
Phylogeny of the *Peronosporomycetes* (*Oomycota*) based on partial sequences of the large ribosomal subunit (LSU rDNA)

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The phylogeny of the nuclear large ribosomal subunit (LSU) rDNA from *Peronosporomycetes* (*Oomycota*) was studied. Five orders *Rhipidiales*, *Leptomitales*, *Saprolegniomycetidae*, *Pythiales* and *Peronosporales* were included in the study in order to reveal phylogenetic relationships within the group. Based on maximum parsimony, neighbour-joining and maximum-likelihood the *Peronosporomycetes* seem to have evolved as two major lineages. One lineage includes members of the *Rhipidiales*, *Leptomitales* and *Saprolegniomycetidae* and the other includes the members of *Pythiales* and *Peronosporales*. However, the inclusion of *Rhipidiales* in *Saprolegniomycetidae* was not well supported. *Leptomitales* were placed basal to the *Saprolegniomycetidae* in the LSU rDNA based tree. In the *Saprolegniaceae sensu lato* the primary zoospore seems to have been lost several times in the evolution of this family. Species of *Aphanomyces* were placed on the most basal branch in this family. The obligate parasite, *Albugo*, was placed on the most basal branch within the *Peronosporomycetes*. *Phytophthora* showed a closer relationship to *Peronospora* and *Peronophythora* than to *Pythium* suggesting that this genus should be removed from *Pythiales* to *Peronosporales*.

INTRODUCTION

The *Peronosporomycetes* (*Oomycota*) are known to be related to the chromophyte algae and other heterokont protists. This relationship between *Peronosporomycetes* and heterokont protists is based on the ultrastructure of the zoospore, e.g. a straminipilous ornamentation of the anterior flagella and similarities in the flagellar rootlet structure (Barr 1981, 1992, Beakes 1987). DNA studies support this relationship and suggest that *Peronosporomycetes* is monophyletic (Gunderson et al. 1987, Förster et al. 1990).

The traditional taxonomy of *Peronosporomycetes* distinguishes four orders: *Lagenidiales*, *Leptomitales*, *Peronosporales*, and *Saprolegniomycetidae* (Sparrow 1960, Dick 1973). The taxonomy of the *Peronosporomycetes* has been reorganized several times (Dick, Wong & Clark 1984, Dick et al. 1989, Dick 1990, 1995). Dick et al. (1984) recognised three additional orders: *Rhipidiales*, previously *Rhipidiaceae*, placed in *Leptomitales*, *Pythiales* including organisms formerly placed in *Lagenidiales* and finally *Sclerosporales* including species of *Sclerosporaceae* formerly placed in *Peronosporales*. In this revision *Peronosporomycetes* was subdivided in two subclasses: *Saprolegniomycetidae* with *Saprolegniales* as a single order, and *Peronosporomycetes* with the orders *Leptomitales*, *Rhipidiales*, *Sclerosporales*, *Pythiales* and *Peronosporales*. *Leptomitales* and *Sclerosporales* were later transferred to *Saprolegniomycetidae* based on DNA studies (Dick et al. 1989, Klassen et al. 1988). Dick et al. (1989) and Dick (1995) proposed a third subclass, the *Rhipidiomycetidae* with members of the *Rhipidiales*, and reorganized *Saprolegniomycetidae* and *Peronosporomycetes*.

The phylogenetic relationships between the groups in *Peronosporomycetes* have long been a matter of controversy (Bessey 1942, Barr 1983, Beakes 1987, Dick et al. 1984, Dick 1988, 1990). Bessey (1942), Barr (1983) and Beakes (1987) proposed a monophyletic origin of the *Peronosporomycetes*. Barr (1983) suggested that the obligate parasites on terrestrial plants evolved from *Saprolegniales* through primitive *Pythium* and *Phytophthora*. According to this theory *Peronosporomycetes* developed from saprobes in aquatic habitats. Terrestrial facultative soil-borne parasites on plant roots developed from these and later obligate parasites of the aerial parts of the plants evolved. The zoospore stages and the dependency on water were gradually reduced during this evolution. Barr (1983) also suggested that *Leptomitales* evolved as a separate lineage from *Saprolegniales*. This idea was based on the presence of chitin in some of the species in *Leptomitales*.

Beakes (1987) supported the theory of a monophyletic origin of *Peronosporomycetes*, and proposed two different evolutionary models for the class. In one of these *Saprolegniales* was considered the main ancestral group due to the similarity between *Saprolegnia* and the alga *Vaucheria*. *Leptomitales*, *Peronosporales* and *Lagenidiales* evolved sequentially or independently from *Saprolegniales*. He further suggested that characteristics of wall synthesis during zoosporogenesis should be regarded as a key character.

A polyphyletic origin of the *Peronosporomycetes* has also
been proposed. Sparrow (1976) suggested two independent evolutionary lineages, a saprolegnian and a peronosporaceous lineage. Dick et al. (1984) and Dick (1988, 1990) adopted this idea, and suggested different evolutionary hypotheses of Peronosporomycetes. Later, however, Dick (1995) stated that it is not yet possible to designate primitive or advanced phylogenetic criteria for Peronosporomycetes.

In order to propose new phylogenetic theories, it is necessary to include additional characters. Additional characters for phylogenetic studies can be obtained from nucleic acid sequences. Such characters have been used in taxonomy and phylogeny of the economically important plant pathogens Pythium and Phytophthora (Lee & Taylor 1992, Briard et al. 1995, Crawford et al. 1996), and for species of the Saprolegniaceae (Daugherty et al. 1998). Ribosomal DNA (rDNA) sequences have been used extensively to reconstruct phylogenetic relationships among fungi and algae, where the evolutionary interpretation of morphological features is ambiguous (Taylor 1993, Bhattacharya & Medlin 1995, Hibbit et al. 1997), and lately Dick et al. (1999) used 18S rDNA to suggest a phylogeny for the Peronosporomycetes.

So far the LSU (28S) rDNA has not been used to develop phylogenetic hypotheses for the major lineages of the Peronosporomycetes. The objective of the present study was to construct a phylogeny of members of the Peronosporomycetes based on partial sequences of LSU rDNA. The molecular phylogeny was compared with morphological and physiological characters in this group. We sequenced 1200 base pairs of LSU rDNA. The objective of the present study was to construct a phylogeny of members of the Peronosporomycetes based on partial sequences of LSU rDNA. The molecular phylogeny was compared with morphological and physiological characters in this group. We sequenced 1200 base pairs of LSU rDNA to suggest a phylogeny for the Peronosporomycetes.

MATERIALS AND METHODS

Fungal material

The taxa/isolates used in the study and the sources of these are given in Table 1. Peronospora farinosa and P. parasitica were obtained as sporangia from infested spinach (Spinacea oleracea var. botrytis) respectively obtained from Dæhnfeldt A/S (Denmark). Albugo candida was obtained as sporangia from infested Shepherds pursse (Capsella bursa-pastoris). Saprolegnia ferax (SIS6) and Achlya americana (am2) were isolated from a lake ( Bagsværd sø, Denmark). Saprolegnia litoralis (SI1) was isolated from forest soil (Grib skov, Denmark). Dictyuchus sp. no. 4.2 and Dictyuchus sp. no. 5.6 were isolated from twigs from fresh water (Smørmosen, Denmark) and wet soil (Hareskoven, Denmark) respectively.

DNA extraction and PCR conditions

The cultures were grown as 100 ml batches in liquid media (Fuller & Jaworski 1987). The batches were inoculated from agar cultures and incubated at 20 °C for 1 week. The resultant mycelia were washed in dilute salt solution (DS) (Dill & Fuller 1971), blotted on filter paper and freeze dried. Total genomic DNA was then extracted by the methods of Lee, Milgroom & Taylor (1988). The extracted DNA was checked on 1% agarose gels, and used for PCR-amplifications of LSU rDNA, except for P. farinosa, P. parasitica and A. candida where resting spores were crushed with a pestle and used directly for PCR-amplifications.

The primers LSU-0025-F and LSU-1170-R (Table 2) were used to amplify the first approximately 1200 bp of the LSU rDNA. PCR conditions were: Initial denaturation for 1 min at 96 °C, followed by 30 cycles of denaturation 1 min at 95 °C, annealing 1 min at 52 °C, extension 2 min at 72 °C and a final extension of 7 min at 72 °C.

DNA sequencing

Amplifications were checked on 2% Nusieve gels, and PCR-products were purified from the amplification mixture with milipore Ultrafree-MC filters (Millipore Corporation, Bedford, MA) and used in cycle-sequencing reactions (ABI Dye Terminator Cycle Sequencing Ready Reactions Kit) together with specific primers (Table 2). Both strands of the LSU rDNA were sequenced on an automatic sequencer (ABI Prism TM 377 DNA Sequencer), and chromatogramms of these sequences were checked using Sequencer 3.1 (Gene Codes Corporations Inc., Ann Arbor).

Sequence alignment and phylogenetic analysis

Twenty-four new sequences from this study were aligned with two published LSU rDNA sequences obtained from GenBank, using the computer program ESEE (vers. 3.1 1997, Cabot, E). The database on the structure of large ribosomal subunit RNA was used for the alignment (De Rijk, van der Peer & De Wachter 1997). The two published sequences were a sequence of Phytophthora megasperma accession no. X75631 and Hlyphochytrium catenoides accession no. X80345. For alignment of variable regions of the LSU rDNA the program Sequence Navigator (Applied Biosystems, version 1.0) was used. The sequences varied substantially and could not be unambiguously aligned. The alignment is available from the authors (soerenr@bot.ku.dk). Analyses were performed that included all organisms but with an exclusion of 180 characters (position 436–507, 580–625, 889–950) with an ambiguous alignment. Phylogenetic analyses were performed with the software PAUP* 4.0b2. All maximum parsimony searches were performed using Fitch parsimony (unordered, multistate characters) with gaps treated as missing data using the heuristic search option, TBR (tree bisection reconnection) branch swapping and random addition sequence with 300 replicates. To assess the robustness of the clades, bootstrap percentages were calculated. Bootstrap values (BV) were calculated for 200 replicates with simple entry of the data. Consistency index (CI) and retention index (RI) were calculated for all parsimony trees (Kluge & Farris, 1969; Farris, 1989). In addition to the parsimony analysis alternative topologies were searched by maximum-likelihood and neighbour-joining methods using PAUP* 4.0b2.

Maximum-likelihood trees were constructed using the heuristic search algorithm. The transition/transversion ratio

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Maximum-likelihood trees were constructed using the heuristic search algorithm. The transition/transversion ratio
Table 1. List of species of the *Peronosporomycetes* sequenced in this study. Taxonomic placement of the species included (Dick 1995) and their source.

<table>
<thead>
<tr>
<th>Major group</th>
<th>Species</th>
<th>Isolate and source</th>
<th>GenBank/EMBL accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Peronosporomycetes</em></td>
<td><em>Peronospora farinosa</em></td>
<td>Spinacea oleracea</td>
<td>AF235955</td>
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<tr>
<td><em>Peronosporomycetidae</em></td>
<td><em>P. parasitica</em></td>
<td>Brassica oleracea var. botrytis</td>
<td>AF235957</td>
</tr>
<tr>
<td><em>Allbuginaceae</em></td>
<td><em>Albugo candida</em></td>
<td>Capsella bursa-pastoris</td>
<td>AF235938</td>
</tr>
<tr>
<td><em>Pythiales</em></td>
<td><em>Pythium aphanidermatum</em></td>
<td>L. Hockenhull, J.</td>
<td>AF235956</td>
</tr>
<tr>
<td><em>Leguminosae</em></td>
<td><em>L. giganteum</em></td>
<td>Novo Nordisk A/S</td>
<td>AF235946</td>
</tr>
<tr>
<td><em>Phytophthoraceae</em></td>
<td><em>Phytophthora infestans</em></td>
<td>Pi-3, Novo Nordisk A/S</td>
<td>–</td>
</tr>
<tr>
<td><em>Peronophythora litchii</em></td>
<td>CBS 100.81</td>
<td>AF235949</td>
<td></td>
</tr>
<tr>
<td><em>Rhipidiomycetidae</em></td>
<td><em>Saprolegnia ferax</em></td>
<td>Sf5.6, Petersen, A. B</td>
<td>AF235953</td>
</tr>
<tr>
<td><em>Saprolegniomycetidae</em></td>
<td><em>S. litoralis</em></td>
<td>SI1, Petersen, A. B</td>
<td>AF235952</td>
</tr>
<tr>
<td><em>Saprolegnia borealis</em></td>
<td>am2, Petersen, A. B</td>
<td>AF235943</td>
<td></td>
</tr>
<tr>
<td><em>Aphanomyces euteiches</em></td>
<td>Tayseir, M. A</td>
<td>AF235940</td>
<td></td>
</tr>
<tr>
<td><em>A. astaci</em></td>
<td>ATCC 201684</td>
<td>AF235939</td>
<td></td>
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<tr>
<td><em>A. piscicida</em></td>
<td>no. 9211, Hatai, K.</td>
<td>AF235941</td>
<td></td>
</tr>
<tr>
<td><em>Dictyuchus sp.</em></td>
<td>no. 5.6, Petersen, A. B</td>
<td>AF235945</td>
<td></td>
</tr>
<tr>
<td><em>Dictyuchus sp.</em></td>
<td>no. 4.2, Petersen, A. B</td>
<td>AF235944</td>
<td></td>
</tr>
<tr>
<td><em>Leptolegnia sp.</em></td>
<td>no. 3.1, Sørensen, D.</td>
<td>AF235954</td>
<td></td>
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<tr>
<td><em>Leptolegnia sp.</em></td>
<td>no. 1.6, Sørensen, D.</td>
<td>AF235948</td>
<td></td>
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<tr>
<td><em>Thraustotheca clavata</em></td>
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<td>AF235951</td>
<td></td>
</tr>
<tr>
<td><em>Brevilegnia bispora</em></td>
<td>CBS 568.67</td>
<td>AF235942</td>
<td></td>
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<tr>
<td><em>Saprolegnia ferax</em></td>
<td>CR-55b</td>
<td>AF235937</td>
<td></td>
</tr>
<tr>
<td><em>S. litoralis</em></td>
<td>61-020b</td>
<td>AF235936</td>
<td></td>
</tr>
</tbody>
</table>

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\*Department of Comparative Physiology, University of Uppsala.\*
\*Division of Fish Diseases, Nippon Veterinary and Animal Science University, Tokyo.\*

Table 2. Primers used for PCR and sequencing of LSU rDNA from organisms of *Peronosporomycetes*.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSU-0025-F</td>
<td>ACCCGCTGAACCTAAGCATAT</td>
<td>van der Auwera et al. (1994)</td>
</tr>
<tr>
<td>LSU-0344-F</td>
<td>CGATAGGCAAACAGACGTGCAGTTAAGCATAT</td>
<td>This study</td>
</tr>
<tr>
<td>LSU-0670-F</td>
<td>GACCTGAGGTTGCTCACCAAC</td>
<td>This study</td>
</tr>
<tr>
<td>LSU-0926-F</td>
<td>CTTGAAACACGGAGCAAAAGGAG</td>
<td>This study</td>
</tr>
<tr>
<td>LSU-1170-R</td>
<td>GCTATCCTGAAGGAAATTGCAG</td>
<td>van der Auwera et al. (1994)</td>
</tr>
<tr>
<td>LSU-0826-R</td>
<td>CTTGAAACACGGAGCAAAAGGAG</td>
<td>This study</td>
</tr>
<tr>
<td>LSU-0670-R</td>
<td>GTCGTGAGCACCTCGTCTCGT</td>
<td>van der Auwera et al. (1994)</td>
</tr>
<tr>
<td>LSU-0344-R</td>
<td>CACGTTACTTGTGCTATCG</td>
<td>This study</td>
</tr>
</tbody>
</table>

was estimated by maximum-likelihood. Standard settings for maximum-likelihood were used, and the Hasegawa-Kishino-Yano (HKY) two parameter model for unequal base frequencies was chosen. Branch support was determined by bootstrap analysis (Felsenstein 1985) calculated using 100 replicates. The settings for the bootstrap analysis were as above, except that the transition/transversion ratio estimated above was used.

*Hyphochytrium catenoides* was chosen as outgroup to the *Peronosporomycetes* based on its sistergroup relationship to this group (van der Auwera et al. 1995).

RESULTS

The 24 sequences obtained were aligned with the published sequences of the large ribosomal subunit of *P. megasperma* and *H. catenoides*, and the length of the sequences obtained,
Fig. 1. Phylogenetic relationships within *Peronosporomycetes* (*Oomycota*) inferred from a maximum parsimony analysis of 981 nucleotides of nuclear LSU rDNA. A strict consensus tree of two equally most parsimonious trees requiring 984 steps with consistency index = 0.559 and retention index = 0.712 is shown. Bootstrap percentages higher than 50% from 200 replicates are shown above each branch. The differences in the two equally parsimonious trees obtained were whether *Peronophythora litchii* clustered with *Phytophthora infestans* or *P. megasperma*. Arrows (↑) indicate where the primary zoospore is suggested to be lost in *Saprolegniales*.

surrounded by the primers LSU-0025-F and LSU-1170-R, varied from 1030 to 1121 bp. The taxa belonging to the *Saprolegniales* had an approx. 60 bp large deletion in helix 2 corresponding to bp 432–492 in *P. megasperma* (van der Auwera, Chapelle & De Wachter 1994) or the excluded positions 436–507 in the alignment.

A maximum parsimony tree based on 981 bp of the partial sequences of the LSU rDNA of the *Peronosporomycetes* is shown in Fig. 1. Of the 981 included sites 361 were variable, and 245 sites of these were parsimony-informative. The tree was a strict consensus tree of two equally parsimonious trees requiring 984 steps. The CI and the RI for the two trees were 0.559 and 0.712 respectively. Bootstrap percentages were assigned above the branches. The tree divided the 25 taxa/isolates of *Peronosporomycetes* included in this study into two major lineages. One lineage consisted of species of *Leptomitales*, *Saprolegniales* and *Sapromyces*, and the other lineage contained the species of the *Pythiales* and *Peronosporales*. These lineages were supported by bootstrap values of 74% and 95% respectively (Fig. 1).

In the former lineage, *Sapromyces* representing *Rhizidiales* was the most basal organism. The two species of *Apodachlya* representing *Leptomitales* were on the next branch to diverge (100% BV). *Saprolegniales* seemed to be the most advanced group in this lineage with high bootstrap support (92%). Three major clusters were seen in *Saprolegniales*. Species of
Aphanomyces formed a cluster on the most basal branch with high bootstrap support (97%), resulting in a cluster containing the remaining species of Saprolegniales with very low bootstrap support (55%). This cluster consisted of two major clusters. One of these contained species of Saprolegnia and Leptolegnia (93% BV), and the other major cluster were composed of species of Achlya, Thraustotheca and Isoachlya in one internal cluster (95% BV) and Brevilegnia and Dictyuchus in another internal cluster (100% BV).

Albugo diverged on the most basal branch within the species representing the Peronosporomycetidae. The species of Peronospora, Phytophthora and Peronophythora formed a strongly supported clade (100% BV), with the species of Peronospora on the most basal line. The positions of Pythium and Lagenidium in the LSU rDNA based tree were less clear. Pythium was resolved as basal to Lagenidium (75% BV) while Lagenidium was resolved as a sister to the clade including Phytophthora but with bootstrap support lower than 50%. Minor differences were seen between the two most parsimonious trees. In the two trees Peronophythora litchii clustered with either Phytophthora infestans or P. megasperma.

The overall topology of the trees obtained by maximum likelihood (Fig. 2) was similar to the tree based on maximum parsimony seen in Fig. 1 except for the position of Lagenidium.
The optimum maximum likelihood tree had a -Ln likelihood of 6366.15977 and the estimated transition/transversion ratio of this tree was 1.651202 (kappa = 3.249579) (Fig. 2). The inclusion of *Sapromyces* as representing the Rhipidiales in the *Saprolegniomycetidae* was supported by only 67% bootstrap in the maximum likelihood tree. *Aphanomyces* was also suggested as basal in the Saprolegniomycetidae in the maximum likelihood tree. The resulting clade including the remaining species of *Saprolegniales* obtained a bootstrap support of 79%. *Isosachlya* clustered with *Achylya* and *Thraustotheca*, but this position was not well-supported (56%). In *Peronosporomycetidae*, *Albugo* was placed on the most basal branch with high bootstrap support (96%) as in the maximum parsimony based trees. The species of *Lagenidium* were placed as basal to a clade including *Pythium*, *Phytophthora* and *Peronospora* but supported by low bootstrap values (57%). In this clade *Pythium* was evolving on a separate line with low bootstrap supports (53%), showing sistergroup relationships to a clade including *Peronospora*, *Phytophthora* and *Peronophythora*. This clade was well-supported (100%).

The topology of the neighbour-joining tree was identical with the trees inferred from maximum likelihood method (data not shown).

**DISCUSSION**

The phylogenetic trees based on partial sequences of the LSU rDNA presented here confirm the two major lineages in the *Peronosporomycetes* representing the *Saprolegniomycetidae* and the *Peronosporomycetidae* as suggested by Dick et al. (1984). One lineage includes *Sapromyces* representing Rhipidiales, Apodachlyla representing Leptomitales, and the species representing *Saprolegniales* (*Saprolegniomycetidae*). The other lineage includes species representing Pythiales and Peronosporales (*Peronosporomycetidae*). In a restriction analysis of rDNA (Klassen et al. 1988), and in the taxonomic revisions by Dick et al. (1989) and Dick (1995), *Peronosporomycetes* consisted of three subclasses: *Saprolegniomycetidae*, *Peronosporomycetidae* and *Rhipidiales*. However, the inclusion of *Sapromyces*, the single representative for the Rhipidiales in this study, in *Saprolegniomycetidae* is only moderately supported in the maximum parsimony analysis (74% BV) and in the maximum-likelihood analysis (67% BV).

Several characters support the existence of two major lineages in the LSU rDNA based phylogenetic trees. The members of the Leptomitales and the Saprolegniomycetidae are able to synthesise sterols de novo while the members of the Pythiales are not (Domnas, Srebo & Hicks 1977, Nes 1987, Weete 1989). K₅-bodies (kinetosome-associated vesicles) present in the zoospore of some members of the *Peronosporomycetes* might also be a key character. K₅-bodies have so far been found in *Sapromyces*, *Apoachlyla* and members of the *Saprolegniomycetidae*, but not in the *Peronosporomycetidae* (Holloway & Heath 1977, Hoch & Mitchell 1972, Olson et al. 1984, Powell, Lehnen & Bortnick 1985, Beakes 1987, Gotelli & Hanson 1987, Randolph & Powell 1992, Powell & Blackwell 1995). The oosporegenesis and oospore organisation is also an important key character. According to Dick (1969, 1995) *Saprolegniomycetidae* and probably *Leptomitales* have a centrifugal oosporogenesis resulting in non-periplasmic oospores, while the members of the *Peronosporomycetidae* have a centripetal oosporogenesis resulting in oospores with a persistent periplasm.

*Sapromyces* has morphological features that place it between the *Saprolegniomycetidae* and the *Peronosporomycetidae*. Gotelli & Hanson (1987) noted that the ultrastructure of *Sapromyces* zoospores suggests that Rhipidiales are more closely related to *Saprolegniales* than to *Pythiales*. Beakes (1987) noted that the secondary cysts of *Sapromyces* are coated with an outer layer of fibrillar material and lack the electron-dense coat similar to the secondary cysts of the *Pythiales*. The oospore morphology is similar to that found in *Peronosporomycetidae*, e.g. the presence of periplasm (Dick 1969, 1996). On the basis of the present study a placement for members of Rhipidiales can not be suggested. Additional and independent molecular markers will be needed to place this group of organisms with certainty.

The phylogenetic trees resulting from sequences of the LSU rDNA strongly suggest that Leptomitales should be included in the *Saprolegniomycetidae* as suggested by Dick et al. (1989). *Leptomitales* and *Saprolegniomycetidae* are placed as sistergroups in all LSU rDNA based trees with very high bootstrap support (100%). Morphological characters also support this placement. In both orders the presence of a pyriform zoospore with a pair of flagella anchored near the spore apex (the primary zoospore) has been described (Sparrow 1960, Dick 1973, Jacobs 1982). *Saprolegniomycetidae* and *Leptomitales* share traits in the oospore morphology as both produce oospores without a periplasm (Dick 1969, 1995), and similarities are seen in the zoospore morphology (Randolph & Powell 1992). A proximal relationship between the Leptomitales and the *Saprolegniomycetidae* was also suggested by Klassen et al. (1988) based on a restriction analysis of ribosomal DNA, and lately by a maximum parsimony analysis based on sequences of the SSU rDNA (Dick et al. 1999).

The species of *Saprolegniales* included in the present study form a monophyletic clade. The genus *Aphanomyces* is suggested to be the first lineage to diverge in the maximum parsimony trees, the neighbour-joining tree and in the maximum likelihood trees. In many ways *Aphanomyces* is different from other genera of *Saprolegniales*. The K₅-bodies examined in the genus *Aphanomyces* are of different morphotypes than the K₅-bodies seen in other species of the *Saprolegniales* (Powell & Blackwell 1995). The genus *Aphanomyces* also has a different oospore organisation. In *Aphanomyces* the ooplasm is non-fluid and shows no Brownian movement of the granules as it is seen in the other genera of *Saprolegniales* (Dick 1971, Howard 1971, Traquair & McKeen 1980, Dick 1995). Antibodies produced against oospores of *A. eutiches* showed almost no cross-reactions with other genera of *Saprolegniales* and *Pythiales*, suggesting that *Aphanomyces* has a different wall chemistry compared to other groups of *Peronosporomycetes* (Petersen, Olson & Rosendahl 1996).

*Aphanomyces* was suggested to be included in a newly erected family of *Saprolegniales*, the Leptolegniaceae, together with species of *Leptolegnia* and *Plectospora*, by Dick et al. (1999) based on morphological and molecular data. In the phylogenetic trees based on LSU rDNA presented here an unnamed
isolate of _Leptolegnia_ clustered with _Saprolegnia_ and not with _Aphanomyces_ in the maximum parsimony trees, in the maximum-likelihood tree and in the neighbour-joining tree. Our data are thus not supporting the erection of the family _Leptolegniacae_. However, we did not include the type species _Leptolegnia caudata_. It would be interesting to include LSU rDNA sequences of this species in future studies to evaluate the proposed family _Leptolegniacae_. Morphologically based studies of these organisms indicate that they should be grouped together (Powell & Blackwell 1998, Dick et al. 1999).

It has been postulated several times that _Saprolegnia_ is the most basal organism in the _Saprolegniaceae_ (Humphrey 1893, Atkinson 1909, Höhn 1933, Barr 1983), mainly because of its mechanism of asexual reproduction. In _Saprolegnia_ two morphologically different zoospores are formed. The first formed zoospore, the primary zoospore, is pyriform and has two flagella anchored in the spore apex. The secondary zoospore is reniform with the flagella inserted laterally in a groove. This zoospore is generally a much better swimmer and forms zoospores on the surface of the sporangia. The _Saprolegnia_ protoplasts are released through individual papilla that project through the sporangial wall. These are reorganized into pyriform spores with stunted axonemes (Money et al. 1987). In _Thraustotheca_ the primary spores encyst in the sporangium before they are released by rupture of the sporangial walls. Later secondary zoospores are released from the cysts. In _Dictyuchus_ the spores encyst in the sporangia. Protoplasts are released through individual papilla that project through the sporangial wall. These are reorganized into secondary zoospores on the surface of the sporangia. The only remnant of the primary zoospore in this genus is the cyst walls in the sporangia (Coker & Matthews 1937). In _Aphanomyces_ protoplasts are cleaved out in hypha-like sporangia. These protoplasts move to the orifice of sporangia and encyst. Later the cysts germinate with a secondary zoospore. It has been shown that the primary spores, the protoplasts, of the plant pathogenic species _Aphanomyces euwhites_ do not possess flagella (Hoch & Mitchell 1972).

Daugherty et al. (1998) confirmed this theory by using internal transcribed spacer sequences (ITS) of four genera of _Saprolegniaceae_. In their phylogenetic analysis _Saprolegnia_ merged as the most basal organism, sister to _Achlya_, _Thraustotheca_ and _Dictyuchus_, with _Achlya_ and _Thraustotheca_ as most closely related, while _Dictyuchus_ appeared to have evolved independently of these.

The topology of the LSU rDNA based phylogenetic trees of the _Saprolegniaceae_ sensu lato is consistent with the topology of the phylogenetic tree based on sequences of ITS published by Daugherty et al. (1998). In both studies a suppression of the flagella of the primary spores is seen during evolution of the family. But our trees include more species, and a new pattern emerges. The LSU based phylogenetic trees suggest several independent losses of the primary zoospore during the evolution of the _Saprolegniaceae_ (Fig. 1). The primary zoospore is first lost at the branch with _Aphanomyces_, then at the branch with _Achlya_ and _Thraustotheca_, and the third time it is lost at the branch with _Brevilegnia_ and _Dictyuchus_. In our trees _Saprolegnia_ is not the most basal organism of _Saprolegniaceae_, but _Aphanomyces_ is suggested to contain the most basal organisms in this family.

Dick et al. (1999) suggests that the primary zoospore is an apomorphic state. According to Dick (1990) the primary zoospore is known only with certainty in _Saprolegnia sensu lato_ (including _Isoachlya_). It is thus most likely that the primary zoospore has been lost several times during evolution. The alternative, that this zoospore with restricted motility has evolved twice, is doubtful.

The topology of the part of the LSU rDNA based phylogenetic trees including members of the _Peronosporomycetidae_ is non-congruent with most phylogenetic theories of the _Peronosporomycetes_. In most theories primitive _Pythium_ and/or _Phytophthora_ are seen as ancestors to the obligate parasites in _Peronosporales_ (Bessey 1942, Shaw 1981, Barr 1983). This is partly based on the view that parasitism represents the apomorphic state in contrast to saprotrophy. In the phylogenetic trees presented here _Albugo_ is seen as basal in the _Peronosporomycetidae_. _Albugo_ is a biotrophic parasite on hosts of relatively early origin like _Amaranthaceae_ and _Convolvulaceae_ (Dick 1988). This might support a basal placement of this genus compared to species of _Pythium_, which contains parasites on monocots. In the true _Fungi_ studies on molecular phylogeny suggest that obligate parasites such as _Taphriniales_ and _Uredinales_ are basal in _Ascomycota_ and _Basidiomycota_ respectively (Swan & Taylor 1993, Nishida & Sugiyama 1994). A basal placement might also be the case for some of the obligate parasites of the _Peronosporomycetes_.

_Phyllosticta_ and _Pythium_ have traditionally been placed together in _Peronosporaceae_ in _Peronosporales_ (Waterhouse 1973) or lately in _Pythiales_ in _Peronosporomycetidae_ based on similarities in morphology and in details of the sexual reproduction (Waterhouse 1973, Dick 1995). In the phylogenetic tree based on partial sequences of LSU rDNA the species of _Phytophthora_ included in this study cluster with _Peronospora_ rather than with _Pythium_, suggesting that _Phytophthora_ should be removed from _Pythiales_ to _Peronosporales_. This alternative placement is strongly supported by bootstrap percentages (100%).

Several differences between _Pythium_ and _Phytophthora_ support such a movement. Species of _Phytophthora_ produce well formed sporangia on distinct sporangiophores, compared to the hypha-like or spherical sporangia of _Pythium_, and the zoosporogenesis of the two genera follows two different patterns. In _Pythium_ the proplasm of the sporangia is emitted into a vesicle, where the differentiation of the zoosporangium takes place, and the zoospores are released by rupture of the vesicle, while in _Phytophthora_ the zoospores are fully differentiated in the sporangia within a boundary plasmamembrane as seen in the _Peronosporales_. Several species of _Phytophthora_, e.g. the type species _Phytophthora infestans_, are dispersed aerially and show parallels to the species of _Peronosporales_ in the production of caducous sporangia and use
of direct germination via a germ-tube. Ecological differences between *Pythium* and *Phytophthora* are well known. The species of *Pythium* are saprobes in soil and water, and they are weak to moderate parasites (Waterhouse 1973). Species of *Phytophthora* are poor competitive saprotrophs but aggressive pathogens mainly on dicots, like the species found in *Peronosporales* (Waterhouse 1973). A key character used to place *Phytophthora* together with *Pythium* in *Pythiales* is the nature of the exospore wall layer of the oospore (Dick 1969, Waterhouse 1973). In *Peronosporales* the mature oospore contains a well-defined exposure wall layer derived from a persistent periplasm. In *Pythium* and *Phytophthora* a thin layer of periplasm is present outside the membrane of the oospore, and this thin layer later disappears in some species. The LSU rDNA based phylogenetic trees do not cluster *Albugo* and *Peronospora* together, but *Albugo* is placed on the most basal branch in *Peronosporomycetidae*. In both genera a conspicuous exospore wall layer is present. The LSU rDNA based trees thus suggest that the exospore is not the key character for uniting these organisms. Instead the exospore wall layer might represent a plesiomorph, shared with the *Rhizidiaceae*, and reduced or lost at some of the internal branches in the *Peronosporomycetidae*.

*Leptogium* is a genus that has been placed differently by several authors. Sparrow (1960) placed it in *Lagenidiales* together with the families *Oidiaceae* and *Sirosporiaceae* based on their endobiotic, simple thalli with sexuality involving fusion of contents of two thalloid bodies. Dick *et al.* (1984) transferred *Lagenidiales* to *Pythiales*, recognising the affinity between *Pythium* and *Leptogium*. *Lagenidium sensu stricto* was later transferred to *Pythiales* (Dick 1995). In both genera zoosporogenesis by external cleavage in a vesicle is seen, and they share several traits in their sexual reproduction. The trees based on maximum parsimony suggest that *Pythium* is basal to *Lagenidium*, but with bootstrap values below 50% (Fig. 1). The maximum likelihood tree (Fig. 2) and the neighbour-joining tree suggest that *Lagenidium* evolved on a line basal to *Pythium*, but this is not well supported by bootstrap values. Both trees suggest that the included species of *Lagenidium* should be placed in a separate order *Lagenidiales*. Unfortunately the present data can not resolve the phylogenetic placement of *Lagenidium* and *Pythium*. Inclusion of sequences of a wide range of species of *Myzocythium*, *Myzocythiopsis* and additional species of *Lagenidium* and *Pythium* might help to suggest a placement of the genus *Lagenidium*.

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