

The Phylogenetic Relationships between Hyphochytridiomycota, specifically *Hyphochytrium catenoides* and *Rhizidiomyces apophysatus*, and other members of Heterokontae through 18S Ribosomal RNA Sequence Analysis

Kelsey Hermick, Gabrielle Van Nest, Michael Terwilliger, Shannon Wood, and Victoria Legere

Department of Biology, Susquehanna University, Selinsgrove, PA 17870.

Abstract

The class Hyphochytriomycetes is problematic and has been classified as Oomycota, as well as a phylum of Heterokontae. Hyphochytriomycetes has common structural characters with Oomycota and Chytridiomycota, leading to contradictory classifications of the problematic taxa. Through 18S ribosomal RNA sequence analysis, 28 taxa from Kingdom Heterokontae and 3 taxa from Kingdom Fungi were chosen to be analyzed. MEGA 5 was used to align these sequences and to generate Maximum Likelihood trees. Tamura-Nei and Kimura 2-parameter models were utilized to compare nucleotides within the 31 sequences obtained from selected taxa. This analysis established that Hyphochytriomycetes do not have a strongly supported phylogenetic relationship with Oomycota. Hyphochytriomycetes should not be considered a part of Oomycota, but as a separate phylum within the heterokonts because of contradicting classification and the molecular analysis that was run.

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Introduction

The purpose of this research is to examine the molecular relationship of Hyphochytridiomycetes and Oomycota through inferred phylogeny and reasoning as to why Hyphochytridiomycetes should be a separate phylum from Oomycota. Van de Auwera (1995) states that there is a monophyletic relationship between Hyphochytridiomycetes, Oomycota and Heterokont algae. However, there is a lack of molecular research of Hyphochytridiomycetes. We analyzed the molecular phylogeny of Hyphochytridiomycetes to see if it agrees with biochemical and structural analysis that state Hyphochytridiomycetes should not be classified as Oomycota. Hyphochytridiomycetes have conflicting morphological and molecular characters from the Oomycota that would support Hyphochytridiomycetes to be a separate phylum (Fuller 1990).

Hyphochytridiomycetes are considered a problematic taxon and the classification is still questioned (Holt and Iudica 2013). Hyphochytridiomycetes are identified as water molds that resemble a reduced form of the Chytrid fungi because of their similar lifestyle and mode of consumption (Holt and Iudica 2013; and Margulis and Schwartz, 1982). The phylum Oomycota has also previously been classified under the Kingdom

Fungi, due to its similarities to fungi in their intake of nutrition and mycelial growth (Rossman and Palm 2006). A recent discovery of a cyanobacterial gene found in the Oomycete *Phytophthora infestans* suggests that Oomycetes may have lost chloroplasts and evolved from photosynthetic Stramenopile algae (Money et al., 2004). This discovery supports that Oomycetes are classified under the Kingdom Heterokontae (Money et al., 2004). Oomycota are classified within the group of Stramenopiles (Heterokontae), together with diatoms, golden-brown, and brown algae.

The similarities are not enough to categorize Hyphochytridiomycetes under the Oomycota phylum because differences in mitosis, centrioles, and the composition of the cell wall all support a separation of class between Oomycetes and Hyphochytridiomycetes (Fuller, 1990). Many studies include Oomycota, but there are relatively few studies that also include Hyphochytridiomycetes. According to Margulis and Schwartz (1988), Dick (1990), and Fuller (1990) Hyphochytridiomycetes should be classified as a separate phylum from Oomycota because of ultrastructure difference and biochemical analyses (Margulis and Schwartz 1988; Dick 1990; and Fuller 1990).

In some recent morphological studies, Oomycetes and Hyphochytridiomycetes are classified

together (Barr and Desaulniers 1989). According to Barr and Desaulniers (1989), Oomycetes and Hyphochytridiomycetes should be grouped together because of the similarity in flagellar structure, even though Oomycetes have two flagella and Hyphochytridiomycetes have one flagellum, which is a derived character. Fuller (1990) also describes the similarities between the mitochondria and lysine synthase of Oomycetes and Hyphochytridiomycetes.

Material and Methods

There were 31 taxa selected from Oomycetes, Bicosoecida, Hyphochytridiomycetes, Labyrinthulomycetes, and Xanthophyceae of the heterokonts and Chytridiomycota of the fungi, which were selected through NCBI (2013). The sequences collected from each taxon were a variation of 18S ribosomal RNA (Table 1). The sequences were acquired from the National Center for Biotechnology Information (NCBI) website. Through the use of 18S

ribosomal RNA sequences, we were able to align and compare sequences from Oomycetes, Bicosoecida, Hyphochytridiomycetes, Labyrinthulomycetes, Xanthophyceae, and the out group Chytridiomycota. The Maximum Likelihood (ML) trees, Figures 1-2, were developed from these sequences.

The taxa's RNA sequences were aligned by Muscle, using the codon alignment feature. The elongated branches of nucleotides were trimmed from the aligned sequences. The final alignment, after cutting, consisted of 1,837 base pairs. Then ML trees, Figures 1-2, were assembled by nucleotide analysis of the 31 taxa. The Kimura 2-parameter model and Tamura-Nei model were used to analyze taxa relationships by nucleotide analysis.

To construct Figures 1 and 2, MEGA 5 (Tamura et al. 2011) was used. There was a bootstrap of 1,000 replications. The figures were constructed and the out group was rooted on both trees.

Table 1: Taxa selected were identified by class, scientific name, accession number, and authority. Taxa were used for phylogenetic analysis. There are five extant classes being investigated from the Kingdom Heterokontae and two extant classes from the Kingdom Fungi. Ribosomal RNA, 18S, sequences were collected from accession numbers through NCBI (2013). All authorities were found in Taxonomicon except for those marked (+), which were found on EOL, and those marked (-) which were found on World Register of Marine Species.

Class	Scientific Name	Accession Number	Authority
Bicosoecida	<i>Pseudobodo tremulans</i>	AF315604	Griessmann, 1913
Bicosoecida	<i>Siluania monomastiga</i>	AF072883	S.A. Karpov, 1998
Bicosoecida	<i>Cafeteria roenbergensis</i>	FJ032655	Fenchel & D.J. Patterson, 1988
Hyphochytridiomycetes	<i>Hyphochytrium catenoides</i>	X80344	Karling, 1939
Hyphochytridiomycetes	<i>Rhizidiomyces apophysatus</i>	JF791062	Kopf, 1884
Labyrinthulomycetes	<i>Thraustochytrium multirudimentale</i>	AB022111	Goldstein, 1963
Labyrinthulomycetes	<i>Aplanochytrium kerquelenense</i>	AB022103	G. Bahnweg and F. K. Sparrow, 1972
Labyrinthulomycetes	<i>Schizochytrium minutum</i>	AB022108	A. Gaertner, 1972 (+)
Labyrinthulomycetes	<i>Aplanochytrium stocchinoi</i>	AJ519935	Bahnweg & Sparrow, 1972 (-)
Labyrinthulomycetes	<i>Botryochytrium radiatum</i>	AB355410	Longcore, Pessier, & D. K. Nichols, 1999 (-)
Labyrinthulomycetes	<i>Thraustochytrium aureum</i>	GU933120	Goldstein, 1963
Labyrinthulomycetes	<i>Schizochytrium limacinum</i>	HM042905	Dick, 2001
Labyrinthulomycetes	<i>Aurantiochytrium limacinum</i>	JN986842	Dick, 2001
Oomycetes	<i>Pythium insidiosum</i>	AF442497	Pringsheim, 1858 (-)
Oomycetes	<i>Phytophthora nicotianae</i>	AY744947	Breda de Haan, 1896 (-)
Oomycetes	<i>Haliphthoros milfordensis</i>	AB178868	Vishniac, 1958 (-)
Oomycetes	<i>Eurychasma dicksonii</i>	AY032607	Wright, 1879 (-)
Oomycetes	<i>Pythium monospermum</i>	AJ238653	Pringsheim, 1858 (-)
Oomycetes	<i>Apodachlya brachynema</i>	AJ238663	Boulenger, 1900 (-)
Oomycetes	<i>Phytophthora capsici</i>	AY742748	Leonian, 1922
Oomycetes	<i>Pythium vexans</i>	AY742759	De Bary, 1876 (+)
Oomycetes	<i>Plasmopara viticola</i>	AY742754	Berl. & De Toni, 1888
Oomycetes	<i>Pseudoperonospora cubensis</i>	AY742760	Berk. & M.A. Curtis, 1903 (+)
Oomycetes	<i>Pythium cylindrosporium</i>	EU199112	B. Paul 1992 (+)
Oomycetes	<i>Lagenidium callinectes</i>	AB284571	Couch, 1857
Oomycetes	<i>Lagenidium thermophilum</i>	AB284572	Schenk, 1857
Xanthophyceae	<i>Tribonema intermixtum</i>	AF083397	Pascher, 1923
Xanthophyceae	<i>Goniochloris sculpta</i>	FJ858970	Geitler, 1928
Chytridiomycetes	<i>Spizellomyces acuminatus</i>	M59759	D. J. S. Barr, 1984
Monoblepharidomycetes	<i>Monoblepharis macrandra</i>	EF014369	Schaffn, 1909
Monoblepharidomycetes	<i>Monoblepharella Mexicana</i>	AF164337	Cornu, 1871

Results

The topology of Figures 1 and 2 is very similar. The Tamura-Nei model, Figure 1, strongly supports the monophyletic clade A. In addition, the monophyletic clade B is strongly supported, with a bootstrap value of 99. Labyrinthulomycetes emerge as a strongly supported paraphyletic clade, C. Clade D is a strongly supported monophyletic group, with a bootstrap value of 95. *Hyphochytrium catenoides* is no more closely related to clade A than to clade B. *Rhizidiomyces apophysatus* is a sister group to clades A, B, C, and D, showing no significant relationship to any particular class. The Kimura 2-parameter model, Figure 2, has the same branching with slightly varying bootstrap values.

Discussion

In Figures 1 and 2, the relationship between *Rhizidiomyces apophysatus* and *Hyphochytrium catenoides* shows Hyphochytridiomycete as being polyphyletic. *Hyphochytrium catenoides* have a monophyletic relationship to Oomycetes and Xanthophyceae. However, *Hyphochytrium catenoides* is not more closely related to either taxon.

Rhizidiomyces apophysatus is a basal group; therefore, *Rhizidiomyces apophysatus* lack phylogenetic relationship to clade A, B, C, and D. Oomycetes appear as a monophyletic clade in Figures 1 and 2, which do not show a strong relationship to either genus of Hyphochytridiomycetes.

According to Richards et al. (2011), molecular phylogenies have also shown Oomycetes and Hyphochytridiomycetes on separate branches. Richards et al. (2011) also describe the relation between Oomycetes, Hyphochytridiomycetes, and Fungi, saying that recent phylogenetic studies show Oomycetes and Hyphochytridiomycetes branch separately from fungi and into their current description as water molds.

According to Van der Auwera (1995) Hyphochytridiomycetes, Oomycetes and heterokont algae form a monophyletic group and share a common ancestor, which resides in the heterokont fungi. Figures 1 and 2 agree with the Van der Auwera (1995) phylogenetic analysis of Hyphochytridiomycetes, Oomycetes and heterokont algae.

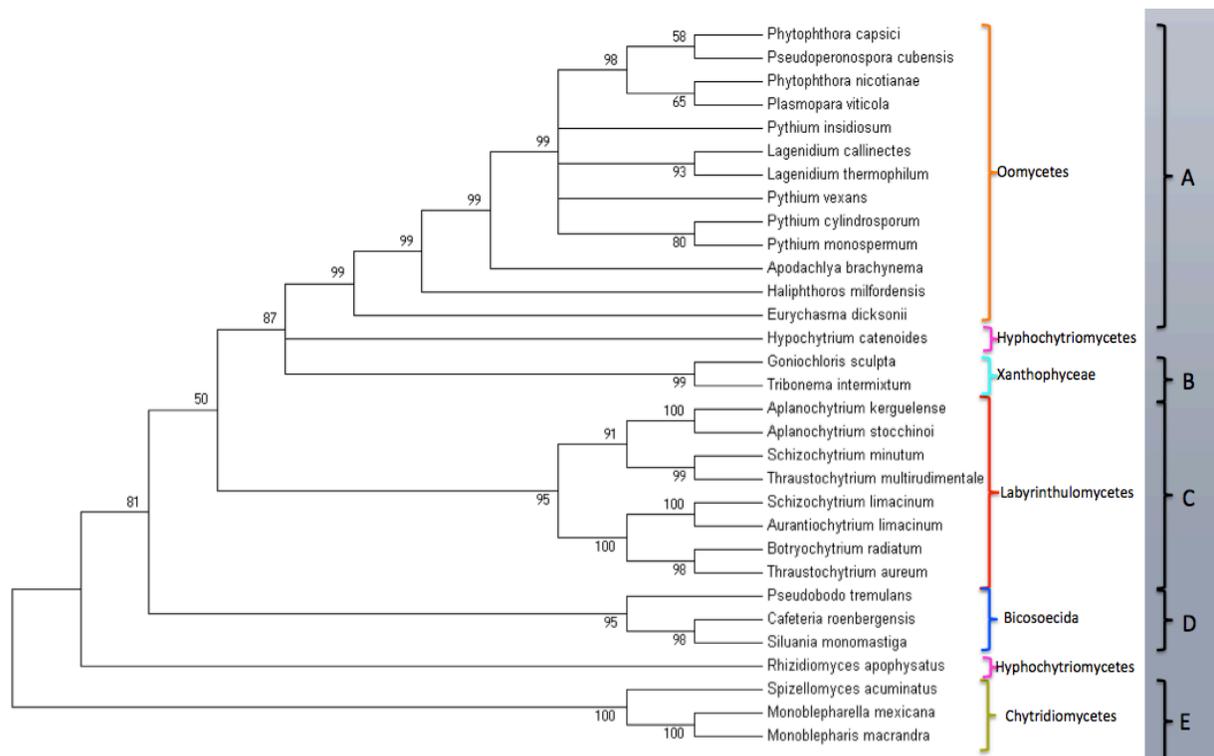


Figure 1: A cladogram generated from the Maximum Likelihood method based on the Tamura-Nei model. The bootstrap consensus tree, inferred from 1,000 replicates, is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). Branches with less than 50 bootstrap replicates represent poorly supported branching and were condensed. The bootstrap values of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches (Hall 2011). Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011). The taxa are grouped by five class classification and one phylum, Chytridiomycetes. Major clades are labeled with letters A-E.

Barr and Desaulniers (1989) stated that Hyphochytridiomycetes should be classified as Oomycota through morphology and biochemistry. Because there is not strong enough information to support that either Hyphochytridiomycetes that was analyzed is monophyletic with Oomycota, we disagree with this statement.

By further investigation though NCBI (2013), Rocke et al. (2012) entered accession number JF791062 as *Rhizidiomyces apophysatus*. However, JF791062 pertains to aquatic fungi such as *Rhizidiomyces apophysitis*. This indicates a misidentification and submission of *Rhizidiomyces apophysatus*, where as *Rhizidiomyces apophysitis* is the correct species (Rocke et al. 2012).

After additional analysis of the species *Hyphochytrium catenoides* and *Rhizidiomyces apophysatus*, we were able to identify that Hyphochytridiomycetes are polyphyletic in relation to each other and are no more strongly related to Oomycota than to any other heterokont phylum that was analyzed. Hyphochytridiomycetes should not be classified under the phylum Oomycota because they lack major defining characters of the Oomycota (Fuller 1990). Due to this significant difference between the two groups, the class Hyphochytridiomycetes should be considered an independent phylum.

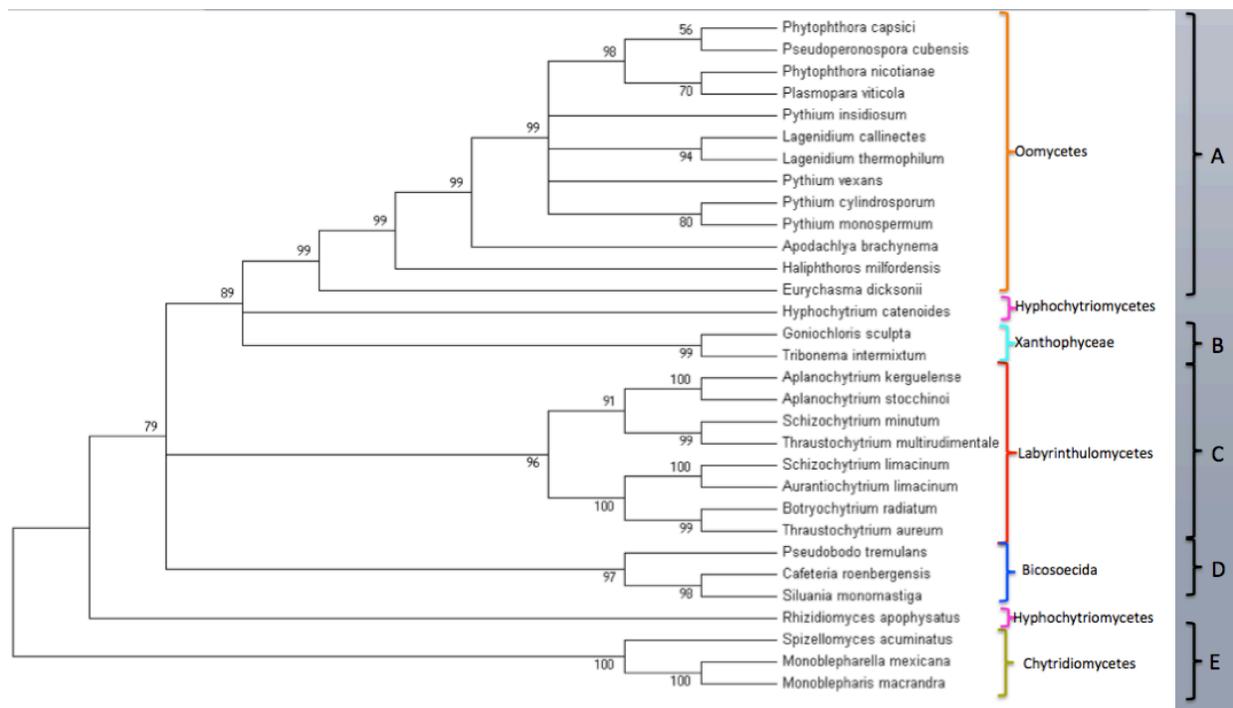


Figure 2: A cladogram generated from the Maximum Likelihood method based on the Kimura 2-paramter model. The bootstrap consensus tree, inferred from 1,000 replicates, is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). Branches with less than 50 bootstrap replicates represent poorly supported branching and were condensed. The bootstrap values of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches (Hall 2011). Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011). The taxa are grouped by five class classification and one phylum, Chytridiomycetes. Major clades are labeled with letters A-E.

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