

The phylogenetic relationships of the anaerobic chytridiomycetous gut fungi (Neocallimasticaceae) and the Chytridiomycota. II. Cladistic analysis of structural data and description of Neocallimasticales ord.nov.

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We investigated the phylogenetic relationships of the Chytridiomycota and the chytridiomycetous gut fungi with a cladistic analysis of 42 morphological, ultrastructural, and mitotic characters for 38 taxa using both maximum parsimony and distance algorithms. Our analyses show that there are three major clades within the Chytridiomycota: the gut fungi, the Blastocladales, and the Spizellomycetales–Chytridiales–Monoblepharidales. Consequently, we elevated the gut fungi to the order **Neocallimasticales** ord.nov. Our results suggest that a modified Chytridiales, including the Monoblepharidales, is a monophyletic group. In contrast the Spizellomycetales are paraphyletic because the Chytridiales arose within them. The separation of the traditional Chytridiales into two orders is thus doubtful. Although the Blastocladales are closer to members of the Spizellomycetales than the Chytridiales, the cladistic analyses of both structural and rRNA sequence data do not support the idea that the Blastocladales were derived from the Spizellomycetales. We suggest emendations to the classification of the Chytridiomycota and note which groupings require further analysis. Our phylogeny for the currently recognized species of gut fungi is inconsistent with the existing classification. Nonetheless, pending further investigations, we prefer to retain the existing, easily defined genera for which a key is provided.

Key words: Chytridiomycota, rumen fungi, phylogeny, morphology, ultrastructure, mitosis.

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Les auteurs ont étudié les relations phylogénétiques des Chytridiomycota et des champignons chytridiomycètes intestinaux à l'aide de l'analyse cladistique de 42 caractéristiques morphologiques, ultrastructurales et mitotiques chez 38 taxons, en utilisant à la fois la parcimonie maximum et les algorithmes de distance. Les résultats montrent qu'il y a trois clades majeurs chez les Chytridiomycota : les champignons intestinaux, les Blastocladales et les Spizellomycétales–Chytridiales–Monoblépharidales. Conséquemment les auteurs élèvent les champignons intestinaux au niveau de l'ordre des **Néocallimasticales** ord.nov. Les résultats suggèrent qu'un groupe Chytridiales modifié, incluant les Monoblépharidales, constitue un groupe monophylétique. Au contraire, les Spizellomycétales sont paraphylétiques parce que les Chytridiales y ont pris naissance. La séparation traditionnelle des Chytridiales en deux ordres apparaît donc douteuse. Bien que les Blastocladales soient plus près de certains membres des Spizellomycétales que des Chytridiales, les analyses cladistiques des données structurales aussi bien que des séquences de rRNA ne supportent pas l'idée que les Blastocladales soient issues des Spizellomycétales. Les auteurs suggèrent des amendements à la classification des Chytridiomycota et notent quels regroupements nécessitent une analyse plus poussée. La phylogénie proposée par les auteurs pour les espèces de champignons intestinaux reconnus diverge des classifications existantes. Cependant, en attendant des études plus poussées, les auteurs préfèrent retenir les genres actuels, faciles à définir, et en proposent une clé.

Mots clés : Chytridiomycota, champignons du rumen, phylogénie, morphologie, ultrastructure, mitose.

[Traduit par la rédaction]

Introduction

A good taxonomic system should reflect the phylogenetic relationships among taxa, not just a series of artificial ranks based on character similarity. Taxonomy of the Chytridiomycota was originally, of necessity, based on morphological characters. However, these criteria were shown to be quite variable owing to environmental change (Miller 1976). As a result, the taxonomy was quite artificial. With the advent of routine electron microscopy, it became possible to utilize zoospore ultrastructure in taxonomy. These features, as well as thallus development, were emphasized in a new taxonomic system proposed by Barr (1980, 1990) and other workers (Lange and Olson 1979) because they were thought to be more conserved and are less influenced by the environment (Barr 1980). Together with mitotic characters, they may more accurately reflect the phylogenetic relationships among species (Barr 1978; Heath 1980, 1986; Powell 1978).

In this new system, orders and genera were defined on

zoospore ultrastructure, and families were defined on thallus development. The traditional Chytridiales was split into two orders, i.e., the Chytridiales *sensu* Barr and Spizellomycetales Barr, based on ribosome configurations, microbody–lipid complexes (MLC), and other criteria (Barr 1980). However, this system is not perfect. First, the differences between the Spizellomycetales and Chytridiales were not distinct. There are some genera that can be assigned to either of the orders since they have characters of both orders, such as the *Karlingia* complex, *Synchytrium*, and *Zygorhizidium* (Barr and Désaulniers 1986; Montecillo et al. 1980; Beakes et al. 1988). Second, the families were defined by developmental characters largely for convenience, and these characters are not reliable. For example, sporangial development may be both endogenous and exogenous in a single species, e.g., *Entophlyctis variabilis*, *Triparticalcar arcticum*, *Spizellomyces* spp., *Piromyces communis*, and *Caecomyces communis* (Powell and Koch 1977; Barr 1984a; Barr et al. 1989; Wubah et al. 1991b). In the present taxonomic system such variation would place each of these single species into two different families. Furthermore, as the number of

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TABLE 1. Taxa used in the cladistic analysis

Order and genus ^a	References
Spizellomycetales	
Neocallimasticaceae	
<i>Neocallimastix</i> ^b	Barr et al. 1989; Heath et al. 1983; Heath and Bauchop 1985; Munn et al. 1987, 1988; Orpin and Munn 1986; Webb and Theodorou 1991; Wubah et al. 1991a
<i>Piromyces</i> ^b	Barr et al. 1989; Breton et al. 1991; Li et al. 1990; Munn et al. 1988
<i>Caecomyces</i> ^b	Gold et al. 1988; Munn et al. 1988; Wubah et al. 1991b
<i>Orpinomyces</i>	Barr et al. 1989; Breton et al. 1989; Li et al. 1991
Spizellomycetaceae	
<i>Spizellomyces</i> ^b	Barr and Hadland-Hartmann 1979; Barr 1984a, 1984b
<i>Tripartalcar</i>	Barr 1984a; Barr and Allan 1981; Chong and Barr 1973
<i>Gaertneriomyces</i> ^b	Barr 1981b; Barr 1984a
<i>Kochiomyces</i>	Barr 1984a; Barr and Allan 1981
<i>Karlingia</i> A	Barr and Hartmann 1977; Barr and Désaulniers 1986
<i>Karlingia</i> B	Barr and Hartmann 1977; Barr and Désaulniers 1986
<i>Karlingia</i> C	Barr and Désaulniers 1986
<i>Karlingia</i> D	Barr and Désaulniers 1986
Olpidiaceae	
<i>Olpidium brassicae</i>	Lange and Olson, 1976a, 1976b
<i>Olpidium radicale</i>	Barr and Hadland-Hartmann 1978b; Lange and Olson 1978b
<i>Rozella</i>	Held 1975
<i>Entophlyctis</i>	Chong and Barr 1974; Heath 1986c; Powell and Koch 1977
Caulochytriaceae	
<i>Caulochytrium</i>	Olive 1980; Powell 1981b
Chytridiales	
Chytridiaceae	
<i>Chytridium</i> ^b	Barr and Hartmann 1976
<i>Rhizoclostratium</i>	Barr and Hartmann 1976
<i>Rhizophydium</i> ^b	Barr and Hadland-Hartmann 1978a; Chong and Barr 1974; Heath 1986c
<i>Allochytrium</i> ^b	Barr 1986; Barr and Désaulniers 1987
<i>Polyphagus</i>	Powell 1981a
Cladochytriaceae	
<i>Nowakowskiella</i>	Heath 1986c; Lucarotti 1981
<i>Cladochytrium</i>	Lucarotti 1981
Harpochytriaceae	
<i>Harpochytrium</i>	Gauriloff et al. 1980a; Heath 1986c; Travland and Whisler 1971
Synchytriaceae	
<i>Synchytrium endobioticum</i>	Lange and Olson 1978a
<i>Synchytrium macrosporum</i>	Montecillo et al. 1980
Uncertain	
<i>Zygorhizidium affluens</i>	Beakes et al. 1988
<i>Zygorhizidium planktonicum</i>	Beakes et al. 1988
Monoblepharidales	
Monoblepharidaceae	
<i>Monoblepharella</i>	Dolan and Fuller 1985; Fuller and Reichle 1968; Gauriloff et al. 1980b; Heath 1986c; Reichle 1972
Oedogoniomycetaceae	
<i>Oedogoniomyces</i>	Gauriloff et al. 1980b; Reichle 1972
Blastocladales	
Blastocladiaceae	
<i>Blastocladia</i>	Heath 1986c; Reichle and Fuller 1967
<i>Blastocladia</i>	Lingle and Barstow 1983
<i>Allomyces</i> ^b	Fuller and Olson 1971; Olson 1973
Catenariaceae	
<i>Catenaria</i>	Chong and Barr 1974; Heath 1986c; Olson et al. 1978
Physodermataceae	
<i>Physoderma</i> ^b	Lange and Olson 1980; Lowry and Sparrow 1978; Olson and Lange 1978
Coelomomycetaceae	
<i>Coelomomyces</i> ^b	Lucarotti and Federici 1984; Travland 1979
Uncertain	
<i>Sorochytrium</i>	Dewell and Dewel 1990
Saprolegniales	
Saprolegniaceae	
<i>Saprolegnia</i>	Barr and Allan 1985; Holloway and Heath 1977; Heath 1978, 1986c

^aOrders and families based on Barr (1980, 1990).^bBased on a number of species.^cThis reference was used as a secondary source for the mitotic data. Original references are contained in it and in Heath (1980).

TABLE 2. Structural characters of the Chytridiomycota used in the cladistic analysis

1. Carpacity	6. Simple spur
0. Holocarpic	7. Complicated system
1. Eucarpic	8. Electron dense arc with fibrous materials
2. Centricity	9. Striated, fibrous rhizoplast
0. Monocentric	17. Organization of mitochondria
1. Polycentric	0. Several, disperse
2. Colonial	1. Several, at the periphery of nuclear cap area
3. Sporangial development	2. Several, posterior
0. Endogenous	3. Several petal-like arrangement
1. Exogenous	4. Single central
4. Thallus morphology	5. Single posterior, surrounding the kinetosome
0. Without rhizoids or hyphae	6. Single, within nuclear cap
1. Sori	7. A single, large, posterior with several small
2. Rhizoids	8. Absent
3. Hyphae	18. Type of MLC (cf. Powell 1978)
5. Rhizoid morphology	0. Absent
0. Without rhizoids	1. Type 3
1. Branched	2. Type 4
2. Bulbous	3. Type 1B
3. Uniaxial with a disklike holdfast	4. Type 1A
6. Hypha morphology	5. Type 2
0. Without hyphae	19. Subtype 1B of MLC
1. Extensively branched	0. Absent
2. Unbranched filament	1. Subtype 1B1
3. Very delicate, interconnecting with sporangia	2. Subtype 1B2
4. Dichotomously branched	20. Subtype 1A of MLC
7. Crystal body in thallus	0. Absent
0. Absent	1. Subtype 1A1
1. Present	2. Subtype 1A2
8. Oxygen requirement	3. Subtype 1A3
0. Primarily aerobic	21. Subtype 4 of MLC (cf. Beakes et al. 1988)
1. Primarily anaerobic	0. Absent
9. Type of flagellum	1. Subtype 4A
0. Heterokont	2. Subtype 4B
1. Whiplash	3. Subtype 4C
10. Number of flagella	22. Type of kinetosomes
0. Biflagellate	0. Two kinetosomes, both functional
1. Mainly uniflagellate	1. Two kinetosomes, one functional, one nonfunctional
2. Polyflagellate	2. One kinetosome, functional
11. Transition zone helix	23. Angle between the functional and vestigial kinetosomes
0. Absent	0. Nonfunctional kinetosome absent
1. Present	1. Right angle
12. Prop	2. Parallel
0. Absent	3. Variable
1. Present	4. Anterior
13. Ribosome configuration	24. Relationship between the functional kinetosome and nonfunctional kinetosome
0. Aggregates and (or) helices	0. Nonfunctional kinetosome absent
1. Disperse	1. No connection between the kinetosomes
2. Loosely packed by membrane	2. Kinetosomes connected by electron opaque or fibrous bridge
3. Nuclear cap	25. The veil-like sheath on the side of nonfunctional centriole
14. Nucleus position	0. Nonfunctional kinetosome absent
0. Variable	1. Absent
1. Anterior	2. Present
2. Center	26. Configuration of endoplasmic reticulum
3. Posterior	0. Dispersed in cytoplasm and not specific patterns
15. Nucleus shape	1. Endoplasmic reticulum stacks and extensive sheets in cytoplasm
0. Spherical or irregular	2. Partially delimiting ribosomes and not traversing ribosomal area
1. Cone-shaped	3. Partially delimiting ribosomes and traversing ribosomal area
2. With an elongated beak	4. Fully delimiting a nuclear cap
16. Type of MTOC or rhizoplast	27. One or more obvious Golgi apparatus
0. No specific structure	0. Absent
1. Compound spur with three arms	1. Present
2. Striated disc	
3. Electron opaque structure	
4. Electron dense plate	
5. Trisegmented spur	

TABLE 2 (concluded)

28. Organization of microtubules	35. Configuration of NAOs
0. Not detected	0. Centrioles oriented at 180°
1. Radiating around nucleus and forming a posterior fan	1. Centrioles oriented at approximately 90°
2. In 9 groups of 3, radiating around nucleus	2. Variable, either centrioles oriented at 90° or discs
3. Bundle to rumposomes	36. Spindle development
4. Along spur to nucleus	0. Poles concomitant with spindle during spindle formation
5. Radiating into cytoplasm and around nucleus	1. Poles migrate prior to spindle formation
6. Dual and complex system	37. Nuclear envelope behaviour
7. Cone-shaped basket around nucleus	0. Remains intact throughout mitosis
29. Electron dense area at the base of flagella	1. Develops polar fenestrae
0. Absent	38. Perinuclear endoplasmic reticulum
1. Present	0. Absent
30. Circumflagellar ring	1. Present
0. Absent	39. Telophase behaviour of nuclear envelope
1. Present	0. Nuclear envelope medianly constricted
31. Number of struts	1. A new envelope formed both inside and independently from the old envelope
0. None	2. Nuclear envelope constrict in two places near the NAOs so that the interzone is cut off from the daughter nucleus
1. One strut	40. Metaphase plate
2. Two struts	0. Absent
3. Three struts	1. Present
32. Connective	41. Arrangement of metaphase chromosomes
0. Absent	0. Uncondensed
1. Present	1. Distinguished separate chromosome
33. Skirt	2. Fused mass of chromatin
0. Absent	42. Nucleolus behaviour
1. Present	0. Persistent
34. Posterior dome	1. Dispersive
0. Absent	2. Discardive
1. Present	

species studied has increased, the data base has expanded significantly, which makes subjective analysis of the data more difficult.

There are now numerous cladistic methods of data analysis designed to aid in phylogenetic reconstructions, but they have yet to be applied to the Chytridiomycota. Also, the advent of DNA sequence data has provided additional criteria that are available for some Chytridiomycota (Bowman et al. 1992; Li and Heath 1992; Doré and Stahl 1991) but that have yet to be correlated with structural data. In addition to these opportunities for clarifying chytrid phylogeny, controversy has developed concerning the chytridiomycetous gut fungi (Heath 1988; Bauchop 1989). Although they were formally assigned to the Neocallimastixaceae in the Spizellomycetales, they differ from both the Spizellomycetales and the Chytridiales in a number of ways such that their assignment to the Spizellomycetales is questionable (Billon-Grand et al. 1991; Heath and Bauchop 1985; Li et al. 1990, 1991; Munn et al. 1987; Yarlett et al. 1986).

We have discussed a limited rRNA sequence analysis of the Chytridiomycota in a previous paper (Li and Heath 1992). A cladistic analysis of the available structural data in conjunction with this work may further clarify the phylogeny and taxonomy of the Chytridiomycota. This paper focuses on such an analysis of the structural data and integrates the results from both approaches in producing a revised phylogeny and taxonomy of the Chytridiomycota.

Methods

Taxa, character selections, and coding

We selected 38 taxonomic units representing genera, species, and isolates from the major groups of the Chytridiomycota for our analysis of the division (Table 1). The units were selected on the basis of the 42 characters used (Table 2) and the availability of adequate data.

In some cases several species in a genus were identical for the selected characters, in which case the genus was the unit used. In other cases, isolates or species within a genus differed, so that these were the units analyzed (Table 3).

In a more detailed analysis of the gut fungi alone, we used an expanded set of 21 more specific characters (Table 4) from 10 species and four genera (Table 5). Ultrastructural data for *Anaeromyces* (Breton et al. 1989) and *Ruminomyces* (Ho and Bauchop 1990) were unavailable, hence these taxa were not included.

Morphological criteria such as operculum and sporangium and zoospore size and shape were not analyzed since they are very variable, even in an isolate (Miller 1976). In contrast, developmental characters, such as sporangial development, are more stable (Whiffen 1944; Barr 1980) and were included. The ultrastructural characters were predominantly from zoospores since they are conserved both within and between isolates (Powell 1978; Barr 1978, 1981a). The kinetosome and its root system and the MLC, which are considered to be good phylogenetic indicators because they are stable, easily identified, and refractory to fixation-induced changes (Powell 1978; Barr 1978, 1981a), were emphasized. Vaguely defined organelles such as γ bodies, various vesicles, and inclusions were excluded, since their homology among the chytrids could not be established.

It is very difficult to justify ordering character states for many characters in the chytridiomycetous fungi. Without a fossil record, a poor knowledge of character function, and little analysis of ontogenetic variation, any proposed ordering of states is pure speculation. Consequently, all characters were treated as unordered. Some characters, such as thallus morphology (characters 4–6), type of MLC (characters 18–21), and type of kinetosomes (characters 22–25), form character state trees that were coded by nonredundant linear coding (O'Grady and Deets 1987; Wiley et al. 1991). Question marks indicate that the data were unavailable.

Outgroup analysis

Although the origin of the Chytridiomycota is uncertain, they are probably monophyletic (Barr 1983; Li and Heath 1992). The Oomycota

TABLE 3. Coded structural data of the Chytridiomycota for 42 characters (see Table 2)

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42			
<i>Saprolegnia</i>	1	1	1	3	0	1	0	0	0	0	0	0	1	0	0	7	0	0	0	0	0	0	0	0	0	1	1	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neocallinastix</i>	1	0	0	2	1	0	1	1	1	2	1	1	0	3	2	6	8	0	0	0	0	2	0	0	0	0	0	0	1	0	1	2	1	1	1	2	1	0	0	0	0	0	2	0	0
<i>Piromyces</i>	1	0	0	2	1	0	1	1	1	1	1	?	0	3	0	6	8	0	0	0	0	2	0	0	0	0	0	1	0	1	3	1	1	1	?	?	?	?	?	?	?	?	?	?	
<i>Orpinomyces</i>	1	1	1	3	0	1	1	1	1	2	1	1	0	2	0	6	8	0	0	0	0	2	0	0	0	0	0	0	1	0	1	1	0	1	1	?	?	?	?	?	?	?	?	?	
<i>Caecomyces</i>	1	0	0	2	2	0	1	1	1	1	1	?	0	3	0	6	8	0	0	0	0	2	0	0	0	0	0	1	0	1	3	1	1	1	?	?	?	?	?	?	?	?	?	?	
<i>Synchytrium endobioticum</i>	0	2	1	1	0	0	0	0	1	1	1	1	1	2	0	0	0	5	0	2	0	1	2	1	1	0	1	5	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Synchytrium macrosporum</i>	0	2	1	1	0	0	0	0	1	1	1	1	1	1	0	0	0	3	1	0	0	1	2	1	1	0	1	3	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Spizellomyces</i>	1	0	0	2	1	0	0	0	1	1	1	1	1	1	2	6	3	4	0	1	0	1	3	2	2	1	0	5	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Tripaticlcar</i>	1	0	0	2	1	0	0	0	1	1	1	1	1	1	0	5	3	4	0	1	0	1	1	2	1	1	0	4	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?		
<i>Gaertneriomyces</i>	1	0	0	2	1	0	0	0	1	1	1	1	1	3	0	8	3	4	0	1	0	1	3	2	1	1	1	5	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?		
<i>Kochiomyces</i>	1	0	0	2	1	0	0	0	1	1	1	1	1	3	0	1	3	4	0	1	0	1	3	2	1	1	1	5	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Entophlyctis</i>	1	0	1	2	1	0	0	0	1	1	1	1	1	2	0	8	3	4	0	2	0	1	1	1	1	0	0	5	0	0	0	0	0	0	0	0	1	1	1	1	1	1	2	?	
<i>Olpidium brassicae</i>	0	0	1	0	0	0	0	0	1	1	1	1	1	0	9	0	4	0	4	0	1	0	1	3	2	1	0	1	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	
<i>Olpidium radicale</i>	0	0	1	0	0	0	0	0	1	1	1	1	1	1	0	0	0	4	0	1	0	1	3	2	1	0	1	5	1	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	
<i>Rozella</i>	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	9	4	4	0	3	0	1	3	1	1	0	1	5	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Caulochytrium</i>	1	0	0	0	0	0	0	0	1	1	1	1	1	1	0	9	0	4	0	?	0	1	?	1	1	0	1	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Karlingia A</i>	1	0	0	2	1	0	0	0	1	1	1	1	1	1	0	9	0	4	0	1	0	1	3	2	1	0	1	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Karlingia B</i>	1	0	0	2	1	0	0	0	1	1	1	1	2	3	0	9	0	4	0	1	0	1	3	2	1	3	?	0	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	
<i>Karlingia C</i>	1	0	0	2	1	0	0	0	1	1	1	1	2	2	0	0	1	4	0	1	0	1	2	2	1	3	1	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Karlingia D</i>	1	0	0	2	1	0	0	0	1	1	1	1	2	2	0	1	4	0	2	0	1	2	2	1	3	?	0	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Zygorhidium affluens</i>	1	0	0	2	1	0	0	0	1	1	1	1	2	2	0	0	6	4	0	2	0	1	2	2	1	3	0	0	1	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Zygorhidium planktonicum</i>	1	0	0	2	1	0	0	0	1	1	1	1	1	1	0	0	4	3	2	0	0	1	3	2	1	0	0	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Chytridium</i>	1	0	0	2	1	0	0	0	1	1	1	1	2	2	0	4	1	3	2	0	0	1	2	2	2	2	1	3	1	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Rhizoclostridium</i>	1	0	0	2	1	0	0	0	1	1	1	1	2	1	0	4	1	3	2	0	0	1	2	2	1	2	1	3	1	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Rhizophydium</i>	1	0	0	2	1	0	0	0	1	1	1	1	2	2	0	4	3	2	0	0	1	2	2	1	2	0	3	0	0	0	0	0	0	1	1	1	1	1	2	1	1	2			
<i>Nowakowskiella</i>	1	1	1	3	0	1	0	0	1	1	1	1	2	2	0	0	1	3	2	0	0	1	2	2	1	2	1	3	1	0	0	0	0	0	1	1	1	1	1	2	1	?	?		
<i>Cladochytrium</i>	1	1	1	3	0	1	0	0	1	1	1	1	2	2	0	0	1	3	2	0	0	1	2	2	1	2	1	3	1	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Allochytrium</i>	1	0	1	2	1	0	0	0	1	1	1	1	2	2	0	0	1	3	2	0	0	1	2	2	1	3	1	3	0	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	
<i>Polyphagus</i>	1	0	1	2	1	0	0	0	1	1	1	1	2	2	0	2	1	3	1	0	0	1	2	2	1	3	0	5	0	0	0	0	0	1	1	0	1	0	1	2	1	1	2		
<i>Harpochytrium</i>	1	0	1	2	3	0	0	0	1	1	1	1	2	3	0	2	0	5	0	0	0	1	2	2	1	3	0	5	1	0	0	0	0	0	1	1	1	0	0	1	1	1	1		
<i>Oedogoniomyces</i>	1	0	1	3	0	2	0	0	1	1	1	1	2	2	0	2	2	1	0	0	0	1	2	2	1	2	1	5	1	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Monoblepharella</i>	1	1	1	3	0	1	0	0	1	1	1	1	2	2	0	2	2	1	0	0	0	1	2	2	1	2	1	5	1	0	0	0	0	1	1	1	1	1	2	1	1	2			
<i>Blastocladiella</i>	1	0	1	2	1	0	0	0	1	1	1	1	3	3	1	3	5	2	0	0	1	1	4	1	1	4	0	2	0	0	0	0	0	1	1	0	0	?	?	?	?	?	?	0	
<i>Blastocladia</i>	1	1	1	3	0	4	0	1	1	1	1	1	3	3	1	3	5	0	0	0	0	1	4	1	1	4	0	2	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Catenaria</i>	1	1	1	3	0	1	0	0	1	1	1	1	3	3	1	3	5	2	0	0	1	1	4	1	1	4	0	2	0	0	0	0	0	1	1	0	0	2	1	2	2	2			
<i>Allomyces</i>	1	1	1	3	0	4	0	0	1	1	1	1	3	3	1	3	7	2	0	0	2	1	4	1	1	4	0	2	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Physoderma</i>	1	1	1	3	0	3	0	0	1	1	1	1	3	3	1	3	5	2	0	0	1	1	4	1	1	4	0	2	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?		
<i>Sorochytrium</i>	0	2	1	1	0	0	0	0	1	1	1	1	3	3	1	3	6	2	0	0	3	1	3	1	1	4	0	7	0	0	0	0	0	?	?	?	?	?	?	?	?	?			
<i>Coelomomyces</i>	1	1	1	0	0	0	0	0	1	1	1	1	3	3	1	3	5	2	0	0	1	2	0	0	0	4	0	7	0	0	0	0	?	?	?	?	?	?	?	?	?	?			

NOTE: ? indicates missing data.

TABLE 4. Morphological and ultrastructural characters of the gut fungi used in the cladistic analysis

1. Thallus morphology	13. Shape of free hydrogenosome
0. Monocentric	0. Without hydrogenosomes
1. Polycentric	1. Tubular
2. Sporangial development	2. More or less spherical
0. Endogenous	14. Megahydrogenosome
1. Exogenous	0. Absent
3. Subsporangial swelling	1. Present
0. Absent	15. Nuclear shape
1. Present	0. Pear shape with a concave end towards the kinetosome
4. Sporangiphore	1. More or less spherical
0. Absent	2. With a beak towards the kinetosome
1. Present	3. Cone-shaped
5. Rhizoid morphology	16. Circumflagellar ring
0. Branched	0. Without circumflagellar ring
1. Coenocytic hyphae	1. Uniform
2. Single bulbous	2. With a C-shaped vertical elongation
3. Several bulbous	3. Inconspicuous or absent in one side
6. Rhizoid with restriction	17. Number of struts
0. Absent	0. Without struts
1. Present	1. One strut
7. Crystal bodies	2. Two struts
0. Absent	3. Three struts
1. Present	18. Connective
8. The mechanism of zoospore release	0. Absent
0. Through multiple differentiated pores	1. Present
1. Through an undifferentiated area	19. Shape of skirt
2. Through one differentiated pore	0. Without skirt
9. Number of flagella	1. Smooth
0. Mainly uniflagellate	2. With a saclike structure
1. Polyflagellate	20. The relationship of microtubules and megatubules in the posterior dome
2. Biflagellate	0. Without posterior dome
10. Nonfunctional kinetosome	1. Microtubules parallel the dome
0. Present	2. Microtubules intersect the dome
1. Absent	21. Sporangia reaction to <i>Sophora japonica</i> lectin
11. Ribosomal configuration	0. Positive
0. Free posterior aggregates	1. Negative
1. Ribosomes dispersed	
2. Free anterior aggregates	
3. Anterior aggregates embedded in uniform substratum	
4. Nuclear cap	
12. Position of nucleus in the zoospore	
0. Anterior	
1. Posterior	
2. Central	
3. Variable	

are related to the Chrysophyta and Xanthophyta (Beakes 1989) and molecular sequence data showed that the Oomycota separated from the Chytridiomycota in early evolutionary history (Gunderson et al. 1987). Both the Chytridiomycota and Oomycota have vegetative and asexual stages that can be compared. Therefore we selected one of the best-studied genera of the Oomycota, *Saprolegnia*, as an outgroup for the Chytridiomycota. For similar reasons, *Spizellomyces punctatus* was selected as an outgroup in the gut fungi analysis.

Tree construction

The PAUP (phylogeny analysis using parsimony) version 3.0 (Swofford 1989) and the FITCH (Fitch–Margoliash algorithm) program from the PHYLIP package version 3.4 (Felsenstein 1991) were used to construct phylogenetic trees. In PAUP, both HEURISTIC and BRANCH AND BOUND algorithms were used in finding the most parsimonious trees. For the chytridiomycetous data set, the following options were used: MAXTREE, 20 000; optimization, ACCTRAN; typeset, ALL UNORDERED; wtsset, EQUAL WEIGHTS; exset, ALL INCLUDED; ancstates, STANDARD; rooting, OUTGROUP; HEURISTIC search algorithm (stepwise addition sequence, RANDOM; branch-swapping, NNI, MULPARS, COLLAPSE, zero-length branches; STEEPEST DESCENT was not in effect). For the gut fungi data

set, similar options were used except that the BRANCH AND BOUND algorithm replaced the HEURISTIC search algorithm. For both data sets, the 50% majority-rule consensus tree option was used to represent the phylogeny. The mean pairwise distances between taxa calculated by PAUP (Tables 6 and 7) were used as input data for FITCH. In the FITCH algorithm, the global search option was used. The PAUP program was run either on a Macintosh Plus with 4 megabytes of RAM or a Macintosh LC computer with 10 megabytes of RAM and a math coprocessor. The latter was approximately 10 times faster than the former. The FITCH program was run on a VAX 4000-300 mainframe computer.

Results

Taxonomy and phylogeny of the Chytridiomycota

A total of 19 055 equally parsimonious trees was generated by PAUP with the NNI option. The tree length was 181, with a consistency index (CI) of 0.56 and a retention index (RI) of 0.77. A 50% majority-rule consensus tree was generated from the above equally parsimonious trees and had a tree length of 182, CI of 0.555, and RI of 0.771 (Fig. 1). Two kinds of pair-

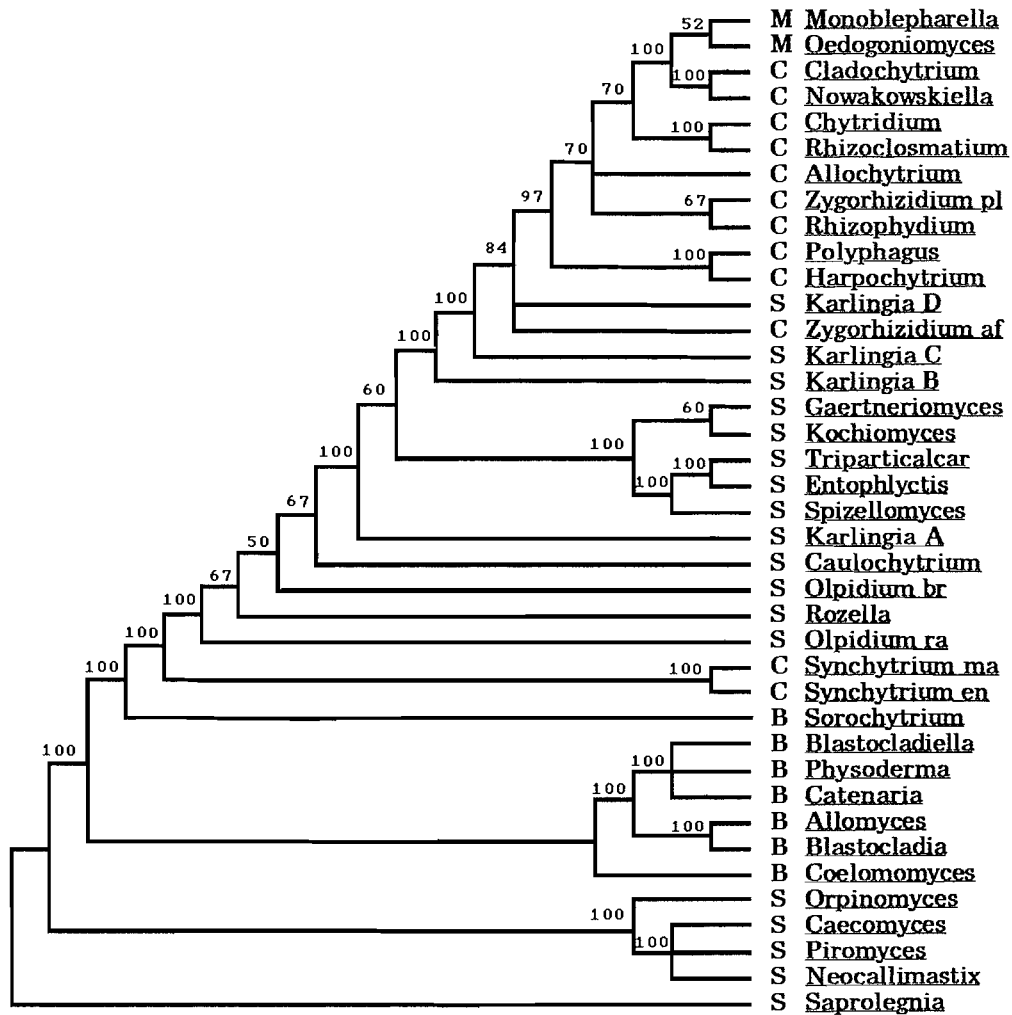


FIG. 1. A majority-rule consensus tree from 19055 parsimony trees generated by PAUP using NNI option, showing the phylogenetic relationships of the genera in the Chytridiomycota. The letters indicate orders from Table 1. The numbers above each node represent percent occurrence of that particular node among the total number of trees. The branch lengths do not represent the evolutionary distance.

TABLE 5. Coded structural data of the chytridiomycetous gut fungi for 21 characters (see Table 4)

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
<i>Neocallimastix patriciarum</i>	0	0	0	0	0	1	1	1	1	1	2	2	2	0	1	1	2	1	1	1	?
<i>Neocallimastix frontalis</i>	0	0	0	0	0	1	1	1	1	1	0	1	2	0	0	1	2	1	1	1	?
<i>Neocallimastix hurleyensis</i>	0	0	0	0	0	?	?	1	1	1	2	2	2	1	1	1	2	1	1	?	?
<i>Piromyces mae</i>	0	?	1	0	0	0	1	2	0	1	2	1	2	1	1	1	3	1	1	1	?
<i>Piromyces dumbonica</i>	0	?	1	0	0	0	1	?	0	1	3	1	1	0	2	2	3	1	1	1	?
<i>Piromyces rhizinflata</i>	0	0	1	0	0	0	1	2	0	1	2	1	2	0	1	1	?	1	1	?	0
<i>Piromyces communis</i>	0	?	1	1	0	0	1	2	0	1	2	?	2	0	?	1	?	1	1	?	1
<i>Caecomyces equi</i>	0	?	0	0	3	0	1	?	0	1	3	1	2	0	0	2	3	1	1	1	?
<i>Caecomyces communis</i>	0	?	0	0	2	0	1	?	0	1	2	?	2	0	?	1	?	1	1	1	?
<i>Orpinomyces jyonii</i>	1	1	0	1	1	1	1	?	1	1	2	2	1	0	1	3	1	0	2	2	?
<i>Spizellomyces punctatus</i>	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	?
<i>Blastocladiella emersonii</i>	0	1	0	0	0	0	?	0	0	0	4	1	0	0	3	0	0	0	0	0	?
<i>Catenaria anguillulae</i>	1	1	0	0	1	0	0	2	0	0	4	1	0	0	3	0	0	0	0	0	?
<i>Saprolegnia ferax</i>	1	1	0	0	1	0	0	2	3	2	1	3	0	0	0	0	0	0	0	0	?

NOTE: ? indicates missing data.

wise evolutionary distances between taxa were generated by PAUP, absolute distance and mean distance (Table 6). The phylogenetic trees constructed by FITCH with the two sets of distances had the same topology and similar branch lengths. Only

the tree constructed with the mean distance is shown (Fig. 2). The phylogenetic trees generated by both methods had similar major groupings. Basically, there were three major distinct groups: the gut fungi, the Blastocladiales, and the Spizello-

TABLE 6. Pairwise distances generated by PAUP

	1	2	3	4	5	6	7	8	9	10	11	12
1. <i>Saprolegnia</i>	0	0.667	0.548	0.429	0.548	0.500	0.476	0.452	0.452	0.452	0.476	0.500
2. <i>Neocallimastix</i>	28	0	0.071	0.214	0.095	0.452	0.500	0.500	0.500	0.500	0.476	0.524
3. <i>Piromyces</i>	23	3	0	0.214	0.024	0.452	0.452	0.452	0.452	0.452	0.429	0.476
4. <i>Orpinomyces</i>	18	9	9	0	0.214	0.571	0.571	0.595	0.595	0.571	0.571	0.571
5. <i>Caecomyces</i>	23	4	1	9	0	0.476	0.476	0.476	0.476	0.476	0.452	0.500
6. <i>Spizellomyces</i>	21	19	19	24	20	0	0.119	0.119	0.119	0.167	0.190	0.238
7. <i>Triparticalcar</i>	20	21	19	24	20	5	0	0.119	0.119	0.143	0.167	0.190
8. <i>Gaertneriomyces</i>	19	21	19	25	20	5	5	0	0.024	0.119	0.119	0.167
9. <i>Kochiomyces</i>	19	21	19	25	20	5	5	1	0	0.119	0.119	0.167
10. <i>Karlingia</i> A	19	21	19	24	20	7	6	5	5	0	0.071	0.143
11. <i>Karlingia</i> B	20	20	18	24	19	8	7	5	5	3	0	0.095
12. <i>Karlingia</i> C	21	22	20	24	21	10	8	7	7	6	4	0
13. <i>Karlingia</i> D	22	20	20	24	21	9	9	9	9	8	6	2
14. <i>Olpidium brassicae</i>	18	25	23	23	23	11	10	9	9	4	7	10
15. <i>Olpidium radicale</i>	19	26	24	24	24	11	11	9	9	7	10	11
16. <i>Rozella</i>	20	24	22	24	22	11	11	9	9	7	10	12
17. <i>Caulochytrium</i>	16	21	19	21	19	10	8	8	8	3	6	8
18. <i>Entophlyctis</i>	27	26	19	21	20	9	7	7	8	9	10	10
19. <i>Chytridium</i>	22	23	21	25	22	13	13	12	12	12	11	8
20. <i>Rhizoclosmatium</i>	22	23	21	26	22	13	11	11	11	10	10	8
21. <i>Rhizophydium</i>	31	27	20	24	21	11	11	12	12	12	10	8
22. <i>Nowakowskiella</i>	23	33	26	20	26	19	17	16	16	16	15	11
23. <i>Cladochytrium</i>	17	28	26	20	26	19	17	16	16	16	15	11
24. <i>Allochytrium</i>	20	23	21	23	22	14	12	11	11	11	9	5
25. <i>Polyphagus</i>	28	28	20	22	21	12	11	11	11	12	9	7
26. <i>Synchytrium endobioticum</i>	18	26	24	22	24	16	15	13	13	12	14	12
27. <i>Synchytrium macrosporum</i>	18	26	24	23	24	17	15	15	15	12	15	14
28. <i>Zygorhizidium affluens</i>	23	22	20	24	21	11	9	10	10	9	6	4
29. <i>Zygorhizidium planktonicum</i>	21	20	18	23	19	9	8	9	9	6	8	9
30. <i>Harpochytrium</i>	26	27	20	23	20	13	12	11	11	12	8	10
31. <i>Oedogoniomyces</i>	18	26	24	21	24	16	15	13	13	14	13	11
32. <i>Monoblepharella</i>	24	34	25	19	25	17	16	14	14	15	14	12
33. <i>Blastocladia</i>	24	22	20	24	21	14	13	13	13	14	12	14
34. <i>Blastocladia</i>	18	22	21	18	21	18	17	17	17	18	16	18
35. <i>Catenaria</i>	24	29	24	20	24	18	17	17	17	18	16	18
36. <i>Allomyces</i>	19	25	24	21	24	18	17	17	17	18	16	18
37. <i>Physoderma</i>	19	25	24	21	24	18	17	17	17	18	16	18
38. <i>Sorochytrium</i>	22	25	24	24	24	17	17	16	16	17	15	18
39. <i>Coelomomyces</i>	17	20	19	18	19	18	18	18	18	19	17	19

NOTE: The lower part shows absolute distances, and the upper part shows mean distances.

mycetales—Chytridiales—Monoblepharidales. *Monoblepharella* and *Oedogoniomyces*, the two Monoblepharidales genera, were clustered with *Nowakowskiella* and *Cladochytrium*, two polycentric genera of the Chytridiales in the trees generated by both algorithms. In the parsimony tree, most Chytridiales, excluding *Synchytrium* spp., formed a distinct group including the Monoblepharidales, whereas the Spizellomycetales did not form a monophyletic group. In contrast, two clusters corresponding roughly to the Chytridiales and Spizellomycetales were found in the distance tree. The most notable anomalies in these trees were (i) *Sorochytrium*, which clustered with the Blastocladiales in the distance tree but not in the parsimony tree, (ii) *Synchytrium* spp., which consistently clustered within, or closer to, the Spizellomycetales than the Chytridiales, and (iii) *Karlingia* C and D, which consistently clustered with the Chytridiales rather than the Spizellomycetales. The Blastocladiales were a well-defined group. They are closer to members of the Spizellomycetales than the Chytridiales in the parsimony tree and show similar affinities to the Spizellomycetales and the Chytridiales in the distance tree. All the gut fungi formed a single clade, which was the first group separated from the other chytrids in both trees.

Phylogeny of chytridiomycetous gut fungi

A total of 162 equally parsimonious trees with length of 41, CI of 0.79, and RI of 0.74 were generated by PAUP with the branch and bound option for the gut fungi data set. The 50% majority-rule consensus tree generated from these equally parsimonious trees was the same as one of the most parsimonious trees (Fig. 3). Similarly, the phylogenetic trees constructed by FITCH with the two sets of distances (Table 7) had the same topology and were similar in branch length. Only the tree constructed with the mean distance is shown (Fig. 4).

Both phylogenetic trees clustered the two polyflagellate genera (*Orpinomyces* and *Neocallimastix*) together (Figs. 3 and 4). The relationships between the uniflagellate genera were unclear. *Piromyces dumbonica* and *Caecomyces equi* consistently clustered together. The remaining species of *Piromyces* are polytomous. However, *C. communis* was consistently isolated and closer to the polyflagellate species than to the other uniflagellate species (Figs. 3 and 4).

Because the outgroup chosen for these analysis (*Spizellomyces*) is a more derived genus than the gut fungi (Figs. 1 and 2), we also analyzed the data set using PAUP with *Blastocladia*, *Catenaria*, and *Saprolegnia*, either singly or in various

based on structural characters of the Chytridiomycota

13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
0.524	0.429	0.452	0.476	0.381	0.643	0.524	0.524	0.738	0.548	0.405	0.476	0.667	0.429	0.429
0.476	0.595	0.619	0.571	0.500	0.619	0.548	0.548	0.643	0.786	0.667	0.548	0.667	0.619	0.619
0.476	0.548	0.571	0.524	0.452	0.452	0.500	0.500	0.476	0.619	0.619	0.500	0.476	0.571	0.571
0.571	0.548	0.571	0.571	0.500	0.500	0.595	0.619	0.571	0.476	0.476	0.548	0.524	0.524	0.548
0.500	0.548	0.571	0.524	0.452	0.476	0.524	0.524	0.500	0.619	0.619	0.524	0.500	0.571	0.571
0.214	0.262	0.262	0.262	0.238	0.214	0.310	0.310	0.262	0.452	0.452	0.333	0.286	0.381	0.405
0.214	0.238	0.262	0.262	0.190	0.167	0.310	0.262	0.262	0.405	0.405	0.286	0.262	0.357	0.357
0.214	0.214	0.214	0.214	0.190	0.167	0.286	0.262	0.286	0.381	0.381	0.262	0.262	0.310	0.357
0.214	0.214	0.214	0.214	0.190	0.190	0.286	0.262	0.286	0.381	0.381	0.262	0.262	0.310	0.357
0.190	0.095	0.167	0.167	0.071	0.214	0.286	0.238	0.286	0.381	0.381	0.262	0.286	0.286	0.286
0.143	0.167	0.238	0.238	0.143	0.238	0.262	0.238	0.238	0.357	0.357	0.214	0.214	0.333	0.357
0.048	0.238	0.262	0.286	0.190	0.238	0.190	0.190	0.190	0.262	0.262	0.119	0.167	0.286	0.333
0	0.286	0.310	0.310	0.214	0.214	0.214	0.214	0.143	0.286	0.286	0.143	0.167	0.286	0.357
12	0	0.071	0.119	0.071	0.262	0.381	0.333	0.381	0.357	0.357	0.310	0.333	0.214	0.214
13	3	0	0.143	0.143	0.262	0.357	0.310	0.381	0.310	0.310	0.310	0.333	0.190	0.214
13	5	6	0	0.071	0.238	0.381	0.333	0.357	0.405	0.405	0.357	0.357	0.214	0.238
9	3	6	3	0	0.190	0.310	0.262	0.310	0.333	0.333	0.286	0.310	0.190	0.190
9	11	11	10	8	0	0.333	0.333	0.333	0.405	0.381	0.262	0.286	0.214	0.310
9	16	15	16	18	14	0	0.048	0.143	0.167	0.167	0.119	0.190	0.381	0.333
9	14	13	14	11	14	2	0	0.143	0.167	0.167	0.119	0.190	0.381	0.286
6	16	16	15	13	14	6	6	0	0.214	0.214	0.119	0.190	0.357	0.310
12	15	3	17	14	17	7	7	9	0	0	0.143	0.262	0.310	0.262
12	15	13	17	14	16	7	7	9	0	0	0.143	0.238	0.310	0.262
6	13	13	15	12	11	5	5	5	6	6	0	0.095	0.286	0.238
7	14	14	15	13	12	8	8	8	11	10	4	0	0.310	0.286
12	9	8	9	8	9	16	16	15	13	13	12	13	0	0.119
15	9	9	10	8	13	14	12	13	11	11	10	12	5	0
3	13	12	14	10	9	9	9	8	12	12	8	8	13	16
9	10	11	10	8	10	10	8	6	13	13	8	9	14	11
10	13	11	15	12	14	11	10	14	14	12	9	10	12	14
12	13	11	14	12	13	10	10	12	7	7	10	9	12	14
13	14	12	15	13	16	11	11	13	5	5	11	11	12	14
13	16	17	16	13	13	16	15	16	20	18	13	14	16	16
17	17	18	17	14	15	20	19	17	15	15	17	16	16	16
17	17	18	17	14	18	20	19	20	16	14	17	18	16	16
17	17	18	17	14	15	20	19	17	15	15	17	16	16	16
17	17	18	17	14	15	20	19	17	15	15	17	16	16	16
17	14	15	14	14	15	20	19	17	18	18	17	16	12	12
18	17	18	18	15	17	20	20	18	18	18	18	17	18	18

combinations, as outgroups. These results (not illustrated) were variable, some were similar to Fig. 3, others closer to Fig. 4 and others placed *Orpinomyces* as the earliest branch in the group, remote from *Neocallimastix*. None generated monophyletic clusters for *Piromyces* and *Caecomyces*.

Discussion

Taxonomy and phylogeny of the Chytridiomycota

Barr (1980, 1990) split the traditional Chytridiales into two orders, the Spizellomycetales and the Chytridiales sensu Barr and proposed that there were two evolutionary lines among the Chytridiomycota, one from the Chytridiales to the Monoblepharidales, the other from the Spizellomycetales to the Blastocladiiales. Our cladistic analysis shows that the Spizellomycetales, Chytridiales, and Monoblepharidales form a monophyletic group, separate from the Blastocladiiales and Neocallimasticeae. This finding is consistent with the limited rRNA based phylogeny that also clustered the Spizellomycetales and the Chytridiales, separate from the Blastocladiiales and gut fungi (Bowman et al. 1992; Li and Heath 1992). The distinction between the Spizellomycetales, Chytridiales, and Monoblephar-

idales is not obvious, especially in the parsimony tree, which disagrees with Barr's (1980) separation of the traditional Chytridiales. This was not totally unexpected (as previously recognized; Barr 1980; Barr and Désaulniers 1986; Beakes et al. 1988) since some genera, such as the *Karlingia* complex, *Synchytrium*, and *Zygorhizidium*, have characters of both orders.

However, in the distance tree, the traditional Chytridiales are divided into two groups, most members of which do correspond to Barr's (1980) Chytridiales and Spizellomycetales, and in the parsimony tree most of Barr's Chytridiales do occur in a single group. However, some revisions are necessary. *Karlingia* types C and D are close to the Chytridiales, while previously they were assigned to the Spizellomycetales (Barr and Désaulniers 1986). On the other hand, *Synchytrium* is close to the Spizellomycetales and probably deserves ordinal status of its own. Previously it was assigned to the Chytridiales (Barr 1980, 1990), but it was pointed out that its relationship with other chytrids was unclear (Barr 1980, 1981a). Overall, our analyses do support a taxonomy that recognizes a modified Chytridiales as a group, but the best treatment of the paraphyletic Spizellomycetales requires further investigation, possibly by use of rRNA sequence analysis of a greater range of

TABLE 6 (concluded)

	28	29	30	31	32	33	34	35	36	37	38	39
1. <i>Saprolegnia</i>	0.548	0.500	0.619	0.429	0.571	0.571	0.429	0.571	0.452	0.452	0.524	0.405
2. <i>Neocallimastix</i>	0.524	0.476	0.643	0.619	0.810	0.524	0.524	0.690	0.595	0.595	0.595	0.476
3. <i>Piromyces</i>	0.476	0.429	0.476	0.571	0.595	0.476	0.500	0.571	0.571	0.571	0.571	0.452
4. <i>Orpinomyces</i>	0.571	0.548	0.548	0.500	0.452	0.571	0.429	0.476	0.500	0.500	0.571	0.429
5. <i>Caecomycetes</i>	0.500	0.452	0.476	0.571	0.595	0.500	0.500	0.571	0.571	0.571	0.571	0.452
6. <i>Spizellomyces</i>	0.262	0.214	0.310	0.381	0.405	0.333	0.429	0.429	0.429	0.429	0.405	0.429
7. <i>Triparticalcar</i>	0.214	0.190	0.286	0.357	0.381	0.310	0.405	0.405	0.405	0.405	0.405	0.429
8. <i>Gaertneriomyces</i>	0.238	0.214	0.262	0.310	0.333	0.310	0.405	0.405	0.405	0.405	0.381	0.429
9. <i>Kochiomyces</i>	0.238	0.214	0.262	0.310	0.333	0.310	0.405	0.405	0.405	0.405	0.381	0.429
10. <i>Karlingia</i> A	0.214	0.143	0.286	0.333	0.357	0.333	0.429	0.429	0.429	0.429	0.405	0.452
11. <i>Karlingia</i> B	0.143	0.190	0.190	0.310	0.333	0.286	0.381	0.381	0.381	0.381	0.357	0.405
12. <i>Karlingia</i> C	0.095	0.214	0.238	0.262	0.286	0.333	0.429	0.429	0.429	0.429	0.429	0.452
13. <i>Karlingia</i> D	0.071	0.214	0.238	0.286	0.310	0.310	0.405	0.405	0.405	0.405	0.405	0.429
14. <i>Olpidium brassicae</i>	0.310	0.238	0.310	0.310	0.333	0.381	0.405	0.405	0.405	0.405	0.333	0.405
15. <i>Olpidium radicale</i>	0.286	0.262	0.262	0.262	0.286	0.405	0.429	0.429	0.429	0.429	0.357	0.429
16. <i>Rozella</i>	0.333	0.238	0.357	0.333	0.357	0.381	0.405	0.405	0.405	0.405	0.333	0.429
17. <i>Caulochytrium</i>	0.238	0.190	0.286	0.286	0.310	0.310	0.333	0.333	0.333	0.333	0.333	0.357
18. <i>Entophlyctis</i>	0.214	0.238	0.333	0.310	0.381	0.310	0.357	0.429	0.357	0.357	0.357	0.405
19. <i>Chytridium</i>	0.214	0.238	0.262	0.238	0.262	0.381	0.476	0.476	0.476	0.476	0.476	0.476
20. <i>Rhizoclosmatium</i>	0.214	0.190	0.238	0.238	0.262	0.357	0.452	0.452	0.452	0.452	0.452	0.476
21. <i>Rhizophyidium</i>	0.190	0.143	0.333	0.286	0.310	0.381	0.405	0.476	0.405	0.405	0.405	0.429
22. <i>Nowakowskiella</i>	0.286	0.310	0.333	0.167	0.119	0.476	0.357	0.381	0.357	0.357	0.429	0.429
23. <i>Cladochytrium</i>	0.286	0.310	0.286	0.167	0.119	0.429	0.357	0.333	0.357	0.357	0.429	0.429
24. <i>Allochytrium</i>	0.190	0.190	0.214	0.238	0.262	0.310	0.405	0.405	0.405	0.405	0.405	0.429
25. <i>Polyphagus</i>	0.190	0.214	0.238	0.214	0.262	0.333	0.381	0.429	0.381	0.381	0.381	0.405
26. <i>Synchytrium endobioticum</i>	0.310	0.333	0.286	0.286	0.286	0.381	0.381	0.381	0.381	0.381	0.286	0.429
27. <i>Synchytrium macrosporum</i>	0.381	0.262	0.333	0.333	0.333	0.381	0.381	0.381	0.381	0.381	0.286	0.429
28. <i>Zygorhizidium affluens</i>	0	0.214	0.190	0.262	0.286	0.333	0.429	0.429	0.429	0.429	0.405	0.452
29. <i>Zygorhizidium planktonicum</i>	9	0	0.286	0.357	0.381	0.310	0.405	0.405	0.405	0.405	0.381	0.429
30. <i>Harpochytrium</i>	8	12	0	0.190	0.286	0.333	0.357	0.452	0.357	0.357	0.357	0.381
31. <i>Oedogoniomyces</i>	11	15	8	0	0.048	0.381	0.357	0.357	0.357	0.357	0.405	0.429
32. <i>Monoblepharella</i>	12	16	12	2	0	0.476	0.333	0.381	0.333	0.333	0.405	0.405
33. <i>Blastoclaadiella</i>	14	13	14	16	20	0	0.167	0.119	0.143	0.095	0.190	0.190
34. <i>Blastocladia</i>	18	17	15	15	14	7	0	0.095	0.095	0.095	0.238	0.238
35. <i>Catenaria</i>	18	17	19	15	16	5	4	0	0.071	0.024	0.190	0.167
36. <i>Allomyces</i>	18	17	15	15	14	6	4	3	0	0.071	0.190	0.214
37. <i>Physoderma</i>	18	17	15	15	14	4	4	1	3	0	0.190	0.167
38. <i>Sorochytrium</i>	17	16	15	17	17	8	10	8	8	8	0	0.214
39. <i>Coelomomyces</i>	19	18	16	18	17	8	10	7	9	7	9	0

the species for which we have ultrastructural data.

At a lower level, Barr (1980, 1990) used thallus development to define families but pointed out that the families in the Chytridiomycota should also be defined on zoospore ultrastructure (Barr 1988). Only two of Barr's families are relatively well defined in the parsimony tree. One is the Cladochytriales, which consists of *Cladochytrium* and *Nowakowskiella*, and the other is the Spizellomycetaceae, which needs revisions. It should include the exogenous *Entophlyctis* and exclude the endogenous *Karlingia* complex. The remaining families do not form monophyletic groups.

Olpidium, *Rozella*, *Caulochytrium*, and *Karlingia* A form a monophyletic group in the distance tree but not in the parsimony tree. These species are related by a striated rhizoplast and dispersed ribosomes in the zoospores, although they belong to different families according to Barr (1980, 1990). However, the frequencies of the branch node of these species in the 50% majority-rule consensus tree are not very high (50–67%), which indicates that their relationships are ambiguous. Similarly, the Chytridiaceae form a monophyletic group in the distance tree, while the parsimony tree shows that they are paraphyletic. Clearly, more data are needed to clarify these problems.

Based on zoospore ultrastructure, the Harpochytriales were abandoned, *Oedogoniomyces* was transferred to the Monoblepharidales, and *Harpochytrium* was transferred to the Chytridiales (Barr 1990; Gauriloff et al. 1980a, 1980b). Our analysis supports these moves. It shows that *Oedogoniomyces* and *Monoblepharella* are closely related to the two polycentric genera of Chytridiales (i.e., *Cladochytrium* and *Nowakowskiella*), which supports the Chytridiales–Monoblepharidales line proposed by Barr (1981a). The ordinal status of the Monoblepharidales seems unwarranted, and they should perhaps be assigned as a family of the Chytridiales. However, it may be justifiable to retain them as an order since the sexual life cycle of at least *Monoblepharella*, which is completed by fertilization of a non-motile female gamete by a motile male gamete, is very distinct from the Chytridiales (cf. Alexopoulos and Mims 1979).

The Blastocladiales is a well-defined group except for *Sorochytrium*. Although it was assigned to the Blastocladiales by Dewell and Dewell (1990), it does not consistently cluster with the rest of the order. Both distance tree analysis and rRNA sequence analysis (Li and Heath 1992; Bowman et al. 1992) show that the Blastocladiales have similar relationships with the Spizellomycetales and Chytridiales. In the parsimony tree, the Blastocladiales are closer to members of the Spizellomyce-

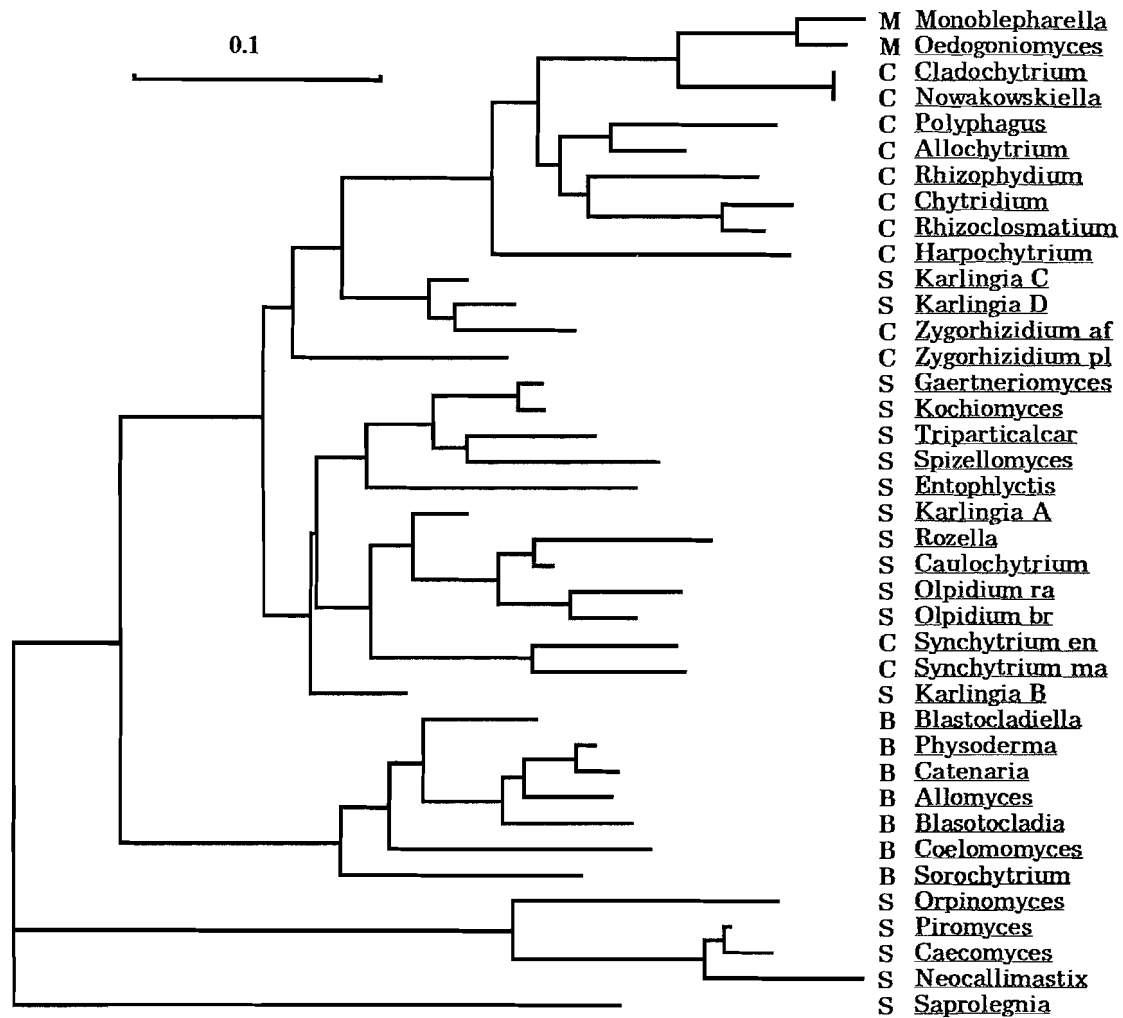


FIG. 2. An unrooted distance tree generated by the FITCH-MARGOLIASH algorithm from the PHYLIP package, showing the phylogenetic relationships of the genera in the Chytridiomycota. The letters indicate orders from Table 1. The branch lengths represent the evolutionary distance between genera.

tales than to those of the Chytridiales. In either case, the cladistic analysis does not support the idea that the Blastocladales were derived from the Spizellomycetales. In the Blastocladales, the relationships between the genera are unclear, with little support for existing families.

Originally, the gut fungi were assigned to the Spizellomycetales as the Neocallimasticaceae (Heath et al. 1983) based on zoospore ultrastructure. Later Heath and Bauchop (1985) found that the mitotic characters of *Neocallimastix*, the type genus, were different from those of other chytrids; consequently they concluded that the gut fungi had a distant relationship with the chytrids and noted that the taxonomic position of the gut fungi was problematic (Heath 1986). Munn et al. (1987) suggested that a new order with affinities to both the Chytridiales and Spizellomycetales might be justified for the gut fungi. The cladistic analysis of structural data, as well as rRNA data (Li and Heath 1992; Bowman et al. 1992; Doré and Stahl 1991), show that the gut fungi are monophyletic and clearly members of the Chytridiomycota (Li and Heath 1992), but they are not closely related to the existing orders. Consequently, a new order, the Neocallimasticales, is established for all the chytridiomycetous gut fungi.

Neocallimasticales ord. nov.

Thallus monocentricus vel polycentricus; zoosporae uniflagellatae vel polyflagellatae; ribosomata zoosporae aggregata et

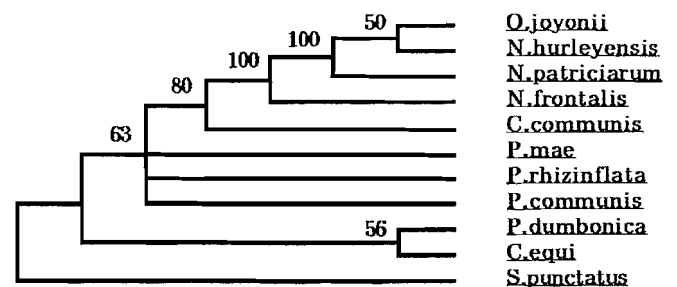


FIG. 3. A majority rule consensus tree from the 162 most parsimonious trees generated by PAUP using the BRANCH AND BOUND algorithm and showing the phylogenetic relationships of the gut fungi. The numbers above each node represent the percent occurrence of that particular node among the total number of trees. The branch lengths do not represent the evolutionary distance between genera.

helictica; kinetosomata circumvallata ab apparatu perkinetosomatico includenti proprium anulum circumflagellarem, limbum et calcar; mitochondria, microcorpus et guttulae consociata et rumposomata absunt; microcorpora (hydrogenosomata) et cupola posterior praesunt.

Polycentric or monocentric thallus, uniflagellate or polyflagellate zoospores; ribosomes in zoospores occur in aggregates and helices; kinetosomes surrounded by perikinetosomal appa-

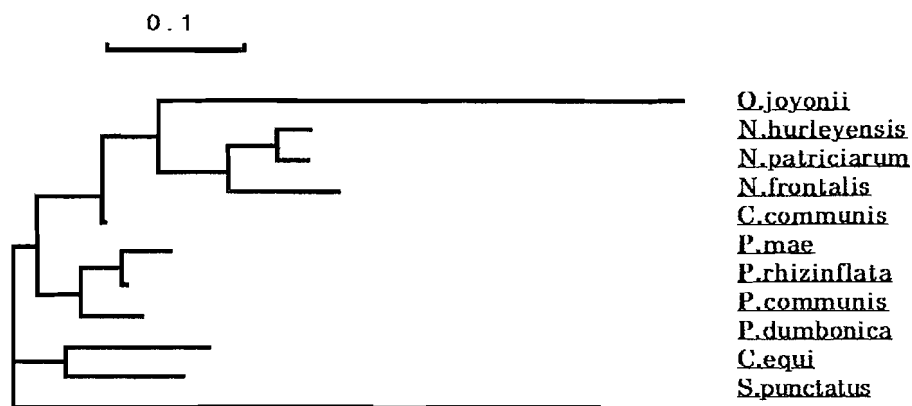


FIG. 4. An unrooted distance tree generated by the FITCH-MARGOLIASH algorithm from the PHYLIP package, showing the phylogenetic relationships of the gut fungi. The branch lengths represent the evolutionary distance between species.

TABLE 7. Pairwise distances generated by PAUP based on structural characters of the gut fungi

	1	2	3	4	5	6	7	8	9	10	11
1. <i>Neocallimastix patriciarum</i>	0	0.143	0.048	0.333	0.429	0.238	0.238	0.381	0.143	0.476	0.714
2. <i>Neocallimastix frontalis</i>	3	0	0.190	0.381	0.381	0.286	0.286	0.286	0.190	0.619	0.667
3. <i>Neocallimastix hurleyensis</i>	1	4	0	0.238	0.429	0.238	0.238	0.381	0.143	0.476	0.619
4. <i>Piromyces mae</i>	7	8	5	0	0.238	0.048	0.095	0.286	0.143	0.667	0.619
5. <i>Piromyces dumbonica</i>	9	8	9	5	0	0.190	0.190	0.190	0.238	0.667	0.524
6. <i>Piromyces rhizinflata</i>	5	6	5	1	4	0	0.095	0.238	0.095	0.524	0.476
7. <i>Piromyces communis</i>	5	6	5	2	4	2	0	0.238	0.143	0.429	0.429
8. <i>Caecomyces equi</i>	8	6	8	6	4	5	5	0	0.143	0.667	0.571
9. <i>Caecomyces communis</i>	3	4	3	3	5	2	3	3	0	0.476	0.476
10. <i>Orpinomyces joyonii</i>	10	13	10	14	14	11	9	14	10	0	0.810
11. <i>Spizellomyces punctatus</i>	15	14	13	13	11	10	9	12	10	17	0

NOTE: The lower part shows absolute distances, and the upper part shows mean distances.

tus including characteristic circumflagellar ring, skirt, and spur; mitochondria, microbody-lipid complex, and rumposomes absent; microbodies (hydrogenosomes) and posterior dome present.

HABITAT: Anaerobically inhabit the digestive system of herbivorous animals.

NOTE: The order includes a single family, the Neocallimasticeae and five or six genera.

We note that all of the above characters, excluding thallus type and number of flagella are apomorphic.

The interrelationships between the orders of the Chytridiomycota requires further clarification because in addition to the questions about the Spizellomycetales-Chytridiales-Monoblepharidales group, the present morphological data suggest that the Blastocladales are more closely related to this assemblage than are the Neocallimasticeales, whereas the limited molecular data suggest the opposite (Li and Heath 1992).

Taxonomy and phylogeny of gut fungi

At present, the taxonomy of the chytridiomycetous gut fungi is based on simple but undoubtedly artificial characters such that genera are defined on the basis of thallus morphology, rhizoid type, and number of flagella alone, and species are mostly based on details of zoospore ultrastructure. In principle the present analysis of all available morphological and ultrastructural characters should provide a more natural and consistent grouping of the isolates, which should be comparable with the limited rRNA sequence data previously published; however,

such is not the case. For example, on one set of characters *Orpinomyces* consistently is most distant from all other genera (Figs. 1 and 2), but with the narrower set of characters its position varies substantially depending on the outgroup chosen. Each of the tested outgroup species is a valid choice, and consequently we are unable to determine the most valid tree. The rRNA sequences are similarly confusing, placing *Orpinomyces* either with *Piromyces*, remote from *Neocallimastix* (Doré and Stahl 1991; Li and Heath 1992) or with *Neocallimastix*, more remote from *Piromyces* (Li and Heath 1992). The three species of *Neocallimastix* frequently form a single cluster (with or more distant from *Orpinomyces*) on morphological characters (Figs. 3 and 4), suggesting that it may be a good genus, but *Piromyces* and *Caecomyces*, while consistently mostly separate from *Neocallimastix* and *Orpinomyces*, do not form two separate clusters along current generic lines. As noted previously, physiological characters seem unlikely to help resolve these questions (Philips and Gordon 1988), and based on the *Orpinomyces*-*Neocallimastix*-*Piromyces* examples above, it is doubtful that rRNA analyses will be unequivocal. Indeed, we have analyzed data from Doré and Stahl (1991, Table 2) with an artificial outgroup by using FITCH and NEIGHBOR algorithms from the PHYLIP package. The results show that *Caecomyces* is the sister group to the remaining three genera, and the phylogenetic relationships among *Neocallimastix*, *Piromyces*, and *Orpinomyces* are controversial.

Although the current taxonomic scheme may not be ideal, for example, rhizoid morphology is variable within a single

isolate (Gold et al. 1988; Wubah et al. 1991b), it is easy to use. For example, no isolates producing predominantly myceloid rhizoids have ever been shown to produce bulbous rhizoids, there does seem to be a clear break between a minimum of one

and a maximum of four versus many (> 10) flagella per zoospore, and monocentric thalli are clearly distinct from polycentric ones. Consequently at present, we prefer to retain these existing genera and present a key to aid in their identification.

Key to current genera of the chytridiomycetous gut fungi

- | | |
|---|-----------------------|
| 1. Polycentric thalli | 2 |
| 1. Monocentric thalli | 3 |
| 2. Predominantly polyflagellate | <i>Orpinomyces</i> |
| 2. Predominantly uniflagellate | <i>Anaeromyces*</i> |
| 3. Predominantly polyflagellate | <i>Neocallimastix</i> |
| 3. Predominantly uniflagellate | 4 |
| 4. Mostly with bulbous rhizoids, occasionally with myceloid rhizoids | <i>Caecomyces</i> |
| 4. Mostly with extensively branched rhizoids, never with bulbous rhizoids | <i>Piromyces</i> |

*Both *Anaeromyces* and *Ruminomyces* have polycentric thalli and uniflagellate zoospores. They were described independently by Breton et al. (1990) and Ho and Bauchop (1990) and appear to be synonymous.

In conclusion, cladistic analysis of structural data shows that (i) the Chytridiales, Spizellomycetales, and Monoblepharidales are closely related, and their separation into individual orders is questionable; (ii) the Blastocladiales are a monophyletic group separate from both the Spizellomycetales and Chytridiales, with no special relationship with the Spizellomycetales; and (iii) gut fungi are distinct from both the Spizellomycetales and Chytridiales on morphological, ultrastructural, mitotic, and molecular characters. Consequently a new order, the Neocallimasticales, is established for them.

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