

opportunity to benefit from his experience. Over 80 isolates of over 60 species were available for examination over the 5 days of the workshop. The isolates were grouped into themes, and our examination was guided by Dr Simmons' commentary. Most of us came away from the workshop exhausted, and having confirmed that *Alternaria* is as difficult as we thought it was. The difference now is that we know how difficult it is. We now know that much of what we have been calling *A. alternata* is not that species. We know also that cultural conditions are critical in determining the morphology of the fungus. During the workshop it was discovered that the slightly higher humidity in the workshop lab, had caused several changes in the cultures, when compared with conditions in Simmons' own lab. Critical characters such as conidiophore branching patterns and conidial ornamentation were affected to such an extent that isolates no longer conformed to their descriptions.

I also visited the National Fungus collections at Beltsville, and had useful discussions with Mary Palm, Amy Rossman, Gary Samuels and David Farr.

## CLADISTIC ANALYSIS IN THE MACROFUNGI USING MORPHOLOGICAL CHARACTERS

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Studies of the Australian taxa of the Hygrophoraceae suggested that cladistic analysis might be useful when applied to morphological data. Puttock (1992) applied cladistic analysis to morphological characters found in the Australian Gardenieae and obtained apparently useful results, so the application of similar analysis to fungal data seemed to have good possibilities. The 57 taxa established for the Australian Hygrophoraceae and 51 associated morphological characters were used to set up the required data matrices and both PAUP 3.1 (Swofford 1993) and HENNIG86 (Farris 1988) employed for their processing. Although the experience has been interesting (if occasionally very frustrating), the results can only be said to have limited value—at least thus far.

To underscore one of the main problems, fungal taxa are extremely variable...this is probably the understatement of the year. Arnolds (1985) wrote: 'It is inherent to nature that some species are more variable than others, in other words that there is variation in variability'. The results of this study of the Hygrophoraceae concur absolutely. The extreme homoplasy created by fungal characters appearing and disappearing in both related and unrelated taxa creates a very fluid situation which is most definitely not conducive to producing data that is readily processed by cladistics programs.

One of the stated aims of cladistic analysis is the attempt to reduce the intuitive nature of phenetic taxonomy (Steussy 1990). An essential part of such analysis is the allocation of primitive and derived states for the various characters. In the fungi, this is extremely difficult or impossible when using macro/micro-characters for without a fossil record, primitive states can only be set by opinion...but then that is what traditional, phenetic systematics is so often all about. The selection of the outgroup taxon to best mirror the primitive states is also a matter of opinion. Finally, just to give the opinion knife a little twist, which morphological characters are useful for a cladistic analysis and which are not, and should any of them be weighted? This is not in any way intended to decry the value of cladistic analysis. On the contrary, such analyses are already shedding extremely valuable light on the derivation of fungal taxa and their geographical distributions. The problems raised do, however, point out to the cladist intending to use morphological characters some of the difficulties involved when they are applied to the macrofungi.

A recently published procedure for analysing the value of phylogenetic data is the 'phylogenetic signal to random noise' test proposed by Hillis (1991). This involves using PAUP to set up a frequency distribution plot of at least 1000 randomly produced trees. (PAUP has this facility built in.) Tree numbers greater than 1000 will probably give better results. Hillis postulated that a purely random set of data with no phylogenetic signal would produce a normal distribution of generated tree lengths and consequently there would be no skew. This would also mean large numbers of equal length, minimum parsimony trees. PAUP's measure of skew ( $g_1$ ) would then give  $g_1 = 0$ . If a data matrix did contain a phylogenetic signal, then there would be a definite skew,  $g_1$  would have a larger absolute value and there would be fewer minimum parsimony trees. Hillis' proposal seems to have been confirmed by Hopple & Vilgalys (1994) using DNA mapping, where a strong left skew ( $g_1$

= -0.64) produced only 6 trees of equal minimum parsimony.

Using all the data from the Australian Hygrophoraceae, initial runs using PAUP were very disappointing. With only taxa from genus *Hygrocybe* retained, a bootstrap analysis of 100 replicates was halted during day 3 when the computer aborted the procedure after having completed only three replicates. The reason was insufficient memory through too many trees having been produced. (These results are similar to those of Chris Puttock where only 1 replicate was completed for the same reason.) As a consequence of this situation, only simple heuristic searches could be made, and these do not guarantee the most parsimonious trees.

A first search with all taxa of the Australian Hygrophoraceae took nearly 4 hours and produced nearly 1500 trees of equal minimum parsimony. A frequency distribution plot showed  $g_1 = -0.11$  which implied minimal skew and little or no phylogenetic value in the data. The next step was to include only taxa from genus *Hygrocybe*. This gave  $g_1 = -0.27$  and only 8 trees of equal minimum parsimony. While this result was still comparatively dubious, it did at least give a consensus tree that had a little value for comparison with the structure proposed by traditional morphological systematics.

The consensus tree has been compared with the structure produced by phenetic methods and there is some agreement in the groups of taxa but also much disagreement. An interesting aspect is that one group of taxa is emphasised as possibly being a separate and new subgenus and phenetic analysis had already suggested that if future data was consistent then this would be quite feasible. It is also possible that further refining of the data matrix could take place. By omitting various characters, possibly some taxa, and doing distribution plots as the omissions proceeded, it would be possible to find which combinations gave greater values for  $g_1$  and hence greater data reliability. This procedure has yet to be tested.

Currently, the conclusion can only be that cladistic analysis has uncertain benefits when applied to analysis of morphological data for at least one family of the macrofungi. If the analysis supports the proposed systematic structure, well and good, but such analyses should be approached with a good deal of caution. There is a plethora of material available on cladistics and increasing amounts of material on research done on the macrofungi, but most (if not all) published material concerns cladistic analysis of genetic data. Cladistic programs were designed to deal with precisely that sort of data input.

Mitchell *et al.* (1995) argued cogently that both classical biologists and molecular biologists have a part to play in formulating systematic structures. It seems that morphological taxonomy is excellent for describing and identifying taxa while simultaneously formulating what can only be said to be a 'draft' systematic structure. Cladistic analysis of genetic and biochemical information, then gives mycologists a chance to test and refine the draft structure; there is no conflict between the two disciplines—each complements the work of the other and is of equal importance. So far, the work completed on the Australian Hygrophoraceae seems to be in agreement with the above premise.

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