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ARTICLE

IMA Genome-F 8

Draft genome of *Cercospora zeina*, *Fusarium pininemorale*, *Hawksworthiomyces lignivorus, Huntiella decipiens* and *Ophiostoma ips*

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Abstract: The genomes of *Cercospora zeina*, *Fusarium pininemorale*, *Hawksworthiomyces lignivorus, Huntiella decipiens,* and *Ophiostoma ips* are presented in this genome announcement. Three of these genomes are from plant pathogens and otherwise economically important fungal species. *Fusarium pininemorale* and *H. decipiens* are not known to cause significant disease but are closely related to species of economic importance. The genome sizes range from 25.99 Mb in the case of *O. ips* to 4.82 Mb for *H. lignivorus*. These genomes include the first reports of a genome from the genus *Hawksworthiomyces*. The availability of these genome data will allow the resolution of longstanding questions regarding the taxonomy of these species. In addition these genome sequences through comparative studies with closely related organisms will increase our understanding of how these species or close relatives cause disease.

Key words:
Gray Leaf Spot
maize
insect vectored fungi
Fusarium fujikuroi species complex
Ophiostomatales
wood decay
Ceratocystidaceae
blue stain

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IMA Genome-F 8A

Draft genome of an African isolate of the maize fungal pathogen, *Cercospora zeina*

Maize (*Zea mays*) is one of the most important staple food crops, especially in Sub-Saharan Africa. In the last three decades, Gray Leaf Spot (GLS) has become a widespread foliar disease of maize globally (Latterell & Rossi 1983, Ward *et al.* 1999, Meisel *et al.* 2009, Berger *et al.* 2014). Since GLS leads to substantial yield losses, it poses a threat to food security, especially in Africa (Meisel *et al.* 2009). Two causal agents of GLS have been identified, with *Cercospora zeae-maydis* the predominant pathogen in the USA (Wang *et al.* 1998), and to date, only *Cercospora zeina* reported from Africa (Crous *et al.* 2006, Meisel *et al.* 2009). These species belong to the class *Dothidiomycete*, that contains several important plant pathogens (Ohm *et al.* 2012). However, little is known about pathogenicity mechanisms of *C. zeina*.

SEQUENCED STRAIN

Zambia: *Central region* (*Mkushi*): isol. ex *Zea mays* (maize), March 2007, *F.J. Kloppers & B. Meisel* (CMW25467, MUCL 51677, CBS142763, PREM 61898 – dried culture).

NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The draft genome has been deposited at DDBJ/ENA/GenBank and is available under the accession number MVDW00000000; Biosample SAMN06067857; Bioproject PRJNA355276. This paper describes the first version of the genome. RNA sequencing data have been deposited in the NCBI Gene Expression Omnibus (Accession number GSE90705).

MATERIALS AND METHODS

Conidia of *Cercospora zeina* were grown on V8 agar (20 % (v/v) Campbells V8 juice, 2 % (w/v) Bacterial Agar, 0.349 % (w/v) $CaCO_3$) at ambient room temperature in constant darkness to promote conidiation. Conidia were collected and $1x10^5$ conidia/ml cultured in Potato Dextrose Broth (PDB) at 25 °C with gentle shaking. Prior to cultures reaching the melanized stage DNA was isolated as described previously (Ma *et al.* 2010).

For RNA isolations *C. zeina* was cultured under seven different *in vitro* growth conditions. For solid media, conidia were transferred from V8 agar onto sterile cellophane sheets overlain onto the particular media by subculturing. For liquid media, conidia were washed from the V8 agar using the relevant media and a sterile L-spreader, and transferred to a flask containing the growth media. Media used included Complete media (1 % (w/v) glucose, 0.1 % (w/v) yeast extract, 0.1 % (w/v) casein hydrolysate, 0.1 % (w/v) $Ca(NO_3)_2.4H_2O$, 1 % (v/v) mineral solution [2 % (w/v) KH_2PO4, 2.5 % (w/v) $MgSO_4.7H_2O$, 1.5 % (w/v) NaCl]); 0.2x PDA (0.3 % (w/v) Potato Dextrose Agar (PDA), 1.2 % (w/v) Bacterial Agar), 0.2x PDA supplemented with 10mM $NH_4H_2PO_4$; Cornmeal agar (1.7 % (w/v)); PDA pH8 (3.9 % PDA, pH adjusted with 0.29 % (w/v) Na_2CO_3 and 0.76 % $NaHCO_3$); PDB pH3 (2.4 % (w/v) PDB, pH adjusted with 1.67 % (w/v) citric acid and 0.58 % (w/v) Na_2HPO_4); and Yeast Extract Peptone Dextrose (YPD, 0.05 % (w/v) peptone, 0.05 % (w/v) yeast extract, 0.5 % (w/v) glucose, 1.8 % (w/v) NaCl). With the exception of V8 agar, the cultures were kept in constant light at 25 °C for 7 d.

RNA was isolated using the Qiagen QIAzol Lysis Reagent and on-column DNase treatment and RNA purification was performed with the Qiagen RNeasy Mini kit, all according to the manufacturer's specifications. Illumina HiSeq2000 100 bp read-length sequencing was performed on three DNA libraries (paired-end (PE), 3 kb and 8 kb mate-pair). Sequence reads were quality filtered and trimmed using Trimmomatic v. 0.30 (Bolger *et al.* 2014) using the default parameters. Due to nucleotide content inconsistencies in the PE and 3 kb libraries the 8 kb mate-pair library reads were assembled as single-end reads using Velvet (Zerbino & Birney 2008), with optimal k-mer size of 57 nt determined with VelvetOptimizer. Contigs were scaffolded using SSPACE v. 2.0 (Boetzer *et al.* 2011) and scaffold gaps filled with Gapfiller v. 1.11 (Boetzer &

Table 1. Species and GenBank accessions for the Translation Elongation Factor 1-alpha (TEF 1-alpha) and Internal Transcribed Spacer (ITS) sequences used for Maximum Parsimony inference.

	GenBank accession	
Species	**TEF 1-alpha**	**ITS**
Cercospora alchemillicola strain CPC 5259	JX143279.1	JX143525.1
Cercospora althaeina strain CBS 126.26	JX143282.1	JX143528.1
Cercospora apii strain CBS 116504	AY840487.1	AY840520.1
Cercospora beticola strain CBS 116502	AY840496.1	AY840529.1
Cercospora chinensis strain CBS 132612	JX143334.1	JX143578.1
Cercospora mercurialis strain CBS 549.71	JX143386.1	JX143627.1
Cercospora olivascens strain CBS 253.67	JX143391.1	JX143632.1
Cercospora ricinella strain CBS 132605	JX143405.1	JX143646.1
Cercospora sp. F JZG-2013 strain CPC 12062	DQ185083.1	DQ185071.1
Cercospora vignigena strain CBS 132611	JX143493.1	JX143734.1
Cercospora violae strain CBS 251.67	JX143496.1	JX143737.1
Cercospora zeae-maydis strain CBS 117755	DQ185084.1	DQ185072.1
Cercospora zeae-maydis strain CBS 117756	DQ185085.1	DQ185073.1
Cercospora zeae-maydis strain CBS 117757	DQ185086.1	DQ185074.1
Cercospora zeae-maydis strain CBS 117758	DQ185087.1	DQ185075.1
Cercospora zeae-maydis strain CBS 117759	DQ185088.1	DQ185076.1
Cercospora zeina strain CMW 25445	EU569217.1	EU569225.1
Cercospora zeina strain CMW 25459	EU569215.1	EU569226.1
Cercospora zeina strain CMW 25462	EU569210.1	EU569224.1
Cercospora zeina strain CMW 25467	EU569218.1	EU569227.1
Cercospora zeina strain CMW 25467 genome	MVDW00000000	
Mycosphaerella thailandica strain CBS 116367	AY840476.1	KF901776.1
Mycosphaerella thailandica strain CPC 10548	AY840477.2	AY752157.1

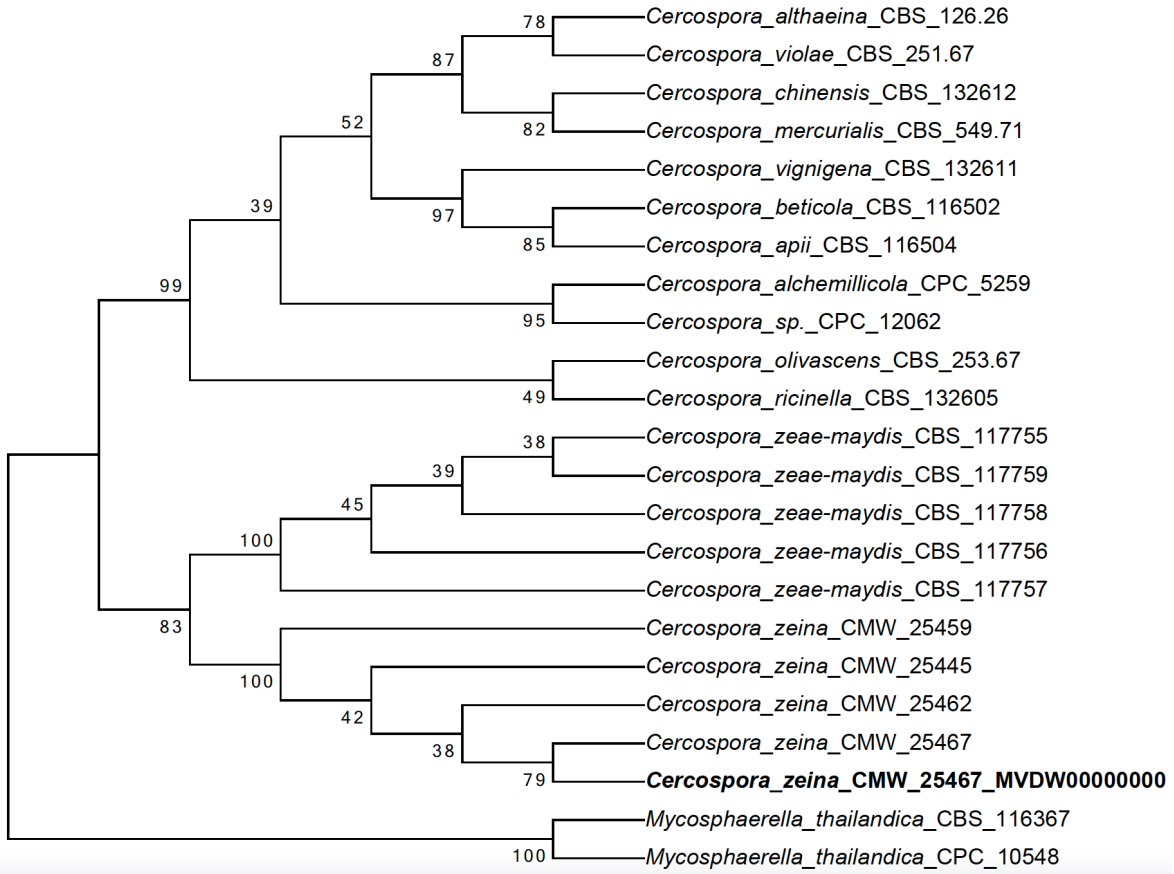

Fig.1. Cladogram to classify the sequenced *Cercospora zeina* genome with reference to related *Cercospora* species. Maximum parsimony analysis based on translation elongation factor 1-alpha and ITS sequences was performed, with percentage bootstrap (1000) values shown. The cladogram was rooted using *Mycosphaerella thailandica*.

Pirovano 2012) using all the sequenced libraries and allowing a 25 % error in library insert sizes. Completeness of the assembly was evaluated using BUSCO (Simao *et al.* 2015).

Transcriptome sequencing was performed at BGI (HK) using HiSeq2000 100bp PE sequencing. RNAseq reads were mapped to the *C. zeina* reference genome using TopHat2 (Kim *et al.* 2013). Manual gene finding of 150 *C. zeina* genes using Genomeview (Abeel *et al.* 2012) was guided by BLAST (Altschul *et al.* 1990) alignments of *C. zeae-maydis* (JGI) genes to the *C. zeina* reference genome together with *C. zeina* transcriptome mapping information for gene and splice site identification. Automated gene prediction was performed using the MAKER (Cantarel *et al.* 2008) pipeline incorporating the SNAP (Korf 2004) and AUGUSTUS (Stanke *et al.* 2006) *ab initio* gene predictors. The SNAP training data were the 150 manually curated genes, while the AUGUSTUS training data comprised of high-confidence predicted genes from MAKER using only SNAP predictions (AED score <0.2).

To verify the species identity of the sequenced strain, the Translation Elongation Factor 1-alpha and ITS sequences for selected *Cercospora* species (Table 1) were concatenated and aligned with ClustalW (Thompson *et al.* 1994). The relevant sequences were extracted from the genome assembly using blastn from the *C. zeina* strain CMW 25467 data, and are listed under the genome assembly accession. The analysis workflow was similar to Meisel *et al.* (2009). The species relationships were inferred using the Maximum Parsimony method in MEGA7 (Kumar *et al.* 2016), with confidence at nodes gained using bootstrap analysis (Felsenstein 1985). Branches corresponding to partitions reproduced in less than 50 % bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The MP tree was obtained using the Tree-Bisection-Regrafting algorithm (Nei & Kumar 2000) with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). All positions containing gaps and missing data were eliminated. Two strains of *Mycosphaerella thailandica* (CBS 116367 and CPC 10548) were used to root the cladogram (Fig. 1).

RESULTS AND DISCUSSION

Assembly of the sequenced reads yielded a draft genome 37 Mb in size in 10 027 scaffolds >200 bp with N50 of 161 kb. The average scaffold size was 4 059 bp with the largest contig of 938 kb. BUSCO evaluation using the Ascomycota dataset yielded a completeness report of C: 95.4 %: (95.4 % Complete and single-copy BUSCOs, D: 0.0 % duplicated BUSCOs, 2.1 % fragmented BUSCOs, M: 2.5 % missing BUSCOs, total 1315 genes evaluated).

Following manual gene curation, 10 193 protein-coding gene-models were predicted. The genome size and number of

genes are similar to other genomes in the order Capnodiales (Stanke *et al.* 2006).

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IMA Genome-F 8B

Draft genome sequence of *Fusarium pininemorale*

INTRODUCTION

The *Fusarium fujikuroi* species complex (FFSC) represents an assemblage of diverse fungal species. The so-called "American" clade of the FFSC contains known emerging pathogens of many cultivated crops and trees including pine (*F. circinatum*; Hepting & Roth 1946), maize (*F. temperatum*; Desjardins *et al.* 2000), and mango (*F. parvisorum*; Liew *et al.* 2016). *Fusarium pininemorale*, a recently recognized member of this clade, was isolated from diseased pine trees in plantations of Colombia (Herron *et al.* 2015). Even though this species shares numerous morphological and biological traits with other FFSC pine pathogens, *F. pininemorale* itself was not found to cause significant disease symptoms on pine as is the case for *F. circinatum* (Herron *et al.* 2015).

Overall, little is known regarding the biology and genetics of this species, even less so for the genetic and determinants of host range in the broader "American" clade. The exact genetic basis and genomic processes underlying pathogenicity also remains elusive. The aim of this study was to determine the full genome sequence for *F. pininemorale,* which will allow further studies to investigate genomic aspects of not only pathogenicity but also of its biology and evolution.

SEQUENCED STRAIN

Colombia: Angela Maria (Santa Rosa), Risaralda 75°360.2100' W 4°490.1800' N, Isolated from *Pinus tecunumanii,* 2012, (CBS 137240 = CMW 25243; FCC 5383 – cultures; Herron *et al.* 2015).

NUCLEOTIDE ACCESSION NUMBER

The *Fusarium pininemorale* whole genomic sequence data has been deposited at DDBJ/EMBL/GenBank under the accession NFZR00000000. The version described in this paper is version NFZR00000000.

METHODS

The *Fusarium pininemorale* isolate was grown on ½ PDA for 7 d and DNA was extracted as described previously (Möller *et al.* 1992). A pair-end library (350 bp average insert size) and a mate-pair library (5 kb average insert size) were prepared and sequenced using Illumina HiSeq platforms at Macrogen (Seoul, Korea). All sequences had an average read length of 58 bp. Poor quality reads, terminal nucleotides, as well as duplicate reads were removed using CLC Genomics Workbench v. 8.0.1 (CLCBio, Aarhus). *De novo* genome assembly was performed using ABySS v. 1.3.7 (Simpson *et al.* 2009), further scaffolding was performed using SSPACE v. 2. 0 (Boetzer *et al.* 2011) and gapped genomic regions were closed using GapFiller v. 1.11 (Boetzer & Pirovano 2012). Genome completeness was assessed using the software BUSCO (Benchmarking Universal Single-Copy Orthologs) v2 with the *Sordariomycete* dataset (Simão *et al.* 2015). Chromosome-sized scaffolds were compared using LASTZ alignments (Harris 2007) against genomes of *F. circinatum* (Wingfield *et al.* 2012) and *F. temperatum* (Wingfield *et al.* 2015a). Lastly, gene prediction was performed using AUGUSTUS 2.5.5 (Hoff & Stanke 2013) based on gene models for *F. graminearum* (http://bioinf.uni-greifswald.de/augustus), together with mRNA sequence data from the *F. circinatum* genome (Wingfield *et al.* 2012).

RESULTS AND DISCUSSION

The draft nuclear genome of *Fusarium pininemorale* had an estimated genome size of 47 778 776 bp. The N50 value was 1 358 616 bp and the GC content was 46.0 %. The assembly consisted of 200 contigs which ranged between 200 bp to 6 144 005 bp in size. The average scaffold size was determined to be 310 252 bp in length of which 13 contigs were larger than 1 000 000 bp. The assembled genome was predicted to encode for 14 640 open reading frames (ORFs) with an average gene length of 1472 bp, and an overall gene density of 306 ORFs/MB was observed. BUSCO suggested that the assembly was 99 % complete (i.e., complete BUSCOs = 99 %; complete and single-copy BUSCOs = 98.2 %; complete and duplicated BUSCOs = 0.8 %; fragmented BUSCOs = 0.6 %; missing BUSCOs = 0.4 %; number of BUSCOs searched = 3725). The taxonomic identity of the genome was confirmed using a phylogenetic analysis using authenticated sequences (Fig. 2).

The GC content and number of identified ORFs is comparable to that of other FFSC genomes (Ma *et al.* 2010, Wingfield *et al.* 2012, Wiemann *et al.* 2013, Wingfield *et al.* 2015a, 2015b, Niehaus *et al.* 2016). However, the genome assembly of *F. pininemorale* is notably larger than those of other species in the "American" clade for which genome sequences are available. In comparison, the genome sizes of *F. circinatum* and *F. temperatum* is 4 534 176 bp and 2 319 967 bp smaller than that of *F. pininemorale*, respectively. Nevertheless, sequence comparisons based on chromosome-sized scaffolds suggests that *F. pininemorale* harbours 12 chromosomes similar to that of species in the FFSC. The putative 12th chromosome of *F. pininemorale* is 968 722 bp size and spans two scaffolds in the current assembly. Also, the reciprocal chromosome translocation (involving parts of chromosomes 8 and 11) confirmed previously in *F. circinatum* and *F. temperatum* (De

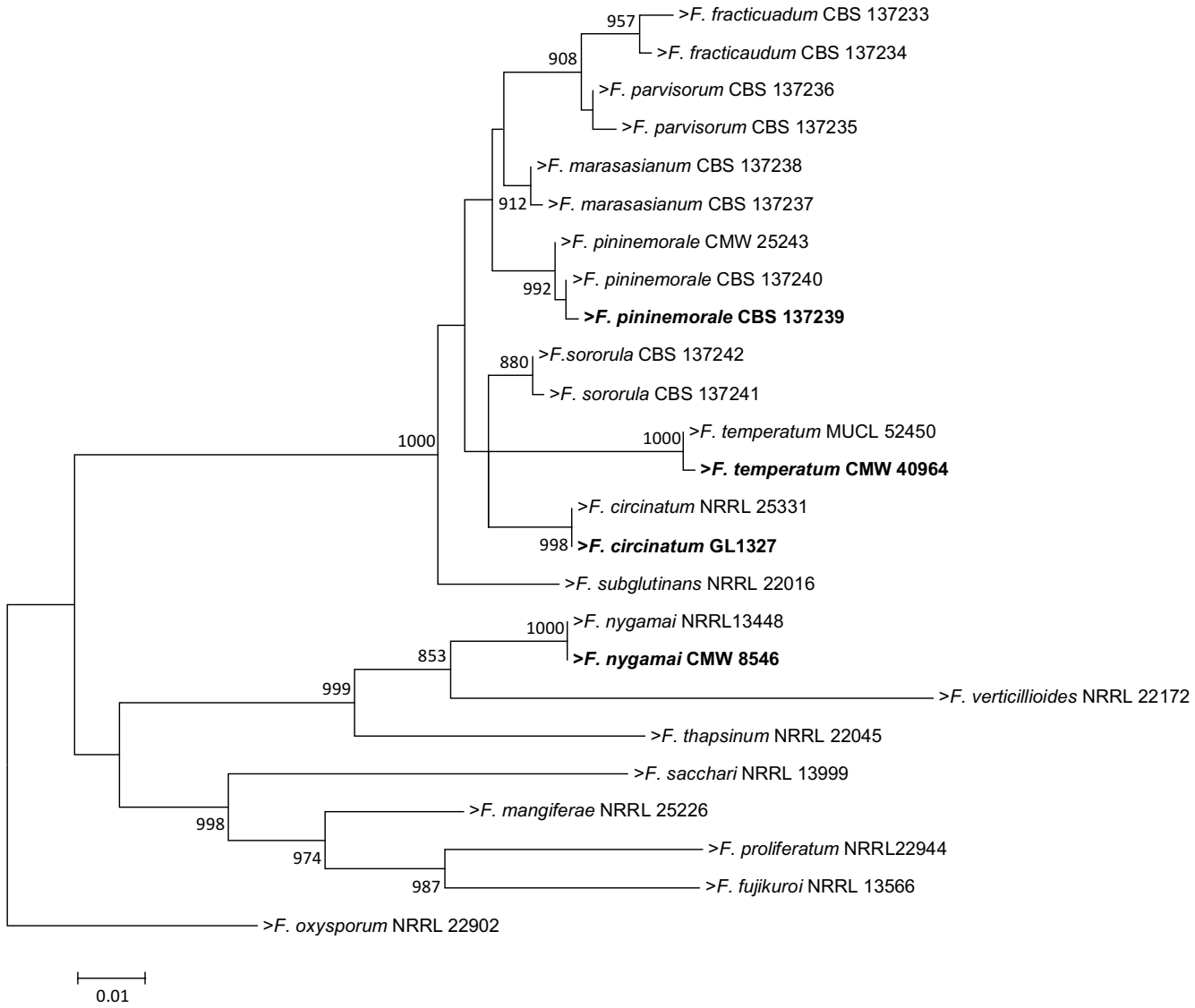

Fig. 2. Maximum likelihood (ML) tree based on partial gene sequences of β-tubulin and translation elongation factor 1-α. Sequence alignments were assembled with MAFFT version 7 (Katoh & Standley 2013). The program jModelTest v 2.1.7 (Guindon & Gascuel 2003, Darribo *et al.* 2012) was used to determine the best-fit substitution model (GTR+I+G substitution model) with gamma correction (Tavaré 1986). The ML phylogenetic analysis was performed using PhyML v 3.1 (Guindon & Gascuel 2010). Values at branch nodes are the bootstrapping confidence values with those ≥ 85 % shown. Indicated in bold are sequences from the *Fusarium* genomes of *F. pininemorale* and those of *F. nygamai, F. temperatum* and *F. circinatum* strain GL1327 which have been previously published in IMA fungus (Wingfield *et al.* 2012, 2015a, 2015b).

Vos *et al.* 2014, Wingfield *et al.* 2015a) is also present in *F. pininemorale*. Clearly, the addition of the *F. pininemorale* genome assembly would facilitate detailed studies of genome evolution, host adaption and pathogenicity in the "American" clade as well as the broader FFSC.

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IMA Genome-F 8C

Draft genome sequence of *Hawksworthiomyces lignivorus*

INTRODUCTION

Hawksworthiomyces lignivorus (*Ascomycota*: *Ophiostomatales*) was first described from decaying *Eucalyptus* utility poles in South Africa (De Meyer *et al.* 2008). It was initially described in the genus *Sporothrix* which, under the dual nomenclature system, accommodated mycelial asexual morphs of *Ophiostomatales* (De Beer & Wingfield 2013,

De Hoog 1993). However, DNA sequences of multiple genes and more inclusive phylogenies showed that that this species was distinct from all other species residing in *Sporothrix* as redefined by De Beer *et al.* (2016a), and from other genera in *Ophiostomatales* (De Beer & Wingfield 2013). De Beer *et al.* (2016b) therefore described the new genus *Hawksworthiomyces,* to accommodate *H. lignivorus* together with four other newly described species, *H. crousii*, *H. hibbettii*, *H. sequentia,* and *H. taylorii*.

Species of *Hawksworthiomyces* differ from all other ophiostomatoid fungi in their biology and ecology. Most species residing in the numerous genera of *Ophiostomatales* are associates of bark or ambrosia beetles that inhabit the cambium and sapwood of trees (De Beer & Wingfield 2013). Many species of *Sporothrix* are exceptions to this norm and are found in soil and *Protea* infructescences (De Beer *et al.* 2016a, Roets *et al.* 2013). All species of *Hawksworthiomyces* have either been isolated from decaying wood or environments associated with degrading plant materials (De Beer *et al.* 2016b). An inoculation study conducted by De Beer *et al.* (2006) also suggested that *H. lignivorus* is capable of degrading lignocellulose components of wood. This is different to other species in *Ophiostomatales* that specifically degrade resinous compounds in wood (Farrell *et al.* 1993) and that do not display any marked lignocellulose degradation (Seifert 1993).

In this study, we generated the draft genome sequence for *H. lignivorus*, which will provide basal data required to explore its unique biology and ecology. As the type species of *Hawksworthiomyces*, the genome sequence of *H. lignivorus* will be useful for future phylogenomic studies aimed at a better understanding the evolutionary history of this genus and other genera in *Ophiostomatales*.

SEQUENCED STRAIN

South Africa: *Western Cape*: Stellenbosch: isolated from *Eucalyptus* pole at soil level, Oct. 2003, *E.M. de Meyer* (CMW 18600 = CBS 119148 = MUCL 55926 – living culture, PREM 59284 – dried culture).

NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The genomic sequence of *Hawsworthiomyces lignivorus* (CMW 18600, CBS 119148) has been deposited at DDBJ/EMBL/GenBank under accession no. NTMA00000000. The version described in this paper is version NTMA01000000.

MATERIALS AND METHODS

The ex-holotype culture of *Hawsworthiomyces lignivorus* (CMW 18600 = CBS 119148) was obtained from the culture collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria (CMW). Genomic DNA was extracted using the method described by Duong *et al.* (2013). Two pair-end libraries of approximately 350 bp and 530 bp were prepared and sequenced using the Illumina HiSeq 2000 platform with 100 bp read length. Reads obtained were subjected to quality and adapters trimming using Trimmomatic v. 0.36 (Bolger *et al.* 2014). De-novo genome assembly was performed using SPAdes v. 3.9.0 (Bankevich *et al.* 2012). The scaffolds obtained from SPAdes were subjected to further scaffolding with SSPACE-Standard v. 3.0 (Boetzer *et al.* 2011). Assembly gaps were filled with GapFiller v. 1.10 (Boetzer & Pirovano 2012). The quality and completeness of the assembly was validated with Benchmarking Universal Single Copy Orthologs (BUSCO v. 2.0.1) program using the *Soradiomyceta* odb9 dataset (Simão *et al.* 2015). The number of protein coding genes was determined using AUGUSTUS v3.2.2 (Stanke *et al.* 2006).

RESULTS AND DISCUSSION

Sequencing of the *Hawksworthiomyces lignivorus* DNA libraries yielded 14 203733 paired-end reads with average read length of around 100 bp. Trimming recovered 12 704 558 pair-end reads and 1 362 623 single reads. *De-novo* genome assembly with SPAdes resulted in an assembly of 43.81 Mb in size, distributed in 280 scaffolds larger than 500 bp. The number of scaffolds was further reduced to 214 after scaffolding with SSPACE and filling gaps with GapFiller. The current genome assembly of *H. lignivorus* has a total sequence length of 43 822 585 bases, with an N50 value of 383 563 and an average GC content of 51.27 %. *Hawsworthiomyces lignivorus* had the largest genome size when compared to all species of *Ophiostomatales* for which whole genome data are available; the smallest genome reported was that of *Ceratocystiopsis minuta* (21.3 Mb) (Wingfield *et al.* 2016a), and the second largest to *H. lignivorus* was that of of *Sporothrix pallida* (37.8 Mb) (D'Alessandro *et al.* 2016).

The assembly had a BUSCO completeness score of 95.7 %. Out of the 3725 BUSCO groups searched, 3556 were complete single-copy BUSCOs, nine were complete duplicated BUSCOs, 64 were fragmented BUSCOs, and 96 were missing BUSCOs. AUGUSTUS predicted a total of 11 216 protein-coding genes encoded by *H. lignivorus* genome. The taxonomic identity of the genome was confirmed using a phylogenetic analysis using authenticated sequences (Fig. 3).

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Draft genome assembly for *Huntiella decipiens*

INTRODUCTION

The family *Ceratocystidaceae* as defined by De Beer *et al.* (2014) includes economically important plant pathogens, as

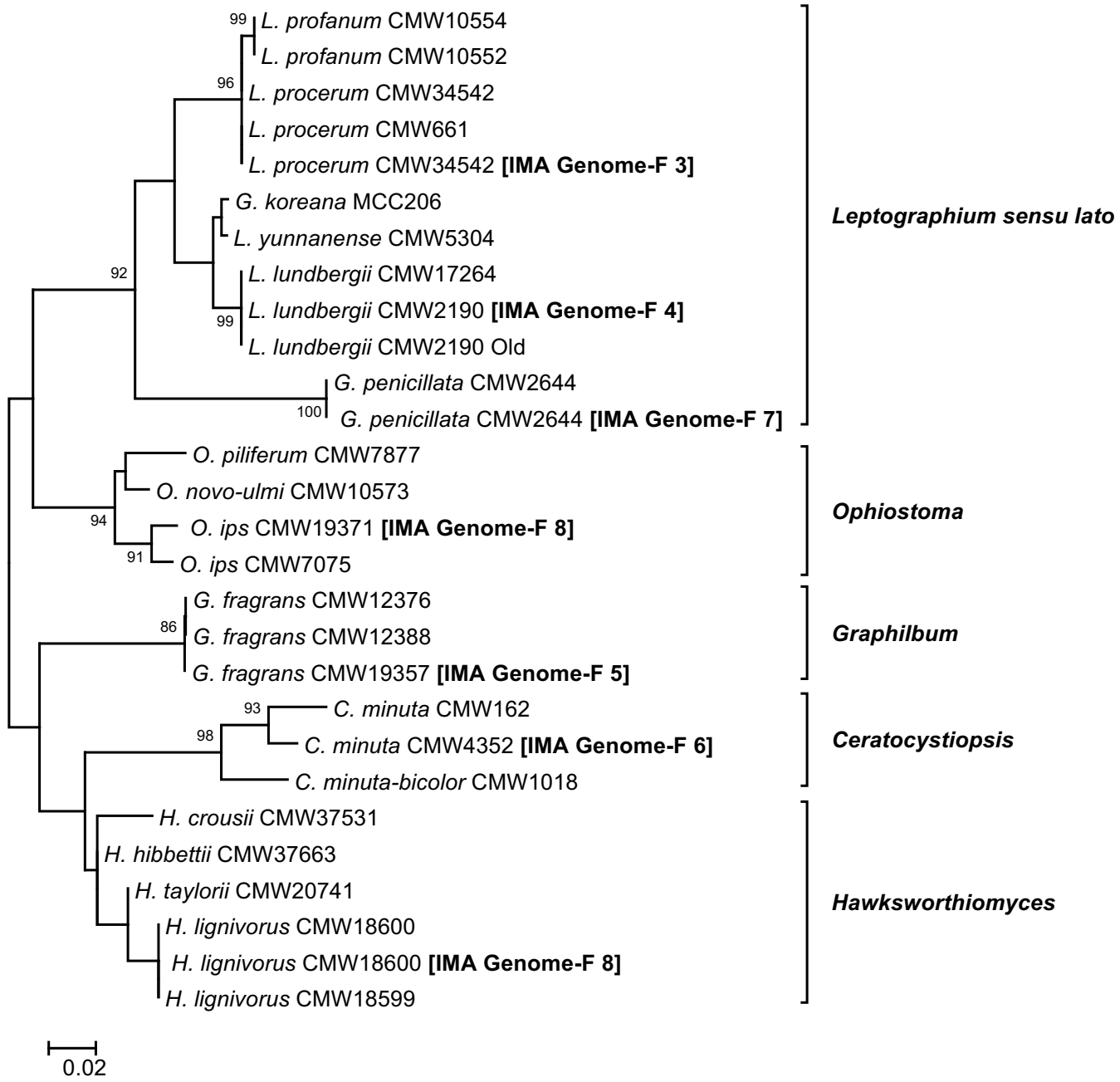

Fig. 3. Identity verification of *Ophiostomataceae* isolates sequenced in this study and in all previous IMA Genome Announcements (IMA Genome-F: 3–7; Van der Nest *et al*. 2014a, Wingfield *et al*. 2015a, 2016a, 2015b, 2016b). Gene regions (LSU, βT) used for verification were extracted from assembled genomes. Other reference isolates and their corresponding sequences were obtained from published papers (De Beer *et al*. 2016, Linnakoski *et al*. 2012, Yin *et al*. 2015, Zipfel *et al*. 2006). The phylogeny was constructed using RAxML with the GTRGAMMA substitution model. Bootstrap values greater than 75 are indicated at the nodes.

well as agents of blue stain in timber, many of which result in substantial economic losses (Roux *et al*. 2000, Baker *et al*. 2003, Barnes *et al*. 2003, Van Wyk *et al*. 2007, Heath *et al*. 2009). De Beer *et al*. (2014), Fig. 4, revised this fungal family based on morphological, phylogenetic and ecological evidence and it now includes numerous clearly circumscribed genera such as *Ceratocystis*, *Endoconidiophora,* and *Huntiella*.

Huntiella species previously formed part of the *Ceratocystis moniliformis* complex (De Beer *et al*. 2014). Species of *Huntiella* are generally weak pathogens or saprobes, although some cause sapstain which reduces the value of timber (Van Wyk *et al*. 2006, Kirisits *et al*. 2013). These fungi are associated with insects, particularly sap-feeding beetles (*Nitidulidae*) that are thought to primarily aid in their spread and distribution (Kirisits 2004). An unusual example of a *Huntiella* species is *H. bhutanensis* that lives in association with the bark beetle *Ips schmutzenhoferi (Scolytidae)* (Van Wyk *et al.* 2004, Kirisits*et et al*. 2013). In general, however, little is known regarding the biology or ecology of *Huntiella* species. For example, *H. decipiens,* that forms the basis of the present study, is known only from one region in the Limpopo Province of South Africa, where it was isolated from wounds on plantation-grown *Eucalyptus*

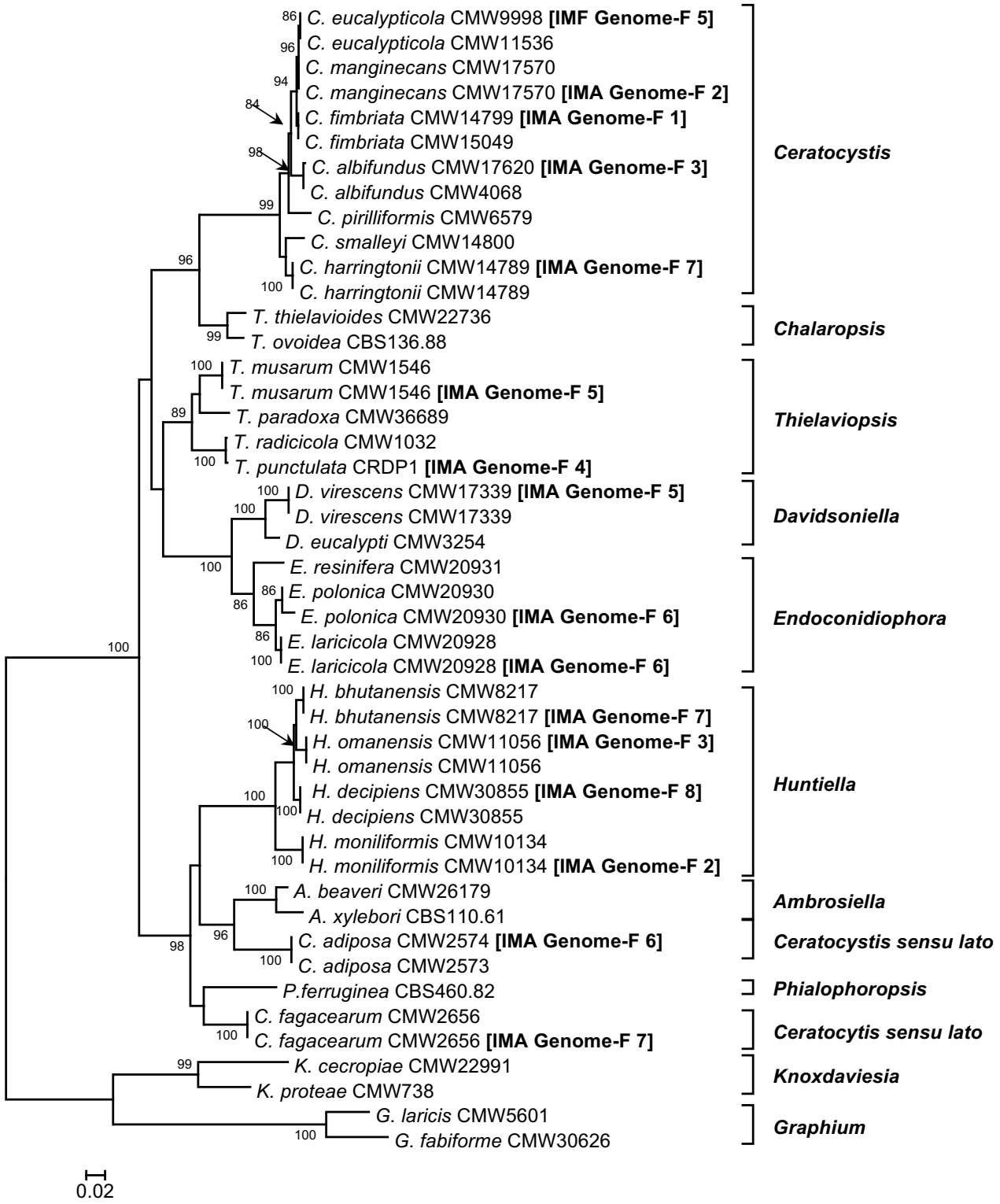

Fig. 4. Identity verification of *Ceratocystidaceae* isolates sequenced in this study, and in all previous IMA Genome Announcements (IMA Genome-F: 1–7; Van der Nest *et al*. 2014a, 2014b, Wilken *et al*. 2013, Wingfield *et al*. 2015a, 2016a, 2015b, 2016b). Gene regions (60S, LSU, MCM7) used for verification were extracted from assembled genomes. Other reference isolates and their corresponding sequences were obtained from De Beer *et al*. (2014). The phylogeny was constructed using RAxML with the GTRGAMMA substitution model. Bootstrap values greater than 75 are indicated at the nodes.

species and from a *Staphilinid* (*Staphylinidae*) beetle found on freshly cut *E. saligna* stumps (Nkuekam *et al*. 2012).

The aim of this study was to produce a good quality draft genome assembly for *H. decipiens*. The genomes of several members of Ceratocystidaceae are already available in the public domain (Belbahri 2015, Van der Nest *et al*. 2014a, 2014b, Wilken *et al*. 2013, Wingfield *et al*. 2015a, 2015b, 2016) and the genome sequence for *H. decipiens* will provide valuable opportunities for comparative genomic studies on this important group of fungi.

SEQUENCED STRAIN

South Africa: Soutpansberg, isol. *Staphilinid* spp. infesting *Eucalyptis saligna,* Dec. 2008, *K. Nkuekam* (CMW 30855, CBS 129736 – cultures; PREM 60560 – dried culture).

NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The *Huntiella decipiens* isolate CMW 30855 Whole Genome Shotgun project has been deposited in GenBank under accession no. NETU00000000.

MATERIALS AND METHODS

Genomic DNA was isolated from *H. decipiens* isolate CMW 30855 (Barnes *et al*. 2001) and sequenced using the Genomics Analyzer IIx, Illumina platform from the UC Davis Genome Centre (University of California, Davis, A). For this purpose, paired-end libraries of 350- p and 600 bp insert sizes were prepared and sequenced following the Illumina protocol. The CLC Genomics Workbench v. 8.0.1 (CLCBio, Aarhus) was employed to quality trim reads and *de-novo* assemble a draft genome sequence using the default parameters. Thereafter, the assemblies were scaffolded using SSPACE v. 2.0 (Boetzer *et al*. 2011). GapFiller v. 2.2.1 (Boetzer & Pirovano 2012) was used to fill the gaps created during the scaffolding. The number of putative open reading frames (ORFs) was predicted with the web-based *de novo* gene prediction software AUGUSTUS using the *Fusarium graminearum* gene models (Stanke *et al.* 2008). The "create detailed mapping report" command of the CLC Genomics Workbench was used to produce statistics for the draft sequence. The Benchmarking Universal Single-Copy Orthologs (BUSCO v. 1.22) tool was used to assess the genome completeness (Simão *et al*. 2015) using the fungal data set.

RESULTS AND DISCUSSION

The estimated size of the assembled *Huntiella decipiens* draft genome was 26.7 Mb, with 638 scaffolds larger than 500 bases. AUGUSTUS analysis predicted 7254 ORFs, which corresponds to an average gene density of 271.7 ORFs/Mb. The assembly contained 1 403 Complete Single-Copy BUSCOs, 90 Complete Duplicated BUSCOs, 11 Fragmented BUSCOs and 24 missing BUSCOs. The taxonomic identity of the genome was confirmed using a phylogenetic analysis using authenticated sequences (Fig. 4).

Relative to other species in *Ceratocystidaceae*, *H. decipiens* has a similar size genome than those of *H. bhutanensis* (26.7 Mb, 7261 ORFs) (Wingfield *et al*. 2016) and *H. moniliformis* (25.4 Mb, 6832 ORFs) (Van der Nest *et al*. 2014b). Compared to other species in the family, however, these three *Huntiella* genomes appeared to be smaller, encoding fewer genes. For example, the *H. decipiens* genome is smaller than those of *H. omanensis* (31.5 Mb, 8395 ORFs), *Ceratocystis manginecans* (31.7 Mb, 7494 ORFs), *C. fimbriata* (29.4 Mb, 7 266 ORFs), *E. laricicola* (33.3 Mb, 6 897 ORFs), and *D. virescens* (33.7 Mb, 6953 ORFs) (Van der Nest *et al*. 2014a, 2014b, Wilken *et al*. 2013, Wingfield *et al*. 2015, 2016). Whether these differences in genome size are linked to the different life-styles of these fungi requires further research, which will be the subject of future studies.

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IMA Genome-F 8E

Draft genome sequence of *Ophiostoma ips*

INTRODUCTION

Ophiostoma ips is an ascomycete fungus of the *O. ips* species complex (*Ophiostomatales*, *Ascomycota*) (De Beer & Wingfield 2013). The fungus was first described as the causal agent of blue stain on *Pinus* lumber in the USA (Rumbold 1931). *Ophiostoma ips* is commonly associated with conifer-infesting bark beetles in the genera *Ips*, *Orthotomicus,* and *Hylurgus*, which are native to the Northern Hemisphere. Outside of its native range, *O. ips* has been reported in various countries of the Southern Hemisphere including Australia, Chile, New Zealand, and South Africa, where it has been accidentally introduced with its bark beetle vectors (Wingfield & Marasas 1980, Zhou *et al.* 2001, 2004). A population genetic study using microsatellite markers (Zhou *et al.* 2007) revealed a high level of admixture between *O. ips* populations, suggesting frequent movement of this species between countries and continents. Although there have been some studies suggesting weak pathogenicity on conifers (Lieutier *et al.* 1989, Zhou *et al.* 2002), like most other *Ophiostoma* spp. and their relatives (Six & Wingfield 2011), *O. ips* is generally considered not to be a primary pathogen and its relevance is usually as a consequence of the reduced value of the wood due to sap stain (Seifert 1993).

We generated the genome sequence for *O. ips*, which adds to a growing number of genomes available from species of *Ophiostomatales.* Together, these genomes will serve as a valuable resource, enabling future comparative genomics studies seeking to gain insight into the biology, ecology and evolution of species in *Ophiostomatales*.

SEQUENCED STRAIN

USA: *Louisiana*: isol. *Pinus taeda*, 2004, *X. Zhou* (CMW 19371 = CBS 138721 – culture).

NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The genomic sequence of *Ophiostoma ips* (CMW 19371 = CBS 138721) has been deposited at DDBJ/EMBL/GenBank under the accession NTMB00000000. The version described in this paper is version NTMB01000000.

METHODS

An isolate of *Ophiostoma ips* (CMW 19371 = CBS 138721) was obtained from the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute, University of Pretoria. DNA was extracted from a single conidium culture following a method described previously (Duong *et al.* 2013). Two pair-end libraries (350 bp and 550 bp medium insert sizes) were prepared and sequenced using the Illumina Hiseq 2500 platform. The program Trimmomatic v. 0.36 (Bolger *et al.* 2014) was used for quality and adapters trimming of pair-end reads. The genome was assembled from trimmed reads using Spades v. 3.10 (Bankevich *et al.* 2012) and was further placed into scaffolds using SSPACE Standard v. 3.0 (Boetzer *et al.* 2011). Gaps were filled or reduced with GapFiller v. 1.10 (Boetzer & Pirovano 2012). Several runs of genome assembly were conducted using different parameters. Assemblies obtained from these runs were subjected to quality and completeness assessment using the program BUSCO v. 2.0 (Simão *et al.* 2015) using the dataset for *Sordariomycetes*. The best assembly based on the best BUSCO statistics in term of completeness, was selected and presented in this study. The program AUGUSTUS v. 3.2.2 (Stanke *et al.* 2006) was used to estimate the number of protein coding genes encoded by the genome using the species model for *Neurospora crassa*.

RESULTS AND DISCUSSION

The genome of *Ophiostoma ips* was assembled into 351 scaffolds. The genome is 25.99 Mb and the assembly has an N50 of 140.6 Kb. The average coverage across the whole genome was 80 times. The assembled *O. ips* genome has an average GC content of 56 %. The genome of *O. ips* has smaller genome size and higher GC genome content when compared to that of other *Ophiostoma* species such as *O. ulmi* (31.5 Mb, GC = 50.02 %; Khoshraftar *et al.* 2013), *O. novo-ulmi* (31.8 Mb, GC = 50.01 %; Forgetta *et al.* 2013) and *O. piceae* (32.8 Mb, GC = 52.8 %; Haridas *et al.* 2013). The taxonomic identity of the genome was confirmed using a phylogenetic analysis using authenticated sequences (Fig. 3). Assessment of the assembly using BUSCO with the *Sodariomycetes* dataset resulted in a completeness of 96.8 % (C:3606 [S:3603, D:3], F:42, M:77, n:3725), indicating that the assembly should cover most of the organism's gene space. AUGUSTUS prediction using species model for *Neurospora crassa* resulted in 7607 protein coding genes, which is slightly lower than that of *O. ulmi* (8639 genes; Khoshraftar *et al.* 2013), *O. novo-ulmi* (8640 genes; Comeau *et al.* 2015), and *O. piceae* (8884 genes; Haridas *et al.* 2013).

The draft genome sequence from *O. ips* presented in this study will be a valuable addition to a number of genomes already available for species in *Ophiostomatales*. These will enable further studies to better understand this interesting group of fungi.

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