Molecular Phylogenetics and Evolution 114 (2017) 199–211

Phylogenomic analysis supports multiple instances of polyphyly in the oomycete peronosporalean lineage

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Running Head: Oomycete phylogenomics

Keywords: Oomycetes, Genomics, Core orthologs, Pythium, Phylogenomics, Lifestyles.

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ABSTRACT

The study of biological diversification of oomycetes has been a difficult task for more than a century. Pioneer researchers used morphological characters to describe this heterogeneous group, and physiological and genetic tools expanded knowledge of these microorganisms. However, research on oomycete diversification is limited by conflicting phylogenies. Using whole genomic data from 17 oomycete taxa, we obtained a dataset of 277 core orthologous genes shared among these genomes. Analyses of this data-set resulted in highly congruent and strongly supported estimates of oomycete phylogeny when we used concatenated maximum likelihood and coalescent-based methods; the one important exception was the position of Albugo. Our results supported the position of *Phytopythium vexans* (formerly in Pythium clade K) as a sister clade to the *Phytophthora-Hyaloperonospora* clade. The remaining clades comprising *Pythium* sensu lato formed two monophyletic groups. One group was composed of three taxa that correspond to Pythium clades A, B and C, and the other group contained taxa representing clades F, G and I, in agreement with previous Pythium phylogenies. However, the group containing Pythium clades F, G and I was placed as sister to the *Phytophthora-Hyaloperonospora-Phytopythium* clade, thus confirming the lack of monophyly of *Pythium* sensu lato. Multispecies coalescent methods revealed that the white blister rust, *Albugo laibachii*, could not be placed with a high degree of confidence. Our analyses show that genomic data can resolve the oomycete phylogeny and provide a phylogenetic framework to study the evolution of oomycete lifestyles.

1. Introduction

Oomycetes are fungal-like non-photosynthetic eukaryotic microorganisms, many of which are pathogenic members that are responsible for costly diseases of plants and animals. Some oomycetes are even emerging pathogens of humans. These unique organisms are members of the major eukaryotic group Heterokonta (also called stramenopiles) that includes brown algae and diatoms as well (Baldauf et al., 2000; Cavalier-Smith and Chao, 2006; Dick, 2001). One major difference

from true fungi is that oomycetes remain diploid throughout their life cycles, with meiosis occurring in the gametangia before fertilization and resulting in the formation of oospores, which are sexual spores that have a thick wall for surviving desiccation and over-seasoning. Many oomycetes produce asexual swimming spores (zoospores) containing a tinsel flagellum, which is absent from the zoospores of true fungi. For many soil-borne, aquatic, and some foliar-inhabiting oomycetes, zoospores are the major infectious propagule.

There are two major lineages of oomycetes: the Saprolegniomycetidae and Peronosporomycetidae. Many saprolegnialean species are animal pathogens infecting fish, fish eggs, amphibians and crustaceans, and also plant and algal pathogens as well as non-pathogenic saprobes (Hussein et al., 2001; Johnson et al., 2002; Densmore and Green, 2007; Pelizza et al., 2011). The other major oomycete lineage, the agriculturally relevant peronosporaleans, includes the plant pathogenic *Phytophthora* and *Pythium* species, downy mildews and white blister rusts. *Phytophthora*, which means 'plant destroyer', include some of the most devastating plant pathogens such as *Ph. infestans*, an aerially dispersing pathogen that causes late blight of potatoes and tomatoes. The emergence of *Ph. infestans* in Europe in the 1840s contributed to the great Irish famine, which resulted in around one million deaths and massive emigration from Ireland (Austin Bourke, 1964; Woodham-Smith, 1991). Currently, this pathogen is responsible for worldwide losses in the billions of US dollars per year (Haverkort et al., 2008).

Other economically significant members of the peronosporaleans include the host-specific soil-borne root rot pathogen of soybean, *Ph. sojae*, the wide host range pathogens *Ph. cinnamomi*, known for jarrah dieback in western Australia, and *Ph. ramorum*, the causal agent of sudden oak/larch death in the western US and UK (Judelson, 1996; Maseko et al., 2007; Grünwald et al., 2008; Poimala and Lilja, 2013). Downy mildews comprise more than 800 species including *Hyaloperonospora arabidopsis* (formerly *H. parasitica* and *Peronospora parasitica*), a pathogen of the model plant *Arabidopsis thaliana*, other pathogens responsible for costly diseases of basil, impatiens, lettuce, and cucurbits, and biosecurity threats such as the maize downy mildews

Peronosclerospora philippinesis and *Sclerophthora rayssiae* (Spencer, 1981; Hall, 1996; Choi et al., 2003, 2015b; Göker et al., 2007). The downy mildews are obligate biotrophs and cannot be cultured apart from their hosts. In contrast, *Phytophthora* species are hemibiotrophic, in which there is an initial phase of biotrophic growth is followed by a necrotrophic phase and can generally be cultured on agar media. Despite early controversy, molecular phylogenies have shown that *Phytophthora* species and the downy mildews form a monophyletic group, but that *Phytophthora* is paraphyletic (Cooke et al., 2000; Voglmayr, 2003; Göker et al., 2007). However, which of the *Phytophthora* species are closely related to downy mildews is still in debate because multigene phylogenies have mostly included members of only one group or the other.

Obligate biotrophy is also present in another lineage of the peronosporaleans, the white blister rusts (WBR). Within this group, *Albugo laibachii*, like *Hyaloperonospora arabidopsis*, is also a pathogen of the model plant *Arabidopsis thaliana*. Downy mildews and white blister rusts share massive gene losses in primary and secondary metabolism that relates to their biotrophic life styles (Baxter et al., 2010; Kemen et al., 2011; Links et al., 2011), but these groups are phylogenetically distant (Riethmüller et al., 2002; Hudspeth et al., 2003). Some phylogenies have shown *Albugo* within clades of species belonging to *Pythium* (Uzuhashi et al., 2010; Robideau et al., 2014) and genomic analysis of *Albugo laibachii* showed the highest overall amino acid identity to the necrotrophic plant pathogen *Py. ultimum* and the hemibiotroph *Ph. infestans* (Kemen et al., 2011). *A multilocus* time-calibrated phylogeny based on conserved regulators of gene expression provided evidence that *Albugo* is sister to a clade comprising *Py. ultimum*, *Phytophthora*, and *Hyaloperonospora* (Matari and Blair, 2014), although that study did not include a number of important *Pythium* species (including *Py. insidiosum*). Other phylogenies have placed white blister rusts sister to *Pythium* and *Phytophthora* (Thines and Kamoun, 2010; McCarthy and Fitzpatrick, 2017), suggesting that the position of Albuginaceae deserves further investigation.

The genus *Pythium* was originally described by Pringsheim in 1858 and it comprises more than 250 species that include plant pathogens (e.g., *Py. aphanidermatum*), invertebrate pathogens (e.g., *Py. guiyangense*), algal pathogens (e.g., *Py. porphrae*), mycoparasites (e.g., *Py. oligandrum*), saprophytes (e.g., *Py. intermedium*) and an emerging mammal pathogen (*Py. insidiosum*) (Van der Plaats-Niterink, 1981). Most *Pythium* species are soil-borne saprobes or facultative plant pathogens that cause seed rot and damping-off, root, stem and fruit rot, foliar blight, and postharvest decay. Little is known about the native ecology of *Pythium* species, but they are hypothesized to mediate forest community structure through seedling damping off (Packer and Clay, 2000; Augspurger and Wilkinson, 2007). *Pythium* species cause economically costly root diseases of food crops, lawns, young trees, and ornamental plants, particularly when soil becomes water-saturated (Van der Plaats-Niterink, 1981). The taxonomy of this genus was mainly based on morphological characters, such as the form, shape and size of sporangia and oogonia, the extent of the oospore in the oogonium (plerotic or aplerotic), and the number of antheridia per oogonium, among others (Waterhouse, 1968; Van der Plaats-Niterink, 1981). It is well-established that *Pythium* is composed of two morphological groups differentiated by filamentous versus globose sporangia, however, distinguishing between species remains exceedingly difficult because morphological characteristics are often very similar among species and may not be apparent when specimens are cultivated on agar medium.

Analyses of sequence data have led to substantial progress in *Pythium* systematics. *Pythium* has been divided into 11 clades of designated A to K that were delineated using sequences of the large subunit (LSU) ribosomal RNA (rRNA) gene and the internal transcribed spacers (ITS) of the rRNA repeats (Lévesque and de Cock, 2004). Molecular phylogenies support the major morphological clustering of *Pythium* into two groups: one monophyletic group composed of Clades A through D characterized by filamentous sporangia, while the other monophyletic group formed by Clades E-J characterized by globose sporangia. However, Clade K was found to be phylogenetically distant from the other 10 clades (Lévesque and de Cock, 2004). Molecular and

morphological studies of Clade K, together with improved taxon sampling, led to its reassignment as genus *Phytopythium* (Bala et al., 2010; De Cock et al., 2015; Marano et al., 2014; Jesus et al., 2016). *Phytopythium* species have been described as morphologically intermediate between *Phytophthora* and *Pythium*, because they show internal proliferation as in some *Phytophthora* species, yet zoospore development and release external (De Cock et al., 2015) or partly internal and partly external to sporangia (Marano et al., 2014; Jesus et al., 2016). Uzuhashi et al. (2010) showed that *Pythium* clade K was a sister group of the *Phytophthora*-downy mildew monophyletic clade, based on the nuclear LSU gene and the mitochondrial cytochrome oxidase II (COII) gene. Similar phylogenetic relationships were shown in previous analyses based on LSU (Briard et al., 1995), ITS (Villa et al., 2006), and beta-tubulin (Villa et al., 2006; Belbahri et al., 2008). However, based on the small subunit gene (SSU), these relation-ships were not supported (De Cock et al., 2015). Thus, there is a general consensus that *Pythium* clade K is a new genus *Phytopythium*, but its evolutionary relationship with other peronosporaleans has not been fully resolved. Uzuhashi et al. (2010) proposed that *Pythium* clades E to J be transferred to two new genera based on molecular and morphological characters. However, there was not phylogenetic support for any particular arrangement of these clades (Uzuhashi et al., 2010; De Cock et al., 2015) and therefore, reassignment of clades has not been widely accepted by the oomycete community. Here we refer to clades A-J as Pythium sensu lato.

Despite the progress in oomycete systematics using rRNA and mitochondrial sequences many questions remain regarding the evolutionary relationships among the major clades of peronosporaleans and within each of those clades. The absence of a robust phylogeny for the oomycetes has hampered testing hypotheses of genome evolution, because species trees provide a null hypothesis of vertical descent (orthology) versus alternative modes of evolution such as horizontal gene transfer (HGT) or paralogy (gene duplications). Robust phylogenies are also needed to provide a framework to study the evolution of morphological and chemical characters, including complex pathways involved in plant and animal pathogenicity.

In the last 10 years, whole genome sequences of oomycetes have led to breakthroughs in research on the molecular basis of host-oomycete interactions. However, these data have not been optimally used for phylogenomic analysis to resolve some of the above questions regarding the relationships among the peronosporalean genera. In this study, we gathered 16 representative oomycete genomes (Table 1), adding a new genome sequence for a critical peronosporalean lineage (*Py. insidiosum*). We obtained hundreds of orthologous coding regions from these genomes for phylogenomic analyses. The goal of this study is to provide a phylogenomic framework that future studies can build upon by including more genomes from different taxa. We identified 277 one-to-one orthologs present in all 17 genomes analyzed, hereafter core oomycete orthologs (COO), that were used for phylogenomic analyses. The specific phylogenetic questions we focused on were the clarification phylogenetic relationships among *Pythium* clades and their relationships with *Phytophthora*, *Hyaloperonospora*, and *Albugo*. Our analyses provided a strong phylogenomic framework to study oomycete evolution through the use of core oomycete orthologs.

2. Methods

2.1. Genome sequencing of *Pythium insidiosum*

The genome sequence for a U.S. clinical isolate of *Py. insidiosum* was previously reported as a data package (Ascunce et al., 2016). Here we briefly additional details regarding the methods used to assemble the *Py. insidiosum* genome.

2.1.1. Sample information and culturing

Pythium insidiosum strain CDC-B5653 (ATCC 200269), was originally isolated from necrotizing lesions on the mouth and eye of a 2-year-old boy in Memphis, Tennessee, USA. The organism was subcultured on Sabouraud Dextrose Agar (SDA) and incubated at 37 °C. Agar plugs containing mycelia were grown in Sabouraud Dextrose Broth (SDB) at 37 °C until a mycelial mat was obtained.

2.1.2. Illumina sequencing

Genomic DNA was extracted from fresh mycelia using a modi-fied CTAB method (Saghai-Maroof et al., 1984). Illumina paired-end (2 x 300 bp) sequencing libraries were prepared using the Nextera sample prep kit (Illumina Inc., San Diego, CA) at UCLA Sequencing and Genotyping Core. Libraries were sequenced in a single flow cell lane in a paired-end manner on an Illumina Genome Analyzer (Illumina Inc., San Diego, CA) following the manufacturer's protocols. Illumina MiSeq sequencing yielded 14 million reads.

2.1.3. PacBio sequencing

Mycelia were taken directly from subculture plates and lyophilized. Dried material was placed in a tube with a glass bead (2 mm diameter) and ground into fine powder in a Mini-Beadbeater-1 (BioSpec Products, Inc., Oklahoma, US) for 20 s. DNA extraction was conducted using a CTAB extraction buffer with modifications (Saghai-Maroof et al., 1984). For PacBio sequencing, we conducted a further purification step using MoBio PowerClean DNA kit following the manufacturer's recommendations (MO BIO Laboratories Inc., Carlsbad, CA, USA). High-quality genomic DNA was submitted to the University of Florida's Interdisciplinary Center for Biotechnology Research (ICBR) Nextseq core for generation of a 10-kb fragment library that was sequenced using SMRT cells providing a total of 356,001 PacBio reads.

2.1.4. Assembly and genome annotation

Read quality was assessed with FastQC. For assembly, we used SPAdes assembler version 3.5.0 (Nurk et al., 2013) to conduct a hybrid de novo assembly using the illumina reads for assembly and PacBio reads for scaffolding. Our procedure produced a final assembly of 45.6 Mb contained in 8,992 contigs with an average coverage of 28X, N50 of 13 Kb, maximum contig length of 148 Kb, and 57% G+C content. We used Augustus version 3.0.1 (Stanke et al., 2008) to predict genes *ab initio*. A gene model for prediction was created using transcripts of *Py. insidiosum* previously reported (Krajaejun et al., 2014).

2.2. Phylogenomic analysis

2.2.1. Homology determination - orthologous groups

Reciprocal blast was conducted using OrthoMCL v1.4 (Chen et al., 2006) with default parameters to delineate groups of orthologous coding regions among 17 oomycetes. We refer to these groups of coding sequences as the core oomycete orthologs (COO). OrthoMCL used the *Py. insidiosum* genome sequence generated for this study along with a set of 16 published genomes (Table 1) (Haas et al., 2009; Baxter et al., 2010; Lévesque et al., 2010; Kemen et al., 2011; Adhikari et al., 2013; Jiang et al., 2013; Ascunce et al., 2016). We included all published *Pythium* genomes, except for *Pythium oligandrum*, which was published after completion of our analyses (Berger et al., 2016). We generated three data-sets using the COOs. Dataset 1 included all one-to-one orthologs present in all of the taxa analyzed. Dataset 2 was based created a composite outgroup in which we required a saprolegnialean ortho-log to be present in any one of *Saprolegnia parasitica*, *Saprolegnia diclina*, or *Aphanomyces astaci* genomes. We selected sequences for the ‘‘composite outgroup” in the following order: (1) *Saprolegnia parasitica*; (2) *Saprolegnia diclina*; and (3) *Aphanomyces astaci*. Dataset 3 has the same composite outgroup, however we removed the two obligate biotroph peronosporaleans, *Albugo laibachii* and *Hyaloperonospora arabidopsidis*, because they have reduced gene contents relative to *Phytophthora* and *Pythium* species and this reduced the number of orthologous groups available to address our focal questions regarding the placement of *Pythium*.

2.2.2. Alignment of nucleotide sequence from one-to-one orthologous groups

Nucleotide sequences of common orthologous groups (OGs) were extracted using the original fasta files from genome sequence projects. We used ProbAlign (Roshan and Livesay, 2006), which employs maximum expected accuracy as an objective function, and introduces a partition function to calculate posterior probabilities for alignment columns, to align COOs for each of the three datasets. We used the pipeline described in Lefebure and Stanhope (2009) to automatically clean and filter alignments using the posterior probabilities emitted by ProbAlign. Briefly, DNA sequences were translated to amino acids, aligned using ProbAlign, and then poorly

aligned sites (those with posterior probabilities less than 0.7) were removed. After quality trimming, only alignments that conserved at least 50% of their original length were considered for further analysis. Finally, the aligned amino acids were used to guide the nucleotide alignments.

We also generated alignments for COO Dataset 1 using PRANK aligner (Loytynoja and Goldman, 2008) to test the sensitivity of our conclusions to the alignment program and the trimming and filtering strategy. For alignment with PRANK, DNA sequences were used and neither trimming nor filtering of sites was conducted. PRANK is a probabilistic multiple alignment program that aims at an evolutionarily correct alignment. We used the default parameters and author recommendations for PRANK. Specifically, we used the HKY model (Hasegawa et al., 1985) with empirical base frequencies and kappa = 2 and option –F, which specifies that the inference of insertions should be trusted and sites appearing as insertions should not be aligned at the later stages of the process.

2.2.3. Individual gene trees

For this analysis we used the alignments obtained using ProbAlign for the three datasets. Individual phylogenetic trees were con-structed for each COO alignment by maximum likelihood (ML) using the PhyML software (Guindon et al., 2010) with the general time reversible model with gamma distributed rates across sites (the GTR+G model). We assessed support by bootstrapping (100 replicates) using the nearest-neighbor interchange (NNI) branch-swapping method. We summarized all trees in a majority-rule consensus using the consense program in the phylip package (Felsenstein, 2005).

For COO Dataset 1, we conducted a deeper analysis that included model testing and comparisons of different methods for tree topology searches. Although gene tree searches are sometimes conducted using a single model in many phylogenomic studies (e.g., Jarvis et al., 2014), either due to limited computation time or the constraints of the analytical programs used, there is some possibility that GTR+G is over-parameterized. Likewise, some NNI searches may not escape local optima. Thus, we tested whether the use of a single model (GTR+G) and NNIs had any effect

on our conclusions. We used the 277 COOs in Dataset 1 and selected the best-fitting model of nucleotide substitution for each OG using the Akaike information criterion in ModelGenerator v0.85 (Keane et al., 2006). Then we constructed ML trees using the PhyML 3.0 software (Guindon et al., 2010) using the best fitting substitution model and the more thorough subtree pruning and regrafting (SPR) branch-swapping method (Swofford et al., 1996) and assessed nodal support by bootstrapping (100 replicates). We con-ducted the NNI and SPR analyses using the alignments generated using each method (ProbAlign and PRANK) and summarized the four sets of gene trees to yield four majority-rule extended (MRE) consensus trees (also called ‘‘greedy” consensus trees) using consense program in the phylip package (Felsenstein, 2005).

2.2.4. Phylogenetic congruence – analysis of bipartition spectra

A bipartition represents the division of a phylogeny into two parts connected by a single internal branch. It does not consider the relationships within each of the two groups. Consideration of bipartitions enables us to capture strongly supported parts of phylogenies even if other parts remain unresolved and to examine signals in the data that do not conform to the majority of trees. Bootstrap support for each bipartition (branch or split) of each individual OG tree was evaluated using the consense program in the phylip package (Felsenstein, 2005). Each bootstrapped tree was used as input to obtain a majority rule consensus tree considering different bootstrap support values. We evaluated percentages of bootstrap value cutoffs of 70%, 80% and 90%. The number of observed bipartitions at specific cutoff value were used to generate ‘‘Lento” plots (Lento et al., 1995). Topological distances among trees were calculated using the symmetric distance, which corresponds to 2 times the Robinson and Foulds (1981) distance, implemented in PAUP⁄4.0a152 (Swofford, 2017).

2.2.5. Concatenated analysis

The Dataset 1 supermatrices comprised the alignments of 277 COOs from all 17 taxa; the supermatrix obtained using ProbAlign was 249,830 bp in length whereas the PRANK supermatrix was 478,755 bp in length. We estimated the ML tree for each supermatrix using RAxML v7.3.5

(Stamatakis, 2006). We conducted unpartitioned analyses using the GTRGAMMA (GTR+G) model and assessed nodal support for all trees using the rapid bootstrapping option in RAxML with 1000 bootstrap replicates. Our analyses of datasets 2 and 3 only used the trimmed ProbAlign alignments. The supermatrix for Dataset 2 included data from 424 orthologs found in all 14 ingroup taxa and at least one outgroup (a composite outgroup was generated as described above). The supermatrix for Dataset 3 comprised 874 orthologs that we could find in 12 ingroup taxa and at least one outgroup. Phylogenetic analyses of the supermatrices for Datasets 2 and 3 were conducted in RAxML as described above (i.e., using the GTR + G substitution model and 1000 rapid bootstrap replicates).

2.2.6. Species trees

To address the concern that parts of oomycete phylogeny might fall in the part of parameter space where concatenated analyses are inconsistent we used ASTRAL-II (Accurate Species TRee ALgorithm) (Mirarab and Warnow, 2015) to estimate the species tree from gene trees. ASTRAL finds the species tree that agrees with the largest number of quartet trees obtained by the gene trees. We conducted a total of four species tree ASTRAL analyses using the gene trees for the Dataset 1 COOs. The four sets of gene trees correspond to those generated by PhyML using the two different alignment methods (ProbAlign and PRANK) and the two types of tree searches (NNI and SPR), using the best fitting substitution model for each ortholog. In all four cases we used the exact search option in ASTRAL-II; we assessed support using local posterior probabilities (Sayyari and Mirarab, 2016).

3. Results

3.1. Orthologous groups

To identify common genes in all oomycete genomes, we compared the predicted protein-coding genes of 17 pathogenic oomycetes using OrthoMCL v1.4 (Chen et al., 2006). A total of 277 orthologs were present in one copy in all 17 oomycetes analyzed in this study, core oomycete orthologs (COO) (Dataset 1) (Supplementary Material Table S1). The use of only 277 COOs in

Dataset 1 reflect the fact that we only used genes that were present in all taxa in order to avoid any potential biases due to missing data (cf. Hosner et al., 2016). Datasets 2 and 3 were constructed in a way that allowed us include more loci but still avoid missing data. A total of 424 orthologs were obtained when we used a composite outgroup (ortholog from any of one of *Saprolegnia parasitica*, *Saprolegnia diclina*, or *Aphanomyces astaci*) (Dataset 2). *Removing Albugo laibachii* and *Hyaloperonospora arabidopsidis* and using the composite outgroup more than doubled the number of orthologous groups to 874 (Dataset 3). This dramatic increase in the number of orthologs is consistent with previous genomic studies that have shown that both *Albugo* and *Hyaloperonospora*, which are obligate biotrophs, have significantly smaller numbers of genes compared to *Phytophthora* and *Pythium* species (Baxter et al., 2010; Kemen et al., 2011; Links et al., 2011). The use of these three datasets allowed us to test whether the patterns noted for the 277 COOs held for larger samples of genes.

3.2. Assessing conflict among oomycete gene trees

Phylogenomics has revealed that many estimated gene trees are incongruent with each other and with the putative species tree. Indeed, some phylogenomic studies (e.g., Salichos and Rokas, 2013; Jarvis et al., 2014) reported that none of the individual gene trees were congruent with the tree obtained by concatenation. This incongruence could reflect stochastic error in gene tree estimation (Patel et al., 2013; Gatesy and Springer, 2014), bias associated with specific genes (e.g., accelerated evolution for a specific gene that leads long branch attraction; Felsenstein, 1978), or it could reflect accurate gene tree estimation combined with biological processes that result in individual genes that have evolutionary histories that differ from the species history (Maddison, 1997; Galtier and Daubin, 2008).

Processes that result in discordance among true gene trees include gene duplication and loss, HGT, and incomplete lineage sorting (ILS). The existence of processes that result in a ‘‘cloud” of gene trees that can differ from the species tree was appreciated long before the phylogenomic era (Brower et al., 1996; Maddison, 1997). However, the full scale of conflict between individual

gene trees and species trees were ultimately revealed by phylogenomic studies (e.g., Rokas et al., 2003). Multispecies coalescent (MSC) models, which address discordance due to ILS, have attracted special attention (e.g., Heled and Drummond, 2010; Tonini et al., 2015; Edwards et al., 2016; Mirarab et al., 2016). Incongruence among gene trees due to the MSC is expected even under neutral models of evolution and the MSC can be problematic because there is a part of parameter space where ML analyses of concatenated data are inconsistent (Kubatko and Degnan, 2007; Roch and Steel, 2015; Warnow, 2015). However, HGT is also an important process and there are many examples HGT of between oomycetes and fungi (Götesson et al., 2002; McLeod et al., 2003; Richards et al., 2011; Torto et al., 2002; Tyler et al., 2006), some of which may be functionally important. Below, we report the results of analyses focused on examining incongruence among gene trees and estimating the species tree for the oomycete taxa we selected.

3.2.1. Analysis of bipartition spectra

In order to evaluate phylogenetic congruence among orthologs and the possibility of conflicting topologies, we examined the number of common bipartitions (splits) among all gene trees. Consideration of bipartitions enables the capture of strongly supported parts of phylogenies, even if other parts remain unresolved. It can also reveal conflicting (i.e., non-treelike) patterns in the data. The number of all possible bipartitions for N taxa is equal to [2(N"1) " N – 1]. For our COO dataset of 277 orthologs from 17 taxa (Dataset 1), there were a total of 65,518 possible bipartitions, many of which were not supported by any orthologous groups. This dataset recovered a total of 53 bipartitions that were supported by 70% or higher bootstrap values in at least one gene (Fig. 1; Table 2). Of those 53 bipartitions, 14 (26%) were supported by a majority of gene trees. Splits that are supported by >50% of gene trees are expected to be present in the species tree (Degnan et al., 2009).

For Dataset 2 (15 taxa, 424 orthologs and 16,368 possible bipartitions) and Dataset 3 (13 taxa, 874 orthologs and 4082 possible bipartitions), there were 47 and 36 bipartitions supported by at least one gene at 70% bootstrap support, respectively (Supplemental Material Figs. S1 and S2).

These datasets revealed patterns similar to Dataset 1, with 12 (25%) and 10 (28%) splits strongly supported across gene trees. Because these datasets produced essentially the same topologies but contained fewer taxa, we focus our attention on the 17 taxa COO Dataset 1.

Splits uniting closely related species (splits: a, b, d, g, h; Fig. 1) were found in many gene trees. The downy mildew *Hyaloperonospora* grouped within *Phytophthora* (splits: e, j, k, l), with many gene trees including a bipartition grouping *Hyaloperonospora* with *Phytophthora infestans* and *Phytophthora parasitica*. The sister relationship between *Phytopythium vexans* and the *Phytophthora–Hyalo peronospora* group (split: j) was also supported. In contrast, monophyly of the genus *Pythium* was not supported (splits: m, n; ortho-logs are given in Supplementary Material Table S2).

3.2.2. Sensitivity of the COO gene trees to substitution model

We compared estimates of gene trees for Dataset 1 obtained using the GTR+G model for all genes to gene trees estimated using the best-fitting model for each gene. The number of bipartitions with #90% bootstrap support that contradicted the consensus tree remained the same at 19 bipartitions, with only a slight difference in the number of genes. Using GTR, there were a total of 47 contradictory genes, while for the best fit model there were 42 contradictory genes. There were two genes that placed *Py. insidiosum* in alternative positions: beta-tubulin united *Py. insidiosum* with the two *Saprolegnia* species and the TFIIH basal transcription factor complex helicase subunit placed *Py. insidiosum* sister to all the other peronosporaleans (Supplementary Material Table S3). The split uniting *Phytophthora species* (i.e., the bipartition uniting the four *Phytophthora* species but excluding *Hyaloperonospora*) had 90% bootstrap support in the trees for two loci (Supplementary Material Table S3). Overall, there were a fairly limited number of gene trees that included strongly supported bipartitions that conflict with the consensus tree.

3.2.3. Sensitivity of the COO gene trees to alignment and tree search

We also examined the impact of model, alignment method (ProbAlign vs. PRANK), and branch-swapping algorithm (NNI vs. SPR). To do this we conducted ML analyses using the best-

fitting substitution model and each combination of alignment and tree search method. In general, the results of these analyses were consistent with the first set of analyses we conducted using NNI searches and a single model (GTR+G) for all genes. The MRE consensus trees obtained using all four methods had very similar number of gene trees supporting each split (Supplementary Material Fig. S3) and the distributions of distances between the MRE consensus tree (Fig. 1b) and the gene trees obtained using all methods were similar (Supplementary Material Fig. S4). One potentially important finding, however, was that the consensus of SPR trees generated using the alignment generated by ProbAlign had slightly more trees that place *Pythium* clades A-C sister to all other peronosporaleans (i.e., a plurality of gene trees includes a bipartition that includes *Albugo* and all peronosporaleans except *Py. insidiosum*, *Py. aphanidermatum*, and *Py. arrhenomanes*; Supplementary Material Table S4). This remained true for the SPR+ProbAlign trees even if we focused only on those trees with splits with 90% bootstrap support, though there were only two gene trees with that level of support that place *Pythium* clades A-C sister to all other peronosporaleans. However, all four combinations of tree search and alignment methods had nearly equal numbers of gene trees supporting three possible splits relevant to the position of Albugo (Supplementary Material Table S4). Taken as a whole, these analyses suggest most of the splits supported by gene trees are robust to alignment and tree search method, with the notable exception of the split placing *Albugo* sister to all other peronosporaleans.

3.3. Concatenated analysis

Phylogenomic analysis of concatenated genes revealed congruent topologies across the three datasets obtained using ProbAlign (Fig. 2, Supplemental Material Fig. S5). All nodes had 100% boot-strap support and all 14 highly supported splits in the gene tree analysis (Fig. 1) were also present in the tree for the concatenated Dataset 1. The concatenated PRANK alignments provided almost twofold more data than the conservatively-trimmed ProbAlign supermatrix (For Dataset 1, PRANK had 478,755 bp whereas Pro-bAlign had 249,830 bp) but we found that the results of analysis using PRANK (Supplemental Material Fig. S6) were identical to the ProbAlign

results (Fig. 2). The limited conflict among gene trees is consistent with a strong vertical signal and all analyses of concatenated data recovered the same topology. This topology has 100% bootstrap support for all nodes and is identical to the MRE consensus tree of gene trees (Fig. 1b). While the analyses of concatenated data consistently placed *Albugo* sister to the other peronosporalean taxa, unlike the consensus trees where that bipartition is present in a limited number of gene trees, the addition of more OGs in Dataset 2 produced lower support for this split (Supplemental Material Fig. S5).

3.4. Phylogenomic inference using multispecies coalescent methods

Species tree analyses of Dataset 1 using the set of trees generated from ProbAlign alignments, best fit model of evolution and SPR tree searching resulted in a topology nearly identical to that obtained using the concatenated analysis. The one important exception was that *Pythium* clades A, B, and C were placed sister to all other peronosporaleans instead of *Albugo* (Fig. 3). The same pattern was observed with the species tree obtained from PRANK alignments, best fit model of evolution and SPR tree search methods (Supplemental Material Fig. S7D). For the NNI+ProbAlign species tree, *Albugo* appeared sister to *Pythium* clades A-C, although with very low nodal support (Supplemental Material Fig. S7A). Finally, species trees estimated using PRANK alignments, best fit model, and NNI tree search method resulted in estimates of the species tree identical to that obtained using the concatenated analysis however the support for the ‘‘m” bipartition was low (Supplemental Material Fig. S7C). Overall, there was substantial variation regarding the support and position of *Albugo* (Fig. 3; Supplemental Material Fig. S7). Although the MRE consensus is not a consistent estimator of the species tree (Degnan et al., 2009), we emphasize that these results are consistent with the variation in the position of *Albugo* in those analyses. Nonetheless, the observation that there is strong concordance among all approaches we used to examine the oomycete gene tree indicates that much of the oomycete phylogeny estimated using the COO dataset is robust; the one important exception is position of *Albugo*.

4. Discussion

Oomycetes exhibit a wide range of lifestyles from free-living saprophytes in aquatic and soil environments, to above ground endophytes, to hemibiotrophic and obligate biotrophic parasites of plants, fungi, invertebrates and vertebrates. This great diversity of host-oomycete interactions has led to increasing research on the evolution of these parasitic life styles (Thines and Voglmayr, 2009; Baxter et al., 2010; Links et al., 2011; Thines, 2014). However, research efforts have focused on the mechanisms that oomycetes use to successfully infect economically important plants. To gain a deeper understanding of the evolution of pathogenesis in oomycetes, comparisons among species should be conducted using a phylogenetic framework. This has been hampered by the absence of a strongly-supported, multilocus oomycete phylogeny. Here we obtained a robust phylogeny for taxa representing the available genomes of the major personosporalean clades. Published oomycete phylogenies have largely focused on a limited set of traditional genetic markers such as LSU, SSU, and COI. These single-locus and the available multi-locus datasets have not contained enough phylogenetic information to resolve relationships among the major clades of oomycetes (reviewed in Choi et al., 2015a). Because comparable single locus phylogenies with our taxon sampling were not available in the literature for comparisons to our phylogenomics approach, we evaluated the performance of two single locus markers, LSU and COI using the same taxon sampling in this phylogenomic study for illustrative purposes only (Supplementary Material Section: Single locus phylogenies). The branch support values in these trees were sometimes low, particularly for COI, and topologies differed due to the occurrence of polytomies and different clustering of some taxa. These single locus phylogenies were not able to resolve the relationships between major clades. Here we showed that analyses 277 single copy orthologous genes shared among 17 taxa from major lineages of the oomycetes can produce a robust oomycete phylogeny. The position of *Albugo* did, however, exhibit uncertainty in our MSC analyses. During the process of revising this current version of the manuscript another manuscript was published with a

phylogenomic reconstruction of 37 oomycetes using amino acid sequences (McCarthy and Fitzpatrick, 2017). These new results are included in the discussion below.

4.1. How much confidence should we have in the oomycete species tree?

This study largely focused on analyses of a phylogenomic data matrix (COO Dataset 1) comprising 277 loci, although we confirmed that similar distributions of gene trees could be obtained from larger datasets including up to 874 OGs (albeit with more limited taxon samples). We focused exclusively on complete data matrices to avoid any problems that might be associated with missing data (e.g., Hosner et al., 2016; Brower and Garzón-Orduña, 2017). Although our phylogenomic analyses emphasized that establishing the position of *Albugo* in the oomycete tree remains challenging, they yielded an estimate of oomycete phylogeny that is otherwise strongly supported. This phylogeny will provide a useful framework for other analyses of oomycete molecular evolution.

This study had two major goals: (1) to examine the degree of congruence among individual gene trees; and (2) to infer the topology of the oomycete species tree for our taxon sample (Table 1). There are several reasons that gene trees can exhibit conflict with each other. One of the most interesting would be HGT, because it can have functional implications. Of the 48 HGT events described in oomycetes, 40 of them showed a fungal origin and those genes seem to be involved in pathogenicity and fungal-like life history traits (Götesson et al., 2002; McLeod et al., 2003; Richards et al., 2011; Torto et al., 2002; Tyler et al., 2006; Savory et al., 2015). Moreover, most of the HGT events appear to have occurred after the oomycete lineage diversified (Savory et al., 2015). Our observation that a limited number of gene trees included strongly supported bipartitions that conflict with an MRE consensus of the gene trees is in agreement with previous findings about HGT in oomycetes. Indeed, our analyses extend the findings regarding the limited amount of HGT to genes that are placed in one-to-one ortholog groups by OrthoMCL. Many genes implicated in HGT in other studies are present only in a subset of oomycetes, sometimes in multiple copies (cf. Fig. 1 in Savory et al., 2015). We also note that our analyses only provide an upper limit on HGT;

other explanations for incongruence among gene trees include ILS and gene tree estimation error. Both ILS and estimation error could contribute to the relatively limited conflict we observed, although the fact that we observed some variation in the topologies obtained when different substitution models, tree search methods, and alignments were used for analyses suggests that estimation error could be important.

The general congruence between estimates of the oomycete species tree obtained by analyses of concatenated data and an MSC approach (ASTRAL-II) suggests that we have obtained a robust tree for our taxon sample. In fact, we find gene trees that match the concatenated tree exactly (Supplementary Material Fig. S4). The notable exception to this robust support for the oomycete tree is the position of *Albugo*, which we could not place with confidence in the ASTRAL tree. This raises the question of whether we should embrace the strongly supported placement of *Albugo* in the concatenated analysis (100% bootstrap support) or view the node as unresolved. We note that *Albugo* is the longest branch for our taxon sample and it also has a somewhat divergent base composition (Supplemental Material Table S5). Thus, there are reasons to believe that it might be the most difficult taxon to place. Moreover, the position of *Albugo* in the tree generate by analysis of concatenated data could reflect long branch attraction (Felsenstein, 1978). There are three relatively long branches in the tree: (1) *Albugo*; (2) *Hyaloperonospora*; and (3) the branch between the outgroup and ingroup. The tree generated by concatenation includes the bipartition (split m) separating *Albugo* and the saprolegnialean outgroups. There is evidence that species tree methods are robust to the simultaneous effects of ILS and long branches (Liu et al., 2015), and this could be the basis for the uncertainty suggested by the ASTRAL trees. Ultimately, we believe it is more conservative to view the placement of *Albugo* as uncertain; resolving this position is likely to require breaking up the long-branch to *Albugo* with additional members of Albuginaceae. Indeed, adding undersampled genera deep in the oomycete phylogeny is likely to be valuable.

Regardless of the position of *Albugo*, the remaining phylogeny appears robust. When we examined Dataset 3, which was composed of 874 orthologs for 12 ingroup taxa (the divergent bio-

trophs *Albugo* and *Hyaloperonospora* were removed), and a composite outgroup we recovered an MRE tree congruent with the COO Dataset 1 trees for the overlapping taxa.

During the course of revising the manuscript describing our analyses McCarthy and Fitzpatrick (2017) published a phylogenomic study of oomycetes that was, in many ways, complementary to our study. McCarthy and Fitzpatrick (2017) conducted analyses of amino acid sequences and they used two different gene tree reconciliation methods [gene tree parsimony (GTP; Goodman et al., 1979; Guigo et al., 1996) and matrix representation with parsimony (MRP; Baum, 1992; Ragan, 1992)]. Their analyses were consistent with our results and they strongly confirmed the position of the oomycete root between Saprolegniomycetidae and Peronosporomycetidae. Notably, they recovered *Albugo* in a position identical to our concatenated tree with 100% bootstrap support. However, neither GTP nor MRP are consistent given the MSC [Than and Rosenberg (2011) demonstrated that the ‘‘minimizing deep coalescences” variant of GTP is inconsistent and Wang and Degnan (2011) proved that MRP is inconsistent]. The most important factors to consider when choosing among phylogenetic methods in light of the MSC remain a topic of vigorous debate (Patel et al., 2013; Gatesy and Springer, 2014; Chou et al., 2015; Tonini et al., 2015; Warnow, 2015; Edwards et al., 2016; Meiklejohn et al., 2016). However, we note that all gene tree reconciliation methods, including GTP and MRP, are likely to be sensitive to concerns regarding the use of poorly-resolved gene trees as input (e.g., Gatesy and Springer, 2014; Simmons and Gatesy, 2015; Xi et al., 2015; Meiklejohn et al., 2016). Thus, it seems reasonable to assert that we should consider the results of methods, like ASTRAL, that are statistically consistent (Mirarab et al., 2014).

4.2. Parallel evolution of biotrophy on plants

Molecular phylogenetics have shown that the downy mildew lineage is nested within the genus *Phytophthora* (Göker et al., 2007; Thines et al., 2009; Runge et al., 2011) rather than sister to *Phytophthora* as long thought. However, this result has been marker-dependent. Recent analyses of concatenated markers placed *Hyaloperonospora* as sister to *Phytophthora* (Seidl et al., 2012) and showed the downy mildews to be polyphyletic (Sharma et al., 2015). Our bipartition analyses

placed *Hyaloperonospora* within *Phytophthora*. Specifically, *Hyaloperonospora* was sister to the *Phytophthora* clade 1 species *Ph. infestans* and *Ph. parasitica* (Fig. 1), and this relationship was strongly supported in both the concatenated and ASTRAL trees (Figs. 2 and 3). While there was strong support for a *Hyaloperonospora-Phytophthora* clade among individual orthologs, a relatively small fraction of orthologs strongly supported the specific placement of *Hyaloperonospora* within *Phytophthora*. Future analysis using the many downy mildew and *Phytophthora* genome sequencing projects currently in progress should help clarify these relationships.

Downy mildews are interesting models to study the evolution of parasitism because they are highly host-specific (e.g. Göker et al., 2007), and their biotrophy seems to be derived from hemibiotrophy (Baxter et al., 2010). Comparative genomics indicate that downy mildews present a reduced number of RXLR effectors, which are specific to the peronosporalean lineage and facilitate infection by suppressing plant immunity (Pel et al., 2014). Another characteristic trait of these obligate biotrophs is the formation of specialized hyphae called haustoria during infection. Because effector content and formation of haustoria vary among *Phytophthora* species, the phylogenetic placement of downy mildews relative to *Phytophthora* species must be clarified for study of the evolution of virulence in the Peronosporales.

The genus *Albugo* (Albuginales), causal agents of white blister rust disease (WBR), also contains obligate biotrophs. While both *Hyaloperonospora* and *Albugo* show similar infection structures within the host, *Albugo* releases motile asexual zoospores, while *Hyaloperonospora* lacks all motile stages (Coates and Beynon, 2010). Single locus phylogenies have placed *Albugo* close to or within *Pythium* clades using flagellar genes (Robideau et al., 2014). As discussed above, we found that the placement of *Albugo* varied with the method used. Our concatenated analysis placed it as a sister group to the other peronosporalean taxa in our analyses, in agreement with the idea that WBR diverged early from other major oomycete plant pathogens (Thines and Voglmayr,

2009). However, our MSC results suggest that its placement relative to *Pythium* should remain uncertain.

4.3. Phylogenomic placement of *Phytopythium vexans* as sister group to the *Phytophthora-Hyaloperonospora* clade

Members of the traditional *Pythium* clade K show intermediate morphologies between the genera *Phytophthora* and *Pythium*, and phylogenetic reconstructions have assigned this group to the genus *Phytopythium* (Lévesque and de Cock, 2004; Bala et al., 2010; De Cock et al., 2015). *Phytopythium vexans* shares important traits with Pythium (Bala et al., 2010), however our phylogenomic approach showed *Phytopythium vexans* clustering with *Phytophthora*. This placement was previously suggested using sequences of the ribosomal SSU gene and COI (Adhikari et al., 2013; De Cock et al., 2015). Because both *Phytopythium vexans* and *Pythium* species lack haustoria and RXLR-class effectors, we can hypothesize that these traits evolved in the Phytophthora-downy mildew group after diverging from *Phytopythium*. By placing *Phytopythium* in the oomycete phylogeny, genomic comparisons can now be used to examine the evolutionary processes that led to hemibiotrophy and biotrophy in the *Phytophthora*-downy mildew lineage.

4.4. Polyphyly in *Pythium* sensu lato

With as many as 250 described species, *Pythium* is one of the most ecologically diverse genera of oomycetes occupying terrestrial ecosystem to salt water estuaries (e.g., Van der Plaats-Niterink, 1981; Martin and Loper, 1999; Martin, 2009). Some of the phytopathogenic *Pythium* species have broad host ranges, whereas others infect a narrow spectrum of plants. In general, *Pythium* species are considered primary saprophytic colonizers because they rapidly colonize fresh organic substrates in the soil (Garrett, 1970). *Pythium* species are usually easily grown and have the potential to be valuable experimental systems for necrotrophic and saprotrophic oomycete lifestyles. Genetic diversity underlies the ecological diversity of *Pythium*, exemplified by 10 distinct phylogenetic clades, named A to J, which comprise *Pythium* sensu lato (Lévesque and de

Cock, 2004; De Cock et al., 2015). This is the same number of clades currently delimited in the intensively studied genus *Phytophthora* (Cooke et al., 2000).

With this phylogenomic approach, we found two monophyletic groups within *Pythium*, which is consistent with previous molecular phylogenies and morphological clustering: one monophyletic group composed of Clades A-D characterized by filamentous sporangia, and another monophyletic group formed by Clades E-J characterized by globose sporangia. Our analysis included three species that correspond to *Pythium* clades A, B and C (*Py. aphanidermatum*, *Py. arrhenomanes*, and *Py. insidiosum*, respectively) and three species representing clades F, G and I (*Py. irregular*, *Py. iwayamai*, and *Py. ultimum*, respectively). Our topology was also consistent with a previous *Pythium* phylogeny based on ITS and 5.8S ribosomal gene (Lévesque and de Cock, 2004). However, a recent study using a different taxon sampling and single locus phylogenies using LSU ribosomal, COI and SSU ribosomal genes showed different clustering of *Pythium* clades and species (De Cock et al., 2015). Furthermore, in our analyses, the *Pythium* species representing Clades F, G, and I with globose sporangia formed a sister group to the *Phytophthora-Hyaloperonospora-Phytopythium* clade, thus confirming the lack of monophyly of the genus *Pythium* sensu lato highlighting the need for genomic, morphological and physiological studies that include representatives from across the peronosporalean lineage.

5. Concluding remarks

A growing number of genomic resources allowed us to gather a large number of coding sequence markers as a first step towards a robust phylogeny of the oomycetes. Although still limited in taxon sampling, we were able to clarify important aspects of the oomycete phylogeny and provide a phylogenomic framework to test evolutionary hypotheses using nucleotide data and both supermatrix ML and MSC approaches. Almost 15 years ago, phylogenomic studies were limited to model organisms with complete (or relatively complete) genome sequences, but since, improvements in technology and methods have allowed an exponential growth in the availability of genomic data from non-model organisms (Ellegren, 2014). However, the complexity of these

datasets could potentially introduce significant phylogenetic noise and conflict into the analyses that follow. The sources of conflict may include incomplete lineage sorting, horizontal gene transfer, and gene or genome duplications (Smith et al., 2015). For the oomycetes, we have shown that one-to-one orthologous gene regions found in whole genome data produce robust phylogenomic results. We applied the widely used methods of analyzing concatenated sequences and gene tree reconciliation assuming the multispecies coalescent and both methods yielded congruent and well-supported phylogenies. Our analyses supported the clustering of *Phytopythium vexans* with the *Phytophthora-Hyaloperonospora* clade, and the lack of monophyly of *Pythium* sensu lato. Although our analysis of individual gene trees identified some loci that conflict with the consensus phylogeny, the evolution of the genes we selected for analyses was largely tree-like. Phylogenomic data for more oomycete taxa are needed to test hypotheses regarding the evolution of parasitic lifestyles in the oomycetes.

Acknowledgments

We wish to thank Jackson Presser for technical support and Jeff Rollins (University of Florida) for guidance and laboratory resources. The University of Florida High Performance Computing Center and the HiPerGator cluster support staff provide computer assistance. We thank the editor and the reviewers for their comments and suggestions on an earlier version of the manuscript. This research was funded by the University of Florida Research Opportunity Incentive Seed Fund from the UF Office of Research, the UF Emerging Pathogens Institute, and the UF IFAS Department of Plant Pathology.

Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2017.06.013.

References

Adhikari, B.N., Hamilton, J.P., Zerillo, M.M., Tisserat, N., Lévesque, C.A., Buell, C.R., 2013. Comparative genomics reveals insight into virulence strategies of plant pathogenic oomycetes. PloS One 8, e75072. doi:10.1371/journal.pone.0075072

Ascunce, M.S., Huguet-Tapia, J.C., Braun, E.L., Ortiz-Urquiza, A., Keyhani, N.O., Goss, E.M., 2016. Whole genome sequence of the emerging oomycete pathogen Pythium insidiosum strain CDC-B5653 isolated from an infected human in the USA. Genomics Data 7, 60–61. doi:10.1016/j.gdata.2015.11.019

Augspurger, C.K., Wilkinson, H.T., 2007. Host Specificity of Pathogenic Pythium Species: Implications for Tree Species Diversity. Biotropica 39, 702–708. doi:10.1111/j.1744-7429.2007.00326.x

Austin Bourke, P.M., 1964. Emergence of Potato Blight, 1843–46. Nature 203, 805–808. doi:10.1038/203805a0

Bala, K., Robideau, G.P., Désaulniers, N., de Cock, A.W. a. M., Lévesque, C.A., 2010. Taxonomy, DNA barcoding and phylogeny of three new species of Pythium from Canada. Persoonia 25, 22–31. doi:10.3767/003158510X524754

Baldauf, S.L., Roger, A.J., Wenk-Siefert, I., Doolittle, W.F., 2000. A kingdom-level phylogeny of eukaryotes based on combined protein data. Science 290, 972–977.

Baxter, L., Tripathy, S., Ishaque, N., Boot, N., Cabral, A., Kemen, E., Thines, M., Ah-Fong, A., Anderson, R., Badejoko, W., Bittner-Eddy, P., Boore, J.L., Chibucos, M.C., Coates, M., Dehal, P., Delehaunty, K., Dong, S., Downton, P., Dumas, B., Fabro, G., Fronick, C., Fuerstenberg, S.I., Fulton, L., Gaulin, E., Govers, F., Hughes, L., Humphray, S., Jiang, R.H.Y., Judelson, H., Kamoun, S., Kyung, K., Meijer, H., Minx, P., Morris, P., Nelson, J., Phuntumart, V., Qutob, D., Rehmany, A., Rougon-Cardoso, A., Ryden, P., Torto-Alalibo, T., Studholme, D., Wang, Y., Win, J., Wood, J., Clifton, S.W., Rogers, J., Van den Ackerveken, G., Jones, J.D.G., McDowell, J.M., Beynon, J., Tyler, B.M., 2010. Signatures of adaptation to obligate biotrophy in the Hyaloperonospora arabidopsidis genome. Science 330, 1549–1551. doi:10.1126/science.1195203

Belbahri, L., McLeod, A., Paul, B., Calmin, G., Moralejo, E., Spies, C.F.J., Botha, W.J., Clemente, A., Descals, E., Sánchez-Hernández, E., Lefort, F., 2008. Intraspecific and within-isolate sequence variation in the ITS rRNA gene region of Pythium mercuriale sp. nov. (Pythiaceae). FEMS Microbiol. Lett. 284, 17–27. doi:10.1111/j.1574-6968.2008.01168.x

Belhaj, K., Cano, L.M., Prince, D.C., Kemen, A., Yoshida, K., Dagdas, Y.F., Etherington, G.J., Schoonbeek, H., van Esse, H.P., Jones, J.D.G., Kamoun, S., Schornack, S., 2016. Arabidopsis late blight: infection of a nonhost plant by *Albugo laibachii* enables full colonization by *Phytophthora infestans*: Arabidopsis co-infection by two oomycetes. Cell. Microbiol. doi:10.1111/cmi.12628

Berger, H., Yacoub, A., Gerbore, J., Grizard, D., Rey, P., Sessitsch, A., Compant, S., 2016. Draft Genome Sequence of Biocontrol Agent Pythium oligandrum Strain Po37, an Oomycota. Genome Announc. 4. doi:10.1128/genomeA.00215-16

Briard, M., Dutertre, M., Rouxel, F., Brygoo, Y., 1995. Ribosomal RNA sequence divergence within the Pythiaceae. Mycol. Res. 1119–1127.

Cavalier-Smith, T., Chao, E.E.-Y., 2006. Phylogeny and megasystematics of phagotrophic heterokonts (kingdom Chromista). J. Mol. Evol. 62, 388–420. doi:10.1007/s00239-004-0353-8

Chen, F., Mackey, A.J., Stoeckert, C.J., Roos, D.S., 2006. OrthoMCL-DB: querying a comprehensive multi-species collection of ortholog groups. Nucleic Acids Res. 34, D363-368. doi:10.1093/nar/gkj123

Choi, Y.-J., Beakes, G., Glockling, S., Kruse, J., Nam, B., Nigrelli, L., Ploch, S., Shin, H.-D., Shivas, R.G., Telle, S., Voglmayr, H., Thines, M., 2015a. Towards a universal barcode of oomycetes - a comparison of the *cox* 1 and *cox* 2 loci. Mol. Ecol. Resour. 15, 1275–1288. doi:10.1111/1755-0998.12398

Choi, Y.-J., Hong, S.-B., Shin, H.-D., 2003. Diversity of the Hyaloperonospora parasitica complex from core brassicaceous hosts based on ITS rDNA sequences. Mycol. Res. 107, 1314–1322.

Choi, Y.-J., Klosterman, S.J., Kummer, V., Voglmayr, H., Shin, H.-D., Thines, M., 2015b. Multi-locus tree and species tree approaches toward resolving a complex clade of downy mildews (Straminipila, Oomycota), including pathogens of beet and spinach. Mol. Phylogenet. Evol. 86, 24–34. doi:10.1016/j.ympev.2015.03.003

Chou, J., Gupta, A., Yaduvanshi, S., Davidson, R., Nute, M., Mirarab, S., Warnow, T., 2015. A comparative study of SVDquartets and other coalescent-based species tree estimation methods. BMC Genomics 16, S2. doi:10.1186/1471-2164-16-S10-S2

Coates, M.E., Beynon, J.L., 2010. Hyaloperonospora Arabidopsidis as a pathogen model. Annu. Rev. Phytopathol. 48, 329–345. doi:10.1146/annurev-phyto-080508-094422

Cooke, D.E.L., Drenth, A., Duncan, J.M., Wagels, G., Brasier, C.M., 2000. A molecular phylogeny of Phytophthora and related oomycetes. Fungal Genet. Biol. FG B 30, 17–32. doi:10.1006/fgbi.2000.1202

De Cock, A.W., Lodhi, A.M., Rintoul, T.L., Bala, K., Robideau, G.P., Abad, Z.G., Coffey, M.D., Shahzad, S., Lévesque, C.A., 2015. Phytopythium: molecular phylogeny and systematics. Persoonia - Mol. Phylogeny Evol. Fungi 34, 25–39. doi:10.3767/003158515X685382

Densmore, C.L., Green, D.E., 2007. Diseases of amphibians. ILAR J. Natl. Res. Counc. Inst. Lab. Anim. Resour. 48, 235–254.

Dick, M.W., 2001. The Peronosporomycetes, in: McLaughlin, D.J., McLaughlin, E.G., Lemke, P.A. (Eds.), Systematics and Evolution, The Mycota. Springer Berlin Heidelberg, pp. 39–72.

Edwards, S.V., Xi, Z., Janke, A., Faircloth, B.C., McCormack, J.E., Glenn, T.C., Zhong, B., Wu, S., Lemmon, E.M., Lemmon, A.R., Leaché, A.D., Liu, L., Davis, C.C., 2016. Implementing and testing the multispecies coalescent model: A valuable paradigm for phylogenomics. Mol. Phylogenet. Evol. 94, 447–462. doi:10.1016/j.ympev.2015.10.027

Ellegren, H., 2014. Genome sequencing and population genomics in non-model organisms. Trends Ecol. Evol. 29, 51–63. doi:10.1016/j.tree.2013.09.008

Feau, N., Taylor, G., Dale, A.L., Dhillon, B., Bilodeau, G.J., Birol, I., Jones, S.J.M., Hamelin, R.C., 2016. Genome sequences of six Phytophthora species threatening forest ecosystems. Genomics Data 10, 85–88. doi:10.1016/j.gdata.2016.09.013

Felsenstein, J., 2005. PHYLIP (Phylogeny Inference Package) version 3.6. Department of Genome Sciences, University of Washington, Seattle, Washington.

Felsenstein, J., 1978. Cases in which parsimony or compatibility methods will be positively misleading. Syst. Zool. 401–410.

Galtier, N., Daubin, V., 2008. Dealing with incongruence in phylogenomic analyses. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 363, 4023–4029. doi:10.1098/rstb.2008.0144

Garrett, S.D., 1970. Pathogenic root-infecting fungi. Cambridge Univ Press, Cambridge.

Göker, M., Voglmayr, H., Riethmüller, A., Oberwinkler, F., 2007. How do obligate parasites evolve? A multi-gene phylogenetic analysis of downy mildews. Fungal Genet. Biol. FG B 44, 105–122. doi:10.1016/j.fgb.2006.07.005

Götesson, A., Marshall, J.S., Jones, D.A., Hardham, A.R., 2002. Characterization and evolutionary analysis of a large polygalacturonase gene family in the oomycete plant pathogen Phytophthora cinnamomi. Mol. Plant-Microbe Interact. MPMI 15, 907–921. doi:10.1094/MPMI.2002.15.9.907

Grünwald, N.J., Goss, E.M., Press, C.M., 2008. *Phytophthora ramorum* : a pathogen with a remarkably wide host range causing sudden oak death on oaks and ramorum blight on woody ornamentals. Mol. Plant Pathol. 9, 729–740. doi:10.1111/j.1364-3703.2008.00500.x

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321. doi:10.1093/sysbio/syq010

Haas, B.J., Kamoun, S., Zody, M.C., Jiang, R.H.Y., Handsaker, R.E., Cano, L.M., Grabherr, M., Kodira, C.D., Raffaele, S., Torto-Alalibo, T., Bozkurt, T.O., Ah-Fong, A.M.V., Alvarado, L., Anderson, V.L., Armstrong, M.R., Avrova, A., Baxter, L., Beynon, J., Boevink, P.C., Bollmann, S.R., Bos, J.I.B., Bulone, V., Cai, G., Cakir, C., Carrington, J.C., Chawner, M., Conti, L., Costanzo, S., Ewan, R., Fahlgren, N., Fischbach, M.A., Fugelstad, J., Gilroy, E.M., Gnerre, S., Green, P.J., Grenville-Briggs, L.J., Griffith, J., Grünwald, N.J., Horn, K., Horner, N.R., Hu, C.-H., Huitema, E., Jeong, D.-H., Jones, A.M.E., Jones, J.D.G., Jones, R.W., Karlsson, E.K., Kunjeti, S.G., Lamour, K., Liu, Z., Ma, L., Maclean, D., Chibucos, M.C., McDonald, H., McWalters, J., Meijer, H.J.G., Morgan, W., Morris, P.F., Munro, C.A., O'Neill, K., Ospina-Giraldo, M., Pinzón, A., Pritchard, L., Ramsahoye, B., Ren, Q., Restrepo, S., Roy, S., Sadanandom, A., Savidor, A., Schornack, S., Schwartz, D.C., Schumann, U.D., Schwessinger, B., Seyer, L., Sharpe, T., Silvar, C., Song, J., Studholme, D.J., Sykes, S., Thines, M., van de Vondervoort, P.J.I., Phuntumart, V., Wawra, S., Weide, R., Win, J., Young, C., Zhou, S., Fry, W., Meyers, B.C., van West, P., Ristaino, J., Govers, F., Birch, P.R.J., Whisson, S.C., Judelson, H.S., Nusbaum, C., 2009. Genome sequence and analysis of the Irish potato famine pathogen Phytophthora infestans. Nature 461, 393–398. doi:10.1038/nature08358

Hall, G.S., 1996. Modern approaches to species concepts in downy mildews. Plant Pathol. 45, 1009–1026. doi:10.1046/j.1365-3059.1996.d01-191.x

Hasegawa, M., Kishino, H., Yano, T., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22, 160–174.

Haverkort, A.J., Boonekamp, P.M., Hutten, R., Jacobsen, E., Lotz, L.A.P., Kessel, G.J.T., Visser, R.G.F., van der Vossen, E.A.G., 2008. Societal Costs of Late Blight in Potato and Prospects of Durable Resistance Through Cisgenic Modification. Potato Res. 51, 47–57. doi:10.1007/s11540-008-9089-y

Heled, J., Drummond, A.J., 2010. Bayesian Inference of Species Trees from Multilocus Data. Mol. Biol. Evol. 27, 570–580. doi:10.1093/molbev/msp274

Hosner, P.A., Faircloth, B.C., Glenn, T.C., Braun, E.L., Kimball, R.T., 2016. Avoiding Missing Data Biases in Phylogenomic Inference: An Empirical Study in the Landfowl (Aves: Galliformes). Mol. Biol. Evol. 33, 1110–1125. doi:10.1093/molbev/msv347

Hudspeth, D., Stenger, D., Hudspeth, M., 2003. A cox2 phylogenetic hypothesis for the downy mildews and white rusts. Fungal Divers. 13, 47–57.

Hussein, M.M., Hatai, K., Nomura, T., 2001. Saprolegniosis in salmonids and their eggs in Japan. J. Wildl. Dis. 37, 204–207. doi:10.7589/0090-3558-37.1.204

Jesus, A.L. de, Gonçalves, D.R., Rocha, S.C.O., Marano, A.V., Jerônimo, G.H., Souza, J.I.D., Castilho Boro, M., Pires-Zottarelli, C.L.A., 2016. Morphological and Phylogenetic Analyses of Three *Phytopythium* Species (Peronosporales, Oomycota) from Brazil. Cryptogam. Mycol. 37, 117–128. doi:10.7872/crym/v37.iss1.2016.117

Jiang, R.H.Y., de Bruijn, I., Haas, B.J., Belmonte, R., Löbach, L., Christie, J., van den Ackerveken, G., Bottin, A., Bulone, V., Díaz-Moreno, S.M., Dumas, B., Fan, L., Gaulin, E., Govers, F., Grenville-Briggs, L.J., Horner, N.R., Levin, J.Z., Mammella, M., Meijer, H.J.G., Morris, P., Nusbaum, C., Oome, S., Phillips, A.J., van Rooyen, D., Rzeszutek, E., Saraiva, M., Secombes, C.J., Seidl, M.F., Snel, B., Stassen, J.H.M., Sykes, S., Tripathy, S., van den Berg, H., Vega-Arreguin, J.C., Wawra, S., Young, S.K., Zeng, Q., Dieguez-Uribeondo, J., Russ, C., Tyler, B.M., van West, P., 2013. Distinctive expansion of potential virulence genes in the genome of the oomycete fish pathogen Saprolegnia parasitica. PLoS Genet. 9, e1003272. doi:10.1371/journal.pgen.1003272

Johnson, T., Seymour, R., Padgett, D., 2002. Biology and Systematics of the Saprolegniaceae. University of North Carolina at Wilmington, Wilmington, NC.

Judelson, H., 1996. Quantitation of *Phytophthora cinnamomi* in Avocado Roots Using a Species-Specific DNA Probe. Phytopathology 86, 763. doi:10.1094/Phyto-86-763

Keane, T.M., Creevey, C.J., Pentony, M.M., Naughton, T.J., Mclnerney, J.O., 2006. Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified. BMC Evol. Biol. 6, 29. doi:10.1186/1471-2148-6-29

Kemen, E., Gardiner, A., Schultz-Larsen, T., Kemen, A.C., Balmuth, A.L., Robert-Seilaniantz, A., Bailey, K., Holub, E., Studholme, D.J., Maclean, D., Jones, J.D.G., 2011. Gene gain and loss during evolution of obligate parasitism in the white rust pathogen of Arabidopsis thaliana. PLoS Biol. 9, e1001094. doi:10.1371/journal.pbio.1001094

Krajaejun, T., Lerksuthirat, T., Garg, G., Lowhnoo, T., Yingyong, W., Khositnithikul, R., Tangphatsornruang, S., Suriyaphol, P., Ranganathan, S., Sullivan, T.D., 2014. Transcriptome analysis reveals pathogenicity and evolutionary history of the pathogenic oomycete Pythium insidiosum. Fungal Biol. 118, 640–653. doi:10.1016/j.funbio.2014.01.009

Kubatko, L.S., Degnan, J.H., 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. Syst. Biol. 56, 17–24. doi:10.1080/10635150601146041

Lento, G.M., Hickson, R.E., Chambers, G.K., Penny, D., 1995. Use of spectral analysis to test hypotheses on the origin of pinnipeds. Mol. Biol. Evol. 12, 28–52.

Lévesque, C.A., Brouwer, H., Cano, L., Hamilton, J.P., Holt, C., Huitema, E., Raffaele, S., Robideau, G.P., Thines, M., Win, J., Zerillo, M.M., Beakes, G.W., Boore, J.L., Busam, D., Dumas, B., Ferriera, S., Fuerstenberg, S.I., Gachon, C.M.M., Gaulin, E., Govers, F., Grenville-Briggs, L., Horner, N., Hostetler, J., Jiang, R.H.Y., Johnson, J., Krajaejun, T., Lin, H., Meijer, H.J.G., Moore, B., Morris, P., Phuntmart, V., Puiu, D., Shetty, J., Stajich, J.E., Tripathy, S., Wawra, S., van West, P., Whitty, B.R., Coutinho, P.M., Henrissat, B., Martin, F., Thomas, P.D., Tyler, B.M., De Vries, R.P., Kamoun, S., Yandell, M., Tisserat, N., Buell, C.R., 2010. Genome sequence of the necrotrophic plant pathogen Pythium ultimum reveals original pathogenicity mechanisms and effector repertoire. Genome Biol. 11, R73. doi:10.1186/gb-2010-11-7-r73

Lévesque, C.A., de Cock, A.W.A.M., 2004. Molecular phylogeny and taxonomy of the genus Pythium. Mycol. Res. 108, 1363–1383.

Links, M.G., Holub, E., Jiang, R.H.Y., Sharpe, A.G., Hegedus, D., Beynon, E., Sillito, D., Clarke, W.E., Uzuhashi, S., Borhan, M.H., 2011. De novo sequence assembly of Albugo candida reveals a small genome relative to other biotrophic oomycetes. BMC Genomics 12, 503. doi:10.1186/1471-2164-12-503

Lippincott-Schwartz, J., Roberts, T.H., Hirschberg, K., 2000. Secretory protein trafficking and organelle dynamics in living cells. Annu. Rev. Cell Dev. Biol. 16, 557–589. doi:10.1146/annurev.cellbio.16.1.557

Loytynoja, A., Goldman, N., 2008. Phylogeny-Aware Gap Placement Prevents Errors in Sequence Alignment and Evolutionary Analysis. Science 320, 1632–1635. doi:10.1126/science.1158395

Marano, A.V., Jesus, A.L., De Souza, J.I., Leão, E.M., James, T.Y., Jerônimo, G.H., De Cock, A., Pires-Zottarelli, C.L.A., 2014. A new combination in Phytopythium: P. kandeliae (Oomycetes, Straminipila). J. Fungal Biol. 5, 510–522.

Martin, F., 2009. Pythium genetics. Oomycete Genet. Genomics Divers. Interact. Res. Tools Lamour K Kamoun Seds Wiley Blackwell E. U. América 213–239.

Martin, F.N., Loper, J.E., 1999. Soilborne Plant Diseases Caused by Pythium spp.: Ecology, Epidemiology, and Prospects for Biological Control. Crit. Rev. Plant Sci. 18, 111–181. doi:10.1080/07352689991309216

Maseko, B., Burgess, T.I., Coutinho, T.A., Wingfield, M.J., 2007. Two new Phytophthora species from South African Eucalyptus plantations. Mycol. Res. 111, 1321–1338. doi:10.1016/j.mycres.2007.08.011

Matari, N.H., Blair, J.E., 2014. A multilocus timescale for oomycete evolution estimated under three distinct molecular clock models. BMC Evol. Biol. 14, 101. doi:10.1186/1471-2148-14-101

McCarthy, C.G.P., Fitzpatrick, D.A., 2017. Phylogenomic Reconstruction of the Oomycete Phylogeny Derived from 37 Genomes. mSphere 2, e00095-17. doi:10.1128/mSphere.00095-17

McLeod, A., Smart, C.D., Fry, W.E., 2003. Characterization of 1,3-beta-glucanase and 1,3;1,4-beta-glucanase genes from Phytophthora infestans. Fungal Genet. Biol. FG B 38, 250–263.

Mirarab, S., Bayzid, M.S., Warnow, T., 2016. Evaluating summary methods for multilocus species tree estimation in the presence of incomplete lineage sorting. Syst. Biol. 65, 366–380.

Mirarab, S., Warnow, T., 2015. ASTRAL-II: coalescent-based species tree estimation with many hundreds of taxa and thousands of genes. Bioinforma. Oxf. Engl. 31, i44-52. doi:10.1093/bioinformatics/btv234

Nurk, S., Bankevich, A., Antipov, D., Gurevich, A., Korobeynikov, A., Lapidus, A., Prjibelsky, A., Pyshkin, A., Sirotkin, A., Sirotkin, Y., Stepanauskas, R., McLean, J., Lasken, R., Clingenpeel, S., Woyke, T., Tesler, G., Alekseyev, M., Pevzner, P., 2013. Assembling Genomes and Mini-metagenomes from Highly Chimeric Reads, in: Deng, M., Jiang, R., Sun, F., Zhang, X. (Eds.), Research in Computational Molecular Biology, Lecture Notes in Computer Science. Springer Berlin Heidelberg, pp. 158–170.

Packer, A., Clay, K., 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. Nature 404, 278–281. doi:10.1038/35005072

Pel, M.J.C., Wintermans, P.C.A., Cabral, A., Robroek, B.J.M., Seidl, M.F., Bautor, J., Parker, J.E., Van den Ackerveken, G., Pieterse, C.M.J., 2014. Functional Analysis of Hyaloperonospora arabidopsidis RXLR Effectors. PLoS ONE 9, e110624. doi:10.1371/journal.pone.0110624

Pelizza, S.A., Cabello, M.N., Tranchida, M.C., Scorsetti, A.C., Bisaro, V., 2011. Screening for a culture medium yielding optimal colony growth, zoospore yield and infectivity of different isolates of Leptolegnia chapmanii (Straminipila: Peronosporomycetes). Ann. Microbiol. 61, 991–997.

Ploch, S., Thines, M., 2011. Obligate biotrophic pathogens of the genus Albugo are widespread as asymptomatic endophytes in natural populations of Brassicaceae. Mol. Ecol. 20, 3692–3699. doi:10.1111/j.1365-294X.2011.05188.x

Poimala, A., Lilja, A., 2013. NOBANIS – Invasive Alien Species Fact Sheet – Phytophthora ramorum. –. European Network on Invasive Alien Species, Online Database of the European Network on Invasive Alien Species – NOBANIS.

Richards, T.A., Soanes, D.M., Jones, M.D.M., Vasieva, O., Leonard, G., Paszkiewicz, K., Foster, P.G., Hall, N., Talbot, N.J., 2011. Horizontal gene transfer facilitated the evolution of plant parasitic mechanisms in the oomycetes. Proc. Natl. Acad. Sci. U. S. A. 108, 15258–15263. doi:10.1073/pnas.1105100108

Riethmüller, A., Voglmayr, H., Göker, M., Weiß, M., Oberwinkler, F., 2002. Phylogenetic relationships of the downy mildews (Peronosporales) and related groups based on nuclear large subunit ribosomal DNA sequences. Mycologia 94, 834–849.

Robideau, G.P., Rodrigue, N., André Lévesque, C., 2014. Codon-based phylogenetics introduces novel flagellar gene markers to oomycete systematics. Mol. Phylogenet. Evol. 79, 279–291. doi:10.1016/j.ympev.2014.04.009

Roshan, U., Livesay, D.R., 2006. Probalign: multiple sequence alignment using partition function posterior probabilities. Bioinforma. Oxf. Engl. 22, 2715–2721. doi:10.1093/bioinformatics/btl472

Runge, F., Telle, S., Ploch, S., Savory, E., Day, B., Sharma, R., Thines, M., 2011. The inclusion of downy mildews in a multi-locus-dataset and its reanalysis reveals a high degree of paraphyly in Phytophthora. IMA Fungus 2, 163–171. doi:10.5598/imafungus.2011.02.02.07

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., Allard, R.W., 1984. Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. U. S. A. 81, 8014–8018.

Salichos, L., Rokas, A., 2013. Inferring ancient divergences requires genes with strong phylogenetic signals. Nature 497, 327–331. doi:10.1038/nature12130

Seidl, M.F., Van den Ackerveken, G., Govers, F., Snel, B., 2012. Reconstruction of oomycete genome evolution identifies differences in evolutionary trajectories leading to present-day large gene families. Genome Biol. Evol. 4, 199–211. doi:10.1093/gbe/evs003

Sharma, R., Xia, X., Cano, L.M., Evangelisti, E., Kemen, E., Judelson, H., Oome, S., Sambles, C., van den Hoogen, D.J., Kitner, M., Klein, J., Meijer, H.J.G., Spring, O., Win, J., Zipper, R., Bode, H.B., Govers, F., Kamoun, S., Schornack, S., Studholme, D.J., Van den Ackerveken, G., Thines, M., 2015. Genome analyses of the sunflower pathogen Plasmopara halstedii provide insights into effector evolution in downy mildews and Phytophthora. BMC Genomics 16. doi:10.1186/s12864-015-1904-7

Smith, S.A., Moore, M.J., Brown, J.W., Yang, Y., 2015. Analysis of phylogenomic datasets reveals conflict, concordance, and gene duplications with examples from animals and plants. BMC Evol. Biol. 15. doi:10.1186/s12862-015-0423-0

Spencer, D.M. (Ed.), 1981. The Downy mildews. Academic Press, London ; New York.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinforma. Oxf. Engl. 22, 2688–2690. doi:10.1093/bioinformatics/btl446

Stanke, M., Diekhans, M., Baertsch, R., Haussler, D., 2008. Using native and syntenically mapped cDNA alignments to improve de novo gene finding. Bioinforma. Oxf. Engl. 24, 637–644. doi:10.1093/bioinformatics/btn013

Sukumaran, J., Holder, M.T., 2010. DendroPy: a Python library for phylogenetic computing. Bioinforma. Oxf. Engl. 26, 1569–1571. doi:10.1093/bioinformatics/btq228

Thines, M., 2014. Phylogeny and evolution of plant pathogenic oomycetes—a global overview. Eur. J. Plant Pathol. 138, 431–447.

Thines, M., Voglmayr, H., 2009. An Introduction to the White Blister Rusts (Albuginales), in: Lamour, K., Kamoun, S. (Eds.), Oomycete Genetics and Genomics. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 77–92.

Thines, M., Voglmayr, H., Gker, M., 2009. Taxonomy and Phylogeny of the Downy Mildews (Peronosporaceae), in: Lamour, K., Kamoun, S. (Eds.), Oomycete Genetics and Genomics. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 47–75.

Tonini, J., Moore, A., Stern, D., Shcheglovitova, M., Ortí, G., 2015. Concatenation and Species Tree Methods Exhibit Statistically Indistinguishable Accuracy under a Range of Simulated Conditions. PLoS Curr. 7. doi:10.1371/currents.tol.34260cc27551a527b124ec5f6334b6be

Torto, T.A., Rauser, L., Kamoun, S., 2002. The pipg1 gene of the oomycete Phytophthora infestans encodes a fungal-like endopolygalacturonase. Curr. Genet. 40, 385–390. doi:10.1007/s00294-002-0272-4

Turner, J., O'Neill, P., Grant, M., Mumford, R.A., Thwaites, R., Studholme, D.J., 2017. Genome sequences of 12 isolates of the EU1 lineage of Phytophthora ramorum, a fungus-like pathogen that causes extensive damage and mortality to a wide range of trees and other plants. Genomics Data 12, 17–21. doi:10.1016/j.gdata.2017.02.006

Tyler, B.M., Tripathy, S., Zhang, X., Dehal, P., Jiang, R.H.Y., Aerts, A., Arredondo, F.D., Baxter, L., Bensasson, D., Beynon, J.L., Chapman, J., Damasceno, C.M.B., Dorrance, A.E., Dou, D., Dickerman, A.W., Dubchak, I.L., Garbelotto, M., Gijzen, M., Gordon, S.G., Govers, F., Grunwald, N.J., Huang, W., Ivors, K.L., Jones, R.W., Kamoun, S., Krampis, K., Lamour, K.H., Lee, M.-K., McDonald, W.H., Medina, M., Meijer, H.J.G., Nordberg, E.K., Maclean, D.J., Ospina-Giraldo, M.D., Morris, P.F., Phuntumart, V., Putnam, N.H., Rash, S., Rose, J.K.C., Sakihama, Y., Salamov, A.A., Savidor, A., Scheuring, C.F., Smith, B.M., Sobral, B.W.S., Terry, A., Torto-Alalibo, T.A., Win, J., Xu, Z., Zhang, H., Grigoriev, I.V., Rokhsar, D.S., Boore, J.L., 2006. Phytophthora genome sequences uncover evolutionary origins and mechanisms of pathogenesis. Science 313, 1261–1266. doi:10.1126/science.1128796

Uzuhashi, S., Kakishima, M., Tojo, M., 2010. Phylogeny of the genus Pythium and description of new genera. Mycoscience 51, 337–365. doi:10.1007/S10267-010-0046-7

Van der Plaats-Niterink, A.J., 1981. Monograph of the genus Pythium. Stud. Mycol. 21, 1–242.

Villa, N.O., Kageyama, K., Asano, T., Suga, H., 2006. Phylogenetic relationships of Pythium and Phytophthora species based on ITS rDNA, cytochrome oxidase II and beta-tubulin gene sequences. Mycologia 98, 410–422.

Voglmayr, H., 2003. Phylogenetic relationships of Peronospora and related genera based on nuclear ribosomal ITS sequences. Mycol. Res. 107, 1132–1142.

Waterhouse, G., 1968. The genus Pythium. Diagnoses (or descriptions) and figures from the original papers. Mycol. Pap. 1–50.

Woodham-Smith, C., 1991. The Great Hunger: Ireland 1845-1849. Penguin.

Table 1. Taxa publicly available at the time of analysis and thus included in the present study, with life style, host/substrate and sequence sources.

Species name	Life style	Host/ Substrate	Accession number	Reference
Pythium insidiosum	Necrotroph	Animal	JRHR00000000	Ascunce et al. (2016)
Pythium aphanidermatum	Necrotroph	Plant	AKXX00000000	Adhikari et al. (2013)
Pythium arrhenomanes	Necrotroph	Plant	AKXY00000000	Adhikari et al. (2013)
Pythium irregulare	Necrotroph	Plant	AKXZ00000000	Adhikari et al. (2013)
Pythium iwayamai	Necrotroph	Plant	AKYA00000000	Adhikari et al. (2013)
Pythium ultimum var. sporangiiferum	Necrotroph	Plant	AKYB00000000	Adhikari et al. (2013)
Pythium ultimum var. ultimum	Necrotroph	Plant	ADOS00000000	Lévesque et al. (2010)
Pythium vexans (now Phytopythium vexans)	Necrotroph	Plant	AKYC00000000	Adhikari et al. (2013)
Phytophthora infestans	Hemibiotroph	Plant	NZ_AATU00000000.1	Haas et al. (2009)
Phytophthora parasitica	Hemibiotroph	Plant	AGFV00000000.2	(*Ph. parasitica* INRA-310 Sequencing Project, 2011)
Phytophthora ramorum	Hemibiotroph	Plant	AAQX00000000	Tyler et al. (2006)
Phytophthora sojae	Hemibiotroph	Plant	AAQY00000000	Tyler et al. (2006)
Hyaloperonospora arabidopsidis	Obligate biotroph	Plant	ABWE00000000.2	Baxter et al. (2010)
Albugo laibachii	Obligate biotroph	Plant	ERP000440	Kemen et al. (2011)
Saprolegnia parasitica	Necrotroph - facultative biotroph	Animal	ADCG00000000	Jiang et al. (2013)
Saprolegnia diclina	Necrotroph	Animal	NZ_AIJL00000000.1	(*Saprolegnia* genome Sequencing Project, 2009)
Aphanomyces astaci	Necrotroph	Animal	AYTG00000000.1	(*Aphanomyces* WGS initiative, 2013)

Table 2. Order of the genomes as they are listed in the bipartitions for Fig. 1 and Table S3, and in Supplementary materials.

Order	Taxa
1	*Pythium arrhenomanes*
2	*Pythium aphanidermatum*
3	*Phytophthora sojae*
4	*Phytophthora ramorum*
5	*Phytophthora infestans*
6	*Phytophthora parasitica*
7	*Pythium insidiosum*
8	*Pythium vexans (now Phytopythium vexans)*
9	*Saprolegnia parasitica*
10	*Pythium iwayamai*
11	*Pythium irregulare*
12	*Pythium ultimum var. sporangiiferum*
13	*Pythium ultimum var. ultimum*
14	*Saprolegnia diclina*
15	*Aphanomyces astaci*
16	*Hyaloperonospora arabidopsidis*
17	*Albugo laibachii*

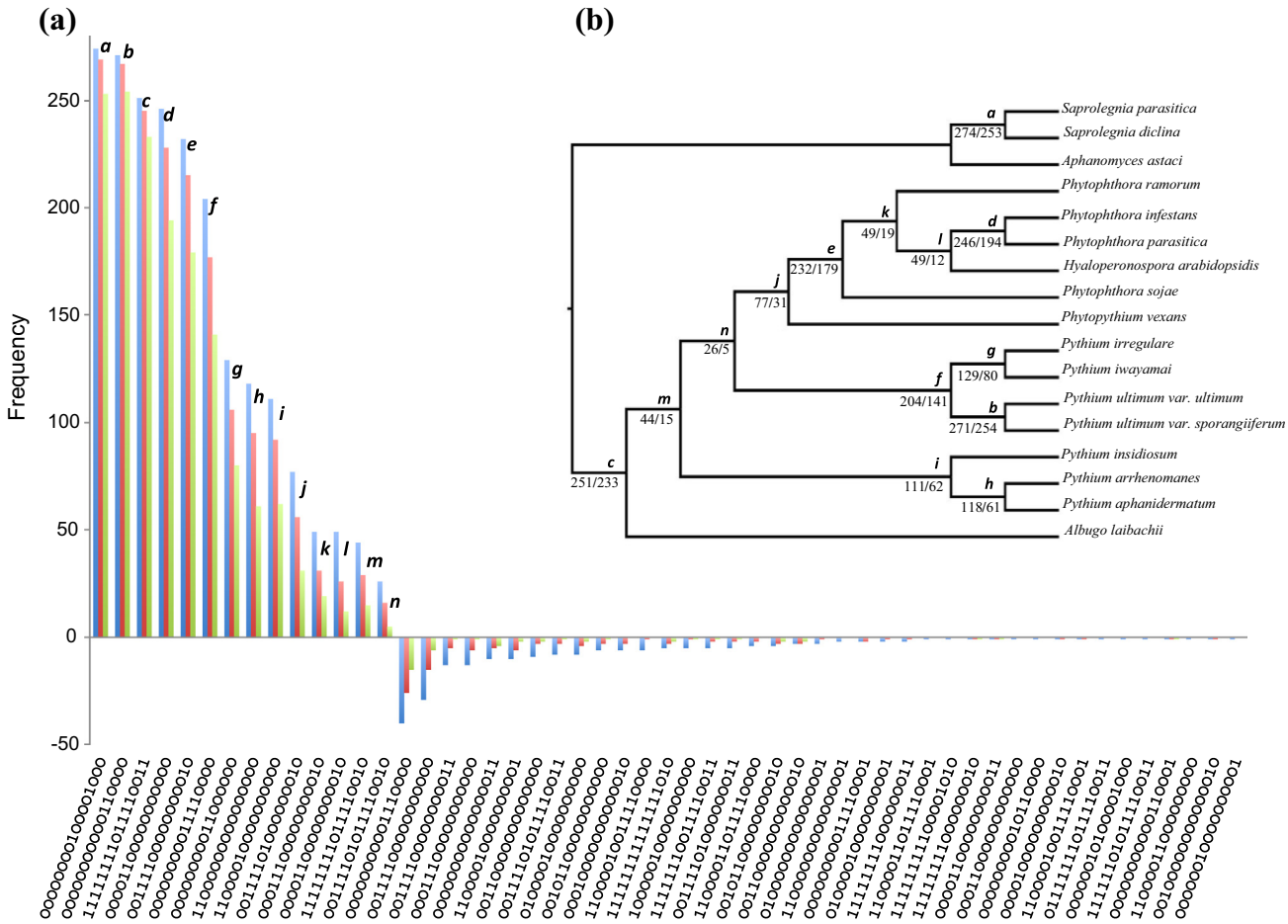

Fig. 1. Spectral analysis for COO Dataset 1: (a) Lento plot displaying the frequency of support of the OGs for each of 53 splits that were supported by at least one gene family with more than 70% bootstrap support. Splits are ordered from left to right according to the number of supporting families at the 70% bootstrap support level. For each bipartition, the bar above the x-axis gives the number of gene families that support the bipartition with the indicated (color coded) bootstrap support value, the bars in the negative direction give the number of supported bipartitions found in all gene families that are in conflict with the bipartitions found in the consensus tree. The bipartitions are ranked in order of the number of supporting OGs at the 70% bootstrap support level color coded blue, 80% color coded red, and 90% color coded green. The 14 bipartitions that support the splits found in the consensus tree are labeled with letters a to n. Under the x-axis bipartitions are described using 1 and O to indicate the taxa involved in the split (1) or absent (O) following the order of taxa as given in Table 2. (b) Majority-rule consensus tree showing bipartitions a to n. Letters at the nodes correspond to the letters in the lento-plot. Below branches are the numbers of OGs that support each bipartition out of 277, based on bootstrap support of 70% (left) and 90% (right), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

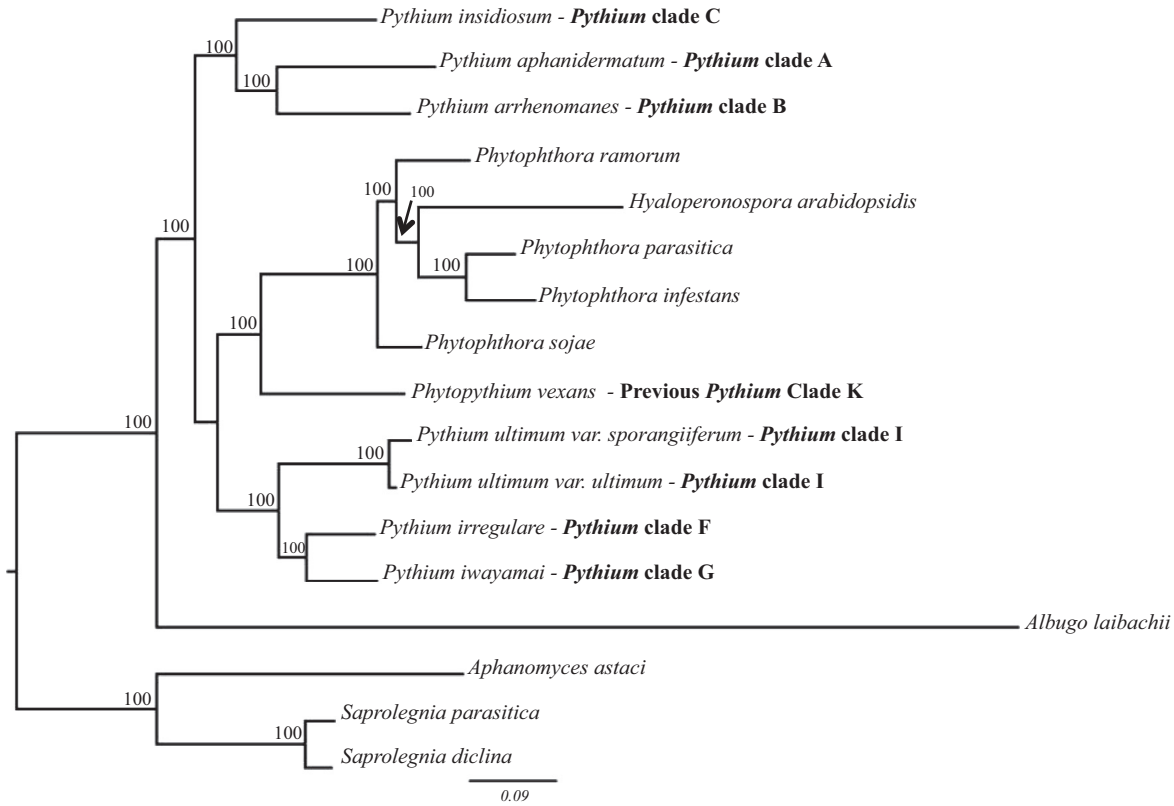

Fig. 2. Maximum likelihood phylogeny reconstructed using a concatenated ProbAlign alignment of 277 oomycete genes (COO Dataset 1). The concatenated alignment contained 17 taxa and a total length of 249,830 bases. Numbers above branches represent bootstrap support values. Trees were rooted using *Saprolegnia parasitica*, *Saprolegnia diclina*, and *Aphanomyces astaci* as outgroups. The scale bar is in units of substitutions per site.

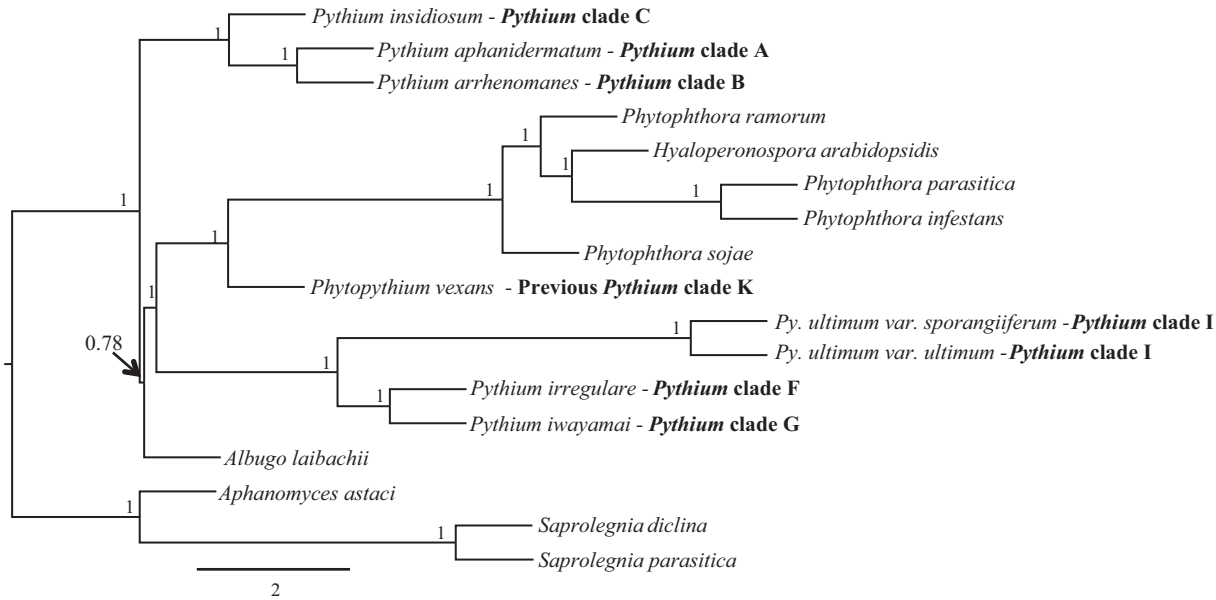

Fig. 3. ASTRAL-II species tree estimated for 17 oomycetes using 277 loci. Input trees were generated using ProbAlign alignment, best fit model of evolution and SPR tree searching. Numbers below branches are the local Bayesian posterior probabilities obtained through the exact tree estimation. Trees were rooted using Saprolegnia parasitica, Saprolegnia diclina, and Aphanomyces astaci as outgroups. The scale bar represents two coalescent units; terminal branch lengths are arbitrary.