Genome Resources for Seven Fungal Isolates That Cause Dollar Spot Disease in Turfgrass, Including *Clariireedia jacksonii* and *C. monteithiana*

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Abstract

Fungi in the genus *Clariireedia* are widespread and destructive pathogens of grasses worldwide, and are best known as the causal agents of dollar spot disease in turfgrass. Here, we report genome assemblies of seven *Clariireedia* isolates, including ex-types of the two most widespread species, *Clariireedia jacksonii* and *C. monteithiana*. These datasets provide a valuable resource for ongoing studies of the dollar spot pathogens that include population diversity, host–pathogen interactions, marker development, and disease control.

Dollar spot is a destructive and widespread fungal disease affecting turfgrasses worldwide (Smiley et al. 2005). Outbreaks of dollar spot disease compromise the health of at least 35 species of warm- and cool-season grasses (Smiley et al. 2005). However, turfgrass species used for golf course fairways and greens incur the greatest economic losses; these include cool-season grasses such as bentgrasses, bluegrasses, and fescues (*Agrostis*, *Poa*, and *Festuca* spp., respectively) and warm-season grasses such as bermudagrass and seashore paspalum (*Cynodon dactylon* and *Paspalum vaginatum*, respectively). Symptoms of dollar spot disease are very similar regardless of host, and include leaf lesions that coalesce into distinctive sunken circular patches of turf the approximate size of a silver dollar coin (Smiley et al. 2005).

Dollar spot can be caused by at least five sister species of fungi in the genus *Clariireedia* (*Rutstroemiaceae*, Ascomycota) (Hu et al. 2019; Salgado-Salazar et al. 2018), formerly

Funding

This work was supported by the United States Department of Agriculture–Agricultural Research Service (USDA-ARS) project 8042-22000-279-00D, Rutgers Center for Turfgrass Science, North Carolina State Center for Turfgrass Environmental Research and Education, The Ohio State University, and the Ohio Turfgrass Foundation.

Keywords

Agrostis stolonifera, *Cynodon dactylon*, dollar spot, *Festuca rubra*, fungi, *Paspalum vaginatum*, pathogen diversity, Rutstroemiaceae, *Sclerotinia homoeocarpa*, turf, turfgrass

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The author(s) declare no conflict of interest.

Accepted for publication 17 July 2020.

known collectively as *Sclerotinia homoeocarpa* (Salgado-Salazar et al. 2018). *Clariireedia jacksonii* and *C. monteithiana* are the most widespread species, affecting cool-season and warm-season grasses, respectively (Salgado-Salazar et al. 2018). Three additional species have been described, each known only from a relatively narrow geographic region or host range: *C. homoeocarpa*, known only from *Festuca rubra* in the United Kingdom; *C. bennettii*, known from Poaceae hosts in the United Kingdom, United States, and The Netherlands (Salgado-Salazar et al. 2018); and *C. paspali*, known only from *P. vaginatum* in China (Hu et al. 2019). Two distinct, single-isolate *Clariireedia* lineages from *F. rubra* turfgrass in the United Kingdom have also been identified but have not been named (Salgado-Salazar et al. 2018).

The recent taxonomic account of the genus *Clariireedia* resolves a long unanswered question about the identity of the dollar spot pathogen. At the same time, the recognition that several distinct *Clariireedia* spp. can infect turfgrasses and cause dollar spot creates a unique opportunity for scientists to dissect the complex cellular underpinnings of disease and host resistance. Disease prediction models, control strategies, population assessments, breeding, diagnostic tools, and other resources are largely based around the concept that just one organism causes dollar spot (Bonos et al. 2006; Green et al. 2016; Rioux et al. 2014; Smith et al. 2018) but some evidence is emerging that phenotypic differences are connected to different *Clariireedia* spp. (Aynardi et al. 2019).

Here, we report the genome sequences of seven *Clariireedia* isolates as a resource to enable advanced research of dollar spot disease in turfgrass. Genome sequences from seven isolates of *Clariireedia* spp. taken from four turfgrass hosts were generated (Table 1). Briefly, three isolates of the globally widespread cool-season grass pathogen *C. jacksonii* were sequenced, including the species ex-type LWC-10 (CBS 138618) (Salgado-Salazar et al. 2018) and the well-studied strain MB-01 (CBS 139392) (Groben et al. 2020; Orshinsky et al. 2012; Ostrander et al. 2014; Putman et al. 2015; Salgado-Salazar et al. 2018; Venu et al. 2011). All *C. jacksonii* isolates originated from diseased *Agrostis stolonifera*. Two isolates of the warm-season turf pathogen *C. monteithiana* were sequenced, including the species ex-type RB-19 (CBS 136376). The *C. monteithiana* isolates originated from diseased *Cynodon dactylon* and *P. vaginatum* turfgrass. Two unnamed *Clariireedia* spp. isolated from diseased *F. rubra* were also sequenced. Fungal biomass for DNA extraction was harvested by vacuum filtration after 5 days of growth in liquid cultures of yeast extract peptone dextrose (YEPD) media, incubated under constant light and shaking at 150 rpm. Genomic DNA (100 µg) was extracted from *Clariireedia jacksonii* MB-01 with the Qiagen Blood & Cell Culture DNA Kit (Qiagen, Gaithersburg, MD, U.S.A.) following the manufacturer's instructions; for all other isolates, DNA was extracted using the Omni-Pure Genomic DNA Extraction Kit (G-Biosciences, St. Louis, MO, U.S.A.), then purified using the Zymo Genomic DNA Clean & Concentrator kit (Zymo Research, Irvine, CA, U.S.A.). For RNA extractions, cultures of DRR09 and MB-01 were grown at room temperature under constant light and shaking at 150 rpm for 5 days. Isolate DRR09 was grown in potato dextrose broth (PDB; Thermo Fisher Scientific) and in PDB amended with 2.5 mM ascorbic acid. Isolate MB-01 was grown in YEPD medium (yeast extract at 3 g liter⁻¹, peptone at 10 g liter⁻¹, and dextrose at 20 g liter⁻¹). Total RNA was extracted for use for expressed sequence tags (ESTs) using the ZR Fungal/Bacterial RNA MiniPrep kit (Zymo Research). RNA extracted from isolate DRR09 under the two growth conditions was normalized and pooled into a single tube for RNA sequencing.

For the six isolates sequenced using Illumina technology alone, DNA was fragmented using the Covaris M220 ultrasonicator (Covaris, Inc., Woburn, MA, U.S.A.), then used to construct libraries using the TruSeq DNA PCR-free kit (Illumina, Inc., San Diego, CA, U.S.A.). Libraries were sequenced as paired ends using the 600-cycle MiSeq reagent kit on an Illumina MiSeq (Illumina, Inc.). RNA libraries for ESTs were constructed using the TruSeq RNA LT preparation kit (Illumina, Inc.). Libraries were quantified as previously described (Malapi-Wight et al. 2015) and sequenced in two independent runs on the Illumina MiSeq platform to generate 1 × 50 and 2 × 150 reads. Post-run read processing was performed using CLC Genomics Workbench v. 7.5.1 (CLC Bio, Germantown, MD, U.S.A.). After adapter removal, bases with low Phred quality scores (limit 0.05) and strings of ambiguous bases >2 were trimmed, and reads <15 bp were removed from the dataset. Between 9.9 and 28.8 million reads were retained after quality filtering. Assembly of Illumina reads was performed in CLC Genomics (kmer = 61, bubble size of 50), with scaffolds ≥500 bp retained for these six assemblies generated entirely from Illumina reads. The CLC Microbial Genome Finishing Module was used to join scaffolds based on paired-end data and BLAST alignments of scaffold ends (e-value 10⁻⁵, minimum match size 50 bp; Qiagen).

Table 1. Summary of genome assemblies from seven isolates of *Clariireedia*^a

Variables	<i>Clariireedia</i> sp.		<i>Clariireedia monteithiana</i>		<i>C. jacksonii</i>		
	CPB17	SE16F-4	DRR09	RB-19 ^b	LWC-10 ^b	MB-01	SH44
CBS accession ^c	CBS 136371	CBS 136372	CBS 136373	CBS 136376	CBS 138618	CBS 139392	CBS 138617
Host	<i>Festuca rubra</i>	<i>F. rubra</i>	<i>Paspalum vaginatum</i>	<i>Cynodon dactylon</i>	<i>Agrostis stolonifera</i>	<i>A. stolonifera</i>	<i>A. stolonifera</i>
Geographic origin	United Kingdom	United Kingdom	Dominican Republic	Mississippi, United States	North Carolina, United States	Ohio, United States	Canada
Year collected	2008	2008	2008	2008	2003	2001	2000
Assembly size (Mb)	36.4	36.0	48.7	36.1	37.2	39.5	35.9
Scaffolds ≥500 bp (<i>n</i>)	3,873	4,789	15,133	3,610	3,606	1,637	4,289
Scaffolds ≥1 kb (<i>n</i>)	2,975	3,725	9,341	2,955	2,820	1,637	3,468
Average coverage	41	38	109	33	58	70	41
Scaffold N ₅₀	20,651	15,633	8,758	20,763	25,161	168,655	17,322
Maximum scaffold length	119,633	100,512	78,171	116,952	122,690	727,903	110,593
Average scaffold length	9,392	7,518	3,219	10,023	10,328	24,110	8,367
Illumina reads (<i>n</i>)	10,448,372	9,897,838	28,807,323	8,031,180	15,204,474	–	10,256,106
GC%	44.6	45.0	44.8	44.7	41.6	39.7	45.1
Gene models	11,134	11,035	16,244	10,821	11,089	10,979	10,975
BUSCO, eukaryote (%) ^d	96	95	94	96	97	97	96
BUSCO, fungus (%) ^e	97	98	97	98	98	98	98
GenBank accession	LLKJ000000000	LLKF000000000	LLKI000000000	LLKG000000000	LLKH000000000	LLKD000000000	LLKE000000000

^a *Clariireedia jacksonii* MB-01 was sequenced using a combination of Sanger/Illumina technologies; all other isolates were sequenced using Illumina technology. All genome data are based on scaffolds ≥500 bp, except for *C. jacksonii* MB-01, which is limited to scaffolds ≥1 kb.

^b Ex-type for the species.

^c All isolates are deposited in the CBS-KNAW Collection, Westerdijk Fungal Biodiversity Institute, The Netherlands.

^d BUSCO analysis based on a set of 438 eukaryote orthologs.

^e BUSCO analysis based on a set of 1,438 fungal orthologs.

Paired-end (Illumina) and mate-paired (Sanger; 12 kb) DNA libraries of *C. jacksonii* isolate MB-01 were constructed, sequenced, and assembled by the J. Craig Venter Institute (Rockville, MD, U.S.A.). Multiple assemblies using multiple assemblers and parameter variations were performed to obtain the highest-quality assembly. This included one CLC assembly using only Illumina paired-end reads, three CLC assemblies using both Illumina reads and Sanger-sequenced mate pairs with varying parameters of mate-pair distance and treatment of nonspecific matches, and one Celera Assembler (CA) assembly using the raw, untrimmed Illumina reads combined with Sanger mate-pair information. At the contig level, both CA and CLC performed similarly, with CA yielding an N₅₀ of about 27 kb while CLC yielded an N₅₀ between 27 and 43 kb for the different runs (scaffolds ≥1 kb). Overall, CA produced the best MB-01 assembly, comprising 1,637 scaffolds, a scaffold N₅₀ of about 168 kb, and an average genome coverage of 70×.

Relative to other members of the Rutstroemiaceae and Sclerotiniaceae families, the seven *Clariireedia* genome assemblies fell within the overall size range of 38.3 to 51.9 Mb (Amselem et al. 2011; Blanco-Ulate et al. 2013; Green et al. 2016; Mardanov et al. 2014; Nest et al. 2014). With the exception of *C. monteithiana* DRR09, genome assembly sizes ranged from 35.9 to 39.5 Mb. In contrast, the *C. monteithiana* DRR09 assembly was 48.7 Mb and highly fragmented (9,341 scaffolds >1 kb). The difference in size observed between DRR09 and the other isolates is likely due to the completeness of the assemblies; scaffold accumulation plots generated in QUAST (Gurevich et al. 2013) showed that a plateau was reached for all isolates except DRR09. This is despite the fact that DRR09 was sequenced at a higher depth of coverage than the other six isolates (109× versus 33× to 70×). Gene models were predicted using AUGUSTUS v3.0.2 (Stanke and Morgenstern 2005) with *S. sclerotiorum* gene models (Amselem et al. 2011) and using *C. jacksonii* MB-01 and *C. monteithiana*

DRR09 RNA sequences generated from this study (52,893 and 83,517 ESTs, respectively) and previous work (29,542 ESTs) (Orshinsky et al. 2012). With the exception of DRR09, the number of predicted gene models ranged from 10,821 to 11,134.

The genome assemblies described herein are contained within a relatively large number of scaffolds (1,637 to 3,725 scaffolds for all isolates except DRR09). However, comparisons with a hybrid 231-scaffold genome assembly of *C. jacksonii* (formerly known as *S. homoeocarpa*) isolate HRS10 (Illumina/PacBio hybrid) (Green et al. 2016) shows that, although the scaffold numbers are larger, these new genome resources provide a largely complete representation of the overall gene space. BUSCO (Simão et al. 2015) assessment of conserved single-copy orthologs identified 97 to 98% of the fungal orthologs and 94 to 97% of the eukaryotic orthologs from the new assemblies. In contrast, the *C. jacksonii* HRS10 assembly contained 98 and 100% of the BUSCO eukaryote and fungal ortholog sets, respectively. Similarly, the number of gene models predicted from the new genome resources are comparable with the 11,285 gene models we predicted from the *C. jacksonii* HRS10 assembly using the same AUGUSTUS pipeline described above.

The genome assemblies described in this article were deposited in the NCBI GenBank under the accessions listed in Table 1. All datasets described herein are permanently curated and publicly available online by the U.S. National Agricultural Library AgData Commons at <https://data-nal.usda.gov/dataset/data-genome-resources-seven-fungal-isolates-cause-turfgrass-dollar-spot-disease-including-clarireedia-jacksonii-and-c-monteithiana>. All *Clarireedia* isolates were deposited in the CBS-KNAW Collection, Westerdijk Fungal Biodiversity Institute, The Netherlands.

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