

CLASSIFICATION OF FUNGI IN THE AGE OF DNA

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There once was a time when the classification of mushroom-like fungi was relatively straightforward. One could go out into the field with a group of beginners and say with confidence “This is a jelly fungus” without too much fear of being challenged. Fungi with gills were called “agarics” and generally put into the Order Agaricales, whereas those lacking gills were put into one of the several other Orders. One of these was sometimes referred to as Aphyllophorales, which means “without gills” and included the brackets, leather fungi (thelephores), teeth fungi (hydroids), coral fungi (clavarioids), and various other sorts to form a loose collection of unrelated fungi. Even here, one could tell the novice “This is a hydroid species”, if the hymenial surface consisted of teeth, and the novice would happily nod in agreement.

This simplistic and very user-friendly approach to taxonomy persisted for almost two centuries, starting with the publication by Persoon (1801) of his *Synopsis methodica fungorum* and later augmented by the work of Fries. In this “Friesian System”, which had the advantage of being easy to apply in the field, the taxa of the higher homobasidiomycetes (mushroom-forming fungi) were classified by their gross morphology, i.e. whether they had gills, pores, teeth, etc. Spore print colour also played an important role in setting limits to the families. Towards the end of the 19th Century, it became apparent that an anatomically based approach was not going to form a basis for a phylogenetic system, that is, one that would reflect the evolutionary pathways linking the taxa. Nevertheless, systems based on anatomy survived throughout most of the 20th Century, sometimes accepting compromises that allowed taxa of dissimilar appearance to be classified into the same family and Order. The last decade of the 20th century, with the rapid development of technology for sequencing DNA, has had a marked effect on the phylogeny of plants, and the same has been true for fungi. This has led to the “falling apart” of the Agaricales, as predicted by Rolf Singer in 1951, so that it is now recognised that gilled mushrooms have evolved on more than one occasion from morphologically unrelated ancestors. Technically, the word for this evolution from unrelated sources is called “polyphyletic”, rather than the monophyletic structure that is the preferred goal of hierarchical-based classification systems. Systematists like to see species arranged in groups that reflect their evolution (phylogeny) rather than their outward similarity of form. The recent use of nuclear and mitochondrial large and small subunit ribosomal RNA genes has led to a breakdown of taxa erected on artificial characters and a bringing together into monophyletic groups, called clades, of what traditional taxonomists would have seen as disparate groups.

The results at the higher taxonomic levels when mushroom-forming fungi are subjected to rDNA work are not all that straightforward. The inferred relationships depend to some extent upon the regions from which the rDNA sequences are extracted. The combinations of nuclear and mitochondrial with large subunit and small subunit genes, give four distinct regions for inferring relationships at higher classification levels. Binder and Hibbett (*Molec. Phylogen. Evol.* 22: 76-90, 2002) found that when information from all four regions were combined, there was strong support (using resampling techniques such as bootstrap analysis) for eight clades, viz. (i) euagarics (“true agarics”), (ii) boletes, (iii) russuloid, (iv) polyporoid, (v)

hymenochaetoid, (vi) thelephoroid, (vii) cantharelloid, and (viii) gomphoid-phalloid. The euagarics clade was close to the bolete clade with which it forms a “sister group” relationship. When less than four regions were used, the support for these clades was not as strong. Another recent study (Moncalvo et al., *Molec. Phylogen. Evol.* 23: 357-400, 2002) took nucleotide sequences for 877 mushroom-forming taxa, including ca. 700 species of gilled mushrooms traditionally placed in Agaricales, and concentrated on the nuclear large subunit gene. Basically, their results support the eight clades defined above, but the russuloid clade and the polyporoid clade were recovered in only one of the two analyses performed (either maximum parsimony or bootstrap). Thus, DNA work to date is still far from assuring unanimity.

The composition of some of the Orders is astonishing, even dismaying, if viewed through the eyes of the classical taxonomist. For example, the Order Agaricales, in addition to containing many, although not all, of the gilled fungi, also contains the puffball genus *Lycoperdon*, the coral fungi genera *Clavaria* and *Macrotiophula*, and the polypore genus *Fistulina*. The Order Boletales not only houses the boletes, but also the “Pagoda Fungus” *Podoserpula pusio* and the puffball genus *Scleroderma*. The Cantharellales not only contains *Cantharellus*, but also the coral fungus genus *Clavulina* and the “tooth” fungus genus *Hydnum*. The Order Phallales includes phalloid fungi like *Aseröe* and *Mutinus*, but is also home to the puffball genus *Geastrum* and the coral fungus genus *Ramaria*. Russulales contains *Russula* and *Lactarius*, to be sure, but also the coral fungi *Clavicornia* and *Mucronella*, the leather fungus genus *Stereum*, and the jagged-toothed gilled fungus genus *Lentinellus*. In this phylogenetic approach, coral fungi are spread out among four Orders, puffballs and hydroids are in three Orders each, and jelly fungi and thelephores in two Orders each.

Has the emergence of DNA-based research into fungi overthrown forever the Friesian system and made life unbearable for those of us who prefer to continue to think of fungi in the relatively simple, traditional way? Or will we wake up from our slumbers and realise that it has all been a bad dream? Will the phylogeny change drastically if the focus of the phylogeneticists shifts from their present preoccupation with nuclear and mitochondrial large and small subunit genes to protein-forming genes? Only time will tell. Meanwhile, I expect that I will continue to tell the participants who come out on our “Fungal Frolics” that “this is a hydroid fungus”, because, “as you can see, the fertile surface has teeth”, and hope I won’t get bitten.