



Taxonomic Resolution of the Nematophagous Fungal Isolate ARF18 via Genome Sequencing

Sandeep Sharma,^a Alex Z. Zaccaron,^a John B. Ridenour,^a Amy Bradshaw,^b Terry L. Kirkpatrick,^a Kelly Cartwright,^{a,c} Burton H. Bluhm^a

Department of Plant Pathology, University of Arkansas, Division of Agriculture, Fayetteville, Arkansas, USA^a; Southern Arkansas University, Magnolia, Arkansas, USA^b; Agricultural Research Initiatives, Inc., Fayetteville, Arkansas, USA^c

ABSTRACT The taxonomically uncharacterized nematophagous fungus ARF18, which parasitizes cysts, juveniles, and adults of the soybean cyst nematode (*Heterodera glycines*), was proposed as a nematode biological control agent in 1991. A 46.3-Mb draft genome sequence of this fungus is presented, and a tentative taxonomic identification as a novel species of *Brachyphoris* is proposed.

Plant-parasitic nematodes are destructive pathogens of crop plants worldwide and cause estimated losses in excess of \$150 billion annually (1). Control of plant-parasitic nematodes relies on chemical nematicides and cultural practices, including crop rotation and using resistant cultivars. The manufacture and use of several key chemical nematicides has been discontinued due to human health risks and environmental concerns. Resistant cultivars do not currently exist for all crops, and effective crop rotation schemes are lacking for many cropping systems due to economic concerns. Alternative nematode control tactics are urgently needed for many major economic crops (2, 3). The hyphomycete fungus ARF18 was first isolated from infected cysts of *Heterodera glycines* nearly 30 years ago (4). Because the fungus parasitizes all stages of the nematode, including eggs, juveniles, and adults in both soil and culture media (5), it was suggested as a potential biological control organism. Culture conditions have not yet been identified that induce conidiation or other morphological features that are required for classical taxonomic identification. Additionally, nothing is yet known about nematophagy in ARF18 at the molecular level.

The genome of ARF18 was sequenced with Pacific Biosciences (PacBio) technology, which generated 142,598 reads. Lengths varied from 35 bp to 43,743 bp with an average length of 7,686 bp. A draft genome assembly of the fungus was obtained with Canu version 1.1 (6), following the program instructions for low coverage data sets. The ARF18 draft genome assembly was improved by merging contigs into scaffolds with AHA from the SMRTanalysis suite version 2.3.0 (<http://www.pacb.com/products-and-services/analytical-software/devnet/devnet-analysis-tools>). The resulting genome assembly had 46,639,970 bp organized into 412 scaffolds with an N_{50} value of 177 kb, an L_{50} value of 76, and a GC content of 44.6%. Compared to the genome of *Arthrobotrys oligospora* and many other ascomycetes, ARF18 had a slightly larger genome (7, 8).

Gene prediction was performed with the Maker version 2.31.6 pipeline (9) with homology evidence proteins from *A. oligospora* ATCC 24927, *Monacrosporium haptotylum* CBS 200.50, and *Drechslerella stenobrocha* 248. A total of 14,461 protein-encoding genes, with an average length of 1,028 bp, were predicted in the ARF18 genome assembly. Through BLAST analyses, several genes were identified that could play roles in nematode pathogenesis, including cuticle-degrading serine proteases, alkaline serine proteases, and chitinases (10–12). Further examination of the ITS1-5.8s to ITS2 rDNA

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Address correspondence to Burton H. Bluhm, bbluhm@uark.edu.

region suggested that ARF18 belongs to a distinct monophyletic clade within *Brachyphoris*, a genus of nematophagous fungi that belongs to the ascomycete family *Orbiliaceae* (13–15). Based on BLAST analyses, most of the genes analyzed showed high identity to *A. oligospora* and *Dactylellina haptotyla*, both nematophagous fungi within the *Orbiliaceae* family, supporting the taxonomic placement of ARF18 within the *Orbiliaceae* family.

Currently, only a few nematophagous fungal genomes are publicly available. Thus, the genome sequence of this fungus will provide a useful resource to study the biology of nematophagous fungi, especially within the *Brachyphoris* genus. Further analyses of the genome of ARF18 will also provide important information regarding the molecular basis of fungal nematophagy and guide the potential development of this nematode pathogen as a biological control agent.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. [AZLU00000000](https://www.ncbi.nlm.nih.gov/nuccore/AZLU00000000). The version described in this paper is the first version, AZLU01000000.

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