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THE COLLECTION OF CAVE FUNGI

by

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Collecting cave animals has long been regarded as a legitimate pastime among Speleologists, however very few have so far ventured to collect fungi, the only notable exceptions being a few Cave Research Group members working in the 1950's; even then, very little of their results has been published, indeed some is only being written up for the first time this year. Mason-Williams and Benson-Evans,[1] dealing mainly with the Bacteria published a short species list of fungi from caves in South Wales.

This lagging behind in our knowledge of fungal species found in caves is undoubtedly due to the difficulty of identifying the finds, which requires specialist knowledge, and the fact that sterile technique is required for the handling of Micro-fungi, including their collection from the field. The larger fungi, Macro-fungi as they are sometimes rather misleadingly called, such as Toadstools and Bracket Fungi often found on old mine timbers, can be conveniently collected into any suitably sized container, such as a tin or polythene bag, and can then be identified at home by the interested amateur using one of the popular handbooks, e.g. Lange & Hora[2] or Massee.[3]

It is quite true that some of the smaller fungi can be identified from preserved specimens collected into 40% Alcohol or 5% Formalin, but it should be stressed that when fungi are collected in this fashion there is no chance of culturing them afterwards, and that most identifications of micro-organisms are done by means of examination of laboratory cultures.

In fact, the principles of the special sterile technique necessary for the handling of fungi are quite easy to learn, and with a little practice the operations involved may come quite naturally, depending on the person, though perseverance coupled with a little manual dexterity is usually rewarded. The necessity for these special methods stems from the fact that the air everywhere is full of bacteria and the spores of fungi in suspension; and that these may easily fall into the mouth of any container when the lid is removed. Such contaminating organisms could greatly complicate the picture in subsequent subculturings of the specimen. It should be noted that it frequently happens, even with sterile working, that original field isolates are contaminated. This is mentioned in a little more detail further on, but for now we may

return to the principles of sterile work. ‘The way to eliminate chance contaminants from outside is to always use instruments which have been dipped in Alcohol and flamed for handling specimens; and to always pass the open end of the tube or bottle through the flame after removing the lid, i.e. before the specimen is introduced, and to repeat this flaming after Withdrawing the instrument used for transfer before replacing the lid. A little manual dexterity ensures that these operations are carried out smoothly, in correct sequence and keeping close to the flame, within the area of upward convection. A simple collecting kit can be got together very easily – a few robust glass bottles with screw on lids (2oz. McCartney bottles are ideal), a small scalpel or mounted needle, forceps, a bottle of Industrial Methylated Spirit into which the instruments can be dipped and some kind of burner, are the basic essentials. A good burner to use in the Camping-Gaz type, though the author has used a spirit lamp, and even a Carbide for this purpose. A further necessity is a stout box or bag into which everything stows neatly for transport. Pencil and notebook are handy for noting the details of collection sites. pH papers and a thermometer should also be included if available, since the more information we have the better. In Springs Wood Level there are thermometers and hygrometers permanently installed – an excellent facility for the cave biologist. The McCartney bottles used for collecting must be clean, and require to be sterilized before each trip. This ensures that all the stray organisms which may be present on or in the bottles are killed off, and it is normally accomplished in an autoclave; though a small domestic pressure cooker serves perfectly well for this if about a quarter of an hour at full pressure is used.

This then is the basic set-up, but for some cave systems, notably those without a draught blowing through them, the author has saved time by collecting small scrapings of the fungal colony direct on to sterile slants of a nutrient agar in the McCartney bottles, (Plate 16, Fig.1). Some of the more commonly used nutrient agars are conveniently available from the Biological suppliers in tablet form, requiring only to be put into the bottle with a little distilled water, in which they dissolve during autoclaving. More frequently though, the fungus and its substrate, or a sample thereof, is collected into an empty sterile McCartney bottle for sub culturing on to agar slants in the laboratory. A number of substances can be added to the agar to keep down any contaminating bacteria which sometimes arise due to the presence of more that one organism at the site of collection. The antibiotic Aureomycin, for instance, can be used at a level of 1.25 micrograms per millilitre of agar. Alternatively, mixed cultures can be separated into their component organisms by physical means, e.g. by making a suspension of the organisms scraped from the surface of the agar into a little sterile water, diluting this suspension, and spreading a little of it evenly over the surface of fresh agar in a Petri dish. The act of making a

dilute suspension separates the fragments of the organisms, which then give rise to distinct colonies when allowed to grow in the dish. A little skill is required for this, since one must work with sterile precautions throughout.

Having obtained ones cultures, or obtained the larger specimens whole, whichever is the case, it is then necessary to complete an identification. As has been mentioned already, this presents few problems in the case of the larger Badidiomycetes (the Toadstools and Bracket Fungi); and even some of the Entomogenous forms (living on insects) can be recognised quite easily e.g. Stilbellum (Stilbum) sp., but the vast majority require the attention of someone with an expert knowledge of Mycology. Practically all that can be said here of the processes of identification is that the presence, type and method of formation of the fungal spores is vital, whether they be sexual spores resulting from nuclear fusion, or of the asexual variety. Many fungi will form both kinds of spore under different conditions, and this may sometimes contribute to their identification. other important factors are the presence or absence of cell walls in the threads (hyphae) of the fungal body, and the distribution and colour of any pigments. Another matter of vital importance in fungal identification, and one which concerns us here, is that unpublished results are virtually worthless; even the person who did the determinations does not derive full benefit from his work, since he deprives himself of the exchange of information with other workers. The Cave Research Group of Great Britain, (to which the N.C.M.R.S. is affiliated) is undertaking to provide for the identification of material, and the publication of results in Hypogean flora lists.

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Of particular interest to the Society are the fungi that have been taken out of Springs Wood Level over the last year, by the author. The remainder of this paper presents these records in full, and mentions some interesting forms from other Sites, which might well come out of Springs Wood Level also, with further collecting.

1st Collection from Springs Wood Level - 28. 9.68.

1. From a sample of damp mud on wall 264m. in. Temp. 9C, R.H.98%. Sterile mycelium of a PHYCOMYCETE.

A colony of white non-sporing mycelium without cross walls in the hyphae, which is characteristic of the group Phycomycetes. A common member of this group of fungi is Rhizobus, which is often the first fungus to colonise damp stale bread. It bears dark coloured spherical sporangia at the ends of upright sporangiophores, and has been referred to as ‘pin’ mould.

2. Sample taken directly on to nutrient agar from muddy wall at 262m. from entrance.
Overgrown by Bacteria. NO IDENTIFICATION was possible.

3. White fungus on dead wood at the 'Magnesium' pool at 244m. Taken directly on to agar. Temp. 9C.
sterile white mycelium with crosswalls, possible a BASIDIOMYCETE. Many common toadstools do not produce their fruiting bodies in laboratory culture, indeed there are some isolates which have never been known to spore. The correct name for such isolates is MYCELIA STERILIA.

4. Branching structure growing upon a dead fly (Leria serrata), on wall at 231m. Temp. 9C., R.H. 98%.
Fungi Imperfecti
Moniliales, STILBELLACEAE
Hirsutella dipterigena (Petch).
Hot really difficult to identify. The identification was done on the fresh specimen as collected. The characteristic asexual spores (Plate 16, Fig. 2) were being formed in profusion.

5. Fungal covering on dead fly (L.serrata), collected on to nutrient agar. In wall crevice 161m. from entrance. Temp. 9C., R.H.98%.
Fungi Imperfecti
Moniliales (Dermatophyta).
? Trichophyton sp. (Fig. 3). (Plate 16)

A difficult fungus to identify correctly, the literature on Dermatophytes being confused. More attention has apparently been paid to the diseases they cause than to the organisms themselves. Dermatophytes are responsible for many skin diseases of man and animals, e.g. Ringworm, growing on and attacking the Keratin layers of the skin. It is therefore difficult to see what this one was doing on a fly, with its exoskeleton of Chitin. One presumes that the fly had picked up the fungus outside, and this had commenced to grow after the death of the fly. It seems improbable that the fungus was actually using Chitin as a food source. It is perhaps worth noting that several reports exist from other parts of the world, of Dermatophytes from the soil and air in caves[4,5] and also from fresh Bat guano.[6]

6. Compact colony covering a dead fly in roof crevice at 111m. from entrance. Temp. 9C.
Fungi Imperfecti
MONILIACEAE.
Beauveria globulifera (Speg) Pic.

Fungi of this genus frequently parasitise flies, and the spread of infection is known to be favoured by low temperature - high humidity conditions. It is therefore interesting to speculate as to whether

PLATE 16

Fig. 1. Colony of wall fungus being sampled on to a slant of nutrient agar. Note method of holding equipment.

Photo: E.W. Blackwell (M.N.R.C.)

Fig. 2. Hirsutella dipterigena (Petch)
Spores are formed on tapering sterigmata and have a mucoid coating. Microscope preparation made from a fresh specimen in Lacto-Phenol Cotton Blue stain.

Mag. x 400 approx.
Photo: B.D. Cubbon.

Fig. 3. ? Trichophyton sp.
Sporing structures from fresh specimen mounted in Lacto-Phenol Cotton Blue.
Material from Specimen No.7. (See text).

Mag. x 400 approx.
Photo: B.D. Cubbon.

Fig. 4. Tilachlidium sp.
Growth from a new isolate on agar slant.

Mag. x 4 approx.
Photo: A.E.McR. Pearce (M.N.R.C.)

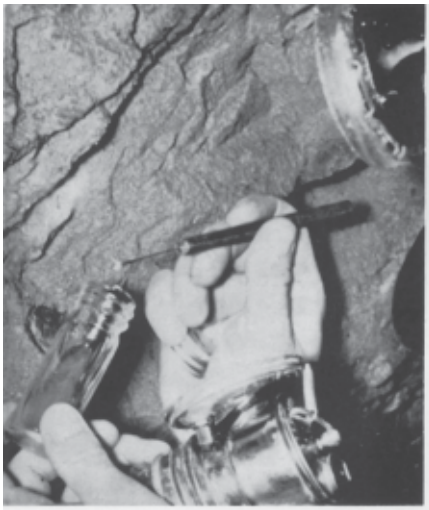

Fig 1 COLONY OF WALL FUNGUS BEING SAMPLED
Photo: E.W. BLACKWELL (MMRC)

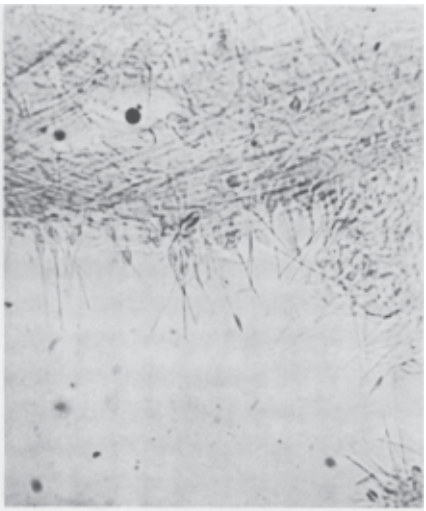

Fig 2 HIRSUTELLA DIPTERIGENA (Petch)
X400 Apx Photo B.D. Cubbon

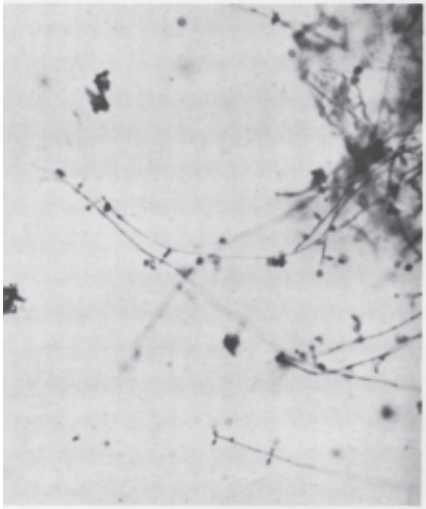

Fig 3 ? TRICHOPHYTON Sp. X400 Apx
Photo: B.D. Cubbon

Fig 4 TILACHLIDIUM SP. X4 Apx
Photo: A.E. Mc.R Pearce

Plate 16

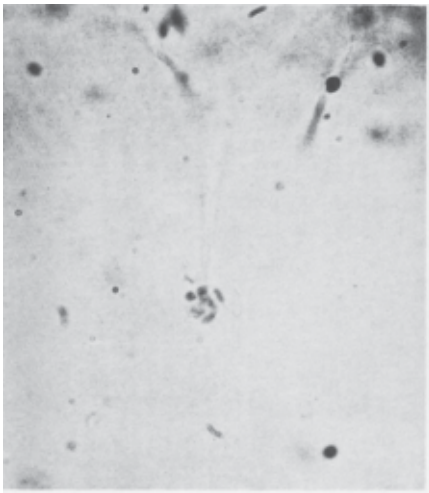

Fig. 5 TRACHELIDIUM Sp x 900 Apx
Photo. B. D Cubbon

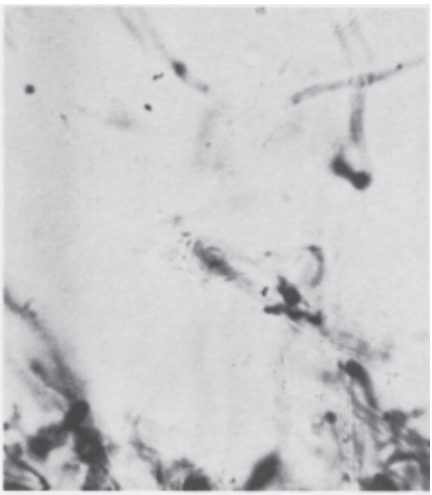

Fig. 6 OEDOCEPHALUM Sp x 900 Apx
Photo: B D Cubbon

Fig 7 SYNNEMATIUM Sp.
Apx Life size Photo A E. Mc R Pearce (MNRC)

Fig 8 MYXOMYCETE x 5 Apx
Photo A E. Mc R Pearce (MNRC)

Plate 17

PLATE 17

Fig. 5. Tilachlidium sp.
Spores aggregated into a spherical head at the tip of the tapering sterigma. Preparation in Lacto-Phenol Cotton Blue made from a new sub-culture on nutrient agar.

Mag. x 900 approx.
Photo: B.D. Cubbon.

Fig. 6. Oedocephalum sp.
Sporehead from agar culture of organism. Stained in Tryphan Blue and mounted in water.

Mag. x 900 approx.
Photo: B.D. Cubbon.

Fig. 7. Synnematium sp.
In situ, approx. life size.

Photo: A.E.McR. Pearce (M.N.R.C.).

Fig. 8. Myxomycete
Similar to T. favoginea but in this case the fruiting bodies are stalked. Specimen photographed in Sandford Lervy.

Mag. x 5 approx.
Photo: A.E.McR. Pearce (M.N.R.C.).

Beauveria infections are endemic among the flies in cave systems. It is certainly true that the gems is frequently isolated from the underground habitat, having occurred 7 times out of 16 flies collected, in both the North and South of the country.

7. Fungal covering on dead fly. On wall at approx. 70m. Collected into dry tube. Temp. 9C.
   IDENTIFICATION as No.5.

8. On dead fly close to No.7
   Fungi Imperfeoti
   Moniliales, MONILIACEAE.
   Aspergillus sp.

These are very common fungi, and produce a great proliferation of dry powdery spores easily dispersed by draughts. Their appearance underground is probably only to be expected. The same applies to the Penicillia.

2nd Collection from Springs Wood Level - 27.12.68

9. On dead fly on wall 50m. from entrance. A compact creamy coloured growth.
   Fungi Imperfecti
   MONILIACEAE
   Beauveria Bassiana (Vuill).

Another common member of this genus on flies. It gives a flat compact colony type on nutrient agar.

10. From dead fly on wall 200m. in.
    Identification as No.9.

Differs from No.9 in being a more flocculent (fluffy) type of growth, even under the same conditions in the laboratory. This dimorphism is not unusual in Beauveria spp. The actual method of bearing the spores, which is diagnostic, remains identical in the two forms.

11. Compact white growth on dead fly in crevice 50m. in. Quite a dry situation.
    Fungi Imperfecti
    Moniliales, STILBELLACEAE.
    Tilachlidium sp. (Figs. 4, 5). (Plates 16 and 17).

An interesting fungus, giving in new cultures an erect branching growth, which diminishes on repeated sub-culturing. Microscopic examination shows that round heads of spores are being formed at the end of a conidiophore. Among the Entomogenous fungi this is characteristic of Tilachlidium, but very old cultures tend to give chains of spores as well, which would indicate a member of the genus Coremium when found coupled with an erect growth habit of the particular antler-like form

shown. This illustrates very well one of the difficulties of fungal taxonomy, namely the inherent variability of some forms.

12. In roof crevice facing away from entrance, 50m. in. A dead fly in advanced state of decomposition with a 'halo' of fungus on the rock around it.
Cultures heavily contaminated by Bacteria, NO IDENTIFICATION possible.

This preliminary dozen isolates from Springs Hood Level shows that there are obvious possibilities in the isolation of species from underground, and that a failure rate of about 15-20% can be anticipated in the growth of these cultures successfully in the laboratory, (as represented by the loss of two cultures out of twelve). However, it should be noted that in the case of these Springs Wood Level collections made by the author, all specimens had to wait up to 10 days before being dealt with in the laboratory, due to being collected whilst on holiday and away from laboratory facilities. It is highly desirable as a general rule that fungi should be got into the laboratory as soon after collection as possible. In the case of cultures collected on Mendip, and transferred to the laboratory within 48 hrs., none were lost out of the first 20 collected.

Consideration will now be given to a few species from other localities, since they show interesting points, and may possibly be expected from Springs Wood Level, or from other sites within the N.C.M.R.S.'s field of interest, if more collecting is done.

Oedocephalum sp. This interesting fungus (Fig. 6) (Plate 17) was collected from an obvious colony growing on the wall of a Level very similar to Springs Wood, but situated on Mendip, being driven for about 1000ft. into the side of Blackdown, for the collection of underground water. It runs through the limestone to penetrate the sandstone core of the hill for a considerable distance. There is a variable water level, from about 4ft. down to a few inches. Oedocephalum is not a difficult fungus to identify, and it has previously only been reported from Britain in the tunnels bored by the Ambrosia beetles in the wood of forest trees.[7] In this association the fungus is literally farmed by the beetles for food. They grow beds of it in special parts of the galleries, carefully planting spores, and tending the fungus until the young shoots are at the right stage for grazing. The present find seems to prove that the fungus is quite capable of looking after itself in the absence of the beetles. Some authorities think that it is the imperfect stage of the bracket fungus Fomes annosus.

Synnematium sp. (Fig. 7) (Plate 17). This was collected in the same level by an interested amateur. It is very similar to Hirsutella,

but the spores are not borne the whole way along the fungal branches, but only close to the apices. There are other differences too, but these are again all microscopic.

Penicillium sp. Like the Aspergilli mentioned from Springs Wood Level these are very common, and liable to be found almost anywhere. They are related to the fungus from which the antibiotic Penicillin is made.

PEZIZALES. These are sexual forms, producing spores in a characteristic sac-like structure called an Ascus. They are known therefore as Ascomycetes. Small PEZIZALES have recently been found on decaying pit-props in Old Ham Iron Mine (Glos.), and in the Box Freestone Mines (Wilts.). They are common fungi on decaying wood above ground as well, and related species grow on dung. The spores are formed on the upper surface of a small disc or trumpet shaped growth. Other Ascomycetes e.g. the SPHAERIALES, form their spores within a more or less spherical body like a tiny flask, and these have also been met with underground in Box Freestone Mines.

MYXOMYCETE. Again collected by an interested amateur, the specimen was identified at Kew Herbarium as Trichia favoginea (Batsch) Pers. The specimen was found as small orange-yellow blobs crowded together on the surface of a pit-prop in the Box Mines. These are the sporangia of a most odd organism which spends half its life creeping about as an Amoeba, ingesting the Bacteria and bits of organic matter on which it feeds, then when conditions are right, it heaps itself up in the middle and differentiation of cells takes place to produce the spore bearing apparatus and spores. These Myxomycetes then, are half animal and half fungal in nature, and are in fact quite common organisms. Fig. 8, Plate 17, shows a closely related Myxomycete, but in this case a stalked fruiting body is formed.

ACTINOMYCETES. These are related to the organism which makes the antibiotic streptomycin. They are frequently met with in caves as small round patches of wall fungus (Fig. 9), which some to some people appear lichen like, and they have a most characteristic pungent earthy smell. They have been noted in Springs Wood Level and many other systems, but are exceedingly difficult to get out into pure culture. The only success in this direction is one identification of a probable Streptomyces sp. found growing on a sample of Kidney Ore in the Daylight Hole Iron Mine at Lindal-in-Furness.

It is evident from all this that not only is the collection of interesting micro-organisms possible by the interested amateur mycologist, but that the results from such Hypogean collections, few

though they be, are already giving rise to some interesting observations on the Biology of the organisms, and speculations as to their distribution and Ecology. There remain almost limitless possibilities for valuable research projects within the underground environment, and it is hoped to be able to enlarge on some of these at a future date, as well as to publish further results for the systems coming directly within the Society's sphere of interest.

Many thanks are due to the following for their help during these investigations:

Society members, especially D.T. Richardson, for trips to Springs Wood Level.

Roy Pearce of the Mendip Nature Research Committee for taking, and for copying, a host of photographs.

Bristol Water Works, for permit to visit their Level in Blackdown Hill.

Stoke Poges, July 1969.

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