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Porocercospora seminalis gen. et comb. nov., the causal organism of buffalograss false smut

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Abstract: False smut caused by *Cercospora seminalis* is an important disease of buffalograss (*Buchloë dactyloides*) affecting seed production. The pathogen prevents normal caryopsis development and causes considerable yield loss and reduced seed germination. The current taxonomic placement of the false-smut causal pathogen in the genus *Cercospora* is incorrect based on its morphological characteristics and DNA phylogeny. In the present study the phylogenetic position of *C. seminalis* is clarified based on DNA sequence analysis of three loci namely the internal transcribed spacer (ITS) region, partial nuclear ribosomal large subunit (LSU) and partial sequences of the RNA polymerase II second largest subunit (RPB2). A collection of *C. seminalis* isolates was made from buffalograss sites near Lincoln, Nebraska. DNA sequence data indicated that *Cercospora seminalis* is phylogenetically close to but distinct from species of *Bipolaris* and *Curvularia* (Pleosporaceae, Pleosporales). *Cercospora seminalis* morphologically had unique characteristics, namely densely aggregated and repeatedly branched conidiophores arising from a brown stroma, monotretic conidiogenous cells with inconspicuous loci, and scolecosporous conidia with distosepta, and thickened, darkened hila. *Porocercospora* is introduced as a new genus to accommodate the buffalograss false-smut pathogen.

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Key words: *Buchloë dactyloides*, *Cercospora seminalis*, false smut, multigene phylogeny, taxonomy

Introduction

Buffalograss (*Bouteloua dactyloides* [Nutt.] Columbus; syn. *Buchloë dactyloides* [Nutt.] Engelm.) is a low-maintenance turfgrass species with exceptional drought, heat and cold tolerance. It is native to the Great Plains of North America (Beetle 1950, Reeder 1971), having a geographic distribution from Mexico to Canada. The diversity of wild buffalograss can be leveraged to develop new turf-type cultivars to partially replace introduced turfgrass species especially in the semi-arid and arid regions of the United States (Riordan et al. 1993). Replacing non-native turfgrasses with turf-type buffalograss may conserve resources such as water, pesticides and fertilizer (Riordan et al. 1993).

Buffalograss is a cross-pollinated, dioecious turfgrass with high genetic and morphological variation. The female inflorescence usually consists of 1–3 spikes on a single short peduncle within two inflated sheaths of upper leaves. Each spike may contain up to five spikelets, which remain intact through ripening and falling. Spikelets and caryopses of buffalograss are enclosed inside a hard bur, which acts as a dispersal unit (Riordan and Browning 2003). Buffalograss can be propagated vegetatively by stolons, sod and plugs, or sexually by seed (Riordan et al. 1993, Wu and Lin 1994). Propagation by seed is relatively inexpensive compared to sod or stolons. However compared to other turfgrasses, buffalograss produces fewer seed heads (Riordan et al. 1993), resulting in lower seed yields.

Inherently low seed yield of buffalograss can be reduced further by false smut caused by *Cercospora seminalis* Ellis & Everh. (Gernert 1936, Weihing 1954). This disease first was reported in Manhattan, Kansas, by Ellis and Everhart (1888). *Cercospora seminalis* is an asexual fungus, which attacks the unfertilized ovaries of buffalograss (Gernert 1936, Weihing 1954) and reduces the germination percentage of burs. The mycelial mat growing inside the caryopsis emerges from the tips of the palea and forms a black, spongy spherical mass of hyphae atop the spike. Once the spike is dissected, infected blackened ovaries within the spikelets are visible. It is common to have only one ovary cavity of an

infected bur to be filled with the pathogen's mycelium. The fungal mass of an infected bur has the shape of a flask with a bulbous structure at the top. The structure acts as a stroma in bearing conidiophores and conidia at the top. The hyphae at the base of the mature stroma contain numerous terminal and intercalary chlamydospores. The pathogen has the ability to infect the host by conidia, hyphae and germinating chlamydospores.

The genus *Cercospora* represents one of the largest genera of hyphomycetes, with more than 3000 names, of which 659 were recognized as morphologically distinct by Crous and Braun (2003). Although *Cercospora* in the past has been linked to the teleomorph genus *Mycosphaerella* (Mycosphaerellaceae, Capnodiales; Stewart et al. 1999, Crous et al. 2000, Goodwin et al. 2001), the latter has been shown to be polyphyletic (Crous et al. 2007) and subsequently split into numerous genera, correlating with its different asexual genera (Crous et al. 2009a, b). The genus *Mycosphaerella* is now restricted to taxa that form *Ramularia* asexual morphs (Verkley et al. 2004) and in all probability will cease to be used in the future (Hawksworth et al. 2011, Wingfield et al. 2012) in that the genus *Ramularia* is well defined and recently has been monographed (Braun 1998). In contrast, *Mycosphaerella* stems from a confused concept harboring numerous unrelated taxa.

False smut of buffalograss is poorly studied. Studies were confined mainly to reporting disease symptoms and pathogen morphology (Ellis and Everhart 1888, Gernert 1936, Weihing 1954, Braun 1999). Since the mid-1950s no detailed studies have been done on *C. seminalis*. Furthermore, no molecular work has been performed to clarify its taxonomy. *Cercospora seminalis* cannot be accommodated in the genus *Cercospora* s. str. (Groenewald et al. 2013) in that it has pigmented conidia resembling *Pseudocercospora* (Crous et al. 2013). It is distinct from *Pseudocercospora*, however, in that it has a different mode of conidiogenesis, with conidiogenous cells having inconspicuous monotretic pores and conidia having hila that are thickened, darkened with a central pore. *Cercospora seminalis* also has tretic conidiogenous cells and similar conidia are produced by many fungal genera with diverse phylogenetic affinities (Seifert et al. 2011, Manamgoda et al. 2012). Therefore, DNA sequence data must be used to assess the definitive taxonomic placement of *C. seminalis*. In an effort to improve our understanding of the taxonomy of this pathogen, we used multilocus DNA sequence data to assess the phylogenetic placement of a set of *C. seminalis* isolates recently obtained from buffalograss near Lincoln, Nebraska.

MATERIALS AND METHODS

Isolate collection and culturing techniques.—False smut-infected buffalograss burs were collected from six sites in eastern Nebraska during summer 2011. All sites were either commercial or experimental buffalograss fields with no history of fungicide application. Five fields were in the Mead area of Saunders County, while the sixth field was in Waverly, Lancaster County. Pure cultures of *C. seminalis* were isolated with both single-spore cultures and pieces of stromata according to the following methods. Infected burs were dissected and flask-shaped stromata were removed. Several spherical masses on the top of the stromata were decapitated and crushed in 100 µL water to extract conidia. The concentrated spore solution was diluted 10 × with water and 60 µL were spread on a large (9 cm diam) Petri dish containing 0.25 × potato dextrose agar (PDA; according to Crous et al. 2009c). When single-spore colonies started to appear, they were transferred to fresh PDA plates. For the second method, the mycelial structures of stromata without the spherical head were surface-sterilized by placing them in 85% ethanol for 1 min and transferred to 2% tap water agar. After 10 d growth, a piece of mycelial mat from each plate was transferred to a fresh PDA plate to obtain pure cultures. Colony growth of *C. seminalis* were determined by incubating five isolates on large Petri plates with PDA at room temperature (21–22 C) and recording numbers of days needed for the mycelium mats to reach the edge of the plates. A total of 86 isolates was collected from the six fields, and the number of isolates representing each field varied 11–16. An arbitrary selection of eight isolates was made for DNA sequence analyses. Vials of sterile wheat seeds were inoculated with *C. seminalis* cultures for long-term preservation. Once isolates had fully colonized the substrate, 15% glycerol was added to the vials and they were stored at −75 C for later use.

Pathogenicity.—Because cultures on PDA medium did not produce conidia, a pathogenicity test was done with isolates 6B_Bs and 109_Bs to verify their ability to cause false smut. A solution of finely blended mycelial mat was thoroughly sprayed onto female buffalograss flowers. The treated buffalograss pots were covered with clear plastic bags to provide high humidity. After 2 d bags were removed and buffalograss pots were maintained in a greenhouse 3 wk before harvesting. The plants were grown under long daylight (16 h) in the greenhouse with daytime and nighttime temperature of 25–30 C and 20–22 C respectively. Harvested burs were examined for the disease by dissection. The black stromata formed in the infected caryopses were transferred to water agar plates for re-isolation of the pathogen to fulfill Koch's postulates.

DNA sequence analyses.—Genomic DNA was extracted from mycelium of eight representative isolates growing on MEA with the UltraClean® Microbial DNA Isolation Kit (Mo Bio Laboratories Inc., Solana Beach, California). Amplification and sequencing of the internal transcribed spacer (ITS) region and partial nuclear ribosomal large subunit (LSU) and RNA polymerase II second largest subunit (RPB2)

were done with primers V9G (de Hoog and Gerrits van den Ende 1998) + ITS4 (White et al. 1990), LR0R + LR5 (Vilgalys and Hester 1990) and 5F2 + 7cR (O'Donnell et al. 2007) respectively. PCR was performed in a 2720 thermalcycler (Applied Biosystems, Foster City, California) in a total volume of 12.5 µL. The PCR mixtures contained 1 µL diluted genomic DNA, 0.2 µM each primer, 1 × colorless GoTaq Flexi Buffer (Promega, Mannheim, Germany), 1 mM (ITS and LSU) or 2 mM (RPB2) $MgCl_2$, 20 µM each dNTP and 0.25 U GoTaq DNA polymerase (Promega). Conditions for amplification of ITS and LSU were an initial denaturation step of 5 min at 94 C followed by 35 cycles of 45 s at 94 C, 45 s at 48 C and 2 min at 72 C and a final extension step of 7 min at 72 C. The PCR program of RPB2 included an initial denaturation step of 5 min at 95 C followed by five cycles of 45 s at 95 C, 45 s at 60 C and 2 min at 72 C; followed by five cycles with an annealing temperature at 58 C and 30 cycles at 54 C, finalized with an extension for 8 min at 72 C. PCR products were sequenced with the same primers used for amplification following application of the BigDye Terminator Cycle Sequencing Kit 3.1 (Applied Biosystems). The software SeqMan Pro 9.0.4 (39), 418 (Lasergene, Madison, Wisconsin) was used to obtain consensus sequences from the complementary sequences of each isolate. BLAST queries (Altschul et al. 1990) were performed to compare data of the isolates studied with those of other fungi deposited in GenBank. Nucleotide sequence alignments were performed with Clustal X 1.81 (Thompson et al. 1997), followed by manual adjustments with a text editor. Incongruence among datasets was tested with the partition homogeneity test (PHT) (Farris et al. 2005) as implemented in PAUP 4.0b10 (Swofford 2003). The concatenated dataset was analyzed with maximum likelihood (ML) with gamma model of rate heterogeneity using the RAxML BlackBox online server (http://phylobench.vital-it.ch/raxml-bb/). The maximum likelihood search option was used to find the best-scoring tree after bootstrapping. By default, the RAxML BlackBox calculates statistical support for branches by rapid bootstrap analyses of 100 replicates (Stamatakis et al. 2008). Bootstrap support (bs) values ≥ 70% were considered significant. A Markov chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities with MrBayes 3.1.1 (Ronquist and Huelsenbeck 2003). The best models of nucleotide substitution for each locus were determined with MrModeltest 2.3 (Nylander 2004). Two analyses of four MCMC chains were run from random trees for 100 000 000 generations and sampled every 1000 generations, resulting in at least 100 000 trees, of which 25% were discarded as burn-in. Posterior probabilities (pp) were determined from the remaining trees. The sequences generated in this study and the alignments used in the phylogenetic analyses were deposited in GenBank (TABLE I) and TreeBASE (Submission ID 14026), respectively.

Morphology.—Morphological examination was based on fungal cultures sporulating in vitro on sterilized burs of *Bouteloua dactyloides* placed on synthetic nutrient-poor agar (SNA; according to Crous et al. 2009c) and incubated under continuous near-ultraviolet light 2–4 wk. Wherever possible, 30 measurements (1000 × magnification) were made of structures mounted in clear lactic acid, with the extremes of spore measurements in parentheses. Colony colors (surface and reverse) were assessed after 2 wk on MEA, PDA and OA at 21–22 C in the dark. Colony and conidial color were determined with the charts of Rayner (1970).

RESULTS

DNA sequence analysis.—Sequences of the ITS, LSU and RPB2 loci were identical for the eight isolates studied. BLAST queries revealed that *Cercospora seminalis* is relatively close to species of *Curvularia* and *Bipolaris* (Pleosporaceae, Pleosporales). Sequence identities of the closest matches were ≤ 91% for ITS (e.g. *Curvularia* sp. GenBank HM371207, identities = 574/634 [91%] and *C. inaequalis* GenBank HM101095, identities = 558/627 [89%], ≤ 99% for LSU [e.g. *Bipolaris microstegi* GenBank JX100808], identities = 884/893 [99%] and *Curvularia hawaiiensis* GenBank JN941532, identities 879/894 [98%] and ≤ 92% for RPB2 [e.g. *Bipolaris maydis* GenBank DQ247790, identities 793/866 [92%] and *Bipolaris sorokiniana* DQ677939, identities 788/863 [91%]). In the phylogenetic study, 11 species of *Curvularia*, eight of *Bipolaris*, four of *Exserohilum* and one of *Pyrenophora* (designated outgroup) were included (TABLE I). The latter two genera were considered in the analysis because they are morphologically similar and phylogenetically close to members of the *Bipolaris-Curvularia* complex (Sivanesan 1987, Berbee et al. 1999).

After removing ambiguously aligned regions, we obtained RPB2, ITS and LSU alignments of 855, 499 and 853 positions of which 278 (32.5%), 56 (11.2%) and 29 (3.4%) were variable respectively. The result of the PHT test ($P = 0.67$) indicated that the three datasets were congruent and therefore could be combined. For Bayesian analysis, a SYM + I + G model was proposed by MrModeltest for RPB2 and ITS, and GTR + I for LSU. These models were incorporated in the analysis. The consensus tree obtained from the Bayesian analysis agreed with the topology of the best-scoring ML tree for the concatenated three-locus dataset (FIG. 1). These trees revealed three main lineages representing (i) *Exserohilum* (96% bs/0.97 pp), (ii) *Pyrenophora* and (iii) *C. seminalis/Bipolaris/Curvularia* (98%/0.99). In the latter clade there were three main strongly supported subclades, composed of *Curvularia* spp. (100%/0.99), *Bipolaris* spp. (98%/0.99) and *C. seminalis* (100%/0.99) respectively. *Cercospora seminalis* and *Bipolaris* appeared as sister groups. The internode that related them had 60% bs and 0.96 pp. These groups were separated by a

TABLE I. Isolates included in phylogenetic the study, their origins and GenBank accession number

Taxon	Isolate nos.	Source	GenBank accession no.		
			ITS	LSU	RPB2
Bipolaris chloridis	CBS 242.77B	*Chloris gayana*, Australia	HF934928	HF934869	HF934830
Bipolaris cynodontis	CBS 285.51	*Cynodon transvaalensis*, Kenya	HF934929	HF934874	HF934831
	CBS 305.64	*Cynodon dactylon*, USA	HF934930	HF934883	HF934832
Bipolaris maydis	CBS 130.26	Unknown	HF934923	HF934873	HF934825
	CBS 136.29	*Zea mays*, Japan	HF934926	HF934879	HF934828
	CBS 307.64	*Zea mays*, USA	HF934925	HF934875	HF93482
	CBS 573.73	*Zea mays* leaf, USA	HF934924	HF934881	HF934826
Bipolaris microlaenae	CBS 280.91^{T}	*Microlaena stipoides* leaf, Australia	HF934933	HF934877	HF934835
Bipolaris oryzae	CBS 157.50	*Oryza sativa* grain, Indonesia	HF934931	HF934870	HF934833
	CBS 199.54	*Oryza sativa* grain, New Guinea	HF934932	HF934884	HF934834
Bipolaris sorghicola	CBS 249.49	*Sorghum vulgare* var. *sudanense*, Locality unknown	HF934927	HF934868	HF934829
Bipolaris sorokiniana	CBS 140.31	Substrate unknown, Japan	HF934935	HF934876	HF934837
	CBS 145.32	*Triticum durum*, Locality unknown	HF934934	HF934885	HF934836
Bipolaris sorokiniana	CBS 148.56	Substrate unknown, USA	HF934937	HF934872	HF934839
	CBS 149.56	Substrate unknown, USA	HF934936	HF934880	HF934838
Bipolaris zeicola	CBS 316.64	*Zea mays*, USA	HF934938	HF934871	HF934840
	CBS 317.64	*Zea mays*, USA	HF934939	HF934878	HF934841
	CBS 247.49	*Zea mays*, Locality unknown	HF934940	HF934882	HF934842
Curvularia aeria	CBS 294.61^{T}	Air, Brazil	HF934910	HF934902	HF934812
Curvularia akaii	CBS 318.86	Substrate unknown, Japan	HF934921	HF934897	HF934823
	CBS 127728	Substrate unknown, Japan	HF934920	HF934898	HF934822
	CBS 127730	Substrate unknown, Japan	HF934922	HF934899	HF934824
Curvularia australiensis	CBS 172.57	*Oryza sativa* seed, Vietnam	HF934912	HF934901	HF934814
Curvularia coicis	CBS 192.29^{T}	*Coix lacrima-jobi* var. *typica*, Japan	HF934917	HF934895	HF934819
Curvularia ellisii	CBS 193.62	Air, Pakistan	HF934913	HF934896	HF934815
Curvularia heteropogonis	CBS 284.91^{T}	*Heteropogon contortus* leaf, Australia	HF934919	HF934893	HF934821
	CBS 511.91	*Heteropogon contortus* leaf, Australia	HF934918	HF934894	HF934820
Curvularia lunata	CBS 730.96^{NT}	Lung biopsy, USA	HF934911	HF934900	HF934813
Curvularia oryzae	CBS 169.53^{T}	*Oryza sativa* seed, Vietnam	HF934906	HF934867	HF934808
Curvularia spicifera	CBS 198.31	*Capsicum anuum*, Cyprus	HF934916	HF934905	HF934818
	CBS 199.31	*Cucurbita maxima*, Cyprus	HF934915	HF934903	HF934817
	CBS 246.62	Cotton pulp, Scotland	HF934914	HF934904	HF934816
Curvularia tuberculata	CBS 146.63^{T}	*Zea mays* leaf, India	HF934907	HF934866	HF934809
Curvularia verruculosa	CBS 149.63^{T}	*Elaeis guineensis*, Nigeria	HF934909	HF934891	HF934811
	CBS 150.63	*Punica granatum* leaf, India	HF934908	HF934892	HF934810
Drechslera graminea	CBS 280.31	*Hordeum vulgare*, Locality unknown	HF934954	HF934857	HF934856
Exserohilum monoceras	CBS 209.78	*Echinochloa colonum*, Australia	HF934951	HF934888	HF934853
Exserohilum oryzicola	CBS 502.90^{T}	*Oryza sativa* leaf, Colombia	HF934949	HF934886	HF934851
Exserohilum pedicellatum	CBS 322.64	*Triticum aestivum*, USA	HF934952	HF934889	HF934854
	CBS 375.76	*Oryza sativa*, Turkey	HF934953	HF934890	HF934855
Exserohilum turcicum	CBS 330.64	*Zea mays*, USA	HF934950	HF934887	HF934852
Porocercospora seminalis gen. et comb. nov.	CBS 134906^{ET} (= CPC 21305)	*Bouteloua dactyloides*, Nebraska, Mead, site 3 USA	HF934942	HF934865	HF934847
	CPC 21329	*Bouteloua dactyloides*, Nebraska, Mead, site 3 USA	HF934943	HF934858	HF934848
	CPC 21330	*Bouteloua dactyloides*, Nebraska, Mead, site 3 USA	HF934948	HF934863	HF934849
	CBS 134907 (= CPC 21332)	*Bouteloua dactyloides*, Nebraska, Mead, site 3 USA	HF934941	HF934862	HF934843

TABLE I. Continued

Taxon	Isolate nos.	Source	GenBank accession no.		
			ITS	LSU	RPB2
	CPC 21333	*Bouteloua dactyloides*, Nebraska, Mead, site 3 USA	HF934946	HF934859	HF934850
	CPC 21336	*Bouteloua dactyloides*, Nebraska, Mead, site 4 USA	HF934947	HF934864	HF934844
	CPC 21349	*Bouteloua dactyloides*, Nebraska, Waverley, site 6 USA	HF934945	HF934861	HF934845
	CBS 134908 (= CPC 21350)	*Bouteloua dactyloides*, Nebraska, Waverley, site 6 USA	HF934944	HF934860	HF934846

All sequences were generated during this study. [T] ex-type strain, [ET] ex-epitype strain, [NT] ex-neotype strain (Manamgoda et al. 2012), CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; CPC: culture collection of Pedro Crous, housed at CBS.

considerable genetic distance, which suggested that they are different genera. The combined dataset confirmed that *C. seminalis* is relatively close to the genera *Bipolaris* and *Curvularia* but represents a distinct, novel lineage of pleosporalean fungi.

Pathogenicity.—The artificially infected plants had black mycelial structures (stromata) in dissected burs. Few burs showed the black spongy bulbous top characteristic of natural false-smut disease. On average, 25–30% harvested burs were diseased. The control plants did not have any burs with disease symptoms either superficially or after dissection. The black stromatal pieces cultured on PDA produced a mycelial mat having macroscopic and microscopic features of the original isolate used for buffalograss inoculation. The bulbous tops contained conidia, which were similar morphologically to those in naturally infected burs.

Taxonomy.—Because of its pigmented conidia with thickened hila, the buffalograss false-smut pathogen cannot be accommodated in *Cercospora* or *Pseudocercospora* (Capnodiales). Furthermore, the presence of central monotretic pores on its conidiogenous cells and distoseptate, elongate conidia confirm the clear relationship to *Curvularia/Bipolaris* (Pleosporales) as revealed by DNA sequence data. Based on the multigene dataset (FIG. 1) and differences observed in morphology, *Cercospora seminalis* is allocated to a new genus.

Porocercospora Amaradasa, Amundsen, Madrid & Crous, gen. nov. FIG. 2
MycoBank MB803981

Conidiophores densely aggregated, arising from brown stroma. Conidiophores intermingled among hyphae, subcylindrical, medium brown (umber sensu Rayner 1974), smooth to finely verruculose, branched above, thin-walled, septate. Conidiogenous cells subcylindrical, medium brown (umber sensu Rayner 1974), smooth to finely verruculose, apex rounded, monotretic, with a central pore, indistinct, not darkened or thickened. Conidia solitary, medium brown (umber sensu Rayner 1974), thick-walled, finely verruculose, obclavate to cylindro-obclavate, with short conidia obovoid to subcylindrical, transversely multi-distoseptate; apex subobtuse, base obconically truncate, with hila having a distinct central brown pore, thickened and darkened.

Etymology: Named after the typical pores present in its conidiogenous cells and its morphological similarity to the genus *Cercospora*.

Type species: Porocercospora seminalis (Ellis & Everh.) Amaradasa, Amundsen, Madrid & Crous

Porocercospora seminalis (Ellis & Everh.) Amaradasa, Amundsen, Madrid & Crous, comb. nov. FIG. 2
MycoBank MB803982

Basionym. Cercospora seminalis Ellis & Everh., J. Mycol. 4(1):4 (1888).

≡ *Sporidesmium seminale* (Ellis & Everh.) U. Braun, Cryptog. Mycol. 20(3):175 (1999).

On sterile Buchloë dactyloides seeds on SNA. Conidiophores densely aggregated in brown stroma, enclosed by spines of involucre, forming a black, spongy spherical mass of hyphae atop spike. Conidiophores intermingled among hyphae, subcylindrical, medium-brown, smooth to finely verruculose, branched above, thin-walled, non-geniculate, 1–10-septate, up to 500 μm long, 5–8 μm diam. Conidiogenous cells subcylindrical, medium brown (umber sensu Rayner 1974), smooth to finely verruculose, apex rounded, monotretic, with a central pore, indistinct, not darkened or thickened, 20–30 × 5–7 μm. Conidia solitary, medium brown (umber sensu Rayner 1974),

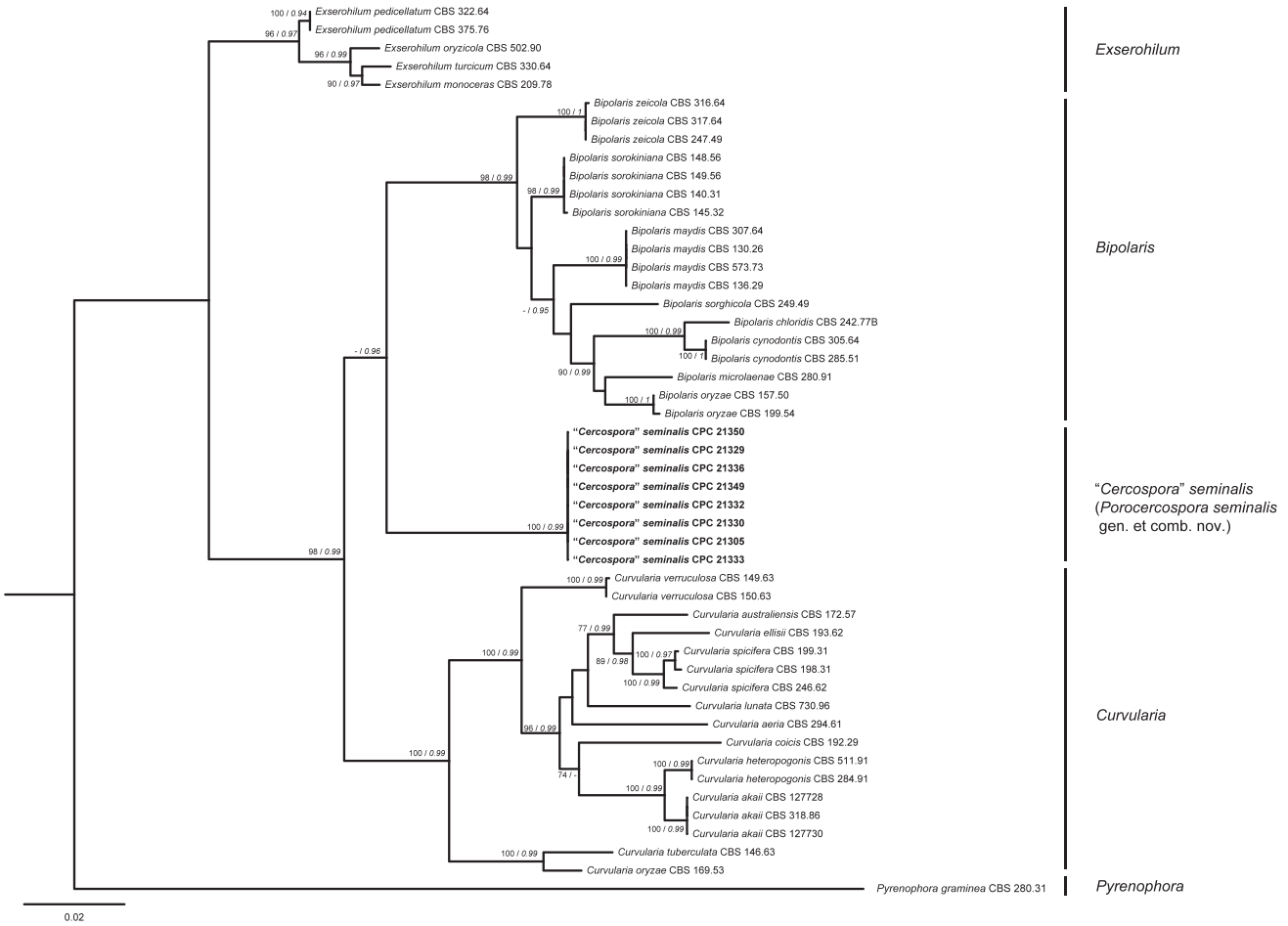

FIG. 1. Best-scoring maximum likelihood tree obtained from the combined RPB2, ITS and LSU dataset of *Porocercospora seminalis* and related taxa. Bootstrap values ≥ 70% and posterior probabilities ≥ 0.95 (in italics) are near the internodes.

thick-walled, finely verruculose, obclavate to cylindro-obclavate, with short conidia obovoid to subcylindrical, 0–12-distoseptate, but some appearing eusepta; apex subobtuse, base obconically truncate, with hila having a distinct central brown pore (1 μm diam), thickened, darkened, (20–)60–90(–160) × (6–)7–8(–11) μm.

Culture characteristics: Colonies on PDA produced an olivaceous green to black, appressed mycelial mat toward periphery of plate. White aerial hyphae dominated center of plate. Growth 2.5–3.3 cm/wk. Conidia not produced. Hyphae developing numerous septa with age; terminal, intercalary chlamydospores in old cultures. Chlamydospores globose to cylindrical, solitary or arranged in chains.

Specimens examined: USA, KANSAS: Manhattan, on seeds of *Buchloë dactyloides*, Jul 1887, *W.T. Swingle*, (HOLOTYPE NY); NEBRASKA: Mead, on seeds of *B. dactyloides*, 5 Sep 2011, *B.S. Amaradasa*, (EPITYPE designated here, CBS H-21149, culture ex-epitype CBS 134906 = CPC 21305 = 21_BS).

DISCUSSION

False smut is a serious disease of buffalograss that limits its seed production, and an improved understanding of the pathogen thus might help buffalograss breeders identify sources of host resistance and improved methods of disease control. Little research has been done on false smut since the 1950s, and its biology remains largely unknown.

As mentioned previously, conidiogenesis and conidial morphology of *P. seminalis* clearly differ from that of *Cercospora* s.str. Based on its pigmented structures and unique mode of conidiogenesis, Braun (1999) placed this fungus in *Sporidesmium*. This genus, however, later proved to be a heterogeneous assemblage of taxa belonging to different orders of Dothideomycetes and Sordariomycetes (Shenoy et al. 2006). Although the phylogenetic position of its type species, *S. atrum*, is unknown, the genus currently is circumscribed as having species with simple or

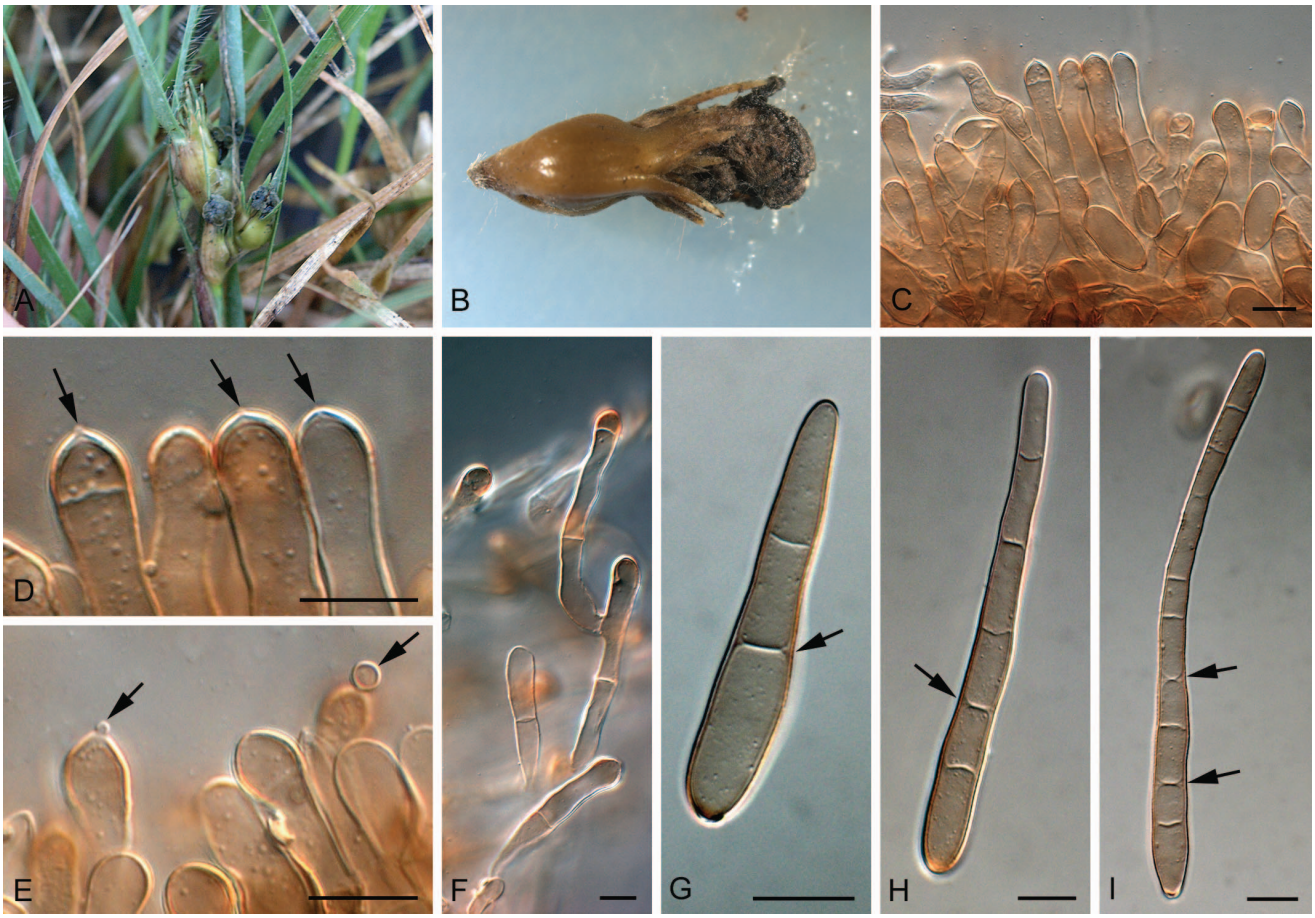

FIG. 2. Macroscopic and microscopic images of the false-smut pathogen in vivo and in vitro. A. Buffalograss burs infected by *Porocercospora seminalis*. B. Black stroma with spongy spherical top. C–E. Aggregated monotretic conidiogenous cells with inconspicuous loci (arrows). F. Branched conidiophore. G–I. Conidia with arrows indicating distosepta (other septa are less clearly distoseptate, almost appearing to be eusepta).

sparingly branched conidiophores, monoblastic (but not tretic) conidiogenous cells with wide scars and euseptate conidia (Seifert et al. 2011). These morphological features clearly differ from those of *P. seminalis*, and thus *Sporidesmium* is not a suitable genus for the buffalograss false-smut pathogen.

This study was aimed mainly at enhancing our understanding of the phylogenetic relationships of the false-smut causal agent. No prior investigations used molecular techniques to assess the phylogenetic position of *P. seminalis*. To resolve the phylogenetic relationship of *P. seminalis* we generated trees based on RPB2, LSU and ITS DNA sequence data (FIG. 1). These data clearly revealed that the buffalograss false-smut pathogen was unrelated to cercosporoid fungi (Capnodiales; Crous et al. 2013, Groenewald et al. 2013) but proved to be phylogenetically close to four fungal genera that produce tretic conidiogenous cells and elongate, distoseptate conidia (i.e. *Bipolaris*, *Curvularia*, *Exserohilum* and *Pyrenophora*). The buffalograss false-smut fungus is distinguished from the latter four genera (Sivanesan 1987, Manamgoda et al. 2012) by its aggregated conidiophores arising from a brown, spongy stroma, lacking a conspicuous geniculate rachis, and its inconspicuous (non-darkened) monotretic conidiogenous loci. Furthermore, conidia of *Bipolaris, Exserohilum* and *Pyrenophora* are usually conspicuously distoseptate (two conidial cell walls are clearly visible and the septa originate from the inner one) and possess a protuberant hilum in *Exserohilum* (Sivanesan 1984, 1987).

Based on an analysis of the RPB2, LSU and ITS sequence data generated for the eight isolates collected in Nebraska (TABLE I), no variation was observed, suggesting that *P. seminalis* is a well defined species. Further studies incorporating more loci or DNA fingerprinting techniques like AFLPs should be employed to study variation at the population level. These studies ideally also should include collections from other states in USA, covering the native range of buffalograss. Because both *Curvularia* and *Bipolaris*

species cause leaf spot on buffalograss (Sivanesan 1987), identification of *P. seminalis* as a close relative provides important information for plant breeders tasked with developing new turf-type cultivars resistant to these pathogens. Future studies should be directed toward understanding whether buffalograss host resistance is similar among these diseases.

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