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ABSTRACT

Dollar spot is one of the most destructive and economically important fungal diseases of amenity turfgrasses. The causal agent was first described in 1937 as the ascomycete *Sclerotinia homoeocarpa*. However, the genus-level taxonomic placement of this fungus has been the subject of an ongoing debate for over 75 y. Existing morphological and rDNA sequence evidence indicates that this organism is more appropriately placed in the family *Rutstroemiaceae* rather than the *Sclerotiniaceae*. Here we use DNA sequence data from samples of the dollar spot fungus and other members of the *Rutstroemiaceae* (e.g. *Rutstroemia, Lanzia, Lambertella*) collected throughout the world to determine the generic identity of the turfgrass dollar spot pathogen. Phylogenetic evidence from three nucleotide sequence markers (CaM, ITS and Mcm7; 1810-bp) confirmed that *S. homoeocarpa* is not a species of *Sclerotinia*; nor is it a member of any known genus in the *Rutstroemiaceae*. These data support the establishment of a new genus, which we describe here as *Clarireedia* gen. nov. The type species for the genus, *Clarireedia homoeocarpa* comb. nov., is described to accommodate the dollar spot fungus, and a neotype is designated. Three new species in this clade, *Clarireedia bennettii* sp. nov., *Clarireedia jacksonii* sp. nov., and *Clarireedia monteithiana* sp. nov. that also cause dollar spot disease are described. *Clarireedia homoeocarpa* and *C. bennettii* occur primarily on *Festuca rubra* (C3 grass) hosts and appear to be restricted to the United Kingdom. *Clarireedia jacksonii* and *C. monteithiana* occur on a variety of C3 and C4 grass hosts, respectively, and appear to be globally distributed. This resolved taxonomy puts to rest a major controversy amongst plant pathologists and provides a foundation for better understanding the nature and biology of these destructive pathogens.

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1. Introduction

Dollar spot is a debilitating fungal disease of cool- and warm-season turfgrass species (Smiley et al., 2005). The disease is widespread and persistent, with more money and effort spent on its control than any other disease affecting golf course turf (Goodman and Burpee, 1991). Despite the aesthetic and economic impact of dollar spot on turfgrass, the taxonomy and nomenclature of the fungus responsible for the disease has been in a state of flux for almost eight decades. The first report of dollar spot disease on turfgrass occurred in 1927, when John Monteith referred to it as a 'small brown patch', characterized by straw colored patches that did not become larger than a silver dollar (Fig. 1A–D) (Monteith, 1927). The term 'small brown patch' to describe the disease was subsequently changed to 'dollar spot' to avoid confusion with another disease affecting turfgrass: 'large brown patch' caused by the fungus *Rhizoctonia solani* (Monteith and Dahl, 1932). Bennett identified the causal agent of dollar spot disease on turfgrass as a new species, *Rhizoctonia monteithiana* (Bennett, 1935); however, the name was not validly published, as a Latin description was not provided in the protolog. The omission was almost certainly due to the timing of new rules implemented by the Cambridge Code of the International Code of Botanical Nomenclature, with the requirement for Latin descriptions only taking effect in January 1935, and the description of *R. monteithiana* published in February 1935. The omission was never corrected, and as such *R. monteithiana* is not a valid basionym for the fungus.

In 1937, Bennett provided a valid name for the fungus responsible for dollar spot disease, withdrawing his earlier proposal for *R. monteithiana* based on new observations and describing the ascomycete *Sclerotinia homoeocarpa* (Bennett, 1937). Three phenotypes were documented from four cultured isolates of the fungus, based on differences in spore production: a 'perfect strain', producing ascospores and conidia; an 'ascigerous strain', producing both ascospores and microconidia; and two 'non-sporing strains' (Bennett, 1937). Bennett observed that the structures from which sporophores arose resembled aggregates of microsclerotia, and classified the fungus in the genus *Sclerotinia* (*Sclerotiniaceae*) (Bennett, 1937). In the years following Bennetts description, Whetzel reviewed the taxonomy of the family *Sclerotiniaceae* and, in doing so, restricted the genus *Sclerotinia* to include only those fungi producing apothecia from tuberoid sclerotia, a characteristic not exhibited by *S. homoeocarpa* (Whetzel, 1945). Instead of sclerotia, *S. homoeocarpa* produces an indeterminate substratal stroma. Whetzel concluded from this morphological characteristic that *S. homoeocarpa* resembled species such as *Rutstroemia* and *Lambertella* (Whetzel, 1945) – organisms that would later be classified as part of a new family, the *Rutstroemiaceae* (Holst-Jensen et al., 1997). Whetzel later proposed that *S. homoeocarpa* was a species of *Rutstroemia*, but never formally reclassified the fungus (Whetzel, 1946). As such, the pathogen retained a generic name that was taxonomically incorrect, but valid from a nomenclatural standpoint (Whetzel, 1946).

In the years following Whetzel's exclusion of the dollar spot fungus from the genus *Sclerotinia*, prospects for re-classification of *S. homoeocarpa* were limited by the absence of fruiting bodies or other taxonomically informative morphological characters. The fungus exists almost exclusively in the vegetative state, as sterile hyphae or substratal stromata. Spore production is exceedingly uncommon, and apothecial fruiting bodies are rarely documented (Smiley et al., 2005). For thirty-six years following Bennett's original description of ascospore production by *S. homoeocarpa*, reproductive structures were not observed *in vitro* or in natural populations of the fungus (Jackson, 1973). Apothecia production was not reported from naturally occurring North American populations of *S. homoeocarpa* until 1970; yet these structures were sterile (Fig. 1E) (Fenstermacher, 1970). In 1973, ascospores were observed from a fresh collection of *S. homoeocarpa* isolated from cool-season turfgrasses in the U.K (Jackson, 1973). The fruiting bodies and spores observed from these newer collections closely resembled the

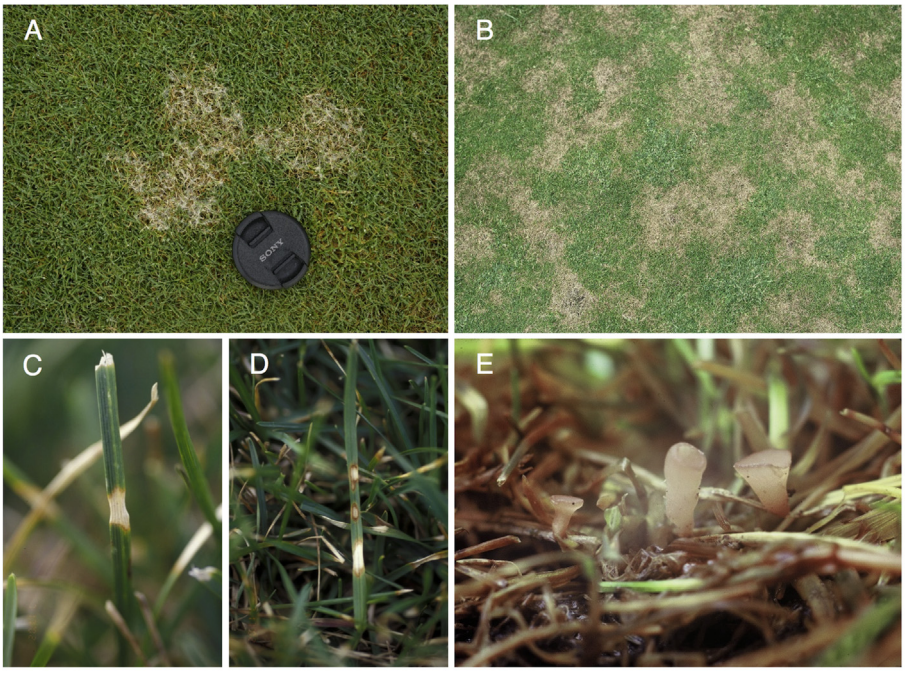

Fig. 1. Symptoms of dollar spot disease; (A) dollar spot disease on creeping bentgrass (*Agrostis stolonifera*) (photo courtesy of Charles J. Schmid); (B) dollar spot disease on red fescue (*Festuca rubra*) in the United Kingdom (photo courtesy of Noel Jackson); (C–D) characteristic hourglass shaped lesion of dollar spot disease on Kentucky bluegrass (*Poa pratensis*); (E) apothecia on sea marsh fescue (*Festuca* sp.) in the United Kingdom (photo courtesy of Noel Jackson).

S. homoeocarpa sexual state described by Bennett (Jackson, 1973). Jackson believed that the fruiting bodies resembled those of a *Rutstroemia* species (Jackson, 1973), but because this genus was deemed unacceptable by taxonomists at the time (Dumont, 1971), he did not seek to reassign *S. homoeocarpa* to a new taxon.

As the number of *Sclerotinia* species described in the mycological literature soared to over 250 by the late 1970s, a new generation of researchers set out to make sense of the taxonomic confusion within the genus and related taxa (Kohn, 1979a; b). Kohn's seminal monographs of the *Sclerotiniaceae* provided additional evidence for the exclusion of *S. homoeocarpa* from the genus *Sclerotinia*. From assessments of morphological and cultural characteristics, Kohn suggested that *S. homoeocarpa* might be placed within the genus *Lanzia* or the genus *Moellerodiscus* (Kohn, 1979a; b). Stromal histology supported this theory (Kohn and Grenville, 1989), however, in the absence of definitive evidence aligning the species with a single genus, formal reclassification of *S. homoeocarpa* was once again deferred (Kohn and Grenville, 1989). More recent investigations have drawn the use of stromatal characters for family level distinctions into question (Baral and Bemmann, 2014; Zhao et al., 2016).

With the advent of molecular technologies in the 1990s, researchers set out yet again to pinpoint the taxonomic identity of *S. homoeocarpa*. These studies produced a series of contradictory results. Electrophoretic analysis of stromatal proteins showed isolates of *S. homoeocarpa* sharing similarity with fungi in the *Rutstroemiaceae* genus *Poculum* (Novak and Kohn, 1991). In contrast, sequence analysis of rDNA markers showed that the relationship of *S. homoeocarpa* isolates with other *Rutstroemiaceae* genera could be quite variable, with generic affinities differing from one study to the next. The first DNA-based phylogenetic analysis of this group of fungi using rDNA internal transcribed spacer (ITS) sequences showed clustering of *S. homoeocarpa* isolates with fungi in the genus *Rutstroemia* (Carbone and Kohn, 1993). Subsequent analysis of DNA sequences from the ITS and portions of the rDNA large and small subunits grouped *S. homoeocarpa* isolates with fungi in the genus *Poculum* (Holst-Jensen et al., 1997). However, type specimens of the genus *Poculum* were not included in this study, and reclassification of *S. homoeocarpa* was deferred for the fifth time (Holst-Jensen et al., 1997). Subsequent analysis of the ITS1 region grouped *S. homoeocarpa* isolates with two fungal isolates from the genus *Rutstroemia* (Powell, 1998). Phylogenetic analysis of the ITS1 dataset using parsimony tests showed *S. homoeocarpa* isolates clustering into two subclades corresponding with geographic origin, although the sample size was small ($n = 7$). Powell suggested reclassification of *S. homoeocarpa* into two new species of *Rutstroemia*: *Rutstroemia festucae* as a new species limited to the U.K., and *Rhizoctonia floccosum* as a new species found outside the U.K., however, these conclusions were not validly published in accordance with fungal nomenclature requirements (http://www.iapt-taxon.org/nomen/main.php).

Despite more than 70 years of accumulated evidence that the dollar spot fungus is not a true *Sclerotinia* species, in the absence of a valid taxonomic and nomenclatural revision, this economically important plant pathogen continues to be referred to as *S. homoeocarpa*, the only legitimate name currently available. Due to morphological and molecular variation and possible host specialization between isolates of *S. homoeocarpa* associated with symptoms of dollar spot, some researchers have proposed the idea that more than just a single organism may cause this disease (Jackson, 1973; Baldwin and Newell, 1992; Putman, 2013; Espevig et al., 2015, 2017). In this study, we use multi-locus molecular phylogenetic analysis, expanded taxon sampling, and morphological evaluations to resolve the identity of the fungi responsible for dollar spot disease on cool- and warm-season turfgrass.

2. Materials and methods

2.1. Fungal isolates

Sixty-seven cultured fungal isolates were used in this study. The samples included members of the *Rutstroemiaceae* (e.g. *Lambertella*, *Rutstroemia, Lanzia*) and *Sclerotiniaceae* (e.g. *Ciboria*, *Monilinia*, *Sclerotinia*) families. Exemplar isolates of *S. homoeocarpa* were selected for inclusion through preliminary variation screening of a worldwide sample of ~1170 dollar spot isolates using ITS sequence data and SSR genotypes (Putman, 2013). Three living samples of *S. homoeocarpa* deposited in the CBS culture collection by Bennett in 1937 (CBS accession numbers CBS 309.37, CBS 310.37, CBS 311.37) were also included. No known documentation directly connects the Bennett CBS isolates to the *S. homoeocarpa* protolog. However, the fact that these three isolates were deposited at the same time as the publication suggests that they may be the same three isolates described in the publication, but this cannot be concluded with certainty. A complete list of isolates used in the present study is found in Table 1.

2.2. Apothecia production and morphological examinations

A subset of isolates of *S. homoeocarpa* were evaluated for the production of apothecia *in vitro*, both individually and in crosses performed between isolates of different mating types (*MAT1-1* × *MAT1-2*) (Supplementary Table 1). Apothecia formation was initiated using techniques described by Orshinsky and Boland (2011). Briefly, isolates were grown on potato dextrose agar (PDA, Difco, Sparks, MD) or wheat meal (Bob's Red Mill, Milwaukie, OR) agar amended with 2.5 mM ascorbic acid at 25 C under continuous light. A minimum of eight plates were prepared per isolate. Plates were inoculated with the fungus by spreading a 300 μl mycelia/sterile water slurry. Morphological assessments were made using a Zeiss V20 dissecting microscope, with images captured utilizing Zeiss Zen software (Carl Zeiss Microscopy, Thornwood, NY). Co-inoculations of isolates of differing mating type were produced by preparing slurries of mycelia and sterile water from actively growing *S. homoeocarpa* cultures that were previously genotyped as either *MAT1-1* or *MAT1-2* (Putman et al., 2015) or by genotyping using the methods of Putnam et al. (2015), followed by plating on ascorbic acid amended PDA. Specifically, a 300 μl slurry of a *MAT1-2* isolate was spread over the surface of the plates using a sterile glass rod, allowed to grow for one day, then reinoculated by 300 μl of a *MAT1-1* mycelia/sterile water slurry. Co-inoculated plates were incubated under the aforementioned conditions. Ten plates per mating cross were used to evaluate apothecia production.

2.3. DNA extractions, PCR amplification, and sequencing

DNA was extracted using a standard phenol/chloroform procedure (Crouch et al., 2005) or the OmniPrep DNA kit (G-Biosciences, St. Louis, MO) according to the manufacturer's protocol. DNA concentration and purity were determined using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). Nucleotide sequence data for phylogenetic analyses was generated from three standard molecular markers: the rDNA internal transcribed spacer (ITS) region, calmodulin (CaM), and DNA replication licensing factor Mcm7. PCR amplification to generate sequencing templates was performed using an Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany) and published primer pairs: ITS4/ITS5 (White et al., 1990), CAL-228F/CAL-737R (Carbone and Kohn, 1999) and Mcm7-709for/Mcm7-1348rev (Schmitt et al., 2009). PCR primers were synthesized as oligonucleotides by Integrated DNA Technologies (Coralville, IA). PCR

Table 1
List of isolates used in the study.

Fungal Specimens	Name	Type	Host/Substrate	MAT1 idiomorph	Locale	Year	CaM	ITS	Mcm7
Botrytis cinerea	B05.10	N/A	N/A	N/A	Germany	N/A	[a]	[a]	[a]
Ciboria aestivalis	CBS 119.47	N/A	N/A	N/A	Australia	1947	KF545281	KF545326	KF545470
Ciboria amentacea	CBS 110.160	N/A	*Alnus glutinosa*	N/A	Netherlands	2002	KF545282	KF545317	–
Ciboria amentacea	CBS 130.31	N/A	*Alnus glutinosa*	N/A	England	1931	–	KF545318	–
Ciboria amentacea	CBS 526.90	N/A	*Alnus incana*	N/A	Switzerland	1990	–	KF545325	–
Ciboria americana	CBS 117.24	N/A	*Castanea sativa*	N/A	N/A	1924	–	KF545327	–
Ciboria cistophila	CBS 773.95	Holotype	*Cistus laurifolius*	N/A	Spain	1995	KF545241	KF545324	–
Ciboria viridifusca	CBS 654.92	N/A	*Alnus sp.*	N/A	Germany	1987	KF545283	KF545322	–
Clarireedia bennettii	CBS 309.37	Holotype	N/A	MAT1 & 2	United Kingdom	1937	MF964270	MF964321	–
Clarireedia bennettii	CBS 311.37	N/A	N/A	MAT1-1	United Kingdom	1937	MF964272	MF964323	MF964284
Clarireedia bennettii	CBS 464.73	N/A	*Symplocarpus foetidus*	N/A	NY, USA	1973	KF545266	KF545316	KF545446
Clarireedia bennettii	CBS 854.97	N/A	Poaceae	N/A	Netherlands	1997	KF545265	KF545314	KF545467
Clarireedia homoeocarpa	CBS 310.37	Ex-epitype	N/A	MAT1-2	United Kingdom	1937	MF964271	MF964322	KF545451
Clarireedia homoeocarpa	CPB-5	N/A	*Festuca rubra*	MAT1-2	United Kingdom	2008	KF545272	KF545313	KF545449
Clarireedia homoeocarpa	IMI 167641	N/A	*Festuca sp.*	MAT1 & 2	United Kingdom	1972	MF964261	MF964312	MF964276
Clarireedia homoeocarpa	PSFFB-3	N/A	*Festuca rubra*	MAT1-2	United Kingdom	2008	KF545268	–	KF545448
Clarireedia jacksonii	A4	N/A	*Agrostis stolonifera*	MAT1-2	OH, USA	2001	KF545243	KF545295	KF545458
Clarireedia jacksonii	CBS 510.89	N/A	dying grass of golf green	N/A	Netherlands	1989	KF545261	KF545289	KF545453
Clarireedia jacksonii	D19	N/A	*Poa pratensis*	N/A	OH, USA	2002	KF545252	KF545298	–
Clarireedia jacksonii	HP-50	N/A	*Agrostis stolonifera*	MAT1 & 2	NJ, USA		KF545247	KF545291	–
Clarireedia jacksonii	LEF17T-21	N/A	*Agrostis stolonifera*	MAT1-2	Italy	2008	KF545250	KF545293	–
Clarireedia jacksonii	LWC-10	Holotype	*Agrostis stolonifera*	MAT1-1	NC, USA	2003	MF964269	MF964320	MF964283
Clarireedia jacksonii	MAFF 235854	N/A	*Agrostis stolonifera*	MAT1 & 2	Japan	1987	KF545242	KF545301	KF545454
Clarireedia jacksonii	MAFF 235856	N/A	*Agrostis stolonifera*	MAT1 & 2	Japan	1987	KF545246	KF545302	KF545456
Clarireedia jacksonii	MAFF 235858	N/A	*Agrostis stolonifera*	MAT1 & 2	Japan	1988	MF964273	MF964324	–
Clarireedia jacksonii	MAFF 236941	N/A	*Lolium perenne*	MAT1 & 2	Japan	1991	KF545248	KF545296	KF545455
Clarireedia jacksonii	MB-01	N/A	*Agrostis stolonifera*	MAT1-1	OH, USA	2001	KF545244	KF545290	MF964289
Clarireedia jacksonii	RCCPG-1	N/A	*Agrostis stolonifera*	MAT1-2	NC, USA	2003	KF545253	KF545297	–
Clarireedia jacksonii	RE18G-38	N/A	*Agrostis stolonifera*	MAT1 & 2	NC, USA	2003	KF545254	KF545292	KF545457
Clarireedia jacksonii	SE16F-4	N/A	*Festuca rubra*	MAT1-2	United Kingdom	2008	MF964268	MF964319	MF964282
Clarireedia jacksonii	SH44	N/A	*Agrostis stolonifera*	MAT1-2	Canada	2000	KF545251	KF545299	KF545459
Clarireedia jacksonii	SH80	N/A	*Agrostis stolonifera*	MAT1-2	Canada	2000	KF545245	KF545294	–
Clarireedia monteithiana	BC-14	N/A	*Cynodon dactylon* x *transvaalensis*	MAT1-1	NC, USA	2008	KF545255	KF545307	–
Clarireedia monteithiana	DRR-9	N/A	*Paspalum vaginatum*	MAT1-1	Dominican Republic	2008	KF545260	KF545303	MF964290
Clarireedia monteithiana	LFDF-14	N/A	*Cynodon dactylon* x *transvaalensis*	MAT1-1	NC, USA	2007	KF545256	KF545308	–
Clarireedia monteithiana	MAFF 236938	N/A	*Cynodon dactylon*	MAT1-2	Japan	1991	KF545258	KF545305	KF545460
Clarireedia monteithiana	RB-19	Holotype	*Cynodon dactylon* x *transvaalensis*	MAT1-2	MS, USA	2008	KF545257	KF545306	MF964291
Clarireedia monteithiana	TEKP-2	N/A	*Paspalum vaginatum*	MAT1-2	HI, USA	2008	KF545259	KF545304	–
Clarireedia sp.	CBS 465.73	N/A	dung of rabbit	N/A	England	1973	KF545264	KF545315	KF545445
Clarireedia sp.	CPB-17	N/A	*Festuca rubra*	MAT1-2	United Kingdom	2008	KF545240	KF545310	KF545447
Clarireedia sp.	PSFFB-1	N/A	*Festuca rubra*	MAT1-2	United Kingdom	2008	KF545263	KF545312	KF545450
Lambertella corni-maris	CBS 184.93	N/A	*Pyrus malus*	N/A	USA	1992	KF545262	KF545336	–
Lambertella corni-maris	CBS 774.95	N/A	*Cornus mas*	N/A	Croatia	1967	–	KF545339	–
Lambertella hicoriae	CBS 294.54	N/A	N/A	N/A	WI, USA	1954	–	KF545337	KF545473
Lambertella himalayensis	CBS 230.77	N/A	*Cassia siamea*	N/A	Burma	1977	KF545285	KF545335	–
Lambertella pruni	CBS 199.47	N/A	*Prunus avium*	N/A	OR, USA	1947	KF545277	KF545338	KF545472
Lambertella subrenispora	CBS 811.85	Paratype	*Aster ageratoides* var. *ovata*	N/A	Japan	1983	–	KF545329	KF545466
Lanzia echinophila	CBS 111.547	N/A	*Quercus castaneifolia*	N/A	Netherlands	2002	KF545239	KF545332	–
Lanzia echinophila	CBS 111.549	N/A	*Castanea sativa*	N/A	Netherlands	2002	KE545271	KF545333	KF545463
Monilinia vaccinii-corymbosi	SSI-1	N/A	*Vaccinium sp.*	N/A	NJ, USA	2009	MF964274	MF964325	MF964285
Monilinia vaccinii-corymbosi	SSI-2	N/A	*Vaccinium sp.*	N/A	NJ, USA	2009	MF964275	MF964326	MF964286
Rutstroemia firma	CBS 115.86	N/A	*Quercus robur*	N/A	Netherlands	1985	KF545286	–	KF545462
Rutstroemia firma	CBS 341.62	N/A	N/A	N/A	France	1962	KF545275	KF545334	KF545461
Rutstroemia sydowiana	CBS 115,975	N/A	N/A	N/A	Netherlands	2002	KF545276	KF545331	KF545465
Rutstroemia sydowiana	CBS 115,928	N/A	green leaf	N/A	Netherlands	2002	–	KF545330	KF545464
Sclerotinia asari	CBS 139.91	NA	*Asarum europaeum*	N/A	Germany	N/A	MF964262	MF964313	MF964277
Sclerotinia matthiolae	CBS 111.17	N/A	*Matthiola vallesiaca*	N/A	Switzerland	N/A	MF964263	MF964314	MF964278
Sclerotinia minor	7440–203	N/A	Unknown		NJ, USA	2009	–	MF964327	MF964287
Sclerotinia minor	CBS 112.17	N/A	*Lactuca sativa*	N/A	Netherlands	N/A	MF964264	MF964315	MF964279
Sclerotinia sclerotiorum	1980 UF-70	N/A	*Phaseolus vulgaris*	N/A	NE, USA	N/A	[a]	[a]	[a]
Sclerotinia sclerotiorum	SS1	N/A	*Solanum lycopersicum*	N/A	NJ, USA	2009	KF545279	KF545320	KF545469
Sclerotinia sclerotiorum	SS4	N/A	*Solanum lycopersicum*	N/A	NJ, USA	2009	–	MF964328	MF964288
Sclerotinia sclerotiorum	SS5	N/A	*Solanum lycopersicum*	N/A	NJ, USA	2009	KF545280	KF545319	KF545468
Sclerotinia sp.	CBS 518.75	N/A	*Alnus glutinosa*	N/A	Netherlands	1975	KF545278	KF545323	KF545471
Sclerotinia spermophila	CBS 219.46	N/A	*Trifolium repens* seed	N/A	N/A	N/A	MF964265	MF964316	–
Sclerotinia sulcata	CBS 303.31	N/A	*Carex hudsonii*	N/A	Denmark	1930	MF964266	MF964317	MF964280
Sclerotinia trifoliorum	CBS 171.24	N/A	*Trifolium incarnatum*	N/A	N/A	1917	MF964267	MF964318	MF964281

[a] CaM, ITS and Mcm7 sequences mined from whole genome assemblies deposited at GenBank: *Botrytis cinerea* B05.10 accession PRJNA15632; *Sclerotinia sclerotiorum* 1980 UF-70 accession PRJNA20263.

reactions were performed using ChromaTaq DNA polymerase (Denville Scientific, Metuchen, NJ) in 25 μl volumes containing 10x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, and 12.5 ng/μl of each primer. PCR amplicons were visualized on 0.8 % agarose gels and purified using the NucleoSpin Gel and PCR Clean-up kit (Macherey–Nagel, Duren, Germany). Purified amplicons were sequenced in both directions using Sanger sequencing technology by GeneWiz, Inc. (South Plainfield, NJ) or in-house using ABI BigDye 3 Terminator Cycle sequencing chemistry on an ABI3130 Genetic Analyzer (Life Technologies, Grand Island, NY). All sequences were assembled using Lasergene Sequence Analysis Software (DNASTAR, Madison, WI) or Sequencher (Gene Codes Corporation, Ann Arbor MI).

2.4. Alignments and phylogenetic analyses

DNA sequences were aligned with the MAFFT program online version 7 (http://mafft.cbrc.jp/alignment/server/) (Katoh and Standley, 2013) using the algorithm G-INS-i. jModeltest version 2.1.7 (Darriba et al., 2012) was used to determine the best nucleotide substitution models using the Akaike Information Criterion (AIC). Individual gene trees were produced for each of the marker regions sequenced from the fungal isolates with the model parameters previously estimated (Supplementary Figs. 1–3). A combined phylogenetic analysis was performed from all sampled taxa using aligned datasets from all sequenced regions and a partitioned approach. Phylogenetic analysis were performed using maximum likelihood (ML) and Bayesian (BI) approaches. Bayesian phylogenetic trees were obtained using MrBayes version 3.2.5 (Ronquist et al., 2012) with a TIM2 + I + γ model for ITS and Mcm7 datasets, and a TPM1 + I + γ model for the CaM dataset. MrBayes analyses were initiated from random starting trees, run for 10 million generations with four chains (Metropolis-coupled Markov Chain Monte Carlo) (Huelsenbeck and Rannala, 2004) and sampled every 1000^{th} generations for a total of 10,000 tree samples per run. Default priors were used on all analyses and two independent BI analyses were run. To evaluate stationarity and convergence between runs, log-likelihood scores were plotted using TRACER version 1.6 (Rambaut et al., 2013). After stationarity evaluation, 25 % of the trees were removed from the analyses. The remaining trees were used to calculate posterior probabilities (PP) at all nodes using the "sumt" command. ML analyses were performed using RaxML (Stamatakis, 2006) implemented in RaxML GUI version 1.5b1 (Silvestro and Michalak, 2012). Branch support was assessed with 1000 nonparametric bootstrapping replicates using the model parameters described above. Clades with PP ≥0.95 and bootstrap values ≥70 % were considered well supported (Huelsenbeck and Rannala, 2004). Finished tree files were visualized in FigTree version 1.4.3 (Rambaut, 2014).

2.5. Data and specimen curation

All sequence data from this study was deposited in NCBI GenBank (Table 1). Sequence alignments are available through the National Agricultural Library AgData Commons (https://doi.org/10.15482/USDA.ADC/1429061). Fungal specimens used for taxonomic descriptions, along with select representative isolates, have been deposited at CBS-KNAW culture collections (Utrecht, The Netherlands); vouchers and type specimens were deposited in the U.S. National Fungus Collections, Beltsville, MD, USA (Table 1). Nomenclature descriptions have been deposited in MycoBank (accession numbers MB807153, MB823934, MB823935, MB823936, MB823937).

3. Results

3.1. Morphological and cultural assessments

When young (~2–10 d), all *S. homoeocarpa* cultures grown on unamended PDA exhibited white, rapidly growing, floccose mycelium (Fig. 2). As cultures matured (>3 weeks), hyphae gradually exhibited a darker coloration, ranging from off-white to olive or brown. Aerial mycelium gradually collapsed, and flat, dark brown/black stroma was formed by some *S. homoeocarpa* isolates on the underside (back) of the colony (Fig. 2). No spores were present in any cultures.

Two individual *S. homoeocarpa* isolates (SE16F-4, RCCPG-1) produced apothecia without the presence of the opposite mating type after four weeks of growth on PDA amended with ascorbic acid (PDA-AA; Fig. 3A–D). Apothecia also formed from the following co-inoculations on PDA-AA: SE16F-4 × MAFF 235856, SE16F-4 × MAFF 235858, SE16F-4 × BC-14, SE16F-4 × RE18G-38, SE16F-4 × LWC-10, SE16-F4 × DRR-9 (Supplementary Table 1). In all instances, regardless of whether isolates of both mating types were present or not, apothecia were sterile, as evidenced by the absence of asci and ascospores (Fig. 3E–G), suggesting that any apothecia visible in crosses might be a result of isolate SE16F-4 producing individual apothecia. Apothecia were, on average, 2.73 by 1.91 mm. Apothecia were not observed on any of the remaining isolates.

3.2. Molecular phylogeny

Sequencing of three molecular markers generated 1810 bp of DNA sequence data, with PCR success rates from DNA templates as follows: CaM = 87 %, Mcm7 = 68 %, ITS = 97 %. Fifty-seven percent of the DNA produced PCR amplicons from all three markers, 37 % of samples produced amplicons from just two markers, and 6 % of samples produced amplicons from only one marker (Table 1).

The phylogenetic tree constructed from the combined dataset produced a topology similar to those constructed from individual marker datasets, although with variation in branch support observed across the trees (Fig. 4, Supplementary Figs. 1–3). The three single gene genealogies did not conflict with each other, although some individual clades had low PP and bootstrap support. As outgroup to the *Rutstroemiaceae* ingroup, *Sclerotinia* species (*Sclerotinia asari*, *Sclerotinia sclerotiorum*, *Sclerotinia matthiolae*, *Sclerotinia minor*, and *Sclerotinia trifoliorum*) and *Ciboria* species (*Ciboria amentacea*, *Ciboria aestivalis*, *Ciboria spermophila*, *Ciboria americana*), together with *Botrytis cinerea*, formed their own well supported monophyletic group, consistent with their placement in the *Sclerotiniaceae* (Fig. 4). Consistent with previous research, *S. homoeocarpa* clustered as a member of the *Rutstroemiaceae*, alongside species of *Rutstroemia*, *Lambertella*, and *Lanzia*. Phylogenetic analyses of the three loci combined showed high bootstrap and PP support values for the majority of the branches, except for a few internal branches (Fig. 4).

In the multilocus phylogenetic tree, the *S. homoeocarpa* isolates clustered into a well-supported clade that was distinct from other species in the family *Rutstroemiaceae* such as *Lambertella*, *Lanzia* and *Rutstroemia* (Fig. 4; PP = 1.0, bootstrap = 73 %). Based on this phylogenetic distinctiveness, we propose to erect a new genus, *Clarireedia*, to accommodate these fungi, as detailed below in the Taxonomy section. All three single gene genealogies recovered the proposed new genus as monophyletic with fully supported bootstrap and PP values (Supplementary Figs. 1–3).

The fungal isolates within the proposed genus *Clarireedia* were subdivided into two main groups with high PP and bootstrap support values in the combined phylogeny; these were designated

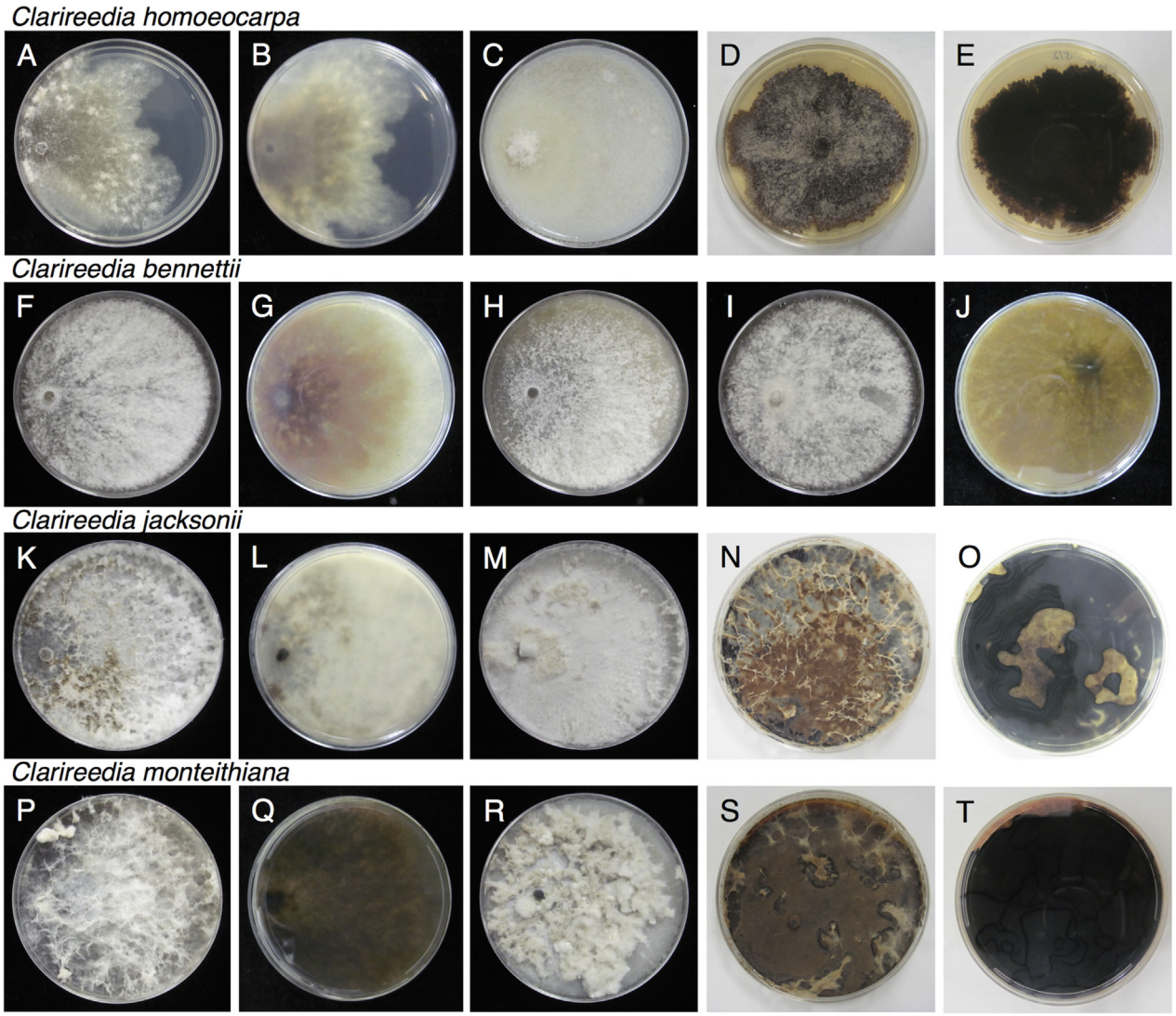

Fig. 2. Colony morphology of species in the genus *Clarireedia* at 8 d old (unless otherwise indicated). (A–E) *C. homoeocarpa*: (A) colony front, PDA + ascorbic acid; (B) colony back, PDA + ascorbic acid; (C) colony front, wheat meal agar; (D) three-week old colony on PDA + ascorbic acid, front; (E) three-week old colony on PDA + ascorbic acid, back; (F–J) *C. bennettii*: (F) colony front, PDA + ascorbic acid; (G) colony back, PDA + ascorbic acid; (H) colony front, wheat meal agar; (I) three-week old colony on PDA + ascorbic acid, front; (J) three-week old colony on PDA + ascorbic acid, back; (K–O) *C. jacksonii*: (K) colony front, PDA + ascorbic acid; (L) colony back, PDA + ascorbic acid; (M) colony front, wheat meal agar; (N) three-week old colony on PDA + ascorbic acid, front; (O) three-week old colony on PDA + ascorbic acid, back; (P–T) *C. monteithiana*: (P) colony front, PDA + ascorbic acid; (Q) colony back, PDA + ascorbic acid; (R) colony front, wheat meal agar; (S) three-week old colony on PDA + ascorbic acid, front; (T) three-week old colony on PDA + ascorbic acid, back.

Group A and Group B (PP = 0.98–1.0; bootstrap = 77–100). Basal to Group A and Group B were three single isolate lineages: CBS 465.73 from rabbit dung; CPB-17 and PSFFB-1 from *Festuca rubra*. These single isolate lineages grouped most closely to Group A. *Clarireedia* Group A included the type species (*C. homoeocarpa* comb. nov.) and a new species to be designated *Clarireedia bennettii.* The clades designated as *C. homoeocarpa* and *C. bennettii* were recovered from all three individual gene genealogies, although with variable bootstrap and PP support values. Although two of the single isolate lineages (CPB-17 and PSFFB-1) clustered as part of *C. homoeocarpa* in the ITS and Mcm7 phylogenies, the other single isolate lineage (CBS 465.73) aligned with *C. bennettii* (Supplementary Figs. 2–3). *C. bennettii* was recovered in the CaM and ITS phylogenies with high bootstrap and PP support values, but was not supported (albeit not contradicted) in the Mcm7 phylogeny. All members of Group A originated from the United Kingdom, and were isolated from *F. rubra* and one isolate from *Symplocarpus foetidus*. The three isolates deposited in the CBS culture collection by Bennett in 1937 (accession numbers CBS 309.37, CBS 310.37, CBS 311.37) fell within Group A, but were not all members of the same species. CBS 310.37 was a member of *C. homoeocarpa*, and CBS 309.37 and CBS 311.37 were members of *C. bennettii*.

Clarireedia Group B contained two new species, to be designated *Clarireedia jacksonii* and *Clarireedia monteithiana* (Fig. 4; see Taxonomy section). *C. jacksonii* was only identified from C3 turfgrasses, including species such as *Agrostis stolonifera*, *F. rubra*, *Lolium perenne* and *Poa pratensis* (Table 1). *C. monteithiana* was identified solely from the C4 turfgrasses *Cynodon dactylon* × *transvaalensis* and *Paspalum vaginatum*.

4. Taxonomy

The results obtained from the phylogenetic analyses showed that fungi previously described as *S. homoeocarpa* form a lineage

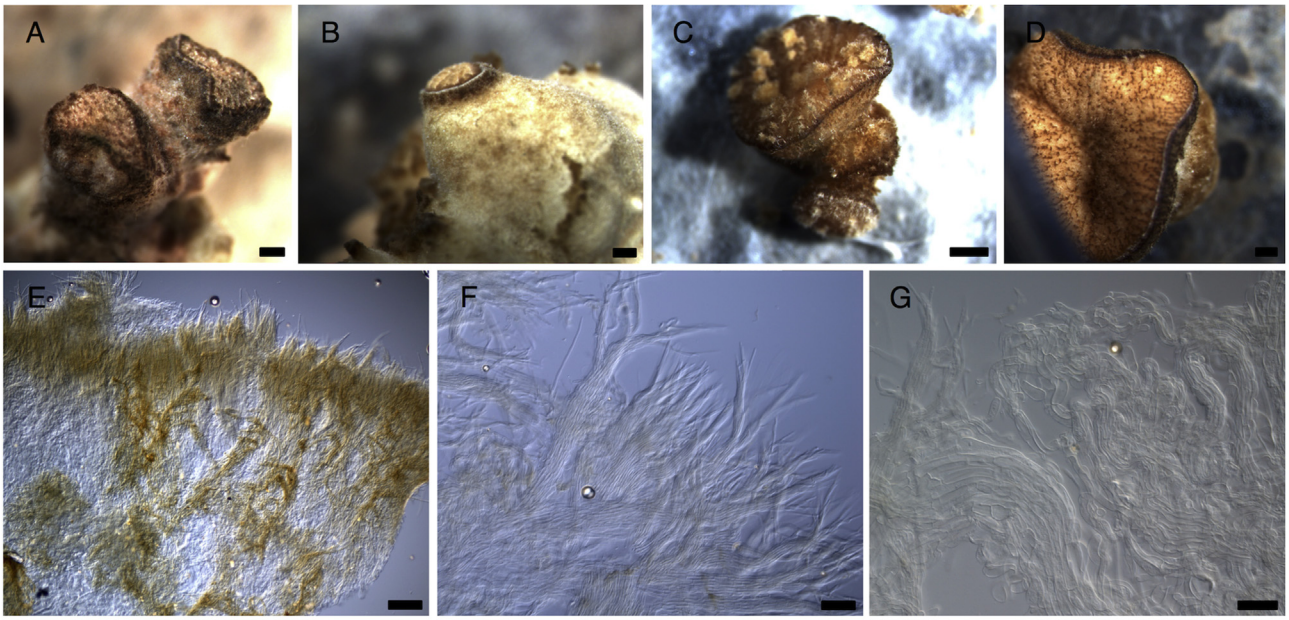

Fig. 3. Infertile apothecia formed by *Clarireedia* spp. on PDA + ascorbic acid. (A–B) apothecia from *C. monteithiana* isolate DRR-9; (C–D) apothecia from *C. jacksonii* isolate SE16F-4 (E–G) microscopic view of cross section of apothecia from *C. jacksonii* isolate SE16F-4. Scale bars: A–B, D = 500 μm; C = 1000 μm; E = 100 μm; F–G = 50 μm.

within the family *Rutstroemiaceae*, distinct from currently recognized species and constituting a new undescribed genus (Fig. 4). Four species, including the type species for the genus are described here. Because these new species do not produce reproductive structures or other distinct characters that allow morphological identification; species recognition within the genus is dependent upon molecular phylogenetic analyses. A list of variable molecular characters found within the CaM, ITS and Mcm7 regions that can be used to discriminate species between and within groups A and B in *Clarireedia* is provided in Table 2.

Clarireedia L.A. Beirn, B.B. Clarke, C. Salgado & J.A. Crouch **gen. nov.**

MycoBank No.: MB807153.

Etym.: "Clarus" is Latin for famous, "reedia" in honor of Dr. C. Reed Funk's seminal contributions to turfgrass science and development of turfgrass cultivars with resistance to dollar spot disease.

A genus of the *Rutstroemiaceae*. Thalli at first aerial, white to off-white, later collapsing and turning brown, tan, olive or grey, sometimes slightly pink. Hyphae septate, hyaline. Apothecia arising from a substratal stroma, cupulate to discoid, brown, cinnamon, or light orange, receptacle pubescent.

Type species: **Clarireedia homoeocarpa** (F.T. Benn.) L.A. Beirn, B.B. Clarke, C. Salgado, & J.A. Crouch **comb. nov.**

MycoBank No.: MB823934 Fig. 2A–E.

Basionym: *Sclerotinia homoeocarpa* F.T. Benn., *Ann. Appl. Biol.* **24**: 254 (1937).

Synonyms: *Rhizoctonia monteithiana nomen invalidum* F.T. Benn., *Gard. Chron.* **3**:129 (1935).

Rutstroemia festucae nomen invalidum J.F. Powell *[doctoral dissertation]* p. 53 (1998).

Morphological description: Thalli at first aerial, white to off-white, later collapsing and turning brown, tan, olive or grey, sometimes slightly pink. Colonies on PDA raised, aerial mycelium white to off-white, collapsing and turning brown, tan, olive, or grey, with undulate margins. Colony reaches 4 cm radial growth after 6 d 25 C under continuous light on PDA + ascorbic acid. Colonies >15 d old do not form a dark stroma on PDA + ascorbic acid. Hyphae septate, hyaline. Apothecia 0.5–1.5 mm in diameter (from Bennett, 1937), arising from a dark substratal stroma, cupulate to discoid, brown, cinnamon, or light orange, receptacle pubescent (Fig. 3A–D). Ascus 162.9 × 12.5 μm, on average (from Bennett, 1937). Ascospores hyaline, oblong to elliptical, mostly unicellular, occasionally with a medium septum, 20.7 × 8.3 μm (from Bennett, 1937). Conidia not observed. Microconidia spherical, hyaline, 2.0 μm in diameter, formed in cream-colored pustules (from Bennett, 1937).

Diagnostic molecular characters: In relationship to the alignment deposited at USDA AgData Commons (https://doi.org/10.15482/USDA.ADC/1429061), *C. homoeocarpa* can be distinguished from the related species *C. bennettii* by molecular characters at three loci (Table 2): CaM: characters 45, 79, 85, 109, 129, 131, 137, 150, 343, 397, 416, 485, 486, 499, 530, 537; ITS: characters 64, 67, 85, 86, 109, 156, 160, 161, 198, 200, 230, 231, 471, 489; Mcm7: characters 60, 69, 364.

Neotype hic designatus: **United Kingdom:** dried sterile apothecia produced on *Festuca rubra* seeds (Fig. 5A–E), 1972, *N. Jackson* (BPI 892697).

Epitype hic designatus: **United Kingdom:** dried mycelium on potato dextrose agar, 1937, *F.T. Bennett* (BPI 910612, marker sequences, CaM: MF964271, ITS: MF964322, Mcm7: KF545451; ex-epitype CBS 310.37).

Habitat: Primarily known as a pathogen of C3 grasses in the genus *Festuca*.

Distribution: United Kingdom.

Notes: No type specimen was ever designated for *S. homoeocarpa*. Through Noel Jackson (Professor Emeritus, University of Rhode Island), we obtained a microscope slide said to originate from Bennett's personal collection from the original collections. The slide was in the possession of Drew Smith at the Sports Turf Research Institute in the U.K., who received it from Bennett at his retirement, and Smith passed the slide on to Jackson during his U.K. sabbatical in 1971. Unfortunately, the material on the slide was degraded, and no recognizable structures were present on the mount. Therefore, we designated a neotype specimen for *C. homoeocarpa* that consists of a dried apothecial specimen, along with a set of 35-mm slides taken by Jackson in 1971 (Fig. 5). The neotype is unique among the *C. homoeocarpa* materials examined in this study. To our knowledge, this is the only sample possessing

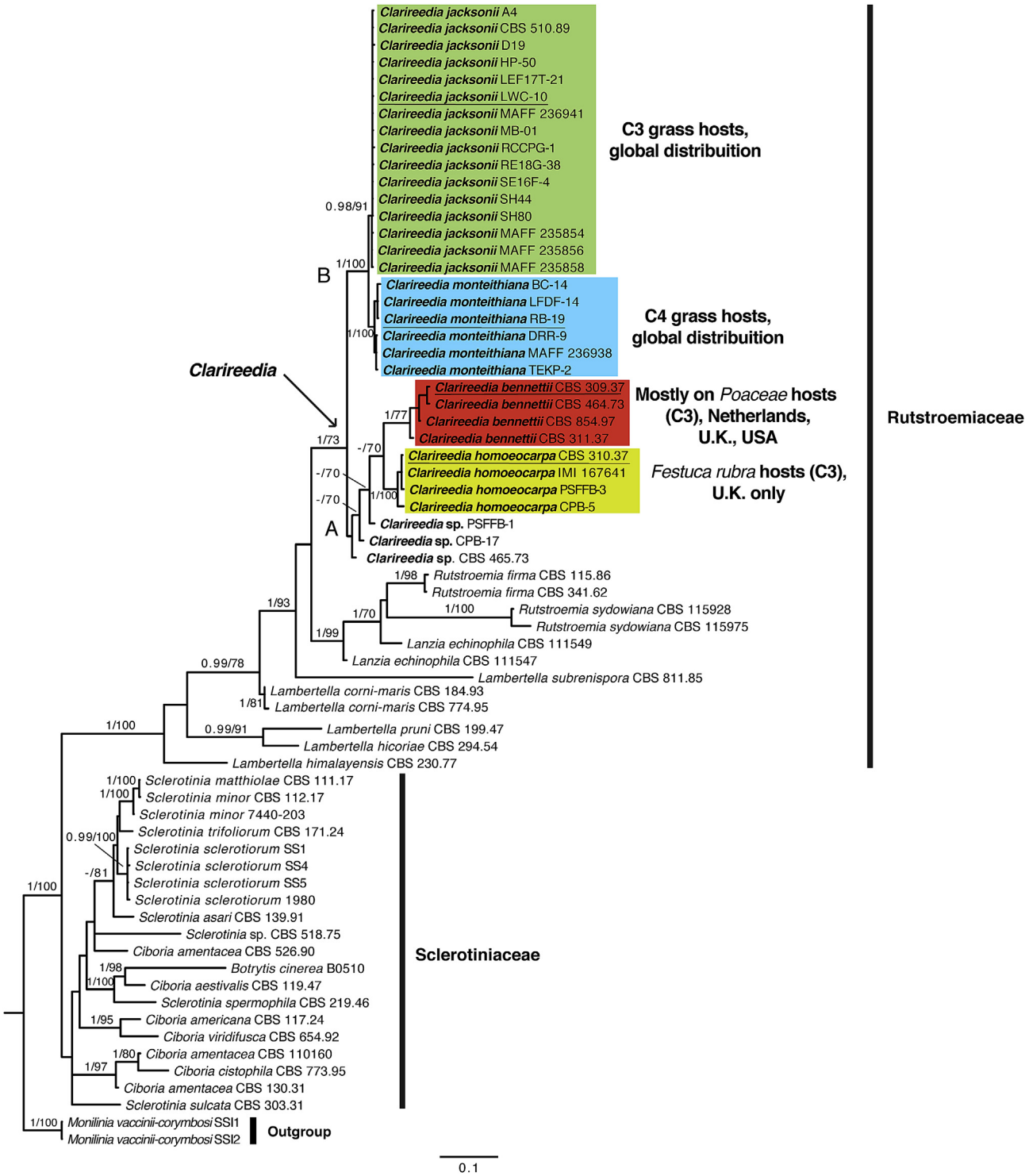

Fig. 4. Majority rule Bayesian phylogenetic tree from the combined three marker analysis showing relationships among fungal isolates in the *Sclerotiniaceae* and *Rutstroemiaceae* families. Support values (posterior probability (PP)/maximum likelihood (ML) bootstrap) are indicated above the branches. No number above the branches indicates that the clade/branch was not supported at values $\geq$ 0.95 PP/70 % ML bootstrap. Underlined isolate names indicate ex-type cultures. *Monilinia vaccinii-corymbosi* was used as outgroup. Branch lengths are proportional to levels of sequence divergence.

morphological characteristics consistent with the protolog, providing a *bona fide* physical specimen of known origin. The geographic and host origin of this specimen (U.K., *F. rubra*) are consistent with those described for *S. homoeocarpa*.

Bennett deposited three cultures with the CBS-KNAW collection in 1937, without any details about host, locale or other origination information. Only one of the original Bennett's isolates, CBS 310.37, is a member of *C. homoeocarpa*; this isolate is designated the epitype for the species. As with all three of the original Bennett isolates, CBS 310.37 produces very sparse and slow growing hyphae. None of the structures described in the protolog were observed from CBS 310.37, even when grown under

Table 2
Single nucleotide polymorphism comparisons between *Clarireedia homoeocarpa*, *C. bennettii*, *C. jacksonii* and *C. monteithiana*.

	CaM																														
Species	**45**	**79**	**85**	**99**	**109**	**118**	**129**	**131**	**137**	**148**	**150**	**159**	**175**	**204**	**343**	**392**	**393**	**397**	**405**	**416**	**438**	**453**	**485**	**486**	**499**	**510**	**530**	**537**			
C. homoeocarpa	C	C	T	A	A	G	T	G	C	T	A	T	–	–	C	–	–	A	A	C	T	C	A	C	C	T	C	C			
C. bennettii	T	T	C	A	C	G	C	A	T	T	G	T	–	–	T	–	–	T	A	T	T	C	C	T	T	T	A	T			
C. jacksonii	C	C	C	A	A	C	T	A	C	G	A	T	C	G	T	C	T	T	A	C	T	C	C	T	T	T	T	C			
C. monteithiana	C	C	C	C	A	G	T	A	C	C	A	G	C	G	T	–	–	T	C	G	C	T	C	T	T	G	T	C			
	ITS																				**Mcm7**										
Species	**44**	**64**	**67**	**82**	**85**	**86**	**109**	**149**	**156**	**160**	**161**	**162**	**164**	**198**	**200**	**230**	**231**	**471**	**472**	**489**	**60**	**69**	**171**	**247**	**295**	**364**	**388**	**400**			
C. homoeocarpa	C	A	T	C	C	G	T	C	G	G	G	C	C	–	C	C	C	G	G	T	G	G	A	T	T	T	T	C			
C. bennettii	C	G	G	C	–	C	A	C	C	A	T	C	C	A	G	T	T	–	G	C	A	A	A	T	T	C	T	C			
C. jacksonii	T	T	C	T	T	T	G	T	T	G	C	T	T	T	A	–	–	T	A	A	A	A	A	C	T	C	T	C			
C. monteithiana	C	T	C	C	T	T	G	C	T	G	C	C	C	T	A	–	–	T	G	A	A	A	T	T	A	C	C	T			

conditions conducive for apothecial formation (Orshinsky and Boland, 2011).

Clarireedia bennettii C. Salgado, L.A. Beirn, B.B. Clarke, & J.A. Crouch **sp. nov.**

Mycobank No.: MB823935 Fig. 2F–J.

Holotype: **United Kingdom**: 1937, *F. T. Bennett* CBS 309.37 (dried specimen BPI 910610, ex-holotype CBS 309.37).

Etym.: in honor of F.T. Bennett, the British mycologist that first described the causal agent of dollar spot disease.

Morphological description: Colonies on PDA + ascorbic acid and wheat meal agar reaching 8 cm (radial growth) after 6 d at 25 C under continuous light, aerial mycelia floccose, colony front white, colony back white to light brown, no pigment diffusing into media. Colonies >15 d old do not form a dark stroma on PDA + ascorbic acid and remain floccose. Hyphae septate, hyaline. Apothecia and conidia not observed.

Diagnostic molecular characters: In relationship to the alignments deposited at USDA AgData Commons (https://doi.org/10.15482/USDA.ADC/1429061), *C. bennettii* can be distinguished from the related species *C. homoeocarpa* by molecular characters at three loci (Table 2): CaM: characters 45, 79, 85, 109, 129, 131, 137, 150, 343, 397, 416, 485, 486, 499, 530, 537. ITS: characters 64, 67, 85, 86, 109, 156, 161, 198, 200, 230, 231, 471, 489. Mcm7: characters 60, 69, 364.

Habitat: Known as a pathogen of an unidentified diseased turfgrass host (Bennett, 1937), found on dead grass and *Symplocarpus foetidus*.

Distribution: The Netherlands, United Kingdom and United States.

Notes: *Clarireedia bennettii* exhibits a higher rate (2X) of radial growth on PDA + ascorbic acid when compared to the sister species *C. homoeocarpa*.

Clarireedia jacksonii C. Salgado, L.A. Beirn, B.B. Clarke, & J.A. Crouch **sp. nov.**

Mycobank No.: MB823936 Fig. 2K–O; Fig. 3A–D

Holotype: **United States**: North Carolina, on *Agrostis stolonifera*, 2008, *L.P. Tredway* LWC-10 (dried specimen BPI 910609, ex-holotype LWC-10 = CBS 138618).

Etym.: in honor of Noel Jackson, turfgrass pathologist and diagnostician renowned for his research on the etiology and control of dollar spot and other important turfgrass diseases throughout a distinguished career that spanned more than 40 y.

Morphological description: Colonies fast growing, cottony, front white to off-white with light brown spots, back white to off-white, later collapsing and turning tan to brown. Colony reaches 8 cm radial growth after 6 d at 25 C under continuous light on PDA + ascorbic acid and wheat meal agar. Colonies >15 d old form thick, flat, black stroma on PDA + ascorbic acid. Hyphae septate, hyaline. Apothecia arising from a substratal stroma, cupulate to discoid, brown, cinnamon, or light orange, receptacle pubescent. Apothecia 2.73 × 1.91 mm arising from dark, substratal stroma (Fig. 3A–D). Asci, ascospores and conidia have not been observed.

Diagnostic molecular characters: In relationship to the alignments deposited at USDA AgData Commons (https://doi.org/10.15482/USDA.ADC/1429061), *C. jacksonii* can be distinguished from the related species *C. monteithiana* by molecular characters at three loci (Table 2): CaM: characters 99, 118, 148, 159, 392, 393, 405, 416, 438, 453, 510. ITS: characters 44, 82, 149, 162, 164, 472. Mcm7: characters 171, 247, 295, 388, 400.

Habitat: Pathogen of C3 grasses such as *Agrostis stolonifera*, *Festuca rubra*, *Lolium perenne* and *Poa pratensis*.

Distribution: worldwide.

Notes: *Clarireedia jacksonii* and *C. monteithiana* appear to be the most important pathogenic species causing dollar spot disease of turfgrasses in North America and perhaps worldwide, as these species affect some of the most important and widely grown cool-season

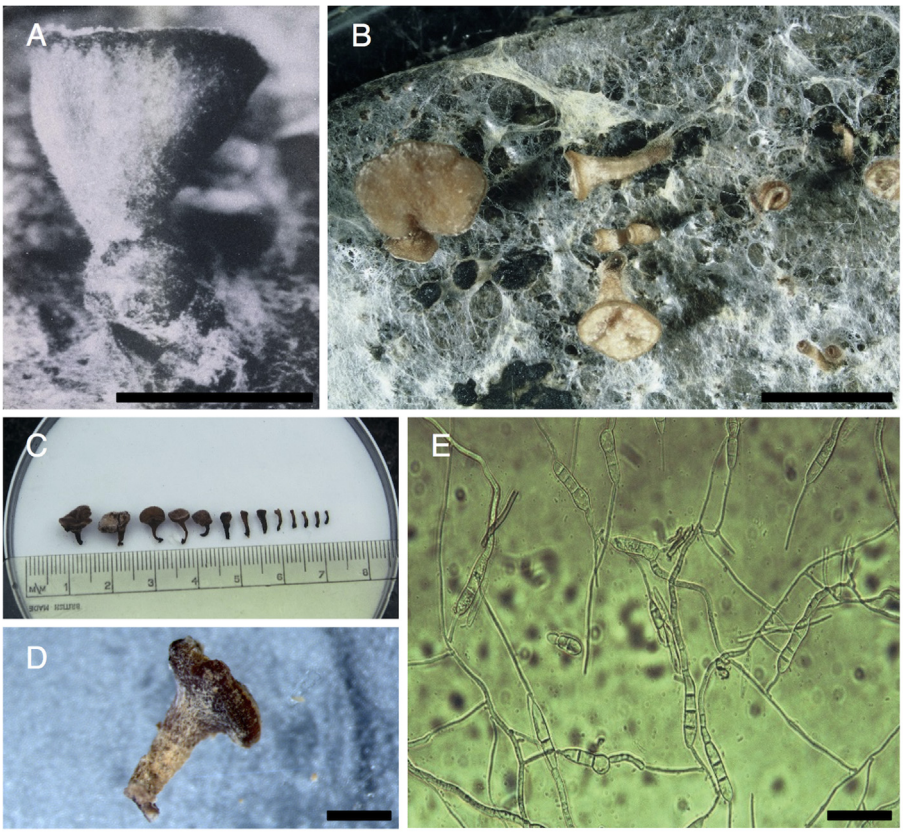

Fig. 5. *Clarireedia homoeocarpa* neotype material. (A) sterile apothecia generated on potato dextrose agar; (B) Close up of apothecia on colonial bentgrass (*Agrostis capillaris*) seeds; (C) apothecia of varying sizes from colonial bentgrass seed culture; (D) apothecia (BPI 892697); (E) Germinating ascospores. Scale bars: A–B = 5 mm; D = 1000 μm; E = 50 μm.

grasses used as turfgrass. The back view of *C. jacksonii* fungal colonies on PDA + ascorbic acid is the same color as the front (Fig 2L), compared to *C. monteithiana* (below), which presents light olive-brown coloration on the back side of the colony (Fig 2Q). Publicly available genome sequences of *Clarireedia* identified as *S. homoeocarpa* (Green et al., 2016) represent isolates of *C. jacksonii* based on sequence identity at the CaM, ITS, and Mcm7 marker regions (data not shown).

Clarireedia monteithiana C. Salgado, L.A. Beirn, B.B. Clarke, & J.A. Crouch **sp. nov.**

Mycobank No.: MB 823937 Fig. 2P–T.

Holotype: **United States**: Mississippi, on *Cynodon dactylon* × *transvaalensis*, 2008, *L.P. Tredway* RB-19 (dried specimen BPI 910611, ex-holotype RB-19 = CBS 136376).

Etym.: in honor of John Monteith, the USDA scientist who first described dollar spot disease of turfgrass in 1928.

Morphological description: Colonies fast growing, cottony, front white to off-white, back light olive-brown, later collapsing and turning medium to dark brown. Colony reaches 8 cm radial growth after 6 d at 25 C under continuous light on PDA + ascorbic acid and wheat meal agar. Colonies >15 d old form thick, flat, black stroma on PDA + ascorbic acid. Hyphae septate, hyaline. Apothecia, asci, ascospores and conidia have not been observed.

Diagnostic molecular characters: In relationship to the alignments deposited at USDA AgData Commons (https://doi.org/10.15482/USDA.ADC/1429061), *C. monteithiana* can be distinguished from the related species *C. jacksonii* by molecular characters at three loci (Table 2): CaM: characters 99, 118, 148, 159, 392, 393, 405, 416, 438, 453, 510. ITS: characters 44, 82, 149, 162, 164, 472. Mcm7: characters 171, 247, 295, 388, 400.

Habitat: Known as a pathogen of C4 grasses such as *Cynodon dactylon* × *transvaalensis* and *Paspalum vaginatum*.

Distribution: Dominican Republic, Japan, United States.

Notes: See notes for *C. jacksonii*. *Clarireedia monteithiana* is currently only known from C4 turfgrasses. It is unknown whether additional species of C4 grasses are parasitized by *C. monteithiana*. Given previous indicators of diversity among isolates from C4 grass hosts (Liberti et al., 2012), this question should be empirically tested using the CaM, ITS and Mcm7 markers rather than assuming the affiliation of isolates with *C. monteithiana* based on host physiology.

5. Discussion

This study marks the first multi-locus phylogenetic analysis of the *Rutstroemiaceae*, a family best known as saprotrophs but also including some necrotrophic plant pathogens and endophytes (Holst-Jensen et al., 1997; Hosoya et al., 2014). Previously, the family *Rutstroemiaceae* was said to include taxa producing substratal stroma represented by the type *Rutstroemia firma* (Holst-Jensen et al., 1997), whereas the *Sclerotiniaceae* was composed of fungi producing apothecia arising from tuberoid sclerotia represented by the type *S. sclerotiorum* (Whetzel, 1945). However, more recent molecular analyses have shown that the substratal stroma is not a reliable character to define the *Rutstroemiaceae* (Baral and Bemmann, 2014; Zhao et al., 2016). While our data supports division between the monophyletic *Sclerotiniaceae* and the paraphyletic *Rutstroemiaceae* families, it also expands on previous rDNA-based studies to uncover these two familial lineages

emerging from a common ancestor (Holst-Jensen et al., 1997; Wang et al., 2006; Zhao et al., 2016).

The primary objective of this study was to determine the identity of the causal agent of dollar spot disease in turfgrass, now named as *Clarireedia homoeocarpa*, the type member of the new genus *Clarireedia*. The multi-locus phylogeny also detected three additional undescribed species within the new genus *Clarireedia*. This study shows that all of the surveyed fungal isolates associated with turfgrass hosts and causing dollar spot disease fall within the genus *Clarireedia*. Our data also shows that earlier attempts to reclassify *C. homoeocarpa* were likely confounded by the fact that genera in the *Rutstroemiaceae* are polyphyletic, and available cultures of the *Rutstroemiaceae* have not always been correctly identified. For example, if we had only included isolates CBS 464.73 and CBS 465.73 alongside the *C. homoeocarpa* isolates from turfgrass, we would have concluded that *C. homoeocarpa* should be placed in the genus *Rutstroemia*, since CBS 464.73 and CBS 465.73 were identified in the CBS culture collection as *Rhizoctonia paludosa* (Groves and Elliot, 1961; synonyms *Poculum paludosa, Sclerotinia paludosa*; isolated from *S. foetidus*) and *Rhizoctonia cunicularia* (Elliott, 1967; synonym = *Peziza cunicularia*; isolated from rabbit dung) based on depositor data. At first glance, the fact that isolate CBS 465.73 was isolated from rabbit dung seems odd, however, the fungal isolate could have been present on grass previous to being eaten by the animal, or could have been transferred to the excrement by close contact with diseased plants. Isolates CBS 464.73 and CBS 465.73 do not appear to be members of the genus *Rutstroemia*, as they do not cluster or are associated with isolates of the type species for the genus *Rutstroemia*, *R. firma* (isolates CBS 115.86, CBS 341.62), but are aligned within *Clarireedia*. This scenario is not unique in the relatively understudied *Rutstroemiaceae*. Another example is found in the recent description of the species *P. pseudosydowiana* in the genus *Poculum* (Hosoya et al., 2014). Identification of *P. pseudosydowiana* was largely based on ITS sequence similarity to isolates of *Rutstroemia sydowiana* CBS 115928 and CBS 115975 that were referred to by the synonym of *P. sydowiana* (Hosoya et al., 2014) by Holst-Jensen et al. (1997). Therefore, in addition to demonstrating the need to re-evaluate many of the currently described species within the *Rutstroemiaceae*, our data also suggests that a taxonomic review at the genus rank may also be necessary for many of the fungi in this family.

Our results confirm that the fungi causing dollar spot disease are not members of the genus *Sclerotinia*, nor are they members of the *Sclerotiniaceae*, consistent with numerous previous studies (Whetzel, 1945; Jackson, 1973; Kohn, 1979a; b; Kohn and Grenville, 1989; Novak and Kohn, 1991; Carbone and Kohn, 1993; Holst-Jensen et al., 1997; Powell and Vargas, 1999). Based on the placement of *C. homoeocarpa* relative to isolates of *Lambertella*, *Lanzia*, and *Rutstroemia* in the multi-locus phylogeny, *C. homoeocarpa* isolates are unique and fall outside of any currently described genus. Thus, rather than placing these fungi in an already established genus, our multi-locus data showed that *C. homoeocarpa* is a member of a singular taxon, unique from all described genera of the *Rutstroemiaceae*. Although representatives of two *Rutstroemiaceae* genera—*Poculum* and *Dicephalospora*—were not included in our work due to the unavailability of *bona fide* isolates, it is exceedingly unlikely that the new genus *Clarireedia* is synonymous with these or other existing genera. Pairwise comparisons between the ITS sequence of *P. hennigsianum* (GenBank Z81442; Holst-Jensen et al., 1997) shows only 77–81 % similarity with *Clarireedia* isolates (data not shown). Similarly, *Clarireedia* isolates share just 82–83 % similarity with isolates of *D. rufocornea* (e.g. GenBank JN033401; Han et al., 2014) and other members of the genus *Dicephalospora* (data not shown). These high levels of dissimilarity with ITS, the most conserved of the three molecular markers employed in the study, supports the distinction of *Clarireedia* from any described genera in the *Rutstroemiaceae*.

Within the new genus *Clarireedia*, in addition to the type species *C. homoeocarpa*, three additional species were recovered in all analyses. This outcome is consistent with previous suggestions by researchers that observed variation in morphological characters, AFLP fingerprints, and ITS data as an indication that more than one fungal species may be responsible for dollar spot disease in turfgrass (Jackson, 1973; Smith et al., 1989; Kohn, 1979a; Liberti et al., 2012; Powell, 1998; Smith et al. 1989; Taylor, 2010; Viji et al., 2004). As early as 1973, Jackson put forth the idea of multiple species causing the disease, citing the morphological differences he observed between isolates from North America and the United Kingdom. Unknowingly, Bennett also worked with two different fungal species, as the three specimens he collected from the United Kingdom fall within *C. homoeocarpa* and *C. bennettii*. These two species appear to represent a minority of the isolates causing dollar spot disease of turfgrass, as 71 % of the remaining isolates examined in this study, which were selected from a larger collection of isolates from around the world (Putnam, 2013), correspond to *C. jacksonii* and *C. monteithiana*. The restriction of *C. jacksonii* and *C. monteithiana* to C3 and C4 grass hosts, respectively, demonstrates a host preference among the most common and widespread incitants of dollar spot disease of turfgrass. It remains unknown whether this host association would be consistently recovered among dollar spot isolates obtained from grass hosts not sampled in this study. However, ITS sequence data from dollar spot isolates recovered from the C4 grass hosts *Zoysia japonica* and *Stenotaphrum secundatum* group with other fungal isolates obtained from C4 grasses (Liberti et al., 2012). Interestingly, Liberti et al. (2012) also reported a unique group of isolates causing dollar spot disease on both C3 and C4 grass hosts restricted to Florida, morphologically and phylogenetically distinct from isolates obtained from northern U.S. locations. A similar finding was also reported in Norway, where isolates obtained from *A. stolonifera* demonstrated only 97.6 % ITS sequence similarity to previously sequenced isolates from the U.S (Espevig et al., 2015). These data suggest that in addition to the four species described herein, additional species of *Clarireedia* responsible for contemporary outbreaks of dollar spot disease may exist, possibly with geographic restrictions, although further analysis of these populations would be required to test this hypothesis. Regardless, the presence of several species within *Clarireedia* demonstrates the unexpectedly high level diversity present within this genus of economically important plant pathogens.

The grouping of the type species *C. homoeocarpa* and three other isolates from *Festuca* species in the U.K. is interesting, since not all isolates from the U.K. clustered together, and some were members of *C. bennettii* and *C. jacksonii*. This suggests that there may also be some form of biological significance to the unique fungal groups reported here. For example, isolates within type species *C. homoeocarpa* not only shared geographic and species origin, but they also exhibited a reduced rate of growth in culture when compared to the other *Clarireedia* species. These attributes, combined with the observation that isolates of *C. homoeocarpa* from this region are routinely found in association with decaying grass substrates (Kate Entwistle, personal communication), suggests that this species may consist of isolates that prefer a saprophytic lifestyle, although additional data is required to test this hypothesis.

Our phylogenetic analyses also discriminated three single isolate lineages (PSFFB-1, CPB-17, CBS 465.73). These lineages constitute additional distinctive evolutionary entities (*Clarireedia* sp.) that

contribute to the diversity of organisms capable of causing dollar spot disease. In the systematics of fungi, there is no consensus on how singleton lineages should be treated (Seifert and Rossman, 2011). In a phylogenetic tree, singleton lineages constitute branches with unknown support (i.e. bootstrap, PP), as a clade should have at least two representatives to obtain statistical significance (Salgado-Salazar et al., 2015). Additional sampling of fungal isolates causing dollar spot disease may help resolve the species status of these singleton lineages.

The CaM, ITS and Mcm7 gene markers performed well for taxonomic delineation at both the genus and species level, and are recommended for use in combination for future phylogenetic and systematic analyses of these pathogens. Additionally, the matrix of molecular characters provided in the taxonomy section can be used to diagnose the species in a practical way. Using the molecular characteristics described herein, a diagnostic assay could be developed to quickly and accurately detect and identify *Clarireedia* to the species level.

The taxonomic resolution of *C. homoeocarpa* and related species after more than 70 y of unresolved identity is an important foundation for ongoing studies of these destructive fungal pathogens. Despite the presumed absence of a sexual cycle in natural populations, our analyses showed considerable diversity within *Clarireedia*. This suggests the potential for more genetic diversity and increased disease problems, particularly if fertile apothecia are formed in nature. Research aimed at understanding the biological significance of this variability may aid in future disease control efforts. For example, recent RNA-Seq analysis of the host pathogen interaction between *C. jacksonii* and creeping bentgrass identified an assortment of fungal enzymes capable of degrading a wide-range of host tissue, as well as ABC transporters that may play a role in fungicide resistance, from a single isolate (MB-01) of *C. jacksonii* (Orshinsky et al., 2012). Expanding these emerging technologies to the population scale may provide insight into how population diversity may impact functional traits required for disease manifestation and control.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.funbio.2018.04.004.

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