Genome analysis of the ubiquitous boxwood pathogen *Pseudonectria foliicola*

Yazmín Rivera¹,²,⁴, Catalina Salgado-Salazar¹,²,³, Daniel Veltri¹,³,⁵, Martha Malapi-Wight¹,⁶ and Jo Anne Crouch¹

¹ Mycology and Nematology Genetic Diversity and Biology Laboratory, US Department of Agriculture, Agriculture Research Service (USDA-ARS), Beltsville, MD, United States of America
² Department of Plant Biology and Pathology, Rutgers, The State University of New Jersey, New Brunswick, NJ, United States of America
³ ARS Research Participation Program, Oak Ridge Institute for Science and Education, Oak Ridge, TN, United States of America
⁴ Current affiliation: Center for Plant Health, Science and Technology, USDA, Animal and Plant Health Inspection Service, Beltsville, MD, United States of America
⁵ Current affiliation: Bioinformatics and Computational Biosciences Branch, Office of Cyber Infrastructure and Computational Biology, National Institute of Allergy and Infectious Diseases, NIH, Rockville, MD, United States of America
⁶ Current affiliation: Plant Germplasm Quarantine Program, USDA, Animal and Plant Health Inspection Service, Beltsville, MD, United States of America

**ABSTRACT**

Boxwood (*Buxus* spp.) are broad-leaved, evergreen landscape plants valued for their longevity and ornamental qualities. Volutella leaf and stem blight, caused by the ascomycete fungi *Pseudonectria foliicola* and *P. buxi*, is one of the major diseases affecting the health and ornamental qualities of boxwood. Although this disease is less severe than boxwood blight caused by *Calonectria pseudonaviculata* and *C. henricotiae*, its widespread occurrence and disfiguring symptoms have caused substantial economic losses to the ornamental industry. In this study, we sequenced the genome of *P. foliicola* isolate ATCC13545 using Illumina technology and compared it to other publicly available fungal pathogen genomes to better understand the biology of this organism. A *de novo* assembly estimated the genome size of *P. foliicola* at 28.7 Mb (425 contigs; N50 = 184,987 bp; avg. coverage 188×), with just 9,272 protein-coding genes. To our knowledge, *P. foliicola* has the smallest known genome within the Nectriaceae. Consistent with the small size of the genome, the secretome, CAzyme and secondary metabolite profiles of this fungus are reduced relative to two other surveyed Nectriaceae fungal genomes: *Dactylonectria macrodidyma* JAC15-245 and *Fusarium graminearum* Ph-1. Interestingly, a large cohort of genes associated with reduced virulence and loss of pathogenicity was identified from the *P. foliicola* dataset. These data are consistent with the latest observations by plant pathologists that *P. buxi* and most likely *P. foliicola*, are opportunistic, latent pathogens that prey upon weak and stressed boxwood plants.

Subjects Genomics, Mycology
Keywords Volutella blight, Boxwood, Comparative genomics, Nectriaceae

Submitted 11 December 2017
Accepted 18 July 2018
Published 24 August 2018
How to cite this article Rivera et al. (2018), Genome analysis of the ubiquitous boxwood pathogen *Pseudonectria foliicola*. PeerJ 6:e5401; DOI 10.7717/peerj.5401
INTRODUCTION

Ascomycete fungi inhabit almost all known ecosystems, and play important roles as plant and insect pathogens, endophytes, mycoparasites, and saprobes (Arnold et al., 2009). Within the Ascomycota, the Nectriaceae family includes 55 genera with approximately 900 species (http://www.indexfungorum.org). Although best known as soil-borne saprobes or weak plant pathogens, several species in this family are responsible for extensive economic losses due to damage incurred to crops or in natural ecosystems (Halleen, Fourie & Crous, 2006; Malapi-Wight et al., 2016a; Windels, 2000). The systematics and taxonomy of the Nectriaceae family has been extensively studied (e.g., Salgado-Salazar et al., 2014; Lombard et al., 2015) however, outside of the genus Fusarium, only a small number of fungal species in this family have genome resources publicly available, including Calonectria pseudonaviculata, C. pseudoteaudii, Dactylonectria macrodidyma and Ilyonectria destructans (http://genome.jgi.doe.gov/Ilysp1/Ilysp1.home.html; Malapi-Wight et al., 2015; Malapi-Wight et al., 2016b; Ye et al., 2017). Whole genome resources are now commonly used to understand evolutionary characteristics of pathogenicity across fungi with different lifestyles (Lo Presti et al., 2015) and could become useful for the characterization and biosecurity analysis of undescribed pathogens (McTaggart et al., 2016).

Pseudonectria foliicola and P. buxi (the latter formerly known as Volutella buxi or P. rousseliana) are nectriaceous species causing a ubiquitous leaf and stem blight disease on boxwood (Buxus spp.), known as volutella blight (Fig. 1). To date, this disease has been reported worldwide, throughout the US, Armenia, Belgium, Bulgaria Canada, China, Greece, Portugal, Spain, UK and Ukraine, among others (Farr & Rossman, 2018), although its distribution may extend further along with the distribution of boxwood plants. Infected plants may lack any disease symptoms, or they may manifest visually discernable physiological changes such as leaf discoloration, stem dieback and extensive pink fungal sporulation on the surface of leaves and twigs (Shi & Hsiang, 2014). The causal agent of volutella blight has been described as the species P. buxi (and synonyms) since the early nineteenth century. However, on the basis of morphological and molecular distinctiveness, Lombard et al. (2015) recently described P. foliicola as a second species of Pseudonectria that infects boxwood in New Zealand and the US. It is currently unclear to what extent previous sightings of volutella blight prior to the discovery of P. foliicola were actually caused by P. buxi, P. foliicola, or both of these pathogens.

The pathogens responsible for volutella blight disease have long been considered saprophytes or secondary invaders, however, recent studies by Shi & Hsiang (2014) identified P. buxi causing primary infection on wounded tissue contributing to boxwood decline. Reports from China and Italy confirm the impact of P. buxi as a primary pathogen of Buxus spp. (Shi & Hsiang, 2014; Garibaldi et al., 2016). Unlike boxwood blight disease caused by C. pseudonaviculata and C. henricotiae (Gehesquière et al., 2016), volutella blight primarily affects the ornamental value of boxwood, as plants are typically not killed by the fungal infection. Nonetheless, financial losses due to volutella blight may be considerable. For example, in 2008, economic losses in a single nursery in southern Ontario due to
volutella blight of boxwood exceeded $60,000 (Shi & Hsiang, 2014), and similar economic burdens could be expected across the ornamental industry in other regions.

Despite being one of the most commonly observed diseases affecting boxwood, little is known about the genetics, biology and etiology of the causal agents of volutella blight. In this study, we report the first draft genome sequence assembly and annotation of *P. foliicola* and compare the genome characteristics against two other plant pathogenic fungi in the Nectriaceae, *Fusarium graminearum* and *Dactylonectria macrodidyma*. *Fusarium graminearum* is the hemibiotrophic pathogen that causes head blight on wheat and barley, responsible for substantial economic losses in these industries (Goswami & Kistler, 2004). *Dactylonectria macrodidyma* is a destructive necrotrophic pathogen that causes the black foot rot of grapevine and root rots of avocado and olive trees (Urbez-Torres, Peduto & Gubler, 2012; Vitale et al., 2012). Our goal in this study was to compare the genome sequence of *P. foliicola* to these organisms to reveal genome-wide characteristics that may help us better understand the lifestyle of this important fungal pathogen.

**MATERIALS AND METHODS**

**Fungal isolate and nucleic acid isolation**

An axenic culture of *P. foliicola* isolate ATCC13545® (also known as isolate A.R. 2711) was used for genome sequencing. This fungal isolate was originally cultured from...
B. sempervirens in Maryland, US. The isolate was grown on potato dextrose agar (BD Difco™, Sparks, MD, USA) for 5-days under 12-h white light photoperiod and then transferred to yeast extract potato dextrose liquid media at 25 °C for 2-days under continuous light. Genomic DNA was extracted from hyphal tissue harvested from liquid media using the OmniPrep DNA kit (G-Biosciences, St. Louis, MO, USA) according to manufacturer’s instructions, and subsequently purified using the Zymo Genomic DNA Clean and Concentrator kit (Zymo Research, Irvine, CA, USA).

**Whole genome sequencing and de novo assembly**

A genomic DNA library was constructed using the TruSeq Nano DNA Library Prep kit (Illumina, Inc., San Diego, CA, USA) and quantified using the Qubit 2.0 fluorometer (Life Technologies, Grand Island, NY) and the LabChipXT DNA 750 (Caliper Life Sciences, Hopkinton, MA, USA). The library was sequenced on an Illumina MiSeq in two independent runs using paired-end 600-cycle reagent cartridge v.3 (Illumina, Inc.). Reads were processed and assembled using CLC Genomics Workbench v.7.5.1 (CLC Bio, Boston, MA, USA) with a k-mer of 24 and a minimum contig length of 500 bp. Illumina adapters were trimmed and low quality reads (Phred score < 0.05) were removed. Summary statistics for the draft genome were generated using CLC Genomics Workbench and QUAST (Gurevich et al., 2013).

Completeness of the P. foliicola draft genome assembly was evaluated using BUSCO v.1.1b1 (Simão et al., 2015). The genome assembly of P. foliicola used in this study was deposited in NCBI GenBank under accession LMTV00000000, and datasets are also available at the US National Agricultural Library on AgData Commons (http://dx.doi.org/10.15482/USDA.ADC/1408094).

**Nuclear genome annotation**

*Ab initio* gene predictions for the draft genome assembly of P. foliicola were performed using the MAKER2 v.2.31.6 annotation pipeline (Holt & Yandell, 2011). Gene training was performed after running three rounds of the program according to the program documentation. Gene boundaries were assigned using protein homology evidence from Fusarium graminearum strain PH-1 (NCBI BioProject Acc: PRJNA13839; Cuomo et al., 2007). Additional *ab initio* gene predictions were made using the program SNAP (http://korflab.ucdavis.edu/software.html) and AUGUSTUS v.3.2.1 (Stanke et al., 2004) with F. graminearum set as the prediction species model organism. New gene predictions using MAKER2 were also performed for the previously published genome assembly of D. macrodidyma (Malapi-Wight et al., 2015) using the same parameters as above.

**Identification of transposable elements and repeat-induced mutations**

The presence of transposable elements (TEs) was evaluated from the P. foliicola and D. macrodidyma genomes using the REPET v2.5 (Flutre et al., 2011) pipeline, along with supporting databases Repbase v.20.05 (Kapitonov & Jurka, 2008; Bao, Kojima & Kohany, 2015) and Pfam v.27.0, and run according to the accompanying program documentation (https://urgi.versailles.inra.fr/Tools/REPET). The TEDeno stage was used first to produce a database of four *de novo* identified TEs: an incomplete helitron, a MITE (miniature
inverted-repeat TE), a TRIM (terminal-repeat retrotransposon in miniature), and one uncategorized TE sequence. These sequences were then used as initial input for the first of two runs of the TEannot pipeline stage, which produced a final genome-wide GFF annotation file using the TE classification scheme described by Wicker et al. (2007). A custom script was used to tabulate counts of TE classes, orders and superfamilies and filtered out fragments less than 80-bp in length according to Wicker et al. (2007) recommendations to avoid misclassification. Results from TEannot based on tblastx and blastx (BLAST+ vr. 2.2.31; Camacho et al., 2009) searches against Repbase were also filtered to remove hits sharing <70% sequence identity.

Individual TE families with ten or more sequences identified, including at least one sequence ≥ 300-bp in length, were checked for signatures of repeat-induced point (RIP) mutation activity using the RIPCAL vr. 2 program (Hane & Oliver, 2008; Hane, 2015). The 300-bp length cutoff was selected based on RIPCAL’s default setting for scanning subsequences and the program was run using both the alignment mode consensus model with ClustalW (Larkin et al., 2007) and di-nucleotide frequency-based methods. Evidence of the signature of RIP mutations was present if di-nucleotide frequencies matched the indexes: (TpA / ApT) ≥ 0.89 and (CpA + TpG) / (ApC + GpT) ≤ 1.03 (Margolin et al., 1998) and visual inspection of RIPCAL alignments showed one or more peaks for (CA ← TA) + (TG ← → TA) mutations (Hane & Oliver, 2008).

Mitochondrial genome
The P. foliicola and D. macrodidyma mitogenomes were identified by performing tBLASTx searches of the complete genome assemblies using the 95.7 kb F. graminearum mitochondrial genome as a query (Al-Reedy et al., 2012), with the genetic code set to four (mold mitochondrial). Mitogenomes were annotated using MITOS (Bernt, Donath & Jühling, 2013). Comparative analysis of genes, rRNA and tRNA was performed in the program SimpleSynteny (Veltri, Malapi-Wight & Crouch, 2016) using the P. foliicola CDS file to annotate all genomes (e-value cutoff 1e-5, minimum query cutoff 10%), with circular genome mode implementation and genomes organized to minimize Euclidean line distance.

Identification of mating type idiomorphs
We assessed the presence of the MAT1-1 and MAT1-2 idiomorphs in the P. foliicola genome by performing a local BLASTn search (e-value cutoff 1e-5) against a MAT gene database. The database contained nucleotide sequences for the highly conserved alpha domain DNA binding motif (MAT1-1-1) and the high mobility group (HMG) box DNA binding motif (MAT1-2-1) for 13 different filamentous fungal species, retrieved from the NCBI GenBank database (Files S1 and S2).

Phylogenetic reconstruction
A phylogenetic analysis was used to illustrate the relationship between P. foliicola and 14 other fungal species. For this analysis, the predicted proteomes of Aspergillus nidulans FGSC A4 (ASM114v1; Galagan et al., 2005), Botrytis cinerea BcDW1 (Assembly GCA000349525; Blanco-Ulate et al., 2013), F. graminearum PH-1 (GCA000240135; Cuomo et al., 2007),
Macrophomina phaseolina MS6 (GCA000302655; Islam et al., 2012), Magnaporthe oryzae 70-15 (MG8; Kim et al., 2010), Neurospora crassa (GCA000786625; Baker et al., 2015), Penicillium oxalicum 114-2 (GCA000346795; Liu et al., 2013), Pyrenophora tritici-repentis (GCA000149985; Manning et al., 2013), Sclerotinia sclerotiorum 1980 UF-70 (ASM1469v1; Amselem et al., 2011), Trichoderma reesei RUT C-30 (GCA000513815; Koike et al., 2013), Ustilago maydis 521 (UM1; Kämper et al., 2006), Verticillium dahliae JR2 (GCA000400815; De Jonge et al., 2012) and Yarrowia lipolytica CLIB122 (GCA000002525; Dujon et al., 2004) were downloaded from either the EnsemblFungi database (https://fungi.ensembl.org/index.html) or the NCBI Genbank Genome database (https://www.ncbi.nlm.nih.gov/genbank/). The predicted proteome of D. macrodidyma generated in our study was also included in the analysis. All proteomes were searched against each other using BLASTp (e-value 0.001) and clustered in orthologous gene sets using OrthoMCL v1.4 in the CYVERSE Discovery Environment (https://de.cyverse.org/de/). Single copy genes (clusters with exactly one member per species) found in all 15 fungal proteomes were extracted from the orthologous dataset and the amino acid alignments were performed using MUSCLE v3.8.31 (Edgar, 2004). Gblocks v.0.91b was used to remove ambiguously aligned regions using relaxed selection parameters following Talavera & Castresana (2007). Maximum likelihood (ML) phylogenetic analyses were performed in RAxML (Stamatakis, 2006), using the RAxML GUI v. 1.5b1 (Silvestro & Michalak, 2012). Phylogenetic trees were constructed using the JTT matrix-based model (Jones, Taylor & Thornton, 1992) and 1,000 bootstrap replicates. Ustilago maydis 521 was used as outgroup in the phylogenetic analyses.

Estimation of evolutionary divergence times
To obtain approximate information on divergence events, the phylogenetic tree was timed using RelTime (Tamura et al., 2012) as implemented in MEGA 7 (Kumar, Stecher & Tamura, 2016). RelTime estimated the relative divergence times for each node of the ML tree using the same outgroup taxa. We used seven confidence intervals obtained from the TreeTime database (http://timetree.org; Hedges et al., 2015) as minimum and maximum times to convert the relative times into absolute times (Table S4). Time estimates were performed using maximum-likelihood branch length, local clocks, a JTT matrix-based model (Jones, Taylor & Thornton, 1992) and a discrete Gamma distribution among sites (five categories).

Comparative genomic analyses
The genome sequences of D. macrodidyma JAC15-245 (NCBI GenBank accession JYGD00000000) and F. graminearum PH-1 were downloaded from NCBI GenBank and Ensembl-Fungi database respectively, and used for comparative analysis against the P. foliicola draft genome assembly generated in this study. The predicted proteome generated from this study was used for D. macrodidyma, while the published proteome dataset for F. graminearum was downloaded from the Ensembl-Fungi database. Genome-wide identification, comparison and visualization of orthologous gene clusters among P. foliicola, D. macrodidyma and F. graminearum were performed using the web platform OrthoVenn (http://www.bioinfogenome.net/OrthoVenn; Wang et al., 2015). The program
Table 1  Genome assembly and annotation statistics for *Pseudonectria foliicola*, *Dactylonectria macrodidyma*, and *Fusarium graminearum*. Values are given in base pairs, unless otherwise specified.

| Genome features     | *Pseudonectria foliicola* | *Fusarium graminearum* | *Dactylonectria macrodidyma* |
|---------------------|---------------------------|------------------------|------------------------------|
| Genome size (Mb)    | 28.7                      | 38.1                   | 58.0                         |
| Average sequence coverage | 188                     | 10                     | 46                           |
| Total number of scaffolds | 425                     | 5                      | 850                          |
| GC content (%)      | 54.3                      | 48.2                   | 49.9                         |
| Transposable elements (%) | 0.7                     | 0.06                   | 6.5                          |
| Predicted proteins  | 9,272                     | 14,164                 | 16,454                       |
| NCBI accession      | LMTV00000000              | AACM00000000           | JYGD00000000                 |

uses a modified OrthoMCL algorithm to identify orthologous gene clusters from the UniProt/Swiss-Prot database. Proteins potentially involved in carbohydrate metabolism were annotated for each proteome by searching against the database of automated Carbohydrate-active enzyme Annotation (dbCAN; [http://csbl.bmb.uga.edu/dbCAN/annotate.php; Yin et al., 2012](http://csbl.bmb.uga.edu/dbCAN/annotate.php)). A $X^2$ test of independence, similar to that used in Martinez et al. (2008), was used to identify statistically significant differences across the CAZyme repertoire. Identification of putative enzymes related to the biosynthesis of secondary metabolites was performed from the predicted proteins using the web-based program AntiSMASH ([http://antismash.secondarymetabolites.org; Medema et al., 2011; Weber et al., 2015](http://antismash.secondarymetabolites.org)). The secretome was predicted by screening the predicted proteins for different features using a bundle of eight different prediction tools implemented in the web-based program SECRETOOL (Cortázar et al., 2014). Pathogenicity associated genes were identified by performing a local BLASTp search of the predicted proteome against the curated pathogen-host interaction database (PHI-base v.4.0; [http://www-phi4.phibase.org; Winnenburg et al., 2008](http://www-phi4.phibase.org)).

**RESULTS**

**Nuclear genome assembly and annotation**

A *de novo* genome assembly of *P. foliicola* ATCC13545® was generated from 19.5 million paired end, 300-bp reads, comprising a total of 5.4 Gb of raw sequence data. The resulting genome assembly for *P. foliicola* was 28.7 Mb, organized in 425 scaffolds (≥500-bp), with an average read depth coverage of 188-fold (Table 1). The N50 scaffold length is 184,987 bp and the three longest scaffolds are 619,696 bp, 551,775 bp, and 524,658 bp. Analysis of the *P. foliicola* genome assembly using BUSCO identified 420 complete genes out of 429 conserved eukaryotic ortholog dataset and 1,416 complete genes out of 1,438 conserved fungal ortholog dataset (genome completeness scores of 97% and 98%, respectively). *Ab initio* gene predictions performed with the MAKER2 pipeline for the *P. foliicola* genome assembly identified 9,272 protein-coding genes with an average predicted protein length of 484 amino acids (Table 1). Meanwhile, gene predictions for the genome of *D. macrodidyma* identified 16,959 protein-coding genes with an average length of 478 amino acids. Of the three genomes evaluated in this study, gene number predictions
were highest for *D. macrodidyma*, which also had the largest estimated genome size at 58 Mb when compared to *P. foliicola* and *F. graminearum* (13,313 genes, 36.1 Mb; Cuomo et al., 2007).

**Transposable elements, repetitive DNA and repeat induced mutations**

Only 0.7% (196,205-bp) of the *P. foliicola* nuclear genome assembly contained TEs based on the REPET pipeline analysis (Table 1). Annotation of TEs across the *P. foliicola* genome assembly identified 191 TE matches. Of these, 141 were Class I (retrotransposons) TEs comprising ~0.4% of genome and 45 were Class II (DNA) TEs making up ~0.3% of the genome. Long terminal-repeats (LTRs) were found to be the most abundant Class I order (~0.3% of genome) and included matches in the Copia, Gypsy and BEL/Pao retrotransposon superfamilies. Almost all Class II TEs were identified as Helitrons, with only ~0.001% of the genome identified with TIR or “unknown” TEs (Wicker code: DXX). Evidence of RIP mutation affecting *P. foliicola* TEs was only found for Helitrons based on alignment of 37 sequences (3.7-kb in length) and di-nucleotide indexes: TpA/ApT = 1.8 and (CpA + TpG)/(ApC + GpT) = 0.3.

The draft genome of *D. macrodidyma* genome as originally published did not include information about TEs. Here, we also analyzed that assembly for the presence of TEs and RIP mutations using the same parameters used for *P. foliicola* for comparative purposes. As REPET identified over 2,000 *D. macrodidyma* TEs of “unknown” superfamily, we performed an additional *tblastx* search (e-value: 0.01, percent query coverage per hsp: ≥70%, minimum percent identity of hits: ≥70%) against Repbase and reclassified five TEs based on the top match if it shared the same class and order. REPET analysis identified ~6.5% (3,794,887-bp) of the *D. macrodidyma* genome to be made up of TEs (2,178 elements). Of these, 1,489 elements were Class I TEs and comprised ~5.3% of the genome and 689 were Class II TEs and comprised ~1.2% of the genome. The most common Class I retrotransposon orders were: LTRs (~3.2%), “Unknown RXX” (~1.1%), DIRS-like (~0.9%), LINE (~0.1%) and SINE (0.001%). For Class II DNA elements, the most common orders were: TIR (~0.7%), Helitron (~0.4%) and “Unknown DXX” (~0.2%). Evidence for RIP mutation affecting *D. macrodidyma* TEs using alignment and di-nucleotide counts was identified in the sets of 91 Helitron and 146 DIRS TEs. For the Helitrons, RIP indexes were calculated as: TpA/ApT = 1.3 and (CpA + TpG)/(ApC + GpT) = 0.9, while for DIRS: TpA/ApT = 1.4 and (CpA + TpG)/(ApC + GpT) = 0.3.

**Mitochondrial genome size and gene content**

The mitogenomes of *P. foliicola* and *D. macrodidyma* were each contained within a single scaffold, measuring 58.3 kb and 44.2 kb respectively (scaffold 56, scaffold 68). Although the mitogenome-containing scaffolds for these two organisms were considerably smaller than that of *F. graminearum* (95.7 kb), a full complement of protein-coding genes was contained: apocytochrome b (*cob*), ATP-synthase subunits (*atp6, atp8, atp9*), cytochrome oxidase subunits (*cox1, cox2, cox3*), and NADH subunits (*nad1, nad2, nad3, nad4, nad4L, nad5, nad6*). This set of 14 protein-coding mitochondrial genes is highly conserved among fungi, and shared with animal mtDNA (Bullerwell & Lang, 2005).
tRNAs necessary for translation was encoded in the mitogenomes (25 total), as were small and large subunit rRNAs (one and three copies, respectively). Neither *P. foliicola* nor *D. macrodidyma* mitogenomes contained copies of the four large unidentified open-reading frames that are encoded by the *F. graminearum* mitogenome (*Al-Reedy et al.*, 2012), accounting for the observed difference in sizes between these species.

**Mitochondrial genome synteny**
The organization of the mitogenomes of *P. foliicola*, *D. macrodidyma* and *F. graminearum* was highly conserved at the gene level. As observed in the mitochondria of most ascomycete fungi, all genes, rRNAs and tRNAs were oriented in the same direction. The three mitogenomes contained a nearly identical ordering of shared genes (Fig. 2). Only a single tRNA (tRNA-G) was positioned differently in the *P. foliicola* and *D. macrodidyma* mitogenomes relative to *F. graminearum*. Two tRNAs (tRNA-R and tRNA-L) were positioned differently between *D. macrodidyma* and *P. foliicola/F. graminearum*. This conserved ordering of the three mitogenomes was consistent with previous observations that the mitochondrial DNA of fungi in the order Hypocreales is highly conserved (*Al-Reedy et al.*, 2012; *Pantou, Kouvelis & Typas, 2008*).

**Identification of mating type idiomorphs in *P. foliicola***
The presence of the conserved alpha domain indicative of the *MAT1-1* mating type idiomorph was identified in *P. foliicola* scaffold 102. In the same scaffold, the *APN2* (encoding DNA lyase) and *SLA2* (encoding cytoskeletal protein) genes were found flanking the mating type idiomorph, as well as the MAT-locus associated gene *COX13* (cytochrome c oxidase subunit VIa homolog). The HMG-box region indicative of the *MAT1-2* mating type idiomorph was not found within the *P. foliicola* genome. Based on these data, *P. foliicola* appears to be a heterothallic fungus, requiring a partner of the alternate mating type in order to initiate the sexual life cycle.

**Phylogeny and divergence estimation**
The phylogenetic relationships and divergence times among the fungal genomes studied is displayed in Fig. 3. Fourteen publicly available fungal genomes were used to examine the phylogenetic placement of *P. foliicola* through the analysis of single copy orthologous genes. The program OrthoMCL identified 16,356 gene clusters, from which 1,884 orthologous genes were shared across all 15 fungal species. From these shared gene clusters, 1,511
orthologous genes present as single copies were used for the phylogenetic analysis. The final dataset after removal of ambiguously aligned regions consisted of 388.7 Mb. The ML analysis identified, with high bootstrap support (>70%), five major clusters representing the ascomycete classes Sordariomycetes, Leotiomycetes, Eurotiomycetes, Dothideomycetes, and Saccharomycetes (Fig. 3). Within the Sordariomycetes, *P. foliicola* was more closely related, albeit appearing basal, to *D. macrodidyma* and *F. graminearum*, all of which belonged to the Nectriaceae in the order Hypocreales. These phylogenetic relationships were consistent with previous reports (Lombard et al., 2015; Yin et al., 2015).

Using RelTime methods and a JTT matrix-based model, the estimated log likelihood value was $-142633.0660$. The divergence of *P. foliicola* from the Nectriaceae species *D. macrodidyma* and *F. graminearum* was estimated to have occurred $\sim$132 Mya. The overall estimates of divergence times in the tree are in agreement with divergence times reported for the order Hypocreales (Sung, Poinar & Spatafora, 2008).

**Comparative genomic analysis of *P. foliicola* and other fungi in the Nectriaceae**

**Gene orthology**

Orthologous gene clusters were identified for *P. foliicola*, *D. macrodidyma* and *F. graminearum* using OrthoVenn (Fig. 4). Proteins from these three fungal species formed 10,403 orthologous clusters, of which 7,135 clusters were shared among all three species.

**Figure 3** Reconstruction of the phylogenetic relationships and divergence times of *Pseudonectria foliicola* relative to other fungal species. The maximum likelihood (ML) tree analysis and time tree (RelTime method) were conducted on the concatenated dataset of 1,511 single copy orthologous genes. Numbers above branches indicate the approximate relative times of divergence (Mya) between two lineages. The seven calibration points used are indicated at the nodes and listed in Table S4. Scale representation under the tree demonstrate divergence times of genes. Statistical support values corresponding to ML are indicated below the branches. The basidiomycete *Ustilago maydis* was used as the outgroup. Full-size DOI: 10.7717/peerj.5401/fig-3
The top three Swiss-Prot annotations among the core shared clusters were the Acyl-CoA-binding domain-containing protein (44 proteins), pleiotropic drug resistance protein 4 (30 proteins) and a short-chain dehydrogenase TIC 32, chloroplastic (12 proteins). Sixteen clusters representing 48 predicted proteins were unique to *P. foliicola*, while 610 and 124 species-specific protein clusters were identified in *D. macrodidyma* and *F. graminearum*, respectively. Only 0.2% of the *P. foliicola* proteome was unique, a low percentage relative to 1.3% for the *F. graminearum* and 6.0% for the *D. macrodydima* proteomes. Although the majority of the 16 clusters unique to *P. foliicola* had no annotations based on the UniProt/Swiss-Prot and Gene Ontology (GO) databases, three of the unique clusters were identified as (1) vegetative cell wall protein Gp1/structural constituent of cell wall (GO:0005199), (2) thioredoxin/protein disulfide oxidoreductase activity (GO:0009507), and (3) leucine-rich repeat extensin-like protein 3/structural constituent of cell wall (GO:0005618). Biological processes and molecular functions annotated by the GO database for the species-unique gene clusters were most abundant in *D. macrodidyma* (66 biological processes, 28 molecular functions), and least abundant in *P. foliicola* (five biological processes, two molecular functions; Table S1). Three GO categories were found enriched (hypergeometric test on OrthoVenn, *p*-value <0.05) in the *P. foliicola* unique clusters: (1) a glycerol ether metabolic process, (2) structural constituent of cell wall and, (3) a protein disulfide oxidoreductase activity. Despite the large number of unique gene clusters found in *D. macrodidyma*, only one GO category, an oxidoreductase activity acting on single donors with incorporation of molecular oxygen, was found to be enriched.

**The CAZyme repertoire**

Annotation of the *P. foliicola* proteome identified 448 CAZyme modules, including domains encoding 179 glycoside hydrolases (GH), 88 glycosyl-transferases (GT), 18 polysaccharide lyases (PL), 64 carbohydrate esterases (CE), 46 carbohydrate-binding modules (CBM), and 53 enzymes with auxiliary activities (AA) (summarized in Table 2, Fig. 5 and Table S2).
Table 2  Summary of the carbohydrate-active enzyme (CAZyme) modules identified from the predicted proteome of Pseudonectria foliicola, Dactylonectria macrodidyma and Fusarium graminearum. RF%: Relative frequency of CAZyme modules over the total number of predicted proteins for the corresponding genome.

| Name | Pseudonectria foliicola | RF% | Dactylonectria macrodidyma | RF% | Fusarium graminearum | RF% |
|------|--------------------------|-----|---------------------------|-----|----------------------|-----|
| AA   | 53                       | 0.52| 147                       | 0.84| 110                  | 0.79|
| CBM  | 46                       | 0.53| 116                       | 0.73| 80                   | 0.65|
| CE   | 64                       | 0.83| 210                       | 1.37| 127                  | 1.08|
| GH   | 179                      | 1.94| 447                       | 2.59| 268                  | 2.01|
| GT   | 88                       | 1.01| 125                       | 0.78| 100                  | 0.82|
| PL   | 18                       | 0.17| 41                        | 0.20| 22                   | 0.16|
| Total| 448                      | 4.83%| 1,086                     | 6.40%| 707                  | 5.31%|

Notes. AA, Auxiliary activity families; CBM, Carbohydrate-binding modules; CE, Carbohydrate esterase families; GH, Glycoside hydrolase families; GT, Glycosyltransferase families; PL, Polysaccharide lyase families.

The D. macrodidyma and F. graminearum genomes encoded 1,086 and 707 CAZyme modules, respectively. The CAZyme encoding genes represented between 4.8 and 6.6% of the predicted proteome for the three fungal species (Table 2).

An increased number of CAZyme modules was identified from the D. macrodidyma proteome when compared to P. foliicola, F. graminearum and a database of 91 other plant pathogenic, facultative pathogenic, saprophytic and symbiotic fungi (Table S2; Zhao et al., 2013). Although not significant in X² tests against P. foliicola and F. graminearum, D. macrodidyma has an increased number of GH and CE enzymes. This is largely due to the GH3 family, which is associated with cellulose degrading activities, the GH28 family, with pectinase activities, as well as the less characterized GH78 and GH109 families. Within the CE and PL module groups, increased numbers in the D. macrodidyma genome were observed in the families CE3, CE5, CE10 and PL1 (Fig. 5). The CE3 and CE5 have acetyl xylan esterase and cutinase activities and are common modules with a higher representation in Ascomycetes relative to Basidiomycetes (Zhao et al., 2013). CE10 families have carboxylesterases activities but can also act on non-carbohydrate substrates (Cantarel et al., 2009). The PL1 family is the most commonly found among fungi, particularly in plant pathogenic species (Zhao et al., 2013). The number of CE families in D. macrodidyma (233) is comparable to that of the pea root pathogen Fusarium solani, previously regarded as having the most CEs with 223 (Zhao et al., 2013).

Secondary metabolite clusters
Annotation of the P. foliicola, F. graminearum and D. macrodidyma proteomes identified key enzyme clusters for the biosynthesis of secondary metabolites such as non-ribosomal peptide synthases (NRPS), polyketide synthases (PKS), terpene synthases (TS), among others (summarized in Fig. 5). The genome of P. foliicola contained 25 secondary metabolite clusters, in contrast with F. graminearum and D. macrodidyma with a total of 41 and 44 clusters, respectively (Fig. 5).
### Secretome

The predicted secretome for *P. foliicola* was also relatively small, comprising just 346 proteins. In comparison, the genomes of *D. macrodidyma* and *F. graminearum* contained 607 and 457 predicted secreted proteins (Table S3). However, for all three species, the secretome made up between 3.4 to 3.7% of the predicted proteome.
Table 3 Summary of the predicted genes associated with virulence in the genome assemblies of *Pseudonectria foliicola*, *Dactylonectria macrodidyma* and *Fusarium graminearum*.

| Name                          | *Pseudonectria foliicola* RF% | *Dactylonectria macrodidyma* RF% | *Fusarium graminearum* RF% |
|-------------------------------|-------------------------------|---------------------------------|---------------------------|
| Chemistry target              | 7                             | 8                               | 9                         | 0.07                      |
| Effector                      | 15                            | 22                              | 19                        | 0.14                      |
| Enhanced antagonism           | 2                             | 2                               | 1                        | 0.02                      |
| Increased virulence           | 7                             | 8                               | 3                         | 0.05                      |
| Increased virulence (Hypervirulence) | 13                        | 20                              | 18                        | 0.14                      |
| Lethal                        | 65                            | 80                              | 87                        | 0.65                      |
| Loss of pathogenicity         | 103                           | 116                             | 99                        | 0.74                      |
| Mixed outcome                 | 112                           | 124                             | 114                       | 0.86                      |
| Reduced virulence             | 459                           | 510                             | 490                       | 3.68                      |
| Unaffected pathogenicity      | 558                           | 712                             | 782                       | 5.87                      |
| Other                         | 2                             | 2                               | 2                         | 0.02                      |
| **Total**                     | **1,343**                      | **1,603**                       | **1,629**                 | **12.24%**                |

**Virulence associated genes**

The genomes of *P. foliicola*, *D. macrodidyma* and *F. graminearum* were screened against PHI-base, a curated database that contains pathogenicity, virulence and effector genes from fungi, oomycete and bacterial pathogens (*Winneburg et al., 2008*). Relative to the total proteome, the frequency of virulence-associated genes was highest in *P. foliicola* (14.5%) compared to *F. graminearum* (12.2%) and *D. macrodidyma* (9.5%) (Table 3). Genes associated with the loss of pathogenicity, reduced virulence and those with mixed outcomes were identified in higher frequencies in the *P. foliicola* proteome than in the *D. macrodidyma* and *F. graminearum* proteomes (Table 3). Genes associated with loss of pathogenicity and with reduced virulence have been identified by PHI-base from transgenic strains of fungal, oomycete and bacterial pathogens that either fail to cause disease or that cause quantitatively lower degrees of disease than the wild-type strains (*Winneburg et al., 2008; Urban et al., 2015*).

**DISCUSSION**

*Pseudonectria* species, *P. foliicola* and *P. buxi*, are economically important fungal pathogens responsible for increased costs in foliar disease management of boxwood plants worldwide. Here, we present a draft genome sequence for *P. foliicola*, including a comparative analysis of this genome against two other plant pathogens in the Nectriaceae. The 28.7 Mb *P. foliicola* draft genome assembly is smaller than the reported size of other fungi in the Ascomycota (average genome size 36.9 Mb; *Mohanta & Bae, 2015*). This assembly represents the smallest genome known from the Nectriaceae, in which genomes range from 36.1 to 58.1 Mb: *D. macrodidyma* (58.0 Mb; *Malapi-Wight et al., 2015*), *Neonectria ditissima* (44.9 Mb; *Gomez-Cortecero, Harrison & Armitage, 2015*) and *F. graminearum* (36.1 Mb; *Cuomo et al., 2007*). Consistent with genome size, the number of predicted gene models from the *P. foliicola* assembly is reduced but comparable to the number of gene models predicted...
in other Ascomycota fungi with similar genome size (e.g., *Patellaria atrata*, 28.7 Mb, 7,794 gene models (JGI); Mohanta & Bae, 2015). *Pseudonectria foliicola* also contains one of the smallest cohorts of TEs reported for filamentous fungi, similar to the genomes of *Trichoderma atroviride*, *T. reesei*, and *T. virens* (ranging from 0.48 to 0.57%; Kubicek et al., 2011; Martinez et al., 2008) as well as *F. graminearum* (<1% of repetitive DNA; Cuomo et al., 2007). A strong correlation between genome size and repeat content was reported in a study of 18 Dothidiomycete genomes (Ohm et al., 2012) however, based on the low percentage of TEs found in *F. graminearum* and *D. macrodidyma*, this correlation may not be sustained within the Nectriaceae.

The uniquely small genome size of *P. foliicola* is similar to the genome assembly recently reported from *Escovopsis weberi* (29.5 Mb), a highly specialized mycoparasitic fungus that also belongs to the order Hypocreales (De Man et al., 2016). Specialized pathogens or those with a narrow host range are predicted to maintain only the essential cohort of genes, and lose those no longer needed in their particular niche (e.g., De Man et al., 2016; Lee & Marx, 2012) and while there are exceptions (e.g., Spanu et al., 2010), the comparative genome analysis of the three Nectriaceae genomes in this study follows that prediction. From the three fungal species analyzed in this study, only *P. foliicola* has a narrow host range (only known from *Buxus* spp.) while *F. graminearum* (reported frequently in a large number of hosts in Poaceae) and *D. macrodidyma* (reported on grapevine, avocado and olive trees) have increasingly broader host ranges and larger genomes and proteomes. Divergence time estimates indicated that *P. foliicola*, *D. macrodidyma* and *F. graminearum* diverged from their common ancestral organism ca. 132 Mya. This relatively distant split may account for the differences observed in pathogenicity and host range between these species, and indicate that ongoing gene loss resulting in a reduced genome size is a major contributor to the genome evolution of *P. foliicola*.

Enzymes that degrade plant cell wall carbohydrates can be essential during the infection and decomposition of host plant tissue, particularly for necrotrophic and hemibiotrophic fungi (Gibson et al., 2011; Zhao et al., 2013). Consequently, CAZyme profiles can be used as indicators of the fungal lifestyle. In previous studies, necrotrophic and hemibiotrophic fungal plant pathogens have been reported to produce a large repertoire of these enzymes (Gibson et al., 2011; Knogge, 1996), and typically exhibit expanded arsenals of CAZymes in their genomes, relative to biotrophic and obligate fungi that typically exhibit the lowest numbers (Zhao et al., 2013). Similarly, the fungal secretome and secondary metabolites, both involved in the host-pathogen interaction process (Van den Burg et al., 2006; Yu & Keller, 2005), can correlate with the lifestyle of a fungal pathogen (Lowe & Howlett, 2012; Ohm et al., 2012). Consistent with its small genome and proteome size, *P. foliicola* has the smallest cohort of CAZymes, SM clusters and secreted proteins relative to *D. macrodidyma* and *F. graminearum*. Despite the reduced number of total CAZyme clusters in *P. foliicola*, all clusters are well represented and comparable to those found in the genomes of fungi with different lifestyles (comparison among the 91 fungal CAZyme profiles by Zhao et al., 2013). Within the Nectriaceae, comparisons among *P. foliicola*, *D. macrodidyma* and *F. graminearum* show increased numbers of CAZymes in several clusters for *D. macrodidyma* relative to the other two fungi, although not significant. The
glycosyl hydrolases (GH), enzymes with an important role in the complete breakdown of the plant cell wall for successful infection (Cantarel et al., 2009), were the most abundant type of secreted protein and CAZymes found across all three species compared.

The pathogenicity profile of *P. foliicola* as predicted by comparisons against the PHI-base database shows a higher relative frequency of genes associated with loss of pathogenicity and reduced virulence, when compared to *D. macrodidyma* and *F. graminearum*. The genome characteristics of *P. foliicola* described in our analyses may help explain the apparent inability of this fungus to penetrate host plant tissue and its dependence on wounding or winter damage for successful infections. Shi & Hsiang (2014) reported primary infection by *P. buxi* on leaves and stems of various *Buxus* species through wounded tissue resulting in general plant decline. A similar strategy may be employed by the closely related species *P. foliicola*; however, due to its recent taxonomic placement, it remains uncertain whether previous disease reports and epidemiology studies correspond to either *Pseudonectria* species.

CONCLUSIONS

Despite the economic importance of fungi in the Nectriaceae family, only a small number of genome resources are currently available. A survey of public databases shows that less than 5% of the estimated 900 fungal species in this family have been sequenced on the whole genome scale (NCBI-GenBank, the Joint Genome Institute Mycocosm and Ensembl databases). To our knowledge, the *P. foliicola* genome is the smallest known genome in the Nectriaceae. Currently, it is unknown if the genome characteristics of other fungal pathogens in the Nectriaceae are similar to those of *P. foliicola*, *D. macrodidyma* or *F. graminearum*, or if these genomes represent the extremes. With the advent and accessibility of next generation sequencing technologies we expect that more in depth comparative genomics studies will characterize fungal groups of great economic and ecological importance. The quality of microbial draft genomes and consequently the predicted size of the associated proteome can also be influenced by the next generation sequencing platform and assembly software (Mavromatis et al., 2012). Even though improvements on sequencing chemistry and better assembly algorithms have reduced the chance of errors, further sampling of genomes of fungi in the Nectriaceae and other families, ideally with a wide range of life styles, would help determine if the differences observed in annotation rates for some protein classes in our study is due to real biological differences or if they might be an artifact of the technology used to generate these draft genomes. Furthermore, the availability of fungal genomes will aid in the resolution of important fungal lineages and explore beyond the commonly used standard molecular markers for taxonomic classification.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This work was supported by funds from the USDA-APHIS 2014 Farm Bill Section 10007 (to Jo Anne Crouch), the USDA-ARS Floricultural and Nursery Research Fund (to
Jo Anne Crouch; project 0500-00059-001) and USDA-ARS (project 8042-22000-279-00D). Martha Malapi Wight was supported by a Class of 2013 USDA-ARS Headquarters Research Associate Award (to Jo Anne Crouch). This research was supported in part by an appointment to the Agricultural Research Service (ARS) Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the US Department of Energy (DOE) and the USDA. ORISE is managed by ORAU under DOE contract number DE-AC05-06OR23100. There was no additional external funding received for this study. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. The USDA is an equal opportunity provider and employer. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures
The following grant information was disclosed by the authors:
USDA-APHIS 2014 Farm Bill Section 10007.
USDA-ARS Floricultural and Nursery Research Fund: 0500-00059-001.
USDA-ARS: 8042-22000-279-00D.
Class of 2013 USDA-ARS Headquarters Research Associate Award.

Competing Interests
The authors declare there are no competing interests.

Author Contributions
• Yazmín Rivera and Catalina Salgado-Salazar conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
• Daniel Veltri analyzed the data, authored or reviewed drafts of the paper, approved the final draft.
• Martha Malapi-Wight analyzed the data, authored or reviewed drafts of the paper.
• Jo Anne Crouch conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.

DNA Deposition
The following information was supplied regarding the deposition of DNA sequences:
The genome assembly of *P. foliicola* used in this study was deposited in NCBI GenBank under accession LMTV00000000, and datasets are also curated by the US National Agricultural Library on AgData Commons (http://dx.doi.org/10.15482/USDA.ADC/1408094).

Data Availability
The following information was supplied regarding data availability:
US National Agricultural Library on AgData Commons http://dx.doi.org/10.15482/USDA.ADC/1408094.
This data are also provided in the Supplemental Files.
Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.5401#supplemental-information.

REFERENCES

Al-Reedy RM, Malireddy R, Dillman CB, Kennell JC. 2012. Comparative analysis of Fusarium mitochondrial genomes reveals a highly variable region that encodes an exceptionally large open reading frame. Fungal Genetics and Biology 49:2–14 DOI 10.1016/j.fgb.2011.11.008.

Amselem J, Cuomo CA, Van Kan JA, Viaud M, Benito EP, Couloux A, Coutinho PM, De Vries DP, Dyer PS, Fillinger S, Fournier E, Gout L, Hahn M, Kohn L, Lapalu N, Plummer KM, Pradier I-M, Quévillon E, Sharon A, Simon A, Ten Have A, Tudzynski B, Tudzynski P, Wincker P, Andrew M, Anthouard V, Beever RE, Beffa R, Benoit I, Bouzid O, Brault B, Chen Z, Choquer M, Collémare J, Cotton P, Danchin EG, Da Silva C, Gautier A, Giraud C, Giraud T, Gonzalez C, Grossetete S, Güldener U, Henrisrat B, Howlett BJ, Kodira C, Kretschmer M, Lappartient A, Leroch M, Levis C, Mauceli E, Neuvéglise C, Oeser B, Pearson M, Poulain J, Poussereau N, Quevillon E, Rascle C, Ségurens B, Sexton A, Silva E, Sirven C, Soanes DM, Talbot NJ, Templeton M, Yarden O, Zeng Q, Rollins JA, Lebrun M-H, Dickman M. 2011. Genomic analysis of the necrotrophic fungal pathogens Sclerotinia sclerotiorum and Botrytis cinerea. PLOS Genetics 7:e1002230 DOI 10.1371/journal.pgen.1002230.

Arnold AE, Miadlikowska J, Higgins KL, Sarvate SD, Gugger P, Way A, Hofstetter V, Kauff F, Lutzoni F. 2009. A phylogenetic estimation of trophic transition networks for ascomycetous fungi: are lichens cradles of symbiotrophic fungal diversification? Systematic Biology 58:283–297 DOI 10.1093/sysbio/syp001.

Baker SE, Schackwitz W, Lipzen A, Martin J, Haridas S, LaButti K, Grigoriev IV, Simmons BA, McCluskey K. 2015. Draft genome sequence of Neurospora crassa strain FGSC 73. Genome Announcements 3:e00074–15 DOI 10.1128/genomeA.00074-15.

Bao W, Kojima KK, Kohany O. 2015. Repbase update, a database of repetitive elements in eukaryotic genomes. Mobile DNA 6:11 DOI 10.1186/s13100-015-0041-9.

Bernt M, Donath A, Jühling F. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. Molecular Phylogenetics and Evolution 69:313–319 DOI 10.1016/j.ympev.2012.08.023.

Blanco-Ulate B, Allen G, Powell AL, Cantu D. 2013. Draft genome sequence of Botrytis cinerea BcDW1, inoculum for noble rot of grape berries. Genome Announcements 1:e00252–13 DOI 10.1128/genomeA.00252-13.

Bullerwell CE, Lang BF. 2005. Fungal evolution: the case of the vanishing mitochondrion. Current Opinions in Microbiology 8:362–369 DOI 10.1016/j.mib.2005.06.009.

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. BMC Bioinformatics 10:421 DOI 10.1186/1471-2105-10-421.
Cantarel B, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. 2009. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Research* 37:D233–D238 DOI 10.1093/nar/gkn663.

Cortázar AR, Aransay AM, Alfaro M, Oguiza JA, Lavin JL. 2014. SECRETOOL: integrated secretome analysis tool for fungi. *Amino Acids* 46:471–473 DOI 10.1007/s00726-013-1649-z.

Cuomo CA, Guldener U, Xu JR, Trail F, Turgeon BG, Di Pietro A, Walton JD, Ma LJ, Baker SE, Rep M, Adam G, Antoniw J, Baldwin T, Calvo S, Chang YL, Decaprio D, Gale LR, Gnerre S, Goswami RS, Hammond-Kosack K, Harris LJ, Hilburn K, Kennell JC, Kroken S, Magnuson JK, Mannhaupt G, Mauceli E, Mewes HW, Mitterbauer R, Muehlbauer G, Münsterkötter M, Nelson D, O’donnell K, Ouellet T, Qi W, Quesneville H, Ronceroy MI, Seong KY, Tetko IV, Urban M, Waalwijk C, Ward TJ, Yao J, Birren BW, Kistler HC. 2007. The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. *Science* 317:1400–1402 DOI 10.1126/science.1143708.

De Jonge R, Van Esse HP, Maruthachalam K, Bolton MD, Santhanam P, Saber MK, Zhang Z, Usami T, Lievens B, Subbarao KV, Thomma BP. 2012. Tomato immune receptor Ve1 recognizes effector of multiple fungal pathogens uncovered by genome and RNA sequencing. *Proceedings of the National Academy of Sciences of the United States of America* 109:5110–5115 DOI 10.1073/pnas.1119623109.

De Man TJ, Stajich JE, Kubicek CP, Teiling C, Chenthamara K, Atanasova L, Druzhinina IS, Bozick BA, Suen G, Currie CR, Gerardo NM. 2016. Small genome of the fungus *Escovopsis weberi*, a specialized disease agent of ant agriculture. *Proceedings of the National Academy of Sciences of the United States of America* 113:3567–3572 DOI 10.1073/pnas.1518501113.

Dujon B, Sherman D, Fischer G, Durrens P, Casaregola S, Lafontaine I, De Montigny J, March C, Neuville C, Talla E, Goffard N, Frangeul L, Aigle M, Anthouard V, Babour A, Barbe V, Barnay S, Blanchin S, Beckerich JM, Beyne E, Bleykasten C, Boisramé A, Boyer J, Cattolico L, Confanioli F, De Daruvar A, Despons I, Fabre E, Fairhead C, Ferry-Dumazet H, Groppi A, Hantraye F, Hennquin C, Janniaux N, Joyet P, Kachouri R, Kerrest A, Koszul R, Lemaire M, Lesur I, Ma L, Muller H, Nicaud JM, Nikolski M, Oztas S, Ozier-Kalogeropoulos O, Pellenz S, Potier S, Richard GF, Straub ML, Suleau A, Swennen D, Tekaia F, Wésolowski-Louvel M, Westhof E, Wirth B, Zeniou-Meyer M, Zivanovic I, Bolotin-Fukuhara M, Thierry A, Bouchier C, Caudron B, Scarpelli C, Gaillardin C, Weissenbach J, Wincker P, Souciet JL. 2004. Genome evolution in yeasts. *Nature* 430:35–44 DOI 10.1038/nature02579.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32:1792–1797 DOI 10.1093/nar/gkh340.

Farr DF, Rossman AY. 2018. Fungal databases. Systematic mycology and microbiology laboratory, USDA ARS. Available at [http://nt.ars-grin.gov/fungal/databases/](http://nt.ars-grin.gov/fungal/databases/) (accessed on 1 April 2018).
Flutre T, Duprat E, Feuillet C, Quesneville H. 2011. Considering transposable element diversification in *de novo* annotation approaches. *PLOS ONE* 6:e16526 DOI 10.1371/journal.pone.0016526.

Galagan JE, Calvo SE, Cuomo C, Ma LJ, Wortman JR, Batzoglou S, Lee SI, Baštürkmen M, Spevak CC, Clutterbuck J, Kapitonov V, Jurka J, Sczzocchio C, Farman M, Butler J, Purcell S, Harris S, Braus GH, Draht O, Busch S, D’Enfert C, Bouchier C, Goldman GH, Bell-Pedersen D, Griffiths-Jones S, Doonan JH, Yu J, Vienken K, Pain A, Freitag M, Selker EU, Archer DB, Peñalva MA, Oakley BR, Momany M, Tanaka T, Kumagai T, Asai K, Machida M, Nierman WC, Denning DW, Caddick M, Hynes M, Paoletti M, Fischer R, Miller B, Dyer P, Sachs MS, Osmani SA, Birren BW. 2005. Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. *Nature* 438:1105–1115 DOI 10.1038/nature04341.

Garibaldi A, Bertetti D, Ortu G, Gullino ML. 2016. First report of volutella blight caused by *Pseudonectria buxi* on Japanese boxwood (*Buxus microphylla*) in Italy. *Plant Disease* 100:1499.

Gehesquière B, Crouch JA, Marra RE, Van Poucke K, Rys F, Maes M, Gobin B, Höfte M, Heungens K. 2016. Characterization and taxonomic reassessment of the box blight pathogen *Calonectria pseudonaviculata*, introducing *Calonectria henricotiae* sp. nov. *Plant Pathology* 65:37–52 DOI 10.1111/ppa.12401.

Gibson D, King B, Hayes M, Bergstrom G. 2011. Plant pathogens as a source of diverse enzymes for lignocellulose digestion. *Current Opinion in Microbiology* 14:264–270 DOI 10.1016/j.mib.2011.04.002.

Gomez-Cortecero A, Harrison RJ, Armitage AD. 2015. Draft genome sequence of a European isolate of the apple canker pathogen *Neonectria ditissima*. *Genome Announcements* 3:10–11.

Goswami RS, Kistler HC. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. *Molecular Plant Pathology* 5:515–525 DOI 10.1111/j.1364-3703.2004.00252.x.

Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075 DOI 10.1093/bioinformatics/btt086.

Hallen F, Fourie PH, Crous PW. 2006. A review of black foot disease of grapevine. *Phytopathologia Mediterranea* 45:S55–S67.

Hane JK. 2015. Calculating RIP mutation in fungal genomes using RIPCAL. In: Van den Berg MA, Maruthachalam K, eds. *Genetic transformation systems in fungi*. Vol. 2. Cham: Springer International Publishing, 69–78.

Hane JK, Oliver RP. 2008. RIPCAL: a tool for alignment-based analysis of repeat-induced point mutations in fungal genomic sequences. *BMC Bioinformatics* 9:478 DOI 10.1186/1471-2105-9-478.

Hedges SB, Marin J, Suleski M, Paymer M, Kumar S. 2015. Tree of life reveals clock-like speciation and diversification. *Molecular Biology and Evolution* 32:835–845 DOI 10.1093/molbev/msv037.

Holt C, Yandell M. 2011. MAKER2: an annotation pipeline and genome-database management tool for second-generation genome projects. *BMC Bioinformatics* 12:491 DOI 10.1186/1471-2105-12-491.
Islam MS, Haque MS, Islam MM, Emdad EM, Halim A, Hossain QM, Hossain MZ, Ahmed B, Rahim S, Rahman MS, Alam MM, Hou S, Wan X, Saito JA, Alam M. 2012. Tools to kill: genome of one of the most destructive plant pathogenic fungi Macrophomina phaseolina. BMC Genomics 13:493 DOI 10.1186/1471-2164-13-493.

Jones D, Taylor W, Thornton J. 1992. The rapid generation of mutation data matrices from protein sequences. Computer Applications in the Biosciences 8:275–282.

Kämper J, Kahmann R, Böker M, Ma LJ, Brefort T, Saville BJ, Banuett F, Kronstad JW, Gold SE, Müller O, Perlin MH, Wösten HA, De Vries R, Ruiz-Herrera J, Reynaga-Peña CG, Snetselaar K, McCann M, Pérez-Martín J, Feldbrügge M, Basse CW, Steinberg G, Ibeas JI, Holloman W, Guzman P, Farman M, Stajich JE, Sentandreu R, González-Prieto JM, Kennell JC, Molina L, Schirawski J, Mendoza-Mendoza A, Greilinger D, Münch K, Rössel N, Scherer M, Vranes M, Ladendorf O, Vincon V, Fuchs U, Sandrock B, Meng S, Ho EC, Cahill MJ, Boyce KJ, Klose J, Klosterman SJ, Deelstra HJ, Ortiz-Castellanos I, Li W, Sanchez-Alonso P, Schreier PH, Häuser-Hahn I, Vaupel M, Koopmann E, Friedrich G, Voss H, Schlüter T, Margolis J, Platt D, Swimmer C, Gninke A, Chen F, Vysotskaia V, Mannhaupt G, Guldener U, Münsterkötter M, Haase D, Oesterheld M, Mauceli EW, DeCaprio D, Wiest A, Butler J, Young S, Jaffe DB, Calvo S, Nusbaum C, Galagan J, Birren BW. 2006. Insights from the genome of the biotrophic fungal plant pathogen Ustilago maydis. Nature 444:97–101 DOI 10.1038/nature05248.

Kapitonov VV, Jurka J. 2008. A universal classification of eukaryotic transposable elements implemented in Repbase. Nature Reviews Genetics 9:411–412.

Kim S, Park J, Park SY, Mitchell TK, Lee YH. 2010. Identification and analysis of in planta expressed genes of Magnaporthe oryzae. BMC Genomics 11:104 DOI 10.1186/1471-2164-11-104.

Knogge W. 1996. Fungal infection of plants. Cell 8:1711–1722.

Koike H, Aerts A, LaButti K, Grigoriev IV, Baker SE. 2013. Comparative genomics analysis of Trichoderma reesei strains. Industrial Biotechnology 9:352–367 DOI 10.1089/ind.2013.0015.

Kubicek CP, Herrera-Estrella A, Seidl-Seiboth V, Martinez DA, Druzhinina IS, Thon M, Zeilinger S, Casas-Flores S, Horwitz BA, Mukherjee PK, Mukherjee M, Kredics L, Alcaraz LD, Aerts A, Antal Z, Atanasova L, Cervantes-Badillo MG, Challacombe J, Chertkov O, McCluskey K, Couplier F, Deshpande N, Von Döhren H, Ebbole DJ, Esquivel-Naranjo EU, Fekete E, Flippi M, Glaser F, Gómez-Rodríguez EY, Gruber S, Han C, Henriussat B, Hermosa R, Hernández-Oñate M, Karaffa L, Kosti I, Le Crom S, Lindquist E, Lucas S, Lübeck M, Lübeck PS, Margeot A, Metz B, Misra M, Nevalainen H, Omann M, Packer N, Perrone G, Uresti-Rivera EE, Salamov A, Schmoll M, Seiboth B, Shapiro H, Sukno S, Tamayo-Ramos JA, Tisch D, Wiest A, Wilkinson HH, Zhang M, Coutinho PM, Kenerley CM, Monte E, Baker SE, Grigoriev IV. 2011. Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of Trichoderma. Genome Biology 12:R40 DOI 10.1186/gb-2011-12-4-r40.
Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**:1870–1874 DOI 10.1093/molbev/msw054.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* **23**:2947–2948 DOI 10.1093/bioinformatics/btm404.

Lee MC, Marx CJ. 2012. Repeated, selection-driven genome reduction of accessory genes in experimental populations. *PLOS Genetics* **8**:e1002651 DOI 10.1371/journal.pgen.1002651.

Liu G, Zhang L, Wei X, Zou G, Qin Y, Ma L, Li J, Zheng H, Wang S, Wang C, Xun L, Zhao GP, Zhou Z, Qu Y. 2013. Genomic and secretomic analyses reveal unique features of the lignocellulolytic enzyme system of *Penicillium decumbens*. *PLOS ONE* **8**:e55185 DOI 10.1371/journal.pone.0055185.

Lo Presti L, Lanver D, Schweizer G, Tanaka S, Liang L, Tollot M, Zuccaro A, Reissmann S, Kahmann R. 2015. Fungal effectors and plant susceptibility. *Annual Review of Plant Biology* **66**:513–545 DOI 10.1146/annurev-arplant-043014-114623.

Lombard L, Van der Merwe NA, Groenewald JZ, Crous PW. 2015. Generic concepts in Nectriaceae. *Studies in Mycology* **80**:189–245 DOI 10.1016/j.simyco.2014.12.002.

Lowe RG, Howlett BJ. 2012. Indifferent, affectionate, or deceitful: lifestyles and secretomes of fungi. *PLOS Pathogens* **8**:e1002515 DOI 10.1371/journal.ppat.1002515.

Malapi-Wight M, Demers D, Velti D, Marra RE, Crouch JA. 2016a. LAMP detection assays for boxwood blight pathogens: a comparative genomics approach. *Scientific Reports* **6**:26140 DOI 10.1038/srep26140.

Malapi-Wight M, Salgado-Salazar C, Demers JE, Clement DE, Rane K, Crouch JA. 2016b. Sarcococca blight: use of whole genome sequencing as a strategy for fungal disease diagnosis. *Plant Disease* **100**:1093–1100 DOI 10.1094/PDIS-10-15-1159-RE.

Malapi-Wight M, Salgado-Salazar C, Demers J, Velti D, Crouch JA. 2015. Draft genome sequence of *Dactylonectria macrodidyma*, a plant-pathogenic fungus in the Nectriaceae. *Genome Announcements* **3**:2014–2015.

Manning VA, Pandelova I, Dhillon B, Wilhelm LJ, Goodwin SB, Berlin AM, Figueroa M, Freitag M, Hane JK, Henrissat B, Holman WH, Kodira CD, Martin J, Oliver RP, Robbertse B, Schackwitz W, Schwartz DC, Spatafora JW, Turgeon BG, Turgeon RL, Yandava C, Young S, Zhou S, Zeng Q, Grigoriev IV, Ma LJ, Ciuffetti LM. 2013. Comparative genomics of a plant-pathogenic fungus, *Pyrenophora tritici-repentis*, reveals transduplication and the impact of repeat elements on pathogenicity and population divergence. *G3: Genes, Genomes, Genetics* **3**:41–63 DOI 10.1534/g3.112.004044.

Margolin BS, Garrett-Engele PW, Stevens JN, Fritz DY, Garret-Engele C, Metzenberg RL, Selker EU. 1998. A methylated *Neurospora* 5S rRNA pseudogene contains a transposable element inactivated by repeat-induced point mutation. *Genetics* **149**:1787–1797.

Martinez D, Berka RM, Henrissat B, Saloheimo M, Arvas M, Baker SE, Chapman J, Chertkov O, Coutinho PM, Cullen D, Danchin EGJ, Grigoriev IV, Harris P,
Jackson M, Kubicek CP, Han CS, Ho I, Larrondo LF, De Leon AL, Magnuson JK, Merino S, Misra M, Nelson B, Putnam N, Robbertse B, Salamov AA, Schmoll M, Terry A, Thayer N, Westerholm-Parvinen A, Schoch CL, Yao J, Barabote R, Nelson MA, Detter C, Bruce D, Kuske CR, Xie G, Richardson P, Rokhsar DS, Lucas SM, Rubin EM, Dunn-Coleman N, Ward M, Brettin TS. 2008. Genome sequencing and analysis of the biomass-degrading fungus Trichoderma reesei (syn. Hypocrea jecorina). *Nature Biotechnology* 26:553–560 DOI 10.1038/nbt1403.

Mavromatis K, Land ML, Brettin TS, Quest DJ, Copeland A, Clum A, Goodwin L, Woyke T, Lapidus S, Klenk PH, Cottingham RW, Kyrpides NC. 2012. The fast changing landscape of sequencing technologies and their impact on microbial genome assemblies and annotation. *PLOS ONE* 7:e4837.

McTaggart AR, Van der Nest MA, Steenkamp ET, Roux J, Slippers B, Shuey LS, Wingfield MJ, Drenth A. 2016. Fungal genomics challenges the dogma of name-based biosecurity. *PLOS Pathogens* 12:e1005475 DOI 10.1371/journal.ppat.1005475.

Medema M, Blin K, Cimermancic P, Jager V, Zakrzewski P, Fischbach MA, Weber T, Takano E, Breitling R. 2011. AntiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Research* 39:339–346 DOI 10.1093/nar/gkr466.

Mohanta TK, Bae H. 2015. The diversity of fungal genome. *Biological Procedures Online* 17:1 DOI 10.1186/s12575-014-0013-3.

Ohm RA, Feau N, Henrissat B, Schoch CL, Horwitz BA, Barry KW, Condon BJ, Copeland AC, Dhillon B, Glaser F, Hesse CN, Kosti I, LaButti K, Lindquist EA, Lucas S, Salamov AA, Bradshaw RE, Ciuffetti L, Hamelin RC, Kema GH, Lawrence C, Scott JA, Spatafora JW, Turgeon BG, De Wit PJ, Zhong S, Goodwin SB, Grigoriev IV. 2012. Diverse lifestyles and strategies of plant pathogenesis encoded in the genomes of eighteen Dothideomycetes fungi. *PLOS Pathogens* 8:e1003037 DOI 10.1371/journal.ppat.1003037.

Pantou MP, Kouvelis VN, Typas MA. 2008. The complete mitochondrial genome of *Fusarium oxysporum*: insights into fungal mitochondrial evolution. *Gene* 419:7–15 DOI 10.1016/j.gene.2008.04.009.

Salgado-Salazar C, Rossman AY, Samuels GJ, Hirooka Y, Sanchez RM, Chaverri P. 2014. Phylogeny and taxonomic revision of *Thelonectria discophora* (Ascomycota, Hypocreales, Nectriaceae) species complex. *Fungal Diversity* 70:1–29.

Shi F, Hsiang T. 2014. *Pseudonectria buxi* causing leaf and stem blight on *Buxus* in Canada. *European Journal of Plant Pathology* 138:763–773 DOI 10.1007/s10658-013-0348-7.

Silvestro D, Michalak I. 2012. raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* 12:335–337 DOI 10.1007/s13127-011-0056-0.

Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31:3210–3212 DOI 10.1093/bioinformatics/btv351.
Spanu PD, Abbott JC, Amselem J, Burgis TA, Soanes DM, Stüber K, Loren van Themaat EV, Brown JKM Butcher, SA, Gurr SJ, Lebrun M-H, Ridout CJ, Schulze-Lefert P, Talbot NJ, Ahmadinejad N, Ametz C, Barton GR, Benjdia M, Bidzinski P, Bindschedler LV, Both M, Brewer MT, Cadle-Davidson L, Cadle-Davidson MM, Collemare J, Cramer R, Frenkel O, Godfrey D, Harriman J, Hoede C, King BC, Klages S, Kleemann J, Knoll D, Koti PS, Kreplak J, López-Ruiz FJ, Lu X, Maekawa T, Mahanil S, Micali C, Milgroom MG, Montana G, Noir S, O’Connell RJ, Oberhaensli S, Pedersen C, Quesneville H, Reinhardt R, Rott M, Sacristán S, Schönh C, Skamnioti P, Sommer H, Stephens A, Takahara H, Thordal-Christensen H, Vigouroux M, Wicker T, Panstruga R. 2010. Genome expansion and gene loss in powdery mildew fungi reveal tradeoffs in extreme parasitism. *Science* **330**:1543–1546 DOI 10.1126/science.1194573.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**:2688–2690 DOI 10.1093/bioinformatics/btl446.

Stanke M, Steinkamp R, Waack S, Morgenstern B. 2004. AUGUSTUS: a web server for gene finding in eukaryotes. *Nucleic Acid Research* **32**:W309–W312.

Sung G-H, Poinar GO, Spatafora JW. 2008. The oldest fossil evidence of animal parasitism by fungi supports a Cretaceous diversification of fungal-arthropod symbioses. *Molecular Phylogenetics and Evolution* **49**:495–502 DOI 10.1016/j.ympev.2008.08.028.

Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systems Biology* **56**:564–577 DOI 10.1080/10635150701472164.

Tamura K, Battistuzzi FU, Billing-Ross P, Murillo O, Filipski A, Kumar S. 2012. Estimating divergence times in large molecular phylogenies. *Proceedings of the National Academy of Sciences of the United States of America* **109**:19333–19338 DOI 10.1073/pnas.1213199109.

Urban M, Pant R, Raghunath A, Irvine AG, Pedro H, Hammond-Kosack KE. 2015. The Pathogen–Host Interactions database (PHI-base): additions and future developments. *Nucleic Acids Research* **43**:D645–D655 DOI 10.1093/nar/gku1165.

Urbez-Torres J, Peduto F, Gubler W. 2012. First report of *Ilyonectria macrodidyma* causing root rot of olive trees. *Plant Disease* **96**:1378.

Van den Burg HA, Harrison SJ, Joosten MH, Vervoort J, De Wit PJ. 2006. *Cladosporium fulvum* Avr4 protects fungal cell walls against hydrolysis by plant chitinases accumulating during infection. *Molecular Plant-Microbe Interactions* **19**:1420–1430 DOI 10.1094/MPMI-19-1420.

Veltri D, Malapi-Wight M, Crouch JA. 2016. SimpleSynteny: a web-based tool for visualization of microsynteny across multiple species. *Nucleic Acids Research* **44**:W41–W45 DOI 10.1093/nar/gkw330.

Vitale A, Aiello D, Guarnaccia V, Perrone G, Stea G, Polizzi G. 2012. First report of root rot caused by *Ilyonectria (= Neonectria) macrodidyma* on avocado (*Persea americana*) in Italy. *Journal of Phytopathology* **160**:156–159 DOI 10.1111/j.1439-0434.2011.01869.x.
Wang Y, Coleman-Derr D, Chen G, Gu YQ. 2015. OrthoVenn: a web server for genome wide comparison and annotation of orthologous clusters across multiple species. *Nucleic Acids Research* **43**:W78–W84 DOI 10.1093/nar/gkv487.

Weber T, Blin K, Duddela S, Krug D, Kim HU, Bruccoleri R, Lee SY, Fischbach MA, Müller R, Wohleben W, Breitling R, Takano E, Medema MH. 2015. antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. *Nucleic Acids Research* **43**:1–7 DOI 10.1093/nar/gku1303.

Wicker T, Sabot F, Hua-Van A, Bennetzen JL, Capy P, Chalhoub B, Flavell A, Leroy P, Morgante M, Panaud O, Paux E, SanMiguel P, Schulman AH. 2007. A unified classification system for eukaryotic transposable elements. *Nature Reviews Genetics* **8**:973–982 DOI 10.1038/nrg2165.

Windels CE. 2000. Economic and social impacts of fusarium head blight: changing farms and rural communities in the northern Great Plains. *Phytopathology* **90**:17–21 DOI 10.1094/PHYTO.2000.90.1.17.

Winnenburg R, Urban M, Beacham A, Baldwin TK, Holland S, Lindeberg M, Hansen H, Rawlings C, Hammond-Kosack KE, Köhler J. 2008. PHI-base update: additions to the pathogen-host interaction database. *Nucleic Acids Research* **36**:572–576.

Ye X, Liu H, Jin Y, Guo M, Huang A, Chen Q, Guo W, Zhang F, Feng L. 2017. Transcriptomic analysis of *Calonectria pseudoreteaudii* during various stages of *Eucalyptus* infection. *PLOS ONE* **12**:e0169598 DOI 10.1371/journal.pone.0169598.

Yin Y, Mao X, Yang J, Chen X, Mao F, Xu Y. 2012. DbCAN: a web resource for automated carbohydrate-active enzyme annotation. *Nucleic Acids Research* **40**:445–451.

Yin Z, Liu H, Li Z, Ke X, Dou D, Gao X, Song N, Dai Q, Wu Y, Xu JR, Kang Z, Huang L. 2015. Genome sequence of *Valsa* canker pathogens uncovers a potential adaptation of colonization of woody bark. *New Phytologist* **208**:1202–1216 DOI 10.1111/nph.13544.

Yu JH, Keller N. 2005. Regulation of secondary metabolism in filamentous fungi. *Annual Reviews of Phytopathology* **43**:437–458 DOI 10.1146/annurev.phyto.43.040204.140214.

Zhao Z, Liu H, Wang C, Xu J-R. 2013. Comparative analysis of fungal genome reveals different plant cell wall degrading capacity in fungi. *BMC Genomics* **14**:274 DOI 10.1186/1471-2164-14-274.