Systematics and molecular pathogenesis of oomycetes with
emphasis on flagellar genes

by

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ABSTRACT

Oomycetes are ubiquitous fungus-like protists which include serious pathogens of agricultural, horticultural, and aquatic commodities. Proper identification to the species level is a critical first step in any investigation of oomycetes, and the use of DNA for oomycete species identification is well established, but DNA barcoding with cytochrome c oxidase (COI) is a relatively new and potentially useful approach that had yet to be assessed over a significant sample of oomycete genera. DNA sequencing of COI from 1205 isolates representing 23 genera and a comparison to internal transcribed spacer (ITS) sequences from the same isolates showed that COI-based identification and the dataset generated are a valuable addition to the currently available oomycete taxonomy resources. Due to the fact that studies of oomycete evolution and taxonomy have traditionally been heavily reliant on ribosomal RNA and mitochondrial cytochrome oxidase sequencing to infer species boundaries and higher level phylogenetic relationships, a new approach to oomycete molecular systematics was undertaken using two single-copy protein-coding flagellar genes, PF16 and OCM1, showing their utility in species delimitation and in phylogenetic reconstruction of oomycete evolution. This approach also demonstrates a recently proposed codon substitution-based phylogenetic method. Using the codon model, the phylogenetic relationships inferred by flagellar genes are largely in agreement with the current views of oomycete evolution, whereas nucleotide and amino acid models failed to reconstruct monophyly in several clades. Interesting parallels exist between the molecular evolution of flagellar genes and zoospore ontology, supporting the tree obtained using the codon model. Sequencing of

OCM1 in particular revealed high variability among oomycetes and indicated a potential role of this protein in host-interaction of zoospores. A hypothesized role of OCM1 in host recognition, possibly through interaction with host C-type lectin was tested, and although the role of OCM1 was not confirmed, plant C-type lectin was shown to contribute to susceptibility of *Arabidopsis* seedlings to *Pythium* zoospores. This is a novel and important finding which helps to understand the unknown function of C-type lectin in plants. As a whole, this thesis contributes new resources and knowledge to the study of oomycetes, molecular evolution, and plant pathology.

DEDICATED TO

My mother, Karen Emily Robideau

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I will start by thanking my father Gerry and my mother Karen (R.I.P.) for their never-ending love and support throughout my life. They constantly went out of their way to help me and they always encouraged me to do the things I loved. They also drilled the idea of higher education into my head at an early age, so I decided to leave no room for disappointment. Thank you mom and dad. My wife Allison is certainly owed a debt of gratitude. Being married to a guy who works long hours for less than minimum wage is not everyone's idea of marital bliss. If she wasn't so loving and understanding I'd be hanging my Ph.D. degree in a bachelor apartment. I also need to thank my daughter Hunter for making me smile so much every day and giving me another good reason to get a solid career started. I know...a solid career in science? Oh well, too late now. My sister Kathy and her husband Hamed are two of my favourite people in the world so I'd like to acknowledge them here for being so awesome. My friends also helped me stay sane throughout this process by always being good company and encouraging me to study the effect of alcohol intake on thesis completion time.

On the business end, I really owe a huge thank you to my supervisor André Lévesque. He took me into his lab and gave me a chance before I even knew how to do a DNA extraction. His technician Nicole Désaulniers (ret.) taught me molecular biology technique and how to work in a lab in general. André as well has always been a great teacher and has offered great opportunities that I'm very grateful for. He has always been supportive of my research ideas even when they went off on tangents, which was fairly often. Since joining his lab for my undergrad thesis project, I've learned practically everything I know, thanks in no small part to the amazing communal atmosphere at ECORC (Agriculture Canada). When everything in the building is considered property of the Queen of England, nobody really hesitates to share. It's the perfect learning environment. There is an entire chapter of this thesis that could not have been done without the help and guidance of our ECORC colleague Gopal Subramaniam. Big thanks to Gopal. I would also like to acknowledge valuable technical support from the following people: Rafik Assabgui, Julie Chapados, Kasia Dadej, and Wayne McCormick for DNA sequencing service at Agriculture and Agri-Food Canada; Kelly Babcock, Modra Kaufert, and Caitlin O'Reilly for providing cultures from CCFC; Dave Chitty, Quinn Eggertson, Zeinab Robleh-Djama, and Tara Rintoul for providing an extra set of hands for lab work, Satpal Bilkhu and Chris Lewis for bioinformatics support.

Grad school has been a great experience for me. It's so much better than undergrad. But I'm still glad that it's over.

PREFACE

The DNA barcoding primers for COI were designed by André Lévesque. Culturing, DNA extraction, PCR, and sequence editing of the majority of the isolates used in Chapter 3 were performed by Gregg Robideau, with assistance from David Chitty and Quinn Eggertson. Additional DNA sequence data and sequence editing were provided by the co-authors of the cited publication. Gregg Robideau performed all the data management and analyses in Chapter 3 except the distance matrix analyses and statistics which were performed by André Lévesque. The publication from Chapter 3 was written by Gregg Robideau. The contribution of Gregg Robideau to the *Pythium ultimum* genome project described in Chapter 4 involved DNA extraction, comparative genomics of flagellar genes, manual annotation of flagellar genes, writing a portion of the manuscript, and manuscript review. Gregg Robideau was one of ten equal contributors from the total 48 authors of the genome publication. Sequencing, assembly, automated annotation of the genome, and transcriptome sequencing were performed by co-authors of the cited publication. The idea of using flagellar genes for a study of oomycete systematics and molecular evolution was devised by Gregg Robideau, inspired by the work of Donald Barr and Nicole Désaulniers. The codon model of substitution described in Chapter 4 was developed and implemented by Nicolas Rodrigue. Analyses which produced the codon model phylogram and the distribution of scaled selection coefficients were performed by Nicolas Rodrigue. All other lab work and analyses described in Chapter 4 were performed by Gregg Robideau, with DNA sequencing assistance from Tara Rintoul. The manuscript cited in Chapter 4 was written by Gregg Robideau. The work described in Chapter 5 was devised and carried out by Gregg Robideau, with technical guidance from

Gopal Subramaniam and André Lévesque. Statistics for Chapter 5 were performed by André Lévesque. Tara Rintoul lent assistance for the plant pathogenicity trials.

Readers are encouraged to cite the published work and forthcoming publications from this thesis using the references provided at the start of Chapters 3 and 4. The results of *Arabidopsis* pathogenicity trials in Chapter 5 are being used to prepare a manuscript for submission in early 2013. The DNA barcoding publication cited in Chapter 3 was reproduced with the permission of the Minister of Agriculture and Agri-Food, Canada, 2012. The *Pythium ultimum* genome publication cited in Chapter 4 is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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List of Abbreviations

3AT	- 3-Amino-1,2,4-triazole
BLAST	- Basic local alignment search tool
BOLD	- Barcode of Life Data systems
BSA	- Bovine serum albumin
CBS	- Centraalbureau voor Schimmelcultures
CCFC	- Canadian Collection of Fungal Cultures
CHAPS	- 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
CMA	- Corn meal agar
COI	- cytochrome <i>c</i> oxidase subunit I
CsCL	- <i>Cucumis sativus</i> C-type lectin
DAOM	- Canadian National Mycological Herbarium
DNA	- Deoxyribonucleic acid
dNTP	- Deoxyribonucleotide triphosphate
dpi	- Days post-inoculation
DTT	- Dithiothreitol
EDTA	- Ethylenediaminetetraacetic acid
EtOH	- Ethanol
HPLC	- High performance liquid chromatography
HRP	- Horseradish peroxidase
IMAC	- Immobilized metal affinity chromatography
IPTG	- Isopropyl β -D-1-thiogalactopyranoside
ISR	- Induced systemic resistance
ITS	- internal transcribed spacer of ribosomal RNA
LB	- Lysogeny broth
LSU	- large subunit 28S ribosomal RNA
MAFFT	- Multiple alignment program for amino acid or nucleotide sequences
MSS	- Mineral salt solution
MUSCLE	- Multiple sequence comparison by log-expectation
NaOCl	- Bleach (Sodium hypochlorite)
OCM1	- tubular mastigoneme protein
PAMP	- Pathogen-associated molecular pattern
PAUP	- Phylogenetic analysis using parsimony (and other methods)
PCR	- Polymerase chain reaction
PF16	- axoneme central apparatus protein
PMSF	- Phenylmethanesulfonyl fluoride
RNA	- Ribonucleic acid
RPKM	- Reads per kilobase per million mapped reads
SDS	- Sodium dodecyl sulfate
SDS-PAGE	- Sodium dodecyl sulfate – polyacrylamide gel electrophoresis

TaCL	-	<i>Triticum aestivum</i> C-type lectin
TBE	-	Tris/Borate/EDTA buffer
TBS	-	Tris-buffered saline
TE	-	Tris, EDTA
TES	-	Tris, EDTA, SDS
Tris	-	Trisaminomethane
UPGMA	-	Unweighted pair group method with arithmetic mean
YEB	-	Yeast extract broth
YPD	-	Yeast peptone dextrose

1 Chapter: INTRODUCTION

1.1 Overview

Oomycetes are microbes which resemble filamentous fungi, but which are actually more closely related to brown algae. In the environment, oomycetes are capable of surviving as saprophytes, living off of dead organic material, but they are often pathogenic, causing diseases of many different plants and animals. They are commonly referred to as water moulds since they typically thrive in water bodies or in wet conditions on land. Common names of oomycete diseases include late blight, downy mildew, cotton wool disease, swamp cancer, sudden oak death, damping off, and root rot. Oomycetes are found all over the world, and they consistently cause serious damage to agricultural, horticultural, and aquatic commodities.

The scientific study of oomycetes has a rich history which laid the foundation for modern plant pathology. Since Anton de Bary's seminal work on the potato late blight organism *Phytophthora infestans* (de Bary, 1861), oomycete research has grown to encompass a wide range of disciplines such as agronomy, pathology, molecular biology, cell biology, biochemistry, genetics, genomics, taxonomy, systematics, phylogenetics, biological control, ecology, and biogeography. In contribution to the scientific knowledge of oomycetes, this thesis describes new research in the areas of taxonomy, systematics, phylogenetics, genomics, biochemistry, and pathology.

Taxonomy is the process of arranging organisms into biological groups based on shared characteristics. With respect to oomycetes, taxonomy entails identification and characterization of environmental isolates, enabling an assessment of the risk or benefit that the isolates pose to human interests. In modern taxonomy, the state of the art relies

heavily on DNA sequence data to augment the traditional taxonomic characters of morphology (Tautz, *et al.*, 2002, Kang, *et al.*, 2010). One approach to the use of DNA sequencing for taxonomy is DNA barcoding (Hebert, *et al.*, 2003). DNA barcoding has become a global initiative which aims to discover a unique and easily detectable DNA reference sequence, a “DNA barcode”, from the genome of every living organism on earth. The idea is that with a database of carefully catalogued DNA barcodes, any organism on earth will be easily identifiable by simply obtaining a small amount of its DNA. In Chapter 3, I summarize the creation and validation of a DNA barcode database for oomycetes, which will enhance the ability to identify currently known species, as well as describe previously undiscovered species.

The taxonomic arrangement of organisms into biological groups naturally leads to the question of how different groups are related to each other. The desire to understand the evolutionary relationships between biological groups is what drives the study of systematics and phylogenetics, with the ultimate goal of creating a “tree of life” in which the intricate relationships between different organisms are defined. Phylogenetic trees create a framework for our modern understanding of biology, where individual organisms in the tree can be better understood by knowledge of the organisms that they are closely related to. Like taxonomy, systematics and phylogenetics have been modernized by the accessibility of DNA sequence data, especially whole genome sequences, giving rise to the disciplines of comparative genomics (Hardison, 2003) and phylogenomics (Eisen & Fraser, 2003). Comparing organisms at the genome level can provide an incredible amount of information, including a list of shared genes whose molecular evolution can be analyzed to better define the evolutionary relationships between the organisms. In

Chapter 4, I describe my contribution to the *Pythium ultimum* genome sequencing project, where I focused on annotation of flagellar genes for comparison to the flagellar gene repertoire of other oomycetes. The comparison of oomycete flagellar genes led to the use of two particular genes, PF16 and OCM1, for a phylogenetic study of oomycetes, where oomycete systematics as well as the molecular evolution of flagellar genes are examined.

In light of the fact that OCM1 is an extracellular flagellar protein (Bouck, 1971) with a highly-variable amino acid sequence (Chapter 4), the potential role of this protein in pathogenicity was investigated. Extracellular proteins that are involved in the infection process of pathogenic microbes often undergo rapid molecular evolution, generating amino acid sequence diversity to evade recognition by host organisms (Deitsch, *et al.*, 1997, Persson, *et al.*, 2006). The diversity of OCM1 sequences among oomycetes and the presence of conserved protein-binding domains within OCM1 indicate that this protein may somehow interact with oomycete hosts. The similarity of OCM1 to animal tenascin protein also gave an indication of the possible host protein that OCM1 may interact with. Tenascins are a family of extracellular glycoproteins that are known to be involved in protein-protein interactions, namely with a family of C-type lectin proteins (Aspberg, *et al.*, 1995, Lundell, *et al.*, 2004). Animals have a wide variety of C-type lectins that are often involved in immune response (Cambi, *et al.*, 2005), whereas plants typically have only one or two C-type lectins (Jiang, *et al.*, 2010) whose function is unknown (Bouwmeester & Govers, 2009). Using plant-oomycete host-pathogen models, I studied the possibility that oomycete zoospores recognize host plants by interacting with their C-

type lectin. The studies conducted on the potential role of OCM1 and C-type lectin in plant-oomycete interaction are presented in Chapter 5.

1.2 Objectives

- i. Sequence COI from a vast array of oomycete strains, encompassing as many genera and as many isolates from closely related species as possible in order to assess the utility of COI in oomycete identification to the species level.
- ii. Use comparative genomics to identify putative flagellar genes in the genome of *Pythium ultimum*. Compare the flagellar gene repertoire of *P. ultimum* to that of other oomycetes and identify candidate genes for further studies in systematics.
- iii. Use advanced phylogenetic methods to reconstruct an oomycete phylogeny based on PF16 and OCM1 sequences. Study the molecular evolution of these genes to gain insight into their biological function.
- iv. Purify heterologously-expressed *Pythium aphanidermatum* OCM1 protein for cucumber inoculation to test potential host response or acquired resistance to subsequent *P. aphanidermatum* infection.
- v. Purify heterologously-expressed plant C-type lectin from cucumber and wheat to test potential recognition of the protein by *Pythium* zoospores.
- vi. Test potential protein-protein interaction between *P. aphanidermatum* OCM1 and plant C-type lectin.
- vii. Test pathogenicity of *Pythium aphanidermatum* zoospores towards transgenic *Arabidopsis thaliana* plants with C-type lectin gene knockout.

2 Chapter: METHODS SUMMARY

2.1 Molecular biology methods

Common molecular biology methods were used. Of particular note is the use of small volume (10 μ l) PCR mixtures with minimal primer concentration, an approach which was optimized by N. Désaulniers, R. Assabgui, and C.A. Lévesque to offset the cost and PCR product purification processing time associated with high-throughput DNA sequencing.

2.2 Phylogenetic methods

Multiple phylogenetic methods were used, depending on the best-suited method for each task with respect to computational intensity and suitability to the scientific question. A recently proposed phylogenetic method developed and implemented by N. Rodrigue is presented in Chapter 4, which employs a codon model of substitution for phylogenetic reconstruction from protein-coding DNA sequences.

2.3 Biochemistry methods

Common biochemistry methods were used for recombinant protein purification and protein-protein interaction assays.

2.4 Plant pathology trials

Pathology trials were carried out in light- and temperature-controlled growth chambers with plants grown in standard potting mix.

3 Chapter: DNA BARCODING OF OOMYCETES

Adapted from:

Robideau, GP, de Cock, AWAM, Coffey, MD, Voglmayr, H, Brouwer, H, Bala, K, Chitty, DW, Desaulniers, N, Eggertson, QA, Gachon, CM, Hu, CH, Kupper, FC, Rintoul, TL, Sarhan, E, Verstappen, EC, Zhang, Y, Bonants, PJ, Ristaino, JB, Lévesque, CA (2011). DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. *Molecular Ecology Resources* 11: 1002-1011. Reproduced with the permission of the Minister of Agriculture and Agri-Food, Canada, 2012.

3.1 Introduction

Oomycetes are fungal-like organisms that are found in a wide range of environments and ecological niches. They are classified among the stramenopiles (=Straminipila), a lineage including brown algae and diatoms that is very distant phylogenetically from the kingdom Eumycota, the true Fungi. Many oomycete species are pathogens of plants and animals. The devastating speed with which they are able to spread makes rapid detection and identification crucial to implementation of control strategies. Biocontrol of oomycetes is an active area of study, and there are examples of oomycete species that are used as biological control against other oomycetes (Jones & Deacon, 1995, Picard, *et al.*, 2000), exemplifying the range of ecological functions between species.

Due to their wide variety of ecological roles, broad distribution and economic impact, proper identification is of great importance in oomycete studies. Identification of species can be a laborious and difficult task requiring time and expertise to cultivate the distinguishing morphological characters and compare them by microscopy. Also the decreasing number of experts able to identify oomycetes by morphological features is an important factor. Although matrix-based Lucid keys are being developed that will improve the speed of identification by morphology (Abad & Coffey, 2008, Ristaino, *et al.*, 2008), DNA-based identification can be done quickly and easily by a non-specialist,

achieving accurate results in a fraction of the time if there is an adequate database of reference strains. DNA-based identification is particularly important for identifying groups where species boundaries are not well delineated. Many oomycetes are self-fertile, making reproductive isolation an inappropriate criterion for species delineation. Species must instead be considered as a monophyletic assemblage of strains where gene flow between the strains is evident.

Currently the most common region of DNA being used for identification of oomycetes to the species level is the internal transcribed spacer (ITS) region of rDNA. The ITS region in oomycetes is easy to amplify for DNA sequencing in most species with the use of universal eukaryotic PCR primers (White, *et al.*, 1990, Ristaino, *et al.*, 1998). Cooke *et al.* (Cooke, *et al.*, 2000) were the first to publish a database of ITS sequences that covered all the known and available species of an oomycete genus. ITS then became the *de facto* DNA barcode for identification of *Phytophthora* species and similar comprehensive databases for *Pythium* (Lévesque & de Cock, 2004) and downy mildews (Voglmayr, 2003) followed. However, due to the apparent lack of functional constraint on this untranslated region of rDNA, alignment of ITS sequences is hampered by large amounts of insertions and deletions (indels), which can be an issue for accurate comparisons. ITS is part of the ribosomal RNA cistron, which occurs in tandem repeats of approximately 200 copies. Indels in the ITS can even be observed within a single strain due to differences in alleles or differences among the multiple copies of the ITS, making direct sequencing of PCR products impossible (Kageyama, *et al.*, 2007). In some species of downy mildews, excessive length due to long insertions can raise difficulties when sequencing the complete ITS region. There are also certain cases where the ITS

sequences of formally described species are extremely similar, particularly when they are evolutionarily closely related such as *Phytophthora infestans*, *P. phaseoli*, *P. ipomoeae*, *Phytophthora* sp. “*andina*”, and *P. mirabilis* (Gomez-Alpizar, *et al.*, 2008) which are 99.9% similar in ITS sequence (Kroon, *et al.*, 2004). Due to these limitations of the ITS region for identification, the use of another region for this purpose may lend more clarity to the molecular depictions of oomycete taxonomy.

Cytochrome c oxidase subunit I (COI, COX1) is a mitochondrially-encoded gene which is recognized as an extremely useful DNA barcode capable of accurate species identification in a very broad range of eukaryotic life forms (Hebert, *et al.*, 2004, Ward, *et al.*, 2005, Hajibabaei, *et al.*, 2006, Seifert, *et al.*, 2007). COI is the default DNA barcode approved by GenBank and the Consortium for the Barcode of Life (CBOL) and it must be proven ineffective as a DNA barcode to be rejected as such. COI has proven useful in phylogenetic studies of the oomycete genus *Phytophthora* (Martin & Tooley, 2003, Kroon, *et al.*, 2004), and the success of COI barcoding in red algae (Saunders, 2005) made it a very intriguing prospect for barcoding of all oomycetes due to their algal ancestry. Because COI is a protein-coding region, alignment of COI sequences is simple and devoid of gaps if introns are absent. With the use of primers that amplify the 5' end of COI, accurate species delimitation has been achieved with sequences of only 650 base pairs (bp) or less (Meusnier, *et al.*, 2008). With the advent of massively parallel sequencing from environmental samples, it is important to compare COI and ITS since the marine and animal science communities appear to have a strong interest in COI, whereas ITS is the established species-level marker in the mycology community (Schoch, *et al.*, 2012), although not implemented by GenBank as a DNA barcode yet. Reported

here is the utility of COI sequence data for accurate species delimitation in oomycetes, and a comparison of COI identification to the benchmark of ITS identification is made with 1205 isolates representing 23 genera including the recently described genus *Phytopythium* (formerly *Pythium* Clade K) (Bala, *et al.*, 2010). Nearly all the currently described species of the two largest genera that can be maintained in culture (*Pythium* and *Phytophthora*) have been included in this study. In addition to COI and ITS, the D1-D3 region of nuclear large subunit (LSU) rDNA, a commonly used marker for phylogeny and identification of oomycetes and Fungi, was sequenced from a subset of 388 isolates from 20 genera and is analyzed in comparison to COI and ITS. The complete list of isolates used for this study is shown in Appendix A.

3.2 Materials and Methods

3.2.1 DNA extraction

Extraction methods varied depending on the source of the cultures. For cultures grown from the Centraalbureau voor Schimmelcultures (CBS), mycelia from 5 - 14 day old liquid cultures grown in pea broth (de Cock, *et al.*, 1992) were harvested by vacuum filtration, freeze dried, and DNA was extracted following the protocol of Möller *et al.* (1992). For cultures grown from the Canadian Collection of Fungal Cultures (CCFC), mycelia from 5 – 14 day old liquid cultures grown in potato dextrose broth (Difco) at room temperature were removed from broth and DNA was extracted following the protocol of Möller *et al.* (1992) with a modification to the tissue lysis step. Instead of grinding mycelia in liquid nitrogen, mycelia were placed in 2 ml screw cap tubes containing 300 mg of zirconium oxide spheres and one ¼” zirconium oxide sphere (Fox

Industries), along with TES buffer (100 mM Tris pH 8.0, 10 mM EDTA, 2% SDS) and proteinase K. Lysis was achieved by placing tubes in a FastPrep® machine (BIO 101) for 45 seconds at speed 4.0. Tubes were incubated at 65°C for 1 h and subsequent steps were performed following the original protocol. At the final step, DNA pellet was resuspended in 0.1x TE buffer containing 20 µg/ml RNase A and tubes were incubated at 65°C for 10 min.

3.2.2 *COI primer design*

In order to design COI barcode primers for a broader range of oomycete genera, the 5' and middle region of COI, overlapping the commonly used barcode primers, was amplified and sequenced using eight different genera of oomycetes with a combination of FM79, FM80, FM82, FM83 and FM85 originally designed for *Phytophthora* (Martin & Tooley, 2003). OomCoxI-Levup was designed to overlap LCO1490 (Folmer, *et al.*, 1994) and GazF1 (Saunders, 2005) primers whereas Fm85mod was a modified version of Fm85 from Martin and Tooley (2003). OomCoxI-Levlo was designed to overlap HCO2198 (Folmer, *et al.*, 1994) and GazR1 (Saunders, 2005).

3.2.3 *DNA amplification*

Sequencing templates were amplified from DNA extracts using the universal eukaryotic primers UN-up18S42 (5'-CGTAACAAGGTTTCCGTAGGTGAAC-3') (Bakkeren, *et al.*, 2000) and the new UN-lo28S1220 (5'-GTTGTTACACACTCCTTAGCGGAT-3') (Bala, *et al.*, 2010) for the combined ITS and LSU regions (Lévesque & de Cock, 2004). In some cases the ITS region alone was

amplified using UN-up18S42 and UN-lo28S22 (5'-GTTTCTTTTCCTCCGCTTATTGATATG-3') (Lévesque & de Cock, 2004). The oomycete-specific primers OomCoxI-Levup (5'-TCAWCWMGATGGCTTTTTTCAAC-3') and Fm85mod (5'-RRHWACKTGACTDATRATACCAAA-3'), modified from Martin and Tooley (2003), were designed to amplify 727 bp from the 5' end of COI mitochondrial DNA. In some cases, an alternative reverse primer, OomCoxI-Levlo (5'-CYTCHGGRTGWCCRAAAAACCAAA-3'), was used with OomCoxI-Levup, amplifying a slightly smaller 680 bp fragment of COI, perfectly overlapping the standard DNA barcode used in other groups. PCR volume was 10 µL containing final concentrations of 1X Titanium Taq buffer (with 3.5 mM MgCl₂), 0.1 mM dNTPs, 0.08 µM each of forward and reverse primer, 0.5X Titanium Taq polymerase, and approximately 1-10 ng/µL of DNA. Reaction volume was brought up to 10 µL with sterile HPLC water. Thermocycler program for amplification of the ITS/LSU region was: 95°C for 3 min followed by 40 cycles of 95°C for 30 s, 68°C for 45 s, 72°C for 2 min. A final extension was made at 72°C for 8 min. Program for ITS alone was identical to that for ITS/LSU, except for a shorter extension time of 90 s at 72°C in each cycle. Program for amplification of the COI region was: 95°C for 2 min followed by 35 cycles of 95°C for 1 min, 55°C for 1 min, 72°C for 1 min. A final extension was made at 72°C for 10 min.

3.2.4 *Cloning PCR products*

In some cases, direct sequencing of PCR products from the ITS/LSU region resulted in poor sequence results due to apparent polymerase slippage at polyT or polyA regions,

or due to the possibility of indels in some copies or alleles of this multicopy locus.

Cloning of PCR products and sequencing directly from the cloning vectors was able to overcome this problem. PCR products were purified by mixing 5 μ L of PCR product with 2 μ L ExoSAP-IT[®] (USB), incubating at 37°C for 15 min, followed by incubation at 80°C for 15 min. Purified PCR products were then poly-adenylated and ligated to the pGEM[®]-T Easy vector (Promega). Transfection of competent cells, selection of transformants, and growth of transformant cultures were performed according to manufacturer's protocols. Isolation of plasmid DNA was performed according to Sambrook and Russel (2001), with omission of the optional phenol/chloroform step. Plasmids isolated from transformants were screened for inserts by PCR with T7 (5'-TAATACGACTCACTATAGGG-3') and SP6 (5'-TATTTAGGTGACACTATAG-3') plasmid primers. Program for screening PCR was: 95°C for 3 min followed by 40 cycles of 95°C for 30 s, 50°C for 45 s, 72°C for 2 min 30 s. A final extension was made at 72°C for 8 min.

3.2.5 Sequencing

Amplification of PCR products for sequencing was done with ABI Big Dye Terminator v3.1 in a reaction volume of 10 μ L, with Big Dye Seq Mix diluted 1:8 with Seq buffer. Final concentrations of each reagent were 0.875X Sequencing buffer, 5% trehalose, 0.125X Big Dye Seq Mix, and 0.16 μ M primer. Reaction volume was brought to 10 μ L with sterile HPLC water and 1 μ L of PCR product was added directly from initial PCR amplification without purification. Thermocycler program for ITS/LSU was: 95°C for 3 min followed by 40 cycles of 95°C for 30 s, 58°C for 40 s, 60°C for 4 min.

Program for COI was: 95°C for 3 min followed by 40 cycles of 95°C for 30 s, 50°C for 20 s, 60°C for 4 min. Sequencing primers for ITS were UN-up18S42 and UN-lo28S22.

Sequencing primers for LSU were UN-up28S40 (5'-GCATATCAATAAGCGGAGGAAAAG-3') (Schurko, *et al.*, 2003), UN-up28S577 (5'-CGTCTTGAAACACGGACCAAGGAG-3') (Bala, *et al.*, 2010), UN-lo28S576B (5'-CTCCTTGGTCCGTGTTTCAAGACG-3') (Bakkeren, *et al.*, 2000), and UN-lo28S1220. Sequencing primers for COI were OomCoxI-Levup and Fm85mod or OomCoxI-Levlo.

DNA sequences were generated from sequencing amplification reactions using the ABI Prism 3130xl Genetic analyzer. DNA sequences have been deposited in the Barcode of Life Data Systems (BOLD) and GenBank. Accession numbers for both databases are found in Appendix A.

3.2.6 Sequence editing, alignment, and cluster analysis

Sequence results were reviewed and edited using Seqman software (DNASar) and alignments were made using MUSCLE for COI and MAFFT for ITS and LSU (Edgar, 2004, Katoh, *et al.*, 2005). MAFFT alignment of LSU was performed with the G-INS-i algorithm on the download Mac OS X version. MAFFT alignment of ITS was performed with the L-INS-i algorithm. The default maximum sequence allowance was raised from 1000 to 2000 by opening the MAFFT script in /usr/local/bin and changing line 762 from `if [ $nseq -gt 1000 -a $iterate -gt 1 ]; then` to `if [ $nseq -gt 2000 -a $iterate -gt 1 ]; then`. Alignments in fastA format were converted to nexus format with MacClade 4.06. Alignment of COI contained 680 characters, alignment of ITS contained 2068 characters, and alignment of LSU contained 1395 characters. No characters were excluded from

analysis of any marker. Calculation of distance matrices and UPGMA hierarchical clustering was performed with PAUP 4.0b10. Bootstrap values were obtained from 1000 reps. Trees were formatted for Fig. 3.2 and Fig. 3.3 using Dendroscope (Huson, *et al.*, 2007).

3.2.7 *Distance matrix statistical analysis*

Uncorrected “p” (percentage) based distance matrices were analyzed using matrix algebra and SAS. The average intraspecific distance was calculated for each species represented by more than one strain and coded as missing data when only one strain could be obtained to avoid having a bias towards zero variation. For each pair of species, the average pairwise distance was calculated for all the possible strain comparisons. A lower triangular uncorrected distance matrix was created with PAUP with the strains shown in Appendix A. The square pairwise distance matrix [PD] was imported into SAS as well as a column for species name and a column for corresponding strain coding. There was no need to keep track of genus names because we did not have the same species name found in two different genera. A 0/1 "dummy" species variable design matrix [SV] was created in SAS using the species name column. The total of the distances [TD] for each species and pairwise comparison was found with the following equation: $[TD] = [SV]^t \times [PD] \times [SV]$, where the diagonal was the number of pairwise comparisons for each species and the lower triangular matrix the total number of possible pairwise comparisons for each pair of species. A lower triangular matrix with a diagonal of 1's [L1] was created with the same number of rows and columns as [PD]. The same equation as above was applied by replacing [PD] by [L1] to find the total number of

pairwise distance comparisons [ND]. The average of all the pairwise comparisons was found by dividing [TD] by [ND], with the diagonal of the matrix giving the averages of all intraspecific comparisons and the lower matrix the averages of all interspecific comparisons. These values were used for distribution analyses.

3.3 Results

3.3.1 PCR primer performance

In an initial trial using the *Phytophthora* primers from Martin and Tooley (2003), consistent amplification of the COI barcode region was not achieved in a set of eight oomycete genera. However, the complete 5' end and middle region of COI was sequenced with various combinations of their primers for *Saprolegnia*, *Achlya* and *Pythium* in addition to *Phytophthora*. Alignment of these sequences allowed design of new COI primers for the current study, OomCoxI-Levup and Fm85mod, which amplified a 727 bp fragment from the 5' end of COI. For 18 isolates that did not amplify well with Fm85mod, an alternative reverse primer (OomCoxI-Levlo) was used. This amplified a smaller fragment of 680 bp compared to using Fm85mod (Fig. 3.1). Introns were not present in any COI sequence of the species studied. ITS fragments of varying length were used; from partial fragments as short as 402 bp from some *Pythium* isolates, up to 1351 bp from *Eurychasma*. In *Basidiophora*, *Plasmopara* and *Plasmoverna*, only the ITS1 region was sequenced due to long insertions in the ITS2 region. The LSU fragments ranged between 1246 bp and 1343 bp, although for three *Saprolegnia* isolates, partial fragments between 700 – 850 bp were used due to lack of high sequence quality for the entire D1-D3 region.

Figure 3.1. Diagram illustrating COI gene region, barcode segment of COI (grey), and COI PCR primer locations. Reprinted with permission of the Minister of Agriculture and Agri-Food, Canada, 2012.

3.3.2 Sequence distances

For each marker, distance matrices were used to calculate intraspecific (within species) variation, as well as interspecific (between species) variation. A graphical representation of the data and a table summarizing the results for all markers is shown in Fig. 3.2. The mean intraspecific variation for COI, ITS, and LSU was 0.0048, 0.0046, and 0.0017, respectively. The mean interspecific variation was 0.1050, 0.2899, and 0.1037, respectively.

3.3.3 Cluster analyses

Trees for each marker are shown in Fig. 3.2. Trees for COI and ITS contain 1205 sequences, including the basal oomycete *Eurychasma dicksonii* as the outgroup (Sekimoto, *et al.*, 2008). The LSU tree contains 388 sequences, including *E. dicksonii* as outgroup. Black squares at branch termini in Fig. 3.2 represent a collapsed subtree containing multiple species or in the case of the order Saprolegniales, multiple genera and species. Black rectangles at branch termini represent a clade of unresolved genera and indicate the presence of multiple species from the genera occupying the clade. Direct comparison between COI and ITS trees is shown in Fig. 3.3. Black squares at branch termini in Fig. 3.3 represent a collapsed subtree containing multiple isolates. Black rectangles at branch termini represent a clade of unresolved species and indicate the presence of multiple isolates from the species occupying the clade. Unresolved species with only single isolates are shown within clades represented by vertical lines at branch termini rather than rectangles. In Fig. 3.3, the genera *Phytophthora* and *Pythium* are divided and displayed by their previously established phylogenetic clades (Lévesque &

de Cock, 2004, Blair, *et al.*, 2008). Genera belonging to the families Saprolegniaceae and Leptolegniaceae are shown under the heading of their respective family. All obligate biotrophs are displayed together. For both COI and ITS, most isolates grouped into conspecific clusters, and the species composition of major clades did not differ between COI and ITS. Exceptions to this trend were *Phytophthora katsurae*, *Phytopythium* aff. *vexans*, *Pythium kunmingense*, and *Pythium okanoganense*, which all appeared in different terminal nodes depending on the marker used. LSU sequences were more highly conserved and did not vary between some closely related species that were distinctly separate with COI and ITS. In some cases, two or more species shared identical or highly similar COI and ITS sequences, consistent across both markers, which invites further discussion of the possible synonymy of those species.

Figure 3.2. Phylograms and distance histograms for each marker. Black boxes at phylogram branch termini indicate multiple species. Branches with less than 50% bootstrap support are greyed out. Branch lengths are not to scale. Histograms display intraspecific variation in grey and interspecific variation in black. Inset table summarizes distance data. Reproduced with the permission of the Minister of Agriculture and Agri-Food, Canada, 2012.

Figure 3.3. Direct comparison of ITS and COI phylograms by clade. ITS is shown on the left and COI on the right of an artificial vertical backbone. Black boxes at phylogram branch termini represent multiple isolates, with number of isolates shown in brackets. Asterisks denote ex-type specimen. Branches with less than 50% bootstrap support are greyed out. Branch lengths are not to scale. Most genus names are abbreviated to the first three letters. See Appendix A (Supporting information) for full names. (a) *Pythium* Clades A and B. (b) *Pythium* Clades C, D, E and J. (c) *Pythium* Clades F and I. (d) *Pythium* Clade G, H and *Phytopyrium*. (e) *Phytophthora* Clades 1, 2, 3, 4 and (= *Halophytophthora*). (f) *Phytophthora* Clades 5, 6, 7, 9 and 10. (g) *Phytophthora* Clade 8, Obligate biotrophs, *Apodachlya* and Leptolegniaceae. Note that grouping of all obligate biotroph isolates is superficial as they do not represent a coherent phylogenetic group. (h) Saprolegniaceae. Reproduced with the permission of the Minister of Agriculture and Agri-Food, Canada, 2012.

3.4 Discussion

The primary purpose of the current study was to compare a validated oomycete *de facto* DNA barcode (ITS) with the default barcode (COI) which is officially accepted as the DNA barcode for eukaryotic groups unless proven ineffective. Our results indicate that both ITS and COI can be valid and useful barcodes for accurate identification of many oomycetes, whereas LSU more often lacks sufficient resolution between species. The genera *Pythium* and *Phytophthora* were almost completely covered by this study, and several other genera representing a wide range of oomycetes, including some obligate biotrophs, were partially covered. Intraspecific variation of COI is at par with that of ITS, although ITS does provide greater interspecific variation than COI. The benefit of COI barcoding is the ease of sequencing and aligning a relatively short fragment which has uniform length and can be amplified with degenerate primers throughout the entire oomycete class. This advantage over ITS is especially evident in the downy mildew genera *Basidiophora*, *Plasmopara*, *Plasmoverna* and relatives, which contain insertions in the ITS2 resulting in ITS sequences often longer than 2 kb (Thines, 2007), this raises difficulty in amplifying, sequencing and aligning the complete ITS region. LSU had the lowest interspecific variation of the three markers (Fig. 3.2), and the

use of LSU as an oomycete barcode does not always provide enough resolution for identification to the species level. LSU appears to be better suited for studying genus- and family-level relationships in oomycetes (Riethmüller, *et al.*, 1999, Petersen & Rosendahl, 2000, Riethmüller, *et al.*, 2002, Voglmayr & Riethmüller, 2006). A large portion of LSU was used to provide sufficient variation but this precludes amplification and sequencing with a single pair of primers. Barcoding with COI on the other hand, can quickly and easily lend additional evidence to identifications and new species descriptions by complementing nuclear DNA sequencing (ITS) with a mitochondrial DNA sequence (Bala, *et al.*, 2010). The speed and ease of ITS and COI sequencing is also enhanced by the method of PCR amplification used in this study, which employed a minimal concentration of primers, thereby eliminating the need for purification of PCR products before sequencing. This approach, which was carried out in small PCR reaction volumes (10 µL), was able to reduce time and cost while still delivering high quality results.

Universality of PCR primers is also an important requirement of DNA barcode-based identification. The primers used for oomycete COI amplification (OomCoxI-Levup and Fm85mod) were able to amplify DNA from the entire range of oomycete genera in this study, including the basal genus *Eurychasma* and genera from the obligate biotrophic white blister rusts (*Albugo*) and downy mildews (*Basidiophora*, *Hyaloperonospora*, *Peronospora*, *Plasmopara*, *Plasmoverna* and *Pseudoperonospora*). There were however, a few exceptional species of *Pythium* and *Phytophthora* (*Py. buismaniae*, *Py. contiguanum*, *Py. kashmirensis*, *Py. ostracodes*, and *Ph. cucurbitacearum*) that did not amplify with Fm85mod, and were instead amplified and sequenced using the alternative reverse primer OomCoxI-Levlo. Standard use of OomCoxI-Levlo is not recommended

though, because our alignment of Fm85mod-derived COI sequences revealed that the 3' end of OomCoxI-Levlo is not conserved throughout all *Pythium*, *Phytophthora*, and *Aphanomyces* species.

Proposition of COI as a complement to ITS for species delimitation is based on the observation that relationships among closely related species and organization of major clades in *Pythium* and *Phytophthora* are concordant with the results of previous multi-locus molecular studies (Kroon, *et al.*, 2004, Lévesque & de Cock, 2004, Blair, *et al.*, 2008). Almost every terminal node on the UPGMA tree was composed of the same isolate(s) regardless of the marker used for sequencing. Replicated DNA sequencing of the isolates that did not follow this trend (*Phytophthora katsurae* P3389, *Phytopythium* aff. *vexans* CBS 261.30, *Pythium kunmingense* CBS 550.88, and *Pythium okanoganense* CBS 315.81) was performed to rule out the possibility of a DNA mix up during COI sequencing. In attempting to explain these situations biologically, the possibility of hybridization exists as has been well documented in *Phytophthora* (Érsek & Nagy, 2008) and recently discovered in *Pythium* (Nechwatal & Mendgen, 2008), but evidence of hybridization based on dimorphism in nuclear DNA sequence chromatograms was not found for any isolate mentioned above. An alternative scenario involving horizontal transfer of mitochondrial DNA (mtDNA) is not implausible based on previous findings in filamentous fungi. Mobile mitochondrial plasmids are prominent in filamentous fungi and they are known to recombine with mtDNA (Griffiths, 1996). The presence of mitochondrial plasmids has not been documented in oomycetes, although it is interesting to note that a mobile plasmid derived from an intron of COI exists in the ascomycete fungus *Podospora anserina* (Osiewacz & Esser, 1984). Fusion of hyphae (anastomosis),

as has been reported in *Phytophthora* (Stephenson, *et al.*, 1974), could be a rare natural event that enables horizontal transfer of mtDNA in oomycetes. Although the true nature of the aforementioned results is unknown, it is worth stating that the use of both ITS and COI rather than one or the other, is recommended for taxonomic identification of oomycetes.

Considering that new species descriptions are a demanding process involving detailed morphological study, the ability to predict candidacy for a new species description with additional DNA sequence data will be very valuable and time-saving, providing more confidence so as to avoid questionable or synonymous species descriptions. Several putative new species are present in the isolates used for this study, denoted by the species epithet “sp. nov.”

The augmented species resolution that COI provides is evident for arguably the most economically important oomycete, *Phytophthora infestans*. This species, which causes late blight of potato and tomato, has an ITS sequence that is indistinguishable from the closely related species *P. sp. “andina”* and *P. mirabilis*. COI on the other hand, separates these three species into individual terminal nodes. The same situation has been seen between the strawberry pathogen *Phytophthora fragariae* and the recently circumscribed raspberry pathogenic species *P. rubi* (Man in 't Veld, 2007), originally classified as *P. fragariae* var. *rubi*. While the ITS sequences do not vary between these two species, a clear distinction exists between their COI sequences. A similar example of species resolution by COI in *Pythium* is between the marine algal pathogens *P. chondricola* and *P. porphyrae*. Other examples of species resolution by COI are listed in Appendix B. The initial recognition of individuality between these species can be credited to examination

of morphological characters and confirmation of these species descriptions by COI sequencing acknowledges the accuracy of morphological observation.

Though the use of COI sequencing is able to reinforce some species boundaries, there are several cases where formally described species are indistinguishable with either ITS or COI. Cases of apparent conspecificity are listed in Appendix C. Our results also implicate the existence of species complexes where gene flow may be occurring between species. In some cases there are several described species included in a complex, or alternatively a single described species may display substantial intraspecific variation, thus suggesting that a complex of multiple species exists within the single described species. For example, there is a large species complex referred to here and in Fig. 3.3a as *Pythium* Cluster B2a which includes *P. coloratum*, *P. diclinum*, *P. cf. dictyosporum*, *P. dissotocum*, *P. lutarium*, *P. sp. "Group F"*, and *P. sp. "tumidum"*. Other examples of species complexes in *Achlya*, *Phytophthora*, *Pythium*, *Phytopythium*, and *Saprolegnia* are listed in Appendix D. Such complicated taxonomic situations are inextricable with a single marker, and it is therefore important to have additional evidence from other markers such as COI for taxonomic identification of oomycetes. Ultimately, multigene phylogenies will be required to resolve taxonomic issues.

The conclusion drawn from this study is that COI sequencing is a very useful addition to the oomycete molecular toolbox which can now be used for identification of many oomycete species using the reference data generated by this study. In some of the most difficult cases of species concept in *Phytophthora* and *Pythium*, COI provides better resolution and support for current taxonomy than ITS does. However, because both markers provide an acceptable resolution when used individually, because of the history

of using ITS in mycology and for oomycetes, and because it is desirable to have the complement of mitochondrial and nuclear markers, we have proposed that ITS be added to COI as a DNA barcode for oomycetes in GenBank. For any oomycete species that is not included in this study or for any new species to be described, both markers should be sequenced and deposited as barcodes.

4 Chapter: COMPARATIVE GENOMICS AND PHYLOGENETIC STUDY OF OOMYCETE FLAGELLAR GENE EVOLUTION

Adapted from:

Lévesque CA, Brouwer H, Cano L, Hamilton JP, Holt C, Huitema E, Raffaele S, **Robideau GP**, Thines M, Win J, Zerillo MM, Beakes GW, Boore JL, Busam D, Dumas B, Ferriera S, Fuerstenberg SI, Gachon CM, Gaulin E, Govers F, Grenville-Briggs L, Horner N, Hostetler J, Jiang RH, Johnson J, Krajaejun T, Lin H, Meijer HJ, Moore B, Morris P, Phuntmart V, Puiu D, Shetty J, Stajich JE, Tripathy S, Wawra S, van West P, Whitty BR, Coutinho PM, Henrissat B, Martin F, Thomas PD, Tyler BM, De Vries RP, Kamoun S, Yandell M, Tisserat N, Buell CR (2010). Genome sequence of the necrotrophic plant pathogen *Pythium ultimum* reveals original pathogenicity mechanisms and effector repertoire. *Genome Biology* 11: R73.

Robideau GP, Rodrigue N, Lévesque CA (to be submitted). Codon substitution modeling of flagellar gene evolution in oomycetes introduces novel molecular markers and a compelling approach to phylogenetic reconstruction with protein-coding genes. *Molecular Biology and Evolution*.

4.1 Introduction

Oomycetes are ubiquitous fungus-like protists which can cause disease in a wide range of plants and animals. Many oomycetes are serious pathogens of agricultural, horticultural, and aquatic commodities. The oomycete lineage belongs to the heterokont phylum among brown algae and diatoms, and includes well-known genera such as *Phytophthora*, *Pythium*, and *Saprolegnia*. Oomycete evolution has been well studied (Sparrow, 1960, Beakes, 1989, Dick, 2001), and continuing efforts are being made towards the most realistic reconstruction of phylogenetic relationships within this group. Studies of molecular evolution and oomycete systematics have generally relied upon mitochondrial cytochrome oxidase subunit II (cox2) (Hudspeth, *et al.*, 2000, Hakariya, *et al.*, 2007, Sekimoto, *et al.*, 2008) and ribosomal RNA (rRNA) sequences from the 18S small subunit (SSU), internal transcribed spacer (ITS), or the 28S large subunit (LSU) to

provide phylogenies (Cooke, *et al.*, 2000, Petersen & Rosendahl, 2000, Riethmüller, *et al.*, 2002, Beakes, *et al.*, 2012).

Prior to DNA sequencing, systematic studies were heavily reliant on ultrastructural characteristics to infer phylogenetic relationships. Some of the most influential work in this area came from studies of the flagellar apparatus (Barr, 1981, Moestrup, 1982, Barr & Allan, 1985). Flagellar hairs, also known as mastigonemes, were consistently cited as an important morphological feature of heterokont flagella (Bouck, 1971, Leadbeater, 1989). The organization of the flagellar rootlet system is also highly conserved throughout stramenopiles (Barr & Désaulniers, 1989), which added to the evidence that led to the eventual distinction of these organisms from true fungi. In fact, the term “stramenopile” used to describe the entire heterokont phylum, is derived from the straw-like hairs present on the so-called “tinsel” flagellum. Since flagellar ultrastructure has traditionally been regarded as such an important character in heterokont systematics, this tradition is now continued on a molecular level using flagellar gene sequences to study oomycete systematics and evolution. This represents the first study of oomycete flagellar evolution on a molecular scale, where the results will be interpreted in comparison to the oomycete evolutionary framework established primarily with ribosomal RNA and mitochondrial genes. Through involvement with the *Pythium ultimum* genome project, available oomycete genomes were searched for putative flagellar genes in order to shed light on *P. ultimum*’s flagellar gene repertoire, and to find suitable candidates for an oomycete-wide study of flagellar evolution.

The oomycete *Pythium ultimum* is a notorious and ubiquitous plant pathogen responsible for root rot and damping off of many different crops and ornamentals. The *P.*

ultimum genome project provided the first *Pythium* genome to be fully sequenced and opened the door to comparison of the *Pythium* gene repertoire with those of related oomycetes *Phytophthora* and *Hyaloperonospora*. Since *P. ultimum* does not produce zoospores in culture, an important question to be asked was whether it is genetically capable of producing zoospores. Has its flagellar gene repertoire been reduced or has transcription of flagellar genes been suppressed? These questions were addressed by comparison to the genomes of known zoospore-producers *Phytophthora infestans*, *Phytophthora ramorum*, and *Phytophthora sojae*, as well as the non-zoospore-producing downy mildew *Hyaloperonospora arabidopsidis*. The results of these comparisons show that *P. ultimum* does not lack any of the compared flagellar genes, but there are several potentially suppressed genes that were not expressed in growth conditions which typically induce zoospore production in oomycetes. The results also provided a list of flagellar genes that are conserved in *Pythium* and *Phytophthora*, of which two genes were found to be particularly amenable to a molecular evolution study: the axoneme central apparatus protein PF16, and the tubular mastigoneme protein OCM1. These genes harbor conserved DNA sequences at their 5' and 3' termini, which enabled the design of PCR primers capable of amplifying these genes from a wide range of oomycetes.

The axoneme central apparatus is involved in flagellar dynein regulation and is a core component of the eukaryotic flagellum (Smith, 2002). It is comprised of several known proteins including PF16 (Smith & Lefebvre, 1996, Smith & Lefebvre, 1997), which is highly conserved among very diverse eukaryotes (Straschil, *et al.*, 2010). Tubular tripartite mastigonemes are a defining feature of heterokont flagella, and their number, thickness, and length have been observed to be consistent for a taxon (Hill &

Outka, 1974, Moestrup, 1982). Most studies of mastigonemes have relied on the golden alga *Ochromonas*, which possesses only tinsel flagella rather than the combination of whiplash and tinsel flagella found in most heterokonts. To date, four distinct proteins named OCM1, OCM2, OCM3, and OCM4 have been isolated from the mastigonemes of *Ochromonas* (Yamagishi, *et al.*, 2007, Yamagishi, *et al.*, 2009). Orthologs of PF16, OCM1, OCM2, and OCM4 proteins are present in the published genomes of *Pythium* and *Phytophthora* (Lévesque, *et al.*, 2010), and the OCM2 ortholog has been studied in detail in *Phytophthora nicotianae* (Blackman, *et al.*, 2011). Access to these flagellar gene sequences now makes the study of their evolution in oomycetes possible.

Since the advent of DNA sequencing, studies of molecular evolution have typically relied on nucleotide or amino acid substitution models, but the analysis of protein coding sequence evolution has significantly advanced since the introduction of codon substitution models (Goldman & Yang, 1994, Muse & Gaut, 1994). In contrast to nucleotide-based or amino acid-based models, codon-based substitution models use coding DNA triplets as the unit of evolution. These models were originally developed to measure selective pressures, and their adoption in phylogenetic frameworks has been slow due to their high computational demands. Now, recent advances in computing technology combined with algorithmic refinements have created renewed interest and utilization of this approach in phylogenetics (Ren, *et al.*, 2005, Rodrigue, *et al.*, 2010, Chang, *et al.*, 2012), since their advantage over nucleotide and amino acid models has been demonstrated (Seo & Kishino, 2009).

Here a genomic comparison of oomycete flagellar genes is reported, as is DNA sequencing of novel markers PF16 and OCM1 from a variety of oomycetes using newly

designed degenerate PCR primers. The coding DNA sequences are analyzed using a codon-based model of sequence evolution to produce a molecular phylogeny. The evolution of PF16 and OCM1 among oomycetes is explored, and their utility in species delimitation is demonstrated.

4.2 Materials and Methods

4.2.1 Comparative genomics and genome annotation

Available genome assemblies were accessed to compare oomycete flagellar gene repertoires and to obtain full length predicted PF16 and OCM1 sequences. Predicted PF16 and OCM1 sequences were retrieved by performing tBLASTN on genomic DNA assemblies using *Chlamydomonas reinhardtii* PF16 protein (GenBank: AAC49169) and *Ochromonas danica* OCM1 protein (GenBank: BAF65668) as queries. Sequences were obtained from the genomes of: *Albugo candida* (Links, *et al.*, 2011), *Albugo laibachii* (Kemen, *et al.*, 2011), *Ectocarpus siliculosus* (Cock, *et al.*, 2010), *Hyaloperonospora arabidopsidis* (Baxter, *et al.*, 2010), *Phytophthora capsici* (These sequence data were produced by the US Department of Energy Joint Genome Institute <http://www.jgi.doe.gov/> in collaboration with the user community), *Phytophthora infestans* (*Phytophthora infestans* Sequencing Project, Broad Institute of Harvard and MIT, <http://www.broadinstitute.org/>), *Phytophthora ramorum*, *Phytophthora sojae* (Tyler, *et al.*, 2006), *Pythium aphanidermatum* (C.R. Buell, unpublished), *Pythium ultimum* (Lévesque, *et al.*, 2010), and *Saprolegnia parasitica* (*Saprolegnia parasitica* Sequencing Project, Broad Institute of Harvard and MIT, <http://www.broadinstitute.org/>).

Using the tBLASTn algorithm to search a translated nucleotide database with protein query sequences, the genome assembly of *Pythium ultimum* DAOM BR 144 was searched for orthologues of known flagellar proteins or flagellum-associated proteins retrieved from GenBank. The majority of query protein sequences were from the green alga *Chlamydomonas reinhardtii*, and others were from the golden alga *Ochromonas danica*, as well as various animals such as *Homo sapiens* and the mouse *Mus musculus*. Significant tBLASTn matches from *P. ultimum* were annotated based on query protein description. Both the whole genome assembly and the predicted gene models of *P. ultimum* were inspected to produce the most accurate annotation of each flagellar gene. Comparative genomics of flagellar gene repertoires was performed using the genome sequences of *P. ultimum*, *Phytophthora infestans*, *Phytophthora ramorum*, *Phytophthora sojae*, and *Hyaloperonospora arabidopsidis*.

4.2.2 *Pythium ultimum* genome assembly and mRNA transcription profiling

P. ultimum (DAOM BR144 = CBS 805.95 = ATCC 200006) was sequenced using a whole-genome shotgun sequencing approach. Sequencing reads from three Sanger libraries and three full runs of 454 FLX pyrosequencing were assembled by a pipeline (Lévesque, *et al.*, 2010) and annotated using the MAKER program (Cantarel, *et al.*, 2008). The gene expression profile of *P. ultimum* was derived from various growth conditions described by Lévesque *et al.* (2010). cDNA libraries were reverse-transcribed from mRNA extract and sequenced by Illumina Genome Analyzer (GA) II. Expression values were calculated using reads per kilobase of exon model per million mapped reads (RPKM) (Mortazavi, *et al.*, 2008).

4.2.3 DNA extraction

For cultures grown from the Centraalbureau voor Schimmelcultures (CBS isolates) and the Canadian Collection of Fungal Cultures (BR isolates), DNA extractions were performed as previously described (Robideau, *et al.*, 2011). DNA extraction from *Plasmopara halstedii* (Lev 5881 isolate) was identical to BR isolate extractions except that the starting material was ~150 µl of crushed dried *Helianthus annuus* leaves that were infected with *P. halstedii* race 2 (Rashid, 1993).

4.2.4 DNA amplification

PCR primers were designed based on alignments of predicted PF16 and OCM1 sequences from assembled *Phytophthora*, *Pythium*, and *Saprolegnia* genomes (*Ph. infestans*, *Ph. ramorum*, *Ph. sojae*, *Py. aphanidermatum*, *Py. ultimum*, and *S. parasitica*). Standard primers designed for PF16 amplification were ACAup13 (5'-TCCGTGCTSCAGCAYTTYGAGGTGTA-3') and ACAlo1475 (5'-TGHGGCGAGTAGTACTCGACGATCTC-3'). Standard primers designed for OCM1 amplification were OCM1up130B (5'-ATGTGCRAMTGCTAYAARAACACTAC-3') and OCM1lo1842B (5'-CARTTRTCGTTVGTGTADCCCTTG-3'). Alternative primers were designed which amplified species that did not amplify well with the standard primers. Alternative OCM1 primers were OCM1up124 (5'-AAGGAYATGTGCAACTGCTA-3') and OCM1lo1853 (5'-TCTGBGTGTYRCARTTGTCGTT-3'). *Pythium insidiosum* OCM1 sequences were amplified using OCM1oomup1 (5'-TWYAARAACACTACVHVGGVAACGACTG-3') and OCM1oomlo1 (5'-

TGBRYRTYRCARTTSTCRRTTVGTGTA-3'). *Pythium porphyrae* OCM1 sequences were amplified with OCM1up145B (5'-RRRSTWCVWRGSSAACGACTGC-3') and OCM1lo1842B. *Apodachlya brachynema* OCM1 sequences were amplified with OCM1Oomup2C (5'-TGYVDVSMNHRNGATATGTGC-3') and OCM1Oomlo1E (5'-TCRYTNGTRTANCCTTTGAAACA-3'). *Dictyuchus monosporus* OCM1 sequences were amplified with OCM1Oomup2D (5'-TGYVDVSMNHRNGATATGTGT-3') and OCM1Oomlo1A (5'-TCRYTNGTRTANCCTTTGAAGCA-3'). PCR volume was 10 μ L containing final concentrations of 1X Titanium Taq buffer (with 3.5 mM $MgCl_2$), 0.1 mM dNTPs, 0.08 μ M each of forward and reverse primer, 0.5X Titanium Taq polymerase, and approximately 0.1 - 1.0 ng/ μ L of DNA. Reaction volume was brought up to 10 μ L with sterile HPLC water. Thermocycler program for amplification of PF16 was: 95°C for 3 min followed by 40 cycles of 95°C for 30 s, 69°C for 45 s, 72°C for 1 min 30 s. A final extension was made at 72°C for 10 min. Program for amplification of OCM1 was: 95°C for 3 min followed by 40 cycles of 95°C for 30 s, 60°C for 45 s, 72°C for 2 min. A final extension was made at 72°C for 10 min.

4.2.5 PCR product isolation

PCR products were run on 1.0% agarose gel in 0.5X TBE buffer at 60 V for 40 min to verify presence of a single band at desired length (~1.5 kb for PF16, ~1.8 kb for OCM1). In a few samples the desired band was not present, or multiple bands were present in the PCR product. In such cases, the PCR was repeated using a more robust PCR mixture containing final concentrations of 1X Titanium Taq buffer (with 3.5 mM $MgCl_2$), 0.2 mM dNTPs, 0.5 μ M each of forward and reverse primer, 1X Titanium Taq

polymerase, and approximately 0.1 - 1.0 ng/uL of DNA. Reaction volume was brought up to 20 µL with sterile HPLC water. The desired band was then isolated from the PCR product using E-Gel® CloneWell™ 0.8% SYBR Safe™ gels run in the E-Gel® iBase™ Power System (Invitrogen).

4.2.6 Sequencing

Sequencing of PCR products was done with ABI Big Dye Terminator v3.1 in a reaction volume of 10 µL, with Big Dye Seq Mix diluted 1:8 with Seq buffer. Final concentrations of each reagent were 0.875X Sequencing buffer, 5% trehalose, 0.125X Big Dye Seq Mix, and 0.16 µM primer. Reaction volume was brought to 10 µL with sterile HPLC water and either 1 µL of PCR product directly from initial PCR amplification or 1 µL of Clonewell™ isolated PCR product was added. Forward and reverse sequences were generated for each gene using internal primers in addition to the standard PCR primers. Sequencing primers used for PF16 were ACAup13, ACAup109 (5'- ATGCAGAAYGCMGGYGTGATGCAG-3'), ACAup156 (5'- CAACGTGCCVAGYATCCAGCAGTC-3'), ACAup829PP (5'- GARRTYGCNAAGCACAC-3'), ACAlo800 (5'- ACRGTGTGGTCRATRTCCTTGAG-3'), ACAlo854PP (5'- AGYTCNCGNGTGTGCTT-3'), ACAlo1445PP (5'- GGGTARCAGTTGTTGAT-3'), ACAlo1453 (5'- TCTCVGGBGGGTARCAGTTGTTGA-3'), ACAlo1472 (5'- GGCGAGTAGTACTCGACGATCTC-3'). Primers designed to sequence PF16 from *Aphanomyces cladogamus* and *A. cochlioides* were ACAsapup1 (5'- TNCCBAGCATCCARCARTCGGCGG-3'), ACAsapup3 (5'-

CAWYACBACMAARGGYATYGC GCC-3'), ACAsaplo1 (5'-
 ACTCGACRATYTC SGGBGGGTAGC-3'), and ACAsaplo3 (5'-
 SGCRC TSGCNGCCTTRATGTGGTC-3'). Sequencing primers used for OCM1 were
 OCM1up124, OCM1up130B, OCM1up145B, OCM1up331 (5'-
 GSSCA YTTYTAYATGGARTGCTC-3'), OCM1up631 (5'-
 TGCAARRTBGGYGTKGATCC-3'), OCM1lo643 (5'-
 GGATCMACRCCVAYYTTGCA-3'), OCM1lo1842B, OCM1lo1853. Primers designed
 to sequence OCM1 from *Pythium insidiosum* and *P. porphyrae* were OCM1ipup2 (5'-
 GATCCTCRKCAACTCGCAGCA-3') and OCM1iplo1 (5'-
 AGCACTCGGASACGTAGTTGTA-3'). OCM1 was sequenced from *Apodachlya*
brachynema and *Dictyuchus monosporus* using their respective OCM1 PCR primers.
 Thermocycler program for both PF16 and OCM1 was: 95°C for 3 min followed by 40
 cycles of 95°C for 30 s, 55°C for 40 s, 60°C for 4 min. Most sequences of LSU used in
 this study have been previously published, otherwise they were amplified and sequenced
 as previously reported (Robideau, *et al.*, 2011).

DNA sequences were generated from sequencing amplification reactions using the
 ABI Prism 3130xl Genetic analyzer. GenBank accession numbers of DNA sequences are
 found in Appendix F.

4.2.7 Sequence assembly and alignment

DNA sequences were assembled using Seqman (DNASar). Introns were detected by
 aligning translated sequences to predicted protein sequences from the *Pythium ultimum*
 genome assembly. All detected introns contained canonical splice sites. Introns were

removed if present and coding DNA sequences were translated using BioEdit (Hall, 1999). PF16 sequences were aligned based on amino acid sequence using ClustalW. Four isolates in the study did not yield PF16 sequences due to lack of PCR amplification. Therefore the final concatenated alignment contained only OCM1 sequences for *Aphanomyces euteiches*, *Apodachlya brachynema*, *Plasmopara halstedii*, and *Pythium grandisporangium*. OCM1 sequences were aligned by first generating a rough alignment of amino acid sequences using MAFFT (G-INS-i algorithm). The relatively conserved amino acid sequence at the 5' end of the alignment (first 212 characters) was used to generate a neighbour joining guide tree in Paup 4.0b10. Guide tree was rooted with *Ectocarpus* as outgroup and then converted to MAFFT format using the newick2mafft ruby script distributed by MAFFT. Guide tree was used in G-INS-i alignment of full OCM1 amino acid sequences. OCM1 amino acid alignment was reverse translated to DNA alignment using RevTrans 1.4 server (Center for Biological Sequence Analysis). Sites containing gaps in > 95% of the taxa were deleted from the OCM1 alignment. After alignment of each gene, PF16 and OCM1 alignments were concatenated for phylogenetic analysis. LSU sequences were aligned using MAFFT under default settings of G-INS-i.

4.2.8 Sequence distances

Amino acid distance matrices were calculated in PHYLIP using the PROTDIST v3.68 program under the Jones-Taylor-Thornton mutation matrix. Mean amino acid distances including standard deviation were calculated from intraspecific and interspecific pairwise comparisons. Intraspecific amino acid distances were calculated for *Phytophthora infestans* (3 isolates), *Phytophthora sojae* (6 isolates), *Pythium irregulare*

(6 isolates), and *Pythium oligandrum* (3 isolates). Interspecific distances were calculated by comparing each of the above mentioned species with the two closest relatives included in this study. Mean amino acid distance of the entire dataset was also calculated from interspecific pairwise comparisons of all isolates. DNA distance matrices were calculated in PHYLIP using the DNADIST v3.68 program under the Kimura 2-parameter model. Mean DNA distance was calculated from interspecific pairwise comparisons of all isolates.

4.2.9 *Phylogenetic inference and distribution of selection coefficients*

We used the site-heterogeneous mutation-selection model based on the Dirichlet process (Rodrigue, *et al.*, 2010), implemented in the MPI version of PhyloBayes (Lartillot *et al.*, submitted), to infer the phylogeny based on the concatenation of the PF16 and OCM1 genes. We ran two independent Markov chain Monte Carlo calculations for 11,000 cycles (each including multiple updates on the tree topology, branch lengths, and substitution model parameters), discarded the first 1,000 draws as burn-in, and used the remaining to obtain the 60% consensus tree. The largest discrepancy of bipartition frequencies between each independent chain (maxdiff) was ~0.2, meaning the two chains had reached reasonable convergence (Lartillot, *et al.*, 2009).

The mutation-selection model utilized allows for the calculation of site-specific distribution of selection coefficients of mutations at stationarity under the evolutionary process (see, e.g., Yang & Nielsen, 2008). We averaged these distributions across sites of the PF16 and OCM1 segments of the alignment separately, in order to summarize the strength of selection operating on each gene.

PF16-OCM1 phylogenies were also constructed using nucleotide and amino acid substitution models in Phylobayes 3.3b (Lartillot, *et al.*, 2009). The PF16-OCM1 alignment was analyzed in both nucleotide and amino acid form under the CAT-GTR model by running two simultaneous chains. Constant sites were deleted, and sites containing gaps for > 95% of the taxa were also deleted. In both the nucleotide and amino acid analyses, the maxdiff was below 0.2. The burn-in used to create the consensus tree was 1/5th of the total cycles. The total number of cycles was 9000 in the nucleotide analysis, and 4500 in the amino acid analysis. LSU phylogeny was constructed using maximum likelihood with Phym1 3.0 (Guindon, *et al.*, 2010) under the GTR model with default settings and bootstrap analysis of 1000 replicates. Additional support of maximum likelihood tree topology was obtained by Bayesian MCMC sampling using Phylobayes. LSU alignment was analyzed under the CAT-GTR model by running two simultaneous chains until the largest discrepancy of bipartition frequencies between the two independent runs was below 0.3. The burn-in used to create the consensus tree was 1/5th of the total 6000 cycles.

4.2.10 Evolutionary rate of codon positions

Evolutionary rates of each codon position in PF16 and OCM1 were calculated in MEGA5. The rates were scaled such that the average evolutionary rate across all sites was 1, meaning that sites showing a rate < 1 are evolving slower than average, and those with a rate > 1 are evolving faster than average. These relative rates were estimated under the General Time Reversible model. Mean evolutionary rate of each codon position in

each gene was determined and rate comparison between genes was performed by unpaired t-test.

4.3 Results

4.3.1 Comparative genomics and *P. ultimum* flagellar gene expression

The 43 megabase genome of *Pythium ultimum* contains over 15,000 genes (Lévesque, *et al.*, 2010). Overall, approximately 80 putative whiplash and tinsel flagellum gene orthologs were identified in *P. ultimum*, with corresponding orthologs present in *P. infestans*, *P. sojae*, and *P. ramorum* (Appendix E). Putative orthologs were defined as those yielding a tBLASTn expect value (E-value) less than $1.0E^{-10}$. The E-value describes the likelihood that another sequence of equivalent match will occur in the database by chance. Low E-values therefore indicate significant matches. Most flagellar genes were absent in *H. arabidopsidis* as evidenced by the relatively high E-value for most flagellar gene queries. Gene expression analysis of *P. ultimum* flagellar orthologs in zoospore-inducing growth conditions of hypoxia and *Arabidopsis* infection showed that many flagellar orthologs were expressed under these conditions, although several putative flagellar orthologs for axonemal dynein and kinesin and intraflagellar transport did not show expression (Appendix E). Expression was quantified by the RPKM value, which is assumed to have a positive correlation to the level of expression, and a value of zero indicates no detectable expression.

Significant matches to OCM1 and PF16 were found in all the oomycetes searched except *H. arabidopsidis*. These genes were found to be particularly amenable to further phylogenetic study due to their length, apparent lack of introns, and conserved terminal

sequences. This enabled design of PCR primers which amplified nearly the entire genes from a wide variety of oomycetes.

4.3.2 *Primer performance*

The standard PF16 primers were very robust in *Pythium*, *Phytophthora*, *Halophytophthora*, *Phytopythium*, *Dictyuchus*, *Lagenidium*, and *Myzocytiopsis*. These primers were less robust in *Achyla*, *Aphanomyces*, and *Saprolegnia*, amplifying some but not all species attempted. The primers did not amplify PF16 from *Apodachlya* or *Plasmopara*. Similar to PF16, the standard OCM1 primers were generally robust in *Pythium*, *Phytophthora*, *Halophytophthora*, *Phytopythium*, *Lagenidium*, and *Myzocytiopsis*, although some *Phytophthora*, *Phytopythium* and *Pythium* species did not amplify. Interestingly, the standard OCM1 primers yielded robust amplification of *Aphanomyces* and *Plasmopara*. They amplified some but not all species attempted from *Achyla* and *Saprolegnia*. The primers did not amplify OCM1 from *Apodachlya* or *Dictyuchus*. Amplification of OCM1 and PF16 from *Albugo* was not attempted, as the sequences used in this study were obtained from whole genome sequencing. The complete list of isolates used along with DNA sequence accessibility information is shown in Appendix F.

4.3.3 *Sequence features*

As determined from the available genome sequences, the predicted amino acid sequence length of PF16 was uniform at 509 amino acids among all species. The predicted sequence length of OCM1 in contrast was variable, ranging from 551 to 706

amino acids. The length variation occurred predominantly in the C-terminal half of the OCM1 amino acid sequences. Sequence alignments of PF16 and OCM1 are depicted in Appendix G. N-terminal signal peptides were not present in PF16 sequences, but were identified in each genomic OCM1 sequence using the SignalP 4.0 server (Petersen, *et al.*, 2011). Size of the OCM1 signal peptide sequences ranged from 17 to 27 amino acids. Stop codons in PF16 or OCM1 were found in three species of *Pythium*. A stop codon was present in the reading frame of PF16 from *Pythium splendens* and *Pythium rhizosaccharum*, as well as in the reading frame of OCM1 from *Pythium violae*. Predicted introns were present in the PF16 sequences of both *Achlya* spp., *Dictyuchus monosporus*, all three *Saprolegnia* spp., *Ectocarpus siliculosus*, and *Aphanomyces astaci*. The full genomic PF16 sequence of *Ectocarpus* contained 11 predicted introns, whereas the PCR amplified partial PF16 sequences of the other species contained two predicted introns, occurring at the same amino acid positions in each species. Predicted intron presence in OCM1 was limited to *Ectocarpus siliculosus* and *Aphanomyces astaci*. The full genomic OCM1 sequence of *Ectocarpus* contained 5 predicted introns, whereas the PCR amplified partial OCM1 sequence of *A. astaci* contained only one predicted intron. The amino acid sequences of PF16 and OCM1 were searched for conserved protein domains using InterProScan 4.8 (European Bioinformatics Institute). PF16 contained Armadillo repeats whereas OCM1 contained four Epidermal Growth Factor - like (EGF) domains. A representative diagram of PF16 and OCM1 amino acid sequences including relative positions of PCR primers is shown in Figure 4.1. The percent homology among the repeats within the different sequences was not determined.

4.3.4 *Sequence evolution*

Estimations of distributions of selection coefficients under the mutation-selection model indicate that PF16 is evolving under stronger purifying selection than OCM1 since the distribution of scaled selection coefficients for PF16 is centered at a lower value of S than that of OCM1 (Fig. 4.2). The majority of codon sites in each gene are subject to purifying selection ($S < 0$), but the values of S were distributed closer to 0 in OCM1. The mean rates of nucleotide evolution at the first and second codon positions were significantly higher in OCM1 than in PF16 ($p < 0.0001$). Conversely, the mean rate of evolution at the third codon position was significantly higher in PF16 ($p < 0.0001$). The rate of evolution *within* each gene was significantly different between all codon positions ($p < 0.0001$) (Fig. 4.3).

4.3.5 *Species delimitation*

Interspecific sequence distances were higher in OCM1 than in PF16. The mean interspecific amino acid distance in OCM1 was 1.354 versus 0.097 in PF16. The mean interspecific nucleotide distance in OCM1 was 0.79 versus 0.223 in PF16. The amino acid sequences of PF16 were highly conserved within and between species, meaning the utility of PF16 in species delimitation is limited to the DNA sequence level. Amino acid sequences of OCM1 were highly conserved within species, but varied significantly between species (Fig. 4.4), showing that OCM1 is a useful marker of species boundaries in oomycetes at both the amino acid and DNA level.

Figure 4.1. Schematic illustration of PF16 and OCM1 protein sequences displaying conserved protein domains and relative positions of PCR primers.

Figure 4.2. Distribution of scaled selection coefficients (S) from sequence alignments of PF16 and OCM1.

Figure 4.3. Mean evolutionary rate of each codon position in PF16 and OCM1. Difference between PF16 and OCM1 was significant ($p < 0.0001$) at all three codon positions. Error bars represent standard deviation of evolutionary rate among sampled codons.

Figure 4.4. Intraspecific and interspecific amino acid distances of PF16 (A) and OCM1 (B). Distances are represented by the distance between grey and black symbols in each pairwise comparison. Scale indicates the fraction of amino acids differing in pairwise sequence alignments. Each displayed distance is an average over pairwise comparisons of multiple isolates from each species and standard deviations are shown with error bars. Only species names are displayed. The species *infestans*, *sojae* and their interspecific counterparts are members of the genus *Phytophthora*. The species *irregulare*, *oligandrum* and their interspecific counterparts are members of the genus *Pythium*.

4.3.6 Phylogeny reconstruction

The Bayesian phylogram produced by the codon-based mutation-selection model using PF16 and OCM1 showed clade structures that reflect zoospore ontology (Fig. 4.5). The distinction between zoospore dimorphism and monomorphism is evident, as is the difference between monomorphic taxa whose zoospore cleavage is intrasporangial versus extrasporangial. Sporangial morphology of *Pythium* species is also represented in the tree topology, and the suppression of zoospore production in *Pythium* species is confined to the globose sporangia clade. The overall topology of the PF16-OCM1 tree is comparable to that of the LSU tree (Fig. 4.6), which represents the currently accepted view of oomycete organismal evolution. The genera *Aphanomyces*, *Phytophthora*, and *Pythium* have been previously divided into phylogenetic clades (Lévesque & de Cock, 2004, Blair, *et al.*, 2008, Dieguez-Uribeondo, *et al.*, 2009), which are represented in the codon model PF16-OCM1 tree. Compared to the codon model tree, the PF16-OCM1 trees constructed by nucleotide and amino acid substitution models introduced alternative clade structures and polyphyly into the tree topology (Fig. 4.7).

Figure 4.5. Illustrated Bayesian codon model phylogram from a concatenated alignment of PF16 and OCM1 DNA sequences. Posterior probabilities are displayed along branches. Branches with posterior probability < 0.6 have been collapsed.

Figure 4.6. Phylogram reconstructed from LSU sequences of each species represented in the study. Tree is based on maximum likelihood analysis, with major nodes showing support values from maximum likelihood bootstrap/bayesian posterior probability.

A) Codon substitution model

B) Amino acid substitution model

C) Nucleotide substitution model

Figure 4.7. Colour-coded comparison between codon (A), nucleotide (B), and amino acid (C) Bayesian substitution model phylogenies from concatenated alignment of PF16 and OCM1. Grey branches in B and C represent unchanged clades with respect to A. Coloured clades in B and C represent clades where polyphyly is introduced in nucleotide and amino acid models.

4.4 Discussion

4.4.1 Overview

Through comparative genomics and identification of putative oomycete flagellar genes in *Pythium* and *Phytophthora*, this study introduces novel protein-coding taxonomic markers to oomycete systematics. Previous studies have used nuclear protein-coding markers to study either a single genus (Blair, *et al.*, 2008) or to study several modestly sampled genera (Blum, *et al.*, 2012). Presented here is the first report of using protein-coding nuclear genes to study molecular evolution across a broad range of well-sampled oomycete genera. Also demonstrated is the utility of a recently developed codon-based model for studying long-range patterns of molecular evolution (Rodrigue, *et al.*, 2010). The biological realism of codon substitution modeling is shown by the congruence between reproductive morphology and flagellar gene evolution. The accuracy of the codon model in phylogenetic reconstruction is also demonstrated by the congruence of the codon model tree with well-established theories of oomycete evolution. In addition to systematic and phylogenetic analyses, the molecular evolution of PF16 and OCM1 was examined to provide context to the discussion of their known and plausible functions.

4.4.2 *Pythium ultimum* flagellar gene repertoire

P. ultimum does not typically exhibit release of zoospores from sporangia in culture (van der Plaats-Niterink, 1981) but zoospore release directly from aged oospores has been reported (Dreschler, 1946). Comparative genomics with well studied whiplash

flagellar proteins from the green algae *Chlamydomonas reinhardtii* and other model organisms indicates that indeed *P. ultimum* does have the necessary genetic complement for flagella. Orthologs of tinsel flagellar mastigoneme proteins have also been identified in *P. ultimum* through comparison to those studied in the golden alga *Ochromonas danica*.

4.4.3 *Phylogram topology and zoospore ontology*

Asexual reproduction via the release of zoospores from sporangia is arguably the most informative and defining morphological feature of oomycetes (Sparrow, 1960, Johnson, *et al.*, 2002). The phylogram reconstructed from our analysis displays congruence between flagellar gene evolution and zoospore ontology in oomycetes (Fig. 4.5). Such a connection between genotype and phenotype has been previously shown in primates, where the evolution of a semen gene is significantly associated with mating systems (O'Connor & Mundy, 2009). Similarly, the molecular evolution of tissue-specific genes in chordates correlates well with the fossil record of morphological tissue evolution (Iwabe, *et al.*, 1996). Correlation of genotypic and phenotypic changes in phylogenetic reconstruction is recognized as a means for detecting functionally evolving genes such as those involved in disease resistance (Habib, *et al.*, 2007) or viral phenotype (Parker, *et al.*, 2008), so the evolution of flagellar genes in our study may reflect a functional evolution of PF16 and/or OCM1.

4.4.4 *Evolution and function of PF16 and OCM1*

The two flagellar genes in our study have been shown to differ in their patterns of molecular evolution. These genes are located on disparate genomic scaffolds, which precludes genetic linkage and implies independent assortment. Despite their differing evolutionary patterns, concatenation of PF16 and OCM1 genes was considered appropriate for analysis with the mutation-selection model and the CAT-GTR model due to the fact that these models account for heterogeneity of the amino acid and nucleotide replacement process across the positions of an alignment, therefore accounting for the heterogeneous regimes of selection that would occur in the concatenated alignment. PF16 is predicted to be highly conserved at the amino acid level, which was evident in the low rates of DNA sequence evolution in the first and second codon positions. The third codon position of PF16 had a much higher rate of evolution, which is expected in genes that are subject to purifying selection since variation at the third codon position can occur with less impact on the amino acid translation (Graur & Li, 2000). It is not surprising that PF16 is under such constraint, given that this protein has an essential role in flagellar bending and is an integral component of the flagellar axoneme, a structure which has been highly conserved over the course of eukaryotic evolution (Inaba, 2011). In contrast to PF16, OCM1 is predicted to be highly variable at the amino acid level. This translated into a scaled selection coefficient distribution with values of S around 0, implying that OCM1 is under relaxed selection. Relaxed selection is a process by which the constraint on genetic mutation is relaxed, allowing divergence to occur. This is different than positive selection, where advantageous mutations are selectively fixed. Under relaxed selection, neutral mutations are more likely to become fixed. Relaxed selection can be the result of gene duplication, whereby the functional redundancy of a duplicated gene

permits mutation, and the mutation may ultimately lead to new interactions of the duplicated gene, causing renewed selective pressure favouring synergistic interactions of the mutated duplicate gene (Deacon, 2010). Relaxed selection therefore contributes to phenotypic plasticity, and genes evolving under relaxed selection are more likely to be differentially expressed between developmental stages (Hunt, *et al.*, 2011). The stage-specific expression of OCM1 during asexual sporulation (Randall, *et al.*, 2005) is therefore a trait that this gene shares with other genes evolving under relaxed selection.

If OCM1 is subject to relaxed selection, this brings into question the true function of this protein in oomycetes. Early work on tinsel flagellum mastigonemes had concluded that they are mechanical appendages, responsible for reversal of thrust to propel zoospores forward (Markey & Bouck, 1977, Cahill, *et al.*, 1996). Further investigation showed calmodulin to be present in tinsel flagella of *Phytophthora* (Gubler, *et al.*, 1990), implying that tinsel flagella may function somehow in calcium signaling. Such signaling could be an indication of a sensory or recognition capacity of tinsel flagella. The ortholog of OCM1 in the diatom *Thalassiosira* is thought to be involved in gamete recognition (Armbrust & Galindo, 2001), and the tinsel flagella of brown algae zoospores are involved in substrate binding (Toth, 1976), while the tinsel flagella of spermatozoids are involved in attachment to the egg surface (Friedmann, 1961). Therefore, it is plausible that OCM1 could be involved in host recognition and/or attachment in oomycete zoospores. The presence of multiple EGF domains in OCM1 also implies this, as the EGF domain is commonly found in extracellular proteins and is involved in cell recognition (Campbell, *et al.*, 1990). Recent work has been done to characterize a mastigoneme

protein in *Phytophthora* (Blackman, *et al.*, 2011), but more research is required to further elucidate the role of tinsel flagella and mastigoneme proteins in the oomycete zoospore.

4.4.5 Oomycete systematics

From a systematics standpoint, the evolution of PF16 and OCM1 as modeled here (Fig. 4.5) is in general accord with the views of oomycete evolution, and the tree sustains previously determined clade organization within the genera *Aphanomyces*, *Phytophthora*, and *Pythium* (Lévesque & de Cock, 2004, Blair, *et al.*, 2008, Dieguez-Uribeondo, *et al.*, 2009). A broad phylogeny of oomycetes using additional protein coding genes still remains to be constructed, since additional genes can alleviate stochastic error caused by lack of signal in the dataset (Rokas & Carroll, 2005). A multigene approach has been used with success in the oomycete genus *Phytophthora* (Blair, *et al.*, 2008), but difficulty in amplifying these protein coding genes, even within *Phytophthora*, is a limiting factor that applies to multigene phylogenies in general. One reason why LSU has proven so useful in phylogenetic studies is its ease of amplification from diverse taxa. Now that PF16 and OCM1 sequences have been obtained from diverse oomycete taxa, our tree illustrates some notable points to consider with respect to current views. The placement of *Albugo* as a sister clade to *Pythium* does not agree with the LSU placement of *Albugo* as an early-diverging Peronosporomycete genus which has been circumscribed to a separate order (Thines & Spring, 2005). This is possibly due to long-branch attraction in the dataset. Addition of another early-diverging genus to the dataset such as *Sapromyces* may have been able to alleviate such long-branch attraction, but attempts to sequence PF16 and OCM1 from *Sapromyces elongatus* were unsuccessful. Also of note is the

placement of *Phytopythium* as a sister clade to *Pythium* (Pythiaceae). This is in contrast to *Phytopythium*'s recent assignment to the family Peronosporaceae (Hulvey, *et al.*, 2010). Although *Phytopythium* does share extrasporangial zoospore cleavage with the Pythiaceae, it has other characteristics such as papillate sporangia that support its inclusion in the Peronosporaceae. Our analyses placed *Halophytophthora operculata* within *Phytopythium*, affirming the known polyphyletic nature of the genus *Halophytophthora*, but *H. operculata* actually exhibits *Phytophthora*-like intrasporangial zoospore cleavage rather than the extrasporangial zoospore cleavage seen in other *Phytopythium* species. This further exemplifies the true intermediary nature of the genus *Phytopythium* between *Phytophthora* and *Pythium*. Like *Phytopythium*, Clade H *Pythium* species are represented as a sister clade to the rest of the genus *Pythium*. This is underlain by the unique sporangial morphology of Clade H, which led to its recent proposition as a new genus *Elongisporangium* (Uzuhashi, *et al.*, 2010). Another interesting observation in Fig. 4.5 is the suppression of zoospore production in many species of *Pythium* within the globose sporangia clade. This is exemplified by the apparent loss of function of PF16 or OCM1 in a few members of this clade, implied by the presence of internal stop codons in these genes. Ecological studies of *Pythium* have shown that freshwater habitats are dominated by species of *Pythium* with filamentous sporangia (Hallett & Dick, 1981, Abdelzaher, *et al.*, 1995), whereas terrestrial habitats are dominated by *Pythium* species with globose sporangia (Ali-Shtayeh, *et al.*, 1986). Apparently the transition of globose *Pythium* species to a terrestrial lifestyle has lessened their dependency on zoospore dissemination to find suitable hosts or substrates. Such evolution and flexibility of dissemination strategies is common in oomycetes (Jeger & Pautasso, 2008). The amino

acid sequences of the flagellar genes containing internal stop codons have not significantly diverged from those of closely-related zoospore-producing species, indicating that these mutations probably occurred recently on an evolutionary timescale. In the phylogenetic framework of the mutation-selection model used in the current study, the mutation of an amino acid to a stop codon violates the assumption that the regime of purifying selection at a coding site is constant over time, although this violation appears to be confined to the individual stop codon sites since sites surrounding the stop codons have not significantly diverged.

In terms of the utility of flagellar genes for species delimitation, OCM1 is clearly a useful addition to the oomycete molecular toolbox, showing low intraspecific variation coupled with high interspecific variation. PF16 also has low variability within species, and its DNA variation between species is at par with that of currently utilized DNA barcodes for oomycetes (Robideau, *et al.*, 2011). Although OCM1 and PF16 are not always as easily PCR-amplified as the barcodes COI and ITS, they do have potential as markers of asexual sporulation in mRNA sequencing/detection assays. The expression of these genes in *Phytophthora infestans* has been shown to occur within developing sporangia, and is induced by nitrogen starvation (Randall, *et al.*, 2005), a condition which is expected to encourage dissemination of zoospores. This stage-specific expression of PF16 and OCM1 could be applicable in monitoring asexual sporulation in the field or greenhouse. For example, the transcription profile (transcriptome) of a diverse oomycete population could potentially be probed using PF16 and OCM1 primers to determine the conditions where species of interest are releasing zoospores, thereby creating disease pressure.

4.4.6 *Conclusion*

The molecular evolution of PF16 and OCM1 provides new insight into oomycete systematics and the evolution of flagellar function in oomycetes. There is potential for these novel molecular markers in future research, as we have demonstrated that PF16 and OCM1 are useful taxonomic and phylogenetic markers whose stage-specific expression in the life cycle could be applicable to studies of oomycete epidemiology. These flagellar genes are expected to be present in the genomes of all zoospore-producing oomycetes, and they can be amplified from a wide range of oomycete genera using the PCR primers introduced here. This study also provides a compelling argument for continued development and implementation of codon model-based phylogenetic tools, which may enable the exploitation of protein-coding genes previously determined to be phylogenetically unreliable.

5 Chapter: INVESTIGATION OF THE POSSIBLE ROLE OF OOMYCETE FLAGELLAR PROTEIN AND PLANT C-TYPE LECTIN IN HOST-PATHOGEN INTERACTION

5.1 Introduction

The plant infection process of oomycete zoospores is a subject of intense interest from two standpoints: how zoospores find host plants in the environment, and how the host is penetrated at the early stage of infection. The former point involves chemotactic and electrotactic zoospore behaviour, while the latter point involves zoospore cyst adhesion and gene-for-gene interaction with the host. Zoospores are known to exhibit directional movement towards concentrated chemical compounds that are found in root exudate such as amino acids, polysaccharides, and organic compounds (Bimpong & Clerk, 1970, Donaldson & Deacon, 1993, Rand & Munden, 1993, Tyler, *et al.*, 1996, Leano, *et al.*, 1998). They are also known to migrate towards weak electric fields such as those generated by plant roots in the rhizosphere (Morris & Gow, 1993, van West, *et al.*, 2002). Upon reaching a suitable host, zoospores swim in close contact to the host before they adhere and encyst on the surface of the host. Adhesion and encystment involves orientation of the zoospore such that the ventral surface from which the flagella emerge is in contact with the host (Hardham & Gubler, 1990). Flagella are then shed or retracted (Reichle, 1969), and vesicles containing adhesive compounds are extruded from the ventral surface while the cyst wall is formed (Sing & Bartnicki-Garcia, 1975, Robold & Hardham, 2005). The orientation of zoospores prior to docking on a host is such that the flagella are expected to be in contact with the host before adhesion and encystment occur. Zoospore flagella have been observed in contact with host stomata in a study of the

downy mildews *Plasmopara* and *Pseudoperonospora* (Royle & Thomas, 1973), so it is plausible that zoospore flagella are involved in a signalling process whereby a suitable docking site on a host is sensed by the flagella, triggering the encystment process. Sensory functions of microbial flagella are well known (Mitchell, 2007, Maric, *et al.*, 2010), and the significant presence of calmodulin in the tinsel flagellum of *Phytophthora* indicates that this organelle may indeed have a sensory role (Gubler, *et al.*, 1990) since calmodulin is involved in proper functioning of sensory hair cells (Seiler & Nicolson, 1999), chemosensory organs (Spehr, *et al.*, 2009), and sensory neurons (Milikan, *et al.*, 2002).

The heterokont tinsel flagellum is somewhat of a mystery compared to the well-studied whiplash flagellum commonly found in eukaryotes. Studies of tinsel flagellum function have been sparse, implying the mechanical nature of this appendage in zoospore motility (Markey & Bouck, 1977, Cahill, *et al.*, 1996), but also implying the role of the tinsel flagellum in substrate attachment and encystment (Friedmann, 1961, Toth, 1976, Hardham & Suzaki, 1986, Jones, *et al.*, 1991). Recently, four mastigoneme proteins from the tinsel flagellum of golden algae were isolated and characterized (Yamagishi, *et al.*, 2007, Yamagishi, *et al.*, 2009), providing much needed molecular evidence of tinsel flagellum composition. Orthologues of three of the four golden algae proteins, OCM1, OCM2, and OCM4, are found in oomycetes. It is assumed that these predicted mastigoneme proteins are extracellular, since the mastigonemes of golden algae are found on the surface of the flagellum (Bouck, 1971). The oomycete orthologues contain epidermal growth factor (EGF) – like domains and share similarity with vertebrate tenascin proteins. Tenascins are extracellular matrix glycoproteins with various functions,

one of which involves calcium-mediated interaction with C-type lectins (Aspberg, *et al.*, 1995, Aspberg, *et al.*, 1997, Lundell, *et al.*, 2004). If the tinsel flagellum is somehow involved in host recognition, then perhaps the tenascin-like proteins of the mastigonemes are involved in an interaction with host C-type lectin.

C-type lectins are calcium-dependent carbohydrate-binding proteins which selectively bind a wide variety of ligands in the presence of calcium (Zelensky & Gready, 2005). C-type lectins are particularly well known for their role in immune systems (Weis, *et al.*, 1998). C-type lectins are responsible for pathogen detection and the initiation of immune response, but in some cases these lectins are targeted by pathogens as a binding site for entry into the host (Alvarez, *et al.*, 2002, Takada, *et al.*, 2004, de Witte, *et al.*, 2006, Gunn, *et al.*, 2012). While there are many different C-type lectins found in animals, plants typically contain only one or two C-type lectins (Jiang, *et al.*, 2010) whose function in the plant is currently unknown (Bouwmeester & Govers, 2009). Plant C-type lectins consist of an extracellular C-type lectin domain, a transmembrane helix, and an intracellular kinase domain. In *Arabidopsis thaliana* there is only one predicted C-type lectin. A representative diagram of the *A. thaliana* C-type lectin is given in Figure 5.1.

The hypothesis that plant C-type lectin is targeted as a binding site by oomycete zoospores is plausible and is substantiated by the fact that during the encystment process, zoospores extrude calcium (Irving & Grant, 1984), which would be necessary for binding with C-type lectin. Recognition of C-type lectin by zoospores may also enable differentiation between host and non-host plants, since lectins have long been suggested as determinants of host specificity (Sequeira, 1978). To test the hypothesized interaction between oomycete mastigoneme protein and plant C-type lectin, several experiments

have been performed involving purification of recombinant OCM1 protein from *Pythium aphanidermatum*, as well as recombinant C-type lectin from cucumber *Cucumis sativus* and wheat *Triticum aestivum*. Recombinant OCM1 protein was used to treat cucumber seedlings to induce a potential defense response. Recombinant C-type lectin from cucumber and wheat were used to test a possible interaction with zoospores of *Pythium aphanidermatum* and *Pythium arrhenomanes*, under the assumption that zoospores of these two species would react differentially to the two lectins since *P. aphanidermatum* is more aggressive on cucumber whereas *P. arrhenomanes* is more aggressive on wheat. A transgenic *Arabidopsis thaliana* knockout line lacking C-type lectin expression was also employed in pathogenicity trials to assess susceptibility to infection by *P. aphanidermatum* zoospores. The experiments described herein test the hypotheses that A) OCM1 may be involved in host-pathogen interaction, and therefore may be recognized by the plant host, inducing a host response, B) plant C-type lectin may be recognized by oomycete zoospores, inducing encystment, possibly in a host-specific manner, C) this recognition may be mediated by OCM1 interaction with C-type lectin, and D) the lack of C-type lectin in a host plant may decrease susceptibility to zoospore infection.

Figure 5.1. Schematic illustration of *Arabidopsis thaliana* C-type lectin gene.

5.2 Materials and Methods

5.2.1 *Pythium* cultures and zoospore production

Pythium aphanidermatum isolate DAOM BR 444 was grown on 2.5% V8 vegetable juice agar and *Pythium arrhenomanes* isolate CBS 324.62 was grown on corn meal agar (CMA). For zoospore production, two agar plugs were transferred to a petri dish containing sterile distilled water and several autoclaved grass blades. Petri dishes were incubated at room temperature under ambient light for 3 days to allow colonization of grass blades. Colonized grass blades were removed from petri dishes, rinsed in sterile distilled water, and single grass blades were placed on a fresh plate of V8 agar for *P. aphanidermatum*, or CMA for *P. arrhenomanes*. Plates were incubated at room temperature in the dark for one week to produce sporangia. To induce zoospore release, plates were flooded with sterile mineral salt solution (MSS) (1.75 mM CaCl₂, 1 mM KCl, 1mM MgSO₄, pH 6.5) and incubated at 16°C under fluorescent light. MSS was changed after one hour, and again after another hour. Plates were left overnight in the third change of MSS. Zoospores were collected the following day by pouring MSS from the plates through a sterile nylon mesh (50 µm pore). Zoospores were quantified by vortexing a 1 mL aliquot of zoospore suspension for 5 minutes to induce encystment. Encysted zoospores were then counted in a haemocytometer to estimate zoospore concentration.

5.2.2 *Plant species and growth conditions*

Plants used were cucumber (*Cucumis sativus* cv National Pickling), wheat (*Triticum aestivum* cv Chinese Spring), and *Arabidopsis thaliana* ecotype Columbia. For cucumber and wheat, seeds were surface sterilized in a 50 mL Falcon tube by gentle rocking in a 40

mL solution of 2.5% Clorox[®] bleach (which contains 6% NaOCl) and 35% EtOH for 5 min. Seeds were then rinsed three times in sterile distilled water. *Arabidopsis* seeds were surface sterilized in a 2 mL tube by inverting to mix in 1 mL of 70% EtOH solution for 5 min. EtOH solution was removed, 1 mL of 30% Clorox[®] bleach, 0.05% Tween 20 solution was added and tube was inverted to mix for 5 min. Bleach-Tween solution was removed and seeds were rinsed in five changes of sterile distilled water for 3 min each change. Seeds of all plants were stratified after sterilization by incubation for 3 days at 4°C without light. For growth of cucumber and wheat seedlings, stratified seeds were placed in sterile Magenta[™] boxes (Magenta Corporation, Chicago, IL) containing two layers of 3MM chromatography paper soaked with 5 mL of sterile distilled water. Seedlings were grown at 24°C with 18h photoperiod for 7 days before further use in pathogenicity trials, or grown for 10 days to be used for RNA extraction. Stratified *Arabidopsis* seeds were sown in autoclaved Pro-Mix BX (Premier Tech Horticulture, Rivière-du-loup, QC) and grown at 24°C with 16h photoperiod for 7 days before further use in pathogenicity trials. Verification of homozygous C-type lectin knockout in mutant *Arabidopsis* was performed by PCR using gene-specific primers and a T-DNA insert-specific primer. DNA was isolated from single leaves of transformant lines by grinding in liquid nitrogen and extracting in 200 mM Tris pH 8.0, 250 mM NaCl, 25 mM EDTA, 0.5% SDS. DNA was precipitated from extract using isopropanol and pellet was resuspended in 0.1x TE buffer containing 20 µg/mL RNase A. At1g52310 N-terminus primer LP1 (5'-TTATGAGTTCATAGCTAGTGGACC-3') and C-terminus primer RP1 (5'-GAACTTTCTTTAATACGACATCGG-3') were each used in PCR with T-DNA

insert primer LBb1 (5'-GCGTGGACCGCTTGCTGCAACT-3') to verify homozygous insertion mutagenesis.

5.2.3 Recombinant protein expression

Recombinant C-type lectin from *Cucumis sativus*, herein referred to as CsCL, and *Triticum aestivum*, herein referred to as TaCL, as well as recombinant OCM1 and PF16 from *P. aphanidermatum*, were expressed using the Gateway® *E. coli* expression system (Life Technologies Inc., Carlsbad, CA). Both CsCL and TaCL were amplified from cDNA of their respective plant, which was reverse transcribed from mRNA extract using SuperScript™ III Reverse Transcriptase (Life Technologies Inc., Carlsbad, CA). mRNA was extracted from 10-day-old seedlings using TRIzol (Life Technologies Inc., Carlsbad, CA). Only the N-terminal extracellular domain of CsCL and TaCL were amplified for expression. The transmembrane domain and intracellular kinase domain were not included in the expression vector. Both OCM1 and PF16 were amplified from total-DNA of *P. aphanidermatum* DAOM BR 444 with PCR-incorporated *c-myc* epitopes (Evan, *et al.*, 1985) at their C-termini. OCM1 primers were designed such that the cloned gene would be lacking the first 23 amino acids of the N-terminus predicted to be a signal peptide. Amplified C-type lectin and flagellar genes were cloned into entry vector pENTR™/D-TOPO® (Life Technologies Inc., Carlsbad, CA). The PCRs for cloning into the pENTR™ vector were performed in two steps to enable PCR-incorporation of the *c-myc* epitope in PF16 and OCM1, or a translation stop codon in CsCL and TaCL, as well as the 5'-CACC nucleotides needed for topoisomerase incorporation into the vector. PCR products from the first step were diluted 100x for use as template DNA in the second

step. PCR primers used for the first step of CsCL amplification were CsCL_cDNA_sense (5'-GATACTATGTTTAACGAGTCAGGAATTGGG-3') with CsCL_cDNA_antisense (5'-CTCCTTGTGACAGTGCATTCGGTGG-3'), and for the second step were CsCL_gate_sense (5'-CACCATGGATACTATGTTTAACGAG-3') with CsCL_gate_antisense (5'-TTACTCCTTGTGACAGTGCATTC-3'). Primers for the first step of TaCL amplification were TaCL_cDNA_sense (5'-CATACCCCGTCATGCCCCGAC-3') with TaCL_cDNA_antisense (5'-CTCCTTGTGGCAGTGATCATGGTAG-3'), and for the second step were TaCL_gate_sense (5'-CACCATGCATACCCCGTCATGC-3') with TaCL_gate_antisense (5'-TTACTCCTTGTGGCAGTGATCATGG-3'). Primers for CsCL and TaCL were based on predicted protein sequences from the genomes of cucumber and wheat (www.plantgdb.org). PCR primers used for the first step of PF16 amplification were PA_ACA_sense1 (5'-ATGGCGTCCACCTCTGTGCTACAG-3') with PA_ACA_gate_antila (5'-GAGTTTTTGTTCCTTCACTTGCGGTTGG-3'), and for the second step were PA_ACA_gate_sense1b (5'-CACCATGGCGTCCACCTCTGTG-3') with CMYC_gate_antilb (5'-TTACAGATCCTCTTCTGAGATGAGTTTTTGTTC-3'). PCR primers used for the first step of OCM1 amplification were PA_OCM1_sense2 (5'-GAGTGCCCGAACGGATGCTCG-3') with PA_OCM1_gate_anti2a (5'-GAGTTTTTGTTCACCGCCAGGATGTTC-3'), and for the second step were PA_OCM1_gate_sense2b (5'-CACCATGGAGTGCCCGAACGG-3') with CMYC_gate_antilb. PCR products from second step were purified using E-Gel[®] Clonewell[™] for PF16 and OCM1 or PureLink[®] for CsCL and TaCL (Life Technologies

Inc., Carlsbad, CA). Concentration of purified PCR products was estimated using a Nanodrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE). Second step PCR products were cloned into the pENTR™ vector via topoisomerase and *E. coli* Mach1™-T1 chemically competent cells were subsequently transformed with pENTR™ constructs according to manufacturer's protocol (Invitrogen/Life Technologies manual part no. 25-0517). Transformant colonies growing on selective media were screened by direct colony-PCR to verify presence of CsCL, TaCL, PF16, and OCM1 constructs. Plasmids were isolated from Mach1™-T1 colonies containing pENTR™ constructs using the QIAprep spin miniprep kit (Qiagen Inc., Toronto, ON). Isolated pENTR™ constructs were subjected to LR recombination reactions with the destination vector pDEST™17 using the LR clonase® II enzyme. pDEST™17 incorporates a 6x-histidine tag at the N-terminus of the recombinant protein. Recombinant pDEST™17 constructs were used for transfection of Top10 *E. coli*. Plasmids isolated from single Top10 colonies containing pDEST™17 constructs were used for transfection of BL21 pLysS *E. coli* and single colonies of BL21-CsCL, BL21-TaCL, BL21-PF16, and BL21-OCM1 were selected for production of recombinant proteins by IPTG induction. Full DNA and amino acid sequences of the recombinant CsCL, TaCL, PF16, and OCM1 proteins are given in Appendix H.

5.2.4 Recombinant protein extraction

Recombinant PF16 and OCM1 were extracted from BL21 *E. coli* by sonication in a surfactant-detergent buffer (100 mM Tris pH 7.5, 50 mM NaCl, 10 mM DTT, 3% Triton X-100, 10 mM CHAPS, protease inhibitor cocktail, 10% N-lauryl sarcosine).

Recombinant CsCL and TaCL were extracted from BL21 *E. coli* by isolation of insoluble inclusion bodies using a modified version of a published protocol (Palmer & Wingfield, 2012). Modifications were the use of sonication rather than French press for cell lysis, centrifugation at 18,500 x g for all steps following lysis, and modified buffers: lysis buffer (100 mM Tris pH 7.5, 50 mM NaCl, 5 mM DTT, protease inhibitor cocktail), wash buffer (100 mM Tris pH 7.5, 50 mM NaCl, 5 mM DTT, 2% N-lauryl sarcosine, 2% Triton X-100), and rinse buffer (step 9) (100 mM Tris pH 7.5, 50 mM NaCl, 5 mM DTT). Recombinant CsCL and TaCL were extracted by resuspending inclusion body pellet in urea (100 mM NaH₂PO₄, 10 mM Tris, 8 M urea).

5.2.5 *Recombinant protein purification*

Recombinant proteins were purified under denaturing conditions using IMAC. Poly-Prep[®] chromatography columns (Bio-Rad Laboratories, Hercules, CA) were filled with 1.5 mL of Ni-NTA agarose beads (Qiagen Inc., Toronto, ON). Protein extracts were passed through the column and non-target proteins were eluted by decreasing pH of urea buffer from 8.0 to 6.3 (100 mM NaH₂PO₄, 10 mM Tris, 8 M urea). Recombinant PF16 and OCM1 were eluted from the column by further decreasing pH to 4.5. Denatured PF16 and OCM1 were refolded by dialysis in 20 mM Tris pH 8.0, 5 mM DTT, 20% glycerol. Recombinant CsCL and TaCL were eluted by pH decrease to 4.5, or alternatively were refolded on-column on the Ni-NTA agarose beads by incubation of column at 4°C in refolding solution (20% glycerol, 20 mM Tris pH 7.5, 500 mM NaCl, 5 mM β-mercaptoethanol, 0.5 mM PMSF, 0.05% Tween 20) containing urea. Concentration of urea was decreased after every hour of incubation, from 6 M urea, to 4

M urea, to 2 M urea, to 1 M urea. Incubation in 1 M urea was left overnight. Following overnight incubation, column was incubated at 4°C for 3 h in TBS (25 mM Tris pH 8.0, 125 mM NaCl). Recombinant CsCL and TaCL remained bound to Ni-NTA agarose beads for subsequent zoospore interaction assay. Recombinant OCM1 was refolded in the same manner to be used as a control in the zoospore interaction assay. Purified recombinant OCM1, PF16, CsCL, and TaCL eluted from columns were verified by SDS-PAGE on 10% gel for OCM1 and PF16, and 14% gel for CsCL and TaCL. 10% gels were run at 150V for 1 h, and 14% gels were run at 150V for 2 h. Gels were fixed in 45% methanol, 10% glacial acetic acid and stained with Coomassie Brilliant Blue R-250.

5.2.6 *Far-western blotting*

Purified CsCL and TaCL were boiled in 1x Laemmli buffer and run on 14% SDS-PAGE at 150V for 2 h in a mini-PROTEAN[®] 3 cell (Bio-Rad Laboratories, Hercules, CA). Two identical gels containing CsCL and TaCL were run concurrently. Transfer from each gel to nitrocellulose was done at 80 mA (7V) for 1 h in an OWL semi-dry electroblotting system (Thermo Scientific, Wilmington, DE). Membranes were rinsed briefly with Milli-Q water and incubated in TBST (TBS containing 0.05% Tween 20) with 2% ECL blocking reagent (GE Healthcare, Baie d'Urfe, QC) for 1 h with gentle shaking. 50 µg of purified *P. aphanidermatum* OCM1 was mixed with 0.5 µg Anti-*myc* antibody (Life Technologies, Carlsbad, CA) and incubated on ice with occasional mixing for 1 h. Membranes were washed twice with TBST, then one membrane was gently shaken for 2 h in 20 mL TBST-ECL containing 50 µg OCM1/Anti-*myc* and 1 mM CaCl₂, while the other membrane was gently shaken for 2 h in 20 mL TBST-ECL containing 1

μg Anti-His tag antibody (Cedarlane, Hornby, ON). Membranes were then washed 3 times in TBST before incubation with shaking for 1 h in 20 mL TBST-ECL containing 0.5 μL Anti-mouse IgG HRP-conjugated antibody solution (GE Healthcare, Baie d'Urfe, QC). Membranes were washed 3 times in TBST and covered with 1 mL Lumigen TMA-6[®] chemiluminescent substrate (Lumigen Inc., Southfield, MI) for 45 sec exposure on Kodak Biomax MS film (Kodak, Rochester, NY). Control western blot of OCM1 with Anti-*myc* primary antibody was performed by the same procedure described above for Anti-His tag blot of CsCL and TaCL, except that the SDS-PAGE of OCM1 was run on 10% gel at 150V for 1 h.

5.2.7 *Yeast two-hybrid assay*

Potential interaction between *P. aphanidermatum* OCM1 and CsCL was tested using the ProQuest™ Two-Hybrid System (Life Technologies, Carlsbad, CA). Interaction between PF16 and CsCL was also tested as a control. Entry vectors pENTR-OCM1, pENTR-CsCL, and pENTR-PF16 described in section 5.2.3 were used in LR recombination reactions with pDEST™32 (bait vector) and pDEST™22 (prey vector). Interactions were tested in duplicate with bait-prey reversal. Bait and prey pDEST™ constructs were isolated from transfected DH5α™ *E. coli* (Life Technologies, Carlsbad, CA) using QIAprep spin minikit. Yeast MaV203 competent cells were co-transfected with bait-prey construct pairs and grown on selective SC-Leu-Trp media for selection of transformants. Transformant colonies were suspended in saline solution (0.85% NaCl) and serially diluted for testing of *HIS3* induction by growth at 30°C for 2 days on SC-Leu-Trp-His media containing varying concentrations of *HIS3* inhibitor 3AT. Testing for

β -galactosidase induction was done by X-gal assay. Transformants were spotted on a nitrocellulose membrane overlaid on YPD agar media and grown at 30°C for 24 h. Membrane was then removed from YPD, frozen briefly in liquid nitrogen, and incubated at 37°C for 24 h saturated with X-gal solution (60 mM Na₂HPO₄, 40 mM NaH₂PO₄, 10 mM KCl, 1 mM MgSO₄, 24 μ M X-gal, 1% DMF, 86 mM β -mercaptoethanol).

5.2.8 *Zoospore interaction with refolded agarose-bound C-type lectin*

Interaction of zoospores from *P. aphanidermatum* and *P. arrhenomanes* with refolded agarose-bound CsCL and TaCL was observed by transferring 500 μ L of CsCL or TaCL agarose beads to size 60 x 15 mm petri dishes. 500 μ L OCM1 agarose beads were transferred to control petri dishes. Each protein bead-containing dish was flooded with zoospore suspension of either *P. aphanidermatum* or *P. arrhenomanes*, whereby one of each *Pythium*-protein combination was tested. Interaction of zoospores with protein-bound beads was observed under a dissecting microscope.

5.2.9 *Cucumber pathogenicity trial – host defense response to flagellar protein*

For growth of cucumber seedlings for pathogenicity trials, seedlings were grown in MagentaTM boxes as described in section 5.2.2, and 7 day – old seedlings were treated with purified protein by dipping the roots in a solution of dialysis buffer (20 mM Tris pH 8.0, 5 mM DTT, 20% glycerol) containing 2 μ g/mL of either PF16, OCM1, or BSA. Negative control treatment was dialysis buffer. Treated seedlings were then planted in Pro-Mix BX in 6” standard pots, with ten seedlings per pot, and grown at 24°C with 18h photoperiod. Twenty seedlings were grown for each treatment. Seedlings were grown for

24h prior to *Pythium* inoculation to allow potential activation of defense response. One day after planting, agar plugs from a 3-day-old V8 agar culture of *P. aphanidermatum* were placed 1/2" deep in the soil of *Pythium* treatments, with one plug adjacent to each cucumber seedling. Control treatments were grown without *Pythium* inoculation. Plants were scored for disease rating and root hair lengths of non-*Pythium* treatments were measured at 14 days post-inoculation (dpi).

5.2.10 *Arabidopsis* pathogenicity trial – C-type lectin knockout

Arabidopsis seedlings were grown in sterile Pro-Mix as described in section 5.2.2, and 7-day-old seedlings were inoculated with *P. aphanidermatum* zoospores suspended in distilled water at a rate of approximately 800 zoospores per seedling in trial 1, and 3000 zoospores per seedling in trial 2. Control seedlings were inoculated with sterile distilled water. A total of 96 seedlings were grown in trial 1, and 384 seedlings were grown in trial 2. Potting mix was kept constantly saturated with sterile distilled water and plants were grown at 24°C with 16h photoperiod for 21 dpi. At 21 dpi, survival was counted and dry weight of survivors was measured following drying at 40°C for 24 h. Two lines of *Arabidopsis thaliana* were used: the wild type Col-0 (Columbia), and a mutant of Col-0 which had T-DNA insertion in the C-type lectin gene At1g52310, rendering this gene knocked-out. At1g52310 insertion mutant seeds of germplasm line SALK_143434C were obtained from the Arabidopsis Biological Resource Center (Ohio State University, Columbus, OH). Wild-type Col-0 seeds were kindly provided by Gislaine Allard at ECORC.

The experiment was set up as a randomized complete block with two trays of seedlings constituting a block. One tray was inoculated with *Pythium* and the other was not. Each tray was placed on a separate shelf in the incubator. The light source was in the door of the incubator and distance from the source was recorded and used as a covariate. Analyses were done with R using GLM and logistic regression with default settings for the survival rate and with the linear model for dry weight and leaf counts (R Development Core Team, 2011).

5.3 Results

5.3.1 Recombinant protein purification

Recombinant OCM1, PF16, CsCL, and TaCL were successfully purified from induced BL21 *E. coli*. SDS-PAGE with purified proteins eluted from Ni-NTA column is shown in Figure 5.2.

5.3.2 Cucumber response to OCM1 inoculation

Cucumber seedlings treated with OCM1 did not have a phenotypic difference in root hair length or *Pythium aphanidermatum* susceptibility compared to controls of PF16, BSA, and dialysis buffer treatment. All treated seedlings grown without *Pythium* inoculation were equally healthy, and root hair lengths were 10 – 20 μm on average with comparable hair density between all treatments (Fig. 5.3). All treated seedlings grown with *Pythium* inoculation were equally unhealthy, with significant yellowing of cotyledons and delayed development compared to treatments lacking *Pythium*. Disease rating for seedling treatments is given in Table 5.1. Rating of 3 indicates 100% cotyledon

yellowing and/or seedling decay, rating of 2 indicates greater than 50% yellowing, rating of 1 indicates less than 50% yellowing, rating of 0 indicates no visible yellowing.

Figure 5.2. Stained SDS-PAGE showing purified PF16 and OCM1 (A) and CsCL and TaCL (B). Predicted molecular weights of recombinant proteins are PF16 55 kDa, OCM1 61 kDa, CsCL 21 kDa, TaCL 18 kDa.

Figure 5.3. Root hairs of cucumber seedlings treated with PF16 (A), OCM1 (B), BSA (C), or dialysis buffer (D). Images are of seedling roots 14 days post-treatment.

Table 5.1. Disease rating of cucumber seedling treatments.

Treatment	<i>P. aphanidermatum</i> inoculation	Average Disease Rating	Std. Dev.
OCM1	no	0	0
PF16	no	0	0
BSA	no	0	0
buffer	no	0	0
OCM1	yes	1.8	0.70
PF16	yes	1.7	0.73
BSA	yes	1.8	0.70
buffer	yes	1.65	0.74

5.3.3 Interaction between OCM1 and C-type lectin

Results of the far-western assay did not indicate any interaction between OCM1 and immobilized CsCL or TaCL due to lack of chemiluminescence (Fig. 5.4 A). The positive control western using Anti-His tag primary antibody showed that protein transfer to nitrocellulose and antibody binding/detection procedure were not at fault (Fig. 5.4 B). Proper binding of Anti-*myc* to OCM1 is not considered to be at fault since this interaction was verified by western blot prior to far-western assay (Fig. 5.4 C). Yeast two-hybrid assay for OCM1 – CsCL interaction showed a weak interaction based on *HIS3* induction (Fig. 5.5 A), but this interaction was not confirmed by β -galactosidase induction in the X-gal assay (Fig 5.5B).

5.3.4 Zoospore recognition of refolded CsCL and TaCL

Zoospores of *P. aphanidermatum* and *P. arrhenomanes* did not respond to contact with agarose-bound refolded CsCL or TaCL, or the control OCM1. Zoospores of both *Pythium* species swam in close contact with the agarose beads, often coming into direct contact with the surface of the beads. No encystment on the beads or taxis towards the beads was noted in periodic microscopic observation over a 6 h duration. An image of *P. aphanidermatum* zoospores swimming among CsCL-bound agarose beads is shown in Figure 5.6.

Figure 5.4. Far-western blot of immobilized CsCL and TaCL using OCM1/Anti-myc as the primary antibody (A). Positive control western blot of CsCL and TaCL using Anti-His tag primary antibody (B). Western blot of OCM1 using Anti-myc primary antibody (C). Chemiluminescence signals in B and C are underlined.

Figure 5.5. Yeast two-hybrid assay. Histidine auxotrophy *HIS3* gene induction by bait-prey interaction in the presence of *HIS3* inhibitor 3AT (A). β -galactosidase induction by bait-prey interaction (B). Controls in B are Strong positive interaction (++), Weak positive interaction (+), and No interaction (-).

Figure 5.6. Petri dish containing refolded CsCL bound to Ni-NTA agarose beads with zoospores of *P. aphanidermatum* swimming among the beads.

5.3.5 *Pythium* susceptibility of C-type lectin knockout *Arabidopsis*

Survival of *A. thaliana* mutants lacking C-type lectin was higher than survival of wild-type Col-0 plants in *Pythium*-inoculated plants. In the first trial with 96 seedlings, this difference was not significant under the logistic regression analysis ($p=0.14$), whereas in the second trial with 384 seedlings the p -value was 0.055. Mortality in inoculated plants was much higher in the second trial because of the higher zoospore concentration where 3000 zoospores were used instead of 800 (Fig. 5.7). However, the difference in survival between knockout mutants (KO) and wild-type plants (WT) was consistent in these two trials, which was confirmed by a non-significant interaction between the WT-KO factor and trial. Therefore, we combined the two trials in one pooled logistic regression analysis which gave a p -value of 0.02 (Fig. 5.7). Knockout and wild-type plants did not differ in their dry weight, or developmental stage as measured by leaf count. There was, however an increase in dry weight and leaf count for plants inoculated with *Pythium* versus non-inoculated (sterile) plants (Fig. 5.8).

Figure 5.7. Survival of C-type lectin knockouts (KO) and wild-type (WT) *Arabidopsis* following inoculation with *P. aphanidermatum* zoospores. Plants in sterile condition were inoculated with sterile water as a control.

Figure 5.8. Dry weight and leaf count of C-type lectin knockouts (KO) and wild-type (WT) *Arabidopsis* following inoculation with *P. aphanidermatum* zoospores. Plants in sterile condition were inoculated with sterile water as a control.

5.4 Discussion

The results of this investigation have shown that C-type lectin contributes to infection of *Arabidopsis thaliana* by *Pythium aphanidermatum* zoospores. The hypothesized mechanism of OCM1-mediated zoospore interaction with C-type lectin was not conclusively shown in this study, nor was an induction of plant defense by OCM1 treatment, but these mechanisms cannot be ruled out of natural plant-zoospore interactions since the recombinant proteins used in this study did not perfectly mimic those found in nature.

Induction of plant defense in response to contact with microbes is a well known phenomenon that often results from plant recognition of specific pathogen-associated molecular patterns (PAMPs) (Zipfel, 2009). PAMPs are well-studied in pathogenic bacteria and can be found in highly variable cell-surface molecules which contain conserved regions such as membrane lipopolysaccharides (Newman, *et al.*, 2007) or flagellin protein (Ramos, *et al.*, 2004). Since oomycete OCM1 is also a highly variable protein with conserved domains that is found on the surface of zoospores, a role of OCM1 in PAMP-triggered immunity was hypothesized. The host-pathogen system of cucumber and *Pythium aphanidermatum* was chosen to test this hypothesis because induced systemic resistance (ISR) to *P. aphanidermatum* has been previously demonstrated in cucumber (Chen, *et al.*, 1998), and there is new evidence of rapid cucumber defense induction in response to infection by a downy mildew oomycete (Adhikari, *et al.*, 2012). In the current experiment, cucumber defense response, or lack thereof, was inferred by overall plant health deduced from tissue colouration (Table 5.1), as well as the augmentation of root hair growth (Fig. 5.3) since this occurs as a result of

defense induction in *Arabidopsis* (Hernandez-Mata, *et al.*, 2010) and rootlet elongation occurs in microbe-inoculated cucumber (De Bellis & Ercolani, 2001). The observations of *Pythium*-inoculated cucumber seedlings did not indicate defense induction, which may have been due to a true lack of OCM1 recognition by cucumber, but it could also have been due to the lack of protein glycosylation of recombinant OCM1.

The tubular mastigonemes of the *Ochromonas* tinsel flagellum are known to be glycosylated (Bouck, 1971, Chen & Haines, 1976), and the amino acid sequence of *P. aphanidermatum* OCM1 contains potential glycosylation sites, so it is reasonable to assume that recognition of OCM1 in a protein-protein interaction would include recognition of OCM1 glycosyl residues. This is true in the case of bacterial flagellin perception by the human immune system, which shows a significantly reduced response to non-glycosylated mutant flagella (Verma, *et al.*, 2005). The expression of OCM1 in bacteria was chosen for ease of use, and the lack of post-translational modification in a prokaryotic expression system was a compromise that could have been circumvented by using a eukaryotic expression system such as yeast, although yeast glycosylation is high in mannose and would not necessarily reproduce oomycete-like glycosylation patterns. Yeast expression was attempted prior to bacterial expression but it proved difficult to optimize so it was not pursued further. In the hypothesized interaction of the tenascin-like protein OCM1 with plant C-type lectin, glycosylation may not have been of necessity, since the interaction between rat tenascin and C-type lectin does not rely on carbohydrate moieties (Aspberg, *et al.*, 1997, Lundell, *et al.*, 2004). The negative results of the far-western assay (Fig. 5.4) may be attributable to the denatured state of the immobilized CsCL and TaCL, but this was not the case in the yeast two-hybrid assay (Fig. 5.5) or the

zoospore recognition assay (Fig. 5.6) where supposed proper protein folding still led to inconclusive results.

Although a direct interaction between OCM1 and C-type lectin was not conclusively demonstrated, the role of C-type lectin in plant susceptibility to *Pythium* infection was shown to be significant. This result is similar to the recent finding that a lectin receptor kinase contributes to infection of *Arabidopsis* by the oomycete *Hyaloperonospora arabidopsidis* (Hok, *et al.*, 2011). This apparent high-jacking of host lectins by oomycete pathogens is actually a common tactic employed by viruses, which are known to target C-type lectins for host entry (Alvarez, *et al.*, 2002, Takada, *et al.*, 2004, de Witte, *et al.*, 2006, Gunn, *et al.*, 2012). With this strategy, the general up-regulation of immune system genes upon pathogen infection would only serve to enhance further infection via up-regulated C-type lectin expression. An example of plant virus-induced C-type lectin up-regulation occurs in the interaction between *Arabidopsis* and Cabbage leaf-curl virus (Ascencio-Ibanez, *et al.*, 2008), so perhaps a similar mechanism is exploited by oomycete pathogens. Alternatively, the lack of C-type lectin may have promoted tolerance to *Pythium* infection in knockout *Arabidopsis* plants. Infection of plants by oomycetes generally involves many gene-for-gene interactions that result in plant hypersensitive response and cell death (van Damme, *et al.*, 2011). Knockout of a gene involved in the hypersensitive response in *Arabidopsis* has been shown to induce systemic acquired resistance, leading to enhanced resistance against pathogenic microbes (Yu, *et al.*, 1998). If C-type lectin is involved in gene-for-gene interaction with pathogens, perhaps a similar defense induction was responsible for the enhanced *Pythium* resistance of C-type lectin knockout *Arabidopsis* plants. In the current study, the lack of C-type lectin in

Arabidopsis had a definite inhibitory effect on *Pythium*, and this effect was consistent over varying zoospore inoculum concentrations (Fig. 5.7). The observation that *Pythium* inoculation yielded greater dry weight and leaf count in surviving plants may be related to the fact that *Arabidopsis* tends to accelerate reproductive development and increase basal branch development in response to oomycete infection (Korves & Bergelson, 2003). Survival rates were correlated to the concentration of zoospore inoculum, as is typical for *Pythium* infection (Chen, *et al.*, 1996), showing that C-type lectin knockout did not confer total resistance, but the findings of this study provide a very interesting step towards further understanding of the role of C-type lectins in plant – pathogen interaction which may be applicable to the development of *Pythium* resistance in crops.

6 Chapter: GENERAL CONCLUSION

It is without doubt that oomycete researchers and society in general will benefit from the ongoing efforts to understand the complex evolutionary relationships between oomycetes, as well as how oomycete evolution relates to their role as pathogenic organisms in the environment. The modern era of molecular biology has revolutionized the way that organisms are studied, where simple sequences of DNA can infer multitudes of biological processes to allow in-depth assessments of ecological roles or evolutionary histories. Now that full genome sequences have been assembled for several important oomycetes, the opportunity to enhance our understanding of these organisms is truly unprecedented.

By using DNA sequence data in a taxonomic and phylogenetic framework, we are able to piece together not only the evolutionary history of oomycetes, but also the puzzle of oomycete ecology. Oomycetes are described ecologically as ubiquitous, cosmopolitan, pervasive, profuse; in other words they're everywhere. But despite their omnipresent nature, we have only a rudimentary understanding of what their ecological niche is. Even when their niche is seemingly obvious, as in the case of plant parasitic oomycetes, there are often alternative habits observed, such as nematode parasitism (nematophagy), that bring into question the primary role of these opportunistic oomycetes in natural ecosystems. Observation and experimentation in the field are imperative to unravelling the ecology of oomycetes, but both the planning and analysis of these experiments can be profoundly supported using molecular data.

The molecular taxonomy and phylogeny of oomycetes described in this thesis aims to augment the resources and knowledge base needed to pursue greater understanding of

these microbes. Next generation sequencing technologies are increasingly being used to study the ecology of oomycetes. We have provided the largest reference dataset ever generated on oomycetes for two markers that can now be used for such purpose, significantly increasing the probability of matching raw sequences from the field with a vouchered reference sequence. New molecular markers specific to zoospore production have also been described which could be exploited in environmental RNA sequencing to determine factors contributing to asexual sporulation. The phylogenetic utility of these markers was also shown, as was the advantage of using a codon modeling approach to phylogenetic reconstruction. In addition to practical resources, the biological understanding that can be achieved from molecular studies is evident in the results of Chapter 5, where the role of C-type lectin in *Pythium* susceptibility was discovered by pursuing the inference of molecular data from Chapter 4. This highlights the potential of molecular data to become a catalyst for functional characterization. Certainly, many discoveries in oomycete biology will continue to be made thanks to the hypnotic suggestions of nucleic acid.

Appendices

Appendix A - List of isolates used and their DNA sequence accession numbers.
Asterisks denote ex-type specimens.

Genus Abbrev	Isolate name	Isolate #	BOLD Process ID	Genbank Accessions
Ach	<i>Achlya ambisexualis</i>	CBS 101.50	OOMYA1184-08	HQ708156, HQ643083
Ach	<i>Achlya ambisexualis</i>	CBS 383.79	OOMYA1185-08	HQ708155, HQ643082
Ach	<i>Achlya americana</i>	CBS 100.52	OOMYA1186-08	HQ708157, HQ643084
Ach	<i>Achlya aquatica*</i>	CBS 103.67	OOMYA1187-08	HQ708158, HQ643085
Ach	<i>Achlya bisexualis</i>	CBS 100.42	OOMYA1188-08	HQ708161, HQ643088
Ach	<i>Achlya bisexualis</i>	CBS 102.62	OOMYA1190-08	HQ708160, HQ643087
Ach	<i>Achlya bisexualis</i>	CBS 103.50	OOMYA1191-08	HQ708159, HQ643086
Ach	<i>Achlya caroliniana</i>	CBS 544.67	OOMYA1192-08	HQ708162, HQ643089
Ach	<i>Achlya colorata</i>	CBS 102.37	OOMYA1193-08	HQ708164, HQ643091
Ach	<i>Achlya colorata</i>	CBS 545.67	OOMYA1194-08	HQ708163, HQ643090
Ach	<i>Achlya conspicua</i>	CBS 103.37	OOMYA1195-08	HQ708165, HQ643092
Ach	<i>Achlya dubia</i>	CBS 101.38	OOMYA1196-08	HQ708167, HQ643094
Ach	<i>Achlya dubia</i>	CBS 546.67	OOMYA1197-08	HQ708166, HQ643093
Ach	<i>Achlya flagellata</i>	CBS 104.37	OOMYA1198-08	HQ708170, HQ643097
Ach	<i>Achlya flagellata</i>	CBS 107.35	OOMYA1199-08	HQ708169, HQ643096
Ach	<i>Achlya flagellata</i>	CBS 528.67	OOMYA1200-08	HQ708168, HQ643095
Ach	<i>Achlya glomerata</i>	CBS 105.50	OOMYA1201-08	HQ708171, HQ643098
Ach	<i>Achlya heterosexuales</i>	CBS 419.65	OOMYA1202-08	HQ708173, HQ643100
Ach	<i>Achlya heterosexuales</i>	CBS 420.65	OOMYA1203-08	HQ708172, HQ643099
Ach	<i>Achlya oligocantha</i>	CBS 101.44	OOMYA1204-08	HQ708174, HQ643101
Ach	<i>Achlya papillosa</i>	CBS 101.52	OOMYA1205-08	HQ708175, HQ643102
Ach	<i>Achlya racemosa</i>	CBS 103.38	OOMYA1206-08	HQ708178, HQ643105
Ach	<i>Achlya racemosa</i>	CBS 541.67	OOMYA1207-08	HQ708177, HQ643104
Ach	<i>Achlya racemosa</i>	CBS 578.67	OOMYA1208-08	HQ708176, HQ643103
Ach	<i>Achlya radiosa</i>	CBS 547.67	OOMYA1210-08	HQ708179, HQ643106
Ach	<i>Achlya recurva</i>	CBS 108.50	OOMYA1211-08	HQ708180, HQ643107
Ach	<i>Achlya sparrowii*</i>	CBS 102.49	OOMYA1213-08	HQ708181, HQ643108
Ach	<i>Achlya spinosa</i>	CBS 576.67	OOMYA1214-08	HQ708182, HQ643109
Alb	<i>Albugo candida</i>	AC2V	OOMYA2013-10	HQ708184, HQ643111, HQ665049
Alb	<i>Albugo candida</i>	AC7A	OOMYA2014-10	HQ708183, HQ643110, HQ665050
Aph	<i>Aphanomyces cladogamus</i>	BR 693	OOMYA1218-08	HQ708187, HQ643114
Aph	<i>Aphanomyces cladogamus</i>	CBS 690.79	OOMYA1217-08	HQ708185, HQ643112
Aph	<i>Aphanomyces cladogamus</i>	CBS 108.29	OOMYA1215-08	HQ708186, HQ643113, HQ665056
Aph	<i>Aphanomyces cochlioides</i>	CBS 477.71	OOMYA1216-08	HQ708188, HQ643115, HQ665241
Aph	<i>Aphanomyces euteiches</i>	BR 694	OOMYA1219-08	HQ708193, HQ643120
Aph	<i>Aphanomyces euteiches</i>	CBS 155.73	OOMYA1221-08	HQ708191, HQ643118
Aph	<i>Aphanomyces euteiches</i>	CBS 157.73	OOMYA1223-08	HQ708189, HQ643116
Aph	<i>Aphanomyces euteiches</i>	CBS 154.73	OOMYA1220-08	HQ708192, HQ643119, HQ665129
Aph	<i>Aphanomyces euteiches</i>	CBS 156.73	OOMYA1222-08	HQ708190, HQ643117, HQ665132
Aph	<i>Aphanomyces iridis*</i>	CBS 524.87	OOMYA1224-08	HQ708194, HQ643121, HQ665248
Aph	<i>Aphanomyces laevis</i>	CBS 478.71	OOMYA1226-08	HQ708195, HQ643122, HQ665242
Aph	<i>Aphanomyces sp.</i>	CBS 583.85	OOMYA1227-08	HQ708196, HQ643123, HQ665276
Apo	<i>Apodachlya brachynema</i>	CBS 184.82	OOMYA1229-08	HQ708197, HQ643124
Apo	<i>Apodachlya brachynema</i>	CBS 557.69	OOMYB040-08	HQ708198, HQ643125
Apo	<i>Apodachlya minima</i>	CBS 185.82	OOMYA1230-08	HQ708199, HQ643126
Bas	<i>Basidiophora entospora</i>	HV 119	DM011-10	HM033184, EF553487
Bas	<i>Basidiophora entospora</i>	HV 123		AY035513
Bre	<i>Brevilegnia gracilis*</i>	CBS 131.37	OOMYA1232-08	HQ708200, HQ643127, HQ665122
Bre	<i>Brevilegnia macrospora*</i>	CBS 132.37	OOMYA1908-08	HQ708201, HQ643128, HQ665124
Bre	<i>Brevilegnia unisporma var. delica</i>	CBS 143.52	OOMYA1233-08	HQ708202, HQ643129, HQ665125
Bre	<i>Brevilegnia variabilis</i>	CBS 110006	OOMYA1234-08	HQ708203, HQ643130, HQ665058
Eur	<i>Eurychasma dicksonii</i>	CCAP 4018/2	OOMYA2124-10	HQ708204, HQ665307
Eur	<i>Eurychasma dicksonii</i>	FI373	OOMYA2126-10	HQ643131
Hal	<i>Halophytophthora exoprolifera</i>	CBS 252.93	OOMYA1236-08	HQ708205, HQ643132, HQ665174
Hal	<i>Halophytophthora kandelii</i>	CBS 111.91	OOMYA006-07	HQ708207, HQ643134, HQ665065
Hal	<i>Halophytophthora kandelii</i>	CBS 113.91	OOMYA1237-08	HQ708206, HQ643133, HQ665079
Hal	<i>Halophytophthora tartarea*</i>	CBS 208.95	OOMYA043-07	HQ708208, HQ643135
Hya	<i>Hyaloperonospora nesliae</i>	HV 203	DM004-10	HM033185, AY198250, EU054892
Hya	<i>Hyaloperonospora sisymbrii-sophiae</i>	HV 276	DM007-10	HM033186, AY198253, EU054910
Lag	<i>Lagenidium caudatum</i>	CBS 584.85	OOMYA199-07	HQ708209, HQ643136, HQ665277
Lag	<i>Lagenidium giganteum</i>	CBS 580.84	OOMYA2112-10	HQ708210
Lag	<i>Lagenidium giganteum</i>	ATCC 36492		AY151183
Lep	<i>Leptolegnia caudata</i>	CBS 680.69	OOMYA213-07	HQ708211, HQ643137, HQ665287
Lep	<i>Leptolegnia sp.</i>	CBS 582.85	OOMYA1238-08	HQ708212, HQ643138, HQ665275
	<i>Peronospora aparines</i>	HV 97	DM001-10	HM033187, AY198300
	<i>Peronospora calotheca</i>	HV 81	DM002-10	HM033188, AY198298
	<i>Peronospora calotheca</i>	HV 83		AY035483
	<i>Peronospora conglomerata</i>	HV 26	DM003-10	HM033189, AY198246

Genus Abbrev	Isolate name	Isolate #	BOLD Process ID	Genbank Accessions
	<i>Peronospora conglomerata</i>	HV 27		AY035489
	<i>Peronospora radii</i>	HV 22	DM005-10	HM033190, AY198296
	<i>Peronospora sherardiae</i>	HV 211	DM006-10	HM033191, AY198301
	<i>Peronospora valerianellae</i>	HV 41	DM008-10	HM033192, AY198293
	<i>Peronospora violae</i>	HV 34	DM009-10	HM033193, AY198240
Phy	<i>Phytophthora aff. citricola</i>	BR 1073	OOMYA1250-08	HQ708271, HQ643204
Phy	<i>Phytophthora aff. infestans</i>	P13660	PHYTO099-10	HQ261238, HQ261491
Phy	<i>Phytophthora aff. meadii</i>	CBS 235.30	OOMYA2081-10	HQ708214, HQ643140
Phy	<i>Phytophthora aff. meadii</i>	CBS 238.28	OOMYA2082-10	HQ708213, HQ643139
Phy	<i>Phytophthora aff. primulae</i>	P6817	PHYTO203-10	HQ261239, HQ261492
Phy	<i>Phytophthora aff. rosacearum</i>	P10678	PHYTO065-10	HQ261240, HQ261493
Phy	<i>Phytophthora alni</i>	CBS 117375	OOMYA2060-10	HQ708217, HQ643143
Phy	<i>Phytophthora alni</i>	CBS 117376	OOMYA2061-10	HQ708216, HQ643142
Phy	<i>Phytophthora alni</i>	IMI 392314	OOMYA2163-10	GU945463, GU993881
Phy	<i>Phytophthora alni</i>	P10564	PHYTO055-10	HQ261244, HQ261497
Phy	<i>Phytophthora alni</i>	P11193	PHYTO084-10	HQ261243, HQ261496
Phy	<i>Phytophthora alni</i>	P11318	PHYTO086-10	HQ261242, HQ261495
Phy	<i>Phytophthora alni</i>	P16202	PHYTO126-10	HQ261241, HQ261494
Phy	<i>Phytophthora alticola</i>	P16052	PHYTO120-10	HQ261245, HQ261498
Phy	<i>Phytophthora arecae</i>	CBS 305.62	OOMYA115-07	HQ708218, HQ643146, HQ665200
Phy	<i>Phytophthora austrocedrae</i>	P15132	PHYTO108-10	HQ261247, HQ261500
Phy	<i>Phytophthora austrocedrae</i>	P16040	PHYTO117-10	HQ261246, HQ261499
Phy	<i>Phytophthora avicenniae</i>	CBS 188.85	OOMYA036-07	HQ708219, HQ643147, HQ665146
Phy	<i>Phytophthora batemanensis*</i>	CBS 679.84	OOMYA212-07	HQ708220, HQ643148, HQ665286
Phy	<i>Phytophthora bisberia</i>	P11311	PHYTO085-10	HQ261249, HQ261502
Phy	<i>Phytophthora bisberia</i>	P7191	PHYTO217-10	HQ261248, HQ261501
Phy	<i>Phytophthora bisberia</i>	P10117	PHYTO013-10	HQ261250, HQ261503, EU080746
Phy	<i>Phytophthora boehmeriae</i>	P1257	PHYTO095-10	HQ261253, HQ261506
Phy	<i>Phytophthora boehmeriae</i>	P1378	PHYTO100-10	HQ261252, HQ261505
Phy	<i>Phytophthora boehmeriae</i>	p43	OOMYA2137-10	GU945465, GU993882
Phy	<i>Phytophthora boehmeriae</i>	P6950	PHYTO211-10	HQ261251, HQ261504, EU080166
Phy	<i>Phytophthora boehmeriae*</i>	CBS 291.29	OOMYA105-07	HQ708221, HQ643149, HQ665190
Phy	<i>Phytophthora botryosa</i>	P1044	PHYTO050-10	HQ261257, HQ261510
Phy	<i>Phytophthora botryosa</i>	P3425	PHYTO169-10	HQ261256, HQ261509
Phy	<i>Phytophthora botryosa</i>	p44	OOMYA2138-10	GU945466, GU993883
Phy	<i>Phytophthora botryosa</i>	P6944	PHYTO209-10	HQ261255, HQ261508
Phy	<i>Phytophthora botryosa</i>	P6945	PHYTO210-10	HQ261254, HQ261507, EU079939
Phy	<i>Phytophthora botryosa*</i>	CBS 581.69	OOMYA197-07	HQ708222, HQ643151
Phy	<i>Phytophthora brassicae</i>	CBS 112277	OOMYA2038-10	HQ708228, HQ643158
Phy	<i>Phytophthora brassicae</i>	CBS 180.87	OOMYA2074-10	HQ708224, HQ643154
Phy	<i>Phytophthora brassicae</i>	CBS 212.82	OOMYA2076-10	HQ708223, HQ643153
Phy	<i>Phytophthora brassicae</i>	CBS 782.97	OOMYA2115-10	HQ708226, HQ643156
Phy	<i>Phytophthora brassicae</i>	CBS 178.87	OOMYA034-07	HQ708225, HQ643155, HQ665144
Phy	<i>Phytophthora brassicae</i>	CBS 686.95	OOMYA2009-09	HQ708227, HQ643157, HQ665289
Phy	<i>Phytophthora brassicae</i>	P10155	PHYTO018-10	HQ261259, HQ261512, EU080794
Phy	<i>Phytophthora brassicae</i>	P3273	PHYTO164-10	HQ261258, HQ261511, EU079812
Phy	<i>Phytophthora cactorum</i>	BR 1067	OOMYA1249-08	HQ708240, HQ643170
Phy	<i>Phytophthora cactorum</i>	BR 194	OOMYA1242-08	HQ708239, HQ643169
Phy	<i>Phytophthora cactorum</i>	BR 672	OOMYA1245-08	HQ708237, HQ643167
Phy	<i>Phytophthora cactorum</i>	BR 673	OOMYA1246-08	HQ708236, HQ643166
Phy	<i>Phytophthora cactorum</i>	BR 675	OOMYA1248-08	HQ708235, HQ643165
Phy	<i>Phytophthora cactorum</i>	CBS 110121	OOMYA2019-10	HQ708238, HQ643168
Phy	<i>Phytophthora cactorum</i>	CBS 112275	OOMYA2037-10	HQ708234, HQ643164
Phy	<i>Phytophthora cactorum</i>	CBS 113344	OOMYA2039-10	HQ708233, HQ643163
Phy	<i>Phytophthora cactorum</i>	CBS 151.88	OOMYA2072-10	HQ708229, HQ643159
Phy	<i>Phytophthora cactorum</i>	CBS 231.30	OOMYA2078-10	HQ708231, HQ643161
Phy	<i>Phytophthora cactorum</i>	CBS 279.37	OOMYA2087-10	HQ708232, HQ643162
Phy	<i>Phytophthora cactorum</i>	CBS 294.29	OOMYA2089-10	HQ708230, HQ643160
Phy	<i>Phytophthora cactorum</i>	P10365	PHYTO043-10	HQ261260, HQ261513
Phy	<i>Phytophthora cactorum</i>	P0714	PHYTO008-10	HQ261261, HQ261514, EU080282
Phy	<i>Phytophthora cajani</i>	P3105	PHYTO157-10	HQ261262, HQ261515, EU080105
Phy	<i>Phytophthora cambivora</i>	CBS 111329	OOMYA2023-10	HQ708248, HQ643179
Phy	<i>Phytophthora cambivora</i>	CBS 114085	OOMYA2045-10	HQ708247, HQ643178
Phy	<i>Phytophthora cambivora</i>	CBS 114086	OOMYA2046-10	HQ708246, HQ643177
Phy	<i>Phytophthora cambivora</i>	CBS 114087	OOMYA2047-10	HQ708245, HQ643176
Phy	<i>Phytophthora cambivora</i>	CBS 114093	OOMYA2048-10	HQ708244, HQ643175
Phy	<i>Phytophthora cambivora</i>	CBS 114094	OOMYA2049-10	HQ708243, HQ643174
Phy	<i>Phytophthora cambivora</i>	CBS 114095	OOMYA2050-10	HQ708242, HQ643173
Phy	<i>Phytophthora cambivora</i>	CBS 356.78	OOMYA2100-10	HQ708241, HQ643172
Phy	<i>Phytophthora cambivora</i>	p64	OOMYA2142-10	GU945467, GU993885
Phy	<i>Phytophthora cambivora</i>	P0592	PHYTO006-10	HQ261263, HQ261516, EU080555
Phy	<i>Phytophthora capsici</i>	CBS 111333	OOMYA2024-10	HQ708256, HQ643188
Phy	<i>Phytophthora capsici</i>	CBS 111334	OOMYA2025-10	HQ708255, HQ643187
Phy	<i>Phytophthora capsici</i>	CBS 111335	OOMYA2026-10	HQ708254, HQ643186
Phy	<i>Phytophthora capsici</i>	CBS 111336	OOMYA2027-10	HQ708253, HQ643185
Phy	<i>Phytophthora capsici</i>	CBS 254.93	OOMYA2084-10	HQ708252, HQ643183
Phy	<i>Phytophthora capsici</i>	CBS 370.72	OOMYA2103-10	HQ708251, HQ643182
Phy	<i>Phytophthora capsici</i>	P3375	PHYTO167-10	HQ261265, HQ261518

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Phy	<i>Phytophthora capsici</i>	P3605	PHYTO172-10	HQ261264, HQ261517
Phy	<i>Phytophthora capsici</i>	p8	OOMYA2144-10	GU945468, GU993886
Phy	<i>Phytophthora capsici</i>	CBS 554.88	OOMYA188-07	HQ708250, HQ643181, HQ665266
Phy	<i>Phytophthora capsici</i>	P10386	PHYTO047-10	HQ261267, HQ261520, EU079548
Phy	<i>Phytophthora capsici</i>	P1319	PHYTO097-10	HQ261266, HQ261519, EU079741
Phy	<i>Phytophthora capsici*</i>	CBS 128.23	OOMYA012-07	HQ708249, HQ643180, HQ665120
Phy	<i>Phytophthora captiosa</i>	P10720	PHYTO073-10	HQ261268, HQ261521
Phy	<i>Phytophthora captiosa</i>	P10719	PHYTO072-10	HQ261269, HQ261522, EU079663
Phy	<i>Phytophthora cinnamomi</i>	CBS 232.30	OOMYA2079-10	HQ708263, HQ643195
Phy	<i>Phytophthora cinnamomi</i>	CBS 249.60	OOMYA2083-10	HQ708262, HQ643194
Phy	<i>Phytophthora cinnamomi</i>	CBS 270.55	OOMYA2085-10	HQ708261, HQ643193
Phy	<i>Phytophthora cinnamomi</i>	CBS 304.36	OOMYA2093-10	HQ708260, HQ643192
Phy	<i>Phytophthora cinnamomi</i>	CBS 319.49	OOMYA2097-10	HQ708266, HQ643198
Phy	<i>Phytophthora cinnamomi</i>	CBS 341.72	OOMYA2098-10	HQ708265, HQ643197
Phy	<i>Phytophthora cinnamomi</i>	CBS 342.72	OOMYA2099-10	HQ708264, HQ643196
Phy	<i>Phytophthora cinnamomi</i>	CBS 402.48	OOMYA2105-10	HQ708259, HQ643191
Phy	<i>Phytophthora cinnamomi</i>	CBS 403.48	OOMYA2106-10	HQ708258, HQ643190
Phy	<i>Phytophthora cinnamomi</i>	P2100	PHYTO147-10	HQ261277, HQ261530
Phy	<i>Phytophthora cinnamomi</i>	P2121	PHYTO148-10	HQ261276, HQ261529
Phy	<i>Phytophthora cinnamomi</i>	P2160	PHYTO151-10	HQ261275, HQ261528
Phy	<i>Phytophthora cinnamomi</i>	P2301	PHYTO152-10	HQ261274, HQ261527
Phy	<i>Phytophthora cinnamomi</i>	P3232	PHYTO162-10	HQ261273, HQ261526, EU079801
Phy	<i>Phytophthora cinnamomi</i>	P6305	PHYTO192-10	HQ261272, HQ261525, EU079898
Phy	<i>Phytophthora cinnamomi</i> var. <i>parvispora</i>	CBS 411.96	OOMYA154-07	HQ708268, HQ643200, HQ665231
Phy	<i>Phytophthora cinnamomi</i> var. <i>parvispora</i>	CBS 413.96	OOMYA155-07	HQ708267, HQ643199, HQ665232
Phy	<i>Phytophthora cinnamomi</i> var. <i>parvispora</i>	P7154	PHYTO216-10	HQ261271, HQ261524, EU080457
Phy	<i>Phytophthora cinnamomi</i> var. <i>parvispora</i>	P8495	PHYTO248-10	HQ261270, HQ261523, EU079953
Phy	<i>Phytophthora cinnamomi</i> var. <i>robiniae</i>	P16351	PHYTO129-10	HQ261278, HQ261531
Phy	<i>Phytophthora cinnamomi*</i>	CBS 144.22	OOMYA017-07	HQ708257, HQ643189, HQ665126
Phy	<i>Phytophthora citricola</i>	BR 519	OOMYA1262-08	HQ708270, HQ643203
Phy	<i>Phytophthora citricola</i>	lp52	OOMYA2127-10	GU945470, GU993888
Phy	<i>Phytophthora citricola</i>	P0716	PHYTO009-10	HQ261281, HQ261534
Phy	<i>Phytophthora citricola</i>	P0767	PHYTO010-10	HQ261280, HQ261533
Phy	<i>Phytophthora citricola</i>	P1805	PHYTO139-10	HQ261279, HQ261532
Phy	<i>Phytophthora citricola*</i>	CBS 221.88	OOMYA059-07	HQ708269, HQ643201, HQ665161
Phy	<i>Phytophthora citrophthora</i>	136	OOMYA2012-10	GU945471, GU993889
Phy	<i>Phytophthora citrophthora</i>	CBS 111338	OOMYA2029-10	HQ708275, HQ643208
Phy	<i>Phytophthora citrophthora</i>	CBS 111339	OOMYA2030-10	HQ708274, HQ643207
Phy	<i>Phytophthora citrophthora</i>	CBS 111726	OOMYA2033-10	HQ708273, HQ643206
Phy	<i>Phytophthora citrophthora</i>	P10368	PHYTO044-10	HQ261282, HQ261535
Phy	<i>Phytophthora citrophthora</i>	CBS 950.87	OOMYA241-07	HQ708272, HQ643205, HQ665305
Phy	<i>Phytophthora citrophthora</i>	P10341	PHYTO041-10	HQ261283, HQ261536, EU080389
Phy	<i>Phytophthora clandestina</i>	P3943	PHYTO182-10	HQ261284, HQ261537
Phy	<i>Phytophthora clandestina</i>	P3942	PHYTO181-10	HQ261285, HQ261538, EU079871
Phy	<i>Phytophthora colocasiae</i>	P6290	PHYTO191-10	HQ261287, HQ261540, EU080128
Phy	<i>Phytophthora colocasiae</i>	P6317	PHYTO194-10	HQ261286, HQ261539, EU079911
Phy	<i>Phytophthora cryptogea</i>	BR 521	OOMYA410-07	HQ708284, HQ643219
Phy	<i>Phytophthora cryptogea</i>	BR 589	OOMYA1259-08	HQ708283, HQ643218
Phy	<i>Phytophthora cryptogea</i>	BR 682	OOMYA1266-08	HQ708282, HQ643217
Phy	<i>Phytophthora cryptogea</i>	CBS 114074	OOMYA2042-10	HQ708280, HQ643215
Phy	<i>Phytophthora cryptogea</i>	CBS 290.35	OOMYA2088-10	HQ708278, HQ643212
Phy	<i>Phytophthora cryptogea</i>	CBS 418.71	OOMYA2108-10	HQ708277, HQ643211
Phy	<i>Phytophthora cryptogea</i>	Lev 1802	OOMYA1268-08	HQ708279, HQ643214
Phy	<i>Phytophthora cryptogea</i>	p13	OOMYA2131-10	GU945473, GU993890
Phy	<i>Phytophthora cryptogea</i>	P16165	PHYTO125-10	HQ261294, HQ261547
Phy	<i>Phytophthora cryptogea</i>	P1693	PHYTO136-10	HQ261293, HQ261546
Phy	<i>Phytophthora cryptogea</i>	P1739	PHYTO137-10	HQ261292, HQ261545
Phy	<i>Phytophthora cryptogea</i>	P1810	PHYTO140-10	HQ261291, HQ261544
Phy	<i>Phytophthora cryptogea</i>	P3700	PHYTO174-10	HQ261289, HQ261542
Phy	<i>Phytophthora cryptogea</i>	P10705	PHYTO070-10	HQ261297, HQ261550
Phy	<i>Phytophthora cryptogea</i>	P1088	PHYTO079-10	HQ261296, HQ261549, EU080451
Phy	<i>Phytophthora cryptogea</i>	P11822	PHYTO090-10	HQ261295, HQ261548, EU080082
Phy	<i>Phytophthora cryptogea</i>	P3103	PHYTO156-10	HQ261290, HQ261543, EU080631
Phy	<i>Phytophthora cryptogea</i>	P3876	PHYTO178-10	HQ261288, HQ261541, EU079851
Phy	<i>Phytophthora cryptogea</i> f. sp. <i>begoniae</i>	CBS 468.81	OOMYA167-07	HQ708276, HQ643210, HQ665238
Phy	<i>Phytophthora cryptogea*</i>	CBS 113.19	OOMYA007-07	HQ708281, HQ643216, HQ665075
Phy	<i>Phytophthora drechsleri</i>	P1087	PHYTO078-10	HQ261299, HQ261552
Phy	<i>Phytophthora drechsleri</i>	P11638	PHYTO087-10	HQ261298, HQ261551
Phy	<i>Phytophthora drechsleri</i>	P10331	PHYTO034-10	HQ261300, HQ261553, EU079511
Phy	<i>Phytophthora epistomium*</i>	CBS 590.85	OOMYA203-07	HQ708285, HQ643220, HQ665279
Phy	<i>Phytophthora erythroseptica</i>	BR 464	OOMYA1272-08	HQ708291, HQ643227
Phy	<i>Phytophthora erythroseptica</i>	BR 664	OOMYA1273-08	HQ708290, HQ643226
Phy	<i>Phytophthora erythroseptica</i>	CBS 111343	OOMYA2031-10	HQ708292, HQ643228
Phy	<i>Phytophthora erythroseptica</i>	CBS 233.30	OOMYA2080-10	HQ708287, HQ643222
Phy	<i>Phytophthora erythroseptica</i>	CBS 951.87	OOMYA2120-10	HQ708289, HQ643225
Phy	<i>Phytophthora erythroseptica</i>	CBS 956.87	OOMYA2121-10	HQ708288, HQ643224
Phy	<i>Phytophthora erythroseptica</i>	P0340	PHYTO003-10	HQ261302, HQ261555
Phy	<i>Phytophthora erythroseptica</i>	p50	OOMYA2140-10	GU945475, GU993891

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Phy	<i>Phytophthora erythroseptica</i>	P10382	PHYTO046-10	HQ261301, HQ261554, EU080780
Phy	<i>Phytophthora erythroseptica</i> *	CBS 129.23	OOMYA013-07	HQ708286, HQ643221, HQ665121
Phy	<i>Phytophthora europaea</i>	BR 1072	OOMYA1302-08	HQ708293, HQ643229
Phy	<i>Phytophthora europaea</i>	P10324	PHYTO031-10	HQ261303, HQ261556, EU079486
Phy	<i>Phytophthora fallax</i>	P10722	PHYTO074-10	HQ261306, HQ261559
Phy	<i>Phytophthora fallax</i>	P10723	PHYTO075-10	HQ261305, HQ261558, EU080033
Phy	<i>Phytophthora fallax</i>	P10725	PHYTO076-10	HQ261304, HQ261557, EU080039
Phy	<i>Phytophthora foliorum</i>	P10969	PHYTO081-10	HQ261308, HQ261561, EU079684
Phy	<i>Phytophthora foliorum</i>	P10971	PHYTO082-10	HQ261307, HQ261560, EU079704
Phy	<i>Phytophthora fragariae</i>	CBS 309.62	OOMYA2095-10	HQ708295, HQ643231
Phy	<i>Phytophthora fragariae</i>	P11808	PHYTO089-10	HQ261312, HQ261565
Phy	<i>Phytophthora fragariae</i>	P6406	PHYTO196-10	HQ261309, HQ261562
Phy	<i>Phytophthora fragariae</i>	CBS 209.46	OOMYA044-07	HQ708294, HQ643230, HQ665150
Phy	<i>Phytophthora fragariae</i>	P1435	PHYTO101-10	HQ261311, HQ261564, EU079748
Phy	<i>Phytophthora fragariae</i>	P3820	PHYTO175-10	HQ261310, HQ261563, EU079839
Phy	<i>Phytophthora frigida</i>	P16051	PHYTO119-10	HQ261316, HQ261569
Phy	<i>Phytophthora frigida</i>	P16053	PHYTO121-10	HQ261315, HQ261568
Phy	<i>Phytophthora frigida</i>	P16054	PHYTO122-10	HQ261314, HQ261567
Phy	<i>Phytophthora frigida</i>	P16059	PHYTO123-10	HQ261313, HQ261566
Phy	<i>Phytophthora gonapodyides</i>	CBS 113346	OOMYA2040-10	HQ708299, HQ643236
Phy	<i>Phytophthora gonapodyides</i>	CBS 117380	OOMYA2063-10	HQ708296, HQ643232
Phy	<i>Phytophthora gonapodyides</i>	IMI 345174	OOMYA2160-10	GU945477, GU993893
Phy	<i>Phytophthora gonapodyides</i>	P7050	PHYTO213-10	HQ261317, HQ261570
Phy	<i>Phytophthora gonapodyides</i>	CBS 363.79	OOMYA134-07	HQ708298, HQ643235, HQ665216
Phy	<i>Phytophthora gonapodyides</i>	CBS 554.67	OOMYA187-07	HQ708297, HQ643233, HQ665265
Phy	<i>Phytophthora hedraiaandra</i>	CBS 118732	OOMYA2068-10	HQ708300, HQ643237
Phy	<i>Phytophthora hedraiaandra</i>	P11678	PHYTO088-10	HQ261318, HQ261571
Phy	<i>Phytophthora heveae</i>	P1000	PHYTO011-10	HQ261321, HQ261574
Phy	<i>Phytophthora heveae</i>	p28	OOMYA2133-10	GU945478, GU993894
Phy	<i>Phytophthora heveae</i>	P3428	PHYTO170-10	HQ261320, HQ261573
Phy	<i>Phytophthora heveae</i>	P8240	PHYTO240-10	HQ261319, HQ261572
Phy	<i>Phytophthora heveae</i>	P0578	PHYTO005-10	HQ261322, HQ261575, EU080701
Phy	<i>Phytophthora heveae</i> *	CBS 296.29	OOMYA111-07	HQ708301, HQ643238, HQ665194
Phy	<i>Phytophthora hibernalis</i>	CBS 119904	OOMYA2068-10	HQ708302, HQ643240
Phy	<i>Phytophthora hibernalis</i>	CBS 522.77	OOMYA176-07	HQ708303, HQ643241
Phy	<i>Phytophthora hibernalis</i>	P3822	PHYTO176-10	HQ261323, HQ261576, EU079518
Phy	<i>Phytophthora himalayensis</i> *	CBS 357.59	OOMYA132-07	HQ708304, HQ643242, HQ665215
Phy	<i>Phytophthora humicola</i>	CBS 114082	OOMYA2043-10	HQ708307, HQ643245
Phy	<i>Phytophthora humicola</i>	CBS 114083	OOMYA2044-10	HQ708306, HQ643244
Phy	<i>Phytophthora humicola</i>	P3826	PHYTO177-10	HQ261325, HQ261578, EU080173
Phy	<i>Phytophthora humicola</i>	P6701	PHYTO200-10	HQ261324, HQ261577, EU079923
Phy	<i>Phytophthora humicola</i> *	CBS 200.81	OOMYA038-07	HQ708305, HQ643243, HQ665148
Phy	<i>Phytophthora idaei</i>	CBS 968.95	OOMYA2123-10	HQ708308, HQ643246
Phy	<i>Phytophthora idaei</i>	P6767	PHYTO202-10	HQ261326, HQ261579, EU080134
Phy	<i>Phytophthora ilicis</i>	P6098	PHYTO184-10	HQ261329, HQ261582
Phy	<i>Phytophthora ilicis</i>	P6099	PHYTO185-10	HQ261328, HQ261581
Phy	<i>Phytophthora ilicis</i>	P3939	PHYTO180-10	HQ261330, HQ261583, EU079864
Phy	<i>Phytophthora ilicis</i>	P6860	PHYTO204-10	HQ261327, HQ261580, EU080140
Phy	<i>Phytophthora infestans</i>	P12022	PHYTO091-10	HQ261335, HQ261588
Phy	<i>Phytophthora infestans</i>	P13198	PHYTO098-10	HQ261334, HQ261587
Phy	<i>Phytophthora infestans</i>	P15168	PHYTO110-10	HQ261333, HQ261586
Phy	<i>Phytophthora infestans</i>	P15938	PHYTO114-10	HQ261332, HQ261585
Phy	<i>Phytophthora infestans</i>	P15941	PHYTO115-10	HQ261331, HQ261584
Phy	<i>Phytophthora infestans</i>	CBS 366.51	OOMYA135-07	HQ708309, HQ643247, HQ665217
Phy	<i>Phytophthora infestans</i>	P10650	PHYTO062-10	HQ261336, HQ261589, EU079630
Phy	<i>Phytophthora inflata</i>	p122	OOMYA2130-10	GU945481, GU993896
Phy	<i>Phytophthora insolita</i>	IMI 288805	OOMYA2157-10	GU945482, GU993897
Phy	<i>Phytophthora insolita</i>	P6195	PHYTO187-10	HQ261338, HQ261591, EU080180
Phy	<i>Phytophthora insolita</i>	P6703	PHYTO201-10	HQ261337, HQ261590, EU080214
Phy	<i>Phytophthora inundata</i>	BR 332	OOMYA1316-08	HQ708312, HQ643252
Phy	<i>Phytophthora inundata</i>	CBS 216.85	OOMYA2077-10	HQ708310, HQ643250
Phy	<i>Phytophthora inundata</i>	P8479	PHYTO246-10	HQ261340, HQ261593
Phy	<i>Phytophthora inundata</i>	CBS 215.85	OOMYA1324-08	HQ708311, HQ643251, HQ665154
Phy	<i>Phytophthora inundata</i>	P8478	PHYTO245-10	HQ261341, HQ261594, EU079946
Phy	<i>Phytophthora inundata</i>	P8619	PHYTO251-10	HQ261339, HQ261592, EU080207
Phy	<i>Phytophthora ipomoeae</i>	PRI 810	OOMYA2153-10	HQ708313, HQ643253
Phy	<i>Phytophthora ipomoeae</i>	P10225	PHYTO022-10	HQ261344, HQ261597, EU080835
Phy	<i>Phytophthora ipomoeae</i>	P10226	PHYTO023-10	HQ261343, HQ261596, EU080842
Phy	<i>Phytophthora ipomoeae</i>	P10227	PHYTO024-10	HQ261342, HQ261595, EU080849
Phy	<i>Phytophthora iranica</i>	P3882	PHYTO179-10	HQ261345, HQ261598, EU080116
Phy	<i>Phytophthora iranica</i> *	CBS 374.72	OOMYA138-07	HQ708314, HQ643254, HQ665219
Phy	<i>Phytophthora katsurae</i>	p45	OOMYA2139-10	GU945485, GU993899
Phy	<i>Phytophthora katsurae</i>	P6921	PHYTO208-10	HQ261346, HQ261599
Phy	<i>Phytophthora katsurae</i>	CBS 587.85	OOMYA201-07	HQ708315, HQ643255, HQ665278
Phy	<i>Phytophthora katsurae</i>	P10187	PHYTO019-10	HQ261348, HQ261601, EU080807
Phy	<i>Phytophthora katsurae</i>	P3389	PHYTO168-10	HQ261347, HQ261600, EU079819
Phy	<i>Phytophthora kernoviae</i>	IMI 393172	OOMYA2164-10	GU945486, GU993900
Phy	<i>Phytophthora kernoviae</i>	PRI 712	OOMYA2149-10	HQ708319, HQ643261

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Phy	<i>Phytophthora kernoviae</i>	PRI 713	OOMYA2150-10	HQ708318, HQ643260
Phy	<i>Phytophthora kernoviae</i>	PRI 714	OOMYA2151-10	HQ708317, HQ643259
Phy	<i>Phytophthora kernoviae</i>	PRI 715	OOMYA2152-10	HQ708316, HQ643258
Phy	<i>Phytophthora kernoviae</i>	P10671	PHYTO064-10	HQ261351, HQ261604, EU080032
Phy	<i>Phytophthora kernoviae</i>	P10681	PHYTO066-10	HQ261350, HQ261603, EU079650
Phy	<i>Phytophthora kernoviae</i>	P10958	PHYTO080-10	HQ261349, HQ261602, EU080057
Phy	<i>Phytophthora lateralis</i>	Lev 1213	OOMYA249-07	HQ708320, HQ643263
Phy	<i>Phytophthora lateralis</i>	p51	OOMYA2141-10	GU945487, GU993901
Phy	<i>Phytophthora litchii</i>	BR 892	OOMYA1239-08	HQ708322, HQ643265
Phy	<i>Phytophthora litchii</i>	BR 893	OOMYA1240-08	HQ708321, HQ643264
Phy	<i>Phytophthora litchii</i>	CBS 100.81	OOMYA002-07	HQ708323, HQ643266
Phy	<i>Phytophthora macrochlamydospora</i>	IMI 351473	OOMYA2161-10	GU945488, GU993902
Phy	<i>Phytophthora macrochlamydospora</i>	P10267	PHYTO026-10	HQ261353, HQ261606, EU080009
Phy	<i>Phytophthora macrochlamydospora</i>	P8017	PHYTO222-10	HQ261352, HQ261605, EU080662
Phy	<i>Phytophthora meadii</i>	p75	OOMYA2143-10	GU945489, GU993903
Phy	<i>Phytophthora meadii</i>	CBS 219.88	OOMYA055-07	HQ708324, HQ643268, HQ665159
Phy	<i>Phytophthora meadii</i>	P6128	PHYTO186-10	HQ261354, HQ261607, EU079878
Phy	<i>Phytophthora medicaginis</i>	BR 610	OOMYA1583-08	HQ708328, HQ643273
Phy	<i>Phytophthora medicaginis</i>	BR 611	OOMYA1584-08	HQ708327, HQ643272
Phy	<i>Phytophthora medicaginis</i>	BR 622	OOMYA1265-08	HQ708326, HQ643271
Phy	<i>Phytophthora medicaginis</i>	CBS 117685	OOMYA2064-10	HQ708325, HQ643270
Phy	<i>Phytophthora medicaginis</i>	P10127	PHYTO014-10	HQ261355, HQ261608
Phy	<i>Phytophthora megakarya</i>	P1664	PHYTO133-10	HQ261358, HQ261611
Phy	<i>Phytophthora megakarya</i>	P1672	PHYTO134-10	HQ261357, HQ261610
Phy	<i>Phytophthora megakarya</i>	p42	OOMYA2136-10	GU945490, GU993904
Phy	<i>Phytophthora megakarya</i>	P8516	PHYTO250-10	HQ261356, HQ261609, EU079974
Phy	<i>Phytophthora megasperma</i>	BR 395	OOMYA1296-08	HQ708335, HQ643282
Phy	<i>Phytophthora megasperma</i>	BR 396	OOMYA1297-08	HQ708334, HQ643281
Phy	<i>Phytophthora megasperma</i>	BR 398	OOMYA1299-08	HQ708333, HQ643280
Phy	<i>Phytophthora megasperma</i>	BR 528	OOMYA413-07	HQ708332, HQ643279
Phy	<i>Phytophthora megasperma</i>	BR 529	OOMYA1300-08	HQ708331, HQ643278
Phy	<i>Phytophthora megasperma</i>	Ip81	OOMYA2129-10	GU945491, GU993905
Phy	<i>Phytophthora megasperma</i>	P6957	PHYTO212-10	HQ261359, HQ261612
Phy	<i>Phytophthora megasperma</i>	CBS 306.36	OOMYA116-07	HQ708330, HQ643277, HQ665201
Phy	<i>Phytophthora megasperma</i>	P10340	PHYTO040-10	HQ261362, HQ261615, EU080383
Phy	<i>Phytophthora megasperma</i>	P1679	PHYTO135-10	HQ261361, HQ261614, EU080337
Phy	<i>Phytophthora megasperma</i>	P3136	PHYTO159-10	HQ261360, HQ261613, EU080063
Phy	<i>Phytophthora megasperma*</i>	CBS 402.72	OOMYA150-07	HQ708329, HQ643275, HQ665228
Phy	<i>Phytophthora melonis</i>	IMI 325917	OOMYA2158-10	GU945492, GU993906
Phy	<i>Phytophthora melonis</i>	P6870	PHYTO205-10	HQ261363, HQ261616
Phy	<i>Phytophthora melonis</i>	P3609	PHYTO173-10	HQ261364, HQ261617, EU080476
Phy	<i>Phytophthora melonis*</i>	CBS 582.69	OOMYA198-07	HQ708336, HQ643283, HQ665274
Phy	<i>Phytophthora menzei</i>	P10139	PHYTO015-10	HQ261366, HQ261619
Phy	<i>Phytophthora menzei</i>	P1275	PHYTO096-10	HQ261365, HQ261618
Phy	<i>Phytophthora mexicana</i>	P0646	PHYTO007-10	HQ261367, HQ261620, EU080707
Phy	<i>Phytophthora mirabilis</i>	p3006	OOMYA2134-10	HQ708338
Phy	<i>Phytophthora mirabilis</i>	CBS 122204	OOMYA2069-10	HQ708337, HQ643285
Phy	<i>Phytophthora mirabilis</i>	P10231	PHYTO025-10	HQ261370, HQ261623
Phy	<i>Phytophthora mirabilis</i>	P3010	PHYTO154-10	HQ261368, HQ261621
Phy	<i>Phytophthora mirabilis</i>	P3005	PHYTO153-10	HQ261369, HQ261622, EU079780
Phy	<i>Phytophthora mirabilis</i>	p3007	OOMYA2135-10	GU993907
Phy	<i>Phytophthora mirabilis*</i>	CBS 678.85	OOMYA211-07	HQ708339, HQ643287, HQ665285
Phy	<i>Phytophthora multivesiculata</i>	IMI 386053	OOMYA2128-10	GU945493, GU993908
Phy	<i>Phytophthora multivesiculata</i>	P10670	PHYTO063-10	HQ261371, HQ261624
Phy	<i>Phytophthora multivesiculata*</i>	CBS 545.96	OOMYA180-07	HQ708340, HQ643288, HQ665257
Phy	<i>Phytophthora multivora</i>	BR 514	OOMYA1244-08	HQ708343, HQ643292
Phy	<i>Phytophthora multivora</i>	CBS 111337	OOMYA2028-10	HQ708344, HQ643293
Phy	<i>Phytophthora multivora</i>	CBS 113347	OOMYA2041-10	HQ708342, HQ643291
Phy	<i>Phytophthora multivora</i>	CBS 119108	OOMYA2067-10	HQ708341, HQ643290
Phy	<i>Phytophthora multivora</i>	P1233	PHYTO093-10	HQ261373, HQ261626
Phy	<i>Phytophthora multivora</i>	P7902	PHYTO221-10	HQ261372, HQ261625, EU080240
Phy	<i>Phytophthora nemorosa</i>	P16352	PHYTO130-10	HQ261374, HQ261627
Phy	<i>Phytophthora nemorosa</i>	PRI 708	OOMYA2145-10	HQ708349, HQ643298
Phy	<i>Phytophthora nemorosa</i>	PRI 709	OOMYA2146-10	HQ708348, HQ643297
Phy	<i>Phytophthora nemorosa</i>	PRI 710	OOMYA2147-10	HQ708347, HQ643296
Phy	<i>Phytophthora nemorosa</i>	PRI 711	OOMYA2148-10	HQ708346, HQ643295
Phy	<i>Phytophthora nemorosa</i>	P10288	PHYTO028-10	HQ261375, HQ261628, EU079479
Phy	<i>Phytophthora nemorosa*</i>	CBS 114870	OOMYA2057-10	HQ708345, HQ643294
Phy	<i>Phytophthora nicotianae</i>	81	OOMYA2011-10	GU945494, GU993910
Phy	<i>Phytophthora nicotianae</i>	CBS 101655	OOMYA2017-10	HQ708354, HQ643303
Phy	<i>Phytophthora nicotianae</i>	CBS 303.29	OOMYA2091-10	HQ708352, HQ643301
Phy	<i>Phytophthora nicotianae</i>	CBS 304.29	OOMYA2092-10	HQ708351, HQ643300
Phy	<i>Phytophthora nicotianae</i>	CBS 310.62	OOMYA2096-10	HQ708353, HQ643302
Phy	<i>Phytophthora nicotianae</i>	CBS 534.92	OOMYA2111-10	HQ708350, HQ643299
Phy	<i>Phytophthora nicotianae</i>	P10297	PHYTO029-10	HQ261379, HQ261632
Phy	<i>Phytophthora nicotianae</i>	P10381	PHYTO045-10	HQ261378, HQ261631
Phy	<i>Phytophthora nicotianae</i>	P6915	PHYTO207-10	HQ261377, HQ261630
Phy	<i>Phytophthora nicotianae</i>	P7146	PHYTO215-10	HQ261376, HQ261629, EU079560

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Phy	<i>Phytophthora palmivora</i>	CBS 179.26	OOMYA2073-10	HQ708355, HQ643305
Phy	<i>Phytophthora palmivora</i>	CBS 274.33	OOMYA2086-10	HQ708358, HQ643308
Phy	<i>Phytophthora palmivora</i>	CBS 299.29	OOMYA2090-10	HQ708356, HQ643306
Phy	<i>Phytophthora palmivora</i>	P16385	PHYTO132-10	HQ261381, HQ261634
Phy	<i>Phytophthora palmivora</i>	P6390	PHYTO195-10	HQ261380, HQ261633
Phy	<i>Phytophthora palmivora</i>	CBS 298.29	OOMYA113-07	HQ708357, HQ643307, HQ665195
Phy	<i>Phytophthora palmivora</i>	P0113	PHYTO001-10	HQ261383, HQ261636, EU080469
Phy	<i>Phytophthora palmivora</i>	P0255	PHYTO002-10	HQ261382, HQ261635, EU080343
Phy	<i>Phytophthora parsiana</i>	P15164	PHYTO109-10	HQ261386, HQ261639
Phy	<i>Phytophthora parsiana</i>	P21281	PHYTO149-10	HQ261385, HQ261638
Phy	<i>Phytophthora parsiana</i>	P21282	PHYTO150-10	HQ261384, HQ261637
Phy	<i>Phytophthora phaseoli</i>	CBS 556.88	OOMYA189-07	HQ708359, HQ643309, HQ665267
Phy	<i>Phytophthora phaseoli</i>	P10145	PHYTO016-10	HQ261389, HQ261642, EU080753
Phy	<i>Phytophthora phaseoli</i>	P10150	PHYTO017-10	HQ261388, HQ261641, EU080766
Phy	<i>Phytophthora phaseoli</i>	P6809	PHYTO199-10	HQ261387, HQ261640, EU079918
Phy	<i>Phytophthora pini*</i>	CBS 181.25	OOMYA035-07	HQ708360, HQ643310, HQ665145
Phy	<i>Phytophthora pinifolia</i>	P16100	PHYTO124-10	HQ261390, HQ261643
Phy	<i>Phytophthora pistaciae</i>	P6196	PHYTO188-10	HQ261392, HQ261645, EU080323
Phy	<i>Phytophthora pistaciae</i>	P6197	PHYTO189-10	HQ261391, HQ261644, EU080329
Phy	<i>Phytophthora plurivora</i>	CBS 117378	OOMYA2062-10	HQ708361, HQ643311
Phy	<i>Phytophthora plurivora</i>	CBS 379.61	OOMYA2104-10	HQ708362, HQ643312
Phy	<i>Phytophthora polonica</i>	P15004	PHYTO104-10	HQ261394, HQ261647, EU080268
Phy	<i>Phytophthora polonica</i>	P15005	PHYTO105-10	HQ261393, HQ261646, EU080261
Phy	<i>Phytophthora polymorphica*</i>	CBS 680.84	OOMYA214-07	HQ708363, HQ643313, HQ665288
Phy	<i>Phytophthora porri</i>	CBS 114100	OOMYA2051-10	HQ708370, HQ643320
Phy	<i>Phytophthora porri</i>	CBS 116662	OOMYA2059-10	HQ708367, HQ643317
Phy	<i>Phytophthora porri</i>	CBS 141.87	OOMYA2070-10	HQ708366, HQ643316
Phy	<i>Phytophthora porri</i>	CBS 142.87	OOMYA2071-10	HQ708365, HQ643315
Phy	<i>Phytophthora porri</i>	CBS 181.87	OOMYA2075-10	HQ708364, HQ643314
Phy	<i>Phytophthora porri</i>	CBS 783.97	OOMYA2116-10	HQ708369, HQ643319
Phy	<i>Phytophthora porri</i>	P7518	PHYTO219-10	HQ261396, HQ261649
Phy	<i>Phytophthora porri</i>	P7899	PHYTO220-10	HQ261395, HQ261648
Phy	<i>Phytophthora porri</i>	CBS 567.86	OOMYA191-07	HQ708368, HQ643318, HQ665271
Phy	<i>Phytophthora primulae</i>	CBS 100531	OOMYA2016-10	HQ708372, HQ643322
Phy	<i>Phytophthora primulae</i>	CBS 110162	OOMYA2021-10	HQ708374, HQ643324
Phy	<i>Phytophthora primulae</i>	CBS 110167	OOMYA2022-10	HQ708373, HQ643323
Phy	<i>Phytophthora primulae</i>	CBS 620.97	OOMYA2113-10	HQ708371, HQ643321
Phy	<i>Phytophthora primulae</i>	P10220	PHYTO021-10	HQ261398, HQ261651, EU080821
Phy	<i>Phytophthora primulae</i>	P10333	PHYTO036-10	HQ261397, HQ261650, EU080403
Phy	<i>Phytophthora pseudosyringae</i>	CBS 111773	OOMYA2034-10	HQ708378, HQ643329
Phy	<i>Phytophthora pseudosyringae</i>	CBS 111774	OOMYA2035-10	HQ708377, HQ643328
Phy	<i>Phytophthora pseudosyringae</i>	CBS 111775	OOMYA2036-10	HQ708376, HQ643327
Phy	<i>Phytophthora pseudosyringae</i>	CBS 114108	OOMYA2053-10	HQ708375, HQ643326
Phy	<i>Phytophthora pseudosyringae</i>	IMI 391716	OOMYA2162-10	GU945496, GU993912
Phy	<i>Phytophthora pseudosyringae</i>	P16355	PHYTO131-10	HQ261399, HQ261652
Phy	<i>Phytophthora pseudosyringae</i>	P10443	PHYTO052-10	HQ261400, HQ261653, EU080026
Phy	<i>Phytophthora pseudotsugae</i>	CBS 445.84	OOMYA2109-10	HQ708380, HQ643331
Phy	<i>Phytophthora pseudotsugae</i>	CBS 446.84	OOMYA2110-10	HQ708379, HQ643330
Phy	<i>Phytophthora pseudotsugae</i>	IMI 331663	OOMYA2159-10	GU945497, GU993913
Phy	<i>Phytophthora pseudotsugae</i>	CBS 444.84	OOMYA160-07	HQ708381, HQ643332, HQ665234
Phy	<i>Phytophthora pseudotsugae</i>	P10218	PHYTO020-10	HQ261402, HQ261655, EU079992
Phy	<i>Phytophthora pseudotsugae</i>	P10339	PHYTO039-10	HQ261401, HQ261654, EU080431
Phy	<i>Phytophthora psychrophila</i>	P10433	PHYTO049-10	HQ261403, HQ261656, EU080521
Phy	<i>Phytophthora quercetorum</i>	P15555	PHYTO111-10	HQ261404, HQ261657
Phy	<i>Phytophthora quercina</i>	CBS 781.95	OOMYA2114-10	HQ708385, HQ643337
Phy	<i>Phytophthora quercina</i>	CBS 786.95	OOMYA2117-10	HQ708384, HQ643336
Phy	<i>Phytophthora quercina</i>	CBS 787.95	OOMYA2118-10	HQ708383, HQ643335
Phy	<i>Phytophthora quercina</i>	CBS 788.95	OOMYA2119-10	HQ708382, HQ643334
Phy	<i>Phytophthora quercina</i>	P10334	PHYTO037-10	HQ261406, HQ261659, EU080494
Phy	<i>Phytophthora quercina</i>	P10441	PHYTO051-10	HQ261405, HQ261658, EU080596
Phy	<i>Phytophthora quininea</i>	P8488	PHYTO247-10	HQ261407, HQ261660
Phy	<i>Phytophthora quininea</i>	P3247	PHYTO163-10	HQ261408, HQ261661, EU079806
Phy	<i>Phytophthora quininea*</i>	CBS 407.48	OOMYA153-07	HQ708386, HQ643338, HQ665230
Phy	<i>Phytophthora ramorum</i>	P10102	PHYTO012-10	HQ261410, HQ261663, EU080734
Phy	<i>Phytophthora ramorum</i>	P10301	PHYTO030-10	HQ261409, HQ261662, EU080688
Phy	<i>Phytophthora ramorum*</i>	CBS 101553	OOMYA246-07	HQ708387, HQ643339, HQ665053
Phy	<i>Phytophthora rosacearum</i>	P3159	PHYTO160-10	HQ261411, HQ261664
Phy	<i>Phytophthora rubi</i>	CBS 109892	OOMYA2018-10	HQ708389, HQ643341
Phy	<i>Phytophthora rubi</i>	P3289	PHYTO165-10	HQ261413, HQ261666
Phy	<i>Phytophthora rubi</i>	P3316	PHYTO166-10	HQ261412, HQ261665
Phy	<i>Phytophthora rubi*</i>	CBS 967.95	OOMYA244-07	HQ708388, HQ643340, HQ665306
Phy	<i>Phytophthora sansomeana</i>	BR 1058	OOMYA1318-08	HQ708391, HQ643343
Phy	<i>Phytophthora sansomeana</i>	BR 623	OOMYA1295-08	HQ708390, HQ643342
Phy	<i>Phytophthora sansomeana</i>	CBS 957.87	OOMYA2122-10	HQ708215, HQ643141
Phy	<i>Phytophthora sansomeana</i>	P8048	PHYTO223-10	HQ261415, HQ261668
Phy	<i>Phytophthora sansomeana</i>	P8049	PHYTO224-10	HQ261414, HQ261667
Phy	<i>Phytophthora sansomeana</i>	P3163	PHYTO161-10	HQ261416, HQ261669, EU080275
Phy	<i>Phytophthora sinensis</i>	P1748	PHYTO138-10	HQ261417, HQ261670

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Phy	<i>Phytophthora sinensis</i>	P1475	PHYTO103-10	HQ261418, HQ261671, EU079754
Phy	<i>Phytophthora sinensis</i> *	CBS 557.88	OOMYA190-07	HQ708392, HQ643344, HQ665269
Phy	<i>Phytophthora siskiyouensis</i>	P16301	PHYTO128-10	HQ261419, HQ261672
Phy	<i>Phytophthora siskiyouensis</i>	PRI 816	OOMYA2155-10	HQ708394, HQ643346
Phy	<i>Phytophthora siskiyouensis</i>	PRI 817	OOMYA2156-10	HQ708393, HQ643345
Phy	<i>Phytophthora siskiyouensis</i>	P15122	PHYTO106-10	HQ261421, HQ261674, HQ665311
Phy	<i>Phytophthora siskiyouensis</i>	P15123	PHYTO107-10	HQ261420, HQ261673, HQ665312
Phy	<i>Phytophthora sojae</i>	BR 1079	OOMYA1311-08	HQ708397, HQ643349
Phy	<i>Phytophthora sojae</i>	BR 582	OOMYA1308-08	HQ708396, HQ643348
Phy	<i>Phytophthora sojae</i>	P0405	PHYTO004-10	HQ261426, HQ261679
Phy	<i>Phytophthora sojae</i>	P10704	PHYTO069-10	HQ261425, HQ261678
Phy	<i>Phytophthora sojae</i>	P6497	PHYTO197-10	HQ261423, HQ261676
Phy	<i>Phytophthora sojae</i>	P7061	PHYTO214-10	HQ261422, HQ261675
Phy	<i>Phytophthora sojae</i>	CBS 382.61	OOMYA146-07	HQ708395, HQ643347, HQ665224
Phy	<i>Phytophthora sojae</i>	P3114	PHYTO158-10	HQ261424, HQ261677, EU079794
Phy	<i>Phytophthora</i> sp.	P6875	PHYTO206-10	HQ261427, HQ261680, EU080548
Phy	<i>Phytophthora</i> sp. "andina"	EC 3163	OOMYA2125-10	GU945464, FJ769180
Phy	<i>Phytophthora</i> sp. "andina"	PRI 814	OOMYA2154-10	HQ708398, HQ643350
Phy	<i>Phytophthora</i> sp. "asparagi"	P10693	PHYTO068-10	HQ261429, HQ261682
Phy	<i>Phytophthora</i> sp. "asparagi"	P10707	PHYTO071-10	HQ261428, HQ261681
Phy	<i>Phytophthora</i> sp. "asparagi"	P10690	PHYTO067-10	HQ261430, HQ261683, EU080569
Phy	<i>Phytophthora</i> sp. "canalensis"	P10456	PHYTO053-10	HQ261431, HQ261684, EU079574
Phy	<i>Phytophthora</i> sp. "cuyabensis"	P8230	PHYTO236-10	HQ261433, HQ261686
Phy	<i>Phytophthora</i> sp. "cuyabensis"	P8232	PHYTO237-10	HQ261432, HQ261685
Phy	<i>Phytophthora</i> sp. "cuyabensis"	P8213	PHYTO230-10	HQ261435, HQ261688, EU080669
Phy	<i>Phytophthora</i> sp. "cuyabensis"	P8218	PHYTO231-10	HQ261434, HQ261687, EU080356
Phy	<i>Phytophthora</i> sp. "glovera"	P10618	PHYTO060-10	HQ261437, HQ261690, EU080220
Phy	<i>Phytophthora</i> sp. "glovera"	P10619	PHYTO061-10	HQ261436, HQ261689, EU080227
Phy	<i>Phytophthora</i> sp. "kelmania"	CBS 307.62	OOMYA2094-10	HQ708399, HQ643351
Phy	<i>Phytophthora</i> sp. "kelmania"	P10614	PHYTO057-10	HQ261438, HQ261691
Phy	<i>Phytophthora</i> sp. "kelmania"	P10613	PHYTO056-10	HQ261439, HQ261692, EU079610
Phy	<i>Phytophthora</i> sp. "lacrimae"	P15880	PHYTO113-10	HQ261440, HQ261693
Phy	<i>Phytophthora</i> sp. "lagoariana"	P8220	PHYTO232-10	HQ261442, HQ261695, EU080362
Phy	<i>Phytophthora</i> sp. "lagoariana"	P8223	PHYTO234-10	HQ261441, HQ261694, EU080369
Phy	<i>Phytophthora</i> sp. "napoensis"	P8221	PHYTO233-10	HQ261445, HQ261698
Phy	<i>Phytophthora</i> sp. "napoensis"	P8225	PHYTO235-10	HQ261444, HQ261697
Phy	<i>Phytophthora</i> sp. "napoensis"	P8233	PHYTO238-10	HQ261443, HQ261696
Phy	<i>Phytophthora</i> sp. "niederhauserii"	P10279	PHYTO027-10	HQ261450, HQ261703
Phy	<i>Phytophthora</i> sp. "niederhauserii"	P10976	PHYTO083-10	HQ261447, HQ261700
Phy	<i>Phytophthora</i> sp. "niederhauserii"	P16237	PHYTO127-10	HQ261446, HQ261699
Phy	<i>Phytophthora</i> sp. "niederhauserii"	P10616	PHYTO058-10	HQ261449, HQ261702, EU080233
Phy	<i>Phytophthora</i> sp. "niederhauserii"	P10617	PHYTO059-10	HQ261448, HQ261701, EU080247
Phy	<i>Phytophthora</i> sp. "novaeguinea"	P1256	PHYTO094-10	HQ261456, HQ261709
Phy	<i>Phytophthora</i> sp. "ohioensis"	P16050	PHYTO118-10	HQ261457, HQ261710
Phy	<i>Phytophthora</i> sp. "sulawesensis"	P6306	PHYTO193-10	HQ261458, HQ261711, EU080349
Phy	<i>Phytophthora</i> sp. "thermophilum"	P1896	PHYTO141-10	HQ261459, HQ261712
Phy	<i>Phytophthora</i> sp. "thermophilum"	P10457	PHYTO054-10	HQ261460, HQ261713, EU079580
Phy	<i>Phytophthora</i> sp. nov.	BR 333	OOMYA1243-08	HQ708403, HQ643355
Phy	<i>Phytophthora</i> sp. nov.	CBS 111346	OOMYA2032-10	HQ708402, HQ643354
Phy	<i>Phytophthora</i> sp. nov.	CBS 114338	OOMYA2056-10	HQ708400, HQ643352
Phy	<i>Phytophthora</i> sp. nov.	CBS 368.79	OOMYA2102-10	HQ708401, HQ643353
Phy	<i>Phytophthora</i> sp. nov.	P1212	PHYTO092-10	HQ261453, HQ261706
Phy	<i>Phytophthora</i> sp. nov.	P3600	PHYTO171-10	HQ261452, HQ261705
Phy	<i>Phytophthora</i> sp. nov.	P10337	PHYTO038-10	HQ261455, HQ261708, EU080535
Phy	<i>Phytophthora</i> sp. nov.	P10728	PHYTO077-10	HQ261454, HQ261707, EU079677
Phy	<i>Phytophthora</i> sp. nov.	P6207	PHYTO190-10	HQ261451, HQ261704, EU079885
Phy	<i>Phytophthora syringae</i>	CBS 110161	OOMYA2020-10	HQ708410, HQ643362
Phy	<i>Phytophthora syringae</i>	CBS 114107	OOMYA2052-10	HQ708409, HQ643361
Phy	<i>Phytophthora syringae</i>	CBS 114109	OOMYA2054-10	HQ708408, HQ643360
Phy	<i>Phytophthora syringae</i>	CBS 114110	OOMYA2055-10	HQ708407, HQ643359
Phy	<i>Phytophthora syringae</i>	CBS 275.74	OOMYA097-07	HQ708405, HQ643357
Phy	<i>Phytophthora syringae</i>	CBS 364.52	OOMYA2101-10	HQ708406, HQ643358
Phy	<i>Phytophthora syringae</i>	P2004	PHYTO146-10	HQ261461, HQ261714
Phy	<i>Phytophthora syringae</i>	CBS 132.23	OOMYA014-07	HQ708404, HQ643356, HQ665123
Phy	<i>Phytophthora syringae</i>	P10330	PHYTO033-10	HQ261463, HQ261716, EU080562
Phy	<i>Phytophthora syringae</i>	P10332	PHYTO035-10	HQ261462, HQ261715, EU079767
Phy	<i>Phytophthora tabaci</i> *	CBS 305.29	OOMYA114-07	HQ708411, HQ643363, HQ665198
Phy	<i>Phytophthora tentaculata</i>	CBS 100411	OOMYA2015-10	HQ708415, HQ643367
Phy	<i>Phytophthora tentaculata</i>	CBS 115458	OOMYA2058-10	HQ708412, HQ643364
Phy	<i>Phytophthora tentaculata</i>	CBS 412.96	OOMYA2107-10	HQ708414, HQ643366
Phy	<i>Phytophthora tentaculata</i>	P10363	PHYTO042-10	HQ261465, HQ261718
Phy	<i>Phytophthora tentaculata</i>	CBS 552.96	OOMYA185-07	HQ708413, HQ643365, HQ665264
Phy	<i>Phytophthora tentaculata</i>	P8497	PHYTO249-10	HQ261464, HQ261717, EU079960
Phy	<i>Phytophthora trifolii</i>	CBS 117688	OOMYA2065-10	HQ708416, HQ643368
Phy	<i>Phytophthora trifolii</i>	P1462	PHYTO102-10	HQ261466, HQ261719, EU080088
Phy	<i>Phytophthora tropicalis</i>	p27	OOMYA2132-10	GU945500, GU993915
Phy	<i>Phytophthora tropicalis</i>	P6522	PHYTO198-10	HQ261467, HQ261720
Phy	<i>Phytophthora tropicalis</i> *	CBS 434.91	OOMYA159-07	HQ708417, HQ643369, HQ665233

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Phy	<i>Phytophthora uliginosa</i>	P10328	PHYTO032-10	HQ261469, HQ261722, EU079697
Phy	<i>Phytophthora uliginosa</i>	P10413	PHYTO048-10	HQ261468, HQ261721, EU080015
Phy	<i>Phytophthora vignae</i>	P7471	PHYTO218-10	HQ261470, HQ261723
Phy	<i>Phytophthora vignae</i>	P3019	PHYTO155-10	HQ261471, HQ261724, EU079787
	<i>Phytophythium aff. cucurbitacearum</i>	Lev 2210	OOMYA450-07	HQ708996, HQ643955
	<i>Phytophythium aff. vexans</i>	Lev 3100	OOMYA1375-08	HQ708995, HQ643954
	<i>Phytophythium aff. vexans</i>	CBS 261.30	OOMYA090-07	HQ708418, HQ643371, HQ665178
	<i>Phytophythium boreale</i>	CBS 551.88	OOMYA183-07	HQ708419, HQ643372, HQ665261
	<i>Phytophythium carbonicum*</i>	CBS 112544	OOMYA1372-08	HQ708420, HQ643373, HQ665073
	<i>Phytophythium chamaehyphon*</i>	CBS 259.30	OOMYA088-07	HQ708421, HQ643374, HQ665177
	<i>Phytophythium citrinum</i>	ADC 9442	OOMYA1649-08	HQ708427, HQ643380
	<i>Phytophythium citrinum</i>	ADC 9512	OOMYA1754-08	HQ708426, HQ643379
	<i>Phytophythium citrinum</i>	ADC 9513	OOMYA1755-08	HQ708425, HQ643378
	<i>Phytophythium citrinum</i>	ADC 9515	OOMYA1756-08	HQ708424, HQ643377
	<i>Phytophythium citrinum</i>	ADC 9519	OOMYA1758-08	HQ708423, HQ643376
	<i>Phytophythium citrinum*</i>	CBS 119171	OOMYA1378-08	HQ708422, HQ643375, HQ665088
	<i>Phytophythium cucurbitacearum</i>	CBS 748.96	OOMYA230-07	HQ708428, HQ643381, HQ665292
	<i>Phytophythium helicoides</i>	ADC 9423	OOMYA1648-08	HQ708431, HQ643384
	<i>Phytophythium helicoides</i>	CBS 167.68	OOMYA1419-08	HQ708429, HQ643382
	<i>Phytophythium helicoides*</i>	CBS 286.31	OOMYA1420-08	HQ708430, HQ643383, HQ665186
	<i>Phytophythium litorale</i>	BR 896	OOMYA1695-08	HQ708434, HQ643387
	<i>Phytophythium litorale</i>	Lev 3002	OOMYA452-07	HQ708721, HQ643677
	<i>Phytophythium litorale</i>	Lev 3006	OOMYA454-07	HQ708720, HQ643676
	<i>Phytophythium litorale</i>	Lev 3011	OOMYA1676-08	HQ708719, HQ643675
	<i>Phytophythium litorale</i>	Lev 3012	OOMYA1677-08	HQ708718, HQ643674
	<i>Phytophythium litorale</i>	Lev 3013	OOMYA1678-08	HQ708717, HQ643673
	<i>Phytophythium litorale</i>	CBS 122662	OOMYA1379-08	HQ708432, HQ643385, HQ665114
	<i>Phytophythium litorale*</i>	CBS 118360	OOMYA1570-08	HQ708433, HQ643386, HQ665082
	<i>Phytophythium megacarpum*</i>	CBS 112351	OOMYA1585-08	HQ708435, HQ643388, HQ665067
	<i>Phytophythium montanum</i>	ADC 9762	OOMYA1591-08	HQ708438, HQ643391
	<i>Phytophythium montanum</i>	ADC 9766	OOMYA1592-08	HQ708437, HQ643390
	<i>Phytophythium montanum*</i>	CBS 111349	OOMYA1593-08	HQ708436, HQ643389, HQ665064
	<i>Phytophythium oedochilum</i>	DAOM 229148	OOMYA1560-08	HQ708756, HQ643712
	<i>Phytophythium oedochilum*</i>	CBS 292.37	OOMYA107-07	HQ708439, HQ643392, HQ665191
	<i>Phytophythium ostracodes</i>	CBS 768.73	OOMYA233-07	HQ708442, HQ643395, HQ665295
	<i>Phytophythium sindhum*</i>	DAOM 238986	OOMYA2165-10	HQ708443, HQ643396, HQ665309
	<i>Phytophythium sp. "amazonianum"</i>	P8239	PHYTO239-10	HQ261476, HQ261729
	<i>Phytophythium sp. "amazonianum"</i>	P8242	PHYTO241-10	HQ261475, HQ261728
	<i>Phytophythium sp. "amazonianum"</i>	P8243	PHYTO242-10	HQ261474, HQ261727
	<i>Phytophythium sp. "amazonianum"</i>	P8246	PHYTO243-10	HQ261473, HQ261726
	<i>Phytophythium sp. "amazonianum"</i>	P8247	PHYTO244-10	HQ261472, HQ261725
	<i>Phytophythium sp. "grandilobatum"</i>	CBS 738.94	OOMYA1415-08	HQ708441, HQ643394
	<i>Phytophythium sp. "grandilobatum"</i>	CBS 739.94	OOMYA1416-08	HQ708440, HQ643393
	<i>Phytophythium sp. nov.</i>	ADC 0004	OOMYA1753-08	HQ708446, HQ643399
	<i>Phytophythium sp. nov.</i>	ADC 9517	OOMYA1757-08	HQ708445, HQ643398
	<i>Phytophythium sp. nov.</i>	ADC 9520	OOMYA1650-08	HQ708444, HQ643397
	<i>Phytophythium vexans</i>	CBS 455.62	OOMYA164-07	HQ708448, HQ643401
	<i>Phytophythium vexans</i>	CBS 119.80	OOMYA011-07	HQ708447, HQ643400, HQ665090
	<i>Phytophythium vexans</i>	P3980	PHYTO183-10	HQ261477, HQ261730, EU080487
Pla	<i>Plasmopara euphrasiae</i>	HV 2226	DM016-10	HM033194, EF553509, EF553465
Pla	<i>Plasmopara nivea</i>	HV 75	DM012-10	HM033195, HM004513
Pla	<i>Plasmopara pusilla</i>	HV 140	DM013-10	HM033196, HM004512, DQ148402
	<i>Plasmoverma anemones-ranunculoidis</i>	HV 125	DM014-10	HM033198, HM004511, HM004514
	<i>Plasmoverma anemones-ranunculoidis</i>	HV 101		EF553471
Ple	<i>Plectospora myriandra</i>	CBS 523.87	OOMYA1328-08	HQ708449, HQ643402, HQ665247
Pro	<i>Protoachlya paradoxa</i>	CBS 100.44	OOMYA1189-08	HQ708451, HQ643404
Pro	<i>Protoachlya paradoxa</i>	CBS 158.45	OOMYA1329-08	HQ708450, HQ643403, HQ665135
Pse	<i>Pseudoperonospora cubensis</i>	HV 222	DM010-10	HM033199, AY198306
Pse	<i>Pseudoperonospora cubensis</i>	HV 221h		AY035496
	<i>Pythiogeton zeae</i>	Lev 3132	OOMYA2166-10	HQ708452, HQ643405, HQ665310
	<i>Pythiopsis terrestris</i>	CBS 110058	OOMYA1816-08	HQ708454, HQ643407
	<i>Pythiopsis terrestris</i>	CBS 110059	OOMYA1817-08	HQ708453, HQ643406
Pyt	<i>Pythium abapressorium*</i>	CBS 110198	OOMYA1331-08	HQ708455, HQ643408, HQ665063
Pyt	<i>Pythium acanthicum</i>	BR 228	OOMYA1332-08	HQ708458, HQ643411
Pyt	<i>Pythium acanthicum</i>	BR 500	OOMYA556-08	HQ708459, HQ643412
Pyt	<i>Pythium acanthicum</i>	Lev 1618	OOMYA342-07	HQ708457, HQ643410
Pyt	<i>Pythium acanthicum</i>	CBS 377.34	OOMYA141-07	HQ708456, HQ643409, HQ665222
Pyt	<i>Pythium acanthophoron*</i>	CBS 337.29	OOMYA128-07	HQ708460, HQ643413, HQ665212
Pyt	<i>Pythium acrogynum*</i>	CBS 549.88	OOMYA181-07	HQ708461, HQ643414, HQ665258
Pyt	<i>Pythium adhaerens</i>	CBS 520.74	OOMYA174-07	HQ708462, HQ643415, HQ665245
Pyt	<i>Pythium fertile</i>	Lev 2066	OOMYA1334-08	HQ708463, HQ643416
Pyt	<i>Pythium aff. attrantheridium</i>	Lev 3004	OOMYA1669-08	HQ708520, HQ643473
Pyt	<i>Pythium aff. diclinum</i>	CBS 229.94	OOMYA1336-08	HQ708464, HQ643417
Pyt	<i>Pythium aff. dictyosporum</i>	ADC 0113	OOMYA1662-08	HQ708466, HQ643419
Pyt	<i>Pythium aff. dictyosporum</i>	ADC 0116	OOMYA1664-08	HQ708465, HQ643418
Pyt	<i>Pythium aff. dissotocum</i>	BR 725	OOMYA1665-08	HQ708470, HQ643423
Pyt	<i>Pythium aff. dissotocum</i>	BR 726	OOMYA1666-08	HQ708469, HQ643422
Pyt	<i>Pythium aff. dissotocum</i>	BR 907	OOMYA1345-08	HQ708467, HQ643420

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Pyt	<i>Pythium</i> aff. <i>dissotocum</i>	BR 992	OOMYA1821-08	HQ708468, HQ643421
Pyt	<i>Pythium</i> aff. <i>hydno sporum</i>	BR 978	OOMYA1697-08	HQ708471, HQ643424
Pyt	<i>Pythium</i> aff. <i>hypogynum</i>	BR 116	OOMYA1636-08	HQ708472, HQ643425
Pyt	<i>Pythium</i> aff. <i>iwayamai</i>	DAOM 229147	OOMYA1452-08	HQ708827, HQ643786
Pyt	<i>Pythium</i> aff. <i>macrosporum</i>	BR 1029	OOMYA1737-08	HQ708729, HQ643685
Pyt	<i>Pythium</i> aff. <i>oopapillum</i>	Lev 1619	OOMYA343-07	HQ708761, HQ643717
Pyt	<i>Pythium</i> aff. <i>perilum</i>	CBS 237.83	OOMYA1626-08	HQ708473, HQ643426
Pyt	<i>Pythium</i> aff. <i>perplexum</i>	BR 925	OOMYA1686-08	HQ708474, HQ643427
Pyt	<i>Pythium</i> aff. <i>pleroticum</i>	ADC 9912	OOMYA1779-08	HQ708475, HQ643428
Pyt	<i>Pythium</i> aff. <i>pleroticum</i>	Lev 2114	OOMYA423-07	HQ708788, HQ643747
Pyt	<i>Pythium</i> aff. <i>polymastum</i>	ADC 9817	OOMYA1652-08	HQ708476, HQ643429
Pyt	<i>Pythium</i> aff. <i>polymastum</i>	Lev 1630	OOMYA350-07	HQ708792, HQ643751
Pyt	<i>Pythium</i> aff. <i>torulosum</i>	BR 626	OOMYA349-07	HQ708479, HQ643432
Pyt	<i>Pythium</i> aff. <i>torulosum</i>	BR 780	OOMYA1683-08	HQ708478, HQ643431
Pyt	<i>Pythium</i> aff. <i>torulosum</i>	BR 897	OOMYA1641-08	HQ708477, HQ643430
Pyt	<i>Pythium</i> aff. <i>volutum</i>	BR 974	OOMYA1355-08	HQ708480, HQ643433
Pyt	<i>Pythium</i> aff. <i>volutum</i>	Lev 1528	OOMYA1594-08	HQ709011, HQ643970
Pyt	<i>Pythium</i> <i>amasculinum</i> *	CBS 552.88	OOMYA184-07	HQ708481, HQ643434, HQ665263
Pyt	<i>Pythium</i> <i>anandrum</i>	Lev 2116	OOMYA424-07	HQ708483, HQ643436
Pyt	<i>Pythium</i> <i>anandrum</i> *	CBS 285.31	OOMYA1335-08	HQ708482, HQ643435, HQ665185
Pyt	<i>Pythium</i> <i>angustatum</i>	CBS 522.74	OOMYA175-07	HQ708484, HQ643437, HQ665246
Pyt	<i>Pythium</i> <i>aphanidermatum</i>	CBS 287.79	OOMYA1337-08	HQ708486, HQ643439
Pyt	<i>Pythium</i> <i>aphanidermatum</i>	Lev 1800	OOMYA385-07	HQ708489, HQ643442
Pyt	<i>Pythium</i> <i>aphanidermatum</i>	Lev 2150	OOMYA1607-08	HQ708488, HQ643441
Pyt	<i>Pythium</i> <i>aphanidermatum</i>	Lev 3014	OOMYA1657-08	HQ708487, HQ643440
Pyt	<i>Pythium</i> <i>aphanidermatum</i>	CBS 118.80	OOMYA010-07	HQ708485, HQ643438, HQ665084
Pyt	<i>Pythium</i> <i>apiculatum</i> *	CBS 120945	OOMYA1341-08	HQ708490, HQ643443, HQ665092
Pyt	<i>Pythium</i> <i>apteroticum</i>	CBS 772.81	OOMYA234-07	HQ708491, HQ643444, HQ665296
Pyt	<i>Pythium</i> <i>aquatile</i>	BR 654	OOMYA1343-08	HQ708493, HQ643446
Pyt	<i>Pythium</i> <i>aquatile</i>	CBS 215.80	OOMYA048-07	HQ708492, HQ643445, HQ665153
Pyt	<i>Pythium</i> <i>aristosporum</i>	BR 166	OOMYA1346-08	HQ708495, HQ643448
Pyt	<i>Pythium</i> <i>aristosporum</i> *	CBS 263.38	OOMYA1347-08	HQ708494, HQ643447, HQ665179
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 1028	OOMYA1361-08	HQ708519, HQ643472
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 122	OOMYA1349-08	HQ708518, HQ643471
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 140	OOMYA1350-08	HQ708517, HQ643470
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 155	OOMYA1412-08	HQ708516, HQ643469
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 605	OOMYA1658-08	HQ708515, HQ643468
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 606	OOMYA1688-08	HQ708514, HQ643467
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 615	OOMYA1689-08	HQ708513, HQ643466
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 618	OOMYA1690-08	HQ708512, HQ643465
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 619	OOMYA1691-08	HQ708511, HQ643464
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 625	OOMYA1413-08	HQ708510, HQ643463
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 667	OOMYA1351-08	HQ708509, HQ643462
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 712	OOMYA1352-08	HQ708508, HQ643461
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 729	OOMYA1667-08	HQ708507, HQ643460
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 817	OOMYA1353-08	HQ708506, HQ643459
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 889	OOMYA1354-08	HQ708496, HQ643449
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 975	OOMYA1659-08	HQ708505, HQ643458
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 980	OOMYA1356-08	HQ708504, HQ643457
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 981	OOMYA1357-08	HQ708503, HQ643456
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 982	OOMYA1358-08	HQ708502, HQ643455
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 983	OOMYA1359-08	HQ708501, HQ643454
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	BR 985	OOMYA1360-08	HQ708500, HQ643453
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	Lev 1538	OOMYA313-07	HQ708498, HQ643451
Pyt	<i>Pythium</i> <i>arrhenomanes</i>	Lev 1728	OOMYA1362-08	HQ708497, HQ643450
Pyt	<i>Pythium</i> <i>arrhenomanes</i> *	CBS 324.62	OOMYA122-07	HQ708499, HQ643452, HQ665208
Pyt	<i>Pythium</i> <i>atrantheridium</i>	DAOM 230386	OOMYA1367-08	HQ708523, HQ643476
Pyt	<i>Pythium</i> <i>atrantheridium</i>	DAOM 230387	OOMYA1368-08	HQ708522, HQ643475
Pyt	<i>Pythium</i> <i>atrantheridium</i>	DAOM 230388	OOMYA1369-08	HQ708521, HQ643474
Pyt	<i>Pythium</i> <i>atrantheridium</i> *	DAOM 230383	OOMYA1364-08	HQ708524, HQ643477, HQ665308
Pyt	<i>Pythium</i> <i>buismaniae</i> *	CBS 288.31	OOMYA102-07	HQ708526, HQ643479, HQ665188
Pyt	<i>Pythium</i> <i>camurandrum</i>	CBS 124096	OOMYA1653-08	HQ708527, HQ643481
Pyt	<i>Pythium</i> <i>camurandrum</i> *	BR 876	OOMYA1715-08	GQ244425, GQ244426
Pyt	<i>Pythium</i> <i>canariense</i> *	CBS 112353	OOMYA1371-08	HQ708528, HQ643482, HQ665069
Pyt	<i>Pythium</i> <i>capillosum</i> *	CBS 222.94	OOMYA061-07	HQ708529, HQ643483, HQ665164
Pyt	<i>Pythium</i> <i>carolinianum</i>	BR 161	OOMYA1637-08	HQ708534, HQ643488
Pyt	<i>Pythium</i> <i>carolinianum</i>	BR 786	OOMYA1692-08	HQ708533, HQ643487
Pyt	<i>Pythium</i> <i>carolinianum</i>	BR 803	OOMYA1714-08	HQ708532, HQ643486
Pyt	<i>Pythium</i> <i>carolinianum</i>	P19301	PHYTO142-10	HQ261478, HQ261731
Pyt	<i>Pythium</i> <i>carolinianum</i>	CBS 122658	OOMYA1605-08	HQ708531, HQ643485, HQ665110
Pyt	<i>Pythium</i> <i>carolinianum</i>	CBS 122659	OOMYA1373-08	HQ708530, HQ643484, HQ665111
Pyt	<i>Pythium</i> <i>catenulatum</i>	CBS 226.94	OOMYA1333-08	HQ708536, HQ643490
Pyt	<i>Pythium</i> <i>catenulatum</i>	CBS 461.75	OOMYA165-07	HQ708535, HQ643489
Pyt	<i>Pythium</i> <i>catenulatum</i>	Lev 1524	OOMYA302-07	HQ708539, HQ643493
Pyt	<i>Pythium</i> <i>catenulatum</i>	Lev 1532	OOMYA308-07	HQ708538, HQ643492
Pyt	<i>Pythium</i> <i>catenulatum</i>	Lev 1533	OOMYA1627-08	HQ708537, HQ643491
Pyt	<i>Pythium</i> <i>catenulatum</i>	CBS 842.68	OOMYA238-07	HQ708540, HQ643494, HQ665302
Pyt	<i>Pythium</i> cf. <i>dictyosporum</i>	ADC 0114	OOMYA1663-08	HQ708541, HQ643495

Genus Abbrev	Isolate name	Isolate #	BOLD Process ID	Genbank Accessions
Pyt	<i>Pythium chondricola</i>	CBS 206.85	OOMYA1376-08	HQ708543, HQ643497
Pyt	<i>Pythium chondricola</i>	CBS 207.85	OOMYA1377-08	HQ708542, HQ643496
Pyt	<i>Pythium chondricola</i>	CBS 312.93	OOMYA1581-08	HQ708545, HQ643499
Pyt	<i>Pythium chondricola*</i>	CBS 203.85	OOMYA040-07	HQ708544, HQ643498, HQ665149
Pyt	<i>Pythium coloratum</i>	BR 1063	OOMYA1391-08	HQ708553, HQ643507
Pyt	<i>Pythium coloratum</i>	BR 176	OOMYA1380-08	HQ708552, HQ643506
Pyt	<i>Pythium coloratum</i>	BR 323	OOMYA1382-08	HQ708551, HQ643505
Pyt	<i>Pythium coloratum</i>	BR 621	OOMYA1383-08	HQ708550, HQ643504
Pyt	<i>Pythium coloratum</i>	BR 677	OOMYA1385-08	HQ708549, HQ643503
Pyt	<i>Pythium coloratum</i>	BR 678	OOMYA1386-08	HQ708548, HQ643502
Pyt	<i>Pythium coloratum</i>	BR 908	OOMYA1389-08	HQ708546, HQ643500
Pyt	<i>Pythium coloratum*</i>	CBS 154.64	OOMYA019-07	HQ708547, HQ643501, HQ665128
Pyt	<i>Pythium conidiophorum</i>	BR 160	OOMYA1399-08	HQ708559, HQ643513
Pyt	<i>Pythium conidiophorum</i>	BR 260	OOMYA1433-08	HQ708558, HQ643512
Pyt	<i>Pythium conidiophorum</i>	CBS 224.88	OOMYA063-07	HQ708554, HQ643508
Pyt	<i>Pythium conidiophorum</i>	Lev 1465	OOMYA279-07	HQ708557, HQ643511
Pyt	<i>Pythium conidiophorum</i>	Lev 1624	OOMYA346-07	HQ708556, HQ643510
Pyt	<i>Pythium conidiophorum</i>	CBS 223.88	OOMYA062-07	HQ708555, HQ643509, HQ665166
Pyt	<i>Pythium contiguanum*</i>	CBS 221.94	OOMYA1392-08	HQ708560, HQ643514, HQ665162
Pyt	<i>Pythium cryptoirregulare*</i>	CBS 118731	OOMYA1393-08	HQ708561, HQ643515, HQ665083
Pyt	<i>Pythium cylindrosporium</i>	DAOM 232335	OOMYA1394-08	HQ708563, HQ643517
Pyt	<i>Pythium cylindrosporium*</i>	CBS 218.94	OOMYA053-07	HQ708562, HQ643516, HQ665157
Pyt	<i>Pythium cystogenes*</i>	CBS 675.85	OOMYA1395-08	HQ708564, HQ643518, HQ665284
Pyt	<i>Pythium debaryanum</i>	CBS 752.96	OOMYA232-07	HQ708565, HQ643519, HQ665294
Pyt	<i>Pythium deliense</i>	CBS 114.84	OOMYA008-07	HQ708566, HQ643520
Pyt	<i>Pythium deliense</i>	Lev 2095	OOMYA018-07	HQ708567, HQ643521
Pyt	<i>Pythium deliense*</i>	CBS 314.33	OOMYA118-07	HQ708568, HQ643522, HQ665204
Pyt	<i>Pythium diclinum</i>	CBS 526.74	OOMYA1398-08	HQ708569, HQ643523
Pyt	<i>Pythium diclinum</i>	CBS 664.79	OOMYA208-07	HQ708570, HQ643524, HQ665282
Pyt	<i>Pythium dimorphum*</i>	CBS 406.72	OOMYA152-07	HQ708571, HQ643525, HQ665229
Pyt	<i>Pythium dissimile</i>	CBS 523.74	OOMYA1401-08	HQ708573, HQ643527
Pyt	<i>Pythium dissimile*</i>	CBS 155.64	OOMYA020-07	HQ708572, HQ643526, HQ665130
Pyt	<i>Pythium dissotocum</i>	BR 127	OOMYA1402-08	HQ708576, HQ643530
Pyt	<i>Pythium dissotocum</i>	DAOM 229134	OOMYA1403-08	HQ708575, HQ643529
Pyt	<i>Pythium dissotocum</i>	CBS 166.68	OOMYA027-07	HQ708574, HQ643528, HQ665139
Pyt	<i>Pythium echinulatum</i>	CBS 281.64	OOMYA099-07	HQ708577, HQ643531, HQ665183
Pyt	<i>Pythium emineosum</i>	BR 836	OOMYA1578-08	GQ244424, GQ244428
Pyt	<i>Pythium emineosum*</i>	BR 479	OOMYA1576-08	GQ244423, GQ244427
Pyt	<i>Pythium erinaceus</i>	Lev 1620	OOMYA344-07	HQ708579, HQ643535
Pyt	<i>Pythium erinaceus*</i>	CBS 505.80	OOMYA172-07	HQ708578, HQ643534, HQ665243
Pyt	<i>Pythium flevoense</i>	CBS 233.72	OOMYA1405-08	HQ708583, HQ643539
Pyt	<i>Pythium flevoense</i>	CBS 236.72	OOMYA076-07	HQ708581, HQ643537
Pyt	<i>Pythium flevoense</i>	CBS 278.81	OOMYA1225-08	HQ708580, HQ643536, HQ665182
Pyt	<i>Pythium flevoense*</i>	CBS 234.72	OOMYA073-07	HQ708582, HQ643538, HQ665170
Pyt	<i>Pythium folliculosum*</i>	CBS 220.94	OOMYA057-07	HQ708584, HQ643540, HQ665160
Pyt	<i>Pythium glomeratum</i>	CBS 119165	OOMYA1406-08	HQ708588, HQ643544, HQ665085
Pyt	<i>Pythium glomeratum</i>	CBS 120914	OOMYA1407-08	HQ708587, HQ643543, HQ665091
Pyt	<i>Pythium glomeratum</i>	CBS 122644	OOMYA1408-08	HQ708586, HQ643542, HQ665097
Pyt	<i>Pythium glomeratum</i>	CBS 122651	OOMYA1410-08	HQ708585, HQ643541, HQ665104
Pyt	<i>Pythium graminicola</i>	CBS 327.62	OOMYA125-07	HQ708589, HQ643545, HQ665211
Pyt	<i>Pythium grandisporangium*</i>	CBS 286.79	OOMYA100-07	HQ708590, HQ643546, HQ665187
Pyt	<i>Pythium helicandrum</i>	CBS 527.74	OOMYA1417-08	HQ708591, HQ643547
Pyt	<i>Pythium helicandrum</i>	CBS 694.79	OOMYA1418-08	HQ708594, HQ643550
Pyt	<i>Pythium helicandrum</i>	CBS 844.68	OOMYA239-07	HQ708593, HQ643549
Pyt	<i>Pythium helicandrum*</i>	CBS 393.54	OOMYA147-07	HQ708592, HQ643548, HQ665225
Pyt	<i>Pythium heterothallicum</i>	ADC 9868	OOMYA1747-08	HQ708607, HQ643563
Pyt	<i>Pythium heterothallicum</i>	BR 440	OOMYA1422-08	HQ708606, HQ643562
Pyt	<i>Pythium heterothallicum</i>	BR 749	OOMYA1712-08	HQ708605, HQ643561
Pyt	<i>Pythium heterothallicum</i>	BR 806	OOMYA1425-08	HQ708604, HQ643560
Pyt	<i>Pythium heterothallicum</i>	BR 828	OOMYA1746-08	HQ708603, HQ643559
Pyt	<i>Pythium heterothallicum</i>	DAOM 229136	OOMYA1429-08	HQ708602, HQ643558
Pyt	<i>Pythium heterothallicum</i>	DAOM 229137	OOMYA1430-08	HQ708601, HQ643557
Pyt	<i>Pythium heterothallicum</i>	DAOM 229138	OOMYA1431-08	HQ708600, HQ643556
Pyt	<i>Pythium heterothallicum</i>	DAOM 229140	OOMYA441-07	HQ708599, HQ643555
Pyt	<i>Pythium heterothallicum</i>	Lev 1590	OOMYA326-07	HQ708598, HQ643554
Pyt	<i>Pythium heterothallicum</i>	CBS 122655	OOMYA1427-08	HQ708596, HQ643552, HQ665107
Pyt	<i>Pythium heterothallicum</i>	CBS 122656	OOMYA1428-08	HQ708595, HQ643551, HQ665108
Pyt	<i>Pythium heterothallicum*</i>	CBS 450.67	OOMYA161-07	HQ708597, HQ643553, HQ665235
Pyt	<i>Pythium hydnosporum</i>	CBS 253.60	OOMYA085-07	HQ708608, HQ643564, HQ665175
Pyt	<i>Pythium hypogynum</i>	CBS 234.94	OOMYA074-07	HQ708609, HQ643565, HQ665171
Pyt	<i>Pythium inflatum</i>	CBS 168.68	OOMYA029-07	HQ708610, HQ643566, HQ665140
Pyt	<i>Pythium insidiosum</i>	CBS 577.85	OOMYA1435-08	HQ708613, HQ643569
Pyt	<i>Pythium insidiosum</i>	CBS 578.85	OOMYA1436-08	HQ708612, HQ643568
Pyt	<i>Pythium insidiosum</i>	CBS 580.85	OOMYA1437-08	HQ708611, HQ643567
Pyt	<i>Pythium insidiosum*</i>	CBS 574.85	OOMYA193-07	HQ708614, HQ643570, HQ665273
Pyt	<i>Pythium intermedium</i>	BR 1042	OOMYA1447-08	HQ708623, HQ643579
Pyt	<i>Pythium intermedium</i>	BR 128	OOMYA1828-08	HQ708622, HQ643578
Pyt	<i>Pythium intermedium</i>	BR 339	OOMYA1438-08	HQ708621, HQ643577

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Pyt	<i>Pythium intermedium</i>	BR 485	OOMYA1439-08	HQ708620, HQ643576
Pyt	<i>Pythium intermedium</i>	BR 707	OOMYA1440-08	HQ708619, HQ643575
Pyt	<i>Pythium intermedium</i>	BR 734	OOMYA1441-08	HQ708618, HQ643574
Pyt	<i>Pythium intermedium</i>	BR 869	OOMYA1800-08	HQ708617, HQ643573
Pyt	<i>Pythium intermedium</i>	BR 924	OOMYA1443-08	HQ708615, HQ643571
Pyt	<i>Pythium intermedium</i>	CBS 266.38	OOMYA091-07	HQ708616, HQ643572, HQ665180
Pyt	<i>Pythium irregulare</i>	BR 1000	OOMYA1533-08	HQ708711, HQ643667
Pyt	<i>Pythium irregulare</i>	BR 1001	OOMYA401-07	HQ708710, HQ643666
Pyt	<i>Pythium irregulare</i>	BR 1002	OOMYA1534-08	HQ708709, HQ643665
Pyt	<i>Pythium irregulare</i>	BR 1003	OOMYA1535-08	HQ708708, HQ643664
Pyt	<i>Pythium irregulare</i>	BR 1004	OOMYA1536-08	HQ708707, HQ643663
Pyt	<i>Pythium irregulare</i>	BR 1005	OOMYA1537-08	HQ708706, HQ643662
Pyt	<i>Pythium irregulare</i>	BR 1006	OOMYA1538-08	HQ708705, HQ643661
Pyt	<i>Pythium irregulare</i>	BR 1008	OOMYA1539-08	HQ708704, HQ643660
Pyt	<i>Pythium irregulare</i>	BR 1009	OOMYA1540-08	HQ708703, HQ643659
Pyt	<i>Pythium irregulare</i>	BR 1013	OOMYA402-07	HQ708702, HQ643658
Pyt	<i>Pythium irregulare</i>	BR 1014	OOMYA1543-08	HQ708701, HQ643657
Pyt	<i>Pythium irregulare</i>	BR 1015	OOMYA1544-08	HQ708700, HQ643656
Pyt	<i>Pythium irregulare</i>	BR 1016	OOMYA1545-08	HQ708699, HQ643655
Pyt	<i>Pythium irregulare</i>	BR 1017	OOMYA1546-08	HQ708698, HQ643654
Pyt	<i>Pythium irregulare</i>	BR 1018	OOMYA1547-08	HQ708697, HQ643653
Pyt	<i>Pythium irregulare</i>	BR 1019	OOMYA1548-08	HQ708696, HQ643652
Pyt	<i>Pythium irregulare</i>	BR 1021	OOMYA1549-08	HQ708695, HQ643651
Pyt	<i>Pythium irregulare</i>	BR 1022	OOMYA1550-08	HQ708694, HQ643650
Pyt	<i>Pythium irregulare</i>	BR 1039	OOMYA1553-08	HQ708693, HQ643649
Pyt	<i>Pythium irregulare</i>	BR 1040	OOMYA1554-08	HQ708692, HQ643648
Pyt	<i>Pythium irregulare</i>	BR 1051	OOMYA1555-08	HQ708691, HQ643647
Pyt	<i>Pythium irregulare</i>	BR 1052	OOMYA1556-08	HQ708690, HQ643646
Pyt	<i>Pythium irregulare</i>	BR 1068	OOMYA1558-08	HQ708689, HQ643645
Pyt	<i>Pythium irregulare</i>	BR 387	OOMYA1456-08	HQ708688, HQ643644
Pyt	<i>Pythium irregulare</i>	BR 469	OOMYA1457-08	HQ708687, HQ643643
Pyt	<i>Pythium irregulare</i>	BR 598	OOMYA1458-08	HQ708686, HQ643642
Pyt	<i>Pythium irregulare</i>	BR 629	OOMYA1460-08	HQ708685, HQ643641
Pyt	<i>Pythium irregulare</i>	BR 630	OOMYA1461-08	HQ708684, HQ643640
Pyt	<i>Pythium irregulare</i>	BR 631	OOMYA1462-08	HQ708683, HQ643639
Pyt	<i>Pythium irregulare</i>	BR 636	OOMYA331-07	HQ708682, HQ643638
Pyt	<i>Pythium irregulare</i>	BR 642	OOMYA1463-08	HQ708681, HQ643637
Pyt	<i>Pythium irregulare</i>	BR 722	OOMYA1465-08	HQ708680, HQ643636
Pyt	<i>Pythium irregulare</i>	BR 733	OOMYA1467-08	HQ708679, HQ643635
Pyt	<i>Pythium irregulare</i>	BR 772	OOMYA1469-08	HQ708678, HQ643634
Pyt	<i>Pythium irregulare</i>	BR 775	OOMYA1470-08	HQ708677, HQ643633
Pyt	<i>Pythium irregulare</i>	BR 778	OOMYA1471-08	HQ708676, HQ643632
Pyt	<i>Pythium irregulare</i>	BR 802	OOMYA1473-08	HQ708675, HQ643631
Pyt	<i>Pythium irregulare</i>	BR 804	OOMYA1474-08	HQ708674, HQ643630
Pyt	<i>Pythium irregulare</i>	BR 808	OOMYA1476-08	HQ708673, HQ643629
Pyt	<i>Pythium irregulare</i>	BR 811	OOMYA1477-08	HQ708672, HQ643628
Pyt	<i>Pythium irregulare</i>	BR 812	OOMYA1478-08	HQ708671, HQ643627
Pyt	<i>Pythium irregulare</i>	BR 815	OOMYA1479-08	HQ708670, HQ643626
Pyt	<i>Pythium irregulare</i>	BR 818	OOMYA1480-08	HQ708669, HQ643625
Pyt	<i>Pythium irregulare</i>	BR 819	OOMYA1693-08	HQ708668, HQ643624
Pyt	<i>Pythium irregulare</i>	BR 820	OOMYA1481-08	HQ708667, HQ643623
Pyt	<i>Pythium irregulare</i>	BR 821	OOMYA1694-08	HQ708666, HQ643622
Pyt	<i>Pythium irregulare</i>	BR 870	OOMYA1482-08	HQ708665, HQ643621
Pyt	<i>Pythium irregulare</i>	BR 900	OOMYA1486-08	HQ708639, HQ643595
Pyt	<i>Pythium irregulare</i>	BR 909	OOMYA1489-08	HQ708638, HQ643594
Pyt	<i>Pythium irregulare</i>	BR 911	OOMYA1490-08	HQ708637, HQ643593
Pyt	<i>Pythium irregulare</i>	BR 912	OOMYA1491-08	HQ708636, HQ643592
Pyt	<i>Pythium irregulare</i>	BR 914	OOMYA1492-08	HQ708635, HQ643591
Pyt	<i>Pythium irregulare</i>	BR 918	OOMYA1495-08	HQ708634, HQ643590
Pyt	<i>Pythium irregulare</i>	BR 920	OOMYA1496-08	HQ708633, HQ643589
Pyt	<i>Pythium irregulare</i>	BR 921	OOMYA1497-08	HQ708632, HQ643588
Pyt	<i>Pythium irregulare</i>	BR 923	OOMYA1498-08	HQ708631, HQ643587
Pyt	<i>Pythium irregulare</i>	BR 933	OOMYA338-07	HQ708630, HQ643586
Pyt	<i>Pythium irregulare</i>	BR 934	OOMYA1500-08	HQ708629, HQ643585
Pyt	<i>Pythium irregulare</i>	BR 940	OOMYA1506-08	HQ708628, HQ643584
Pyt	<i>Pythium irregulare</i>	BR 946	OOMYA1508-08	HQ708627, HQ643583
Pyt	<i>Pythium irregulare</i>	BR 947	OOMYA1509-08	HQ708626, HQ643582
Pyt	<i>Pythium irregulare</i>	BR 948	OOMYA1510-08	HQ708625, HQ643581
Pyt	<i>Pythium irregulare</i>	BR 959	OOMYA1514-08	HQ708624, HQ643580
Pyt	<i>Pythium irregulare</i>	BR 960	OOMYA1515-08	HQ708664, HQ643620
Pyt	<i>Pythium irregulare</i>	BR 961	OOMYA1516-08	HQ708663, HQ643619
Pyt	<i>Pythium irregulare</i>	BR 962	OOMYA339-07	HQ708662, HQ643618
Pyt	<i>Pythium irregulare</i>	BR 963	OOMYA1517-08	HQ708661, HQ643617
Pyt	<i>Pythium irregulare</i>	BR 964	OOMYA1518-08	HQ708660, HQ643616
Pyt	<i>Pythium irregulare</i>	BR 965	OOMYA1519-08	HQ708659, HQ643615
Pyt	<i>Pythium irregulare</i>	BR 966	OOMYA1520-08	HQ708658, HQ643614
Pyt	<i>Pythium irregulare</i>	BR 967	OOMYA1521-08	HQ708657, HQ643613
Pyt	<i>Pythium irregulare</i>	BR 969	OOMYA1522-08	HQ708656, HQ643612

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Pyt	<i>Pythium irregulare</i>	BR 970	OOMYA1523-08	HQ708655, HQ643611
Pyt	<i>Pythium irregulare</i>	BR 971	OOMYA1524-08	HQ708654, HQ643610
Pyt	<i>Pythium irregulare</i>	BR 972	OOMYA1525-08	HQ708653, HQ643609
Pyt	<i>Pythium irregulare</i>	BR 979	OOMYA1698-08	HQ708652, HQ643608
Pyt	<i>Pythium irregulare</i>	BR 994	OOMYA1528-08	HQ708651, HQ643607
Pyt	<i>Pythium irregulare</i>	BR 995	OOMYA1529-08	HQ708650, HQ643606
Pyt	<i>Pythium irregulare</i>	BR 996	OOMYA1530-08	HQ708649, HQ643605
Pyt	<i>Pythium irregulare</i>	BR 997	OOMYA1531-08	HQ708648, HQ643604
Pyt	<i>Pythium irregulare</i>	BR 999	OOMYA1532-08	HQ708647, HQ643603
Pyt	<i>Pythium irregulare</i>	CBS 749.96	OOMYA1559-08	HQ708646, HQ643602
Pyt	<i>Pythium irregulare</i>	DAOM 232336	OOMYA1561-08	HQ708645, HQ643601
Pyt	<i>Pythium irregulare</i>	Lev 1680	OOMYA1762-08	HQ708644, HQ643600
Pyt	<i>Pythium irregulare</i>	Lev 2120	OOMYA1563-08	HQ708643, HQ643599
Pyt	<i>Pythium irregulare</i>	Lev 2151	OOMYA433-07	HQ708642, HQ643598
Pyt	<i>Pythium irregulare</i>	Lev 3105	OOMYA1566-08	HQ708641, HQ643597
Pyt	<i>Pythium irregulare</i>	ADC 0838	OOMYA1453-08	HQ708712, HQ643668, HQ665051
Pyt	<i>Pythium irregulare</i>	CBS 250.28	OOMYA083-07	HQ708640, HQ643596, HQ665172
Pyt	<i>Pythium iwayamai</i>	CBS 156.64	OOMYA021-07	HQ708713, HQ643669, HQ665131
Pyt	<i>Pythium kashmirensense*</i>	CBS 122908	OOMYA1569-08	HQ708715, HQ643671, HQ665118
Pyt	<i>Pythium kunmingense*</i>	CBS 550.88	OOMYA182-07	HQ708716, HQ643672, HQ665259
Pyt	<i>Pythium longandrum</i>	CBS 122660	OOMYA1572-08	HQ708722, HQ643678, HQ665112
Pyt	<i>Pythium longandrum*</i>	CBS 112355	OOMYA1571-08	HQ708723, HQ643679, HQ665071
Pyt	<i>Pythium longisporangium*</i>	CBS 122646	OOMYA1573-08	HQ708724, HQ643680, HQ665099
Pyt	<i>Pythium lucens*</i>	CBS 113342	OOMYA1574-08	HQ708725, HQ643681, HQ665077
Pyt	<i>Pythium lutarium*</i>	CBS 222.88	OOMYA060-07	HQ708726, HQ643682, HQ665163
Pyt	<i>Pythium lycopersicum*</i>	CBS 122909	OOMYA1575-08	HQ708727, HQ643683, HQ665119
Pyt	<i>Pythium macrosporum</i>	ADC 0029	OOMYA1776-08	HQ708730, HQ643686
Pyt	<i>Pythium macrosporum*</i>	CBS 574.80	OOMYA192-07	HQ708728, HQ643684, HQ665272
Pyt	<i>Pythium mamillatum</i>	BR 648	OOMYA1580-08	HQ708733, HQ643689
Pyt	<i>Pythium mamillatum</i>	BR 765	OOMYA340-07	HQ708732, HQ643688
Pyt	<i>Pythium mamillatum</i>	CBS 251.28	OOMYA084-07	HQ708731, HQ643687, HQ665173
Pyt	<i>Pythium marsipium</i>	CBS 773.81	OOMYA235-07	HQ708734, HQ643690, HQ665297
Pyt	<i>Pythium mastophorum</i>	ADC 0158	OOMYA1582-08	HQ708736, HQ643692
Pyt	<i>Pythium mastophorum</i>	CBS 375.72	OOMYA139-07	HQ708735, HQ643691, HQ665220
Pyt	<i>Pythium megalacanthum</i>	DAOM 229154	OOMYA1586-08	HQ708737, HQ643693
Pyt	<i>Pythium middletonii</i>	CBS 528.74	OOMYA177-07	HQ708738, HQ643694, HQ665249
Pyt	<i>Pythium minus</i>	CBS 122657	OOMYA1589-08	HQ708739, HQ643695, HQ665109
Pyt	<i>Pythium minus*</i>	CBS 226.88	OOMYA086-07	HQ708740, HQ643696, HQ665168
Pyt	<i>Pythium monospermum</i>	BR 1031	OOMYA1681-08	HQ708743, HQ643699
Pyt	<i>Pythium monospermum</i>	BR 1032	OOMYA1682-08	HQ708742, HQ643698
Pyt	<i>Pythium monospermum</i>	CBS 158.73	OOMYA025-07	HQ708741, HQ643697, HQ665137
Pyt	<i>Pythium multisporum*</i>	CBS 470.50	OOMYA168-07	HQ708744, HQ643700, HQ665239
Pyt	<i>Pythium myriotylum</i>	CBS 695.79	OOMYA218-07	HQ708748, HQ643704
Pyt	<i>Pythium myriotylum</i>	Lev 1529	OOMYA306-07	HQ708747, HQ643703
Pyt	<i>Pythium myriotylum</i>	Lev 1737	OOMYA376-07	HQ708746, HQ643702
Pyt	<i>Pythium myriotylum</i>	CBS 254.70	OOMYA086-07	HQ708745, HQ643701, HQ665176
Pyt	<i>Pythium nagaii</i>	BR 602	OOMYA330-07	HQ708751, HQ643707
Pyt	<i>Pythium nagaii</i>	BR 646	OOMYA1612-08	HQ708750, HQ643706
Pyt	<i>Pythium nagaii</i>	CBS 779.96	OOMYA1597-08	HQ708749, HQ643705, HQ665299
Pyt	<i>Pythium nodosum</i>	CBS 122661	OOMYA1614-08	HQ708752, HQ643708, HQ665113
Pyt	<i>Pythium nodosum*</i>	CBS 102274	OOMYA1598-08	HQ708753, HQ643709, HQ665055
Pyt	<i>Pythium nunn</i>	Lev 2098	OOMYA1599-08	HQ708754, HQ643710
Pyt	<i>Pythium nunn*</i>	CBS 808.96	OOMYA237-07	HQ708755, HQ643711, HQ665300
Pyt	<i>Pythium okanoganense</i>	CBS 701.83	OOMYA222-07	HQ708757, HQ643713
Pyt	<i>Pythium okanoganense*</i>	CBS 315.81	OOMYA119-07	HQ708758, HQ643714, HQ665205
Pyt	<i>Pythium oligandrum</i>	BR 252	OOMYA1602-08	HQ708760, HQ643716
Pyt	<i>Pythium oligandrum</i>	CBS 382.34	OOMYA145-07	HQ708759, HQ643715, HQ665223
Pyt	<i>Pythium oopapillum</i>	BR 180	OOMYA1381-08	FJ655180, FJ655176
Pyt	<i>Pythium oopapillum</i>	BR 641	OOMYA1342-08	FJ655179, FJ655175
Pyt	<i>Pythium oopapillum*</i>	BR 632	OOMYA1384-08	FJ655178, FJ655174
Pyt	<i>Pythium ornacarpum*</i>	CBS 112350	OOMYA1603-08	HQ708762, HQ643721, HQ665066
Pyt	<i>Pythium ornamentatum</i>	CBS 122665	OOMYA1604-08	HQ708763, HQ643722, HQ665117
Pyt	<i>Pythium orthogonon*</i>	CBS 376.72	OOMYA140-07	HQ708764, HQ643723, HQ665221
Pyt	<i>Pythium pachycaule</i>	BR 679	OOMYA1387-08	HQ708768, HQ643727
Pyt	<i>Pythium pachycaule</i>	CBS 224.94	OOMYA064-07	HQ708767, HQ643726
Pyt	<i>Pythium pachycaule</i>	CBS 225.94	OOMYA1606-08	HQ708766, HQ643725
Pyt	<i>Pythium pachycaule*</i>	CBS 227.88	OOMYA067-07	HQ708765, HQ643724, HQ665169
Pyt	<i>Pythium paddicum</i>	CBS 698.83	OOMYA219-07	HQ708769, HQ643728, HQ665290
Pyt	<i>Pythium papilogynum</i>	CBS 122648	OOMYA1608-08	HQ708770, HQ643729, HQ665101
Pyt	<i>Pythium paroecandrum</i>	ADC 9909	OOMYA1655-08	HQ708778, HQ643737
Pyt	<i>Pythium paroecandrum</i>	ADC 9910	OOMYA1656-08	HQ708777, HQ643736
Pyt	<i>Pythium paroecandrum</i>	BR 601	OOMYA1459-08	HQ708776, HQ643735
Pyt	<i>Pythium paroecandrum</i>	BR 773	OOMYA1852-08	HQ708775, HQ643734
Pyt	<i>Pythium paroecandrum</i>	BR 774	OOMYA1670-08	HQ708774, HQ643733
Pyt	<i>Pythium paroecandrum</i>	BR 807	OOMYA1475-08	HQ708773, HQ643732
Pyt	<i>Pythium paroecandrum</i>	BR 929	OOMYA1499-08	HQ708771, HQ643730
Pyt	<i>Pythium paroecandrum</i>	CBS 157.64	OOMYA022-07	HQ708772, HQ643731, HQ665133
Pyt	<i>Pythium parvum*</i>	CBS 225.88	OOMYA065-07	HQ708779, HQ643738, HQ665167

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Pyt	<i>Pythium pectinolyticum</i> *	CBS 122643	OOMYA1615-08	HQ708780, HQ643739, HQ665096
Pyt	<i>Pythium perillium</i>	CBS 169.68	OOMYA030-07	HQ708781, HQ643740, HQ665141
Pyt	<i>Pythium periplocum</i>	CBS 170.68	OOMYA1618-08	HQ708782, HQ643741
Pyt	<i>Pythium periplocum</i>	CBS 122664	OOMYA1617-08	HQ708783, HQ643742, HQ665116
Pyt	<i>Pythium periplocum</i> *	CBS 289.31	OOMYA103-07	HQ708784, HQ643743, HQ665189
Pyt	<i>Pythium perplexum</i>	BR 984	OOMYA1619-08	HQ708786, HQ643745
Pyt	<i>Pythium perplexum</i>	CBS 674.85	OOMYA210-07	HQ708785, HQ643744, HQ665283
Pyt	<i>Pythium phragmitis</i> *	CBS 117104	OOMYA1620-08	HQ708787, HQ643746, HQ665081
Pyt	<i>Pythium pleroticum</i>	CBS 776.81	OOMYA236-07	HQ708789, HQ643748, HQ665298
Pyt	<i>Pythium plurisporium</i>	ADC 9824	OOMYA1623-08	HQ708791, HQ643750
Pyt	<i>Pythium plurisporium</i> *	CBS 100530	OOMYA1624-08	HQ708790, HQ643749, HQ665052
Pyt	<i>Pythium polymastum</i>	CBS 811.70	OOMYA1625-08	HQ708793, HQ643752, HQ665301
Pyt	<i>Pythium porphyrae</i>	CBS 369.79	OOMYA137-07	HQ708794, HQ643753, HQ665218
Pyt	<i>Pythium prolatum</i> *	CBS 845.68	OOMYA240-07	HQ708795, HQ643754, HQ665303
Pyt	<i>Pythium pyrlobum</i> *	CBS 158.64	OOMYA024-07	HQ708796, HQ643755, HQ665136
Pyt	<i>Pythium radiosum</i> *	CBS 217.94	OOMYA051-07	HQ708797, HQ643756, HQ665156
Pyt	<i>Pythium rhizo-oryzae</i> *	CBS 119169	OOMYA1628-08	HQ708798, HQ643757, HQ665087
Pyt	<i>Pythium rhizosaccharum</i>	CBS 122652	OOMYA1630-08	HQ708800, HQ643759, HQ665105
Pyt	<i>Pythium rhizosaccharum</i>	CBS 122654	OOMYA1632-08	HQ708799, HQ643758, HQ665106
Pyt	<i>Pythium rhizosaccharum</i> *	CBS 112356	OOMYA1629-08	HQ708801, HQ643760, HQ665072
Pyt	<i>Pythium rostratifingens</i>	BR 1061	OOMYA1699-08	HQ708806, HQ643765
Pyt	<i>Pythium rostratifingens</i>	BR 197	OOMYA351-07	HQ708807, HQ643766
Pyt	<i>Pythium rostratifingens</i>	BR 627	OOMYA352-07	HQ708805, HQ643764
Pyt	<i>Pythium rostratifingens</i>	CBS 383.34	OOMYA1634-08	HQ708803, HQ643762
Pyt	<i>Pythium rostratifingens</i>	Lev 3023	OOMYA1635-08	HQ708804, HQ643763
Pyt	<i>Pythium rostratifingens</i> *	CBS 115464	OOMYA1633-08	HQ708802, HQ643761, HQ665080
Pyt	<i>Pythium rostratum</i>	CBS 533.74	OOMYA179-07	HQ708808, HQ643767, HQ665252
Pyt	<i>Pythium salpingophorum</i>	BR 1024	OOMYA1642-08	HQ708811, HQ643770
Pyt	<i>Pythium salpingophorum</i>	BR 750	OOMYA1640-08	HQ708810, HQ643769
Pyt	<i>Pythium salpingophorum</i>	CBS 471.50	OOMYA169-07	HQ708809, HQ643768, HQ665240
Pyt	<i>Pythium scleroteichum</i> *	CBS 294.37	OOMYA109-07	HQ708812, HQ643771, HQ665192
Pyt	<i>Pythium segnitium</i> *	CBS 112354	OOMYA1644-08	HQ708813, HQ643772, HQ665070
Pyt	<i>Pythium senticosum</i> *	CBS 122490	OOMYA1645-08	HQ708814, HQ643773, HQ665093
Pyt	<i>Pythium</i> sp.	CBS 113341	OOMYA1639-08	HQ708818, HQ643777, HQ665076
Pyt	<i>Pythium</i> sp.	CBS 750.96	OOMYA231-07	HQ708817, HQ643776, HQ665293
Pyt	<i>Pythium</i> sp.	CBS 122649	OOMYA1370-08	HQ708525, HQ643478, HQ665102
Pyt	<i>Pythium</i> sp. "balticum"	ADC 0014	OOMYA1706-08	HQ708826, HQ643785
Pyt	<i>Pythium</i> sp. "Group F"	ADC 9425	OOMYA1707-08	HQ708825, HQ643784
Pyt	<i>Pythium</i> sp. "Group F"	ADC 9426	OOMYA1708-08	HQ708824, HQ643783
Pyt	<i>Pythium</i> sp. "Group F"	ADC 9518	OOMYA1709-08	HQ708823, HQ643782
Pyt	<i>Pythium</i> sp. "Group F"	ADC 9989	OOMYA1710-08	HQ708822, HQ643781
Pyt	<i>Pythium</i> sp. "Group F"	BR 1041	OOMYA1716-08	HQ708821, HQ643780
Pyt	<i>Pythium</i> sp. "Group F"	BR 1044	OOMYA1717-08	HQ708820, HQ643779
Pyt	<i>Pythium</i> sp. "Group F"	BR 710	OOMYA335-07	HQ708830, HQ643789
Pyt	<i>Pythium</i> sp. "Group F"	BR 739	OOMYA1711-08	HQ708829, HQ643788
Pyt	<i>Pythium</i> sp. "Group F"	BR 777	OOMYA1713-08	HQ708828, HQ643787
Pyt	<i>Pythium</i> sp. "jasmonium"	DAO/M 229150	OOMYA422-07	HQ708714, HQ643670
Pyt	<i>Pythium</i> sp. "jasmonium"	CBS 101876	OOMYA1568-08	HQ708819, HQ643778, HQ665054
Pyt	<i>Pythium</i> sp. "spiculacarpum"	CBS 122647	OOMYA1781-08	HQ708815, HQ643774, HQ665100
Pyt	<i>Pythium</i> sp. "tumidum"	CBS 223.94	OOMYA1827-08	HQ708816, HQ643775
Pyt	<i>Pythium</i> sp. nov.	ADC 0013	OOMYA1587-08	HQ708876, HQ643835
Pyt	<i>Pythium</i> sp. nov.	ADC 0110	OOMYA1588-08	HQ708875, HQ643834
Pyt	<i>Pythium</i> sp. nov.	ADC 0111	OOMYA1434-08	HQ708874, HQ643833
Pyt	<i>Pythium</i> sp. nov.	ADC 9407	OOMYA1777-08	HQ708873, HQ643832
Pyt	<i>Pythium</i> sp. nov.	ADC 9409	OOMYA1646-08	HQ708872, HQ643831
Pyt	<i>Pythium</i> sp. nov.	ADC 9421	OOMYA1647-08	HQ708871, HQ643830
Pyt	<i>Pythium</i> sp. nov.	ADC 9759	OOMYA1651-08	HQ708870, HQ643829
Pyt	<i>Pythium</i> sp. nov.	ADC 9966	OOMYA1748-08	HQ708869, HQ643828
Pyt	<i>Pythium</i> sp. nov.	ADC 9982	OOMYA1749-08	HQ708868, HQ643827
Pyt	<i>Pythium</i> sp. nov.	BR 1033	OOMYA1822-08	HQ708850, HQ643809
Pyt	<i>Pythium</i> sp. nov.	BR 1034	OOMYA1414-08	HQ708849, HQ643808
Pyt	<i>Pythium</i> sp. nov.	BR 1047	OOMYA1448-08	HQ708848, HQ643807
Pyt	<i>Pythium</i> sp. nov.	BR 1062	OOMYA1613-08	HQ708847, HQ643806
Pyt	<i>Pythium</i> sp. nov.	BR 147	OOMYA1680-08	HQ708867, HQ643826
Pyt	<i>Pythium</i> sp. nov.	BR 205	OOMYA1685-08	HQ708866, HQ643825
Pyt	<i>Pythium</i> sp. nov.	BR 574	OOMYA400-07	HQ708865, HQ643824
Pyt	<i>Pythium</i> sp. nov.	BR 613	OOMYA1720-08	HQ708864, HQ643823
Pyt	<i>Pythium</i> sp. nov.	BR 655	OOMYA1344-08	HQ708863, HQ643822
Pyt	<i>Pythium</i> sp. nov.	BR 688	OOMYA1388-08	HQ708862, HQ643821
Pyt	<i>Pythium</i> sp. nov.	BR 706	OOMYA333-07	HQ708861, HQ643820
Pyt	<i>Pythium</i> sp. nov.	BR 779	OOMYA1472-08	HQ708860, HQ643819
Pyt	<i>Pythium</i> sp. nov.	BR 798	OOMYA1577-08	HQ708859, HQ643818
Pyt	<i>Pythium</i> sp. nov.	BR 879	OOMYA1483-08	HQ708858, HQ643817
Pyt	<i>Pythium</i> sp. nov.	BR 887	OOMYA353-07	HQ708854, HQ643813
Pyt	<i>Pythium</i> sp. nov.	BR 901	OOMYA1487-08	HQ708853, HQ643812
Pyt	<i>Pythium</i> sp. nov.	BR 902	OOMYA1488-08	HQ708852, HQ643811
Pyt	<i>Pythium</i> sp. nov.	BR 951	OOMYA1445-08	HQ708851, HQ643810
Pyt	<i>Pythium</i> sp. nov.	CBS 232.94	OOMYA1616-08	HQ708855, HQ643814

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Pyt	<i>Pythium</i> sp. nov.	CBS 607.81	OOMYA1700-08	HQ708857, HQ643816
Pyt	<i>Pythium</i> sp. nov.	CBS 633.85	OOMYA1702-08	HQ708856, HQ643815
Pyt	<i>Pythium</i> sp. nov.	DAOM 229155	OOMYA1601-08	HQ708846, HQ643805
Pyt	<i>Pythium</i> sp. nov.	Lev 1457	OOMYA275-07	HQ708845, HQ643804
Pyt	<i>Pythium</i> sp. nov.	Lev 1523	OOMYA301-07	HQ708844, HQ643803
Pyt	<i>Pythium</i> sp. nov.	Lev 2156	OOMYA436-07	HQ708843, HQ643802
Pyt	<i>Pythium</i> sp. nov.	Lev 2168	OOMYA440-07	HQ708842, HQ643801
Pyt	<i>Pythium</i> sp. nov.	Lev 3062	OOMYA1672-08	HQ708841, HQ643800
Pyt	<i>Pythium</i> sp. nov.	Lev 3063	OOMYA1673-08	HQ708840, HQ643799
Pyt	<i>Pythium</i> sp. nov.	Lev 3106	OOMYA1567-08	HQ708839, HQ643798
Pyt	<i>Pythium</i> sp. nov.	P16024	PHYTO116-10	HQ261487, HQ261740
Pyt	<i>Pythium</i> sp. nov.	P19400	PHYTO143-10	HQ261486, HQ261739
Pyt	<i>Pythium</i> sp. nov.	P19448	PHYTO144-10	HQ261485, HQ261738
Pyt	<i>Pythium</i> sp. nov.	P19510	PHYTO145-10	HQ261484, HQ261737
Pyt	<i>Pythium</i> sp. nov.	P8201	PHYTO225-10	HQ261483, HQ261736
Pyt	<i>Pythium</i> sp. nov.	P8204	PHYTO226-10	HQ261482, HQ261735
Pyt	<i>Pythium</i> sp. nov.	P8207	PHYTO227-10	HQ261481, HQ261734
Pyt	<i>Pythium</i> sp. nov.	P8209	PHYTO228-10	HQ261480, HQ261733
Pyt	<i>Pythium</i> sp. nov.	P8212	PHYTO229-10	HQ261479, HQ261732
Pyt	<i>Pythium spiculum*</i>	CBS 122645	OOMYA1782-08	HQ708831, HQ643790, HQ665098
Pyt	<i>Pythium spinosum</i>	CBS 276.67	OOMYA098-07	HQ708833, HQ643792
Pyt	<i>Pythium spinosum</i>	Lev 1526	OOMYA304-07	HQ708835, HQ643794
Pyt	<i>Pythium spinosum</i>	CBS 122663	OOMYA1783-08	HQ708832, HQ643791, HQ665115
Pyt	<i>Pythium spinosum</i>	CBS 275.67	OOMYA1784-08	HQ708834, HQ643793, HQ665181
Pyt	<i>Pythium splendens</i>	BR 788	OOMYA1786-08	HQ708838, HQ643797
Pyt	<i>Pythium splendens</i>	Lev 1497	OOMYA1759-08	HQ708837, HQ643796
Pyt	<i>Pythium splendens</i>	CBS 462.48	OOMYA166-07	HQ708836, HQ643795, HQ665237
Pyt	<i>Pythium sukuense*</i>	CBS 110030	OOMYA1787-08	HQ708877, HQ643836, HQ665059
Pyt	<i>Pythium sulcatum</i>	BR 113	OOMYA1788-08	HQ708884, HQ643843
Pyt	<i>Pythium sulcatum</i>	BR 146	OOMYA1789-08	HQ708883, HQ643842
Pyt	<i>Pythium sulcatum</i>	BR 652	OOMYA1791-08	HQ708882, HQ643841
Pyt	<i>Pythium sulcatum</i>	BR 653	OOMYA341-07	HQ708885, HQ643844
Pyt	<i>Pythium sulcatum</i>	BR 708	OOMYA334-07	HQ708881, HQ643840
Pyt	<i>Pythium sulcatum</i>	BR 709	OOMYA1792-08	HQ708880, HQ643839
Pyt	<i>Pythium sulcatum</i>	Lev 3111	OOMYA1794-08	HQ708879, HQ643838
Pyt	<i>Pythium sulcatum*</i>	CBS 603.73	OOMYA205-07	HQ708878, HQ643837, HQ665281
Pyt	<i>Pythium sylvaticum</i>	BR 1045	OOMYA1807-08	HQ708893, HQ643852
Pyt	<i>Pythium sylvaticum</i>	BR 1069	OOMYA1738-08	HQ708892, HQ643851
Pyt	<i>Pythium sylvaticum</i>	BR 171	OOMYA1455-08	HQ708891, HQ643850
Pyt	<i>Pythium sylvaticum</i>	BR 179	OOMYA1797-08	HQ708890, HQ643849
Pyt	<i>Pythium sylvaticum</i>	BR 599	OOMYA1798-08	HQ708889, HQ643848
Pyt	<i>Pythium sylvaticum</i>	BR 647	OOMYA332-07	HQ708888, HQ643847
Pyt	<i>Pythium sylvaticum</i>	Lev 1544	OOMYA317-07	HQ708887, HQ643846
Pyt	<i>Pythium sylvaticum</i>	P15580	PHYTO112-10	HQ261488, HQ261741
Pyt	<i>Pythium sylvaticum*</i>	CBS 453.67	OOMYA163-07	HQ708886, HQ643845, HQ665236
Pyt	<i>Pythium takayamanum</i>	CBS 122492	OOMYA1814-08	HQ708894, HQ643853, HQ665095
Pyt	<i>Pythium takayamanum*</i>	CBS 122491	OOMYA1813-08	HQ708895, HQ643854, HQ665094
Pyt	<i>Pythium tardicrescens</i>	Lev 1534	OOMYA309-07	HQ708896, HQ643855
Pyt	<i>Pythium terrestris</i>	ADC 9906	OOMYA1654-08	HQ708899, HQ643858
Pyt	<i>Pythium terrestris</i>	BR 922	OOMYA1801-08	HQ708897, HQ643856
Pyt	<i>Pythium terrestris*</i>	CBS 112352	OOMYA1818-08	HQ708898, HQ643857, HQ665068
Pyt	<i>Pythium torulosum</i>	CBS 316.33	OOMYA120-07	HQ708900, HQ643859, HQ665206
Pyt	<i>Pythium tracheiphilum</i>	BR 659	OOMYA1823-08	HQ708904, HQ643863
Pyt	<i>Pythium tracheiphilum</i>	BR 931	OOMYA1825-08	HQ708902, HQ643861
Pyt	<i>Pythium tracheiphilum</i>	BR 932	OOMYA1826-08	HQ708901, HQ643860
Pyt	<i>Pythium tracheiphilum*</i>	CBS 323.65	OOMYA121-07	HQ708903, HQ643862, HQ665207
Pyt	<i>Pythium ultimum</i> var. <i>sporangiferum</i>	BR 651	OOMYA1836-08	HQ708921, HQ643880
Pyt	<i>Pythium ultimum</i> var. <i>sporangiferum*</i>	CBS 219.65	OOMYA054-07	HQ708920, HQ643879, HQ665158
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	ADC 9967	OOMYA1718-08	HQ708919, HQ643878
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 1036	OOMYA1882-08	HQ708918, HQ643877
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 1037	OOMYA1883-08	HQ708917, HQ643876
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 1038	OOMYA1884-08	HQ708916, HQ643875
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 1054	OOMYA1886-08	HQ708915, HQ643874
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 1060	OOMYA407-07	HQ708914, HQ643873
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 1064	OOMYA1888-08	HQ708913, HQ643872
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 1065	OOMYA408-07	HQ708912, HQ643871
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 1089	OOMYA1889-08	HQ708911, HQ643870
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 144	OOMYA386-07	HQ708984, HQ643943
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 229	OOMYA1668-08	HQ708983, HQ643942
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 319	OOMYA1829-08	HQ708982, HQ643941
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 511	OOMYA1830-08	HQ708981, HQ643940
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 583	OOMYA1831-08	HQ708980, HQ643939
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 600	OOMYA1832-08	HQ708979, HQ643938
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 612	OOMYA1719-08	HQ708978, HQ643937
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 628	OOMYA1833-08	HQ708977, HQ643936
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 640	OOMYA1834-08	HQ708976, HQ643935
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 656	OOMYA1721-08	HQ708975, HQ643934
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 657	OOMYA1722-08	HQ708974, HQ643933

Genus Abbrev	Isolate name	Isolate #	BOLD Process ID	Genbank Accessions
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 658	OOMYA1723-08	HQ708973, HQ643932
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 718	OOMYA389-07	HQ708972, HQ643931
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 736	OOMYA1840-08	HQ708971, HQ643930
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 737	OOMYA1841-08	HQ708970, HQ643929
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 738	OOMYA1842-08	HQ708969, HQ643928
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 745	OOMYA1843-08	HQ708968, HQ643927
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 747	OOMYA1844-08	HQ708967, HQ643926
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 748	OOMYA1845-08	HQ708966, HQ643925
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 754	OOMYA1847-08	HQ708965, HQ643924
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 755	OOMYA1729-08	HQ708964, HQ643923
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 759	OOMYA1733-08	HQ708963, HQ643922
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 760	OOMYA1734-08	HQ708962, HQ643921
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 763	OOMYA1848-08	HQ708961, HQ643920
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 768	OOMYA1850-08	HQ708960, HQ643919
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 776	OOMYA1853-08	HQ708959, HQ643918
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 781	OOMYA393-07	HQ708958, HQ643917
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 783	OOMYA404-07	HQ708957, HQ643916
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 784	OOMYA1854-08	HQ708956, HQ643915
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 792	OOMYA1855-08	HQ708955, HQ643914
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 793	OOMYA1856-08	HQ708954, HQ643913
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 810	OOMYA387-07	HQ708953, HQ643912
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 813	OOMYA1857-08	HQ708952, HQ643911
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 814	OOMYA1858-08	HQ708951, HQ643910
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 816	OOMYA1859-08	HQ708950, HQ643909
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 822	OOMYA1740-08	HQ708949, HQ643908
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 823	OOMYA1741-08	HQ708948, HQ643907
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 824	OOMYA1742-08	HQ708947, HQ643906
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 825	OOMYA1743-08	HQ708946, HQ643905
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 826	OOMYA1744-08	HQ708945, HQ643904
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 827	OOMYA1745-08	HQ708944, HQ643903
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 833	OOMYA1860-08	HQ708943, HQ643902
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 835	OOMYA1861-08	HQ708942, HQ643901
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 841	OOMYA392-07	HQ708941, HQ643900
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 842	OOMYA1864-08	HQ708940, HQ643899
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 843	OOMYA1865-08	HQ708939, HQ643898
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 844	OOMYA1866-08	HQ708938, HQ643897
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 845	OOMYA1867-08	HQ708937, HQ643896
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 847	OOMYA1868-08	HQ708936, HQ643895
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 848	OOMYA1869-08	HQ708935, HQ643894
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 849	OOMYA1870-08	HQ708934, HQ643893
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 850	OOMYA1871-08	HQ708933, HQ643892
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 851	OOMYA1872-08	HQ708932, HQ643891
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 854	OOMYA1873-08	HQ708931, HQ643890
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 858	OOMYA1739-08	HQ708930, HQ643889
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 861	OOMYA1876-08	HQ708929, HQ643888
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 863	OOMYA1751-08	HQ708928, HQ643887
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 864	OOMYA1752-08	HQ708927, HQ643886
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 867	OOMYA1877-08	HQ708926, HQ643885
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 930	OOMYA1878-08	HQ708923, HQ643882
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 944	OOMYA1696-08	HQ708922, HQ643881
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 987	OOMYA1879-08	HQ708925, HQ643884
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	BR 988	OOMYA1880-08	HQ708924, HQ643883
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	CBS 729.94	OOMYA1890-08	HQ708910, HQ643869
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	DAOM 232337	OOMYA1894-08	HQ708909, HQ643868
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	Lev 1441	OOMYA112-07	HQ708908, HQ643867
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	Lev 1442	OOMYA171-07	HQ708907, HQ643866
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	CBS 122650	OOMYA1409-08	HQ708905, HQ643864, HQ665103
Pyt	<i>Pythium ultimum</i> var. <i>ultimum</i>	CBS 398.51	OOMYA149-07	HQ708906, HQ643865, HQ665227
Pyt	<i>Pythium uncinulatum</i>	ADC 0108	OOMYA1684-08	HQ708986, HQ643945
Pyt	<i>Pythium uncinulatum</i> *	CBS 518.77	OOMYA173-07	HQ708985, HQ643944, HQ665244
Pyt	<i>Pythium undulatum</i>	ADC 9929	OOMYA1899-08	HQ708990, HQ643949
Pyt	<i>Pythium undulatum</i>	CBS 323.47	OOMYA1116-08	HQ708989, HQ643948
Pyt	<i>Pythium undulatum</i>	Lev 1232	OOMYA250-07	HQ708988, HQ643947
Pyt	<i>Pythium undulatum</i>	CBS 157.69	OOMYA023-07	HQ708987, HQ643946, HQ665134
Pyt	<i>Pythium vanterpoolii</i>	CBS 115.77	OOMYA009-07	HQ708991, HQ643950
Pyt	<i>Pythium vanterpoolii</i>	CBS 431.91	OOMYA1400-08	HQ708992, HQ643951
Pyt	<i>Pythium vanterpoolii</i>	Lev 1536	OOMYA311-07	HQ708994, HQ643953
Pyt	<i>Pythium vanterpoolii</i> *	CBS 295.37	OOMYA110-07	HQ708993, HQ643952, HQ665193
Pyt	<i>Pythium viniferum</i> *	CBS 119168	OOMYA1900-08	HQ708997, HQ643956, HQ665086
Pyt	<i>Pythium violae</i>	ADC 9739	OOMYA1901-08	HQ709010, HQ643969
Pyt	<i>Pythium violae</i>	ADC 9754	OOMYA1902-08	HQ709009, HQ643968
Pyt	<i>Pythium violae</i>	ADC 9923	OOMYA1903-08	HQ709008, HQ643967
Pyt	<i>Pythium violae</i>	ADC 9924	OOMYA1904-08	HQ709007, HQ643966
Pyt	<i>Pythium violae</i>	ADC 9974	OOMYA1905-08	HQ709006, HQ643965
Pyt	<i>Pythium violae</i>	ADC 9975	OOMYA1906-08	HQ709005, HQ643964
Pyt	<i>Pythium violae</i>	BR 322	OOMYA1907-08	HQ709004, HQ643963
Pyt	<i>Pythium violae</i>	Lev 1518	OOMYA299-07	HQ709003, HQ643962
Pyt	<i>Pythium violae</i>	Lev 1519	OOMYA300-07	HQ709002, HQ643961

Genus Abbrev	Isolate name	Isolate #	BOLD Process ID	Genbank Accessions
Pyt	<i>Pythium violae</i>	Lev 1604	OOMYA336-07	HQ709001, HQ643960
Pyt	<i>Pythium violae</i>	Lev 1605	OOMYA337-07	HQ709000, HQ643959
Pyt	<i>Pythium violae</i>	CBS 159.64	OOMYA026-07	HQ708999, HQ643958, HQ665138
Pyt	<i>Pythium violae</i>	CBS 178.86	OOMYA033-07	HQ708998, HQ643957, HQ665143
Pyt	<i>Pythium volutum</i>	CBS 699.83	OOMYA220-07	HQ709012, HQ643971, HQ665291
Pyt	<i>Pythium zingiberis</i>	CBS 217.82	OOMYA1909-08	HQ709013, HQ643972
Pyt	<i>Pythium zingiberis</i>	CBS 216.82	OOMYA050-07	HQ709014, HQ643973, HQ665155
Sap	<i>Saprolegnia anisospora</i>	CBS 110060	OOMYA1910-08	HQ709015, HQ643974
Sap	<i>Saprolegnia asterophora</i>	CBS 531.67	OOMYA1911-08	HQ709016, HQ643975, HQ665250
Sap	<i>Saprolegnia delica</i>	CBS 344.62	OOMYA1912-08	HQ709018, HQ643977
Sap	<i>Saprolegnia delica</i>	CBS 345.62	OOMYA1913-08	HQ709017, HQ643976, HQ665214
Sap	<i>Saprolegnia diclina</i>	CBS 282.38	OOMYA1915-08	HQ709020, HQ643979
Sap	<i>Saprolegnia diclina</i>	CBS 326.35	OOMYA1916-08	HQ709021, HQ643980, HQ665209
Sap	<i>Saprolegnia diclina</i>	CBS 536.67	OOMYA1917-08	HQ709019, HQ643978, HQ665254
Sap	<i>Saprolegnia eccentrica</i>	CBS 199.38	OOMYA1918-08	HQ709023, HQ643982, HQ665147
Sap	<i>Saprolegnia eccentrica</i>	CBS 211.35	OOMYA1919-08	HQ709022, HQ643981, HQ665151
Sap	<i>Saprolegnia eccentrica</i>	CBS 551.67	OOMYA1920-08	HQ709024, HQ643983, HQ665260
Sap	<i>Saprolegnia ferax</i>	BR 114	OOMYA1921-08	HQ709029, HQ643988
Sap	<i>Saprolegnia ferax</i>	CBS 283.38	OOMYA1923-08	HQ709027, HQ643986
Sap	<i>Saprolegnia ferax</i>	CBS 534.67	OOMYA1925-08	HQ709026, HQ643985
Sap	<i>Saprolegnia ferax</i>	CBS 173.42	OOMYA1922-08	HQ709025, HQ643984, HQ665142
Sap	<i>Saprolegnia ferax</i>	CBS 305.37	OOMYA1924-08	HQ709028, HQ643987, HQ665199
Sap	<i>Saprolegnia hypogyna</i>	CBS 869.72	OOMYA1927-08	HQ709030, HQ643989, HQ665304
Sap	<i>Saprolegnia lapponica</i>	CBS 284.38	OOMYA1928-08	HQ709031, HQ643990, HQ665184
Sap	<i>Saprolegnia litoralis</i>	CBS 110062	OOMYA1929-08	HQ709033, HQ643992, HQ665060
Sap	<i>Saprolegnia litoralis</i>	CBS 535.67	OOMYA1930-08	HQ709032, HQ643991, HQ665253
Sap	<i>Saprolegnia megasperma</i>	CBS 532.67	OOMYA1932-08	HQ709034, HQ643993, HQ665251
Sap	<i>Saprolegnia mixta</i>	CBS 149.65	OOMYA1933-08	HQ709035, HQ643994, HQ665127
Sap	<i>Saprolegnia mixta</i>	CBS 307.37	OOMYA1934-08	HQ709036, HQ643995, HQ665202
Sap	<i>Saprolegnia monilifera</i>	CBS 552.67	OOMYA1935-08	HQ709038, HQ643997, HQ665262
Sap	<i>Saprolegnia monilifera</i>	CBS 558.67	OOMYA1936-08	HQ709037, HQ643996, HQ665270
Sap	<i>Saprolegnia monoica</i>	CBS 539.67	OOMYA1939-08	HQ709040, HQ643999, HQ665255
Sap	<i>Saprolegnia monoica</i>	CBS 599.67	OOMYA1940-08	HQ709039, HQ643998, HQ665280
Sap	<i>Saprolegnia parasitica</i>	CBS 113187	OOMYA1941-08	HQ709046, HQ644005, HQ665074
Sap	<i>Saprolegnia parasitica</i>	CBS 223.65	OOMYA1942-08	HQ709045, HQ644004, HQ665165
Sap	<i>Saprolegnia parasitica</i>	CBS 300.32	OOMYA1943-08	HQ709044, HQ644003, HQ665196
Sap	<i>Saprolegnia parasitica</i>	CBS 302.56	OOMYA1944-08	HQ709043, HQ644002, HQ665197
Sap	<i>Saprolegnia parasitica</i>	CBS 397.34	OOMYA1945-08	HQ709042, HQ644001, HQ665226
Sap	<i>Saprolegnia parasitica</i>	CBS 540.67	OOMYA1946-08	HQ709041, HQ644000, HQ665256
Sap	<i>Saprolegnia rodrigueziana</i>	CBS 119354	OOMYA1948-08	HQ709047, HQ644006, HQ665089
Sap	<i>Saprolegnia sp.</i>	CBS 632.85	OOMYA1701-08	HQ709048, HQ644007
Sap	<i>Saprolegnia subterranea</i>	CBS 278.52	OOMYA1950-08	HQ709049, HQ644008
Sap	<i>Saprolegnia subterranea</i>	CBS 113343	OOMYA1914-08	HQ709050, HQ644009, HQ665078
Sap	<i>Saprolegnia terrestris</i>	CBS 533.67	OOMYA1952-08	HQ709051, HQ644010
Sap	<i>Saprolegnia terrestris</i>	CBS 110063	OOMYA1951-08	HQ709052, HQ644011, HQ665061
Sap	<i>Saprolegnia turfosa</i>	CBS 110065	OOMYA1953-08	HQ709055, HQ644014
Sap	<i>Saprolegnia turfosa</i>	CBS 313.81	OOMYA1954-08	HQ709054, HQ644013, HQ665203
Sap	<i>Saprolegnia turfosa</i>	CBS 327.35	OOMYA1938-08	HQ709053, HQ644012, HQ665210
Sap	<i>Saprolegnia unispora</i>	CBS 110066	OOMYA1955-08	HQ709057, HQ644016, HQ665062
Sap	<i>Saprolegnia unispora</i>	CBS 213.35	OOMYA1956-08	HQ709056, HQ644015, HQ665152
Thr	<i>Thraustotheca clavata</i>	CBS 343.33	OOMYA1957-08	HQ709059, HQ644018, HQ665213
Thr	<i>Thraustotheca clavata</i>	CBS 557.67	OOMYA1958-08	HQ709058, HQ644017, HQ665268
Thr	<i>Thraustotheca terrestris*</i>	CBS 109851	OOMYA1960-08	HQ709060, HQ644019, HQ665057

Appendix B - Species resolved by COI that are indistinguishable by ITS.

***Phytophthora*:**

- *Phytophthora cambivora* and *Phytophthora alni*

***Pythium*:**

- *Pythium catenulatum* and *Pythium rhizo-oryzae*
- *Pythium graminicola*, *Pythium peritum*, and *Pythium tardicrescens*
- *Pythium sylvaticum* and *Pythium terrestris*
- *Pythium mamillatum* and *Pythium spiculum*
- *Pythium attrantheridium* and *Pythium* sp. “balticum”
- *Pythium aquatile* and *Pythium sukuiense*
- *Pythium capillosum* and *Pythium flevoense*

***Phytopythium*:**

- *Phytopythium boreale* and *Phytopythium megacarpum*
- *Phytopythium* sp. “grandilobatum” and *Phytopythium oedochilum*

Appendix C - Species that are indistinguishable with either COI or ITS.

***Phytophthora*:**

- *Phytophthora capsici* and *Phytophthora mexicana* (Mchau & Coffey, 1995)
- *Phytophthora melonis* and *Phytophthora sinensis* (Erwin & Ribeiro, 1996, Mirabolfathy, *et al.*, 2001, Guharoy, *et al.*, 2006)
- *Phytophthora arecae* and *Phytophthora palmivora*
- *Phytophthora erythroseptica* and *Phytophthora himalayensis*
- *Phytophthora nicotianae* and *Phytophthora tabaci*
- *Phytophthora parsiana* and *Phytophthora* sp. “thermophilum” or *Phytophthora* sp. “lagoariana”

In the last example, one isolate of *P. parsiana* shows conspecificity with *P.* sp. “thermophilum”, one shows conspecificity with *P.* sp. “lagoariana”, and the third *P. parsiana* (P21281) seems separate from the rest.

***Pythium*:**

- *Pythium myriotylum* and *Pythium zingiberis*
- *Pythium aristosporum* and *Pythium arrhenomanes*
- *Pythium amasculinum*, *Pythium hydnosporum*, *Pythium lycopersicum*, and *Pythium ornamentatum*.
- *Pythium folliculosum* and *Pythium torulosum*
- *Pythium conidiophorum* and *Pythium salpingophorum*
- *Pythium debaryanum* and *Pythium viniferum*
- *Pythium irregulare*, *Pythium cryptoirregulare*, and *Pythium cylindrosporum*
- *Pythium acrogynum* and *Pythium hypogynum*
- *Pythium erinaceus* and *Pythium ornacarpum*
- *Pythium minus* and *Pythium pleroticum*

- *Pythium dimorphum* and *Pythium undulatum*

***Saprolegnia*:**

- *Saprolegnia subterranea* and *Saprolegnia rodrigueziana*

***Thraustotheca*:**

- *Thraustotheca clavata* and *Thraustotheca terrestris*

Appendix D - Apparent species complexes based on COI and ITS.

***Achlya*:**

- *Achlya ambisexualis*, *Achlya bisexualis*, and *Achlya heterosexalis*

***Phytophthora*:**

- *Phytophthora citricola*, *Phytophthora inflata*, *Phytophthora pini*, and *Phytophthora plurivora*.
- *Phytophthora cryptogea*, *Phytophthora erythroseptica*, *Phytophthora* sp. "kelmania", and *Phytophthora cryptogea* f. sp. *begoniae*

Other *Phytophthora* species that could be considered a complex on their own are:

- *Phytophthora megasperma* (Hansen & Maxwell, 1991)
- *Phytophthora cryptogea* (Gallegly & Hong, 2008)

***Pythium*:**

- *Pythium glomeratum* and *Pythium heterothallicum*
- *Pythium rhizosaccharum* and *Pythium takayamanum*
- *Pythium buismaniae*, *Pythium megalacanthum*, *Pythium polymastum*, and *Pythium uncinulatum*.

Other *Pythium* species that could be considered a complex on their own are:

- *Pythium irregulare* (Barr, et al., 1997)
- *Pythium ultimum* var. *ultimum* (Barr, et al., 1996)
- *Pythium acanthicum*
- *Pythium okanoganense*
- *Pythium violae* (epithet is currently assigned to two distinct isolates from carrot and soil that occupy different clades in the genus *Pythium*).

***Phytopythium*:**

- *Phytopythium vexans* and *Phytopythium cucurbitacearum*

Saprolegnia

- *Saprolegnia ferax*, *Saprolegnia parasitica*, *Saprolegnia diclina*, *Saprolegnia lapponica*, *Saprolegnia mixta*, *Saprolegnia unisporea*, *Saprolegnia hypogyna*, and *Saprolegnia delica*

Appendix E - Flagellar apparatus-related genes in *Pythium* and *Phytophthora*.
Expression levels of *Pythium ultimum* genes are shown from hypoxia and *Arabidopsis* infection (Lévesque, *et al.*, 2010).

GenBank	Name	Function	Pu E-value	Ps E-value	Pr E-value	Pi E-value	Ha E-value	Hypoxia RPKM	Arabidopsis RPKM
gi159485786	ANK24	flagellar associated protein	e-152	e-157	e-156	e-156	9.0E-07	4.975277	11.836567
gi159482892	VFL2	centrin	5.0E-15	9.0E-11	9.0E-11	7.0E-08	2.0E-06	26.481349	23.834006
gi159474390	VFL3	templated centriole assembly	2.0E-56	4.0E-49	7.0E-48	4.0E-47	3.9	0.898252	0.462289
gi112734843	SNF1	serine/threonine protein kinase	3.0E-35	4.0E-41	1.0E-40	2.0E-41	8.0E-38	10.746487	12.950776
gi156766597	IFT144	intraflagellar transport subunit 144	e-166	e-171	e-170	e-160	2.0E-17	0.242387	1.978383
gi159475992	IFT140	intraflagellar transport subunit 140	1.0E-09	8.0E-14	5.0E-31	2.0E-31	0.71	0.084901	0.646769
gi156630943	KPL2	sperm flagellar protein	3.0E-17	5.0E-18	7.0E-17	7.0E-18	0.015	0.95311	0.670222
gi134084607	An18c0170	sperm tail associated protein	0.064	0.21	0.12	0.32	0.26	6.198063	4.538835
gi30580462	DYH1B	axonemal dynein heavy chain 1-beta	0	0	0	0	e-179	0.050607	0.041306
gi172044682	DYH1A	flagellar inner arm dynein heavy chain 1-alpha	0	0	0	0	0	0.264108	0.479844
gi238231321	OCM2	tubular mastigoneme protein	2.0E-53	3.0E-53	2.0E-48	5.0E-51	1.0E-04	10.328066	0.553628
gi156632335	IFT22	intraflagellar transport subunit 22	0.012	2.0E-06	0.017	6.0E-04	0.045	0.383921	0
gi46361984	Hydin	central pair flagellar protein	0	0	0	0	no hits	0.795243	0.979478
gi172044538	DYH2	axonemal dynein	0	0	0	0	0	0.745752	0.68105
gi2754612	DNAL4	axonemal dynein light chain 4	1.0E-14	2.0E-16	2.0E-16	4.0E-17	4.0E-07	72.773384	34.516482
gi159469750	OPEL	thaumatin-like secretory protein	0.049	0.042	0.055	0.32	0.57	0.619814	0.973757
gi75338601	DNAL1	axonemal dynein light chain 1	4.0E-37	6.0E-32	4.0E-36	7.0E-37	6.0E-11	0.604104	0
gi1169693	FLA10	axonemal kinesin II motor protein	e-154	e-151	e-152	e-152	3.0E-77	0	0
gi158280836	KLP1	axonemal kinesin-like protein	4.0E-81	e-110	1.0E-93	e-109	9.0E-55	0.287059	2.342999
gi159469111	ANK10	flagellar associated protein	1.0E-22	9.0E-22	5.0E-23	2.0E-17	2.0E-16	59.818471	126.131418
gi71658800	AKM1	axonemal kinesin/myosin-type protein	3.0E-05	3.0E-06	3.0E-04	5.0E-04	0.002	0.349882	0.324202
gi15193244	AKM2	axonemal kinesin/myosin-type protein	2.0E-54	2.0E-48	6.0E-31	4.0E-42	3.0E-23	16.747003	7.722479
gi61214500	ANKP1	unknown	2.0E-06	4.0E-07	4.0E-06	7.0E-05	2.0E-06	108.64238	32.518791
gi150371706	OCM1	tubular mastigoneme protein	2.0E-61	9.0E-60	5.0E-55	9.0E-56	0.029	0.069511	3.177184
gi74716342	DYH7	axonemal dynein heavy chain 7	0	0	0	0	0	0.137818	0.385673
gi159483419	ANK19	flagellar associated protein	8.0E-49	4.0E-49	3.0E-49	4.0E-48	5.0E-33	2.839957	7.226302
gi159478625	FAP208	flagellar associated protein	2.0E-75	4.0E-76	3.0E-76	5.0E-77	1.0E-07	94.601084	81.255677
gi29825690	IFT74	intraflagellar transport subunit 74	7.0E-63	4.0E-60	2.0E-61	2.0E-60	3.0E-08	1.272272	3.503662
gi159477026	LRR1	unknown	7.0E-17	3.0E-09	5.0E-06	5.0E-14	0.022	2.170167	0.66489
gi2494209	ODA2	dynein gamma chain	0	0	0	0	0	0.208189	0.733265
gi71384457	LRR2	unknown	0.031	0.099	0.85	3.8	4	0.104301	0.148229
gi2317719	AKM3	axonemal kinesin/myosin-type protein	2.0E-14	7.0E-18	2.0E-12	3.0E-16	1.0E-10	0	0

GenBank	Name	Function	Pu E-value	Ps E-value	Pr E-value	Pi E-value	Ha E-value	Hypoxia RPKM	Arabidopsis RPKM
gi9716372	CALK	serine/threonine protein kinase	4.0E-41	3.0E-31	3.0E-39	2.0E-42	8.0E-43	2.254314	2.823795
gi158513400	FAP189	flagellar associated protein	9.0E-21	2.0E-25	6.0E-21	5.0E-21	3.0E-18	1.668341	0.325047
gi83284725	RSP7	radial spoke protein 7	2.0E-42	3.0E-13	1.0E-37	4.0E-14	4.0E-09	0.182844	0.298478
gi61814636	SPEF1	flagellar associated protein	6.0E-24	4.0E-27	2.0E-28	2.0E-27	2.9	1.908435	0.298478
gi27805726	SPAG4	flagellar associated protein	0.031	7.0E-05	4.0E-06	0.069	0.007	4.752934	4.56398
gi182637563	DYH10	axonemal dynein heavy chain 10	0	0	0	0	0	0.192741	0.629266
gi75142763	GAS8	dynein regulatory complex protein	e-102	e-117	e-106	e-118	4.0E-07	0.080103	0.523047
gi18277872	DYH11A	axonemal dynein heavy chain 11 alpha	0	0	0	0	0	0.047278	0.308712
gi71384428	C1a-18	axoneme central apparatus associated protein	1.0E-16	2.0E-18	5.0E-18	5.0E-17	9.0E-12	22.466426	111.678633
gi33591148	CAMK2D	calcium/calmodulin protein kinase II delta	5.0E-47	2.0E-51	4.0E-47	1.0E-49	7.0E-48	39.548012	78.251099
gi156631028	IFT121	intraflagellar transport subunit 121	0	0	0	0	0.25	2.519586	6.070168
gi117380778	CCRK	cell-cycle related kinase	1.0E-41	2.0E-50	8.0E-50	9.0E-50	2.0E-41	4.241256	1.852766
gi75337416	DYH1B	axonemal dynein 2 heavy chain 1b	0	0	0	0	0	0.222218	2.121905
gi159488240	RSP10	radial spoke protein 10	6.0E-27	6.0E-24	6.0E-24	4.0E-22	2.0E-07	1.056154	4.388576
gi122137042	ODF3	flagellar outer dense fiber 3	7.0E-08	7.0E-09	9.0E-09	8.0E-09	2.4	0.90439	1.476341
gi159466466	ARL3	ADP ribosylation factor 3	1.0E-26	4.0E-25	3.0E-25	2.0E-24	7.0E-18	1.118461	3.605944
gi71063501	AGG2	flagellar associated protein	6.0E-04	0.004	0.009	2.0E-06	0.35	0	0
gi159474288	FAP21	flagellar associated protein	4.0E-12	5.0E-12	2.0E-11	2.0E-11	5.0E-11	0.405764	0.316359
gi148832347	PKD2	polycystin cation channel	1.0E-15	6.0E-12	7.0E-15	8.0E-08	5.0E-08	0	0
gi29648322	DNAJB1	Dna-J class molecular chaperone	1.0E-65	5.0E-65	4.0E-65	4.0E-62	8.0E-60	2478.3068	172.608664
gi14548081	IDA4	axonemal dynein light chain inner arm 4	1.0E-67	8.0E-66	1.0E-66	1.0E-64	0.057	0	0
gi226459514	PPI-1	axonemal protein phosphatase	e-130	e-102	e-135	e-135	e-105	220.74383	478.212376
gi158271520	PPI-2	axonemal protein phosphatase	e-123	e-110	e-127	e-145	e-114	210.63475	442.973938
gi156632333	FAP232	flagellar associated protein	5.0E-16	3.0E-09	2.0E-12	2.0E-07	no hits	1.98547	0
gi82082176	AKM4	axonemal kinesin/myosin-type protein	1.0E-22	5.0E-22	2.0E-18	9.0E-20	4.0E-18	0.03129	0
gi81900317	Rpgrip11	retinitis pigmentosa GTPase regulator interacting protein	2.0E-46	6.0E-46	7.0E-40	3.0E-38	5.0E-12	0.870304	1.584423
gi212286121	PF13	pre-assembly of axonemal dyneins	7.0E-29	2.0E-16	4.0E-26	1.0E-27	2.0E-06	2.307763	1.777213
gi238231323	OCM4	tubular mastigoneme protein	3.0E-66	3.0E-63	1.0E-62	2.0E-64	3.0E-04	3.031403	2.440884
gi159486159	EB1	microtubule plus-end binding protein	5.0E-85	1.0E-71	4.0E-84	4.0E-41	3.0E-39	58.489909	103.594999
gi159467981	IFT52	intraflagellar transport subunit 52	3.0E-94	2.0E-73	3.0E-74	3.0E-73	9.4	0.166882	2.724201
gi61680380	Tctex1	axonemal dynein light chain inner arm 4	1.0E-23	2.0E-23	2.0E-23	1.0E-23	9.0E-09	3.242392	7.913023
gi29839411	ANXE1	annexin E1	1.0E-13	1.0E-10	4.0E-10	1.0E-11	3.0E-09	4195.8408	1425.681039
gi2811014	DNAL6	axonemal dynein light chain outer arm 6	4.0E-46	7.0E-46	6.0E-46	2.0E-45	1.0E-45	306.5572	151.887844

GenBank	Name	Function	Pu E-value	Ps E-value	Pr E-value	Pi E-value	Ha E-value	Hypoxia RPKM	Arabidopsis RPKM
gi125302	FCK	flagellar creatine kinase	0	0	0	0	5.8	20.406949	77.028099
gi122166113	ODA7	outer row flagellar dynein assembly protein	3.0E-43	1.0E-48	2.0E-47	1.0E-48	5.0E-09	0	0.777902
gi13157754	DHC64C	cytoplasmic dynein heavy chain	8.0E-17	6.0E-16	5.0E-16	2.0E-15	8.0E-16	5.139509	13.211344
gi159467108	IFT172	intraflagellar transport subunit 172	0	0	0	0	8.0E-06	0.140336	0.393744
gi159476286	FAP279	flagellar associated protein	0	0	0	0	0.002	0.112806	0
gi1168796	Caltractin	basal body associated calcium binding protein	7.0E-72	3.0E-71	2.0E-71	6.0E-71	5.0E-51	16.715682	17.756905
gi61217633	IFT80	intraflagellar transport subunit 80	0	0	0	0	8.0E-06	0.384307	0.896212
gi111145351	IFT46	intraflagellar transport subunit 46	5.0E-65	2.0E-65	6.0E-65	9.0E-63	6.5	0.069742	1.138472
gi82400100	IFT57	intraflagellar transport subunit 57	9.0E-30	2.0E-39	3.0E-37	4.0E-36	0.016	1.867536	2.956215
gi134041	RSP3	radial spoke protein 3	7.0E-39	1.0E-35	8.0E-36	1.0E-31	8.5	0	0.43726
gi159489466	RSP2	radial spoke protein 2	4.0E-05	4.0E-05	1.0E-05	2.0E-04	0.31	0.084958	0.944803
gi117607157	FBB5	flagellar basal body protein (predicted)	0.42	2.7	1.7	1.2	2	3.804724	5.115736
gi221222440	B9D2	B9 domain-containing protein 2	3.0E-44	1.0E-36	3.0E-37	5.0E-37	0.25	0.926525	0
gi74762434	IFT139	intraflagellar transport subunit 139	e-160	0	0	0	0.008	0.340205	0.749871
gi159484104	IFT88	intraflagellar transport subunit 88	9.0E-21	6.0E-20	2.0E-21	1.0E-18	2.0E-18	0.199851	0.406212
gi42412387	NDPK6	flagellar radial spoke nucleoside diphosphate kinase	2.0E-32	3.0E-33	8.0E-35	1.0E-28	6.0E-16	0.029326	0.191492
gi159470411	RSP1	radial spoke protein 1	1.0E-63	3.0E-33	2.0E-58	8.0E-62	3.0E-07	0.667526	0.476735
gi159486617	VFL1	variable flagella protein 1	0	0	0	0	0	0.472518	0.530301
gi122138665	DC2L1	axonemal dynein 2 light intermediate chain 1	2.0E-36	8.0E-38	4.0E-37	4.0E-34	3.9	0	0.346717
gi2494208	ODA4	axonemal dynein beta chain, flagellar outer arm	0	0	0	0	0	0.414247	0.604407
gi159468277	IMPA2	importin	3.0E-21	2.0E-20	4.0E-21	4.0E-19	3.0E-19	133.28077	55.375525
gi117923377	PRED-UNK1	predicted protein, bacterial flagellin domain	0.036	0.2	0.089	9.0E-11	7.0E-44	0.56433	54.17535
gi158282063	KAP1	kinesin associated protein 1	5.0E-82	1.0E-84	1.0E-84	3.0E-84	3.1	0.133505	0
gi109391151	ODA16	axonemal dynein, flagellar outer arm	2.0E-36	2.0E-38	4.0E-38	9.0E-41	2.0E-30	0.968352	12.399678
gi13157760	DHC8	axonemal dynein heavy chain 8, flagellar inner arm	2.0E-12	4.0E-12	3.0E-12	1.0E-11	9.0E-07	0.382089	0.769707
gi401050	RSP4	radial spoke protein 4	3.0E-17	9.0E-20	9.0E-20	4.0E-21	0.88	1.172117	1.488187
gi32130553	LF4	MAP kinase involved in flagellar length control	e-128	e-129	e-129	e-126	2.0E-46	1.637978	0.852793
gi159465329	RSP16	radial spoke protein 16	2.0E-46	1.0E-51	5.0E-51	6.0E-50	2.0E-51	0	0
gi159469762	FAP134	flagellar associated protein	2.0E-09	6.0E-09	9.0E-10	3.0E-08	7.0E-09	0.920912	0
gi158280659	BLD10	flagellar basal body protein	3.0E-12	2.0E-09	2.0E-08	5.0E-08	3.0E-09	28.940794	11.379463
gi50660932	CPKG1	flagellar cGMP-dependent protein kinase	6.0E-82	7.0E-85	9.0E-85	1.0E-78	5.0E-66	3.564768	13.0312
gi2494215	ODA9	axonemal dynein intermediate chain, flagellar outer arm	e-162	e-161	e-160	e-161	0.004	0	0.380859

GenBank	Name	Function	Pu E-value	Ps E-value	Pr E-value	Pi E-value	Ha E-value	Hypoxia RPKM	Arabidopsis RPKM
gi159483683	PPCT1	peptidyl-prolyl cis-trans isomerase	6.0E-15	9.0E-14	7.0E-11	5.0E-11	4.0E-08	141.80215	102.521256
gi1101777	PF16	axoneme central apparatus	0	0	0	0	1.0E-16	1.180196	1.699421
gi159486599	HSP70A	heat shock protein 70	4.0E-36	4.0E-36	5.0E-35	1.0E-35	5.0E-37	3749.1331	608.983783
gi159487717	RSP8	radial spoke protein 8	0.004	3.0E-07	1.0E-05	3.0E-06	0.39	3.587389	0
gi159478264	IFT81	intraflagellar transport subunit 81	1.3	1.1	0.3	0.27	2.4	0.580911	0.198985
gi1384450	C1a-34	axoneme central apparatus associated protein	3.0E-06	0.021	5.0E-05	0.054	9.3	0.503642	3.956617
gi27922947	FIBH	fibroin heavy chain	2.0E-20	1.0E-17	9.0E-14	3.0E-13	0.001	91.796882	122.161487
gi159487911	IFT20	intraflagellar transport subunit 20	3.3	0.047	0.93	1.2	1.1	0	0

Pu - *Pythium ultimum*, Ps - *Phytophthora sojae*, Pr - *Phytophthora ramorum*, Pi - *Phytophthora infestans*, Ha - *Hyaloperonospora arabidopsidis*, RPKM - Reads Per Kilobase per Million mapped reads.

Appendix F - Isolates used in this study, their host/substrate, and GenBank accessions of DNA sequence data. Footnotes refer to sequences accessed directly from genome assemblies.

Species	Isolate Number	Isolated From	PF16 Accession	OCM1 Accession	LSU Accession
<i>Achlya racemosa</i>	CBS 103.38	unknown	JX115023	JX115117	JX115214
<i>Achlya radiosa</i>	CBS 547.67	unknown	JX115024	JX115118	JX115215
<i>Albugo candida</i>	Ac2VRR	<i>Brassica rapa</i>	JX556328	JX556329	HQ665049
<i>Albugo laibachii</i>	Nc14	<i>Arabidopsis thaliana</i>	Footnote ^a	Footnote ^a	FR825184
<i>Aphanomyces astaci</i>	CBS 121537	unknown	JX115025	JX115116	JX115216
<i>Aphanomyces cladogamus</i>	CBS 108.29	<i>Lycopersicon esculentum</i>	JX115026	JX115119	HQ665056
<i>Aphanomyces cochlioides</i>	CBS 477.71	<i>Beta vulgaris</i>	JX115027	JX115120	HQ665241
<i>Aphanomyces euteiches</i>	CBS 156.73	<i>Pisum sativum</i>		JX115121	HQ665132
<i>Apodachlya brachynema</i>	CBS 557.69	unknown		JX436345	JX436350
<i>Dictyuchus monosporus</i>	Lev 1848	lake water	JX436354	JX436346	JX436351
<i>Ectocarpus siliculosus</i>	Ec32	sea water	Footnote ^b	Footnote ^b	Footnote ^b
<i>Halophytophthora operculata</i>	CBS 241.83	<i>Avicennia marina</i>	JX115028	JX115122	JX115217
<i>Halophytophthora polymorphica</i>	CBS 680.84	<i>Eucalyptus</i> sp.	JX115029	JX115123	HQ665288
<i>Halophytophthora vesicula</i>	CBS 393.81	<i>Prunus laurocerasus</i> leaf in Pacific Ocean	JX436355	JX436347	JX436352
<i>Lagenidium caudatum</i>	CBS 584.85	soil from cattle pen	JX115070	JX115165	HQ665277
<i>Myzocytiopsis</i> sp.	Lev 6103	nematodes in rabbit dung	JX436357	JX436348	JX436353
<i>Phytophthora capsici</i>	LT1534	<i>Cucumis</i> sp./ <i>Cucurbita</i> sp.	Footnote ^c	Footnote ^c	
<i>Phytophthora capsici</i>	CBS 128.23	<i>Capsicum annuum</i>			HQ665120
<i>Phytophthora cinnamomi</i>	CBS 144.22	<i>Cinnamomum burmannii</i>	JX115032	JX115126	HQ665126
<i>Phytophthora cryptogea</i>	CBS 113.19	<i>Lycopersicon esculentum</i> or <i>Petunia</i>	JX115033	JX115127	HQ665075
<i>Phytophthora drechsleri</i>	CBS 291.35	<i>Beta vulgaris</i>	JX115034	JX115128	EU079511
<i>Phytophthora erythroseptica</i>	CBS 380.61	<i>Tulipa</i> sp.	JX115035	JX115129	HQ665121
<i>Phytophthora fragariae</i>	CBS 209.46	<i>Fragaria</i> sp.	JX115036	JX115130	HQ665150
<i>Phytophthora gonapodyides</i>	CBS 554.67	unknown	JX115037	JX115131	HQ665265
<i>Phytophthora heveae</i>	CBS 296.29	<i>Hevea brasiliensis</i>	JX115038	JX115132	HQ665194
<i>Phytophthora hibernalis</i>	CBS 522.77	<i>Aquilegia vulgaris</i>	JX115039	JX115133	EU079518
<i>Phytophthora humicola</i>	CBS 200.81	soil from citrus orchard	JX115040	JX115134	HQ665148
<i>Phytophthora ilicis</i>	CBS 255.93	unknown	JX115041	JX115135	EU079864
<i>Phytophthora infestans</i>	CBS 366.51	<i>Solanum tuberosum</i>	JX115042	JX115136	HQ665217
<i>Phytophthora infestans</i>	Lev 1429	<i>Solanum tuberosum</i>	JX115043	JX115137	
<i>Phytophthora infestans</i>	T30-4	<i>Solanum tuberosum</i>	Footnote ^d	Footnote ^d	
<i>Phytophthora iranica</i>	CBS 374.72	<i>Solanum melongena</i>	JX115044	JX115138	HQ665219
<i>Phytophthora katsurae</i>	CBS 587.85	soil	JX115045	JX115139	HQ665278
<i>Phytophthora lateralis</i>	CBS 168.42	<i>Chamaecyparis lawsoniana</i>	JX115046	JX115140	EU080093
<i>Phytophthora medicaginis</i>	DAOM BR 331	<i>Medicago sativa</i>	JX115047	JX115141	EU080695
<i>Phytophthora megasperma</i>	CBS 306.36	<i>Brassica</i> sp.	JX115048	JX115142	HQ665201
<i>Phytophthora mirabilis</i>	CBS 678.85	<i>Mirabilis jalapa</i>	JX115049	JX115143	HQ665285
<i>Phytophthora multivesiculata</i>	CBS 545.96	<i>Cymbidium</i> sp.	JX115050	JX115144	HQ665257
<i>Phytophthora nicotianae</i>	DAOM BR 235	<i>Euphorbia pulcherrima</i>	JX115051	JX115145	EU079560
<i>Phytophthora phaseoli</i>	CBS 556.88	<i>Phaseolus lunatus</i>	JX115052	JX115146	HQ665267
<i>Phytophthora psychrophila</i>	CBS 803.95	rhizosphere of decaying <i>Quercus robur</i>	JX115053	JX115147	EU080521
<i>Phytophthora ramorum</i>	Pr-102	<i>Quercus agrifolia</i>	Footnote ^e	Footnote ^e	
<i>Phytophthora ramorum</i>	CBS 101553	<i>Rhododendron catawbiense</i>			HQ665053
<i>Phytophthora rubi</i>	CBS 967.95	<i>Rubus idaeus</i>	JX115054	JX115148	HQ665306

Species	Isolate Number	Isolated From	PF16 Accession	OCM1 Accession	LSU Accession
<i>Phytophthora sinensis</i>	CBS 557.88	<i>Cucumis sativus</i>	JX115055	JX115149	HQ665269
<i>Phytophthora sojae</i>	CBS 382.61	<i>Glycine max</i>	JX115061	JX115155	HQ665224
<i>Phytophthora sojae</i>	DAOM BR 1074	<i>Glycine max</i>	JX115056	JX115150	
<i>Phytophthora sojae</i>	DAOM BR 1079	<i>Glycine max</i>	JX115057	JX115151	
<i>Phytophthora sojae</i>	DAOM BR 1081	<i>Glycine max</i>	JX115058	JX115152	
<i>Phytophthora sojae</i>	DAOM BR 198	<i>Glycine max</i>	JX115059	JX115153	
<i>Phytophthora sojae</i>	DAOM BR 556	<i>Glycine max</i>	JX115060	JX115154	
<i>Phytophthora syringae</i>	CBS 275.74	<i>Malus sylvestris</i>	JX115062	JX115156	HQ665123
<i>Phytophthora vignae</i>	CBS 241.73	<i>Vigna sinensis</i>	JX115063	JX115157	EU079787
<i>Phytophythium helicoides</i>	CBS 286.31	<i>Phaseolus vulgaris</i>	JX115030	JX115124	HQ665186
<i>Phytophythium sindhum</i>	DAOM 238986	rhizosphere of <i>Musa</i> sp.	JX436356	JX436349	HQ665309
<i>Phytophythium vexans</i>	CBS 119.80	soil	JX115031	JX115125	HQ665090
<i>Plasmopara halstedii</i>	Lev 5881	<i>Helianthus annuus</i>		JX115158	EF553469
<i>Pythium acanthophoron</i>	CBS 337.29	<i>Ananas sativus</i>	JX115064	JX115159	HQ665212
<i>Pythium anandrum</i>	CBS 285.31	<i>Rheum rhaponticum</i>	JX115065	JX115160	HQ665185
<i>Pythium angustatum</i>	CBS 522.74	soil	JX115066	JX115161	HQ665246
<i>Pythium aphanidermatum</i>	CBS 118.80	unknown	JX115067	JX115162	HQ665084
<i>Pythium aristosporum</i>	CBS 263.38	<i>Triticum aestivum</i>	JX115068	JX115163	HQ665179
<i>Pythium arrhenomanes</i>	DAOM BR 983	<i>Hordeum vulgare</i>	JX115069	JX115164	HQ665208
<i>Pythium coloratum</i>	CBS 154.64	loamy nursery soil	JX115071	JX115166	HQ665128
<i>Pythium cryptoirregulare</i>	CBS 118731	<i>Euphorbia pulcherima</i>	JX115072	JX115167	HQ665083
<i>Pythium cylindrosporum</i>	CBS 218.94	soil	JX115073	JX115168	HQ665157
<i>Pythium cystogenes</i>	CBS 675.85	<i>Vicia faba</i>	JX115074	JX115169	HQ665284
<i>Pythium dimorphum</i>	CBS 406.72	<i>Pinus taeda</i>	JX115075	JX115170	HQ665229
<i>Pythium grandisporangium</i>	CBS 286.79	<i>Distichlis spicata</i>		JX115171	HQ665187
<i>Pythium hydnosporum</i>	CBS 253.60	unknown	JX115076	JX115172	HQ665175
<i>Pythium hypogynum</i>	CBS 234.94	soil	JX115077	JX115173	HQ665171
<i>Pythium insidiosum</i>	CBS 574.85	<i>Equus ferus</i>	JX115078	JX115174	HQ665273
<i>Pythium intermedium</i>	CBS 266.38	<i>Agrostis stolonifera</i>	JX115079	JX115175	HQ665180
<i>Pythium irregulare</i>	CBS 250.28	<i>Phaseolus vulgaris</i>	JX115085	JX115181	HQ665172
<i>Pythium irregulare</i>	DAOM BR 724	<i>Apium graveolens</i>	JX115080	JX115176	
<i>Pythium irregulare</i>	DAOM BR 751	unknown	JX115081	JX115177	
<i>Pythium irregulare</i>	DAOM BR 772	<i>Daucus carota</i>	JX115082	JX115178	
<i>Pythium irregulare</i>	DAOM BR 775	<i>Medicago</i> sp.	JX115083	JX115179	
<i>Pythium irregulare</i>	DAOM BR 812	<i>Triticum</i> sp.	JX115084	JX115180	
<i>Pythium iwayamai</i>	CBS 156.64	soil under <i>Pinus</i> sp.	JX115086	JX115182	HQ665131
<i>Pythium kunmingense</i>	CBS 550.88	soil under <i>Vicia faba</i>	JX115087	JX115183	HQ665259
<i>Pythium lutarium</i>	CBS 222.88	soil	JX115088	JX115184	HQ665163
<i>Pythium mamillatum</i>	CBS 251.28	<i>Beta vulgaris</i>	JX115089	JX115185	HQ665173
<i>Pythium multisporem</i>	CBS 470.50	soil	JX115090	JX115186	HQ665239
<i>Pythium myriotylum</i>	CBS 695.79	<i>Vigna sinensis</i>	JX115091	JX115187	HQ665176
<i>Pythium nagaii</i>	CBS 779.96	soil	JX115092	JX115188	HQ665299
<i>Pythium nodosum</i>	CBS 102274	soil	JX115093	JX115189	HQ665055
<i>Pythium nunn</i>	CBS 808.96	grassland soil	JX115094	JX115190	HQ665300
<i>Pythium okanoganense</i>	CBS 315.81	<i>Triticum sativum</i>	JX115095	JX115191	HQ665205
<i>Pythium oligandrum</i>	CBS 217.46	<i>Viola</i> sp.	JX115097	JX115193	
<i>Pythium oligandrum</i>	CBS 382.34	<i>Viola</i> sp.	JX115098	JX115194	HQ665223
<i>Pythium oligandrum</i>	DAOM BR 252	<i>Satureja hortensis</i>	JX115096	JX115192	
<i>Pythium orthogonon</i>	CBS 376.72	<i>Zea mays</i>	JX115099	JX115195	HQ665221
<i>Pythium paddicum</i>	CBS 698.83	root of <i>Triticum</i> sp. and <i>Hordeum</i> sp.	JX115100	JX115196	HQ665290
<i>Pythium periplocum</i>	CBS 170.68	soil under <i>Saccharum</i> <i>officinarum</i>	JX115101	JX115197	HQ665189

Species	Isolate Number	Isolated From	PF16 Accession	OCM1 Accession	LSU Accession
<i>Pythium phragmitis</i>	CBS 117104	rhizosphere soil of <i>Phragmites australis</i>	JX115102	JX115198	HQ665081
<i>Pythium pleroticum</i>	CBS 776.81	<i>Nymphoides peltata</i>	JX115103	JX115199	HQ665298
<i>Pythium porphyrae</i>	CBS 369.79	<i>Porphyra yezoensis</i>	JX115104	JX115200	HQ665218
<i>Pythium prolatum</i>	CBS 845.68	<i>Rhododendron</i> sp.	JX115105	JX115201	HQ665303
<i>Pythium pyrilobum</i>	CBS 158.64	<i>Pinus radiata</i>	JX115106	JX115202	HQ665136
<i>Pythium rhizosaccharum</i>	CBS 112356	rhizosphere soil of <i>Saccharum officinarum</i>	JX115107	JX115203	HQ665072
<i>Pythium segnitium</i>	CBS 112354	soil	JX115108	JX115204	HQ665070
<i>Pythium splendens</i>	CBS 462.48	unknown	JX115109	JX115205	HQ665237
<i>Pythium sukuiense</i>	CBS 110030	soil	JX115111	JX115207	HQ665059
<i>Pythium sulcatum</i>	CBS 603.73	<i>Daucus carota</i>	JX115112	JX115208	HQ665281
<i>Pythium ultimum</i>	DAOM BR 144	<i>Nicotiana tabacum</i>	Footnote ^f	Footnote ^f	
<i>Pythium ultimum</i>	CBS 398.51	<i>Lepidium sativum</i>			HQ665227
<i>Pythium ultimum</i> var. <i>sporangiferum</i>	CBS 219.65	<i>Chenopodium album</i>	JX115110	JX115206	HQ665158
<i>Pythium undulatum</i>	CBS 157.69	soil under <i>Pinus</i> sp.	JX115113	JX115209	HQ665134
<i>Pythium violae</i>	CBS 178.86	<i>Daucus carota</i>	JX115114	JX115210	HQ665143
<i>Pythium volutum</i>	CBS 699.83	root of <i>Triticum</i> sp. and <i>Hordeum</i> sp.	JX115115	JX115211	HQ665291
<i>Saprolegnia eccentrica</i>	CBS 551.67	unknown	JX115021	JX115212	HQ665260
<i>Saprolegnia ferax</i>	CBS 173.42	unknown	JX115022	JX115213	HQ665142
<i>Saprolegnia parasitica</i>	CBS 223.65	<i>Esox lucius</i>	Footnote ^g	Footnote ^g	HQ665165

^a Nc14 PF16: GenBank FR824088: bp 20191 – 21717, OCM1: GenBank FR824315: bp 16898 – 18652.

^b Ec32 PF16: Ectsi_PROT_LATEST: Esi0080_0014, OCM1: Ectsi_PROT_LATEST: Esi0101_0018, LSU: Ectsi_genome11x: sctg_595: bp 2939-4439.

^c LT1534 PF16: Phycal1: PHYCAscaffold_33: bp 104442 – 105967, OCM1: Phycal1: PHYCAscaffold_109: bp 81981 – 83783.

^d T30-4 PF16: *P. infestans* T30-4: Supercontig 2: bp 419667 – 421193, OCM1: *P. infestans* T30-4: Supercontig 19: bp 180514 – 182223.

^e Pr-102 PF16: ramorum1.allmasked: scaffold_43: bp 28702 – 30219, OCM1: ramorum1.allmasked: scaffold_8: bp 188483 – 192711.

^f DAOM BR 144 PF16: *Pythium ultimum* DAOM BR144 Annotation: PYUI_T013715, OCM1: *Pythium ultimum* DAOM BR144 Annotation: PYUI_T003513.

^g CBS 223.65 PF16: *S. parasitica* CBS 223.65: Supercontig 1: bp 1368869 – 1370535, OCM1: *S. parasitica* CBS 223.65: Supercontig 12: bp 219231 – 221253.

[illegible]

[illegible]

Appendix H - Recombinant flagellar protein and C-type lectin sequences. N-terminal polyhistidine tags and C-terminal *c-myc* epitopes are shown in bold.

>OCM1_P. aphanidermatum_BR444_DNA

ATGTCGTA**CTACCATCACCATCACCATCACATGGAGTGCCCGAACGGATGCTCGGGTAAC**
GGCGATTGTATGGCCAAGGATATGTGCGAATGCTACAAAACTACCAGGGGAACGACTGC
GGTGACCGTACGTGTCTGTTCCGGCCGTGCGCATGTGACACGCCCCAAGGGCGATATCAAC
ATGGACAAGAACACGAAGACTACGGGATTGATCCTCACCACCTCGCAGCAGCACCCAGCG
GGTACGTGGGAGTACTTCCCGGACAGTTCAGCCGACGCGAACGAAGAGGCGCATTTCTAC
ATGGAGTGTCTCGAACAAAGGGCATCTGTGACCGCGGTACGGGGCTATGTCAGTGTCTTGAC
GGCTATGAAGGAAACGGATGCCAGCGCACGACGTGTCCTGGCAAGTGCAATGGCCACGGC
ACGTGCGAGAGCATTCGCGAGCTCGGTCTGAAGGAGGCGGGAACATTGTTTGGGGGTGAG
GGGCCGTGACGGCGAGTGACGTATGACTTGTGGGACAGCAACAGCACTTACGGGTGCCGA
TGCGACCCATGGTACTTTGGTCCGGACTGCAACCGACGCACATGCAAGGTTGGCGTGGAT
CCGTTGTTCTGTGCGGTGGATCTCCCAATTACGAAACGTTTGTCTACATGCATACGTC
GCATCCGGTGGCACTATCACCGACGGATGGGTCCGCCTTCGCTTGTTCGACTACTACGGG
GAGTCTTATATACCAAGAAGATAACAATCGTAGCTGATACTTCAACAGCTAACACAGAA
GCAAATGCCAAGGCCGTGATGACTGCAATCAAAGGAGTGCCGAACCTCACATTCCGAAAT
GTGAAGTGCGAGACACTCGGAGCAAATTTTACGGCTCCGACTTACAAGGCTTCAAGTCG
AAACGAGCAACTGGTACCGTAGGCACGTCCATTATTTGCCAATACGTCGACAACCCCGGG
AAGATGCGAATCCCAGAAGTCGCTGGGTTTGGTGGTGTACGTCAGCCTTTGTGGTGACG
ACTTCGCAGCAGGGCATGAATGATGAATGGTTCACGGTGAAAACCACTAGTATTGTGGAC
ACTGCCACTGACACTCCGACGAGCTTGCCACTCTTGGCTGCGCTTCCAGCGACTGTCACA
TTTCCTGCGCTGGTGAAAATTGGTCAGAATATCGTGCTGGCCAAAACCGGATCTACATCG
ACTTCTCTTACGCTCGAGTACGACCTCACACAGACGCTGGGCACTGCCCCACCTGTTTTC
ATCCAGAAACGGCGTCAGTTGTACGGTCAACGCAGGAGTACCGACGAATGCTATCGCC
GCAGTTGGTGACCAAACTCTCTCTTTCGATTGCTACGAACCCCGCACCAAGCATG
TACTCCGTGCGCGATTTGATCTTCTTCCACAATCAGTTCTTCACGATTACAGAAAGTCGTG
GATGACGGTACAACTATGTGATCACACTCAACAAGCCGTTTGGAGGACAAGCGTCTGAC
GGTGACGCCACTGGAGCTACGTCTCCTGTGTACGAAGTCACAATGGGGGCTGACAAGGCG
AAAGTGTACAACACTACGTCTCCGAGTGCTCAGGCCGTGGCATGTGCAACTACGAGACCGGC
ATCTGCTCGTGCTTCAAGGGCTACACCAACGACAACCTGTAACACACAGAACATCCTGGCG
GTGGAACAAAACTCATCTCAGAAGAGGATCTGTAA

>OCM1_P. aphanidermatum_BR444_amino acid

MSYHHHHHHHMECPNGCSNGDCMAKDMCECYKNYQGND**CGDRTCLF****GRAHVDTPKGDIN**
MDKNTKTTGLILTNSSQHPAGTWEIFPDSSAAANEEAHFYMECSNKGICDRGTGLCQCFD
GYEGNGCQRTTCPGKCNHGTCEISIRELGLKEAGTLFGGEGPSGAVTYDLWDSNSTYGCR
CDPWYFGPDCNRRTCKVGVDPLFLSAGSPNYETFVLHAYVASGGTITDGWVRLRLFDYYG
ESYITKKITIVADTSTANTEANAKAVMTAIGVPNLTFRNVKCETLGANFYGSDLQGFKS
KRATGTVGTSIICQYVDNPGKMRIPEVAGFGGVTSAFVVTTSSQQGMNDEWFTVKTTISVD
TATDTPTSPLLAALPATVTFPALVKIGQNIVLAKTGSTSTSLTLEYDLTQTLGTAPPVF
IPETASVVTVTAGVPTNAIAAVGDQTLSDSTATNPAPSMYSVGDLIFFHNQFFTIQKV
DDGTNYVITLNKPFGGQASDGDATGATSPVYEVTMGADKAKVYNYVSECSGRGMCNYETG
ICSCFKGYTNDNCNTQNILAVEQKLISEEDL*

>PF16_P. aphanidermatum_BR444_DNA

ATGTCGTA**CTACCATCACCATCACCATCACATGGCGTCCACCTCTGTGCTACAGCACTTC**
GAAGTGTATCAGAAAGCACGCGTGACGTTTCGTCCAGGCTGTGGCAGAGGCAGCGACGCGC
CCACAGAACATTGAGGTCATGCAGAACGCTGGTGTGATGCAGCTGCTACGACCACTTTTA
CTCGACAACGTCCCCAGCATCCAACAGTCGGCTGCTCTAGCGCTCGGTCTCGCGAAC
TATAGTGATGACCTCGCTGAGGCCGTAGTTGGAAATGAGATCCTGCCGCAACTCGTGTAC
TCACTATCGGAGCAGAACCGGTTCTACAAGAAGGCGGCTGCGTTTCGTGCTACGTGCTGTC
GCCAAGCACTACCCGAGTTGGCCCAAGCAGTAGTCGACTCGGGTGCCTGGAATCCCTT

GTGCCATGTCTAGAAGAGTTCGATCCAACGGTTAAAGAAGCTGCTGCGTGGGCAATTGGT
TACATCGCTCAGCACACTGGCGAGTTAGCACAGCATGTTGTCGACGCGGGTGGGTGCCC
TTACTTGTGTTGTGTATCCAAGAGCCTGAAGTCGCTCTGAAGCGAGTGGCTGCTTCTGCA
TTAAGTGATATCGCCAAGCACTCGCCAGAGCTAGCTCAGGCTGTGGTTGACCCTGGCACA
GTGGCATAACCTTGCTCCTCTCATTACAGCACCTGACTCTAAGCTGAAGCGTCAAGTATGC
AGCTGTCTGGCACAGGTCGCCAAGCATTCGGTCGATCTTGCTGAGATCGTGGTTCGAAGCC
GAAATTTTCCCCAACATTCTATACAATTTGAAGGACATTGATCACACAGTTTCGCAAAAAC
GCAGCCACATGTGTACGAGAGATTGCCAAGCACACTCCAGAGCTCGCCAAGCTGATCGTG
AATGCCCGGCGGCGCTCAGCGTTGGTTGACTACGTGGCGGAAGCGTCAGGAAACAACAAA
CTGCCCGGCATCATGGCCATCGGCTACATCTCTGCATTTTCGGAGACACTCGCCCTCGCG
GTTATTACATCCAAAGGAATACTACCGGTGAAAAGCGCACTCATCACAGAGCCAGAAGAC
CACATCAAGGCCGCCAGTGGTGGACACTTGGCCAAATTGGGCGTCACACGCCTGATCAC
GCGCGGGCCATTGCCGAGGCAGATGTGCTTCGGCACCTTCTGGCGTCATGGTGCATTCT
AACAGTCCGACGATCTCAGGACAAAATCGAAACGTGCACCTAAGAGCATCTTAGCGAAG
TGCACGCATCTCCAGGCGCTGCAACCACTTCTACGCGAAGCCCCAATGAAGGTGCAGAAG
TATATCCTAAAGCAGTTTGACACAGATGCTACCTCACGACCTGGAAGCCAGGCGATCATTT
GTTTCAAGATGGTGGTCTCGAATTTCTGCAGACACTAGGTGAGGCAGCTGGTGGCAAACTC
ACGGAGTATATCATGGAGATCAACAATTGCTACCCGCCTGAGATTGTGAGTACTACTCG
CCTCACTACAGCAAGACATTACTCGACAAGCTCGACGAATACCAACCGCAAGTGAAGGAA
CAAAAACCTCATCTCAGAAGAGGATCTGTAA

>PF16_P. aphanidermatum_BR444_amino acid

MSYYHHHHHHMASTSVLQHFVYQKARVTFVQAVAEAAATRPQNIEMQNAGVMQLLRPLL
LDNVPSIQQSAALALGRLANYSDDLAEAVVGNEILPQLVYSLSEQNRFYKKAFAFVLRAV
AKHSPELAQAQVVDGSALESVPCLEEDPTVKEAAA WAIGYIAQHTGELAQHVVVDAGAVP
LLVLCIQEPEVALKRVAASALS DIAKHSPELAQAQVVDPGTVAYLAPLIQHPDSKLKRQVC
SCLAQVAKHSDLAIEIVVEAEIFPNILYNLKDIDHTVRKNAATCVREIAKHTEPELAKLIV
NAGGASALVDYVAEASGNNKLPGIMAIGYISAFSETLALAVITSKGILPVKSALITEPED
HIKAASAWTLGQIGRHTPDHARAIAEADVLRHLLACMVHSNSSDDLRTKSKRALKSILAK
CTHLQALQPLLREAPMKVQKYILKQFAQMLPHDLEARRSFVQNGGLEFLQTLGEAAGGKL
TEYIMEINNCPPEIVEYYSPHYSKTLDDKLDLEYQPQVKEQKLISEEDL*

>Cucumber_Cucumis_sativus_C-lectin_CuCsa134550_DNA

ATGTCTGTACTACCATCACCATCACCATCACATGGATACTATGTTTAAACGAGTCAGGAATT
GGGTATTTCGGTTGTTTCCAGAAAGGAAATTTCAAAAGGACGGTGGCCACATGGTTGGATT
ATTAGCCCAAGCAAGACTAAGTGCTTTGGTTTTCATGTCAGCCCTAAATCATGGAATGAT
TCAGAGACCCAATGTAATAGTTTGGTGGGAACCTTAGCAGCTCTGGTAACATATCAAGAG
TTTAGCTATGCACAAAATCTGTGTAATGGAACCTTGGTGGCTGTTGGGTTGGTGGCAGA
GCGTTCAACTCTTTGAATGATTTCTGTTTGGAAAGTGGTCAGATAATGTTTCAAAATGGAAC
GATTCCATTTTTCCAGCGCAACTTTGCAATCGAACTGCAAAAATGCCTCTTGCCACCGG
AATGATTTCGGTTGAAACATGTACATTAATATTTGGTGGACCAGCAACACCATTCTTAGG
GACGAGAAATGCAACAGTTCTCACCCTTTTATATGCATGATAAACCTAGATGATAGATGC
CACCGAATGCACTGTCACAAGGAGTAA

>Cucumber_Cucumis_sativus_C-lectin_CuCsa134550_amino acid

MSYYHHHHHHMDTMFNESGIGYSVVS RKEISKGRCPHGWIISPSKTKCFGMSSPKSWND
SETQCNSFGGNLAALVTYQEF SYAQNLCNGTLGGCWVGGRFNSLND FVWKWSDNVSKWN
DSIFPSATLQSNCKNASCHRND SVETCTLIFGGPATPFLRDEKCNSSHPFICMINLDDRC
HRMHCHKE*

>Wheat_Triticum_aestivum_PUT163b_1816165448_DNA

ATGTCTGTACTACCATCACCATCACCATCACATGCATACCCCGTCATGCCCCGACGGTTGG
CAAATCAGTCCTGATCGGGCCAAATGCTTCATGCACATCTCAACATCCCTTTCTGGGAC
GGATCCGAAGCCCTCTGCCGCAACTTCAGCGGTCACTGGCTGCATTATCATCAGTTCAG
GAGCTGAATTTCCGCGAGGTCTCTGTGGAGCTGCCTCCTCCGGGTGCTGGGTTGGAGGG
CGTCGCCGCAACACCAGTAGTGGTGTGTTGGCTGGAAATGGTCTGACAATTCGTCTTCT

TGGAACGGGACCGCATTTCCTGGTGAGCCGTTGCGTCCCAATTGCACCGGTGCTCGCTGC
GGTCTAGCCACCGGCGACGATATGTGCACTTTGGTGACCAGTAAGCACACTGCACTTACC
GGAAAGAAGTGTGCCGAGTCACATGGGCTGATTTGCATGATGAATCACGAAGATAGGTGC
TACCATGATCACTGCCACAAGGAGTAA

>Wheat_Triticum_aestivum_PUT163b_1816165448_amino acid

MSYYHHHHHMHTPSCPDGWQISPDRAKCFMHISTLSWDGSEALCRNFSGHLAALSSVQ
ELNFARSLCGAASSGCWVGRRRNTSSGVVGWKWSDNSSSWNGTAFPGEPLRPNCTGARC
GLATGDDMCTLVTSKHTALTGKKCAESHGLICMMNHEDRCYHDHCHKE*

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