

# Discovery of novel organisms leads to recognition of a major new basal fungal group: the cryptomycota



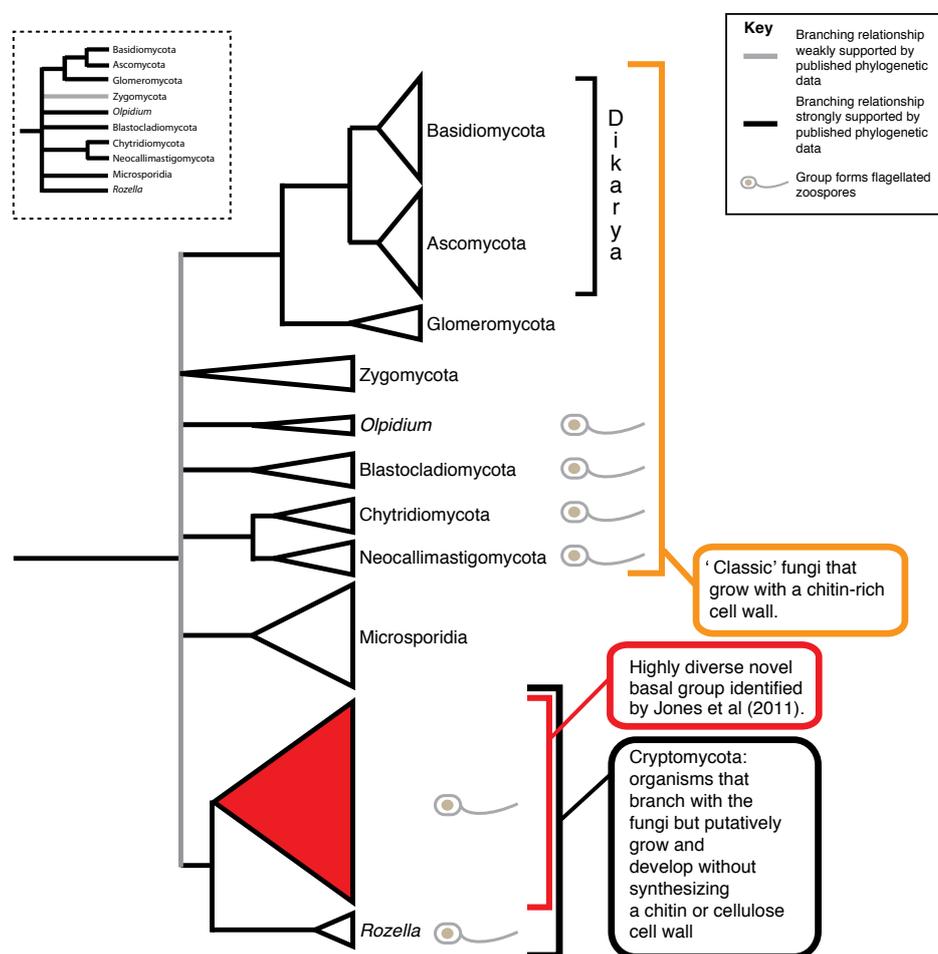
Meredith Jones, discoverer of the 'cryptomycota' in the Exeter University pond. Photo: courtesy T A Richards.

Just as the field mycologist that stays close to the excursion bus often finds the rarest fungus of the day, and the late Terence Ingold discovered aquatic hyphomycetes in streams near where he lived in Leicester, Meredith D. M. Jones obtained some enigmatic organisms from environmental sequences from a pond at the University of Exeter, UK (Jones *et al.* 2011). A comparison with sequence data in GenBank revealed that there were other environmental sequences that fell into the same clade; these were derived from a broad spectrum of aquatic habitats, including marine, polluted, and anaerobic as well as freshwater. Only a single named genus was found to belong to the clade – *Rozella*, represented by *R. allomyces* and *R. sp.* DNA probes were designed to see just how widespread these organisms might be in south-west England, and led to their detection in three Dartmoor reservoirs and four coastal

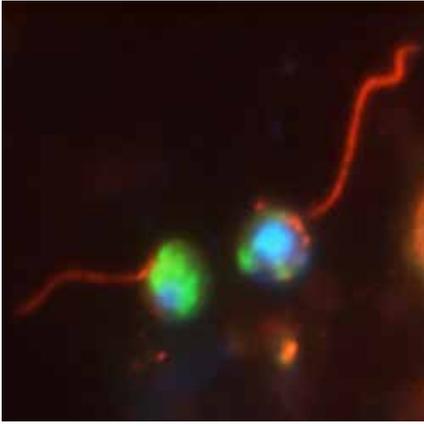
marine surface water samples.

Then, not content with knowing these organisms only from environmental sequences, the team used ten probe sequences conjugated for tyramide signal amplification fluorescence *in situ* hybridization (TSA-FISH). They were not disappointed. These organisms were revealed as unicellular ovoid cells of around 3–5 µm diam, in which some had a single distinct microtubule-based flagellum and others did not. This led the authors to postulate a life-cycle which included a cyst stage, interpreted the flagellate cells as zoospores, with both cysts and zoospores being able to attach themselves to second-party cells such as diatoms.

The issue as to how this evidently huge unexpected but well-supported clade of diversity might be classified then had to be addressed. The position of *Rozella* as a basal branch below the main fungal clade had



Summary of the phylogenetic and cellular context of the cryptomycota (after Jones *et al.* 2011). The tree in the hatched box, shows a common working model of the fungal tree of life. The larger tree shows a diverse uncultured basal fungal clade that branches with *Rozella*, demonstrating that current models of fungal evolution and biodiversity, which are largely based on cultured organisms, have missed a large fraction of the kingdom (perhaps even approaching one half). Furthermore, this group appears to grow without the 'defining' fungal characteristics a chitin-rich cell wall.



A swimming flagellate 'cryptomycotan' zoospore visualized by *in situ* fluorescence hybridization using TSA-FISH. Photo: T A Richards.

been something of an oddity since the study of James *et al.* (2006), but now it could not just be ignored. The authors did not detect any chitin in the walls of their organisms, but while it might be convenient to use the presence of chitin as an essential diagnostic

character for the kingdom *Fungi*, perhaps the circumscription should be emended to allow for a clade that diverged by the loss of this feature? The biology of the organisms remains obscure, though it has suggested that the absence of a firm outer wall might mean they could be phagotrophic rather than osmotrophic.

As an interim measure, the informal name "cryptomycota" was coined for the clade, although some mycologists have objected arguing that these organisms should not be included within the kingdom *Fungi* – to some extent paralleling the continuing debate over the placement of *Microsporidia*. See in particular the comments on the paper posted with the online version of the article on [www.nature.com](http://www.nature.com). It has been pointed out that the higher taxon name *Rozellida* has also been coined in the past, but I could not ascertain whether that was validly published. Also, taxon names above the rank of family under the botanical *Code* are not subject to the principle of priority, and the

practice of having names over the rank of order as in some way descriptive rather than based on an included genus has something to commend it, as for instance in the case of *Basidiomycota*. The suffix "–mycota" denotes the rank of phylum within the kingdom *Fungi*, and a formal validation of the name would stress the basal position of the group whether considered within or outside the kingdom. The debate is sure to continue, especially as the work was only published online on 11 May 2011 and the hard copy version on 9 June 2011.

I am indebted to Tom Richards for discussion and the figures included here.

James TY *et al.* (2006) Reconstructing the early evolution of *Fungi* using a six-gene phylogeny. *Nature* 443: 818–822.

Jones MDM, Forn I, Gadelha C, Egan MJ, Bass D, Massana R, Richards TA (2011) Discovery of novel intermediate forms redefines the fungal tree of life. *Nature* 474: 200–203.

## *Sebacinales*: a hidden force in plant ecosystems



*Sebacina dimitica* basidium. Photo: M Weiß.

It is hard to believe that it was only in 2004 that the *Sebacinales* were discovered (Weiß *et al.* 2004) as paper after paper has since attested to their diversity and ubiquity first as mycorrhizal fungi and then as plant endophytes. Although molecular work shows they belong to the *Agaricomycotina*, basidomes are unknown in most. Now, using a combination of DNA-based detection methods and electron microscopy, Weiß *et al.* (2011) have discovered that these fungi are even more ubiquitous than previously contemplated. They occur as symptomless

endophytes in liverworts, ferns, and all 27 families of herbaceous flowering plants examined – including maize, wheat, and the non-mycorrhizal model of geneticists, *Arabidopsis thaliana*. Further, they were found in roots of freshly collected material from 13 countries dispersed through four continents, and even detected in herbarium specimens collected in North Africa in the 1830s–1840s. The examination by transmission electron microscopy to demonstrate the characteristic septal pores of the order in roots, excludes the possibility that PCR detection methods were only picking up soil-inhabiting fungi present on root surfaces.

In the phylograms, similar or even identical sequences were found in plants from geographically widely separated areas, but at the same time diverse sequences occurred in a single field site, and in some instances in the root system of a single plant specimen. However, host-specificity appears to be rather low, with, for example, one sequence was found in the ericoid *Vaccinium* and in the liverwort *Riccardia*, another in genera from three different plant families (*Cavendishia* in *Ericaceae*, *Melittis* in *Lamiaceae*, and *Calamagrostis* in *Poaceae*).

Experimental work has previously shown that *Sebacinales* endophytes can have positive effects on plant (including crop) growth, yield, stress-resistance, and pathogen-resistance. These studies are significant in that they show that in addition to having important roles in mycorrhizal associations, these fungi may also have key roles in ecosystem processes. The authors also speculate the *Sebacinales* may eventually prove to be of practical value as biofertilizers or biocontrol agents. Investigations on these fungi, as in many others, which initially were curiosity-driven, now emerge to be of more importance than ever could have been contemplated on their first discovery.

Weiß M, Selse M-A, Rexer K-H, Urban A,

Oberwinkler F (2004) *Sebacinales*: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. *Mycological Research* 108: 1003–1010.

Weiß M, Šýkorová Z, Garnica S, Riess K, Martos F, Krause C, Oberwinkler F, Bauer R, Redecker D (2011) *Sebacinales* everywhere: previously overlooked ubiquitous fungal endophytes. *PLoS ONE* 6 (2): e16793. doi: 10.1371/journal.pone.0016793.

## How immune systems recognize fungal pathogens

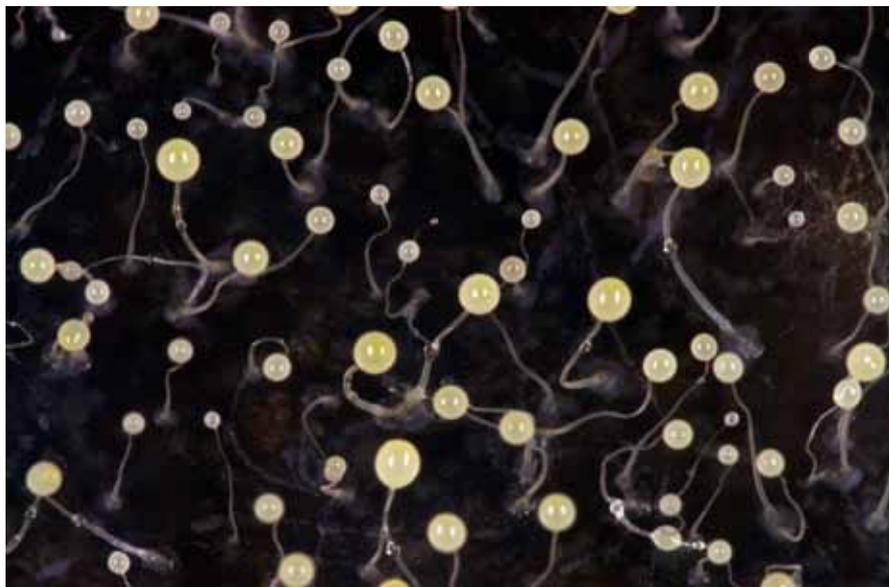
It has been known for some time that Dectin-1, produced by myeloid phagocytes, has some role in anti-fungal defence in humans, stimulating antimicrobial, inflammatory and innate immune responses. When Dectin-1 connects with  $\beta$ -glucans from fungal cell walls, ligation occurs, triggering the responses of the myeloid cells. Now, Goodridge *et al.* (2011) have shown, using *Saccharomyces*

*cerevisiae*, that the response is due only to particulate  $\beta$ -glucans, and that these cluster the receptor in synapse-like structures which also exclude tyrosine phosphatase regulators. This mechanism, referred to as the “phagocytic synapse”, by responding only to particulate  $\beta$ -glucans, means that activation is not prompted by the compound itself which may have been released by distantly located fungi

– i.e. the immune responses are not initiated except when the organism itself is present.

Goodridge HS, Reyes CN, Becker CA, Katsumoto TR, Ma J, Wolf AJ, Bose N, Chan ASH, Magee AS, Danielson ME, Weiss A, Vasilakos JP, Underhill DM (2011) Activation of the innate immune receptor Dectin-1 upon formation of a ‘phagocytic synapse’. *Nature* 472: 471–475.

## Bacterium-farming *Dictyostelium discoideum*



*Dictyostelium discoideum* sporophores. Photo: D A Brock.

be a source of bacteria they could devour when a new surface was reached. The process might be compared with the conjoint simultaneous dispersal of the fungal and algal partners in lichen mutualisms achieved through the production of soredia – but in that case particular genera or species of algae are involved. In the *Dictyostelium* case, however, a variety of bacteria were evidently involved, not all of which could be cultured by the methods used, but in the laboratory they could use *Escherichia coli* and *Klebsiella aerogenes*.

The authors refer to this food-carrying phenomenon as ‘primitive agriculture’, or ‘animal husbandry’, terms which some might consider inappropriate as anthropomorphic, but it could be embraced within the broad concept of ‘symbiosis’. That would be especially so if at least some of the bacteria were having their ranges extended or maintained by this perhaps evolving activity. There is clearly scope for further work on this phenomenon and to see if it is more widespread in other slime moulds.

Brock DA, Douglas TE, Queller DC, Strassmann JE (2011) Primitive agriculture in a social amoeba. *Nature* 469: 393–396.

Brock *et al.*, (2011) investigated the bacterium-feeding habits of different clones of the slime mould *Dictyostelium discoideum* collected from the wild. They had observed that some colonies directly isolated from soil at Biological Stations in Minnesota and Virginia had bacteria as well as *Dictyostelium* spores in the sori. Further isolations revealed that about one-third of the clones

showed this phenomenon. Laboratory studies showed that these did not simply engulf and devour all bacteria they encountered, as might be expected. Instead, they were more prudent, and stopped feeding before all bacteria were eliminated, incorporating them into their spore-masses so that they could be dispersed along with the spores of the slime-mould – thus ensuring there would

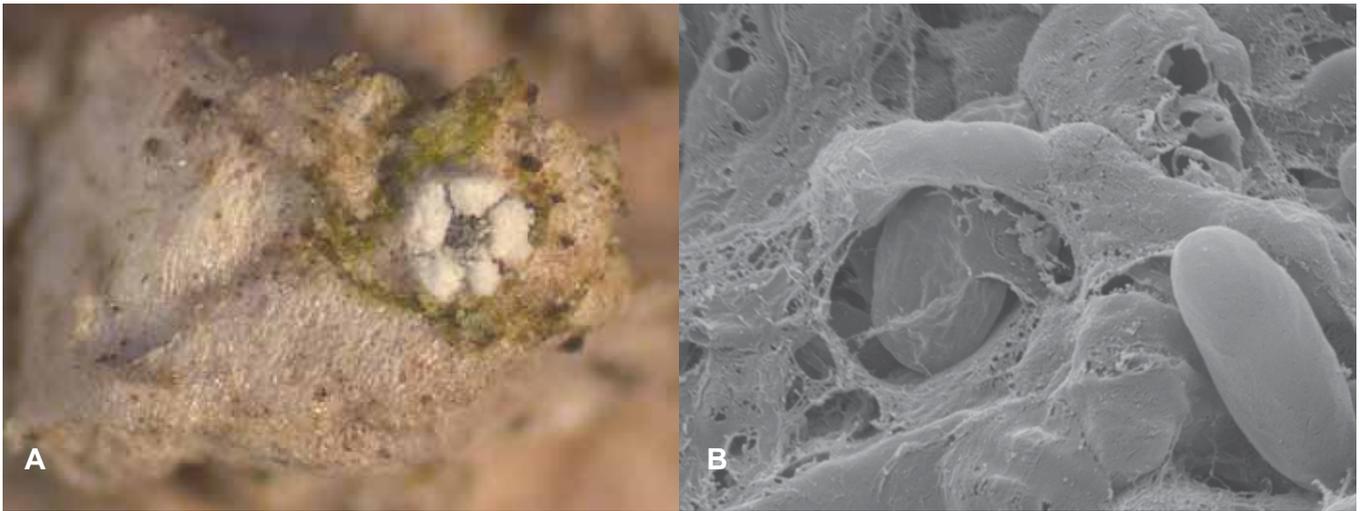
## A model system in which to study lichenization?

The ability of the same fungus species to form a lichen or not depending on whether it developed on bark or wood was first conclusively established with molecular data by Wedin *et al.* (2004), in several species of *Stictis* (syn. *Conotrema*), and reported on in more detail by Wedin *et al.* (2006). The situ-

ation in *Schizoxylon albescens* has now been investigated in more detail by Muggia *et al.* (2011) who examined the genetic distinctness of lichenized and saprobic collections, and also the diversity of the algae involved – and further conducted co-culture laboratory experiments to study the anatomical and

morphological interactions involving isolated algae as well as algal cultures obtained from some other lichens.

Haplotype studies based on 51  $\beta$ -tubulin and 57 ITS rDNA sequences revealed a single haplotype network whereas the 56 GPDH sequences obtained



*Schizoxylon albescens*. **A.** On *Populus* bark, showing an apothecium with algal colonies distributed by them. **B.** Fungal hyphae enveloping *Coccomyxa* algal cells in a co-culture, showing the filamentous mucilaginous material binding them together. Photos: Courtesy L Muggia.

separated into two distinct networks. The lichenized and saprobic collections were not distinguished, but the fungus evidently comprised two cryptic species, each of which could have either life-style. Cryptic speciation seems increasingly to be the norm in lichenized fungi (Crespo & Lumbsch 2010). The situation with the algae was more complex – the algae were allied to *Coccomyxa* and *Pseudococcomyxa*, but those obtained from thallus fragments grouped in a different clade from those represented by sequences obtained directly from specimens on bark. In the co-culture experiments, the *Schizoxylon* hyphae often covered the algal colonies, and microscopically could be seen to be surrounding and closely adhering to the algal cells. However, when *Trentepohlia*

or coccoid algae from *Flakea papillata* were used, such close associations were not formed. The association on bark and in co-cultures was further studied by scanning electron microscopy (SEM); the hyphae secrete an unidentified filamentous matrix that fuses the hyphae to the algal cells – in the samples of bark, bacteria and other fungal spores were also seen to become entangled in the lichenized thallus.

As the fungal and algal partners can be cultured both separately and together, and the fungus can be lichenized or saprobic, the authors commend the system as one for further experimental studies of the nature of lichen symbioses.

- Crespo A, Lumbsch HT (2010) Cryptic species in lichen-forming fungi. *IMA Fungus* 1: 167–168.
- Muggia L, Baloch E, Stabentheiner E, Grube M, Wedin M (2011) Photobiont association and genetic diversity of the optionally lichenized fungus *Schizoxylon albescens*. *FEMS Microbiology, Ecology* 75: 255–272.
- Wedin M, Döring H, Gilenstam G (2004) Saprotrrophy and lichenization as options for the same fungal species on different substrata: environmental plasticity and fungal life-styles in the *Stictis*—*Conotrema* complex. *New Phytologist* 164: 459–465.
- Wedin M, Döring H, Gilenstam G (2006) *Stictis* s. lat. (*Ostropales*, *Ascomycota*) in northern Scandinavia, with a key and notes on morphological variation in relation to life-style. *Mycological Research* 110: 773–789.