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A high-quality draft genome sequence of *Neonectria faginata*, causative agent of beech bark disease of *Fagus grandifolia*

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ABSTRACT The draft genome of *Neonectria faginata* was sequenced with Oxford Nanopore and Illumina 250 bp paired-end sequencing technologies. The assembled genome was 42.9 Mb distributed over 24 contigs, with N50 of 4.4 Mb and 98.6% BUSCO completeness. This genome sequence will aid in understanding *N. faginata* population structure and ecology.

KEYWORDS fungi, neonectria, pathogenic fungi

Beech bark disease (BBD) of American beech trees (*Fagus grandifolia*) in North America has significant ecological, economic, and aesthetic impacts (1). BBD is referred to as a disease complex as it arises from the interaction among an introduced insect (*Cryptococcus fagisuga*) and at least one of two fungi, *Neonectria faginata* and *N. ditissima*. The dominant causal agent, *N. faginata*, has never been observed outside of the BBD complex (2) raising questions about its origin and potential alternative ecological modes or hosts. We report a high-quality draft genome of *N. faginata* that will aid in advancing understanding of the natural history of this organism through population genetic and comparative genomic approaches.

The sample was collected from the bark of an American beech tree in Penobscot County, Maine, USA (44.83071 N, 68.59962 W) on 31 May 2018. A culture was isolated following the single-ascospore isolation protocol of Stauder et al. (3) on malt-yeast agar medium [1% (wt/vol) malt extract, 0.2% yeast extract, 2% agar, 50 mg L^{-1} streptomycin, and 10 mg L^{-1} tetracycline). DNA was isolated following van Diepen et al. (4), including a 30-min RNase A digestion (ThemoScientific EN0531). Taxonomic identification was performed by sequencing the *TEF1-α* and *RPB2* genes, performing a multiple sequence alignment with reference sequences from Castlebury et al. (2), and confirming that our isolate grouped within a monophyletic *N. faginata* clade in a neighbor joining tree.

Long read sequencing was carried out with Oxford Nanopore Technology (ONT) using the SQK-LSK109 ligation sequencing kit and a MinION Mk1B sequencing device with a MIN106 flow cell. DNA was size selected using a 1:1 mixture of AxyPrep MAG beads (Axygen MAG-PCR-CL-5). Total read count was 959,919 comprising 3.25 Gbp (475,527 reads ≥ 1 kbp totaling 2.97 Gbp). Read N50 was 11,647 bp. Base calling was performed in Albacore 2.3.4 (5).

An Illumina sequencing library was prepared using a Kapa HyperPlus DNA library kit, and 250 bp paired-end sequencing was performed on a HiSeq 2500 with Rapid Run chemistry. Raw read count was 33,620,764, of which 5,054,628 remained after quality control using BBTools 38.57 (6) with bbduk options "ktrim = r k = 23 mink = 11 hdist = 1 tpe tbo qtrim = r trimq = 10 minlength = 36."

The ONT reads were assembled with Canu 1.6 (7) with estimated genome size set to 45 Mbp. We polished the assembly with Nanopolish 0.10.2 (5) using the original ONT reads, followed by one round of PILON 1.22 (8) with the trimmed Illumina reads. Assembly contiguity and completeness were assessed with QUAST 4.5 (9) and BUSCO

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3.0.0 (10), respectively. All algorithms were run with default settings unless otherwise noted. The BUSCO lineage was "sordariomyceta_odb9."

The resulting assembly was 42,948,211 bp in length with 24 total contigs, and GC content of 52.47%. Coverage was 65.9× and 25.8× for ONT and Illumina reads, respectively. The assembly N50 was 4.4 Mbp, L50 was 5, and the largest contig was 5,591,828 bp. Completeness was estimated at 98.6% (98.4% complete and single-copy genes, 0.2% duplicates), 0.6% fragmented, 0.8% missing with 3725 BUSCOs tested.

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DATA AVAILABILITY

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAULBG000000000. The version described in this paper is version JAULBG010000000. Raw sequence data are available at NCBI SRA under project number PRJNA994555. Sequences of TEF1- and RPB2 are deposited in GenBank under accession numbers OR338330 and OR338331.

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