coordinator
(djh.somerton@gmail.com)
January 2016

Fungus records

UK Fungus Day events

The PFRN held two events to mark UKFD; both scheduled for Saturday the 10th October to avoid a clash with a Sunday event at the National Botanic Garden of Wales organised by our neighbours in Carmarthenshire.

The morning excursion, a sand dune walk at Poppit Sands in north Pembrokeshire, was organised in conjunction with the Darwin Centre and led by David Harries. In spite of a rather dry period leading up to the weekend, the event was well supported and an enthusiastic group soon started finding interesting species. The highlight for many was the discovery of a fine collection of *Phallus hadriani* - a striking stink-horn which emerges from purple/pink eggs nestling amongst marram grass in the dunes.



Forayers let loose on the dunes

The opportunity was taken to distribute copies of the PFRN guide to sand dune fungi, publication of which was supported by the Pembrokeshire Biodiversity Partnership.

Inset: *Phallus hadriani* (dune stinkhorn)

After a brief interval for lunch, several members of the party moved on to the Welsh Wildlife Centre at Cilgerran to participate in an afternoon event organised in conjunction with the Wildlife Trust of South and West Wales. This was led by Adam Pollard who guided the group along a woodland walk overlooking the Teifi waterway. In addition to a wide range of woodland species, the group was treated to a fine display of *Hygrocybe reidii* alongside the track. A notable woodland find was *Hohenbuehelia fluxilis*; a small white shell-shaped fungus found on dead wood by Adam Pollard.



Hygrocybe reidii (honey waxcap)



Hohenbuehelia fluxilis

Particular thanks are due to Sam Williams (Darwin Centre) and Nia Stephens (WTSWW) for their help in organising the events. The BMS/UKFD handouts were well received, with sufficient available to hand on to the partners for display or distribution within their respective organisations.

Orielton Field Centre - Fungal Bioblitz

Following the success of last year's venture, we returned to Orielton for another fungal Bioblitz. In spite of rather damp conditions the event was very well supported and we were rewarded with some less well known grassland species.

Particular favourites were *Hygrocybe colemanniana* (only the second County record), *Hygrocybe aurantiosplendens* and some fine examples of *Geastrum triplex* (collared earthstar.)

Adam reported some interesting woodland fungi including a first Welsh record for *Inocybe xanthomelas*. This followed a lot of microscopy and subsequent corroboration from Penny Cullington, co-author of "Keys to British species of Inocybe."

FSC staff kindly laid on hot drinks and cake to revive participants after the event.



Woodland Glade

Adam Pollard's regular feature highlighting woodland finds.

Mycorrhizal fungi continue to produce fruitbodies throughout the autumn but we start seeing more saprophytic (rotters) fungi from late October and throughout the winter. Our excursions included:

Little Milford - 27th September

A productive foray led by Adam Pollard resulted in a new county record for *Amanita argentea* (pictured right) spotted by Suzanne Powell.

Also found were a good range of coral fungi, several Tricholomas (Knights) including *Tricholoma ustale* (Burnt Knight) and the rubbery *Lyophyllum decastes* (collection shown below right).



Minwear - 18th October

An excursion into mixed woodland led by Mike Karpaty produced an interesting crop of records including:

Cantharellus tubaeformis: A mycorrhizal species associated with conifers, aptly named the trumpet chanterelle because of its shape.



Pseudohydnum gelatinosum: (toothed jelly fungus), a species found on dead logs and branches.



Tubifera ferruginosa: A myxomycete found on dead wood. Recent studies suggest this is actually a species complex and may comprise at least 7 different species based on DNA sequences and morphological features.

Inocybe petiginosa: one of the fibrecaps which is found in broadleaved woodland, often in a mycorrhizal association with beech.



Pembrokeshire Blackening Waxcap study (DJH)

Background

Molecular studies, such as the analysis of the DNA in biological material, are becoming increasingly important as taxonomists attempt to unravel the relationships between genera, species, varieties and forms of organisms. Nowhere is this more important than in the field of mycology where we largely rely on the collection and inspection of fruit bodies - many of which only appear during favourable fruiting seasons.

The sequencing of a tiny fragment of DNA can be used to generate a “barcode” for a fungus. The extent to which barcodes vary between two samples can be used to help determine how closely related they are. DNA sequencing methods thus provide data which complements the information collected from macroscopic and microscopic characters which was traditionally used to support the construction of phylogenetic (evolutionary) trees.

Such analyses may reveal more than one “cryptic species” hidden under a single species name - leading to interesting challenges for taxonomists seeking to name such species. However, even where molecular studies indicate genetic variation, this alone may be unhelpful unless this variation can be linked to distinguishing morphological characters or ecological traits. Several PFRN enthusiasts became especially interested in this aspect of mycology and sought to learn more about the techniques.

The project

Over the years there have been different interpretations of the blackening waxcap assemblage depending on whether the author was a “lumper” or “splitter”. Recent work at the Royal Botanic Gardens, Kew and Aberystwyth University (Cannon P.F. et. al., 2013) indicates that it should be treated as a species complex which may contain up to 6 individual taxa in the UK. We therefore selected the blackening waxcap (*Hygrocybe conica* sensu lato) as our target species as it is widely distributed and easily identified in the field.



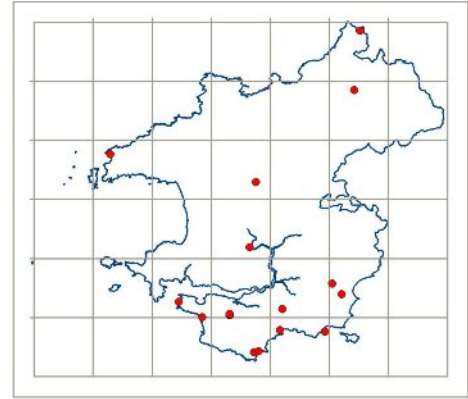
The project breaks down into four key activities:

- (a) Collecting specimens of the blackening waxcap from across the County, examining the specimens and documenting their macro and micro features.
- (b) Assigning the records to different “groups” according to the morphological and habitat preferences of the collections, and mapping their distribution.
- (c) Extracting, purifying and amplifying the DNA from collections in each provisional group to produce material suitable for sequencing.
- (d) Working with specialists at Aberystwyth University to obtain and interpret DNA barcode sequences from selected specimens in the study.

Progress to date

(a) Sample collection

2015 proved to be a difficult year for grassland species with fewer fruit bodies showing at many sites compared with other years. In spite of this, we have 18 collections of fruit bodies from a cross-section of sites across the County: more than enough for us to establish and test our DNA extraction and amplification protocols. Further collections will be made in autumn 2016.



(b) Preliminary evaluation of samples

Work is progressing on documenting the microscopic and macroscopic features in order to group the different apparent forms of blackening waxcap. Macroscopic features appear to show several different morphological types as illustrated below.

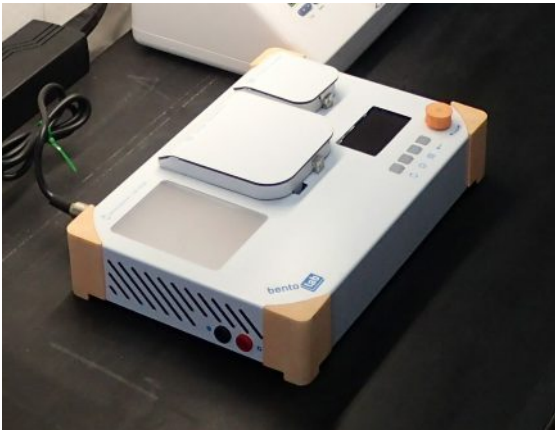


(c) DNA extraction

In late 2014, three members of our working group visited Aberystwyth University (mycology section, IBERS) where we were introduced to the techniques for extracting and preserving fungal DNA. With the invaluable assistance of Dr. Gareth Griffith we subsequently assembled sufficient equipment and consumables to carry out the DNA extraction locally.

In July 2015 we learned of an innovative project looking for participants willing to carry out field trials of a portable DNA extraction unit: Bento Lab. The programme is run by a start-up group based

at University College London with a vision to open up access to biosciences for everyone in the same way that Arduino or Raspberry Pi made electronics and computing accessible.



The product of the Bento Lab development work is a portable mini-lab which incorporates much of the specialist equipment needed to carry out the DNA amplification process and subsequent checking of the DNA product. The test unit is being trialled in 20 citizen science projects worldwide during the latter part of 2015 and early 2016.

We successfully applied to join the pilot programme which provides us with cost-effective access to a prototype instrument, backed up with technical support. The Pembrokeshire Biodiversity Partnership contributed towards the cost of participating in this project.

(d) Interpretation and outcomes

The final stage of the project will involve sending the amplified DNA extracts to Aberystwyth for sequencing. If we successfully generate meaningful sequences, these will be evaluated to see how well they correspond with the different groups previously identified and will be used to generate a distribution map of the various blackening waxcap taxa across Pembrokeshire.

Information collected during the study will be forwarded to the mycology team at Aberystwyth University where it will contribute to the DNA database of grassland fungi being established as part of their ongoing research programme.

Local field mycologists will gain a better understanding of the principles and techniques involved in the molecular study of fungi.

The Bento Lab developers will receive feedback on the operability and suitability of their equipment for citizen science projects.

Acknowledgements

Particular thanks are due to the Pembrokeshire Biodiversity Partnership/Natural Resources Wales for providing financial support, Gareth Griffith and colleagues at Aberystwyth University for their continuing encouragement and technical support, and the Bento Lab team for accepting us onto their pioneer programme.

Reference

Cannon PF, Dentinger BTM, Ainsworth AM & Griffith GW (2013). Systematics, barcoding, and ecology of fungi from waxcap grasslands in England and Wales. Defra Final Project Report (WC0787).

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Fungus Records Database

Stuart Skeates gave a presentation at the BMS Autumn Meeting (Kew) on progress with the replacement recording and data handling package. Current work includes ensuring that the inventory of species names held by the Natural History Museum, and used by the NBN, is brought up to date and a system is in place for its continuing maintenance.

Arrangements for workshops/training for recorders will be established, with the intent that the new FRD will be in operation early in 2016. The system will retain provision for the submission of records by spreadsheet - providing these have been run through the NBN record cleaner which will highlight any invalid names or grid references.

Kew Lost and Found project

The Lost and Found team has been strengthened by the addition of a new member, Lukas Large, who will be assisting Brian Douglas.

Kew vouchers

Following the restructuring at Kew, the fungarium is now under control of the collections department and fungus recorders who submit voucher material to Kew may be asked to complete a declaration regarding the origin of the material. This is to enable Kew, as part of its commitment to the 1992 Convention on Biodiversity, to demonstrate that donated material has been legally acquired.

Paul Cannon is working with colleagues to establish whether or not this should really apply to dried material collected within the UK, or if there are ways to minimise the paperwork - perhaps through a blanket declaration.

BMS - Fungal Education and Outreach

2016 UK Fungus day will cover the weekend of the 9th October.

The BMS has appointed Meg Skully as the Social Media and Outreach Officer. Meg advises that she will facilitate liaison between external agencies and Senior Officers of the BMS and manage all social media and website outputs and content from the Society's committees. Part of her rôle will be to raise the profile of UK Fungus Day with external organisations and the wider audience.

Fungus Conservation Trust (formerly ABFG) - red data assessment

Michael Jordan advises that a team at the FCT has compiled a red data assessment of 80 species of fungi based on the current IUCN guidelines. The assessment has been submitted to the JNCC but has not at this stage been endorsed by them.

[DJH: I understand that there are unresolved issues related to JNCC requirements regarding use of the FCT data held in the CATE database, and also concerns arising as a result of a parallel assessment which has been submitted by other specialists based largely on BMS records held on the FRD.]