Now who would have thought it?

Roy Watling

The classification of fungi is turning out to be more complex than was once thought. Roy Watling considers the inter-relationships of fungi and the changing views of mycologists in the light of rDNA sequencing data.

A knowledge of fungal systematics is fundamental to searches for useful secondary metabolites, antibiotics and other compounds for industry, and for understanding lifestyles, pathogenic development, endophytic growth and exploitation of fungi for biocontrol. Now at last fungal taxonomy has a more scientific basis, with molecular techniques using nuclear 18S and 25S, and mitochondrial 12S rDNA sequences producing some startling revelations. Although species concepts have been consolidated, and some relationships not previously appreciated revealed, major areas of our classical classification have been thrown into turmoil.

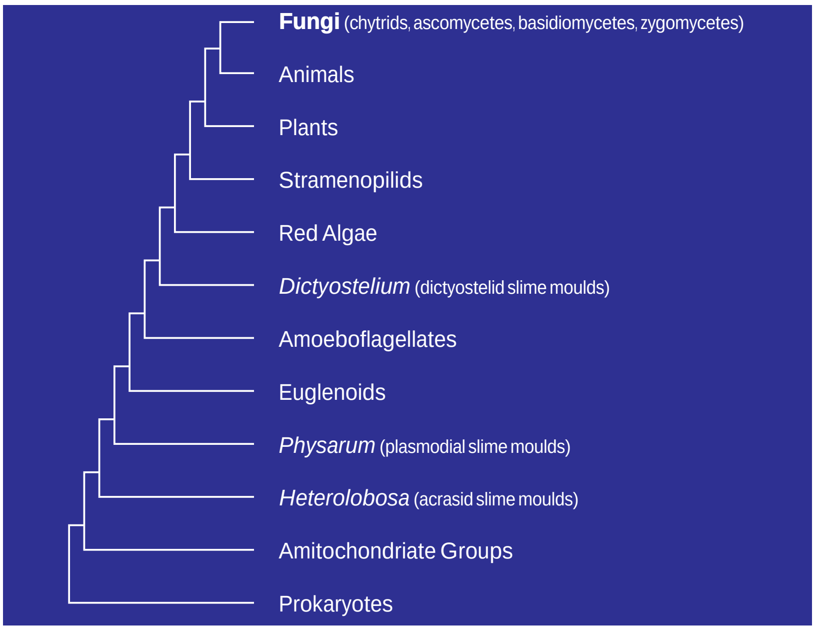
Fungi separated from the main line of organisms in the early Phanerozoic; well over 500 million years ago, according to workers such as Pirozynski. They appear to have branched off from the plants, together with the animals, at an early stage in evolution. This of course contradicts our traditional teaching. Mycology has been taught, and still is, in university plant science departments and fungi are studied in botanic gardens, or in microbiology departments (although their inclusion is a rare event these days and mycology has rather tended to fall between the two stools). The relationship to microbiology is an uneasy one – perhaps because the larger fungi fit incongruously in the context of microscopic organisms – and the fungi are not related to the prokaryotes as put forward in the classical texts. But the edible mushrooms of the supermarkets spend much of their time hidden from view in the soil or woody substrates breaking down complex compounds, and the humble penny bun (*Boletus edulis*), so familiar to those who buy dried mushroom soup, undertakes a very sophisticated root association with trees called sheathing mycorrhiza, before the 'micro-organism' forms its visible fructification: there are similarities in lifestyle between fungi and prokaryotes.

It now appears that the fungi are a mixture of disparate groups each with its own history. Based on small-subunit rDNA sequences, the majority of fungi, including our most familiar forms, can be distributed on four closely related branches (phyla) of the evolutionary tree, with the basidiomycetes and the ascomycetes closest to each other (see Fig. 1). Other organisms studied in the past under the umbrella term fungi can now be located in at least four other isolated groups, including three groups of slime moulds and one termed 'stramenopilids'. The latter, a kingdom in its own right, brings together the brown algae, diatoms and moulds related to the causal organism of potato blight, *Phytophthora infestans*. Thus the fungi can now be located in eight phyla, with some of the groups closer to animals than they are to other fungi. Nevertheless, it is still useful to maintain the old classical term fungi for a group of organisms which have similar modes of life and biological strategies.

The fossil record of fungi is rather sparse, although isolated spores attributable to this group of organisms have been found at a number of geological stages. Fossils of larger fungi are particularly rare, but some good examples have been located recently in ambers. Experimentalists such as Berbee and Taylor have suggested from 18S rDNA sequence comparisons that the origin of the basidiomycetous fungi was 400–300 million years ago and that mushroom ancestors appeared approximately 65 million years ago, as shown by the occurrence of clamp-connected hyphae in coal measures (Carboniferous). An early mushroom referable to the modern genus *Mycena* has been dated at between 15–20 million years and a *Marasmius* at 90–94 million years: both pre-date the separation of the ancient land mass Gondwanaland. These results suggest a rather long history perhaps as far back as the Cretaceous, the very period in which our now familiar flowering plants underwent rapid evolution.

Identification of the larger fungi, especially the mushrooms, toadstools and bracket fungi, has been fraught with difficulties over the years, differences between species being small and often based on subtleties in texture, taste and the like – all very subjective! Although these characters are underpinned by differences in secondary metabolites, anatomy and developmental features, identification of larger fungi has caused disbelief and frustration to those in neighbouring disciplines. Yet surprisingly, each new

BELOW:
Fig. 1. Phylogenetic tree, based on small-subunit rDNA sequence data and incorporating several eukaryotic taxa, showing the polyphyly of organisms known as the fungi.
DATA FROM VARIOUS SOURCES



TOP LEFT:
Dictyophora (petticoat stinkhorn)
COURTESY R. WATLING

LOWER LEFT:
A mushroom market in northern Italy.
COURTESY W. TAIT, EDINBURGH

experimental technique has provided more support for the majority of species recognized on classical grounds. The recent application of molecular techniques has not discredited the use of field characters in species delimitation, but has thrown up relationships between species which few would have imagined only 10 years ago. Interestingly, so-called aberrant groupings within the larger fungi, especially those in the 'Gasteromycetes' or stomach fungi (such as the false truffles, puffballs and stinkhorns) have been brought into the fold. But relationships between what were at one time considered familiar and biologically sturdy groupings have been destroyed. So the species concept has been fairly well supported but our understanding of relationships within the macromycetes needs to go back to the drawing board.

With such a long history there are undoubtedly various elements of the evolutionary pathway which have not become apparent from studying present-day fungi. But intriguingly, results keep emerging in each of the major groupings which suggest new relationships and challenge others. These results often appear in the most unexpected places, genera or even larger groupings of so-called fungi, and the value of features by which we identified a species in the field may have been almost entirely lost. Although for many mycologists, including the present author, the single taxon 'Gasteromycetes', which brought all these forms together in classification, was artificial and needed drastic sorting, in no way could it have been imagined just how fabricated it was!

True, some indication had already been given by developmental and micro-morphological studies which linked, for instance, the boletes with the false truffles. Bolete-like species, which have an intermediate morphology, had been placed in the genus *Gastroboletus* and a morphological series proposed, but even this genus has been shown, by molecular studies, to be based on convergent evolution: the individual species should be assigned to a range of different, existing genera, as suggested also from morphological analysis. Recently, workers at Berkeley, California, using parallel methods have been able to date the supposed separation of one such gastroboletoid fungus from its proposed parent group.

There are some parallel examples in the secotioid fungi or tobacco pouches, e.g. *Weraroa*, which have been studied in the same way as *Gastroboletus* and with similar results, various species being linked to a range of different mushroom families and genera. But these are not the core gasteromycetes. What of the birds nest fungi, the earth stars, the puffballs etc.? The brown-spored toadstool *Paxillus* has been associated by molecular techniques with the first group, but how different they are, one a mushroom and the other a small cup-like receptacle, the nest, containing capsules containing spores. Where is morphology now? Equally, the earth star, *Geastrum*, and cannon ball fungus, *Sphaerobolus*, are associated by molecular techniques with the fairy club fungus, *Ramaria*, and are separated from the puffballs despite the incredible resemblance, apart from the outer ray-like arms from which the common name 'earth star' derives. Puffballs (*Lycoperdon* and relatives) find themselves neatly parked amongst the parasol (*Macrolepiota*) and true mushrooms (*Agaricus*).

Using sequencing data the false truffle, *Melanogaster*, is now firmly within the boletes: this result is of great interest for over the years no true relationships have been proposed for this genus. This contrasts with some other false truffles which have each been associated with a family or genus of mushrooms based on anatomical similarities and which led to the term sequestrate being coined to delimit a morphotype and not a taxonomic entity. *Calsotoma*, a group of stalked puffballs with a stem of interwoven threads, gelatinized tissues and a suture-like apical hole from which the spores escape was also of unknown affinity. Recent work by Hughey and colleagues has shown, by using 12S, 18S and 25S rDNA analysis, that this genus can be also confidently inserted into the framework of the boletes. Horrors! The value of molecular methods is therefore demonstrated for not only does it throw up provocative data, but also it points to relationships in the absence of classical information. The bizarre-shaped and often evil-smelling stinkhorns (*Phallus* and its allies) now nestle amongst the agarics but at the moment the present author is at a loss to explain how the padi straw mushroom (*Volvariella*) sits with the split-gills (*Schizophyllum*), so frequently used in genetic experiments, and the beefsteak fungus (*Fistulina*).

TOP RIGHT:
Pleurotus ostreatus (oyster mushroom)
COURTESY R. WATLING

LOWER RIGHT:
Geastrum rufescens (earth star).
COURTESY R. WATLING

Combining morphological information and molecular techniques allows objectives to be set, testing proposed positions and relationships, e.g. the presence of the phenolic hydroxy pigment pistillarin found in certain club fungi should now be investigated in their newly proposed relatives. Such work would parallel the joint studies by Gill in Melbourne and the author who showed the use of anthraquinone production and pathways in helping to place the devil's club fungus (*Pisolithus*) in the boletes. Molecular support is required for the association of *Gyrophragmium*, a puffball-like fungus with *Agaricus*, demonstrated by the complex Schaeffer's test, which uses the reaction of aniline and nitric acid in the presence of the fungus tissues; also for the association of *Torrendia*, another puffball-like fungus, with *Amanita*, a genus which includes the death cap (*A. phalloides*), each pair only being previously linked through developmental similarities. Equally, on macromorphology alone, *Podaxis* has been linked to the lawyer's wig, a fungus traditionally placed in *Coprinus* although in molecular terms even it is not closely related to most species presently placed in the genus.

The reassessment of relationships is happening in the three other phyla of fungi, *viz.* Ascomycota, Zygomycota and Chytridiomycota, with equally interesting results and from which I could have taken examples. The scene is set for an exciting start to the new millennium, the first task being to consolidate these new studies and bring them into the main structure of classification.

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