Hampshire Fungus Recording Group Survey of Franchises Lodge 2020-2021

Summary

Hampshire Fungus Recording Group (HFRG) were asked to carry out a survey of Franchises Lodge to serve as a baseline taxonomic list for the site and to highlight likely areas of particular fungal interest. It was agreed that, at least initially, the surveys would concentrate on the areas around the veteran trees identified by Gareth Fischer in 2019.

A total of 12 visits were carried out from November 2020, when we regained access to the site, to September 2021. No surveys were conducted in May or June by request of the site managers to minimise disturbance to the nesting birds.

In total, 599 records were made covering 303 named species.

Of these, 220 were new to the site which brings the total number of species recorded on the site to 357.

Fungal Ecology

The fungal kingdom covers almost 20,000 named species within the UK spanning single-celled yeasts to extended mycelial networks. All species ultimately depend on photobionts (plants or cyanobacteria) for their energy demands, either by direct exchange (mycorrhizal or lichenised fungi) or breaking down plant or animal products (saprotrophic fungi).

This survey excluded lichenised fungi which were the subject of a separate survey.

Except in exceptional circumstances, the mycelial networks of fungi cannot be identified to species by direct observation in their normal state, even with a microscope, so identification and recording has been limited to the their spore-baring structures, colloquially known as "fruit-bodies" and the vast majority of the records in this report are of such fruit-bodies.

As most fruit-bodies are ephemeral and their production erratic, multiple visits, often spread over several seasons, are required to record which fruit-body-producing fungi might be present. In addition, many species either produce no fruit-bodies or ones that are too small to be seen without microscopic examination of their substrate. For the most part, such species are not covered by this survey.

Environmental DNA sampling is starting to be used to assess the presence of non-fruiting fungi but these techniques require specialised equipment beyond the scope of this survey (eg Ref 9).

In summary, the fungal species covered by this report are almost entirely restricted to those producing macroscopic (if small) fruiting bodies, mostly from the soil or litter or dead vegetation, again mostly wood or woody litter.

The fruiting of ground inhabiting fungi tends to be inhibited by dense vegetation cover (eg. bracken or grass), which then also hides them from view. Fungi fruiting on large logs or branches may be less affected but the potentially diverse subterranean fungal communities are likely to be underrecorded.

Empirically, the timing of fungal fruit-body production appears to be related to climatic conditions (both current and previous) and possibly independently to season. 2021 was an unusual year in terms of rainfall and sunshine with damp conditions in the summer and drier ones in the early autumn. The former seems to have inhibited or delayed fruiting of the ectomycorrhizal species while the latter reduced the fruiting of litter-inhabiting saprotrophs. Unfortunately, the timing of the survey prevented us from observing whether these trends continued throughout the autumn and early winter.

Methodology

The group employed the classical, opportunistic approach ie a group of surveyors searched the environment for fungal fruit-bodies. Whenever possible, specimens were identified in the field but if this was not possible, samples were taken away for subsequent identification/confirmation. However, in a few cases, the collection could only be identified to genus.

It was agreed that, at least initially, the surveys would concentrate on the areas around the veteran trees identified by Gareth Fischer in 2019.

12 visits were made to the site between the end of November 2020 and the end of September 2021. The dates of these visits, the areas covered and the taxa targetted are shown in Appendix II.

Where possible, the location of every collection was noted to a precision of 10m (8-figure OS grid reference) but field identifications were occasionally reduced to 100m to maximise the number of records collected. Similarly, all collections (specimens taken away for further work) were recorded but in-field records, particularly of common fungi, were only made once. There was no attempt made to record the number of individual fruiting bodies as this is more a measure of each species lifestyle than their distribution.

This opportunistic approach has a number of known limitations:

- 1. Only fruiting fungi are recorded
- 2. There can be a bias towards larger fruit-bodies
- 3. Only genera familiar to the collectors tend to be identified to species
- 4. The area covered depends on the time available and the number and type of fruit-bodies found:

However, it was the only method available given the time and resource constraints and was mitigated as far as possible by involving group members with expertise across a range of genera.

Collected material was subject to normal microscopic examination appropriate to the genus using standard literature (see Appendix I).

In addition, some of the more interesting collections were subject to DNA bar-coding analysis (Ref 8). This approach has only recently become available to field mycologists (Refs 3 & 4), and while it is still a time-consuming and moderately expensive technique, it can provide results not available via standard methods.

The technique consists of 3 distinct components:

- 1. PCR amplification of a small sample of the specimen. Followed by an QA step using gel electrophoresis to confirm that the DNA has (apparently) amplified cleanly.
- 2. Sequencing of the resulting amplicons. This was carried out by the Institute of Biological, Environmental and Rural Sciences (IBERS) at Aberystwyth University as a chargeable service.
- 3. Analysis of the resulting sequences by comparing them against the two public fungal data bases, Genbank (Ref 2) and Unite (Ref 7).

In principle, it could be used to overcome the limitations of item 3 above but it currently has issues beyond the effort and cost involved:

- 1. It can be quite difficult to extract small, clean samples from some specimens, particularly small or thin fruit bodies.
- 2. For unknown reasons, some collections are difficult to amplify
- 3. Matching to published sequences is not always straight-forward, both because of the lack of coverage of known species and the "variable" quality of the identifications associated with

the published sequences but also the fact that only a fraction of the fungal diversity has been described.

Many of these shortcomings were well expressed in Hofstetter et al (Ref 5), who estimated that up to 40% of the identifications published in Genbank are incorrect.

However, it should also be pointed out that the technique can allow identification of collections which may be of undescribed species.

Findings

Examination of the Fungal Records Database of Britain and Ireland (<u>FRDBI</u>) revealed that previous visits to the site by HFRG in August 2018 and September 2019 and the Salisbury Natural History Society around 1970 resulted in 200 records from 137 species. A spread-sheet of these records is included here for completeness (FranchisesRecords201129.xlsx).

The 12 visits between November 2020 and September 2021 resulted in 599 records, comprising 303 named species, 220 of which had not been recorded from the site previously.

In addition to these named species there were a number which, thanks to the DNA work, are "known unknowns" ie collections whose DNA differs from all sequenced species.

At the time of writing, PCR amplification was attempted on 31 specimens, resulting in 13 useable sequences of which 10 could be matched to known species and 3 were "novel" ie from unsequenced or undescribed species. Details of these 13 collections are given in Appendix IV. The more interesting of these sequences will be published on Genbank and the accession numbers will be added to the FRDBI record.

While there were obvious reasons for it, the timing of the survey was hardly ideal for recording macroscopic fungal fruitbodies as it missed the majority of the autumn, which is the usual peak of the season. Comparing the species recorded prior to the survey with the current list, it is clear that a number of commonly observed, particularly ectomycorrhizal, species were absent from the survey and it quite likely that other, less common species will have been missed.

On the other hand, the spring visits lead to better coverage of resupinate fungi fruiting on the underside of fallen wood, including several species considered rare or very rare.

Spread-sheets listing the full records are included (HFRGFranchisesSurvey211031.xlsx and HFRGFranchisesSurvey211031.Detail.xlsx)

The nature of the existing records and these surveys did not begin to allow any indication of trends to be drawn. Although we did attempt to visit different areas of the site, it was not possible to identify particular areas of fungal diversity and it is quite possible that the areas of high diversity, such as the more established woodland, were missed because of the mismatch between the timing of the survey and this years fruiting patterns.

The current species count of 357 may sound quite a respectable for a site of this size reflecting the diversity of habitats. However, it is instructive to compare this with neighbouring areas. As a comparison, the New Forest has been visited by a wide range of expert mycologists over the last century and a half.

The FRDBI lists 572 species from the adjacent Bramshaw Wood and well over 2,000 from the whole of the New Forest with the earliest dating back to 1884.

Clearly some of this difference will reflect the additional habitats found in the New Forest, such as ancient beech woods but much could "just" be the consequence of such sustained recording activity.

Recommendations

Fungal diversity tends evolve over time and to mirror habitat diversity, both in host plants (mycorrhizal and saprophytic fungi) and substrate type (saprophytic fungi). This implies that as wide a range of plant species as possible should be encouraged and dead material should be left in situ.

Gareth Fischer's survey identified a few larger trees but there appear to be very few of the large fallen trees observed in less managed "ancient woodland" which host a wider range of species of all taxa than smaller pieces of wood.

As part of this management process, the use of grazing to control dominant plant species should be encouraged - with the added benefit that their dung provides an additional, fertile substrate.

In addition, in our experience, fruiting of ground-inhabiting fungi is inhibited by rank vegetation found over much of the site (although it is less clear how much this reflects mycelial vigour).

One additional variable that needs to be considered when advising on the conservation of fungi is the extent and longevity of an individual. For the most part, very little work has been done on this topic but the range of values seen across the kingdom is considerable:

Litter-inhabiting saprotrophic species extend through the soil or litter with the largest known being the multi-km Armillaria found in Oregon (Ref 1), which has been estimated to be several thousands of years old.

Ectomycorrhizal species, also spread throughout the soil, forming the so-called "Wood-wide Web", although little is yet known about their extent or life-cycles.

In contrast, some saprotrophic species are restricted to a single piece of substrate; examples being dung-inhabiting fungi, which inhabit a single dung pile or Cordyceps species on a single invertebrate body. Both have lifetimes measured in weeks or months.

Clearly, conserving the former groups requires conservation of a particular location while the latter case the biodiversity of a whole area is more important.

In the attached spreadsheet (HFRGFranchisesSurvey211031.Detail.xlsx), we have given an indication to which class the recorded species belong:

- SL: Saprotrophic-Localised fungi found on a single piece of substrate although they may also have some ability to "forage" for other substrates.
- SW: Saprotrophic-Wide ranging fungi growing in the soil or leaf-litter and extending over an unknown area.
- E: Ectomycorrhizal fungi growing in association with the surrounding vegetation (mainly trees), again extending over an unknown area and number of trees.

It should also be noted that not all tree genera form ectomycorrhizal associations with fungi (Ref 6) and not all fungi can be classed simply as either mycorrhizal or saprotrophic.

In the same spreadsheet, we have also indicated "Notable" species that are either rare (or rarely recorded) or are of some conservation concern.

Appendix I Literature used in Species Determination

- 1. Ascomycetes in Colour, Thompson, P.I., 2013
- 2. British Ascomycetes, Dennis, R.W.G., 1981
- 3. British Boletes, with keys to species, Kibby, G, 2011
- 4. British Chanterelles and Tooth Fungi, Pegler, D.N.; Roberts, P.; Spooner, B.M., 1997
- 5. British Milkcaps Lactarius & Lactifluus, Kibby, G, 2015
- 6. Collins Complete British Mushrooms and Toadstools: The essential photograph guide to Britain's fungi, Sterry, P.; Hughes, B., 2009
- 7. Collins Fungi Guide, Buczacki, S., 2012
- 8. Die Gattung Inocybe in Bayern, Stangl, J., 1989
- 9. Discomycete Workshop keys, Spooner, B.M., 2000
- 10. European Polypores, Ryvarden, L.; Gilbertson, R.L., 1993-4, 1-2
- 11. Flora Agaricina Neerlandica Vol 1 (Entolomataceae), Bas, C., Kuyper, T.W. et al, 1988
- 12. Flora Agaricina Neerlandica Vol 2 (Waxcaps, Pleurotus, Pluteus), Bas, C., Kuyper, T.W. et al, 1990
- 13. Flora Agaricina Neerlandica Vol 3 (Tricholomataceae-i), Bas, C., Kuyper, T.W. et al, 1995
- Flora Agaricina Neerlandica Vol 4 (Tricholomataceae-ii, Strophariaceae), Bas, C., Kuyper,
 T.W. et al, 1999
- 15. Flora Agaricina Neerlandica Vol 5 (Agaricaceae), Noordeloos, M.E., Kuyper, T.W. et al, 2001
- Flora Agaricina Neerlandica Vol 6 (Bolbitiaceae, Coprinus s.l.), Noordeloos, M.E., Kuyper,
 T.W. et al, 2005
- 17. Funga Nordica (1st ed), Knudsen, H.; Vesterholt, J., 2008
- 18. Funga Nordica (2nd ed), Knudsen, H.; Vesterholt, J., 2012
- 19. Fungi Europaei Agaricus, Cappelli, A., 1984, 1 (old)
- 20. Fungi Europaei Corticiaceae s.l., Bernicchia, A. & Gorjón, S.P., 2010, 12
- 21. Fungi Europaei Entoloma s.l. (and supp.), Noordeloos, M.E., 1992, 2004, 5, 5A
- 22. Fungi Europaei Polyporaceae, Bernicchia, A., 2005, 10

- 23. Fungi Europaei Tricholoma (and supp.), Riva, A., 1988, 3, 3A
- 24. Fungi of Switzerland, v 1-6, Breitenbach, J.; Kränzlin, F., 1984-2005, 1-6
- 25. Fungi of Temperate Europe, Læssøe, T., Petersen, J.H., 2019
- 26. Keys to British Species of Inocybe, Outen, A.R.; Cullington, P., 2010
- 27. Microfungi on Land Plants, Ellis, M.B.; Ellis, J.P., 1997
- 28. Microfungi On Miscellaneous Substrates, Ellis, M.B.; Ellis, J.P., 1998
- 29. Monografia Illustrata del genere Russula in Europa (2 vols), Sarnari, M., 1998
- 30. Mushroom and Toadstools of Britain & Europe, Kibby, G., 2017
- 31. Mushrooms & other Fungi of Great Britain & Europe, Phillips, R., 1981
- 32. Mushrooms of Britain and Europe (photoguide), Courtecuisse, R., 1999
- 33. Mycena d'Europa, Robich, G., 2003
- 34. Mycenas of the Northern Hemisphere, Maas Geesteranus, R.A., 1992, 1&2
- 35. Nordic Macromycetes Vol 1 (Ascomycetes), Hansen, L.; Knudsen, H., 2000
- 36. Nordic Macromycetes Vol 2 (Polyp., Bolet., Agaric., Russ.), Hansen, L.; Knudsen, H., 1995
- 37. Nordic Macromycetes Vol 3 (Hetero., Aphylloph., Gastero.), Hansen, L.; Knudsen, H., 1997
- 38. Poroid fungi of Europe, Ryvarden, L., Melo, I., 2017
- 39. Provisional Key to Clavarioid Fungi in British Grasslands, Roberts, P., 1995
- 40. The genus Agaricus in Britain, Kibby, G, 2011
- 41. The genus Amanita in Britain, Kibby, G, 2012
- 42. The Genus Hygrocybe (ed 1), Boertmann, D., 1995
- 43. The Genus Lactarius, Heilmann-Clausen, J.; Verbeken, A.; Vesterholt, J., 1998
- 44. The genus Mycena s.l., Aronsen, A.; Læssøe, T., 2016
- 45. The genus Russula in Great Britain, Kibby, G, 2007
- 46. The genus Tricholoma in Britain, Kibby, G, 2013
- 47. The Resupinates of Hampshire, Hugill, P., Lucas, A., 2017

Appendix II Dates of Site Visits

Date of Visit	Compartments Visited	Target Taxa
29/11/2020	Tuckers Hat, Ransoms Piece, Thorne Hill Copse	General
17/12/2020	Tuckers Hat, Ransoms Piece	General
14/03/2021	Tuckers Hat, Ransoms Piece, Fishpond Birches, Bury Hill	Resupinates
19/03/2021	Burnt Ground Wood, Pound Bottom Wood	Resupinates
27/03/2021	Sunnyside, Pimlico Pasture	Resupinates
04/04/2021	Franchises Common, Cloven Hill North	Resupinates
09/07/2021	Fishpond Birches	General
16/07/2021	Franchises Common	General
23/07/2021	Franchises Bank, Burnt Ground Wood, Pound Bottom Wood	General, Plant Pathogens
13/08/2021	Beaked Wood, Pimlico Pasture, Pimlico Wood	General
04/09/2021	Tuckers Hat, Ransoms Piece	General
30/09/2021	Tuckers Hat, Ransoms Piece, Crow's Nest North, Ashens Hat	General

Appendix III References

- 1. Anderson James B., Bruhn Johann N., Kasimer Dahlia, Wang Hao, Rodrigue Nicolas and Smith Myron L. (2018) Clonal evolution and genome stability in a 2500-year-old fungal individual *Proc. R. Soc. B.***285**
- 2. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic Acids Res. 2013 Jan;41(Database issue):D36-42. doi: 10.1093/nar/gks1195. Epub 2012 Nov 27. PMID: 23193287; PMCID: PMC3531190.
- 3. Harries, D. J. (2017). DNA and the fieldmycologist: part 1. Field Mycology 18(1), 20-23.
- 4. Harries, D. J. (2017). DNA and the field mycologist: part 2. Field Mycology 18(3), 92-96.
- 5. Hofstetter V, Buyck B. Eyssartier G, Schnee S, Gindro K. (2019) The unbearable lightness of sequenced-based identification, Fungal Diversity 96:243–284. doi: 10.1007/s13225-019-00428-3
- 6. Newton AC and Haigh JM (1998), Diversity of ectomycorrhizal fungi in Britain: a test of the species—area relationship, and the role of host specificity, New Phytol. 138, 619–627.
- 7. Nilsson RH, Larsson KH, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, Kennedy P, Picard K, Glöckner FO, Tedersoo L, Saar I, Kõljalg U, Abarenkov K. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. 2019 Jan 8;47(D1):D259-D264. doi: 10.1093/nar/gky1022. PMID: 30371820; PMCID: PMC6324048.
- 8. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, (2012) Barcoding Fungal Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proc Natl Acad Sci USA 109(16):6241–6246.
- 9. van der Linde S et al. Environment and host as large-scale controls of ectomycorrhizal fungi (2018), Nature 558, 243-248.

Appendix IV DNA barcoded samples

Species: Hyphoderma transiens Grid Reference: SU23231663

Date: 29/11/2021

FRDBI Number: 18309315

ITS sequence:

Comment: Unusual collection with encrusted hyphae and cystidia

Species: Postia ceriflua

Grid Reference: SU2328516751

Date: 29/11/2021 FRDBI Number: ITS sequence:

Comment: Unusual cephaloid poroid

Species: Mycena silvae-nigrae Grid Reference: SU2237819139

Date: 14/03/2021

FRDBI Number: 20063397

ITS sequence:

Comment: Unusual collection with 4-spored basidia.

Species: Mycena silvae-nigrae Grid Reference: SU2318118665

Date: 14/03/2021

FRDBI Number: 20063379

ITS sequence:

GGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATCGAATACGATTGGTGC
TGTTGCTGGCTTCTTCACCGAGGCATGTGCACGCCCATCTATTTATCTTCTCTTGTGCACATTTTGTAGTCTT
GAATCGAAACCTCTCGCAGCAATGCGGTTTGGGAGCCTGGCGCTTTAAACGGCCCCTTCTCCTGCTTCTTC
AAGGCTACGTTTTCATATACACGTTAAAGTTACAGAATGTCTTTTTAACGATTGTGCTTGGTCGCAGTCATTA
AACCTATACAACTTTCAGCAACGGATCTCTTGGCTCTCCCATCGATGAAGAACGCAGCGAAATGCGATAAGT
AATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTTGGCCCCTTTTGGTATTCCGAAGGG
CATGCCTGTTTGAGTGTCATTAAATTATCAACCTTGTTCGCTTTTTCGGAGCTTGAGCGAGGCTTGGTGACC
GTCACTTGGTGTGATAAATTATCTATGCCTATTGACTTGCTTAAACGAGAGTCTGCTTATAAACCGTCTCGCAAGG
GACAGCACTTTATTGACAATTGACCTCAAAT

Comment:

Unusual collection with 4-spored basidia.

Species: Conferticium cf. insidiosum Grid Reference: SU2217817338

Date: 19/03/2021

FRDBI Number: 20222465

ITS sequence:

Comment: ITS sequence below is close to published sequences of C. ochraceum but closer to unnamed Chinese collections (Genbank accession numbers: KY860404-KY860412). Specimen matches description of C. insidiosum but there are no published sequences of that species

Species: Hyphoderma pallidum Grid Reference: SU2277918000

Date: 04/04/2021

FRDBI Number: 20184043

ITS sequence:

Species: Rhodonia placenta Grid Reference: SU22631867

Date: 27/03/2021

FRDBI Number: 21369407

ITS sequence:

TTAGAĞGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAATGAATTTTGAA AGGGGTTGTAGCTGGCCTTTTAGGAGGCATGTGCACACCCTGCTCTTCCATTCTTACACCTGTGCACACTCT GTAGGTCGGTTTGAGCGGTTCTCTAACGGGGGATGCTGTTTGGCCTTCCTATGTTTTATAACAAACTCTGT AATGTCATAGAATGTCATCGCGTATAACGCATTATAAATATAAACTTTCAGCAACGGATCTCTTGGCTCTCGCATC GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAAC GCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAATTCTCAACCCTCTTATCCT TGTGGTGAGATTGGGATTTGGAGGTTTATGCTGGCTTGTAATGAGTCGGCTCCTCTTGAATGCATTAG CTTGGACCTTTTGCGGACCAGCTTTTCGGTGTG

Comment: Unusual species with only 15 records on FRDBI and only 8 this century.

Species: Sistotremella perpusilla

Grid Reference: Date: 24/04/2021

FRDBI Number: 20184071

ITS sequence:

Comment: Unusual species with only 11 unique records on FRDBI.

Species: Inocybe salicis Grid Reference: SU23171885

Date: 09/07/2021

FRDBI Number:21011309

ITS sequence:

Comment: Unusual species with only 18 records on FRDBI. DNA evidence suggests that it might be a separate species – I. straminipes – but the taxonomy is still under discussion.

Species: Mallocybe sp (fka Inocybe sp.)

Grid Reference: SU2266319031

Date: 09/07/2021

FRDBI Number: 22467945

ITS sequence:

Comment: ITS sequence is closest to but still far removed from Malocybe sicilliana,

Species: Inocybe sp.

Grid Reference: SU23171885

Date: 09/07/2021

FRDBI Number: 21011331

ITS sequence:

Comment: DNA barcoding suggests that this is an undescribed (or at least unsequenced) species, closest to but separate from I. purpureobadia. There is one matching environmental ectomycorrhizal sequence from Estonia.

Species: Marasmius curreyi Grid Reference: SU23221892

Date: 09/07/2021

FRDBI Number: 22494363

ITS sequence:

Comment: Uncommon species with 54 records on FRDBI.

Species: Russula puellaris Grid Reference: SU22561918

Date: 13/08/2021

FRDBI Number: 21890747

ITS sequence:

Comment: Unusually stout form of the species.