IMA Genome-F 10

Nine draft genome sequences of *Claviceps purpurea* s.lat., including *C. arundinis*, *C. humidiphila*, and *C. cf. spartinae*, pseudomolecules for the pitch canker pathogen *Fusarium circinatum*, draft genome of *Davidsoniella eucalypti*, *Grosmannia galeiformis*, *Quambalaria eucalypti*, and *Teratosphaeria destructans*

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Abstract: This genome announcement includes draft genomes from *Claviceps purpurea* s.lat., including *C. arundinis*, *C. humidiphila* and *C. cf. spartinae*. The draft genomes of *Davidsoniella eucalypti*, *Quambalaria eucalypti* and *Teratosphaeria destructans*, all three important eucalyptus pathogens, are presented. The insect associate *Grosmannia galeiformis* is also described. The pine pathogen genome of *Fusarium circinatum* has been assembled into pseudomolecules, based on additional sequence data and by harnessing the known synteny within the *Fusarium fujikuroi* species complex. This new assembly of the *F. circinatum* genome provides 12 pseudomolecules that correspond to the haploid chromosome number of *F. circinatum*. These are comparable to other chromosomal assemblies within the FFSC and will enable more robust genomic comparisons within this species complex.

Key words: chromosome numbers, ergotism, *Eucalyptus*, pine pitch canker, *Poaceae*

Article info: Submitted: 21 November 2018; Accepted: 26 November 2018; Published: 14 December 2018.

IMA Genome-F 10A

Nine draft genome sequences of *Claviceps purpurea* s.lat., including *C. arundinis*, *C. humidiphila* and *C. cf. spartinae*

INTRODUCTION

*Claviceps purpurea* (Clavicipitaceae, Hypocreales) is a plant pathogen that infects the flowers of cereal crops and grasses (*Poaceae*) causing ergot disease. After floral infection by the pathogen, the seeds of grass hosts are replaced with hard, dark fungal resting bodies called sclerotia or ergots. Consumption of grains contaminated with ergots is harmful to human and animal health, causing ergotism, the result of a spectrum of potent mycotoxins known as ergot alkaloids (Lyons et al. 1986, Miles et al. 1996, Scott 2009). These alkaloids have caused significant health, social and economic concerns at different times in history (Fuller 1968, Caporael 1976, Miles et al. 1996, De Groot et al. 1998, Alm 2003), but are also powerful pharmaceuticals for treating various medical conditions (De Groot et al. 1998, Crosignani 2006, Micale et al. 2006). Understanding the genetic diversity of species, their correlated toxin profiles and molecular backgrounds is important for the agricultural and pharmaceutical sectors, and regulatory agencies.

Intraspecific variations in morphology, alkaloid chemistry, genetics, and ecological niches have revealed the existence...
of several subgroups (Pazoutová et al. 2000) in C. purpurea s. lat. These groups were later identified as cryptic species based on multi-gene phylogenetic and population genetic analyses, i.e. C. arundinis, C. humidiphila, and C. spartinae (Douhan et al. 2008, Pazoutová et al. 2015) and recently a few more species from South Africa were described as a part of the species complex (Van der Linde et al. 2016). In Canada, the incidence of ergot diseases in Alberta, Saskatchewan and Manitoba has been increasing since 2002 (Menzies & Turkington 2014). During a recent investigation of ergot fungi in agriculture areas in Canada, we discovered a few more new phylogenetic lineages: two closely related but different from C. spartinae (G3), one close to C. humidiphila, and another two located as basal branches in the C. purpurea s. lat. complex (Fig. 1). Here, we selected representatives of these new and previously designated lineages, sequenced and assembled their genomes. A complete multigene phylogenetic analysis, including a much greater sampling of strains, will be presented in a separate publication.

**SEQUENCED STRAINS**

*Claviceps purpurea s.str.*  

*Claviceps purpurea s.lat.*  
**Canada:** Alberta: North Star 58.53 N 118.12 W, isolated from *Bromus inermis*, 7 Sep. 1956 [identified by W. P. Campbell] (LM78 = DAOMC 250578); Metiskow, 52.41 N 110.63 W, isolated from *Elymus albicans*, 7 Sep. 1956 [identified by W. P. Campbell] (LM81 = DAOMC 250581); Quebec: Cote Nord, MRC Minganie,Pointe-Parent, 50.13 N, 61.08 W, isolated from *Ammophila sp.*, 8 Sep. 2015, J. Cayouette & Y. Dalpé (LM458 = DAOMC 251898).

*Claviceps cf. spartinae*  

*Claviceps humidiphila*  

*Claviceps arundinins*  
**Czech Republic:** Haklovy Dvory, Stary Vrbensky pond, 49.01 N 14.43 E, isolated from *Phragmites australis*, 11 Jan. 2008, M. Kolářik (LM583 = DAOMC 251724 = CCC933 ex-type).

**NUCLEOTIDE SEQUENCE ACCESSION NUMBERS**

The genome data for the nine strains were deposited at DDBJ/EMBL/GenBank under BioProject PRJNA449361. The versions described in this paper are version QERD01000000, QEQW01000000, QEQX01000000, QEQY01000000, QEQZ01000000, QERA01000000, QERB01000000, QERC01000000, QERE01000000. Raw reads were deposited in NCBI SRA (http://www.ncbi.nlm.nih.gov/sra) under accession number SRP139610.

**MATERIALS AND METHODS**

Fungal mycelia and spores were inoculated onto Potato Dextrose Agar (PDA; BD DifcoTM) by streaking the medium surface in Petri dishes using inoculation loops, to allow the fungus to cover the dish in a short time and incubated for 10 d at 20 °C in the dark. Mycelia of 1–2 dishes were harvested and ground using liquid N2, followed by genomic DNA extraction using a modified cetyltrimethyl ammonium bromide (CTAB) protocol (Doyle & Doyle 1987). The protocol was modified to remove RNA by adding extra RNase and incubating for longer time as follows: (1) after homogenization of ground fungal tissues with 700 µl 2X CTAB, 140 µl RNase Cocktail™ Enzyme Mix (4x recommended volume ratio: 2.5 µl / 50 µl sample; Invitrogen by Thermo Fisher Scientific), and 28 µl RNASE A (4x recommended volume ratio: 1 µl / 100 µl sample) was added, and digested for 6–7 h at room temperature. (2) Afterwards, 10 µl Proteinase K (50 µg/µl stock; Fisher Scientific by Thermo Fisher Scientific) was added to the suspension and incubated for 2 h at 55 °C. (3) Before DNA precipitation, another 35ul RNase Cocktail (recommended volume ratio without extra) was added into the supernatant, incubated for 45 min – 1 h at room temperature, followed by adding 650 µl CHCl3 and centrifuging at 13 000 rpm for 15 min. Potential RNA contamination in the DNA samples was checked by running the samples on 1% agarose gels, and determining the 260/280 ratio using NanoDrop 1000 Spectrophotometer v3.8 (Thermo Fisher Scientific) to ensure no RNA was present. gDNA was quantified using Qubit® 2.0 Fluorometer (Invitrogen by Life Technologies).

The extracted gDNAs were normalized to 400 ng and sheared to a 300 bp insert using a Covaris LE220 instrument. The fragmented inserts were used as a template to construct PCR free Libraries with a NxSeq AmpFREE Low DNA Library kit (Lucigen) following the manufacturer’s instructions. Indexed libraries were pooled into two individual pools and sequencing runs were carried on a NextSeq (Illumina; Molecular Technologies Laboratory, Ottawa Research & Development Centre, Agriculture and Agri-Food Canada) using 2x150 bp NextSeq Mid Output Reagent Kits (Illumina).

The FastQC software v0.11.5 (https://www.bioinformatics. babraham.ac.uk/projects/fastqc/) was used to assess the raw read quality. Poor quality data was removed with Trimmomatic v0.36 (Bolger et al. 2014) using the following parameters: HEADCROP:20 SLIDINGWINDOW:4:20 MINLEN:36. The trimmed reads were error corrected with BayesHammer (Nikolenko et al. 2013). De novo genome assembly was
Fig. 1. One of the two MP trees showing nine strains (in bold) in relation to Claviceps lineages based on EF1-a partial region. 99 informative characters, length = 302, CI = 0.608, RI = 0.807, RC = 0.489, HI = 0.394, G-fit = -75.089. Values on branches are MP bootstrapping/BI posterior probability.

performed using SPAdes v3.10.1 (Bankevich et al. 2012) with the mismatch correction step enabled. Contigs shorter than 1000 bp were discarded. QUAST v4.5 program (Gurevich et al. 2013) was used to evaluate assemblies. Corrected reads
Table 1. Statistics of Claviceps genomes sequenced in this study.

| Species                  | Strain | Voucher # | Version # | Number of
|                        |        |           |           | gene models |
|-------------------------|--------|-----------|-----------|-------------
| Claviceps purpurea s.str. | LM26   | DAOMIC 25688 | CCA000000.000 | 1430 |
| Claviceps purpurea s.str. | LM582  | DAOMIC 251723 | CCA000000.000 | 2027 |
| Claviceps purpurea s.str. | LM78   | DAOMIC 256576 | CCA000000.000 | 2318 |
| Claviceps purpurea s.str. | LM81   | DAOMIC 251631 | CCA000000.000 | 1423 |
| Claviceps purpurea s.str. | LM428  | DAOMIC 251820 | CCA000000.000 | 1630 |
| Claviceps purpurea s.str. | LM218  | DAOMIC 251904 | CCA000000.000 | 2015 |
| Claviceps purpurea s.str. | LM545  | DAOMIC 251717 | CGC2000000.000 | 2015 |
| Claviceps purpurea s.str. | LM576  | DAOMIC 252724 | CGC2000000.000 | 1613 |
| Claviceps purpurea s.str. | LM583  | DAOMIC 253500 | CGC2000000.000 | 1442 |

RESULTS AND DISCUSSION

The strains sequenced include: two strains of *Claviceps purpurea* s.str. and *C. Cf. Spartinae*, one strain each of *C. humidipilosa* and *C. arundinis*, and three strains of *C. purpurea* s.lat. (Table 1, Fig. 1). The genomes were assembled into 1423 to 2321 contigs with a mean assembly size 30.8 Mb ranging from 28.6 Mb to 35.9 Mb. The average GC content was 51.5 %. The N50 ranged from 21.6 kb to 46.6 kb. The assemblies had a BUSCO completeness score ranging from 96.9 % to 98.6 %; the number of gene models ranged from 8410 to 9230.

The genome of *Claviceps purpurea* strain 20.1 (NCBI accession no. CAGA0000000.1) was previously published by (Schardl et al. 2013). They obtained a similar number of contigs (1442), number of gene models (8823), total assembly size (30.9 Mb), N50 (46.5 kb) and GC content (51.6 %), compared to our assemblies, suggesting we obtained reasonable assemblies. However, they additionally ordered contigs into only 191 scaffolds and performed genome annotation. We ran BUSCO on their assembly and obtained a completeness score of 97.6 %. Our aim is to use these sequenced *Claviceps* genomes to further understand the species diversity, genetic variation, and establish correlations with ergot alkaloid chemical profiles.


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IMA Genome-F 10B

Assembling pseudomolecules for the pitch canker pathogen, *Fusarium circinatum*, utilising additional genome sequence data and synteny within the *Fusarium fujikuroi* complex

INTRODUCTION

*Fusarium* includes a diverse group of filamentous ascomycetes (Geiser et al. 2013). Many of these fungi cause diseases on economically important plants, with an estimated 80 % of cultivated crops having at least one associated *Fusarium* disease (Leslie & Summerell 2006). Within the *Fusarium fujikuroi* species complex (FFSC), more than 50 phylogenetically distinct species have been grouped into three biogeographical clades (O’Donnell et al. 1998, 2000). *Fusarium circinatum*, residing in the so-called “American clade”, is the causal agent of the disease known as pine pitch canker that damages susceptible *Pinus* spp. and *Pseudotsuga menziesii* (Douglas Fir). It has a cosmopolitan distribution and is associated with significant economic losses due to widespread seedling mortality in nurseries as well as the reduction of growth in mature trees due to dieback of infected branches (Gordon et al. 2015).

The importance of this pathogen has justified sequencing its genome (GenBank accession AJYJ00000000, version AJYJ01000000) (Wingfield et al. 2012). The isolate sequenced, FSP34 (Gordon et al. 1996), has a genetic linkage map available (De Vos et al. 2007) which has been anchored to the genome (De Vos et al. 2014) enabling localization of quantitative trait loci (QTLs) to the genomic sequence data (De Vos et al. 2011, Van Wyk et al. 2018). The draft assembly was 94.8 % complete (Waterhouse et al. 2017), but it included an exorbitant number of contigs (4145) (Wingfield et al. 2012, De Vos et al. 2014) and this limits its use in comparative genomic studies.

The whole genome sequences of other members of the FFSC are available and their genome complement is present in chromosomes. These include *Fusarium verticillioides* for which the sequences for only 11 chromosomes are available (Ma et al. 2010). This *F. verticillioides* assembly excludes that for the twelfth and smallest chromosome known to exist in members of the FFSC. This is due to the dispensable nature of this chromosome, with it being strain-specific within the FFSC (Xu et al. 1995, Ma et al. 2010, Wiemann et al. 2013, Van der Nest et al. 2014). In contrast, the whole molecule sequences have been determined for the full complement of the twelve chromosomes for *F. fujikuroi* (Wiemann et al. 2013). These two species represent two of the three biogeographical clades of the FFSC. Comparisons among them and *F. temperatum* have shown a significant level of macrosynteny at the genomic sequence level (Wiemann et al. 2013, De Vos et al. 2014). This highlights the fact that the genomic content on chromosomes is highly conserved between various species in the FFSC.

The aim of this study was to improve the available draft assembly of *F. circinatum*, and assemble it into pseudomolecules that are comparable with the chromosomes of other members of the FFSC. For this purpose, we utilized additional genome sequence information (i.e. mate-pair sequence data) to allow for the scaffolding of contigs. We then exploited the macrosynteny that characterizes the genomes of species within FFSC (Wiemann et al. 2013, De Vos et al. 2014), to orientate and order these scaffolds into twelve pseudomolecules. In this study we present the pseudomolecule assemblies for the full chromosomal complement of *F. circinatum*. This improved genome assembly will aid in genome-sequence based studies, particularly those involving chromosomal comparisons. Addition of the *F. circinatum* pseudomolecule complement will furthermore enable comparative studies focusing on genomic synteny and architecture between the three biogeographic clades within the FFSC, as well as more broadly in the genus *Fusarium*.

SEQUENCED STRAIN


NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The improved genome assembly for *Fusarium circinatum*, generated in this study, has been deposited at DDBJ/EMBL/GenBank under the accession AJYJ00000000, version AJYJ02000000.

MATERIALS AND METHODS

*Fusarium circinatum* was grown on half strength potato dextrose broth (20 % potato dextrose broth w/v) and incubated at 25 °C in the dark on an orbital shaker at 120 rpm. After 7 d, DNA was extracted following the protocol outlined (Möller et al. 1992) and the DNA quality was assessed using a Nanodrop™ Spectrophotometer.

Additional genomic sequence data from *F. circinatum* isolate FSP34 and a second isolate, KS17, were utilised for scaffolding the original 4145 contig assembly (Wingfield et al. 2012). Isolate KS17 was cultured from infected root tissue of *P. radiata* nursery seedlings collected from the Western Cape, South Africa in 2005 (Steenkamp et al. 2014). The genomes of *F. circinatum* isolates FSP34 and KS17 were sequenced using mate-pair libraries (1 kb insert size) by making use of the SOLiD™ V4 technology (Applied Biosystems) at Seqcomics (Hungary). In total, 82.45 and 153.95 million mate-pair reads were obtained for the respective isolates. Poor quality reads (below Q20), reads smaller than 36 bp and duplicate reads were removed in CLC Genomics Workbench v.5.1 (CLCbio, Aarhus).
SSPACE v. 2.0 (Boetzer et al. 2011) was utilized to scaffold the pre-assembled contigs of the FSP34 assembly (GenBank accession no. AYJV00000000) (Wingfield et al. 2012), using the trimmed mate-pair data. Default parameters were used, but the minimum number of paired reads linking contigs to form a scaffold was set to 200. The average genome coverage was calculated using the Lander/Waterman equation (number of reads x read length/genome size).

The resulting scaffolds were then assembled into 11 contiguous pseudomolecules (representing the first chromosome 1-11) using F. verticillioides as a reference genome. The scaffolds were ordered and orientated based on BLAST searches (Altschul et al. 1990) against a local database of the F. verticillioides genome (DDBJ/EMBL/GenBank accession number AAMX00000000.2) using CLC Genomics Workbench. To assemble pseudomolecule 12, scaffolds were ordered and orientated to chromosome 12 of F. fujikuroi (Wiemann et al. 2013) and F. temperatum (Wingfield et al. 2015b), as described above. To indicate a break between the various scaffolds comprising a pseudomolecule, 100 N’s were inserted. Synteny maps were generated between the chromosomes of F. verticillioides and F. fujikuroi and the pseudomolecules of F. circinatum using the program MUMmer v. 3.22 (Kurtz et al. 2004).

The assembled genome was annotated using the MAKER annotation pipeline (Cantarel et al. 2008) utilizing Genemark ES (Ter-Hovhannisyan et al. 2008), Augustus (Stanke & Morgenstern 2005), and SNAP (Korf 2004). Manual curation of the predicted annotations was also performed (Wingfield et al. 2012). As additional evidence, genome data from F. verticillioides, Fusarium oxysporum f. sp. lycopersici and F. graminearum (Ma et al. 2010), as well as expressed sequence tag (EST) evidence for F. circinatum (Wingfield et al. 2012) were included.

**RESULTS AND DISCUSSION**

The improved genome assembly for *Fusarium circinatum*, generated in this study, has been deposited at DDBJ/EMBL/GenBank under accession no. AYJV00000000, version AYJV02000000. The *F. circinatum* genome was assembled into 585 scaffolds that cumulatively comprised 43,932,912 bases of DNA and had a N50 of 363,633bp. The genome coverage was 273.82X. A GC content of 47.41% was obtained, which is comparable to other sequenced *Fusarium* species within the FFSC (Ma et al. 2010, Wingfield et al. 2012, Jeong et al. 2015a, IMA FUNGUS 2012). 2013). Van der Nest et al. 2014, Chira et al. 2015, Wingfield et al. 2015a, b, Niehaus et al. 2017a, b, Wingfield et al. 2017, Gardiner 2018, Srivastava et al. 2018, Van Wyk et al. 2018, Wingfield et al. 2018). A total of 14,923 genes were predicted to be protein-coding, yielding a gene density of 339.68 open reading frames (orfs) per million base pairs. Phylogenetic analysis of the sequenced genome confirmed the taxonomic identity as *F. circinatum* (Fig. 2).

Pseudomolecules, corresponding to each of the 11 chromosomes of *F. verticillioides*, were constructed in this study. Pseudomolecule 12 was assembled according to synteny observed with chromosome 12 of *F. fujikuroi*. We managed to genetically anchor 96.97% (ca. 42.60 Mb) of the *F. circinatum* scaffolds to these 12 pseudomolecules. These pseudomolecules harbour 99.09% of the 15,060 genes originally predicted for *F. circinatum* (Wingfield et al. 2012). Genomic alignments of these 12 pseudomolecules to the corresponding *F. verticillioides* and *F. fujikuroi* chromosomes are shown in Fig. 3. These dot-blots are indicative of the observable macrosynteny of *Fusarium* species within the FFSC.
CONCLUSIONS

Resequencing has provided a vastly improved assembly for *Fusarium circinatum* characterized by fewer and significantly larger scaffolds. By making use of the macrosynteny known to characterize the genomes of FFSC species (Wiemann et al. 2013, De Vos et al. 2014), we were able to join these scaffolds into 12 pseudomolecules that represent the haploid chromosome number of this fungus.

This new assembly of the *F. circinatum* genome provides a considerably more robust and complete representation of the whole genome sequence of the pathogen, including pseudomolecules that correspond to the twelve chromosomes of *F. circinatum*. The availability of the sequence for these pseudomolecules will enable future comparative studies at the chromosomal level between/within the three biogeographic clades of the FFSC. In addition, comparisons of chromosomal architecture will expand our knowledge regarding the genomes of species in the FFSC and highlight inter- and intraspecific similarities and differences between them. This would broaden available knowledge regarding the evolution of an important group of plant pathogens.


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IMA Genome-F 10C

Draft genome sequence of *Quambalaria eucalypti*

INTRODUCTION

The genus *Quambalaria* (*Quambaliaceae, Microstromatales, Exobasidiomycetes, Basidiomycotina*) includes mainly leaf and shoot pathogens of trees belonging to *Myrtaceae* (De Beer et al. 2006, Pegg et al. 2008). The exceptions to this are *Q. coycerep* which is a canker pathogen of marri (*Corymbia calophylla*) in Western Australia (Paap et al. 2008), and *Q. cyanescens* which is an opportunistic pathogen of primarily immunocompromised or debilitated humans (Kuan et al. 2015). The latter species is also frequently isolated from galleries of bark beetles infesting hardwoods, although its ecological role in these ecosystems remains enigmatic (Kolarik et al. 2006). Of the leaf and shoot pathogens in the genus, *Q. pitereka* and *Q. eucalypti* are most important. *Quambalaria pitereka* affects *Corymbia* species in Australia (Pegg et al. 2009) and China (Zhou et al. 2008), while *Q. eucalypti* causes disease on *Eucalyptus* spp. in South Africa (Wingfield et al. 1993), Brazil (Alfenas et al. 2001), Uruguay (Perez et al. 2008), Australia (Pegg et al. 2008), and Portugal (Braganca et al. 2015).

Research on species of *Quambalariaeae* has mostly focussed on their classification and taxonomy (De Beer et al. 2006, Kipomonyangpa and Aime 2017, Paap et al. 2008), as well as their pathogenicity and impact on tree health (Pegg et al. 2009, 2011). However, little is understood about the life-cycle and general biology of these fungi, that are related to the smut fungi and human pathogenic members of *Malasseziales* (De Beer et al. 2006, Wang et al. 2015b). For
example, it is not yet known whether *Quambalariaceae* have the ability to reproduce sexually, and if so, what the mating system of these fungi encompasses.

Recently the first genome-based studies started exploring pathogenicity factors in *Ustilaginozymycota*, and although two species in *Microstromatales* were included in the comparative analyses, no representative of *Quambalariaceae* was incorporated (Kijpornspongpan et al. 2018). Whole genome sequences have also been shown to be extremely valuable to study mating systems in smut fungi (Que et al. 2014), the *Malasseziales* (Xu et al. 2007), and the more distantly related *Polyporales* (James et al. 2013).

The aim of this study was to produce a draft genome sequence of *Q. eucalypti*. This genome sequence will allow for the exploration and comparative analyses of genes involved in pathogenicity and mating for this pathogen. Here we report the draft genome sequence of isolate CMW1101, an isolate representing the holotype (PREM 51089) of *Q. eucalypti*.

**SEQUENCED STRAIN**

**South Africa:** KwaZulu Natal: Kwambonambi, *Eucalyptus grandis* clone TAG12, May 1987, M.J. Wingfield (CMW1101=CBS118844 ex-holotype isolate; PREM 51089 – holotype).

**NUCLEOTIDE ACCESSION NUMBER**

The draft genome sequence of *Quambalaria eucalypti* has been deposited at GenBank under accession no. PIRRYC00000000. The version presented here is RRYC01000000.

**MATERIALS AND METHODS**

Genomic DNA was extracted from cultures grown on Malt Yeast Agar (2% Malt extract, 0.5% yeast extract; 2% agar,
Biolab, Midrand, South Africa) using the method described by Duong et al. (2013). The genomic DNA was sent to Macrogen (South Korea), where one pair-end library with 500 bp insert size was prepared and sequenced on Illumina HiSeq 2500 to get 250 bp pair-end reads, aiming for 100 X coverage. The raw sequencing reads were imported into CLC Genomics Workbench v. 7.5.1 (CLCbio, Aarhus), and default settings were used to both trim the reads for quality and to produce a de novo genome assembly using the trimmed reads. The completeness of the assembly was evaluated using the Benchmarking Universal Single-Copy Orthologs (BUSCO v. 1.1b1) tool using the Basidiomycota dataset (Simao et al. 2015). The number of protein coding genes was determined using Augustus v. 3.3.2 (Stanke et al. 2008) using pre-optimised species models for Ustilago maydis.

RESULTS AND DISCUSSION

The paired end sequencing yielded just over 31 million reads. Assembly of the trimmed reads resulted in 966 contigs, with the largest contig being 225 583 bp, the smallest contig being 449 bp, with an average contig size of 24 384 bp and the N50 value was 62 600 bp. The genome size is estimated at around 23.5 Mb, estimated through the sum of the contig sizes, with a GC content of 60 %. This estimated size is in the larger size range of that reported in Exobasidiomycetes, which typically range from 17 Mb to 19 Mb with the exception of Tillettia caries with a genome size of 29.5 Mb (Konishi 2013, Saika et al. 2014, Toome et al. 2014, Wang et al. 2015a, Kijpornsngpan et al. 2018). BUSCO analysis indicated an assembly completeness of 84.5 %. The assembly contained 1128 complete BUSCOs (1093 complete single-copy BUSCOs, 35 complete and duplicated BUSCOs), 129 fragmented BUSCOs and 78 missing BUSCOs out of a total 1335 BUSCO groups searched. AUGUSTUS predicted 7241 putative protein coding regions. Phylogenetic analysis of sequences from the sequenced genome confirmed the taxonomic identity as Q. eucalypti (Fig. 4). The availability of the Q. eucalypti genome will enable the inclusion of this species as representative for the family Quambalariaceae in comparative studies with other members of the class Exobasidiomycetes. Such studies could focus on topics like the factors involved in pathogenicity, mating, evolution and more.

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**IMA Genome-F 10D**

**Draft genome sequence of the Eucalyptus pathogen Teratosphaeria destructans**

**INTRODUCTION**

The genus Teratosphaeria includes numerous economically important tree pathogens of plantation eucalypts in tropical and subtropical areas (Park et al. 2000, Crous et al. 2009, Hunter et al. 2011). The aggressive pathogen *T. destructans* was initially reported from Indonesia in 1996 (Wingfield et al. 1996), followed by reports from Thailand, Vietnam, East Timor, Laos, China, and, most recently, South Africa (Old et al. 2003, Burgess et al. 2006, Barber et al. 2012, Greyling et al. 2016). It causes leaf, bud and shoot blight disease in one- to three-year-old trees of Eucalyptus camaldulensis, *E. grandis* and *E. urophylla* as well as on hybrids of these species (Wingfield et al. 1996, Old et al. 2003, Barber 2004). The rapid spread of *T. destructans* over large distances has been attributed to the anthropogenic movement of germplasm to establish clonal Eucalyptus nurseries (Andjic et al. 2011).

The discovery of *T. destructans* on clonal *E. grandis* × *E. urophylla* plantations in South Africa, coupled with its reported rapid spread to new areas, makes this pathogen of major concern to the forestry industries of southern African countries (Andjic et al. 2011, Greyling et al. 2016). Similarly, the ability of this pathogen to rapidly invade new areas are also a concern to Australia, where the prospect of *T. destructans* spreading to native Eucalyptus species could prove catastrophic to Australia’s commercial and natural vegetation (Old et al. 2003). In this study, the genome sequence of a South African isolate of *T. destructans* is reported. The availability of a complete genome sequence for *T. destructans* will prove beneficial to studies on the genes and pathways involved in virulence, pathogenicity and sexual reproduction. Such studies will increase our understanding of the biology of this fungus, which is crucial for the development of preventative and control measures for this tree pathogen.

**SEQUENCED STRAIN**

**South Africa:** Kwa-Zulu Natal: Kwambonambi,isol. *Eucalyptus grandis* × *E. urophylla*, Apr. 2015, I. Greyling (CMW 44962, PREM 62207 – dried specimen).

**NUCLEOTIDE ACCESSION NUMBER**

The *Teratosphaeria destructans* Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under accession no. RIBY00000000. The version described in this paper is version RIBY01000000.

**MATERIALS AND METHODS**

*Teratosphaeria destructans* isolate CMW 44962 was obtained from the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria. A single-spore culture was generated (Greyling et al. 2016) and grown on sterile cellophane sheets placed onto MEA+V plates (malt extract agar; Biolab, South Africa; amended with 0.3 % yeast extract; Oxoid, Basingstoke). After incubation for four weeks at 25 °C, the mycelia were collected and freeze-dried for DNA extraction. Approximately 30 mg of freeze-dried material, three 5 mm
Fig. 5. A Maximum Likelihood phylogeny showing *Teratosphaeria* species including the genome sequences of *Teratosphaeria destructans* reported here. The β-tubulin and EF1-α gene regions were used and were obtained from previous studies (Quaedvlieg et al. 2014, Aylward et al. 2018).

glass beads and 13 mg polyvinylpyrrolidone (PVP, Sigma Aldrich, Steinheim) were mixed and powdered in a FastPrep FP120 tissue lyzer (Qiogene, Carlsbad, CA). Subsequently, 650 μL of CTAB extraction buffer (100 mM Tris-HCl, pH 8; 25 mM EDTA; 2 M NaCl; 3.5 % CTAB; 2 % SDS), 2 μL of 500 mg/L spermidine (Sigma Aldrich, Steinheim) and 4.5 % (v/v) β-mercaptoethanol were added and the sample was incubated at 60 °C for 20 min. After cooling to room temperature, 1.5 volumes of chloroform:isoamylic alcohol (24:1) was added, the sample was mixed and centrifuged for 15 min at 8100 g. The supernatant was re-extracted with 1.5 volumes of chloroform:isoamylic alcohol (24:1) and centrifuged for 15 min at 16 300 g. The supernatant was combined with potassium acetate to a final concentration of 1.5 M. After incubation for 30 min at –20 °C, 1.5 volumes of cold isopropanol were added, followed by a 30 min incubation at 24 °C. Thereafter, the sample was centrifuged for 20 min at 16 300 g to collect the DNA which was washed twice with 70 % ethanol. The dried DNA pellet was dissolved in 50 μL low TE (Tris-EDTA) buffer (Thermo Fisher Scientific, Wilmington, NC).

The genomic DNA sample was submitted to the Central Analytical Facilities (CAF) of Stellenbosch University (Stellenbosch, South Africa) for whole genome sequencing using the Ion GeneStudio S5 Next-Generation Sequencer. An Ion 530 chip with a capacity to generate 12 million reads of 600 bp was prepared as per the manufacturer’s instructions. The resulting single reads were trimmed and assembled using SPAdes v. 3.12.0 (Nurk et al. 2013) with k-values of 21, 33, 55, 77, 99 and 127. The completeness of the genome assembly was evaluated using the Benchmarking Universal Single-Copy Orthologs (BUSCO v. 2.0.1) tool in conjunction with the fungal data set (Simão et al. 2015). Bowtie2 v. 1.1.2 and SAMtools v. 1.5 were used to calculate the average base coverage by mapping the reads back to the genome assembly (Li et al. 2009, Langmead & Salzberg 2012). QUAST v. 5.0.1 (Mikheenko et al. 2018) was used to calculate general genome statistics.

The β-tubulin and elongation factor 1-alpha (EF1-α) gene regions, commonly used for species delineation in *Teratosphaeria* (Quaedvlieg et al. 2014) were extracted from the *T. destructans* genome sequence. These sequences, together with previously published sequences (Quaedvlieg et al. 2014, Aylward et al. 2018) sourced from the NCBI database (https://www.ncbi.nlm.nih.gov/) were subjected to a “one click” phylogeny analysis using the Phylogeny.fr online tool (Dereeper et al. 2008, 2010). This analysis included a MUSCLE alignment (Edgar 2004) and a Gblocks (Castresana 2000) curation step before phylogenetic analysis was conducted using PhyML (Guindon & Gascuel 2003, Anisimova & Gascuel 2006).

RESULTS AND DISCUSSION

The assembly yielded a genome of 32 316 120 bp assembled into 4132 contigs. Of these, 1837 were 500 bp or larger and contained 31.61 Mb of the genome. The genome had a GC content of 51.83 %, a N50 value of 103 119 bases (with
the largest contig 398 757 bp in size), and a L50 value of 94 contigs. The average coverage of this assembly was 170x. The genome completeness, as assessed by BUSCO analysis, was 95.86 %, with 278 complete, five fragmented and seven missing BUSCO terms out of the 290 searched.

The genomic sequence of *Teratosphaeria destructans* reported here is the first published genome sequence for a member of this genus (Fig. 5). Considering its status as an emerging pathogen (Andjic et al. 2011, Greyling et al. 2016, Burgess & Wingfield 2017) the availability of a genome sequence holds various benefits. These include its use in comparative genomic studies between different *Teratosphaeria* species, many of which are a concern to the global *Eucalyptus* industry (Hunter et al. 2011). Similar studies have already yielded insight into the factors that determine host range, while also elucidating an arsenal of pathogenic effector and virulence factors important for the pathogenicity of fungal species (Klosterman et al. 2011, Condon et al. 2013, Zhao et al. 2013, Deng et al. 2017). The availability of a genome sequence also provides the opportunity to develop species-specific microsatellite markers (Gnocato et al. 2017, Rafiei et al. 2018) that would be important for studying invasive populations of this aggressive, emerging tree pathogen.

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IMA Genome-F 10E

**Draft nuclear genome assembly for *Davidsoniella eucalypti***

**INTRODUCTION**

The family *Ceratocystidaceae* includes an ecologically diverse assemblage of fungi currently classified into 11 distinct genera (De Beer et al. 2014, 2017, Mayers et al. 2015, Nel et al. 2018). The best-known of these is *Ceratocystis*, a genus that includes economically important plant pathogens such as the mango and *Acacia mangium* pathogen *C. manginecans* (Al Adawi et al. 2013, Tarigan et al. 2011) and the causal agent of cacao wilt *C. cacaofunesta* (Baker Engelbrecht & Harrington 2005). Species of the genera *Endoconidiophora* and *Thielaviopsis* are well known as pathogens of conifers and monocot plants, respectively (Mbenoun et al. 2014, Wingfield et al. 2013). The genera *Bretziella* and *Berkeleyomyces* were recently erected to accommodate the casual-agent of oak wilt *Br. fagacearum* (previously *Ceratocystis fagacearum*; De Beer et al. 2017) and the multi-host root pathogen *Be. basiola* (formerly *Thielaviopsis basicola*; Nel et al. 2018). Many *Ceratocystidaceae* species rely on flies, picnic beetles, and ambrosia beetles for spread, forming casual associations with these insects (Van Wyk et al. 2013). In contrast, species in the genera *Ambrosiella*, *Meredithiella*, and *Phialophoropsis* form obligate mutualisms with ambrosia beetles of the tribes *Xyleborini*, *Corthylini* and *Xylotermi*, respectively (Mayers et al. 2015). Three asexual species associated with woody substrates are present in *Chalaropsis*, while members of the genus *Huntiella* are considered saprobes or weak pathogens, with only a handful of species known to cause sapstain of timber (De Beer et al. 2014). The genus *Davidsoniella* consists of four species, three of which (*D. eucalypti*, *D. neocaledoniea* and *D. australis*) are present in Australasian, with *D. virescens* being described from North America (De Beer et al. 2014).

In this study we report a draft nuclear genome assembly for *Davidsoniella eucalypti*, first isolated from stem wounds on living *Eucalyptus* species in Australia (Kile et al. 1996). Although able to colonize wounds made in these trees, the fungus causes limited damage and is not considered pathogenic (Kile et al. 1996). In contrast, *D. virescens*, *D. australis*, and *D. neocaledoniea* are all considered plant-pathogens, causing disease on maple trees (*Acer* spp.), *Nothofagus cunninghamii* trees (Kile 1993), and *Coffea* robusta plants (Dantad 1950), respectively.

*Davidsoniella eucalypti* and *D. virescens* are the only two species in the genus for which a sexual morph is known (De Beer et al. 2014), although the reproductive strategy between these species differ dramatically – homothallism in *D. virescens* and heterothallism in *D. eucalypti* (Harrington et al. 1998). With the genome sequence of *D. virescens* already published (Wingfield et al. 2015b), the addition of the *D. eucalypti* genome brings the total number of *Davidsoniella* sequences to two. This raises the possibility for interesting genomic studies on these two biologically diverse species.

**SEQUENCED STRAIN**


**NUCLEOTIDE SEQUENCE ACCESSION NUMBER**

This Whole Genome Shotgun project for *Davidsoniella eucalypti* isolate CMW 3254 has been deposited at DDBJ/ENA/GenBank under the accession RMBW00000000. The version described in this paper is version RMBW01000000.

**MATERIALS AND METHODS**

*Davidsoniella eucalypti* isolate CMW 3254 was obtained from the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI) and grown on 2 % malt extract agar (MEA: 2 % w/v, Biolab, South Africa) at 25 °C. A 14-d old culture was used for genomic DNA extraction using a previously described phenol-chloroform protocol (Roux et al. 2004). The isolated DNA was submitted to Macrogen (Seoul, Korea) to generate long-read sequences from three cells of the Pacific BioSciences Single-molecule real time (SMRT or
PacBio) sequencing platform. This was complemented by a second round of sequencing on the Illumina HiSeq 2500 instrument at Macrogen. For this, a single library with 550 bp insert size was generated and used to produce pair-end reads of 250 bp target length. The DNA for the Illumina sequencing was extracted as described by Duong et al. (2013).

The reads obtained from the three cells of the SMRT sequencing run were concatenated into a single fastq file which was used for read-correction, trimming and assembly using Canu v1.4 and default settings (Koren et al. 2017). The resulting assembly was scaffolded using SSPACE-LongRead with the default settings (Boetzer et al. 2011, Boetzer & Pirovano 2014), using the corrected long-read sequences produced by Canu. The paired-end Illumina reads were imported into the CLC Genomics Workbench v11.0.1 (Qiagen, South Africa) and trimmed using default settings. These trimmed reads were indexed and aligned to the scaffolded, long-read assembly using BWA (Li & Durbin 2009) and SAMtools (Li et al. 2009). The alignment files were used in three rounds of Pilon corrections (Walker et al. 2014) to improve the long-read assembly by correcting single base differences, small insertions/deletions and other mis-assemblies identified in the draft genome assembly. To produce the best version of the assembly, the trimmed Illumina pair-end reads were used by GapFiller (Boetzer & Pirovano 2012) to fill gaps produced in the assembly during the scaffolding process. The draft genome was assessed for completeness using the Benchmarking Universal Single Copy Orthologs tool (BUSCO v 2.0.1) (Simão et al. 2015) and the Ascomycota database. An estimation of the number of protein coding genes in the genome was made by the de novo prediction software AUGUSTUS using the Fusarium graminearum gene models (Keller et al. 2011, Stanke et al. 2006b), while general genome statistics (genome length, GC content, N50, L50 and largest contig size) were calculated using QUAST v5.0.1 (Mikheenko et al. 2018).

The 60S, LSU and MCM7 gene regions were extracted from the genome and, together with these regions from D. eucalypti, D. virescens, D. neocaledoniae, D. australis, Endoconidiophora polonica, and E. laricocola (De Beer et al. 2014) were used for phylogenetic analysis. The datasets were subjected to a “one click” phylogeny analysis at the Phylogeny.fr online tool (Dereeper et al. 2008, 2010) that included a MUSCLE alignment (Edgar 2004) and a Gblocks (Castresana 2000) curation step before phylogenetic analysis was conducted using PhyML (Guindon & Gascuel 2003). Branch support was calculated using the approximate likelihood ratio test (Anisimova & Gascuel 2006).

RESULTS AND DISCUSSION

The 41 874 515 bp genome assembly of Davidsoniella eucalypti was present in 1219 contigs of 1000 bp or large, the largest of which was 1 065 836 bp. The genome had a G/C content of 45.93 %, an average coverage of 69x, a N50 value of 230 092 bp and a L50 value of 51. AUGUSTUS predicted 9029 protein coding genes, while BUSCO reported a completeness score of 96.1 %. This was based on the analysis of 1315 orthologs, with 1236 present as complete single copies, 27 as complete duplicated copies, 16 as fragmented copies, and 36 copies absent.

The genome assembly of D. eucalypti differed dramatically from that of the sister-species D. virescens (Fig. 6) (Wingfield et al. 2015b). At 33.6 Mb, the latter genome was 8.3 Mb smaller than that of D. eucalypti. The latter genome is also predicted to encode more proteins (9029 vs. 6953 for D. virescens), although the gene densities were comparable at 207 and 215 genes/Mb for D. virescens and D. eucalypti respectively. It is known that the genome sizes of plant-pathogenic filamentous fungi tend to be larger than those of non-pathogenic relatives, mostly due to the presence of high amounts of repetitive DNA and an expansion of the effector repertoire (Frantzeskakis et al. 2018, Möller & Stukenbrock 2017, Raffaele & Kamoun 2012). Therefore, the larger genome size of the non-pathogenic D. eucalypti as compared to the pathogenic species D. virescens was surprising and warrants further study.

The hybrid assembly of D. eucalypti presented here has a N-50 value (230092 bp) twice that of D. virescens (Wingfield et al. 2015b). This improvement in contig contiguity can be attributed to the inclusion of long-read PacBio sequences (English et al. 2012), a trend seen for many other genomes (Huddleston et al. 2014, Koren et al. 2013, Koren & Phillippy 2015). The availability of a highly contiguous genome sequence for one Davidsoniella species could provide the basis for genomic comparative studies. These should be of much interest as the higher gene number and larger genome size of D. eucalypti might point to an interesting evolutionary history for the genome of this species.

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IMA Genome-F 10F

Draft genome sequence of *Grosmannia galeiformis*

**INTRODUCTION**

*Grosmannia galeiformis* was first described as *Ceratocystis galeiformes* from conifer infesting bark and ambrosia beetles in Scotland in 1951 (Bakshi 1951). It was later transferred to *Ophiostoma* (as *Ophiostoma galeiforme*; Mathiesen-Káráík 1953), and thereafter to *Grosmannia* (as *G. galeiformis*; Zipfel et al. 2006).

*Grosmannia galeiformis* is often found associated with conifer-infesting bark beetles and has been reported from Europe (Bakshi 1951, Linnakoski et al. 2012, Mathiesen-Káráík 1953, Zhou et al. 2004), South America (Zhou et al. 2004), and Africa (Zhou et al. 2004). Phylogenetic studies indicated that this species is part of a complex of several closely related species known as the *G. galeiformis* species complex, which is distinct from other species complexes in *Leptographium s. lat.* (Chang et al. 2019, De Beer et al. 2013 Linnakoski et al. 2012; Fig. 7). In this study, we sequenced and assembled the draft genome sequence for *G. galeiformis*, the key species representing the *G. galeiformis* species complex.

**SEQUENCED STRAIN**


**NUCLEOTIDE SEQUENCE ACCESSION NUMBER**

The genomic sequence of *Grosmannia galeiformis* (CMW 5290, CBS 115711) has been deposited at DDBJ/EMBL/GenBank under accession no. RQWE0000000. The version described in this paper is version RQWE01000000.

**METHODS**

Genomic DNA was extracted from freeze-dried mycelium obtained from a single spore culture of *G. galeiformis* (CMW 5290). DNA extraction was done following a previously described method (Duong et al. 2013). Genome sequencing was carried out on the Illumina HiSeq 2000 platform (University of California Davis, CA). Two libraries (350 and 550 bp insert sizes) were prepared and sequenced to obtain 100 bp pair-end reads. Obtained pair-end reads were trimmed using Trimmomatic v. 0.36 (Bolger et al. 2014), and *de novo* assembled using SPAdes v. 3.9.0 (Bankevich et al. 2012). Scaffolds obtained from SPAdes was further placed into larger scaffolds using SSPACE-Standard v. 3.0 (Boetzer et al. 2011), and assembly gaps were filled using GapFiller v. 1.10 (Boetzer & Pirovano 2012). The completeness of the resulting assembly was estimated using Benchmarking Universal Single Copy Orthologs (BUSCO) program v. 2.0.1 with the Sordariomycetesodb9 dataset (Simão et al. 2015). Protein coding gene models were predicted using the MAKER genome annotation pipeline (Cantarel et al. 2008) with the combination of GeneMark v. 4.32 (using self-training; Lomsadze et al. 2005) and AUGUSTUS v. 3.2.2 (using species models optimised for *Neurospora crassa*; Stanke et al. 2006a) as gene predictors.

**RESULTS AND DISCUSSION**

Over 25 million 100 bp pair-end reads were obtained after filtering and trimming. The final draft assembly consisted of 869 scaffolds that were over 500 bp in size. The assembly had a N50 of 67.79 Kb and a genome size of around 26.44 Mb. BUSCO reported the score for the assembly of 97 %[D:5.8 %], F:1.8 %, M:0.6 %, n = 1348 (C: complete; D: duplicated; F: fragmented; M: missing, n = number of genes), which is comparable to that from other species of *Leptographium s. lat.* generated from previous studies (Wingfield et al. 2015a, 2016). Genome annotation using MAKER pipeline with GeneMark and AUGUSTUS as gene predictors resulted in 8527 protein-coding gene models. The genome of *G. galeiformis* generated in this study will add to the already growing genome resources for species of ophiostomatoid fungi (Wingfield et al. 2015a, b, 2016), which will facilitate future comparative genomic and evolutionary studies of these fungi (Fig. 7).
ACKNOWLEDGEMENTS

The authors of the *Claviceps purpurea* s.lat. study thank Zlatko Popovic, Rafik Assabgui, and the Molecular Technologies Laboratory (MTL) at the Ottawa Research & Development Centre specifically Julie T. Chopados, Wayne McCormick and Lisa Kozioł for technical assistance. They also thank the Canadian Collection of Fungal Cultures (CCFC), Canadian National Mycological Herbarium (DAOM), and Jacques Cayouette for providing ergot samples or cultures. Genome sequencing was funded by Agriculture and Agri-Food Canada’s Growing Forward 2 for a research network on Emerging Mycotoxins (EmTox, project # J-000048).

The authors of the *Davidsoniella eucalypti, Fusarium circinatum, Grosmannia galeiformis and Teratosphaeria destructans* study acknowledge funding received from the Department of Science and Technology (DST)/National Research Foundation (NRF) Centre of Excellence in Tree Health Biotechnology (CTHB), South Africa and the DST-NRF SARChI chair in Fungal Genomics. The *F. circinatum* study was also supported by an NRF Scarce Skills Postdoctoral Fellowship awarded to the first author. P.M.W. received a scholarship from the Claude Leon foundation.

The authors of the *Quambalaria eucalypti* study thank the University of Pretoria, the Tree Protection Co-operative Programme (TPCP), the DST-NRF Centre of Excellence in Tree Health Biotechnology the National Research Foundation and the DST-NRF SARChI chair in Fungal Genomics for their financial support.

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