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Resource Announcement

Genome Sequence Resources from Three Isolates of the Apple Canker Pathogen *Neonectria ditissima* Infecting Forest Trees

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Abstract

Neonectria ditissima is a generalist ascomycete plant pathogen causing canker diseases on a variety of hardwood tree species and can cross-infect many of them. The fungus enters the plants through wounds throughout the year. *N. ditissima* is considered a major threat to apple production responsible for the fruit tree canker disease, which damages trees and causes rotting of fruits in storage. Nearby forests and shelter belts can serve as sources of inoculum for well-managed apple orchards. Thus, knowledge about the *N. ditissima* isolates infecting different host species is essential for designing integrated pest management strategies. Here, we describe the genomes of three *N. ditissima* isolates, Nd_iso34, Nd_iso35, and Nd_iso36, infecting European beech, American tulip tree, and American beech, respectively. We obtained genome assemblies of approximately 45 megabases for all isolates, covering 94% of the *N. ditissima* reference annotation, and 97% of the universal single-copy orthologs (BUSCOs). We conclude that these genome assemblies are a highly relevant resource considering the scarcity of genomic data available for *N. ditissima*.

Keywords: apple canker, fungal pathogens, genome sequencing, Illumina, *Neonectria ditissima*, tree pathogen

Genome Announcement

The fungus *Neonectria ditissima* (formerly *N. galligena*) causes a disease known as European canker or fruit tree canker, which is a serious threat to apple production in Sweden and globally (Garkava-Gustavsson et al. 2016; Weber 2014). *N. ditissima* is a wound pathogen that can infect trees throughout the year and fruits in orchards and storage (Holthusen and Weber 2021; Weber 2014). The fungus has a wide range of hosts (Flack and Swinburne 1977; Walter et al. 2015). *N. ditissima* can attack other fruit trees such as pear (*Pyrus communis*) and quince (*Cydonia oblonga*) (Walter et al. 2015), as well as many different species of forest trees, e.g., *Acer, Betula, Fagus, Populus, Quercus*, and *Salix* (Ward et al. 2010). Canker damages caused by *N. ditissima* have been recorded on forest trees in Europe including Austria, Germany, and Slovakia (Cech 2010; Metzler et al. 2002; Mihál 2011). In North America, *N. ditissima* is known to be associated with

beach bark disease but was also found on yellow birch (*Betula alleghaniensis*), striped maple (*Acer pensylvanicum*), and red maple (*Acer rubrum*) (Kasson and Livingston 2009). Hence, *N. ditissima* also has the propensity to cause damage to Swedish and European forestry through the extensive scarring and damage that leads to reductions in quality and value of timber (Metzler et al. 2002).

We sequenced three isolates, Nd_iso34, Nd_iso35, and Nd_iso36, infecting European beech (*Fagus sylvatica*), American tulip tree (*Liriodendron tulipifera*), and American beech (*Fagus grandifolia*), respectively. Fungal cultures were obtained from The Centraalbureau voor Schimmelcultures (CBS) and the Fungal Biodiversity Centre (Royal Netherlands Academy of Arts and Sciences, KNAW). Nd_iso34 (synonym CBS 118920, AR 3690, and BPI 870951) was isolated in 2001 from European beech in Stiavnickey vrchy (Slovakia). Nd_iso35 (synonym CBS 118919, GJS 04-350, and BPI 864075) was isolated in 2004 from the American tulip tree in the Great Smokey Mountains National Park (Tennessee, U.S.A.). Finally, Nd_iso36 (synonym CBS 118923, AR 4196, and BPI 870950) was isolated in 2005 from American beech near Bass Lake (Michigan, U.S.A.).

High molecular weight genomic DNA was extracted from mycelium using QIAGEN genomic-tips according to the instructions from the manufacturer (QIAGEN, The Netherlands). DNA concentration and integrity were evaluated with the Qubit dsDNA quantification assay kits according to instructions from the manufacturer (Invitrogen, Waltham, MA, U.S.A.) and were further visualized on a 0.8% agarose gel with tris-acetate-ethylenediaminetetraacetic acid (TAE) buffer (0.4 M tris acetate and 0.01 M EDTA, pH = 8.3) and a high DNA mass ladder (Invitrogen). Sequencing libraries were prepared from 1 µg of DNA using the TruSeq DNA PCR-Free sample preparation kit (Illumina) targeting an insert size of 350 bp. The library preparation was performed according to the manufacturers' instructions. Libraries were applied to 150-bp paired-end sequencing on an Illumina HiSeq X sequencing system at the SNP&SEQ Technology Platform at Uppsala University (Uppsala, Sweden). The quality of the reads was checked using FASTQC, adapters were filtered, and sequences were quality trimmed using Trimmomatic v0.39 with default parameters (Bolger et al. 2014). Paired-end reads were applied to ABySS, a de novo parallel assembler particularly adapted for paired-end sequences (Simpson et al. 2009), with default parameters. The resulting genome assemblies ranged from 44.51 to 45.71 Mb, with an N_{50} ranging from 200,252 to 438,025 bp (see summary in Table 1).

Gene prediction was carried out on contigs with a minimum size of 1,000 bp (Table 1) using Funannotate v1.8.15 (Palmer and Stajich 2020). A total of 11,984 (Nd_iso34), 11,890 (Nd_iso35), and 11,960 (Nd_iso36) high confidence genes were predicted, indicating that approximately 94% of the reference *N. ditissima* genome annotation (Gómez-Cortecero et al. 2015) is covered by our genome assemblies (Fig. 1A). Furthermore, protein size distribution was consistent over all isolates (Fig. 1B), further indicating that there is no isolate-specific bias in gene completeness with respect to differences in assembly (Table 1) (Gómez-Cortecero et al. 2015). Finally, analysis of universal fungal single-copy orthologs (BUSCOs) (Kim et al. 2023) using the Funannotate-annotate pipeline shows that approximately 97% of the BUSCO terms were recovered for isolates (Fig. 1C). We thus conclude that the resulting genome assemblies are of good qual-

TABLE 1

Summary of the genome assemblies of the *Neonectria ditissima* isolates Nd_iso34, Nd_iso35, and Nd_iso36

Isolate	Number of contigs ≥ 1,000 bp	N_{50} contig length (bp)	Maximum contig length (bp)	Genome assembly size (Mb)
Nd_iso34	463	438,025	1,618,518	44.51
Nd_iso35	854	200,252	956,856	45.71
Nd_iso36	622	375,853	1,216,142	44.89

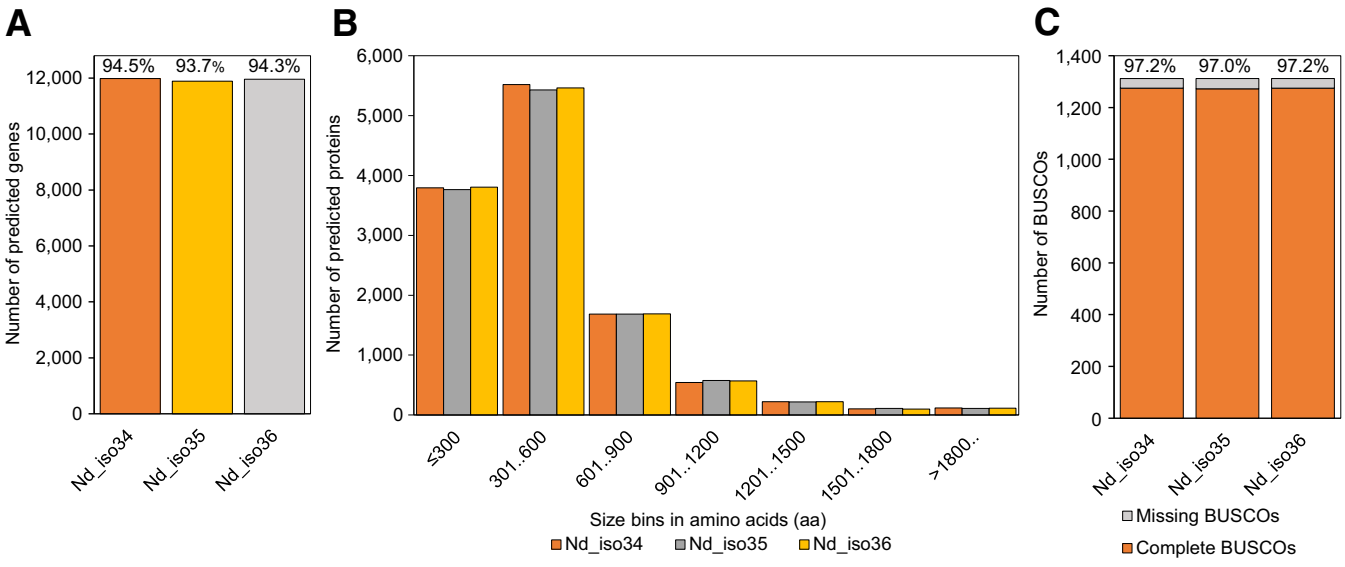

FIGURE 1

Genome annotation summary for the *Neonectria ditissima* isolates Nd_iso34, Nd_iso35, and Nd_iso36. **A,** Total number of predicted genes in every genome with the percentage of gene coverage as compared with the *N. ditissima* reference genome indicated as a percentage. **B,** Protein size distribution in bins of 300 amino acids (aa). **C,** Functional annotation of universal single-copy orthologs (BUSCOs) with the percentage of complete BUSCOs indicated.

TABLE 2

Gene functional annotation summary for the *Neonectria ditissima* isolates Nd_iso34, Nd_iso35, and Nd_iso36

Isolate	Number of annotated Pfams[a]	Number of annotated CAZymes[b]	Number of proteins with signalP[c]	Number of proteins with mTP[d]
Nd_iso34	4,042	554	962	386
Nd_iso35	4,042	558	955	381
Nd_iso36	4,032	564	977	373

[a] Protein families annotated from the Pfam database.
[b] Carbohydrate-active enzymes (CAZymes) annotated from dbCAN.
[c] Proteins with a predicted signal peptide annotated with signalP 6.0.
[d] Proteins with a predicted mitochondrial targeting peptide (mTP) annotated with targetP 2.0.

ity considering the use of short reads for genome sequencing and assembly.

Further functional annotation of the predicted proteins was performed using a combination of Funannotate-annotate v1.8.15, dbCAN (https://bcb.unl.edu/dbCAN2/), SignalP v6.0 for signal peptide prediction (https://services.healthtech.dtu.dk/services/SignalP-6.0/), and TargetP v2.0 for targeting to the mitochondrion (https://services.healthtech.dtu.dk/services/TargetP-2.0/) (see summary in Table 2). Here also, results were consistent among all isolates and within the expected range with an average of 4,038 protein families (Pfams), 559 carbohydrate-active enzymes (CAZymes), 965 putative secreted proteins with a predicted signal peptide (SignalP), and 380 putative mitochondrially targeted proteins (mTP, TargetP) (Table 2). We conclude that, overall, the resulting genome annotations have a high level of completeness, so these resources will be valuable for the study of *N. ditissima*.

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