E5.3.

Progress toward a phylogenetic classification of the Polyporaceae through parsimony analysis of mitochondrial ribosomal DNA sequences

David S. Hibbett and Michael J. Donoghue

Abstract: We used sequence data from mitochondrial small-subunit ribosomal DNA to infer phylogenetic relationships of the Polyporaceae. We examined 62 species representing 14 families of Aphyllophorales and Agaricales. Parsimony analyses of these sequences suggest that the Polyporaceae are polyphyletic. Higher order relationships are poorly resolved, but seven groups of species are generally well supported (as measured by bootstrapping) or are congruent with previous taxonomic hypotheses. Group 1 includes *Polyporus* s.str., seven other genera of Polyporaceae, *Lentinus*, and *Ganoderma*. Because this clade contains the type species of *Polyporus*, it may serve as the core for a future recircumscription of the Polyporaceae. Group 2 is morphologically and ecologically diverse, but all members have amyloid, ornamented spores (with the possible exception of *Heterobasidion*). This group includes *Bondarzewia, Heterobasidion, Hericium, Echinodontium, Lentinellus, Auriscalpium*, and *Russula*. Group 3 includes five exemplars of the Hymenochaetaceae, as well as *Oxyporus* and *Trichaptum*. *Trichaptum* and members of the Hymenochaetaceae are unusual among the holobasidiomycetes in their possession of imperforate parenthosomes. Group 4 represents the Boletaceae and includes *Boletus* and *Suillus*. Group 5 includes *Bjerkandera*, which is a polypore, and *Pulcherricium* and *Phanerochaete*, which are corticioid. In Group 5 the corticioid habit may have been derived by reduction. Group 6 includes *Fomitopsis, Piptoporus*, and *Daedalea*, all of which are brown rot polypores with bipolar mating systems. Group 7 includes *Laetiporus* and *Phaeolus*. *Laetiporus* is classified in Polyporaceae, but *Phaeolus* has been placed in both the Polyporaceae and Hymenochaetaceae. In general, our results suggest that macromorphology is evolutionarily flexible, but that certain anatomical and physiological characters, while not free from homoplasy, contain clues to higher order relationships of polypores.

Key words: Aphyllophorales, basidiomycetes, classification, phylogeny, Polyporaceae, ribosomal DNA.

Résumé : Les auteurs ont utilisé les données de la petite sous-unité de l'ADN mitochondrial pour déduire les relations phylogénétiques chez les Polyporaceae. Ils ont examiné 62 espèces représentant 14 familles d'Aphyllophorales et d'Agaricales. Les analyses en parcimonie de ces séquences suggèrent que les Polyporaceae sont polyphylétiques. Les relations d'ordres supérieurs sont mal résolues, mais 7 groupes d'espèces sont généralement bien supportés (tel que mesuré par le « bootstrapping ») ou sont congruents avec les hypothèses taxonomiques précédentes : le group 1 inclut les *Polyporus* s.str., sept autres genres de Polyporaceae, les *Lentinus* et les *Ganoderma*. Parce que ce clade comporte l'espèce type du genre *Polyporus*, il pourrait servir comme noyau en vue d'un nouveau regroupement des Polyporaceae. Le groupe 2 est morphologiquement et écologiquement diversifié, mais tous les membres ont des spores amyloïdes et ornementées (avec l'exception possiblement du genre *Heterobasidion*). Ce groupe inclut les *Bondarzewia, Heterobasidion, Hericium, Echinodontium, Lentinellus, Auriscalpium* et *Russula*. Le groupe 3 inclut cinq exemples chez les Hymenochaetaceae, ainsi que les genres *Oxyporus* et *Trichaptum*. Les *Trichaptum* et les membres des Hymenochaetaceae sont inhabituels au sein des holobasidiomycètes en ce qu'ils possèdent des parenthésomes imperforés. Le groupe 4 représente les Boletaceae et inclut les *Boletus* et les *Suillus*. Le groupe 5 inclut les *Bjerkandera*, appartenant aux polypores, et les *Pulcherricium* et *Phanerochaete* appartenant aux corticoïdes. Dans le groupe 5 le port corticoïde pourrait provenir d'une réduction. Le groupe 6 inclut les *Fomitopsis, Piptoporus* et *Daedelea*, tous des polypores associés aux pourritures brunes avec un système de compatibilité bipolaire. Le group 7

Received August 17, 1994.

D.S. Hibbett[1] **and M.J. Donoghue.** Harvard University Herbaria, 22 Divinity Avenue, Cambridge, MA 02138, U.S.A.

[1] Author to whom all correspondence should be addressed.

inclut les *Laeptiporus* et *Phacolus*. Les *Laetiporus* sont classés dans les Polyporaceae aussi bien que les Hymenochaetaceae. En général, les résultats des auteurs suggèrent que la macromorphologie est evolutivement flexible, mais que certains caractères anatomiques et physiologiques, bien qu'ils ne soient pas libres d'hoplasie, contiennent des indices sur les relations d'ordres supérieurs chez les polypores.

Mots clés : Aphyllophorales, basidiomycètes, classification, phylogénie, Polyporaceae, ADN ribosomal.
[Traduit par la rédaction]

Introduction

Polypores are a major component of the fungi in forest ecosystems. As wood decayers and tree pathogens, polypores play important ecological roles and have a significant impact on timber-based industries (16). Understandably, polypores have been the subject of much applied and basic research. Despite their ecological and economic importance, classification of polypores remains controversial, especially at the family level. In this study we used parsimony analyses of mitochondrial small-subunit ribosomal DNA (mt-rDNA) to assess the phylogenetic status of the Polyporaceae and to approach an understanding of the evolution of the polypore habit in other families.

The Polyporaceae was originally described by Fries (15) as a famiy of the Aphyllophorales that included all fungi with poroid hymenophores. The macromorphologically based Friesian system has long been regarded as artificial (49), and we have not adopted it for this study. Instead, we follow the classification of Donk (9), who used anatomical, biochemical, and other characters to divide the Friesian families of the Aphyllophorales into smaller, putatively natural families. Modern families of polypores that Donk derived from the Polyporaceae sensu Fries (at least in part) include the Ganodermataceae, Bondarzewiaceae, and Hymenochaetaceae. Many of Donk's segregate families are well accepted and have distinctive, possibly synapomorphic characters. However, what Donk left behind in the Polyporaceae was a set of residual taxa grouped not by any putative synapomorphies, but rather by the lack of distinguishing features by which they might have been segregated. Thus, even in its much reduced form, Donk conceded that "the Polyporaceae is merely a container of all 'polypores' not (yet) assigned to other families" (Ref. 9, p. 282).

The Polyporaceae sensu Donk are indeed heterogeneous. Macromorphological variation is great; fruiting bodies range from resupinate to pileate–stipitate, and hymenophores may be poroid, lamellate, or hydnoid. Anatomically, the Polyporaceae includes monomitic, dimitic, and trimitic species. Both brown and white rot wood decay types are present, as are both bipolar and tetrapolar mating systems (17, 18). Even septal pore ultrastructure is variable; most polypores have perforate parenthosomes, but *Trichaptum* has imperforate parenthosomes (40, 64).

In contrast to Donk, Singer (56) employed a highly restricted concept of the Polyporaceae that was centered on the type genus *Polyporus*. Singer's decision to place the Polyporaceae in the Agaricales was strongly influenced by the anatomical similarity of *Lentinus* and other agaric genera to certain polypores (4, 22, 50, 56). Faced with the apparent close relationship between *Lentinus* and *Polyporus*, Singer chose to put both genera into the Polyporaceae in the Agaricales, rather than move the lentinoid agarics into the Aphyllophorales. Thus, Singer's classification exemplifies two trends in taxonomy of Polyporaceae that have been manifested elsewhere: (*i*) reduction by removal of certain distinctive polypores and (*ii*) expansion by inclusion of the lentinoid agarics. For reviews of other classifications, the reader is directed to Overholts (46), Donk (9, 10), Jülich (27), and Ryvarden (54).

Molecular characters have helped to resolve many problems in fungal systematics (2, 21, 28), but so far there have been no molecular phylogenetic studies aimed specifically at the Polyporaceae. However, Hibbett and Vilgalys (24) did include nine species of polypores in analyses centered on *Lentinus* that used sequence data from nuclear large subunit rDNA. Although certain terminal groups of species were well supported, many internal nodes were weak, which suggested that the nuclear large subunit rDNA does not contain appropriate variation for higher level phylogenetic analyses. For the present study we looked to the small-subunit mt-rDNA, which was used by Bruns and Szaro (3) in studies in the Boletales. The resolution that Bruns and Szaro found suggested that this molecular region might also provide insight into phylogeny of the polypores.

Materials and methods

The 62 species examined in this study are listed in Table 1, with family-level classifications based primarily on Donk (9) and Singer (56). Sequence data for *Boletus satanas, Suillus cavipes*, and *Suillus sinuspaulianus* were previously published and obtained from Genbank (3). *Auricularia auricula-judae* was chosen as an outgroup. This choice is supported by phylogenetic analyses at more inclusive levels than the present study, which suggest that the Auriculariales are the sister group to the holobasidiomycetes (61).

Genomic DNA was isolated from field-collected basidiocarps or cultured mycelia following protocols that we have used previously (24). Mitochondrial rDNA was amplified and sequenced with the primer pair MS1–MS2 (67). Amplified mt-rDNAs were purified using Geneclean II (Bio 101, La Jolla, Calif.) and cycle sequenced using dye terminator sequencing kits (Applied Biosystems, Foster City, Calif.). Sequencing reactions were purified with Centrisep columns (Princeton Separations, Adelphia, N.J.). Polyacrylamide gel electrophoresis and data collection were performed on Applied Biosystems 370A or 373A automated DNA sequencers. Sequences were proofread, edited, and merged into composite sequences using SeqEd 1.01 (Applied Biosystems).

Alignments were performed using CLUSTAL V (25) running on a Sun workstation, followed by manual adjustments. Mitochondrial rDNA contained hypervariable regions, within which only certain subsets of the taxa could be aligned. In the

Table 1. Taxa examined.

Classification	Source[a]	GenBank accession
Auriculariaceae		
Auricularia auricula-judae (L.:Fr.) J. Schröt.	FPL 11504	U27022
Auriscalpiaceae		
Auriscalpium vulgare S.F. Gray	DAOM 197828	U27024
Lentinellus omphalodes (Fr.) Karst	DSH-9	U27048
L. ursinus (Fr.) Kühner	VT 237	U27051
Boletaceae		
Boletus satanas[b]		M91009
Suillus cavipes[b]		M91016
S. sinuspaulianus[b]		M91017
Bondarzewiaceae		
Bondarzewia berkeleyi (Fr.) Bond. & Sing.	DSH 93-190	U27026
B. montana (Fr.) Sing.	DAOM 415	U27027
Corticiaceae		
Peniophora nuda (Fr.) Bres.	FPL 4756	U27063
Phanerochaete chrysosporium Burds.	FPL 5175	U27058
Pulcherricium caeruleum (Fr.) Parm.	FPL 7658	U27057
Echinodontiaceae		
Echinodontium tinctorium Ellis & Ever.	DAOM 16666	U27035
Fistulinaceae		
Fistulina hepatica Schaeff.:Fr.	DSH 93-183	U27037
Ganodermataceae		
Ganoderma lucidum (Fr.) Karst.	DAOM 73245	U27039
G. lucidum B	SAR s.n.	U27040
Hericiaceae		
Hericium ramosum (Bull.:Merat) Let.	DSH 93-199	U27043
Hymenochaetaceae		
Coltricia perennis (Fr.) Murr.	DSH 93-198	U27028
Inonotus hispidus (Bull.:Fr.) Karst.	FPL 3597	U27044
Phaeolus schweinitzii (Fr.) Pat.	DSH 93-196	U27066
Phellinus gilvus (Schw.) Pat.	FPL 5528	U27060
P. igniarius L.:Fr.) Quél.	FPL 5599	U27061
Phylloporia ribis (Fr.) Ryv.	FPL 10677	U27065
Polyporaceae		
Antrodia carbonica (Overh.) Ryv. & Gilbn.	DAOM 19782	U27023
Bjerkandera adusta (Willd.:Fr.) Karst.	DAOM 21586	U27025
Ceriporia purpurea (Fr.) Donk	DAOM 21316	U27029
C. viridans (Berk. & Br.) Donk	FPL 7440	U27030
Cryptoporus volvatus (Pk.) Shear	DAOM 21179	U27031
Daedalea quercina Fr.	DAOM 14247	U27034
Daedaleopsis confragosa (Bolt.:Fr.) Schroet.	DAOM 18049	U27032
Datronia mollis (Sommerf.:Fr.) Donk	DAOM 21179	U27033
Fomes fomentarius (L.:Fr.) Kickx.	DAOM 12903	U27036
Fomitopsis pinicola (Swartz:Fr.) Karst.	DAOM 18913	U27038
Gloeophyllum sepiarium (Fr.) Karst.	DAOM 13786	U27041
Heterobasidion annosum (Fr.) Bref.	DAOM 73191	U27042
Laetiporus sulphureus (Bull.:Fr.) Murr.	DSH 93-194	U27049
Lentinus tigrinus (Bull.:Fr.) Fr.	DSH 93-181	U27050
Lentinus sp.	DSH 92-162	U27052
Lenzites betulina (Fr.) Fr.	DAOM 180504	U27045
Meripilus giganteus (Fr.) Karst.	DSH 93-193	U27053
Oxyporus sp.	DSH 93-188	U27054
Piptoporus betulinus (Bull.:Fr.) Karst.	DSH 93-186	U27056
Polyporus arcularius Batsch:Fr.	DSH 92-144	U27055
P. melanopus Fr.	DAOM 21226	U27062
P. squamosus Huds.:Fr.	FPL 6846	U27068
P. tuberaster Jacq.:Fr.	DAOM 7997B	U27070

Table 1 (*concluded*).

Classification	Source[a]	GenBank accession
P. varius Fr.	DSH 93-195	U27072
Pycnoporus cinnabarinus (Jacq.:Fr.) Karst.	DAOM 72065	U27059
Trametes suaveolens L.:Fr.	DAOM 19632	U27079
T. versicolor (L.:Fr.) Pilát	DSH 93-197	U27080
Trichaptum abietinum (Dicks.:Fr.) Ryv.	FPL 8973	U27078
Russulaceae		
Russula ballouii Pk.	DUKE 36	U27073
R. compacta Frost in Pk.	DUKE s.n.	U27074
Sparassidaceae		
Sparassis spathulata (Schw.:Fr.) Fr.	DSH 93-184	U27077
Stereaceae		
Stereum annosum Berk. & Br.	FPL 8562	U27075
S. hirsutum (Willd.:Fr.) S.F. Gray	FPL 8805	U27076
Tricholomataceae *sensu lato*		
Lentinula boryana (Berk. & Mont.) Pegler	R-39	U27046
L. lateritia (Berk.) Pegler	DSH 92-143	U27047
Panellus serotinus (Pers. in Hoffmann:Fr.) Kühner	DSH 93-218	U27067
P. stipticus (Bull.:Fr.) Karst.	DSH 93-213	U27069
Pleurotus aff. ostreatus (Jacq.:Fr.) Kummer	DSH 93-214	U27064
P. tuberregium (Fr.) Sing.	DSH 92-155	U27071

[a]DAOM isolates from Canadian Collection of Fungus Cultures, DAOM, Ottawa, Ont.; FPL isolates from USDA Forest Products Laboratory, Madison, Wis.; VT isolates from Orson K. Miller, Jr., Virginia Polytechnic Institute, Blacksburg, Va.; SAR isolate from Stephen A. Rehner; DUKE isolates from Rytas Vilgalys, Duke University, Durham, N.C.; R isolate from Mike Nicholson, Pennsylvania State University Mushroom Culture Collection, University Park, Pa.; DSH isolates in personal collection of D.S. Hibbett.
[b]Sequences from Bruns and Szaro (3).

final data set (available on request), mt-rDNA was interleaved in alternating blocks of aligned complete sequence sets, aligned partial sequence sets (with unaligned taxa scored as missing data), and unaligned sequences. The unalignable regions and all positions within 20 bases of the ends of the sequences were omitted from analyses.

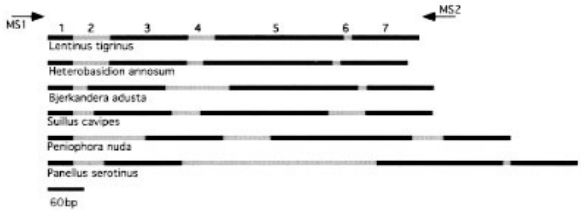
Phylogenetic analyses were performed using PAUP 3.1 (62) running on Macintosh computers. All transformations were weighted equally and gaps were scored as missing. Owing to the size of the data set, we were limited to heuristic searches, which are not guaranteed to find all shortest trees (35, 62, 63). To improve our chances of finding the shortest tree(s), we followed a search protocol based on strategies designed by Maddison et al. (36) and Olmstead et al. (45). In both strategies, exploration of the set of all possible trees is initiated from many starting points (initial tree topologies) in the hopes of finding at least one starting point from which the shortest tree(s) can be reached. Briefly, our searches proceeded as follows. (*i*) An initial set of 100 heuristic searches was performed using TBR swapping on starting trees generated with random taxon addition sequences, MAXTREES unrestricted, and keeping no more than two shortest trees from each replicate. (*ii*) From the set of shortest trees generated in step 1, pairs of trees, corresponding to the results of one replicate search, were input into complete TBR swapping, keeping all most parsimonious trees. (*iii*) Trees generated in step 2 were compared using the GETTREES function in PAUP to trees found in step 1. If unique trees remained in the set found in step 1, then they were input into complete TBR swapping. This process continued until all the trees found in step 1 had either been rediscovered by swapping on other trees or used as input trees for swapping.

Relative robustness of individual branches was estimated by bootstrapping (13, 26) (100 replicates, MAXTREES = 5, simple addition sequences, NNI branch swapping). These settings doubtlessly compromised the ability of PAUP to find the shortest trees in each bootstrap replicate, but they allowed us to run the bootstrap in 4–6 days on a Macintosh Quadra 800.

Results

Mitochondrial rDNA amplification products ranged in length from approximately 600 to over 850 base pairs (Fig. 1). From the aligned sequences, it was apparent that most of the length differences were due to insertions and deletions in three distinct hypervariable regions that alternate with four relatively conserved regions, which nonetheless had numerous small length mutations scattered throughout (Fig. 1). For the purpose of discussion, we have labeled these regions 1–7 consecutively from the 5′ to 3′ direction (Fig. 1). Regions 4 and 6 correspond to the divergent regions V7 and V8 that were defined by Gray et al. (20) and Cummings et al. (7). These regions were also found to be highly divergent within the Boletales (3). In our taxa, they range from 18 to 306 and 5 to 290 bases, respectively. Region 2 ranges from 19 to 107 bases (Fig. 1). The more conservative regions 1, 3, 5, and 7 total on average around 430 bases (aligned length of 500 bases) and were aligned across all taxa. The blocks of sequence that could only be aligned for subsets of the taxa

Fig. 1. Maps of mt-rDNA from six representative species. Primer sites indicated by arrows (pointing in primer 3′ direction). Hypervariable regions 2, 4, and 6 are shaded.



or that were deemed totally unalignable were limited to regions 2, 4, and 6. There were a total of 314 putatively informative positions: 247 from regions 1, 3, 5, and 7 combined; 67 from regions 2 and 4; and none from region 6.

A single most parsimonious tree of 1542 steps and consistency index (CI) = 0.361 was found (Fig. 2). Polypores occurred on numerous branches of the tree, often along with nonporoid taxa (Fig. 2). Seven polypore-containing clades were identified that were strongly supported by bootstrapping (Fig. 2) or were significant in terms of previous taxonomic hypotheses. To facilitate discussion, we have designated these as groups 1–7 (Fig. 2). Five genera of polypores were of uncertain placement and have not been referred to the groups defined above: *Meripilus, Antrodia, Ceriporia, Gloeophyllum*, and *Fistulina*.

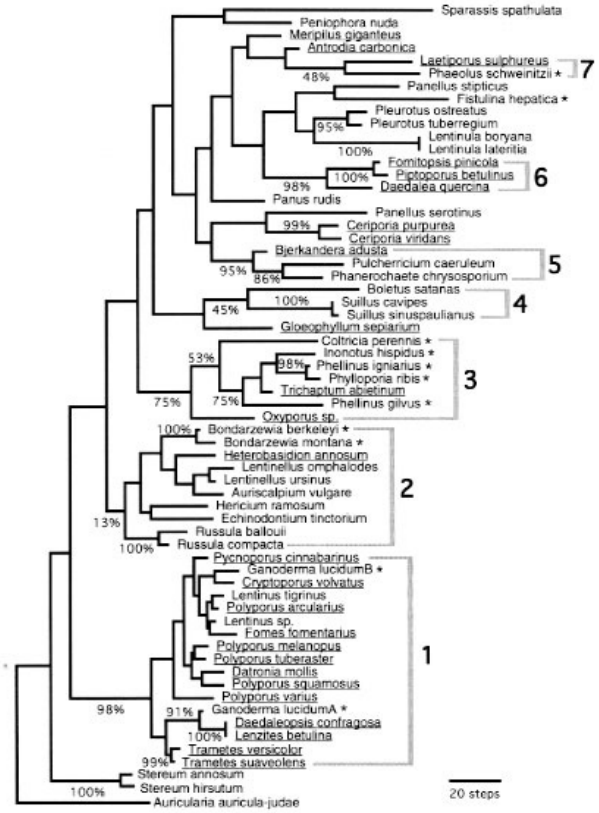
Discussion

The mitochondrial rDNA data set produced a single, fully resolved cladogram (Fig. 2), but bootstrap support for many nodes is weak. Sources of error in our estimate of the phylogeny may include (*i*) saturation by multiple substitutions at variable positions, (*ii*) inadequate taxon sampling, or (*iii*) incorrect hypotheses of homology for individual nucleotide positions owing to uncertainty in sequence alignment. Delimiting the conserved regions from the hypervariable regions and making manual adjustments to alignments were subjective exercises. The hypervariable regions could not be compared across all taxa in our study, but they were alignable for subsets of the taxa, and might be useful for studies at low taxonomic levels. However, we caution that even within the Boletales, Bruns and Szaro (3) were unable to align all V7 (region 4) sequences or any V8 (region 6) sequences. Our observations suggest that alignment difficulties will limit the utility of small-subunit mt-rDNA for broad phylogenetic comparisons in fungi.

In future work, addition of more characters from slowly evolving molecules (such as the nuclear small-subunit rDNA) could improve resolution of deep branches. Expanding the set of taxa to include representatives from other families might help to reduce internode lengths and thereby counteract long branch attraction (12). In the meantime, we view the overall topology in Fig. 2 as a working hypothesis of relationships that requires further testing. The remaining discussion focuses on the taxonomic significance of groups 1–7.

Group 1 includes *Lentinus* and 16 species in 10 genera from the Polyporaceae and Ganodermataceae sensu Donk (9). There are five species of *Polyporus* s.str., including the type species *P. tuberaster*. Monophyly of this clade is

Fig. 2. Phylogenetic relationships of polypores inferred from mt-rDNA sequences. Single most parsimonious tree, length = 1542 steps, CI = 0.361. Names of Polyporaceae sensu Donk are underlined. Other polypores indicated by asterisks. Bootstrap frequencies are shown for branches that are well supported or discussed in the text. Bracketed groups correspond to those in the text. Branch lengths are proportional to number of character state transformations inferred using ACCTRAN optimization.



strongly supported by bootstrapping (98%). We suggest that this clade serve as the core group for a future recircumscription of the Polyporaceae.

The group 1 taxa range from pileate–sessile (e.g., *Trametes*) to pileate–stipitate (e.g., *Polyporus melanopus*). Most are poroid, except for *Lentinus* and *Lenzites*, which are lamellate, and *Daedaleopsis*, which is daedaleoid. In contrast to its macromorphological variation, group 1 is remarkably uniform in anatomical, physiological, and genetic characters. All are either dimitic or trimitic and all have binding hyphae or at least branched skeletal hyphae (17, 18). All of the species in group 1 that have been evaluated produced a white rot (15 species known) and have a tetrapolar mating system (13 species known) (17, 18, 43, 52, 58). Except for *Ganoderma*, all have smooth, inamyloid, cylindric spores.

The two isolates of *Ganoderma lucidum* that we examined came out in two different locations in group 1 (Fig. 2). At this time, we cannot determine whether this problematic result reflects technical error or lineage sorting, heteroplasmy, or other phenomena. Although its exact placement is unclear, our results suggest that *G. lucidum*, which is the

type species of the Ganodermataceae, belongs within group 1. This agrees with Ryvarden, who characterized the Ganodermataceae as "an advanced and fairly young family" (Ref. 54, p. 100), and also supports Moncalvo et al. (personal communication) who observed low levels of divergence in nuclear large subunit rDNA sequences and therefore concluded that *Ganoderma* is of recent origin. In contrast, Corner (5) proposed that the Ganodermataceae is a primitive family from which other polypores have been derived. If *Ganoderma* is indeed nested within group 1 and if group 1 eventually comes to be recognized as the Polyporaceae, then it will be necessary to reduce the Ganodermataceae to subfamilial rank. Donk (9) segregated the Ganodermataceae largely on the basis of its unique, thick-walled, dark spores, but also clearly indicated in his writings that the Polyporaceae is the logical alternative placement for *Ganoderma* if the spore characters are discounted (Ref. 9, p. 267; Ref. 10, p. 408). Our results suggest that *Ganoderma*-type spores are apomorphic within the Polyporaceae, having been derived from smooth, cylindric spores.

The presence of *Lentinus* s.str. in group 1 and the exclusion of *Panus* s.str., *Pleurotus*, and *Lentinula* (Fig. 2) is congruent with previous molecular systematic studies (24). The derivation of *Lentinus* from *Polyporus*, and the nonmonophyly of *Lentinus* and *Panus* is also supported by comparative developmental morphology (22, 23). Taken together, these results strongly support Pegler's (50) hypothesis that *Lentinus* is derived from the Polyporaceae, but also contradict previous classifications in which the lentinoid–pleurotoid fungi are placed in a small number of closely related genera (e.g., Refs. 4, 50, and 56).

Group 2 is a weakly supported clade (bootstrap 13%; Fig. 2) composed of 10 species from 7 genera in the Russulaceae, Bondarzewiaceae, Polyporaceae, Echinodontiaceae, Hericiaceae, and Auriscalpiaceae. This clade encompasses a tremendous range of morphological diversity, including pileate–stipitate or sessile polypores (*Bondarzewia, Heterobasidion*), pileate–stipitate agarics (*Lentinellus, Russula*), pileate or coralloid toothed fungi (*Hericium, Echinodontium*), and the unusual pileate, laterally stipitate, toothed *Auriscalpium*. Nutritional modes and substrate preferences are also highly variable, including saprophytes (*Hericium, Lentinellus*, and *Auriscalpium*, which is restricted to pine cones), pathogens (*Bondarzewia, Heterobasidion*), and ectomycorrhizal fungi (*Russula*). Group 2 is morphologically and ecologically heterogeneous, but there are certain anatomical features that suggest that these species are related, and their placement together is not unprecedented.

Members of group 2 are unique among the ingroup taxa in their possession of spores with amyloid ornamentations (Refs. 9, 38, 51, 56, and 59, but see Refs. 17, 53, and 56 with regard to *Heterobasidion*). All taxa in this study outside of group 2 have smooth spores except *Ganoderma*, which has a unique spore morphology. *Panellus* and *Stereum* have amyloid spores, but they are smooth (37). *Spiniger* type anamorphs are also limited to group 2, where they are found in *Bondarzewia berkeleyi* and *Heterobasidion* (57). *Laurilia sulcata*, a member of the Echinodontiaceae, is also reported to produce a *Spiniger* anamorph (57).

There are other anatomical features that tend to distinguish group 2, but none that are invariant or unique to group 2. Spore shapes in group 2 range from globose to broadly elliptical. However, similarly shaped spores are also found in *Fistulina, Meripilus, Oxyporus*, and certain members of the Hymenochaetaceae (17, 18). All members of group 2 except *Heterobasidion* and *Echinodontium* have gloeoplerous hyphae or cystidia, but so do *Phaeolus, Peniophora*, and *Fistulina* (1, 11, 17, 18, 42, 56, 59, 60). Skeletal hyphae are found in all members of group 2 except *Hericium*. However, skeletal hyphae are also found in no less than 24 other species in this study (1, 9, 17, 18).

Reflecting on this uneven, yet suggestive distribution of characters, Donk described the amyloid-spored Aphyllophorales as resembling "a protean-shaped rhizopod that withdraws or extends one or more of its 'feet' whenever a particular character is properly studied" (Ref. 9, p. 270). As "feet," Donk listed the Auriscalpiaceae, Bondarzewiaceae, Echinodontiaceae, Hericiaceae, and others. Jülich's (27) phylogeny of the basidiomycetes shows the Hericiales, including *Hericium, Lentinellus*, and *Auriscalpium*, as basal to both the Bondarzewiales and Russulales. Other mycologists have also proposed relationships between Bondarzewiaceae and Russulaceae (55), Auriscalpiaceae and Russulaceae (29), *Heterobasidion* and Bondarzewiaceae (59), and *Lentinellus* and Auruscalpiaceae (34). Ongoing phylogenetic studies of nuclear large subunit rDNA sequences suggest that *Bondarzewia* and *Russula* together form a monophyletic group (S.A. Rehner, personal communication).

Certain other fungi have amyloid, ornamented globose to subglobose spores, and gloeoplerous hyphae. These may be related to group 2 and would further extend the range of morphological variation. Examples include *Clavicorona*, which is coralloid, and *Aleurodiscus* and *Gloeocystidiellum*, which are resupinate (9, 27, 32).

Group 3 includes four genera of the Hymenochaetaceae, as well as *Trichaptum* and *Oxyporus*, which are generally classified in the Polyporaceae (e.g., Refs. 9 and 17). This clade received moderate support, judging by bootstrapping (75%) (Fig. 2). Group 3 taxa range from pileate–sessile (e.g., *Oxyporus*) or occasionally resupinate (*Trichaptum*), to pileate–stipitate (*Coltricia*), and include both annual (e.g., *Phylloporia*) and perennial species (*Phellinus igniarius*). All are saprophytic wood decayers, except *Coltricia* which is ectomycorrhizal (8).

The Hymenochaetaceae has long been regarded as a natural family (e.g., Refs. 6, 9, and 49). Important characters in the description of the Hymenochaetaceae include clampless generative hyphae, darkening upon treatment with KOH (the xanthochroic reaction), production of a white rot, and presence of setae. Of these characters, only setae are unique to the Hymenochaetaceae and even these are not invariant (9, 48).

Oxyporus and *Trichaptum* both lack xanthochroic reactions and setae, and their inclusion in group 3 is, therefore, at odds with the accepted delimitation of the Hymenochaetaceae. In addition, *Trichaptum* has clamped hyphae, whereas clampless hyphae were considered of great importance in delimiting the Hymenochaetaceae by both Corner (6) and Donk (9). Similarities of *Oxyporus* and *Trichaptum* to the Hymenochaetaceae include production of a white rot and annual to perennial poroid, irpicoid, or lamellate fruiting bodies (18, 43). These characters might be taken as consis-

tent with placement in the Hymenochaetaceae, but they are also found in many other wood decay fungi, and do not provide positive support.

However, septal ultrastructure, which is generally considered a conservative character (40), suggests that *Trichaptum* may indeed be closely related to the Hymenochaetaceae. The vast majority of holobasidiomycetes have perforate parenthosomes, but *Trichaptum* and members of the Hymenochaetaceae (*Phellinus, Inonotus, Onnia*) have imperforate parenthosomes (39, 40, 64). The only exception in the Hymenochaetaceae is *Coltricia*, which has perforate parenthosomes (39). Other genera in this study for which parenthosome structure has been determined include *Pleurotus* (41), *Phaeolus, Fomes, Polyporus* (39), and *Lentinula* (65). All have perforate parenthosomes. Imperforate parenthosomes are found in the outgroup *Auricularia auricula-judae* and other heterobasidiomycetes (33, 66), as well as certain corticioid holobasidiomycetes (30, 31). It is tempting to speculate that imperforate parenthosomes are plesiomorphic for holobasidiomycetes, with parallelism accounting for the perforate parenthosome in *Coltricia*. However, relationships among the major clades within the ingroup are too poorly resolved and septal ultrastructure is known from too few taxa to infer pathways of parenthosome evolution at this time.

Group 3 represents an incomplete sample of morphological diversity in the Hymenochaetaceae, which also includes species that are stipitate–pendent (*Coltriciella dependens*), fully resupinate (*Hymenochaete* spp.), or concentrically lamellate (*Cyclomyces*). Owing to limited taxon sampling, we can say little about patterns of morphological evolution and classification within the Hymenochaetaceae. However, in Fig. 2, *Phellinus igniarius* and *Phylloporia ribis* are strongly supported as monophyletic, to the exclusion of *Phellinus gilvus*, which suggests that *Phellinus* is polyphyletic, as was suggested by Fiasson and Niemalä (14).

Group 4 represents the Boletaceae. It is weakly supported as monophyletic (bootstrap = 45%) and its relationship to other taxa in this study remains unclear (Fig. 2).

Group 5 includes *Bjerkandera*, which is classified in the Polyporaceae (9, 17), and *Pulcherricium* and *Phanerochaete*, which are traditionally classified in the Corticiaceae (9, 15, 47) but have also been segregated into the Vuilleminiaceae (e.g., Ref. 19), and Phanerochaetaceae (e.g., Refs. 19 and 27). *Pulcherricium* and *Phanerochaete* are resupinate and have smooth hymenophores, whereas *Bjerkandera* is a polypore that typically is pileate–sessile, but can also be effused/reflexed or occasionally resupinate when growing on the underside of logs (17).

Bjerkandera, Pulcherricium, and *Phanerochaete* have never been classified in the same family (although all are Corticiomycetes sensu Parmasto; Ref. 47). Nevertheless, monophyly of the group is strongly supported by the molecular characters (bootstrap = 95%; Fig. 2). In addition, all three have smooth, cylindric, inamyloid spores, are monomitic, produce white rots, and, when heterothallic, are bipolar (1, 11, 17, 42, 43, 58).

The Corticiaceae s.l. is generally regarded as an artificial taxon. Indeed, the other corticioid fungus in this study, *Peniophora nuda*, is widely separated from group 5 (Fig. 2) and has been placed in a separate family, the Peniophoraceae (e.g., Refs. 19 and 47). Corticioid fungi have been hypothesized to be a paraphyletic group that is primitively simple (47) or a polyphyletic group that has been derived by reduction from more complex forms (6). The most parsimonious interpretation of our results suggests that the corticioid habit of *Pulcherricium* and *Phanerochaete* is derived by reduction from pileate, poroid ancestors. However, inclusion of more resupinate and pileate taxa is needed to understand pathways of morphological evolution in group 5.

Group 6 is a strongly supported clade (bootstrap = 98%; Fig. 2) that includes *Fomitopsis, Piptoporus*, and *Daedalea. Daedalea* has a labyrinthine hymenophore, but the others are poroid, and all are classified in the Polyporaceae (9, 17). Although the three species are distinct in outward appearance, they all have similar trimitic construction that enables the fruiting bodies to be persistent (*Daedalea, Piptoporus*) to truly perennial (*Fomitopsis*). In addition, all produce a brown rot and have a bipolar mating system (17, 18, 43). The three species also share cylindric to elliptical, smooth, inamyloid spores and clamped generative hyphae, but these are not unique to this group. *Fomitopsis* has been compared to *Trametes* (group 1), which has similar spores and hyphal construction (17, 54), but our results do not support a close relationship.

Group 7 includes *Laetiporus* and *Phaeolus. Laetiporus* is generally classified in the Polyporaceae (17), but *Phaeolus* has previously been classified in both the Polyporaceae (e.g., Ref. 17) and the Hymenochaetaceae or Hymenochaetales (9, 14). *Phaeolus* is a brown polypore that stains darkly in KOH, and thus it superficially resembles the Hymenochaetaceae. However, the xanthochroic reaction has been shown to be common outside of the Hymenochaetaceae (48). Furthermore, *Phaeolus* lacks setae (18), has a perforate parenthosome (39), and produces a brown rot. Molecular characters provide no evidence that *Laetiporus* is related to the Hymenochaetaceae, which is here represented by group 3 (Fig. 2).

Monophyly of *Phaeolus* with *Laetiporus* received low support from molecular characters (bootstrap = 48%; Fig. 2), but is corroborated by certain morphological characters. Both *Phaeolus* and *Laetiporus* produce a brown rot and have gloeoplerous hyphae (17, 18, 43). *Laetiporus* fruiting bodies are bright orange and in this way contrast with the mature fruiting bodies of *Phaeolus*, which are brown. However, when young, *Phaeolus* fruiting bodies also are orange. Ryvarden (54) considered *Phaeolus* and *Laetiporus* to be very similar but held open the possibility that *Phaeolus* was derived from the Hymenochaetaceae. Our results support the view that *Phaeolus* and *Laetiporus* are monophyletic, but because of limited taxon sampling, their closest relatives remain unclear. One likely candidate is *Pycnoporellus*, which is a brown rot genus that Gilbertson and Ryvarden (17) suggested is closely related to *Phaeolus*.

Conclusions

Members of the Polyporaceae occur in groups 1, 3, and 5–7. The unplaced polypores *Meripilus, Antrodia, Ceriporia*, and *Gloeophyllum* are also classified in the Polyporaceae. Thus, our results support Donk's (9) prediction that the Polyporaceae are polyphyletic, and will ultimately have to be subdivided into groups that better reflect phylogeny. Molecular

characters will be of great importance in constructing a truly phylogenetic classification of polypores.

A general implication of this work is that there has been extensive convergence and parallelism in macromorphological features. For example, perennial fruiting bodies are formed by members of groups 1–3, and 6, the polypore habit occurs in all groups except group 4 (boletes), and pileate–stipitate agarics occur in groups 1, 2, and 4. Within-group variation is also high, with five of the seven groups containing more than one type of hymenophore configuration. At the same time, our results suggest that certain anatomical or physiological features may provide better clues to higher order relationships (cf. Refs. 9, 44, and 49). As examples, we cite binding hyphae in group 1; amyloid, ornamented spores in group 2; imperforate parenthosomes in group 3; and brown rot and bipolar mating systems in group 6. Nevertheless, the distribution of variation in these characters both within and between groups suggests that there is considerable homoplasy. There are also many missing data, especially for characters that are difficult to study, such as septal ultrastructure and mating systems. Ironically, the growth of fungal molecular phylogenetic data threatens to outpace development of morphological data bases, even as molecular phylogenies make morphological characters more interesting and intelligible than ever before. We hope that the results presented here will not only spur on more molecular phylogenetic studies but will also provide a framework for studies that will refine hypotheses of homology of non-molecular characters that may be useful in understanding the evolution of polypores.

Acknowledgments

We are grateful to the individuals and institutions who donated isolates for this project (Table 1), especially James H. Ginns (DAOM, Ottawa, Ont.) and Harold H. Burdsall and Karen K. Nakasone (USDA Forest Products Laboratory, Madison, Wis.). We also thank Jean-Marc Moncalvo and Stephen A. Rehner for sharing prepublication results, Elizabeth Pine for her technical assistance, and Bart Buyck, James H. Ginns, Maria Nuñez, Erast Parmasto, Scott Redhead, Joost Stalpers, and two anonymous reviewers for their comments on early versions of this paper. This research was supported by National Science Foundation Postdoctoral Fellowship DEB-9303268 to D.S.H.

References

1. Breitenbach, J., and Kränzlin, F. 1986. Fungi of Switzerland. Vol. 2. Verlag Mykologia, Lucerne.
2. Bruns, T.D., White, T.J., and Taylor, J.W. 1991. Fungal molecular systematics. Annu. Rev. Ecol. Syst. **22**: 525–564.
3. Bruns, T.D., and Szaro, T.M. 1992. Rate and mode differences between nuclear and mitochondrial small-subunit rRNA genes in mushrooms. Mol. Biol. Evol. **9**: 836–855.
4. Corner, E.J.H. 1981. The agaric genera *Lentinus*, *Panus*, and *Pleurotus*. Beih. Nova. Hedwigia, **69**: 1–169.
5. Corner, E.J.H. 1983. Ad Polyporaceas I: *Amauroderma* and *Ganoderma*. Beih. Nova Hedwigia, **75**: 1–182.
6. Corner, E.J.H. 1991. Ad Polyporaceas VII: the xanthochroic polypores. Beih. Nova Hedwigia, **101**: 1–173.
7. Cummings, D.J., Domenico, J.M., Nelson, J., and Sogin, M.L. 1989. DNA sequence, structure, and phylogenetic relationships of the small subunit rRNA coding region of mitochondrial DNA from *Podospora anserina*. J. Mol. Evol. **28**: 232–241.
8. Danielson, R.M. 1984. Ectomycorrhizal associations in jack pine stands in northeastern Alberta. Can. J. Bot. **62**: 932–939.
9. Donk, M.A. 1964. A conspectus of the families of the Aphyllophorales. Persoonia, **3**: 199–324.
10. Donk, M.A. 1971. Multiple convergence in the polyporaceous fungi. *In* Evolution in the higher basidiomycetes. *Edited by* R.H. Petersen. University of Tennessee Press, Knoxville. pp. 393–422.
11. Eriksson, J., Hjortstam, K., and Ryvarden, L. 1978. The Corticiaceae of North Europe. Vol. 5. Fungiflora, Oslo.
12. Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Syst. Zool. **27**: 401–410.
13. Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, **39**: 783–791.
14. Fiasson, J.-L., and Niemalä, T. 1984. The Hymenochaetales: a revision of the European poroid taxa. Karstenia, **24**: 14–28.
15. Fries, E. 1874. Hymenomycetes Europaei. Upsaliae.
16. Gilbertson, R.L. 1980. Wood-rotting fungi of North America. Mycologia, **72**: 1–49.
17. Gilbertson, R.L., and Ryvarden, L. 1986. North American polypores. Vol. 1. Fungiflora, Oslo.
18. Gilbertson, R.L., and Ryvarden, L. 1987. North American polypores. Vol. 2. Fungiflora, Oslo.
19. Ginns, J.H. 1993. Lignicolous corticioid fungi (Basidiomycotina) of North America. Mycol. Mem. **19**: 1–247.
20. Gray, M.W., Sankoff, D., and Cedergren, R.J. 1984. On the evolutionary descent of organisms and organelles: a global phylogeny based on a highly conserved structural core in small subunit ribosomal RNA. Nucleic Acids Res. **12**: 5837–5852.
21. Hibbett, D.S. 1992. Ribosomal RNA and fungal systematics. Trans. Mycol. Soc. Jpn. **33**: 533–556.
22. Hibbett, D.S., Murakami, S., and Tsuneda, T. 1993. Hymenophore development and evolution in *Lentinus*. Mycologia, **85**: 428–443.
23. Hibbett, D.S., Murakami, S., and Tsuneda, T. 1993. Sporocarp ontogeny in *Panus* (Basidiomycotina): evolution and classification. Am. J. Bot. **80**: 1336–1348.
24. Hibbett, D.S., and Vilgalys, R. 1993. Phylogenetic relationships of *Lentinus* (Basidiomycotina) inferred from molecular and morphological characters. Syst. Bot. **18**: 409–433.
25. Higgins, D.G., Bleasby, A.J., and Fuchs, R. 1992. CLUSTAL V: improved software for multiple sequence alignment. Comput. Appl. Biosci. **8**: 189–191.
26. Hillis, D.M., and Bull, J.J. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. **42**: 182–192.
27. Jülich, W. 1981. Higher taxa of basidiomycetes. Cramer, Vaduz, Lichtenstein.
28. Kohn, L.M. 1992. Developing new characters for fungal systematics: an experimental approach for determining the rank of resolution. Mycologia, **84**: 139–153.
29. Kühner, R. 1980. Les Hyménomycètes agaricoïdes (*Agaricales, Tricholomatales, Pluteales, Russulales*). Etude générale et classification. Numéro spécial du Bulletin de la Société Linnéenne de Lyon.
30. Langer, E., and Oberwinkler, F. 1993. Corticioid

basidiomycetes. I. Morphology and ultrastructure. Windahlia, **20**: 1–28.
31. Langer, G. 1994. Die Gattung *Botryobasidium* DONK (Corticiaceae, Basidiomycetes). Bibl. Mycol. **158**: 1–459.
32. Lemke, P.A. 1964. The genus *Aleurodiscus* (sensu stricto) in North America. Can. J. Bot. **42**: 213–282.
33. Lü, H., and McLaughlin, D.J. 1991. Ultrastructure of the septal pore apparatus and early septum initiation in *Auricularia auricula-judae*. Mycologia, **83**: 322–334.
34. Maas Geesteranus, R.A. 1963. Hyphal structures in Hydnums. II. Proc. K. Ned. Akad. Wet. Ser. C, **66**: 426–436.
35. Maddison, D.R. 1991. The discovery and importance of multiple islands of most-parsimonious trees. Syst. Zool. **40**: 315–328.
36. Maddison, D.R., Ruvolo, M., and Swofford, D.L. 1992. Geographic origins of human mitochondrial DNA: phylogenetic evidence from control region sequences. Syst. Biol. **41**: 111–124.
37. Miller, O.K., Jr. 1970. The genus *Panellus* in North America. Mich. Bot. **9**: 17–30.
38. Miller, O.K., Jr., and Stewart, L. 1971. The genus *Lentinellus*. Mycologia, **63**: 333–369.
39. Moore, R.T. 1980. Taxonomic significance of septal ultrastructure in the genus *Onnia* Karsten (Polyporineae/Hymenochaetaceae). Bot. Not. **133**: 169–175.
40. Moore, R.T. 1985. The challenge of the dolipore/parenthosome septum. *In* Developmental biology of higher fungi. *Edited by* D. Moore, L.A. Casselton, D.A. Wood, and J.C. Frankland. Cambridge University Press, Cambridge, U.K. pp. 175–212.
41. Moore, R.T., and Patton, A.M. 1975. Parenthosome fine structure in *Pleurotus cystidiosus* and *Schizophyllum commune*. Mycologia, **67**: 1200–1205.
42. Nakasone, K.K. 1990. Cultural studies and identification of wood-inhabiting Corticiaceae and selected Hymenomycetes from North America. Mycol. Mem. **15**: 1–412.
43. Nobles, M.K. 1965. Identification of cultures of wood-inhabiting Hymenomycetes. Can. J. Bot. **43**: 1097–1139.
44. Nobles, M.K. 1971. Cultural characters as a guide to the taxonomy of the Polyporaceae. *In* Evolution in the higher basidiomycetes. *Edited by* R.H. Petersen. University of Tennessee Press, Knoxville. pp. 169–196.
45. Olmstead, R.G., Bremer, B., Scott, K.M., and Palmer, J.D. 1993. A molecular systematic analysis of the Asteridae *sensu lato* based on *rbc*L sequences. Ann. Mo. Bot. Gard. **80**: 700–722.
46. Overholts, L.O. 1953. The Polyporaceae of the United States, Alaska, and Canada. University of Michigan Press, Ann Arbor.
47. Parmasto, E. 1986. On the origin of the Hymenomycetes (what are corticioid fungi?) Windahlia, **16**: 3–20.
48. Parmasto, E., and Parmasto, I. 1979. The xanthochroic reaction in Aphyllophorales. Mycotaxon, **8**: 201–232.
49. Patouilliard, N. 1900. Essai taxonomique sur les familles et les genres des Hyménomycètes. Lons-le-Saunier, Paris.
50. Pegler, D.N. 1983. The genus *Lentinus*. Kew Bull. Additional Ser. **10**: 1–281.
51. Pegler, D.N., and Young, T.W.K. 1972. Reassessment of the Bondarzewiaceae (Aphyllophorales). Trans. Br. Mycol. Soc. **58**: 49–58.
52. Redhead, S.A., and Ginns, J.H. 1985. A reappraisal of agaric genera associated with brown rots of wood. Trans. Mycol. Soc. Jpn. **26**: 349–381.
53. Redhead, S.A., and Norvell, L.L. 1993. Notes on *Bondarzewia, Heterobasidion*, and *Pleurogala*. Mycotaxon, **48**: 371–380.
54. Ryvarden, L. 1991. Genera of polypores. Fungiflora, Oslo.
55. Singer, R. 1984. Tropical Russulaceae II. *Lactarius* section *Panuoidei*. Nova Hedwigia, **40**: 435–447.
56. Singer, R. 1986. The Agaricales in modern taxonomy. 4th ed. Koeltz Scientific Books, Koenigstein, Germany.
57. Stalpers, J.A. 1974. *Spiniger*, a new genus for imperfect states of basidiomycetes. Proc. K. Ned. Akad. Wet. Ser. C, **77**: 402–407.
58. Stalpers, J.A. 1978. Identification of wood-inhabiting Aphyllophorales in pure culture. Stud. Mycol. **16**: 1–248.
59. Stalpers, J.A. 1979. *Heterobasidion* (*Fomes*) *annosum* and the Bondarzewiaceae. Taxon, **28**: 414–417.
60. Stalpers, J.A. 1992. *Albatrellus* and the Hericiaceae. Persoonia, **14**: 537–541.
61. Swann, E.C., and Taylor, J.W. 1993. Higher taxa of basidiomycetes: an 18S rRNA perspective. Mycologia, **85**: 923–936.
62. Swofford, D.L. 1993. PAUP: phylogenetic analysis using parsimony, version 3.1 edition. Illinois Natural History Survey, Champaign.
63. Swofford, D.L., and Olsen, G.J. 1990. Phylogeny reconstruction. *In* Molecular systematics. *Edited by* D.M. Hillis and C. Moritz. Sinauer Associates, Sunderland, Mass. pp. 411–501.
64. Traquair, J.A., and McKeen, W.E. 1978. Ultrastructure of the dolipore septum in *Hirschiporus paragamenus* (Polyporaceae). Can. J. Microbiol. **24**: 767–771.
65. Tsuneda, A. 1983. Fungal morphology and ecology. Tottori Mycological Institute, Tottori, Japan.
66. Wells, K. 1994. Jelly fungi, then and now! Mycologia, **86**: 18–48.
67. White, T.J., Bruns, T., Lee, S., and Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR protocols. *Edited by* M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T. White. Academic Press, San Diego, Calif. pp. 315–322.