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PII: S1878-6146(18)30065-5
DOI: 10.1016/j.funbio.2018.04.004
Reference: FUNBIO 919

To appear in: Fungal Biology

Received Date: 19 January 2018
Revised Date: 27 March 2018
Accepted Date: 3 April 2018


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Clarireedia: A new fungal genus comprising four pathogenic species responsible for dollar spot disease of turfgrass

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Keywords - Ascomycota, Leotiomycetes, molecular phylogenetics, Rutstroemia, Sclerotinia
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 years. 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, *C. bennettii* sp. nov., *C. jacksonii* sp. nov., and *C. 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.
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 Bennett’s 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 *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*: *R. festucae* as
a new species limited to the U.K., and *R. floccosum* as a new species found outside the U.K.,
however, these conclusions were not validly published in accordance with fungal nomenclature

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 Sclerotinia homoeocarpa were selected for inclusion through preliminary variation screening of a worldwide sample of ~1,170 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.
PCR primers were synthesized as oligonucleotides by Integrated DNA Technologies (Coralville, IA). PCR reactions were performed using ChromaTaq DNA polymerase (Denville Scientific, Metuchen, NJ) in 25 µl volumes containing 10x PCR buffer, 1.5 mM MgCl₂, 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 Fig 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 1000th 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 1,000 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 (http://dx.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 to 10 days), all *Sclerotinia 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 3 E-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 1,810 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 Fig 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 (S. asari, S. sclerotiorum, S. matthiolae, S. minor, and S. trifoliorum) and Ciboria species (C. amentacea, C. aestivalis, C. spermophila, C. 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 Fig 1-3).

The fungal isolates within the proposed genus \textit{Clarireedia} were subdivided into two main groups with high PP and bootstrap support values in the combined phylogeny; these were designated 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 \textit{Festuca rubra}. These single isolate lineages grouped most closely to Group A. \textit{Clarireedia} Group A included the type species (\textit{C. homoeocarpa} comb. nov.) and a new species to be designated \textit{C. bennettii}. The clades designated as \textit{C. homoeocarpa} and \textit{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 \textit{C. homoeocarpa} in the ITS and Mcm7 phylogenies, the other single isolate lineage (CBS 465.73) aligned with \textit{C. bennettii} (Supplementary Fig 2-3). \textit{Clarireedia 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 \textit{Festuca rubra} and one isolate from \textit{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 \textit{C. homoeocarpa}, and CBS 309.37 and CBS 311.37 were members of \textit{C. bennettii}.

\textit{Clarireedia} Group B contained two new species, to be designated \textit{C. jacksonii} and \textit{C. monteithiana} (Fig 4; see Taxonomy section). \textit{Clarireedia jacksonii} was only identified from C3 turfgrasses, including species such as \textit{Agrostis stolonifera, F. rubra, Lolium perenne} and \textit{Poa}.

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pratensis (Table 1). Clarireedia 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 Sclerotinia homoeocarpa form a lineage 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.


**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 days 25 C under continuous light on PDA + ascorbic acid. Colonies > 15 days old do not form a dark stroma on PDA + ascorbic acid. Hyphae septate, hyaline. Apothecia 0.5 to 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 x 12.5 µm, on average (from Bennett 1937). Ascospores hyaline, oblong to elliptical, mostly unicellular, occasionally with a medium septum, 20.7 x 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 (http://dx.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;

Neotype hic designatus: United Kingdom: dried sterile apothecia produced on Festuca rubra seeds (Fig 5A-E), 1972, N. Jackson (BPI892697).


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 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 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 days 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 days 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 [http://dx.doi.org/10.15482/USDA.ADC/1429061], *C. bennettii* can be distinguished

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

Distribution: 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


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 years.

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 days at 25 C under continuous light on PDA + ascorbic acid and wheat meal agar. Colonies > 15 days 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 x 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 http://dx.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, 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 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 days at 25 C under continuous light on PDA + ascorbic acid and wheat meal agar.

Colonies > 15 days 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 http://dx.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 R. 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
\textit{Rutstroemia}, since CBS 464.73 and CBS 465.73 were identified in the CBS culture collection as \textit{R. paludosa} (Groves and Elliot 1961; synonyms \textit{Poculum paludosa}, \textit{Sclerotinia paludosa}; isolated from \textit{Symplocarpus foetidus}) and \textit{R. cunicularia} (Elliott 1967; synonym=\textit{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 \textit{Rutstroemia}, as they do not cluster or are associated with isolates of the type species for the genus \textit{Rutstroemia}, \textit{R. firma} (isolates CBS 115.86, CBS 341.62), but are aligned within \textit{Clarireedia}. This scenario is not unique in the relatively understudied \textit{Rutstroemiaceae}. Another example is found in the recent description of the species \textit{P. pseudosydowiana} in the genus \textit{Poculum} (Hosoya et al. 2014). Identification of \textit{P. pseudosydowiana} was largely based on ITS sequence similarity to isolates of \textit{R. sydowiana} CBS 115928 and CBS 115975 that were referred to by the synonym of \textit{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 \textit{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 \textit{Sclerotinia}, nor are they members of the \textit{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 \textit{C. homoeocarpa} relative to isolates of \textit{Lambertella}, \textit{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 to 81% similarity with *Clarireedia* isolates (data not shown). Similarly, *Clarireedia* isolates share just 82 to 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. bennetti*. 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 years 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.

Acknowledgements

This work was supported by USDA-ARS project 8042-22000-279-00D and the Center for Turfgrass Science, Rutgers University. We thank Shamal Budhdev for laboratory support; Christian Feuillet and Amy Rossman for assistance with the Latin language and botanical nomenclature; Stacy Bonos, Brad Hillman, William Meyer, and Tom Molnar for helpful naming discussions; Noel Jackson for sharing original research materials from his personal collection; and Manish Parashar, Moustafa AbdelBaky, Melissa Romanus, and Bryan Rabin for access to computing servers and technical assistance. This research was supported in part by an appointment of C. Salgado-Salazar to the Agricultural Research Service (ARS) Research.
Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and the USDA. ORISE is managed by ORAU under DOE contract number DE-AC05-06OR23100. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. USDA is an equal opportunity provider and employer.

References


Fenstermacher JM, 1970. Variation within *Sclerotinia homoeocarpa* F. T. Bennett [Master’s Thesis]. University of Rhode Island.


Phylogenetics and Evolution 41: 295–312.


List of Tables

Table 1. List of isolates used in the study.

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

Figure Legends

Figure 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).

Figure 2. Colony morphology of species in the genus *Clarireedia* at 8 days 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.

**Figure 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.

**Figure 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 ≥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.

**Figure 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.
Supplementary Figures.

**Supplementary Figure 1.** Majority rule Bayesian phylogenetic tree based on the CaM region analysis showing relationships among fungal isolates in the *Sclerotinia* and *Rutstroemia* families. Support values (posterior probability (PP) / maximum likelihood (ML)) are indicated above the branches. No number above the branches indicates that the clade/branch was not supported at values ≥0.95 PP / 70% bootstrap. *Monilinia vaccinii-corymbosi* was used as outgroup. Branch lengths are proportional to levels of sequence divergence.

**Supplementary Figure 2.** Majority rule Bayesian phylogenetic tree based on the ITS region analysis showing relationships among fungal isolates in the *Sclerotinia* and *Rutstroemia* families. Support values (posterior probability (PP) / maximum likelihood (ML)) are indicated above the branches. No number above the branches indicates that the clade/branch was not supported at values ≥0.95 PP / 70% bootstrap. *Monilinia vaccinii-corymbosi* was used as outgroup. Branch lengths are proportional to levels of sequence divergence.

**Supplementary Figure 3.** Majority rule Bayesian phylogenetic tree based on the Mcm7 region analysis showing relationships among fungal isolates in the *Sclerotinia* and *Rutstroemia* families. Support values (posterior probability (PP) / maximum likelihood (ML)) are indicated above the branches. No number above the branches indicates that the clade/branch was not supported at values ≥0.95 PP / 70% bootstrap. *Monilinia vaccinii-corymbosi* was used as outgroup. Branch lengths are proportional to levels of sequence divergence.
Supplementary Tables

Supplementary Table 1. Mating type crosses performed with *Clarireedia* MAT1-1 x MAT1-2 isolates. All crosses were made with each strain serving as both a donor and recipient.
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