Phylogenetic implications of generic concepts in fungal taxonomy: The impact of molecular systematic studies.

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Introduction

One of the major challenges faced by systematists is the circumscription of taxa. At the species level, mycologists have the option of employing a variety of genetic criteria based on mating behavior and population genetics. At higher taxonomic levels, however, it is not possible to apply classical genetic criteria toward systematics. Different genera of fungi simply do not form hybrids, and so other methods are necessary to obtain comparative data for systematic studies. For understanding relationships above the species level, it is quite clear now that molecular sequence data can provide an almost unlimited source of genetic characters that are useful for systematic studies. The aim of this paper is to discuss how molecular data may be applied toward understanding generic and other higher taxonomic concepts in fungi[1].

Our genus concept is one which emphasizes evolutionary history:For taxonomy to reflect evolutionary relationships, genera need to be based on monophyly. Non-monophyletic taxa should be discarded in favor of generic concepts which reflect descent from a common ancestor. The strict application of the criterion of monophyly will certainly lead to short-term nomenclatorial instability in many groups of fungi. The predictive power and long-term stability of monophyletic classifications, however, justifies their use.

There is little doubt that molecular systematic studies will have an impact on fungal taxonomy and how it is practiced. The "revolution" in molecular systematics, however, is actually two separate revolutions: one technological and the other intellectual. The technological revolution is discussed in a recent review by Bruns et al. (1991), which summarizes different applications of molecular methods for fungal systematics. The full significance of molecular data, however, can only be fully appreciated within the broader analytical framework to which it is applied. One of the truly exciting aspects of using molecular data is the way in which it lends itself to analysis using modern principles of systematics based on phylogenetic theory. Viewed in this manner,

[1] Details concerning methods used for molecular analyses described in this paper have or will eventually be published in a more complete form elsewhere; they are also available upon request from the autors.

molecular systematics is an integral part of the broader realm of modern systematics that seeks to understand all aspects of organismal evolution, based on data from morphology, development, ecology and molecular biology.

The taxonomic process

Major advances in systematic biology are the result of several processes, which include description of new taxa, as well as the application of new methods of data collection and analysis. The history of mycology can also be viewed in terms of the methods and taxonomic principles applied by different schools. For example, most students of agaricology trace the development of modern taxonomy as an episodic series of refined classification systems, beginning with the relatively simple macroscopic system of Fries and culminating with our current Singerian system, which includes information from a variety of sources including microanatomy, ultrastructure, and (to a lesser extent) biochemistry. Viewed in this regard, the comparative study of molecular data based on analysis of DNA is certainly part of a continuing systematic tradition.

The purpose of taxonomy also dictates the type of classification system one might choose. Classification systems serve a variety of needs in biology. For example, **utilitarian** purposes dictate that taxonomy needs to be based on characteristics which are easily recognized by persons other than the expert for a given group. Such an approach values taxonomic schemes which are "user friendly". The **scientific** purpose of a classification system, however, is philosophically independent of the user-defined goals of taxonomy and thus may appear to be sometimes diametrically opposite. The scientific aim of taxonomy is to accurately reflect what is known about the biology of the organisms as completely as possible, including their evolutionary relationships and other relevant characteristics. Most modern classification systems reflect this second aim, while still trying to conform to the first one.

In essence, a single "perfect" classification for fungi already exists: their **phylogeny**. Fungi, as all organisms, share a unique evolutionary history which reflects the origins of their biological diversity in a most complete fashion. One primary goal of taxonomy can, therefore, be viewed as an attempt to reflect the phylogeny of a group as best as possible. Although taxonomists have been advocating phylogenetic classifications since Darwin's time, methods for determining phylogenetic affinities have not been available until only recently. As a result, most earlier hypotheses regarding the origins of different fungal groups still rest largely on the authority of individual taxonomists who are familiar with them.

Recent advances in the way systematists view and analyze taxonomic data now make it feasible to address phylogenetic questions within a common theoretical framework. In fungi, the explicit application of phylogenetic principles to classification has only been attempted in a few groups, mostly using conventional morphological characters (Crisci et al., 1988, Tehler, 1988; Oberwinkler, this volume). We have also been employing such a phylogenetic approach in our own studies on the molecular systematics of fungi. As we will demonstrate below, molecular data are particularly amenable for developing explicit phylogenetic hypotheses. In contrast, the highly variable and plastic morphological features commonly used to classify fungi are not always easily studied using phylogenetic criteria, particularly since their homology is not well understood (Vilgalys, 1986).

Molecular data for systematic analysis

One principle advantage of molecular data for systematics is the larger size of most molecular data sets. For most fungi whose average haploid genome size is 3×10^8 nucleotides, fungal molecular systematists shall certainly be busy collecting DNA sequence data for some time. To date, most molecular studies have concentrated on only a few genes, such as ribosomal RNA, using a restricted number of taxa. In the future, however, mycologists will likely be able to expand and combine comparative data sets to include a truly representative sample of the fungal genome. For this purpose, actual nucleotide sequence data will be most useful in the future, since they can be collected into an ever growing data base.

The type of DNA molecule and gene under study provide different levels of information about fungal evolution. Evolutionary patterns may vary depending on whether one samples data from the mitochondrial or nuclear genome. Rates of substitution and other changes may also vary considerably from one gene to the next, and even among different regions within the same gene (Bruns et al., 1991). The variable rates of change can be an advantage, since they can provide phylogenetic resolution over a wide range of taxonomic levels being studied.

In spite of claims to the contrary, phylogenetic analysis of molecular data relies on the same principles applied toward other systematic data. The same phylogenetic criteria applied toward morphological characters, such as homology and polarity, apply equally for molecular sequence data. Because DNA sequences consist of a discrete string of nucleotides, each a potential character, homology of DNA characters is often inferred from their relative positions and identities. Similar positional homology is commonly inferred for

morphological characters (e.g., caulocystidia located near a stipe apex). However, the complex features of fungal anatomy and development sometimes make it difficult to apply morphological characters for phylogenetic analysis in a straightforward fashion. For example, morphological structures such as cystidia or spore ornamentation often intergrade, making it difficult to define character states.

Many other common characters used for agaric systematics may simply not be homologous to begin with. Characters such as cheilocystidia, universal veil, and spore wall ornamentation have each evolved numerous times in various lineages of agarics. Often times, a potentially useful character, such as pleurocystidia, may develop in some closely related taxa but not in others, while in other taxa it may have never appeared. As a result, phylogenetic interpretation of morphological characters may rely more heavily on the experience of the investigator. Morphological data will remain to be critical for understanding the phylogeny of fungi; however, it is also clear that additional evidence from developmental and molecular studies will be required to completely appreciate the phylogenetic information contained within the morphological characters used by systematists.

USE OF MOLECULAR DATA FOR IDENTIFYING TAXONOMIC GROUPS

The first challenge faced by any practicing systematist is to identify meaningful groups within a larger set of organisms. Traditional characters based on ecology and morphology have provided us with a basic framework for recognizing most higher taxa. As taxonomic systems become refined, however, additional data are sought to provide new characters which can be used to support existing taxonomic concepts or erect new ones. For this purpose, molecular characters are already being used to reassess taxonomy in a variety of fungi. By providing an independent data set, molecular characters provide needed information about evolutionary relationships for making informed decisions about classification.

As with other types of systematic data, molecular data are already being used in two different ways to clarify taxonomic relationships. As a first approach, molecular data may be applied in a phenetic manner to group different species (and strains) based on genetic criteria. Examples of using molecular data for phenetic grouping include the application of DNA/DNA hybridization evidence as well as restriction fragment length polymorphisms (RFLPs) based on either nuclear or mitochondrial DNA. In such instances, evidence for genetic similarity (or non-similarity, as may be the case) provides the main criterion for recognizing members of a common group.

Similarity, however, is not the optimal criterion for classification, since taxa recognized in this manner may not necessarily share the same evolutionary history. For this purpose, an explicitly phylogenetic method of analysis based on discrete characters (i.e., cladistics) is more appropriate. Molecular data are ideally suited for cladistic analysis (Bruns et al., 1991). However, not all kinds of molecular data can be analyzed cladistically (e.g., DNA hybridization or RFLP data). For this reason, phenetic approaches will still provide a useful preliminary approach to many basic taxonomic questions about generic groupings.

Most taxonomic applications of RFLP data in molecular mycology have only been primarily useful at lower taxonomic levels in fungi, usually below the rank of species. However, by studying molecules with lower rates of evolution, comparisons may still be made at higher taxonomic levels as well. Of the many molecules chosen for higher level taxonomic studies, the genes coding for ribosomal RNA have been found to be most generally applicable at a variety of taxonomic levels ranging from Kingdoms down to genera. As an initial approach toward molecular-based classification, we have been using RFLP obtained from enzymatically amplified regions of ribosomal DNA (rDNA) to assess taxonomic relationships in a variety of basidiomycetous groups (Cubeta et al., 1991; Hibbett and Vilgalys, 1991; Vilgalys and Hester, 1990). This approach, which we call "PCR fingerprinting", involves the use of the polymerase chain reaction (PCR) to selectively amplify a region of ribosomal DNA known to vary among even closely related taxa. By employing frequent-cutting restriction enzymes having 4-base recognition sequences, genetic differences between strains and groups of strains may be reliably diagnosed with a minimum amount of time and effort. Because this technique does not require the use of radioactive reagents and gives immediate results (in the form of directly visible restriction patterns), PCR fingerprinting provides an extremely rapid view of genetic variability within a particular study group. By indicating what levels of overall genetic variability exist within the group, results of initial phenetic analyses based on PCR fingerprinting also provide a useful guide toward which sorts of additional molecular data ought to be sought. When sufficient numbers of restriction enzymes are employed, we have found that the initial results of PCR fingerprinting yield similar results to more rigorous cladistic analyses based on nucleotide sequence data involving the same groups of taxa (Hibbett, 1991).

Linking of anamorph and teleomorph classification systems: the genus *Cryptococcus* and its teleomorphic affinities in the Filobasidiaceae

One of the most powerful applications of molecular systematics will be for establishing biological identity between asexual and sexual stages of the fungal life cycle, finally permitting adoption of a universal holomorphic classification scheme. The complex life history of many fungal groups has resulted in the adoption of diverse classification systems, which rely on features of the teleomorph or anamorph, but rarely both. We recently employed a molecular approach for establishing genetic identity and anamorph-teleomorph relationships of several yeast strains classified within the genus *Cryptococcus* (Vilgalys and Hester, 1990). Most species of *Cryptococcus* are known to be associated with a *Filobasidium* teleomorph (Kreger-van Rij, 1984). One species, *C. neoformans*, has been shown to have a different teleomorph described as *Filobasidiella* (Kwon-Chung, 1975). Although *C. neoformans* is important as an opportunistic human pathogen in victims of acquired immune deficiency syndrome (AIDS), its relationship with other *Cryptococcus* species is not known. Furthermore, since most *Cryptococcus* species do not produce their teleomorph in culture, correct taxonomic placement of clinical strains is often problematic.

Recently, molecular approaches have provided a convenient means for distinguishing between strains of *Cryptococcus* having different teleomorph associations (Vilgalys and Hester, 1990). In that study, we employed PCR fingerprinting to analyze a part of the rDNA gene from a variety of *Cryptococcus* strains. Restriction analysis of the resulting PCR fragments using frequent-cutting enzymes results in the production of restriction patterns that may be used to distinguish among genera, species and even individual strains in some cases. Using only four restriction enzymes, 13 distinct rDNA genotypes could be distinguished among a sample of over 30 strains (Vilgalys and Hester, 1990). By assessing patterns of similarity among the diverse restriction phenotypes (fingerprints), a rough estimate of genetic (and phylogenetic) relationships among strains was obtained. The tree depicted in Figure 1 shows genetic relationships among the 11 major rDNA types which were detected for different strains of *Cryptococcus*. All strains of *C. neoformans* were found to share identical "fingerprints" for their rDNA, and were clearly distinguished from other *Cryptococcus* strains. These molecular data support the association of all *C. neoformans* strains with a *Filobasidiella* teleomorph. Similarly, several other strains of *Cryptococcus* are also shown to be associated within a more heterogeneous teleomorph genus *Filobasidium* (Fig. 1).

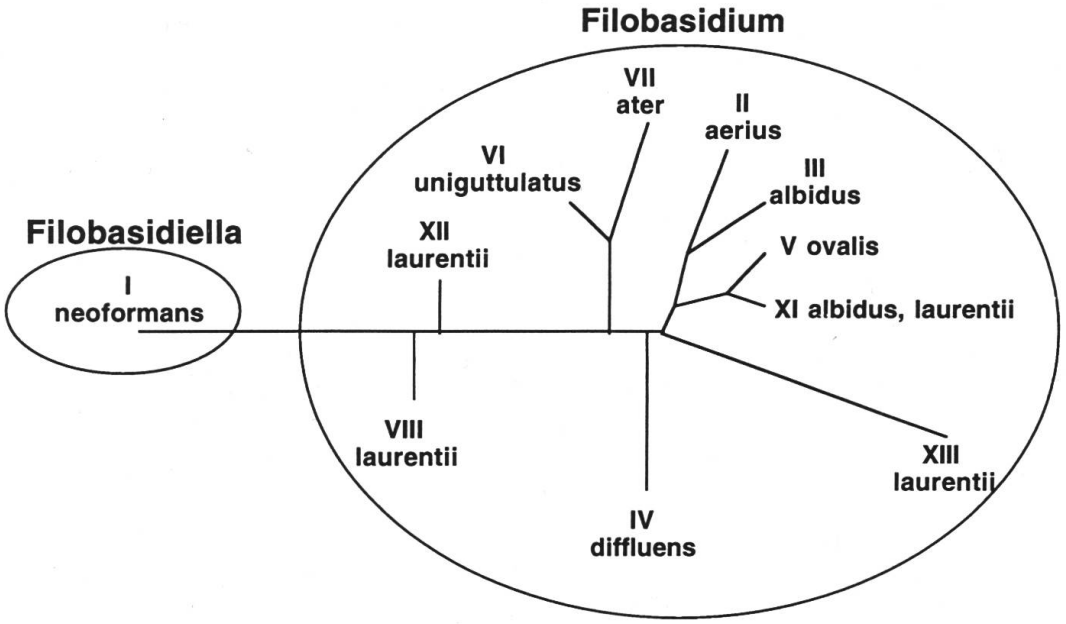

Figure 1. Genetic relationships between strains of the anamorphic genus *Cryptococcus* and its basidiomycetous teleomorphs in *Filobasidium* and *Filobasidiella* (adapted from Vilgalys and Hester, 1990).

The applicability of molecular data for establishing anamorph-teleomorph relationships again emphasizes the importance of using a single phylogenetically based classification system for all fungi, and questions the continued reliance on separate classification schemes for imperfect fungi (Bruns et al., 1991).

THE GENUS CONCEPT: THE SIGNIFICANCE OF MONOPHYLY

Patterns of character evolution vary according to the taxonomic level being investigated (Wiley, 1981). At the species level and below, several patterns of evolution are possible that do not occur at higher taxonomic levels. For example, hybridization between divergent populations or closely related species may result in introgression of characters from one lineage to another. The resulting pattern of reticulate evolution makes phylogenetic analysis difficult if not impossible. Mechanisms of rapid speciation (e.g., through allopolyploidy) may also produce a situation whereby a progenitor species and its derived taxa are all extant and represented within a phylogenetic tree. Examples of progenitor-derivative species pairs are commonly encountered among land plants, but have not yet been clearly documented for any of the

fungi. These patterns of evolution present serious problems for phylogenetic analysis at the species level; it is not clear whether they present a problem for phylogenetic studies at higher taxonomic levels.

In contrast to the variety of phylogenetic situations that are possible at the species level, genera and other higher taxa have a much more restricted range of possible evolutionary relationships. Higher taxa represent historical entities, and as such cannot be directly ancestral to other higher taxa (Wiley, 1981). Instead, higher taxa represent groupings (of lower taxa) which are based on systematic criteria. The criteria employed by taxonomists for defining higher taxa vary considerably, and are often not explicitly stated. In many groups of fungi the proliferation of segregate taxa on the basis of only one or two characters has not necessarily increased our understanding of systematic relationships (Parmasto, and Kuyper, this volume). The choice of which classification scheme to use often rests on authority rather than on any explicit pattern of evolutionary relationships. For this reason, the application of phylogenetic criteria ought to be especially important for establishing and evaluating taxonomic concepts.

The criterion of monophyly is one of the most basic concepts in systematics. Its application is critical in identifying natural relationships among taxa (Wiley, 1981). Monophyletic groups are delimited by unique character states (synapomorphies) which unite taxa descended from, and including, a common ancestor (Fig. 2) (Farris, 1974). In contrast, paraphyletic taxa may include a common ancestor but not all of its descendents, while

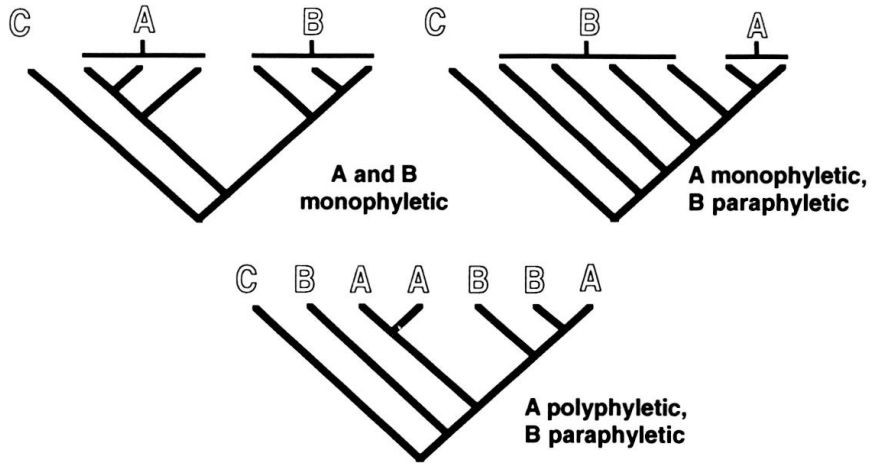

Figure 2. Possible phylogenetic relationships between two hypothetical taxa (A and B) with respect to an outgroup (C), and their implications for taxonomy. Most higher taxa are normally assumed to be monophyletic. However, the evolutionary basis for classification is less clear when taxa are paraphyletic or polyphyletic.

polyphyletic taxa lack a most recent common ancestor altogether. Differences between paraphyly and polyphyly could be interpreted as being relative, however, since all taxa can be eventually traced back to a common ancestor.

TAXONOMIC IMPLICATIONS OF PARAPHYLY AND POLYPHYLY IN FUNGI

Polyphyletic origin of the genus *Ustilago*

Mounting evidence suggests that the smut fungi are a polyphyletic group. Traditional classifications of the Ustilaginales stressed their importance as plant pathogens (Durán, 1976); however, it is now clear that the haploid yeast phase of most smut species is saprophytic, and in fact many basidiomycetous yeasts may be completely saprophytic through their entire life cycle (Fell et al., 1970). Within the type genus *Ustilago*, studies documenting differences in morphology, patterns of parasitism, host preference and biochemistry led Deml and Oberwinkler (1982) to erect a new genus, *Microbotryum*, to accommodate those species of *Ustilago* occurring on dicot hosts belonging to the Caryophyllaceae.

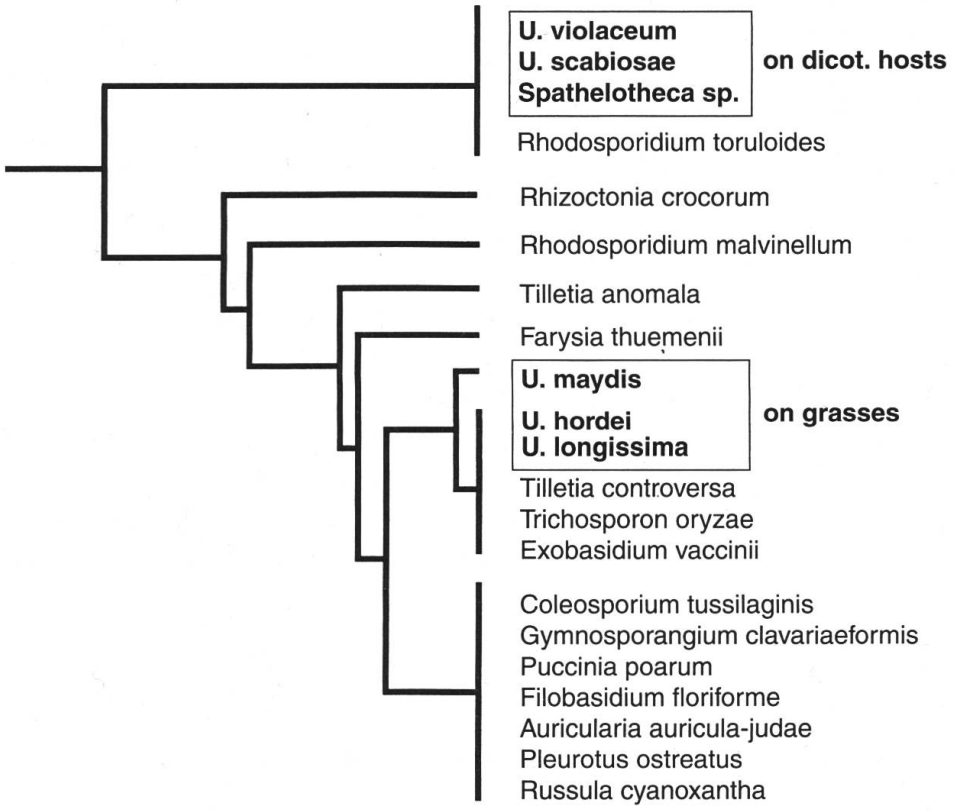

Figure 3. Phylogenetic tree for the Basidiomycotina based on 5S rRNA (after Blanz and Gottschalk, 1984). The polyphyly of several *Ustilago* species is also supported by their differences in host preference.

Some of the earliest molecular phylogenetic investigations on any group of fungi are the studies of 5S ribosomal RNA (rRNA) from basidiomycetes (Walker and Doolittle, 1982; Huysmans et al., 1983; Blanz and Gottschalk, 1984). Based on 5S rRNA evidence, these studies all suggest that the basidiomycetes may be grouped into at least two and possibly five distinct phylogenetic groups. Although all of the early analyses were based on distance (phenetic) methods, similar conclusions regarding the major phylogenetic groups of basidiomycetes were also inferred by cladistic analyses (Wolters and Erdmann, 1986). In all of these analyses, species previously assigned in the genus *Ustilago* were placed on at least two well-separated branches of the 5S rRNA tree (Fig. 3). One branch includes *Microbotryum violaceum* (previously in *Ustilago*) along with two other smut fungi which occur solely on dicotyledenous hosts (*U. scabiosae* and an unnamed species of *Sphacelotheca*). The remaining species of *Ustilago* (*U. maydis, U. hordei,* etc.) occur solely on graminicolous hosts, and were found to belong to a different group of basidiomycetes based on 5S rRNA data.

These results strongly suggest, as originally suggested by Deml and Oberwinkler (1982), that the genus *Ustilago* is polyphyletic, and supports the recognition of dicot smuts into separate taxa. Recently, however, the utility of 5S rRNA data for phylogenetic inference has been questioned (Mishler et al., 1988; Steele et al., 1991). Much of the criticism directed at 5S rRNA is due to its small size (approximately 100 nucleotides) and highly constrained secondary structure, which make rigorous phylogenetic analysis using cladistic methods difficult (Bruns et al., 1991). Therefore, additional evidence from other DNA sequences will still be necessary before any conclusions regarding the polyphyletic origin of smut fungi are certain.

The genus *Coprinus* and its secotioid relatives

The genus *Coprinus* has always been viewed as one of the more natural genera in the Agaricales. Criteria for delimiting members of *Coprinus* include the presence of deliquescent basidiomata, thin parallel lamellae, and a unique inaequihymenial pattern of lamellar development (Kühner and Romagnesi, 1978; Orton and Watling, 1979). This distinctive set of characters is not found together in any other genus, and strongly suggests that the group is monophyletic (Smith, 1971). In spite of these data, however, there is a growing body of evidence that the genus *Coprinus* is polyphyletic.

The strongest suggestion that *Coprinus* is not monophyletic is based on evidence for a close association with at least two secotioid genera, *Podaxis* and *Montagnea*. Both of these taxa grow in xeric habitats and produce gastroid

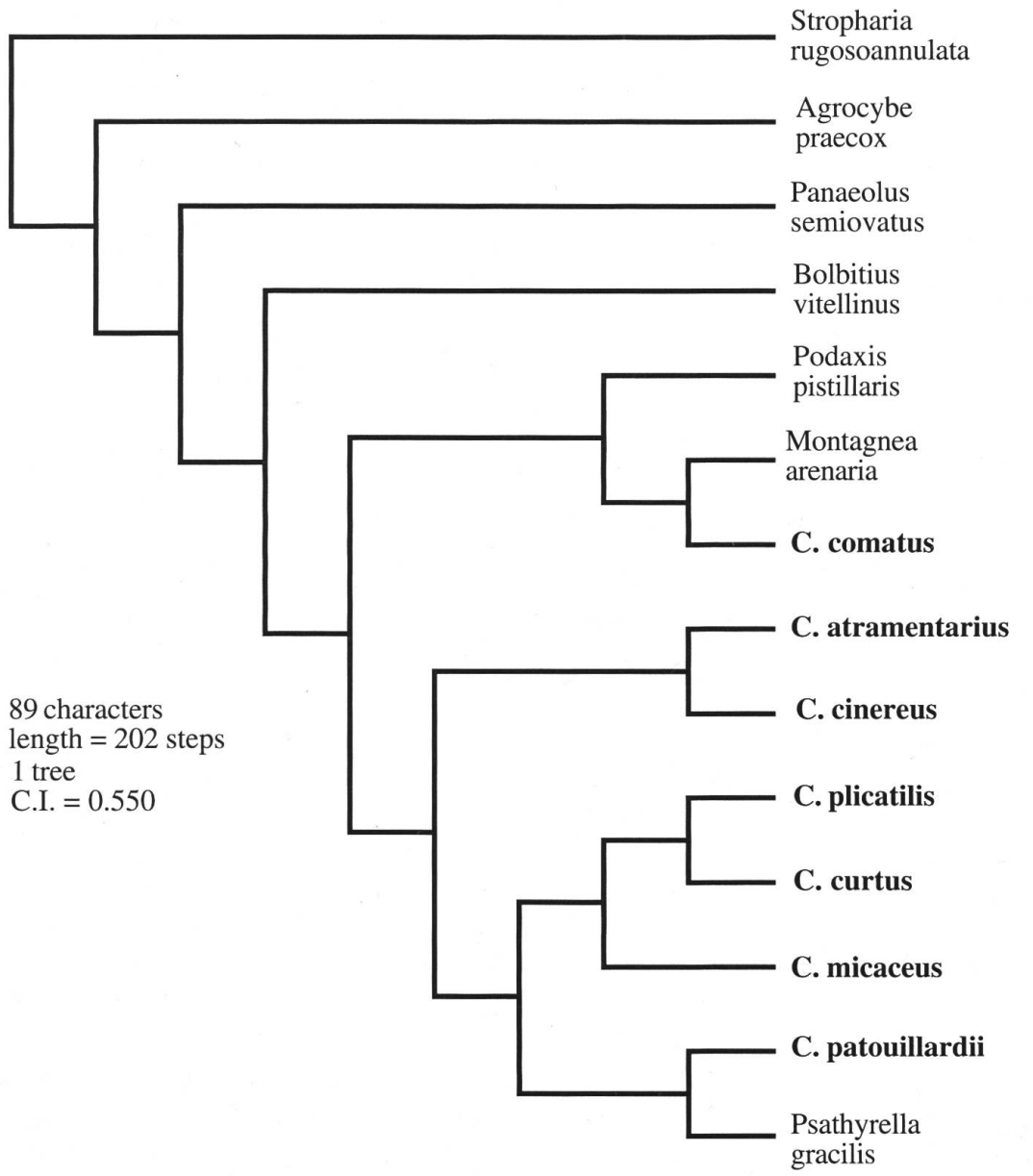

Figure 4. Phylogenetic relationships of representative taxa in *Coprinus* and related taxa, based on sequence data consisting of approximately 860 bases from the nuclear gene encoding 25S ribosomal RNA. The tree was rooted using *S. rugosoannulata* as the outgroup. Taxa in *Coprinus* are indicated in bold script to the right of the tree.

basidiomata lacking forcible spore discharge. Nevertheless, both still possess dark thick-walled basidiospores similar to many other coprinoid fungi, and show at least superficial resemblance to taxa in *Coprinus* in particular. For example, a striking macroscopic resemblance has often been noted for the

gleba-filled basidiomata of *Podaxis pistillaris* with the shaggy mane, *Coprinus comatus* (Morse, 1933; Miller and Miller, 1988). Until recently, however, the close relationship between these secotioid taxa and the genus *Coprinus* has been difficult to prove, because many critical morphological characters are either absent or else have been grossly modified by evolution (Thiers, 1984).

Recent studies in our laboratory have attempted to integrate molecular and morphological information to reexamine phylogenetic relationships in the Coprinaceae. These studies have demonstrated a unique ancestry for certain taxa in *Coprinus* with both *Podaxis pistillaris* and *Montagnea arenaria*, indicating that *Coprinus* is polyphyletic (Hopple and Vilgalys, in prep.). Sequence data representing approximately 860 nucleotide positions from the nuclear gene encoding the large ribosomal subunit RNA was analyzed for fourteen taxa representing *Coprinus* and potential sister groups. When these data are analyzed cladistically utilizing the PAUP computer package (Swofford, 1991), the secotioid taxa are clearly shown to be part of a natural group sharing common ancestry with taxa in *Coprinus* (Fig. 4). Supporting the predictions of several mycologists, *Podaxis* is seen to be closely allied to *C. comatus* (Miller and Miller 1988, Thiers 1984). In addition, a close relationship between *Montagnea* and *C. comatus* is also apparent. A final surprising result suggested by our preliminary molecular phylogenetic investigations is that some members of the genus *Psathyrella*, represented in Figure 4 by *P. gracilis*, may have also arisen at least in part from within *Coprinus*. This would indicate that even the removal of *C. comatus* from *Coprinus* ss. str. would leave a paraphyletic grouping of *P. gracilis* with the remaining coprini. Continuing investigations in our lab with a more complete sampling of taxa in *Coprinus* and related taxa support and further reinforce the above conclusions.

The taxonomic problems surrounding the development of a phylogenetic classification in *Coprinus* present fertile ground for those interested in the legalistic aspects associated with nomenclature. As *C. comatus* is recognized as the type species for the genus (Singer, 1986), the nomenclatoral consequences in this instance are especially significant. Under the scenario presented in Figure 4, the polyphyletic genus *Coprinus* gives rise in part to at least three other genera. Assuming that *Coprinus* is the oldest name for the group, under a strict application of monophyletic criteria either both *Montagnea* and *Podaxis*, as well as *Psathyrella* would need to be synonymized under *Coprinus,* or the name *Coprinus* would need to be applied to *C. comatus* and the two secotioid taxa with the remainder of the genus and *P. gracilis* classified under a different generic heading. Alternatively, phylogenetic classification might recognize unique status for the secotioid genera, either as form genera (as with many

Deuteromycotina) or as specially sanctioned taxa. What is certain, however, is that as more data becomes available from DNA sequences and morphology, the taxonomic structure of *Coprinus* and other genera of agarics with secotioid relatives will doubtlessly need to be reconsidered.

The genus *Lentinus:* Polypore or Agaric?

In contrast to *Coprinus*, the monophyly of the genus *Lentinus* has been the subject of much more controversy in modern systematics. Along with *Panus* and *Pleurotus*, *Lentinus* species are all white-spored wood rotters which possess decurrent lamellae and tough basidioma consistency. Although most systematists now agree that these three genera are closely related, there is still little consensus about their generic limits and evolutionary relationships to other hymenomycetes. The most recent comprehensive treatment of *Lentinus* strongly emphasizes characters of the mitic system for segregating the three genera (Pegler, 1983): *Lentinus* includes *Panus* (as a subgenus), and is restricted to strictly dimitic species, while *Pleurotus* species are only secondarily monomitic. Competing concepts of *Lentinus* have also emphasized characters of the hymenophoral trama (Singer, 1986), mitic system (Corner, 1981), and type of wood decay (Redhead and Ginns, 1985). Nomenclatoral arguments of the type species in *Lentinus* have also contributed to taxonomic instability (Singer, 1986 p. 183; Pegler, 1983 p. 2).

The higher taxonomic affinities of the *Lentinus* group are also controversial. Under the Friesian system of classification, *Lentinus* is placed within the Agaricales. However, anatomical features of the basidioma suggest that *Lentinus* may be closely related to certain polypore fungi. Whereas the Agaricales are typically monomitic, many polypores and most species of *Lentinus* ss. lato are dimitic (Corner, 1932, 1981; Pegler, 1975, 1983). Furthermore, some *Lentinus* species possess hyphal pegs which are also found in certain polypore genera (Gilbertson and Ryvarden, 1986). These characters have been the basis for hypotheses that *Lentinus* is derived from the polypores (Pegler, 1983), or is ancestral to the polypores (Corner, 1981).

We have used molecular and morphological characters to address two basic phylogenetic questions about *Lentinus*: 1) is *Lentinus* monophyletic?, and 2) is *Lentinus* most closely related to the polypores or agarics? The answer to the first question is clearly no; as a result, the answer to the second question is a qualified yes!

We undertook phenetic analyses of RFLPs in ribosomal DNA (Hibbett and Vilgalys, 1991), and cladistic analyses of rDNA sequence data (Hibbett, 1991) to address questions about the phylogeny of *Lentinus*. The results of the

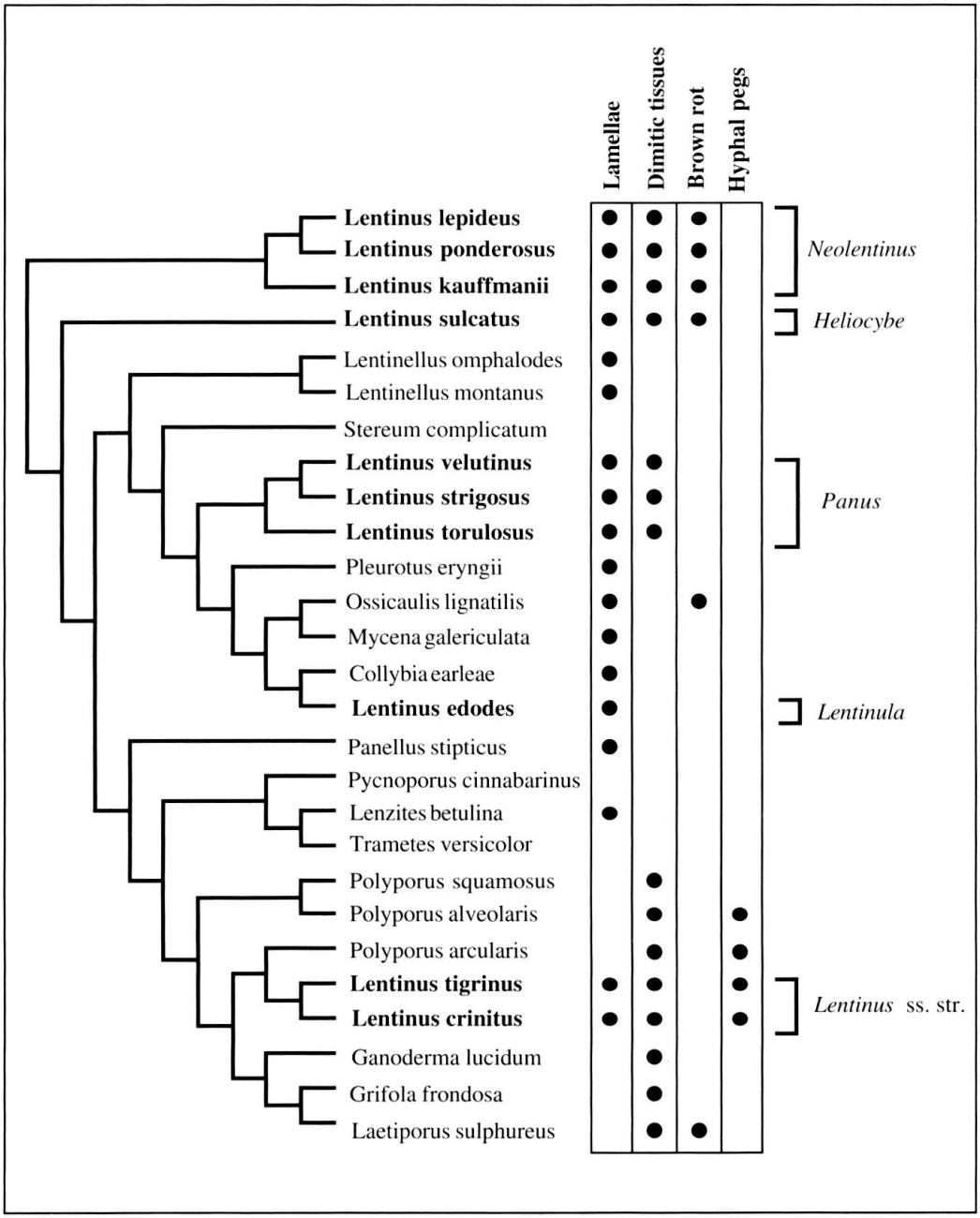

Figure 5. Phylogenetic relationships of representative members of the genus *Lentinus* and related taxa based on 790 bases from part of the the nuclear gene encoding 25S ribosomal RNA. Taxa tradionally placed in *Lentinus* are indicated in bold script; their current generic disposition is indicated on the right.

cladistic analyses based on 704 nucleotide positions from the large subunit (25S) ribosomal RNA gene are summarized in Figure 5. The topology shown in Figure 5 is also consistent with trees obtained through RFLP analysis of the same genes (Hibbett and Vilgalys, 1991). These results suggest that *Lentinus* sensu Pegler is not monophyletic Nevertheless, several significant aspects of Pegler's (1983) evolutionary hypotheses are supported by our data. For example, molecular evidence also indicates that *Lentinus* is derived at least in part from the genus *Polyporus*. In addition, the monomitic shiitake fungus *Lentinula edodes* was found to be unrelated with the rest of *Lentinus*, and, as suggested, appears to be closely allied with the Tricholomataceae (Pegler, 1983).

Information about character evolution in Figure 5 supports a number of previous suggestions that lamellae have probably arisen numerous times from poroid ancestors. Furthermore, dimiticism appears to have evolved independently in several different lineages of these fungi, and thus is not a synapomorphy for the lentinoid fungi. While most of the taxa included in this study produce a characteristic white-rot pattern of wood decay, the presence of brown rot fungi (e.g., *Laetiporus sulphureus, Lentinus lepideus*) in at least two separate lineages in Figure 5 also suggests that different decay chemistries have arisen numerous times during evolution.

Using the criterion of monophyly as a basis for classification, our results support recognition of several segregate genera as distinct from *Lentinus*: 1) *Neolentinus* Redhead and Ginns, 2) *Heliocybe* Redhead and Ginns, and 3) *Panus* Fr. A number of morphological and/or physiological features are also consistent with this view. The former two genera include species known to produce brown rot type of wood decay, while the latter is distinguished by having dimitic tissues. Based upon strict monophyly, *Lentinus* can be restricted to species within subgenus *Lentinus*, which includes those species known to possess hyphal pegs. Based on the phylogenetic hypothesis presented in Figure 5, hyphal pegs are known in several species of *Polyporus* as well, and were therefore probably also present in the polypore ancestor of *Lentinus*. Two of these species, *L. tigrinus* and *L. crinitus*, have each been proposed as the type species of *Lentinus* (Donk, 1962; Clements and Shear, 1931; Hawksworth, 1984). From a phylogenetic standpoint, both species are closely related, and the choice of either species as the lectotype would not affect the classification of *Lentinus*.

While the classification of groups within a once-polyphyletic *Lentinus* can be satisfactorily resolved by recognizing several segregate genera, this approach still presents new problems for other existing taxa. In Figure 5, a

monophyletic *Lentinus* appears nested within a larger group of polypores which are paraphyletic. Given the current confusing state of polypore taxonomy (Ryvarden, this volume), additional phylogenetic studies, including morphological as well as molecular data, will be necessary for sorting out phylogenetically meaningful taxa within the polypores.

GENERAL CONCLUSIONS

The points made in this paper emphasize a clear need for applying phylogenetic principles toward classification. For practical taxonomy, a clear distinction needs to be made between which taxa are "natural" (and therefore monophyletic), and those in need of further study. Clearly, nomenclatoral issues will need to be addressed in the interest of stability; however, taxonomic stability should never be an excuse for poor taxonomic judgement. A number of nomenclatoral alternatives for phylogenetic classification have been proposed by Wiley (1981). The issues raised above apply equally whether one is studying morphological characters or molecular data, since the eventual goal is the same in both cases.

Recognition of monophyletic groups is a necessary first step in understanding the patterns and processes of evolution. However, the application of phylogenetic classifications will also need to be weighed against the costs associated with increased taxonomic instability for some groups. For many groups of fungi with uncertain patterns of evolution, molecular characters have been indispensable for identifying monophyletic taxa. Application of phylogenetic criteria has many benefits for taxonomic and evolutionary studies. One direct benefit is a more predictive taxonomy for many groups of fungi. By concentrating on monophyletic genera, it should now be possible to gain insight into the evolution of important fungal character complexes such as hymenial configuration and development, wood decay biochemistries, hyphal systems, and host specificity.

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