

Draft genome sequences for *Neonectria magnoliae* and *Neonectria punicea*, canker pathogens of *Liriodendron tulipifera* and *Acer saccharum* in West Virginia

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ABSTRACT The fungal genus *Neonectria* contains many phytopathogenic species currently impacting forests and fruit trees worldwide. Despite their importance, a majority of *Neonectria* spp. lack sufficient genomic resources to resolve suspected cryptic species. Here, we report draft genomes and assemblies for *Neonectria magnoliae* NRRL 64651 and *Neonectria punicea* NRRL 64653.

KEYWORDS fungi, Nectriaceae, canker pathogens, Hypocreales, genomics

Neonectria ditissima, *N. faginata*, and *N. coccinea* cause lethal canker diseases of fruit (*Malus* and *Pyrus* spp.) and forest (*Fagus* spp.) trees and have been studied extensively (1–3). Since 2018, genomic data for these species and six additional *Neonectria* spp. have provided unique insights into their biology, pathogenicity, and host specialization [(4–10, Table 1)]. In 2020, *N. magnoliae*, a native canker pathogen of tulip-poplar (*Liriodendron tulipifera*) and Fraser magnolia (*Magnolia fraseri*), was formally described from isolates from West Virginia (Fig. 1; 11). During follow-up surveys in Pocahontas County, *N. punicea*, a species rarely documented in North America (12), was recovered from dead sugar maple (*Acer saccharum*; Fig. 1). Both *N. magnoliae* (*Nm*) and *N. punicea* (*Np*) co-occur with *N. ditissima* (*Nd*) and *N. faginata* (*Nf*) in eastern U.S. forests on three common hosts, *L. tulipifera* (*Nm,Nd*), *A. saccharum* (*Np,Nd*), and *F. grandifolia* (*Np,Nf*), yet their incidence and impact on these and other hosts are largely unexplored (1, 11–13). *Neonectria* and *Corinectria* canker diseases are of increasing concern (11, 14, 15), emphasizing an urgent need to fill critical data gaps; generating genomic resources for these species is fundamental to these efforts.

Single-ascospore cultures of *Nm* (NRRL 64651) and *Np* (NRRL 64653) were harvested from serial dilution platings of freshly collected individually macerated perithecia (Fig. 1). Cultures were grown out for ~2 weeks on potato dextrose agar at 20°C under ambient light conditions and genomic DNA extracted from harvested tissue with a Qiagen DNeasy PowerSoil Pro Kit using the manufacturer's protocols. An Illumina NextSeq 1000 (Marshall University Genomics Core Facility, Huntington, WV), generated 12.3 M paired sequence reads or 3.7 Gb for *Nm* and 5.8 M paired sequence reads or 1.8 Gb for *Np*. The assembled genome for *Nm* strain NRRL 64651 was 43.67 Mbp (coverage, 77.6 x; *N*₅₀, 246.50 kb; *L*₅₀, 58; G + C content, 51.83%) and 47.36 Mbp (coverage, 34.2 x; *N*₅₀, 121.04 kb; *L*₅₀, 120; G + C content, 51.47%) for *Np* strain NRRL 64653 (Table 1). Both assemblies were cleaned of vector contamination and redundant contigs using SPAdes v3.15.2 running within AATF (v0.4.1) (16) with the steps trim, filter using fastp (17), and vectrim, sourpurge, and rmdup steps to remove contaminating contigs in the assembly. Assemblies were further corrected by five rounds of polishing with Pilon (v1.24) with the Illumina reads. Genome annotation was performed with funannotate (v1.8.15; (18))

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TABLE 1 Genome strain information and statistics for *Neonectria magnoliae* and *Neonectria punicea* with comparisons to other public genome resources

Species ^a	<i>N. magnoliae</i>	<i>N. punicea</i>	<i>N. punicea</i>	<i>N. punicea</i>	<i>N. faginata</i>	<i>N. coccinea</i>	<i>N. hederae</i>	<i>N. neomacrospora</i>	<i>N. ditissima</i>	<i>N. lugdunensis</i>	<i>N. sp.</i>	<i>C. fuckeliana</i>
Strain ID	NRRL 64651 / FERN 10531	NRRL 64653 / WVPC2	CBS 119724 / A.R. 3102	CBS 134246 / A.R. 4307	CBS 119158 / G.J.S. 98–114	CBS 714.97	KNNDK1	CBS 226.31 / IMI 113922	DSM 113088	DH2	CBS 125109 / G.J.S. 02–67	
Year sampled / sequenced	2018/2024	2020/2024	1999/2018	2002/2021	1998/2021	1932/2018	2015/2021	1925/2021	1980/2024	2013/2018	2002/2021	
Plant Host	<i>Liriodendron tulipifera</i>	<i>Acer saccharum</i>	<i>Frangula alnus</i>	<i>Fagus grandifolia</i>	<i>Fagus sylvatica</i>	<i>Hedera helix</i>	<i>Abies nordmanniana</i>	<i>Fagus sylvatica</i>	Unknown woody host	<i>Meconopsis grandis</i>	<i>Pinus radiata</i>	
Location details	U.S.A.	U.S.A.	Austria	U.S.A.	Germany	Netherlands	Denmark	Germany	Slovakia	China: Tibet	New Zealand	
Coordinates (Lat., Long.)	39.034 N; −79.685 W	38.1913 N; −80.1963 W	— ^b	—	—	—	—	—	—	—	—	
Sequencing Technology	Illumina NextSeq	Illumina NextSeq	Illumina MiSeq	Illumina MiSeq	Illumina MiSeq	Illumina MiSeq	PacBio	Illumina MiSeq	PacBio RSII	PacBio Sequel	Illumina MiSeq	
Genome size (Mbp)	43.67	47.36	41.47	42.17	42.74	43.29	37.12	43.53	44.79	45.83	42.25	
No. of scaffolds	408	1,077	1,779	522	571	508	12	1,274	15	43	737	
Longest contig / scaffold (kbp)	1,157 / —	405 / —	— / 342.6	— / 709.7	— / 532.9	— / 802	— / —	— / 515.4	— / —	5,080 / —	822.7	
Scaffold N50 (kbp)	246.50	121.04	69.92	237.13	178.88	248.96	4,617.23	114.09	4,002.58	1,899.89	255.65	
Scaffold L50	58	120	180	53	76	54	4	119	5	8	54	
Avg coverage (x)	77	34	25	20	20	52	65	20	1	80	20	
Total Illumina sequence (Gbp) ^{c,d}	3.7	1.8	—	0.0018	0.0053	—	—	0.0028	—	—	0.005	
Total Illumina reads ^d	12,321,892	5,838,868	—	9,900,000	40,400,000	—	—	16,700,000	—	—	31,500,000	
GC content (%)	52	52	53	53	52	50	53	52	52	53	50	
Complete BUSCOs ascomy-	1,696 (99.4)	1,694 (99.3)	— / 1,412 (98.2)	— / 1,353 (94.1)	— / 1,424 (99.0)	— / 1,429 (99.4)	—	— / 1,415 (98.4)	—	(96.2) / —	— / 1,428 (99.3)	
cota_odb10 (%) / fungi_odb9 (%) ^d	1,686 (98.8)	1,686 (98.8)	—	—	—	—	—	—	—	—	—	
Single-copy BUSCOs	10 (0.6)	9 (0.5)	—	—	—	—	—	—	—	—	—	
Duplicated BUSCOs	3 (0.2)	2 (0.1)	—	—	—	—	—	—	—	—	—	
Fragmented BUSCOs	12,569 (12,394/174)	13,388 (13,207/181)	13,180	12,991	12,941	11,966	11,291	13,669	—	13,606	11,446	
Total genes (protein coding genes / tRNAs) ^d												

(Continued on next page)

TABLE 1 Genome strain information and statistics for *Neonectria magnoliae* and *Neonectria punicea* with comparisons to other public genome resources (Continued)

Species ^a	<i>N. magnoliae</i>	<i>N. punicea</i>	<i>N. punicea</i>	<i>N. faginata</i>	<i>N. coccinea</i>	<i>N. hederiae</i>	<i>N. neomacrospora</i>	<i>N. ditissima</i>	<i>N. lugdunensis</i>	<i>N. sp.</i>	<i>C. fuckeliana</i>
GenBank assembly	GCA_037954305.1	GCA_037954315.1	GCA_03385315.1	GCA_019137275.1	GCA_019137265.1	GCA_003385265.1	GCA_917563905.1	GCA_019137815.1	GCA_041721585.1	GCA_003934905.1	GCA_019137255.1
no.											
WGS master record	JAZAVK0000000000	JAZAVJ0000000000	QGQA01000000	WPDD01000000	WPDF01000000	QGQB01000000	OU830638 -OU830649	WPDG01000000	JBGLZM0100000000	RQWH01000000	WPDH01000000
SRA accession no.	SRR24938461	SRR24938484	--	SRR12873405	SRR12873403	--	--	SRR12873402	--	--	SRR12873401
BioSample accession no.	SAMN35642710	SAMN35646705	SAMN09242091	SAMN13483917	SAMN13483919	SAMN09242090	SAMEA9994693	SAMN13483920	SAMN43221455	SAMN10492166	SAMN13483921
Reference	This study	This study	4	6	6	4	7	6	N/A	5	6
Biosynthetic Gene Clusters (BGC) ^c											
T1PKS	12	10	9	8 (10)	10 (12)	7	12	11 (16)	12	14 (14)	14 (17)
T3PKS	1	1	1	1 (1)	1 (1)	1	1	1 (1)	1	1 (1)	1 (1)
NRPS	11	10	10	10 (18)	10 (20)	10	6	7 (23)	8	13 (13)	9 (22)
Terpenes	6	7	6	5 (5)	6 (7)	7	4	6 (6)	5	5 (5)	5 (5)
Other	5	5	6	7 (2)	6 (1)	7	9	9 (0)	7	11 (14)	4 (0)
Total	35	33	32	31 (337)	33 (377)	32	32	34 (397)	33	44 (47)	33 (407)

^aSeven additional total genomes are publicly available for *N. faginata* (two), *N. coccinea* (one), and *N. ditissima* (four), but are excluded from this table as only single representatives per species are provided for comparison.
^b“--” Denotes missing data except for coordinates as they may exist but were not relevant to this study.
^cAn Agilent 2100 Bioanalyzer system using an Agilent High-Sensitivity DNA Kit was used for sizing and quantitation of DNA sequencing libraries following the manufacturer’s protocols.
^dDenotes rows where data were extracted from the reference listed rather than from the NCBI Genome Assembly Accession page.
^eAntismash results: newly generated for this study (previously published results). Numbers followed by a “?” denote instances where total BGCs reported differ from the sum of the individual categories shown here. Results generated for this study used a “strict” detection strictness, whereas previously published studies did not include detection strictness in methods.

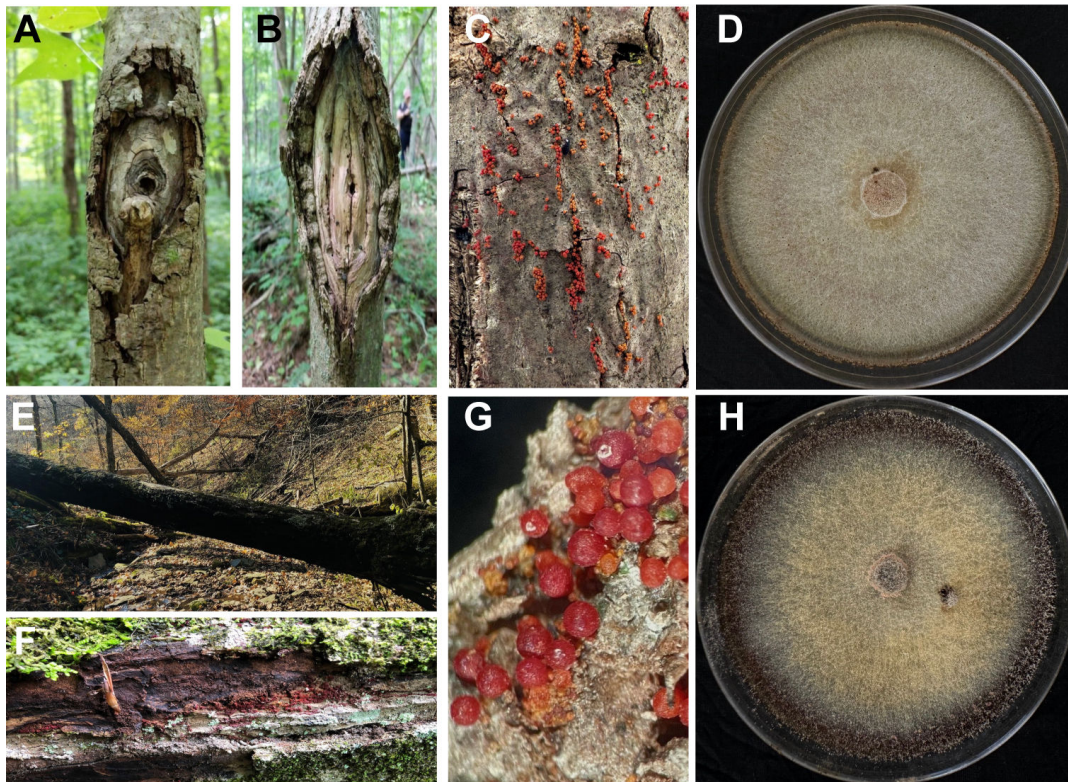


FIG 1 *In situ* and *in vitro* photos of *Neonectria magnoliae* (A–D) and *Neonectria punicea* (E and F) canker pathogens of woody angiosperms. *N. magnoliae* produces both classic target-shaped or fusiform-shaped cankers (A and B) on healthy tulip-poplar (*Liriodendron tulipifera*) trees and inconspicuous cankers (C) on stressed, small-diameter understory trees. *N. punicea* produces inconspicuous cankers on dead and dying sugar maple (*Acer saccharum*) trees (E and F), identifiable primarily based on the occurrence of red perithecia (C and G). Single ascospore-derived cultures of *N. magnoliae* (D) and *N. punicea* (H) with characteristic *Cylindrocarpon*-morph colonies.

utilizing alignment of proteins in UniProt and BUSCO with sordariomycetes_odb10 for training. tRNA genes were predicted using tRNAscan-SE v2.0.9 (19). BUSCO v5.4.4 (20), using the ascomycota_odb10 data set (21), identified 1,696 complete markers (99.4%) in *Nm* and 1,694 (99.3%) in *Np* (Table 1). Default parameters were used or when specified, available in the pipeline code, parameters, and logfiles at GitHub and Zenodo (22). The final genome annotations included a total of 12,394 protein-coding genes (PCGs) and 174 tRNAs for *Nm* and 13,207 PCGs and 181 tRNAs for *Np* (4–9; Table 1). AntiSMASH (v5.0; (23)) predicted 32 and 35 biosynthetic gene clusters (BGCs) for *Nm* and *Np*, respectively. Genome size, gene counts, and BGCs agreed with published statistics for other *Neonectria* spp. (Table 1).

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Hannah M. Petronek, Conceptualization, Funding acquisition, Investigation, Validation, Visualization, Writing – original draft, Writing – review and editing | Matt T. Kasson, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing | Amy M. Metheny, Conceptualization, Investigation, Writing – review and editing | Cameron M. Stauder, Conceptualization, Investigation, Writing – review and editing | Brian Lovett, Conceptualization,

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DATA AVAILABILITY

This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession numbers [JAZAVK010000000](#) and [JAZAVJ010000000](#). Sequence reads were deposited under SRA project accession numbers [SRR24938461](#) and [SRR24938484](#) and BioProject accession numbers [PRJNA980700](#) and [PRJNA980721](#).

REFERENCES

1. Castlebury LA, Rossman AY, Hyten AS. 2006. Phylogenetic relationships of *Neonectria/Cylindrocarpon* on *Fagus* in North America. *Botany* 84:1417–1433. <https://doi.org/10.1139/b06-105>
2. Weber RWS. 2014. Biology and control of the apple canker fungus *Neonectria ditissima* (syn. *N. galligena*) from a Northwestern European perspective. *ErwObstbau* 56:95–107. <https://doi.org/10.1007/s10341-014-0210-x>
3. Cale JA, Garrison-Johnston MT, Teale SA, Castello JD. 2017. Beech bark disease in North America: over a century of research revisited. *For Ecol Manage* 394:86–103. <https://doi.org/10.1016/j.foreco.2017.03.031>
4. Salgado-Salazar C, Crouch JA. 2019. Genome resources for the stem and bark canker pathogens *Corinectria fuckeliana*, *Neonectria hederæ* and *N. punicea*. *Plant Dis* 103:389–391. <https://doi.org/10.1094/PDIS-05-18-0904-A>
5. Lin X, Xu H, Liu L, Li H, Gao Z. 2020. Draft genome sequence of *Neonectria* sp. DH2 isolated from *Meconopsis grandis* Prain in Tibet. 3 Biotech 10. <https://doi.org/10.1007/s13205-020-02345-8>
6. Salgado-Salazar C, Skaltsas DN, Phipps T, Castlebury LA. 2021. Comparative genome analyses suggest a hemibiotrophic lifestyle and virulence differences for the beech bark disease fungal pathogens *Neonectria faginata* and *Neonectria coccinea*. G3 (Bethesda) 11:jkab071. <https://doi.org/10.1093/g3journal/jkab071>
7. Nielsen KN, Sirén K, Petersen B, Sicheritz-Pontén T, Gilbert MTP, Korneliussen TS, Hansen OK. 2021. The pangenome of the fungal pathogen *Neonectria neomacrospora*. *bioRxiv*. <https://doi.org/10.1101/2021.03.11.434922>
8. Morrison EW, Duong TA, Garnas JR. 2024. A high-quality draft genome sequence of *Neonectria faginata*, causative agent of beech bark disease of *Fagus grandifolia*. *Microbiol Resour Announc* 13:e0104823. <https://doi.org/10.1128/mra.01048-23>
9. Bourras S, Véléz H, Ihrmark K, Corrales Gutiérrez MÁ, Elfstrand M, Garkava-Gustavsson L, Dalman Falk K. 2024. Genome sequence resources from three isolates of the apple canker pathogen *Neonectria ditissima* infecting forest trees. *bioRxiv*. <https://doi.org/10.1101/2024.01.13.575195>
10. Stauder CM, Garnas JR, Morrison EW, Salgado-Salazar C, Salgado-Salazar MT. 2020. Characterization of mating type genes in heterothallic *Neonectria* species, with emphasis on *N. coccinea*, *N. ditissima*, and *N. faginata*. *Mycologia* 110:880–894. <https://doi.org/10.1080/00275514.2020.1797371>
11. Stauder CM, Utano NM, Kasson MT. 2020. Resolving host and species boundaries for perithecia-producing nectriaceous fungi across the central Appalachian Mountains. *Fungal Ecol* 47:100980. <https://doi.org/10.1016/j.funeco.2020.100980>
12. Hirooka Y, Rossman AY, Zhuang W-Y, Salgado-Salazar C, Chaverri P. 2013. Species delimitation for *Neonectria coccinea* group including the causal agents of beech bark disease in Asia, Europe, and North America. *Mycosystema* 32:485–517.
13. Lohman ML, Watson AJ. 1943. Identity and host relations of *Nectria* species associated with diseases of hardwoods in the eastern states. *Lloydia* 6:77–108.
14. Nielsen KN, Gopalakrishnan S, Korneliussen TS, Skovrind M, Sirén K, Petersen B, Sicheritz-Pontén T, Thomsen IM, Gilbert MTP, Hansen OK. 2020. Population genomics of the emerging forest pathogen *Neonectria neomacrospora*. *bioRxiv*. <https://doi.org/10.1101/2020.12.07.407155>
15. Pavlov IN, Vasaitis R, Litovka YA, Stenlid J, Jankovsky L, Timofeev AA, Menkis A. 2020. Occurrence and pathogenicity of *Corinectria* spp.—an emerging canker disease of *Abies sibirica* in Central Siberia. *Sci Rep* 10:5597. <https://doi.org/10.1038/s41598-020-62566-y>
16. Stajich JE, Palmer J. 2019. Automatic assembly for the fungi (AAFTF): genome assembly pipeline. *Zenodo*. <https://doi.org/10.5281/zenodo.1620526>
17. Chen S. 2023. Ultrafast one-pass FASTQ data preprocessing, quality control, and deduplication using fastp. *iMeta* 2:e107. <https://doi.org/10.1002/imt2.107>
18. Palmer JM, Stajich JE. 2020. Funannotate: Eukaryotic genome annotation. *Zenodo*. <https://doi.org/10.5281/zenodo.4054262>
19. Chan PP, Lin BY, Mak AJ, Lowe TM. 2021. tRNAscan-SE 2.0: improved detection and functional classification of transfer RNA genes. *Nucleic Acids Res* 49:9077–9096. <https://doi.org/10.1093/nar/gkab688>
20. Seppey M, Manni M, Zdobnov EM. 2019. BUSCO: assessing genome assembly and annotation completeness. *Methods Mol Biol* 1962:227–245. https://doi.org/10.1007/978-1-4939-9173-0_14
21. Manni M, Berkeley MR, Seppey M, Zdobnov EM. 2021. BUSCO: assessing genomic data quality and beyond. *Curr Protoc* 1:e323. <https://doi.org/10.1002/cpz1.323>
22. Stajich JE. 2024. Pipeline for genome assembly and annotation of *Neonectria magnoliae* and *Neonectria punicea* (version v1.0). *Zenodo*. <https://doi.org/10.5281/zenodo.13787582>
23. Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, Weber T. 2019. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. *Nucleic Acids Res* 47:W81–W87. <https://doi.org/10.1093/nar/gkz310>