AMERICAN SOCIETY FOR MICROBIOLOGY

Microbiology®
Resource Announcements

Genome Sequence of the Fungus *Nannizziopsis barbatae*, an Emerging Reptile Pathogen

Daniel Powell,[a] Ashley Jones,[b] Nicola Kent,[a] Parwinder Kaur,[c] Ido Bar,[d] Benjamin Schwessinger,[b] Céline H. Frère[a]

aGlobal Change Ecology Research Group, School of Science, Technology, and Engineering, University of the Sunshine Coast, Sippy Downs, QLD, Australia
bResearch School of Biology, The Australian National University, Acton, Canberra, ACT, Australia
cSchool of Agriculture and Environment, Faculty of Science, University of Western Australia, Perth, WA, Australia
dEnvironmental Futures Research Institute, School of Environment and Sciences, Griffith University, Nathan, QLD, Australia

ABSTRACT *Nannizziopsis barbatae* is an emerging fungal pathogen capable of causing contagious dermatomycosis in reptiles. Here, we report a 31.54-Mb draft genome sequence of an isolate originating from an infected eastern water dragon in Brisbane, Australia.

*N*annizziopsis barbatae* is a keratinophilic ascomycetous fungus of the family *Onygenaceae*. Recently distinguished from the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) complex, this species was originally isolated from outbreaks of infection in captive bearded dragons (*Pogona barbata*) (1, 2). Members of the genus *Nannizziopsis* are capable of infecting a wide range of reptile hosts and are a leading cause of often fatal fungal dermatitis in captive animals, sometimes referred to as "yellow fungus disease" (3). *N. barbatae* has become increasingly prevalent in a free-living population of eastern water dragons (*Intellagama lesueurii*), presenting an emergent threat to populations of wild Australian reptiles.

The isolate of *N. barbatae* was obtained via a skin swab taken from a lesion from an infected dragon, selected using Mycosel agar (Becton, Dickinson), and cultivated further on potato dextrose agar for 2 weeks at 25°C. Genomic DNA for Illumina short-read sequencing was extracted from mycelium from a pure plate culture using the DNeasy blood and tissue kit (Qiagen) following the manufacturer's protocol for cultured cells. A single DNA library was sequenced on the HiSeq platform, generating 221 million 150-bp paired-end reads, which were trimmed and filtered using Trimmomatic v0.39 (4).

High-molecular-weight DNA was extracted for Nanopore sequencing from mycelium from the same isolate using a modified protocol based on Mayjonade et al. (5), which is described in detail on Protocols.io (6). Between 1 and 2 g of mycelium was homogenized and purified using a sorbitol wash solution described by Inglis et al. (7). DNA was size selected for fragments of ≥20 kb using a PippinHT instrument (Sage Science).

A long-read DNA sequencing library was prepared according to the Oxford Nanopore Technologies protocol 1D genomic DNA by ligation (SQK-LSK109). Sequencing was performed on a MinION Mk1B device using a FLO-MIN106 R9.4.1 revD flow cell according to the manufacturer's instructions. Base calling of MinION fast5 reads to FASTQ format was performed with Guppy software v4.0.14 (Oxford Nanopore Technologies). Sequencing yielded 147,481 reads with an N_{50} value of 32.95 kb (read lengths up to 182 kb), which totaled 3.46 Gbp (approximately 110× coverage). Next, the read data were analyzed and filtered using two NanoPack tools (8); quality was inspected with NanoPlot v1.28.2, and long reads were processed with NanoFilt v2.7.1 using the parameters -l 5000 -q 7 –headcrop 200 –tailcrop 200.

Illumina short reads and Nanopore long reads were assembled simultaneously with MaSuRCA v3.4.1 (9) using default parameters except for allowing up to 35× coverage of Nanopore long reads. The resulting *de novo* assembly of 11 contigs was 31.54 Mb in total length with a contig N_{50} value of 6.19 Mb and a GC content of 40.36%. The largest contig was 9.29 Mb in size. Completeness of the assembly was assessed using the BUSCO v4.0.6 (10) tool against the ascomycota_odb10 database of 1,706 genes. This resulted in the identification of 97.8% complete and single-copy genes. Illumina short-read data mapped to the assembly with a 99.87% overall alignment rate and with 89.19% aligning uniquely in pairs. Telomeric repeats ($TTAGGG_N$) were identified using the FindTelomeres script (11) at one end of 3 of the contigs and at both ends of contig 5. The karyotype of this species is currently unknown; however, at least four chromosomes were identified in the haploid *Coccidioides* spp. (12–14), fungal pathogens also from the order *Onygenales*. The assembly was masked for repetitive regions (12.24% of the genome) using RepeatMasker v4.1.0 (15) with default settings and using a custom repeat database built from the draft assembly using RepeatModeler2 v2.0.1 (16), also with default settings.

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number JACVYH000000000. The version described in this paper is version JACVYH010000000. The sequence reads were deposited in association with BioProject PRJNA662660.

REFERENCES

1. Johnson RSP, Sangster CR, Sigler L, Hambleton S, Paré JA. 2011. Deep fungal dermatitis caused by the *Chrysosporium* anamorph of *Nannizziopsis vriesii* in captive coastal bearded dragons (*Pogona barbata*). Aust Vet J 89:515–519. https://doi.org/10.1111/j.1751-0813.2011.00851.x.
2. Stchigel AM, Sutton DA, Cano-Lira JF, Cabañes FJ, Abarca L, Tintelnot K, Wickes BL, García D, Guarro J. 2013. Phylogeny of chrysosporia infecting reptiles: proposal of the new family *Nannizziopsiaceae* and five new species. Persoonia 31:86–100. https://doi.org/10.3767/003158513X669698.
3. Sigler L, Hambleton S, Pare JA. 2013. Molecular characterization of reptile pathogens currently known as members of the *Chrysosporium* anamorph of *Nannizziopsis vriesii* complex and relationship with some human-associated isolates. J Clin Microbiol 51:3338–3357. https://doi.org/10.1128/JCM.01465-13.
4. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
5. Mayjonade B, Gouzy J, Donnadieu C, Pouilly N, Marande W, Callot C, Langlade N, Muños S. 2016. Extraction of high-molecular-weight genomic DNA for long-read sequencing of single molecules. Biotechniques 61:203–205. https://doi.org/10.2144/000114460.
6. Jones A, Torkel C, Stanley D, Nasim J, Borevitz J, Schwessinger B. 2020. Scalable high-molecular weight DNA extraction for long-read sequencing. Protocols.io https://doi.org/10.17504/protocols.io.bnjhmcj6.
7. Inglis PW, Marilia de Castro RP, Resende L. v, Grattapaglia D. 2018. Fast and inexpensive protocols for consistent extraction of high quality DNA and RNA from challenging plant and fungal samples for high-throughput SNP genotyping and sequencing applications. PLoS One 13:e0206085–14. https://doi.org/10.1371/journal.pone.0206085.
8. De Coster W, D'Hert S, Schultz DT, Cruts M, Van Broeckhoven C. 2018. NanoPack: visualizing and processing long-read sequencing data. Bioinformatics 34:2666–2669. https://doi.org/10.1093/bioinformatics/bty149.
9. Zimin A. v, Marçais G, Puiu D, Roberts M, Salzberg SL, Yorke JA. 2013. The MaSuRCA genome assembler. Bioinformatics 29:2669–2677. https://doi.org/10.1093/bioinformatics/btt476.
10. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva E. v, 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.
11. Sperschneider J. 2020. FindTelomeres. https://github.com/JanaSperschneider/FindTelomeres.
12. Kirkland TN, Fierer J. 2018. *Coccidioides immitis* and *posadasii*: a review of their biology, genomics, pathogenesis, and host immunity. Virulence 9:1426–1435. https://doi.org/10.1080/21505594.2018.1509667.
13. Neafsey DE, Barker BM, Sharpton TJ, Stajich JE, Park DJ, Whiston E, Hung CY, McMahan C, White J, Sykes S, Heiman D, Young S, Zeng Q, Abouelleil A, Aftuck L, Bessette D, Brown A, FitzGerald M, Lui A, Macdonald JP, Priest M, Orbach MJ, Galgiani JN, Kirkland TN, Cole GT, Birren BW, Henn MR, Taylor JW, Rounsley SD. 2010. Population genomic sequencing of *Coccidioides* fungi reveals recent hybridization and transposon control. Genome Res 20:938–946. https://doi.org/10.1101/gr.103911.109.
14. Wa S, State W, Teixeira MDM, Barker M. 2019. Improved reference genome sequence of *Coccidioides immitis*. Microbiol Resour Announc 8:e00149-19. https://doi.org/10.1128/MRA.00149-19.
15. Smit AFA, Hubley R, Green P. 2015. RepeatMasker Open-4.0. http://www.repeatmasker.org.
16. Flynn JM, Hubley R, Goubert C, Rosen J, Clark AG, Feschotte C, Smit AF. 2020. RepeatModeler2 for automated genomic discovery of transposable element families. Proc Natl Acad Sci U S A 117:9451–9457. https://doi.org/10.1073/pnas.1921046117.