




Draft Genome Sequence of the Phytopathogenic Fungus *Fusarium euwallaceae*, the Causal Agent of *Fusarium* Dieback

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ABSTRACT Here, we report the genome of *Fusarium euwallaceae* strain HFEW-16-IV-019, an isolate obtained from Kuroshio shot hole borer (a *Euwallacea* sp.). These beetles were collected in Tijuana, Mexico, from elm trees showing typical symptoms of *Fusarium* dieback. The final assembly consists of 287 scaffolds spanning 48,274,071 bp and 13,777 genes.

Fusarium euwallaceae belongs to the ambrosia *Fusarium* clade (1). Species within this clade form symbiotic relationships with ambrosia beetles of the genus *Euwallacea* (Coleoptera: Curculionidae: Scolytinae) (2). An obligate symbiosis exists between *F. euwallaceae* and its *Euwallacea* sp. beetle host (3), and together they cause *Fusarium* dieback, which severely threatens natural forests, landscape trees, and avocado orchards (2–5). The Kuroshio shot hole borer (a *Euwallacea* sp.), an exotic ambrosia beetle native to Asia (1), is established in landscapes and forests in southern California in the United States (6) and was detected recently in Tijuana, Mexico (7).

The fungus was grown in potato dextrose broth (PDB) medium ($29 \pm 2^\circ\text{C}$, 200 rpm), and after 14 days, mycelia were harvested by filtration and cryogenically pulverized. Genomic DNA was isolated from 500 mg of pulverized tissue according to a previously described protocol (8).

Two DNA libraries were constructed using the Nextera XT DNA kit (Illumina). The libraries were quantified with a Qubit version 2.0 fluorometer (Thermo Fisher Scientific), and their quality was evaluated on a 2100 Bioanalyzer instrument (Agilent Technologies) using a 7500 DNA kit. Both libraries were sequenced on a MiSeq sequencer (Illumina) using a MiSeq version 2.0 reagent kit (300 cycles). Before assembly, paired-end reads (12,294,902) were filtered (see <https://github.com/Czh3/NGSTools/blob/master/qualityControl.py>) to obtain high-quality reads that were merged and adapter trimmed with SeqPrep (see <https://github.com/jstjohn/SeqPrep>). Over 11.2 million merged or paired-end reads were *de novo* assembled using Newbler version 3.0. Scaffolds were generated using the reference-based scaffolder MeDuSa (9) with the *Fusarium solani* genome as a guide for alignment. The assembly resulted in 287 scaffolds (788 contigs) totaling 48,274,071 bp (N_{50} , 1,349,055 bp; largest scaffold, 4,967,455 bp; $\sim 62.3\times$ coverage). The genome assembly comprises a size comparable to that of other published genomes of *Fusarium* species (e.g., *F. fujikuroi*, 43.83 Mb [10];

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F. graminearum, 36.44 Mb [11]; *F. oxysporum*, 61.35 Mb [12]; *F. verticillioides*, 41.77 Mb [11, 12] and *F. solani*, 51.21 Mb [13]).

Gene models were identified with the evidence-directed AUGUSTUS predictor (14), which was specially trained for *F. euwallaceae* using WebAUGUSTUS (15) and full coding sequences derived from a previously assembled transcriptome (unpublished data). AUGUSTUS gene models were improved/corrected using the Maker-P pipeline (16) and a database containing all of the proteins from the genomes of *F. fujikuroi*, *F. graminearum*, *F. oxysporum*, *F. verticillioides*, and *F. solani* (latest versions downloaded from the JGI/MycoCosm portal [see <http://genome.jgi.doe.gov/programs/fungi/index.jsf>]). The total number of genes predicted in the *F. euwallaceae* genome was 13,777, which is similar to the number of genes reported for *F. graminearum*, *F. fujikuroi*, *F. solani*, and *F. verticillioides* (13,322, 14,813, 15,705, and 15,869, respectively) and slightly lower than that of *F. oxysporum* (20,925). The genome completeness was assessed using BUSCO (17), which estimated the genome sequence to be 98.4% complete based on the presence of conserved orthologous gene sets specific to *Ascomycota* fungi.

The genomic data reported here will be useful to deepen our understanding of *Fusarium* dieback disease. Our preliminary analyses suggest that the *F. euwallaceae* genome encodes proteins homologous to those involved in the biosynthesis of polyketide-derived mycotoxins. Undoubtedly, *F. euwallaceae* can potentially produce an arsenal of toxins and virulence factors.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. [NHTE00000000](https://accession.ddbj.go.jp/acc/showacc.cgi?accession=NHTE00000000). The version described in this paper is the second version, NHTE02000000.

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REFERENCES

- O'Donnell K, Sink S, Libeskind-Hadas R, Hulcr J, Kasson MT, Ploetz RC, Konkol JL, Ploetz JN, Carrillo D, Campbell A, Duncan RE, Liyanage PN, Eskalen A, Na F, Geiser DM, Bateman C, Freeman S, Mendel Z, Sharon M, Aoki T, Cossé AA, Rooney AP. 2015. Discordant phylogenies suggest repeated host shifts in the *Fusarium-Euwallacea* ambrosia beetle mutualism. *Fungal Genet Biol* 82:277–290. <https://doi.org/10.1016/j.fgb.2014.10.014>.
- Kasson MT, O'Donnell K, Rooney AP, Sink S, Ploetz RC, Ploetz JN, Konkol JL, Carrillo D, Freeman S, Mendel Z, Smith JA, Black AW, Hulcr J, Bateman C, Stefkova K, Campbell PR, Geering ADW, Dann EK, Eskalen A, Mohotti K, Short DPG, Aoki T, Fenstermacher KA, Davis DD, Geiser DM. 2013. An inordinate fondness for *Fusarium*: phylogenetic diversity of fusaria cultivated by ambrosia beetles in the genus *Euwallacea* on avocado and other plant hosts. *Fungal Genet Biol* 56:147–157. <https://doi.org/10.1016/j.fgb.2013.04.004>.
- Freeman S, Sharon M, Maymon M, Mendel Z, Protasov A, Aoki T, Eskalen A, O'Donnell K. 2013. *Fusarium euwallaceae* sp. nov.—a symbiotic fungus of *Euwallacea* sp., an invasive ambrosia beetle in Israel and California. *Mycologia* 105:1595–1606. <https://doi.org/10.3852/13-066>.
- Eskalen A, Stouthamer R, Lynch SC, Rugman-Jones PF, Twizeyimana M, Gonzalez A, Thibault T. 2013. Host range of *Fusarium* dieback and its ambrosia beetle (Coleoptera: Scolytinae) vector in southern California. *Plant Dis* 97:938–951. <https://doi.org/10.1094/PDIS-11-12-1026-RE>.
- O'Donnell K, Libeskind-Hadas R, Hulcr J, Bateman C, Kasson MT, Ploetz RC, Konkol JL, Ploetz JN, Carrillo D, Campbell A, Duncan RE, Liyanage PN, Eskalen A, Lynch SC, Geiser DM, Freeman S, Mendel Z, Sharon M, Aoki T, Cossé AA, Rooney AP. 2016. Invasive Asian *Fusarium-Euwallacea* ambrosia beetle mutualists pose a serious threat to forests, urban landscapes and the avocado industry. *Phytoparasitica* 44:435–442. <https://doi.org/10.1007/s12600-016-0543-0>.
- Boland JM. 2016. The impact of an invasive ambrosia beetle on the riparian habitats of the Tijuana River Valley, California. *PeerJ* 4:e2141. <https://doi.org/10.7717/peerj.2141>.
- García-Avila CDJ, Trujillo-Arriaga FJ, López-Buenfil JA, González-Gómez R, Carrillo D, Cruz LF, Ruiz-Galván I, Quezada-Salinas A, Acevedo-Reyes N. 2016. First report of *Euwallacea* nr. *Fornicatus* (Coleoptera: Curculionidae) in Mexico. *Fla Entomol* 99:555–556.
- Tapia-Tussell R, Lappe P, Ulloa M, Quijano-Ramayo A, Cáceres-Farfán M, Larqué-Saavedra A, Perez-Brito D. 2006. A rapid and simple method for DNA extraction from yeasts and fungi isolated from *Agave fourcroydes*. *Mol Biotechnol* 33:67–70.
- Bosi E, Donati B, Galardini M, Brunetti S, Sagot MF, Lió P, Crescenzi P, Fani R, Fondi M. 2015. MeDuSa: a multi-draft based scaffolder. *Bioinformatics* 31:2443–2451. <https://doi.org/10.1093/bioinformatics/btv171>.
- Wiemann P, Sieber CM, von Bargen KW, Studt L, Niehaus EM, Espino JJ, Huß K, Michiels CB, Albermann S, Wagner D, Bergner SV, Connolly LR, Fischer A, Reuter G, Kleigrew K, Bald T, Wingfield BD, Ophir R, Freeman S, Hippler M, Smith KM, Brown DW, Proctor RH, Münsterkötter M, Freitag M, Humpf HU, Güldener U, Tudzynski B. 2013. Deciphering the cryptic genome: genome-wide analyses of the rice pathogen *Fusarium fujikuroi* reveal complex regulation of secondary metabolism and novel metabolites. *PLoS Pathog* 9:e1003475. <https://doi.org/10.1371/journal.ppat.1003475>.
- Cuomo CA, Güldener U, Xu JR, Trail F, Turgeon BG, Di Pietro A, Walton JD, Ma LJ, Baker SE, Rep M, Adam G, Antoniw J, Baldwin T, Calvo S, Chang YL, Decaprio D, Gale LR, Gnerre S, Goswami RS, Hammond-Kosack K, Harris LJ, Hilburn K, Kennell JC, Kroken S, Magnuson JK, Mannhaupt G, Mauceli E, Mewes HW, Mitterbauer R, Muehlbauer G, Münsterkötter M, Nelson D, O'Donnell K, Ouellet T, Qi W, Quesneville H, Roncero MI, Seong

- KY, Tetko IV, Urban M, Waalwijk C, Ward TJ, Yao J, Birren BW, Kistler HC. 2007. The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. *Science* 317: 1400–1402. <https://doi.org/10.1126/science.1143708>.
12. Ma LJ, van der Does HC, Borkovich KA, Coleman JJ, Daboussi MJ, Di Pietro A, Dufresne M, Freitag M, Grabherr M, Henrissat B, Houterman PM, Kang S, Shim WB, Woloshuk C, Xie X, Xu JR, Antoniw J, Baker SE, Bluhm BH, Breakspear A, Brown DW, Butchko RA, Chapman S, Coulson R, Coutinho PM, Danchin EG, Diener A, Gale LR, Gardiner DM, Goff S, Hammond-Kosack KE, Hilburn K, Hua-Van A, Jonkers W, Kazan K, Kodira CD, Koehrsen M, Kumar L, Lee YH, Li L, Manners JM, Miranda-Saavedra D, Mukherjee M, Park G, Park J, Park SY, Proctor RH, Regev A, Ruiz-Roldan MC, Sain D, Sakthikumar S, Sykes S, Schwartz DC, Turgeon BG, Wapinski I, Yoder O, Young S, Zeng Q, Zhou S, Galagan J, Cuomo CA, Kistler HC, Rep M. 2010. Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature* 464:367–373. <https://doi.org/10.1038/nature08850>.
 13. Coleman JJ, Rounsley SD, Rodriguez-Carres M, Kuo A, Wasmann CC, Grimwood J, Schmutz J, Taga M, White GJ, Zhou S, Schwartz DC, Freitag M, Ma LJ, Danchin EG, Henrissat B, Coutinho PM, Nelson DR, Straney D, Napoli CA, Barker BM, Gribskov M, Rep M, Kroken S, Molnár I, Rensing C, Kennell JC, Zamora J, Farman ML, Selker EU, Salamov A, Shapiro H, Pangilinan J, Lindquist E, Lamers C, Grigoriev IV, Geiser DM, Covert SF, Temporini E, Vanetten HD. 2009. The genome of *Nectria haematococca*: contribution of supernumerary chromosomes to gene expansion. *PLoS Genet* 5:e1000618. <https://doi.org/10.1371/journal.pgen.1000618>.
 14. Stanke M, Diekhans M, Baertsch R, Haussler D. 2008. Using native and syntenically mapped cDNA alignments to improve de novo gene finding. *Bioinformatics* 24:637–644. <https://doi.org/10.1093/bioinformatics/btn013>.
 15. Hoff KJ, Stanke M. 2013. WebAUGUSTUS—a Web service for training AUGUSTUS and predicting genes in eukaryotes. *Nucleic Acids Res* 41: W123–W128. <https://doi.org/10.1093/nar/gkt418>.
 16. Campbell MS, Holt C, Moore B, Yandell M. 2014. Genome annotation and curation using MAKER and MAKER-P. *Curr Protoc Bioinformatics* 48: 4.11.1–4.11.39. <https://doi.org/10.1002/0471250953.bi0411s48>.
 17. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31:3210–3212. <https://doi.org/10.1093/bioinformatics/btv351>.