UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PROGRAMA DE PÓS-GRADUAÇÃO EM FITOPATOLOGIA

Tese de Doutorado

Espécies de *Phaeoacremonium* e *Campylocarpon* associadas ao declínio da videira no Nordeste brasileiro

Marcondes Araújo da Silva

Recife – PE
2014

MARCONDES ARAÚJO DA SILVA

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Tese apresentada ao Programa de Pós-Graduação em Fitopatologia da Universidade Federal Rural de Pernambuco, como parte dos requisitos para obtenção do título de Doutor em Fitopatologia.

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Tese defendida e aprovada pela Banca Examinadora em: 30/07/2014.

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RECIFE – PE
JULHO – 2014

“*O Senhor é o meu pastor e nada me faltará*”

Salmo 23:1

Aos meus pais Laura Brotas e Aprígio Ferreira, aos meus irmãos e sobrinhos e a minha querida Jéssica Rodrigues, pelo amor e carinho, meus eternos agradecimentos.

DEDICO

Ficha catalográfica

S586e Silva, Marcondes Araújo da
Espécies de *Phaeoacremonium* e *Campylocarpon* associadas ao declínio da videira no nordeste brasileiro / Marcondes Araújo da Silva. – Recife, 2014.
91 f. : il.

Orientador: Sami Jorge Michereff.
Tese (Doutorado em Fitopatologia) – Universidade Federal Rural de Pernambuco, Departamento de Agronomia, Recife, 2014.
Referências.

1. *Campylocarpon* 2. *Phaeoacremonium 3. Phaeomoniella* 4. Filogenia 5. Patogenicidade 6. *Vitis vinifera* I. Michereff, Sami Jorge, orientador II. Título

CDD 632

AGRADECIMENTOS

A Deus por estar sempre presente na minha vida, dando força pra sempre seguir em frente em busca de novos horizontes.

À minha família pelo amor, incentivo e apoio durante toda minha trajetória de vida.

Ao professor Dr. Sami Jorge Michereff pela orientação, ensinamentos, confiança e respeito.

Ao professor Dr. Marcos Paz Saraiva Câmara pela orientação e conselhos.

À Universidade Federal Rural de Pernambuco (UFRPE) pela oportunidade da realização do Curso de Doutorado.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE), pelas concessões das bolsas de estudo.

À Embrapa Semi-Árido, especialmente à Drª. Maria Angélica Guimarães Barbosa por todo apoio para realização de parte das coletas.

A todos os viticultores que autorizaram o acesso às áreas de coleta.

À Coordenação e professores do Programa de Pós-Graduação em Fitopatologia, pelos ensinamentos recebidos.

Aos funcionários do Programa de Pós-Graduação em Fitopatologia pelas contribuições necessárias.

A todos os colegas do Programa de Pós-Graduação em Fitopatologia pela convivência.

Aos integrantes dos Laboratórios de Epidemiologia de Doenças de Plantas e do Laboratório de Fungos Fitopatogênicos por sempre estarem dispostos a ajudar.

Aos amigos de república desde a graduação Toni Carvalho e Diego Loiola.

A todos que contribuíram para a realização deste trabalho.

SUMÁRIO

RESUMO GERAL

Nos últimos anos, um declínio de videiras tem sido relatado em parreiras do Nordeste brasileiro e recentemente foi relatada a ocorrência das doenças de Petri e pé-preto. Embora os impactos dessas doenças ainda não tenham sido calculados, produtores têm relatado perdas, principalmente devido aos custos com replantio nas áreas infestadas. Diante disso, os objetivos dessa tese foram: a) identificar as espécies de *Phaeoacremonium* (*Pm.*) e *Campylocarpon* (*Campyl.*) associadas, respectivamente, à doença de Petri e ao pé-preto no Nordeste brasileiro utilizando características morfológicas e análises filogenéticas; b) investigar a prevalência e a distribuição das espécies de *Phaeoacremonium* e *Campylocarpon* nessa região; e c) avaliar a patogenicidade e a virulência das espécies de *Phaeoacremonium* e *Campylocarpon* em ramos destacados de videira. Amostras de plantas de videira com sintomas de declínio foram coletadas em 2012 em viveiros, plantações jovens e parreirais adultos localizados nos vales do São Francisco (BA e PE), Siriji (PE) e Baixo Jaguaribe (CE). As amostras foram submetidas ao isolamento dos fungos e estes foram identificados por métodos morfológicos e moleculares. Baseado nas características morfológicas e nas análises de sequências parciais dos genes β-tubulina (BT) e actina (ACT) foram identificadas três espécies de *Phaeoacremonium* associadas à doença de Petri, sendo duas previamente descritas como *Pm. aleophilum* e *Pm. parasiticum*, e uma nova espécie denominada *Pm. nordesticola* sp. nov. *Phaeoacremonium aleophilum* foi a espécie mais prevalente nas áreas com sintomas da doença de Petri. Todas as espécies de *Phaeoacremonium* foram patogênicas em ramos destacados de videira, sendo *Pm. aleophilum* a espécie mais virulenta. A identificação dos fungos responsáveis pelo pé-preto foi realizada pela combinação da morfologia com análise filogenética baseada na região do espaço transcrito interno (ITS) do rDNA. Foram identificadas três espécies de *Campylocarpon* associadas ao pé-preto, sendo duas previamente descritas como *Campyl. fasciculare* e *Campyl. pseudofasciculare*, e uma nova espécie denominada *Campyl. semiaridus* sp. nov. *Campylocarpon pseudofasciculare* foi a espécie mais prevalente nas áreas com sintomas de pé-preto. Todas as espécies de *Campylocarpon* foram patogênicas em ramos destacados de videira e não houve diferença na virulência entre as espécies.

Palavras-chave: *Vitis vinifera*, *Vitis labrusca, Campylocarpon, Phaeoacremonium,* filogenia, virulência.

GENERAL ABSTRACT

In the last years, a decline of table grape plants has been noticed in vineyards of the Northeastern Brazil, and recently was reported the occurrence of Petri and black foot diseases. Although the impacts of these diseases have not yet been calculated, producers have reported losses, mainly due to the cost of replanting the infested areas. Therefore, the objectives of this thesis were: a) to identify the species of *Phaeoacremonium* (*Pm.*) and *Campylocarpon* (*Campyl.*) associated with Petri disease and black foot disease of table grapes in Norteastern Brazil, using morphological characteristics and phylogenetic analysis; (b) to investigate the distribution of the *Phaeoacremonium* and *Campylocarpon* species in this region; and (c) to evaluate the pathogenicity and virulence of *Phaeoacremonium* and *Campylocarpon* species in excised green shoots of table grapes. Samples of grapevine plants with symptoms of decline were collected in 2012 in nurseries, young and adult vineyard located in the San Francisco (BA and PE), Siriji (PE) and Baixo Jaguaribe (CE) Valleys. The samples were subjected to the isolation of associated fungi and theses were identified by morphological and molecular methods. Based on morphological characteristics and analysis of partial sequences of the β-tubulin (BT) and actin (ACT) genes three species of *Phaeoacremonium* associated with Petri disease were identified, two previously described as *Pm. aleophilum* and *Pm. parasiticum*, and a new species named *Pm. nordesticola* sp. nov. *Phaeoacremonium aleophilum* was the most prevalent species in areas with Petri disease symptoms. All *Phaeoacremonium* species were pathogenic on detached grapevine branches and *Pm. aleophilum* was the most virulent species. The identification of the fungi responsible for black foot was performed by the combination of morphology with phylogenetic analysis based on the internal transcribed space (ITS) of rDNA region. Three species of *Campylocarpon* associated with black foot were identified, two previously described as *Campyl. fasciculare* and *Campyl. pseudofasciculare*, and a new species called *Campyl. semiaridus* sp. nov. *Campylocarpon pseudofasciculare* was the most prevalent species in areas with symptoms of black foot. All *Campylocarpon* species were pathogenic on detached grapevine shoots and there were no difference in virulence among species.

Key-words: *Vitis vinifera*, *Vitis labrusca Campylocarpon, Phaeoacremonium,* phylogeny, virulence.

Capítulo I

Introdução Geral

ESPÉCIES DE *PHAEOACREMONIUM* E *CAMPYLOCARPON* ASSOCIADAS AO DECLÍNIO DA VIDEIRA NO NORDESTE BRASILEIRO

INTRODUÇÃO GERAL

1. A videira

O cultivo da videira (*Vitis vinifera* L.) é muito antigo. Registros fósseis encontrados na Turquia mostraram que a viticultura era praticada há cerca de 3.500 anos a.C. (LEÃO; POSSÍDIO, 2000). Esse cultivo teve início na Ásia Menor, na região entre os mares Negro e Cáspio, acreditando-se ser essa região o berço dessa espécie, da qual a maioria das variedades cultivadas se originaram. Com o passar do tempo, a videira foi difundida e se adaptam a diversas regiões do globo terrestre (HIDALGO, 2002; LEÃO; POSSÍDIO, 2009).

As várias conquistas de novos territórios pelo Império Romano possibilitaram a difusão da viticultura pelos países europeus, sendo introduzida na Espanha, França e Portugal, cerca de 500 a.C., pelas Ilhas das Canárias e da Madeira, de onde foi trazida ao Brasil. A introdução no Brasil foi feita pelos colonizadores portugueses, datando do ano de 1532 na Capitania de São Vicente, hoje Estado de São Paulo. Três anos após a introdução no País, foram levados alguns exemplares para a região do Nordeste brasileiro, mais especificamente para os estados da Bahia e de Pernambuco pela expedição de Duarte Coelho, alcançando algum progresso nas ilhas de Itaparica no estado da Bahia e de Itamaracá, no estado de Pernambuco (LEÃO, 2001).

A viticultura como atividade comercial relevante no Brasil teve início em 1875, com a colonização italiana no estado do Rio Grande do Sul. No início, a produção de uva e vinho era destinada apenas ao consumo regional e, posteriormente, foi expandindo-se gradativamente até atingir o mercado nacional, obtendo-se grande impulso de produção na década de 1970 com o cultivo da uva “Itália”. Desde então, a viticultura tem tido grande incremento na produção, principalmente em regiões onde as condições climáticas são favoráveis (ROSA; SIMÕES, 2004).

A videira tem sido considerada como uma planta adaptada a regiões de clima temperado pelo fato de ter folhas decíduas. No entanto, é cultivada atualmente em enorme diversidade de condições climáticas, a exemplo dos desertos da Califórnia (USA) e no Vale

do Submédio São Francisco, onde temperaturas muito elevadas são comuns. No Brasil, a videira é cultivada desde o extremo Sul, principalmente Rio Grande do Sul e Santa Catarina, até o Nordeste, em regiões anteriormente consideradas climaticamente inaptas. Com o emprego da irrigação, o Vale do Submédio São Francisco, localizado na região semiárida dos estados da Bahia e de Pernambuco, tornou-se excelente região produtora de uva (PEDRO JÚNIOR; SENTELHAS, 2003), destacando-se pela expansão da área cultivada, volume de produção e, principalmente, pelos altos rendimentos alcançados (30 t/ha/ano) e qualidade da uva produzida (SILVA; COELHO, 2010; SILVA; CORREIA, 2000).

A videira é uma planta de porte arbustivo, lenhosa sarmentosa e trepadeira perene, que se apoia e se fixa a tutores naturais ou artificiais, mediante caules modificados (gavinhas). Nas partes opostas às gavinhas, ocorrem às emissões dos primórdios de inflorescências (RAVEN; EVERT; EICHHORN, 2007). Suas bagas são reunidas em cachos, que são formados pelo pedúnculo e pelas ramificações que correspondem ao engaço, cujas extremidades são denominadas pedicelos, nos quais estão presas as bagas. A parte do pedicelo que penetra na baga é denominada pincel. As bagas são constituídas pela película que contém a parte pigmentada e é revestida de uma substância serosa denominada pruína, impermeável à água (CLANCY, 2001). Este arbusto possui alta longevidade, podendo superar em alguns casos os 100 anos de vida (RAVEN; EVERT; EICHHORN, 2007).

A videira pertence à ordem *Vitales*, família *Vitaceae*, que compreende 12 gêneros e 800 espécies (SOUZA; LORENZI, 2008), distribuídas extensivamente nas regiões subtropicais e temperadas, com variantes que se estendem até regiões de clima tropical (LEÃO; BORGES, 2009). Dentre os representantes da família, o gênero *Vitis* é o mais antigo e o único com importância econômica e alimentar (CAMARGO, 2000), já que inclui espécies que são consumidas como fruta fresca ou seca, e também na forma de vinhos e sucos. Está subdividido em dois subgêneros: *Muscadinia* e *Euvitis*, cujas espécies estão agrupadas de acordo com a morfologia e a origem geográfica (LEÃO; BORGES, 2009). Destacam-se *V. vinifera* (*Euvitis*) com frutos apropriados para a produção de vinhos, de origem européia, e *Vitis labrusca* L. (*Muscadinia*) que são adequadas para produzir uvas de mesa e servir de porta-enxerto, com origem na América do Norte (POMMER; MAIA, 2003). Os outros gêneros são empregados para fins ornamentais (THIS; LACOMBE; THOMAS, 2006).

Existem cerca de 10 mil cultivares de videira, adaptadas a vários tipos de solo e clima, o que possibilita o cultivo em quase todas as regiões do mundo. Embora amplamente cultivadas, as uvas são bastante sensíveis e variam de acordo com as condições edafoclimáticas em que se desenvolvem, apresentando características diferenciadoras como

sabor, acidez, doçura, formato, coloração, resistência da casca, tamanho, quantidade de sementes e formato dos cachos (QUEIROZ-VOLTAN; PIRES, 2003).

As uvas de mesa no Brasil podem ser divididas em dois grupos: um formado pelas uvas finas (*V. vinifera*), representado principalmente por cultivares como a Itália e suas mutações (Rubi, Benitaka e Brasil), Red Globe, Red Meire e as sem sementes (Centennial Seedless, Superior Seedless ou Festival, Thompson Seedless, Perlette, Catalunha e Crimson Seedless); e outro pelas uvas comuns ou rústicas (*V. labrusca*), cujas representantes principais são as cultivares Niagara Rosada e Isabel (PROTAS; CAMARGO, 2011). A videira pode ser cultivada para diferentes finalidades, sendo classificada comercialmente em: uvas para mesa (consumo *in natura*), para vinificação, para passas e para sucos doces (LEÃO, 2000).

2. Importância econômica e distribuição geográfica

A videira é uma das mais importantes espécies frutíferas cultivadas (SENTELHAS, 1998) e atualmente ocupa a terceira posição em produção total (65.584.233 t) entre as principais frutas no mundo inteiro. Em primeiro lugar está a banana (105.815.354 t), seguida pelos citros (105.440.168 t) (FAO, 2014).

A China é o principal país produtor de uva, com 9,6 milhões de toneladas produzidas em 2012, seguida dos Estados Unidos da América (6,6 milhões de toneladas) e a Itália, que até recentemente era o principal produtor, agora ocupa a terceira posição (5,8 milhões de toneladas). O Brasil ocupa a 12ª posição entre os países produtores de uva, com uma produção de 1,5 milhões de toneladas (FAO, 2014).

A região Sul destaca-se como a maior produtora de uva no Brasil, tendo participado com 65% da produção (989.8 mil toneladas), seguida da região Nordeste, com 19% da produção (289,9 mil toneladas) (IBGE, 2014). Muito embora a região Sul apresente-se como a maior produtora brasileira, vale ressaltar que a uva produzida nessa região destina-se, principalmente, à produção de vinho, enquanto na região Nordeste predomina a produção de uvas de mesa. A produção brasileira de uva destinada ao consumo *in natura* representa cerca de 56% da uva produzida, enquanto o restante é utilizado na elaboração de vinhos, sucos, destilados e outros derivados (SILVA; COELHO, 2010).

A uva de mesa é uma das principais frutas frescas exportadas pelo Brasil. Em 2011, foram exportadas 52 mil toneladas de uvas de mesa, com faturamento de 121,9 milhões de dólares (SECEX, 2014). A região Nordeste é responsável por 99% das exportações brasileiras

de uvas finas de mesa, onde são cultivados cerca de 9.500 ha de videira. Nesta região, o Vale do São Francisco é o principal pólo produtor de uvas finas de mesa, sendo responsável por 98% da produção (ARAÚJO; RAMALHO, 2009; LAZZAROTTO; FIORAVANÇO, 2013). Apesar da importância da participação do Vale do São Francisco nas exportações de uva de mesa, vale ressaltar que cerca de 70% da produção regional é absorvida pelo mercado interno (SILVA; COELHO, 2010).

A produção de uva no Vale do São Francisco concentra-se, principalmente, nos municípios de Petrolina, Lagoa Grande e Santa Maria da Boa Vista, em Pernambuco, com 80% da área cultivada, e Juazeiro, Curaçá, Sento Sé e Casa Nova, na Bahia, que detém os 20% restantes de área. O município de Petrolina foi o principal produtor de uva de mesa em 2012, com área cultivada de 4.650 ha e produção de 153,4 mil toneladas (IBGE, 2014).

Além do Vale do São Francisco, existem outros pequenos pólos de produção de uvas de mesa no Nordeste brasileiro, com destaque para o localizado no Vale do Siriji, na região úmida do estado de Pernambuco, onde é cultivada videira há mais de 40 anos em pequenas propriedades e a produção de uvas comuns (Isabel) se destina ao mercado interno, não sendo exportada (TAVARES; LIMA, 2009). O município de São Vicente Férrer se destaca como principal produtor de uva no Vale do Siriji, com 500 hectares cultivados e produção de 9,5 mil toneladas em 2012 (IBGE, 2014). No vale do Jaguaribe o cultivo da videira é incipiente (teve inicio em 2011), sendo o perímetro irrigado de Tabuleiro de Russas o principal produtor.

O Nordeste brasileiro possui uma característica ímpar na produção de uva, pelo fato de ser capaz de produzir mais de duas safras por ano devido suas condições climáticas. O repouso vegetativo, nessa região, é regulado pela época seca, e o manejo de irrigação, aliado ao clima quente, permite ao viticultor obter duas colheitas sucessivas no mesmo ano (PEDRO JÚNIOR; SENTELHAS, 2003). Esse fato tem levado investidores para essa região, fomentando a produção de uvas nas duas últimas décadas. A região ainda se encontra em estágio de contínua expansão, e hoje se volta para investimentos na produção de cultivares de uvas apirênicas e uvas para vinhos e sucos. Todavia, com a intensificação do cultivo de videira, o plantio de variedades suscetíveis, as condições climáticas prevalentes no Vale do São Francisco, como também a aquisição de mudas sem certificação, têm propiciado o surgimento de problemas fitossanitários que afetam diretamente a produção e a produtividade nesse pólo agrícola (TAVARES, 1995).

Entre as doenças que afetam a videira nesta região destacam-se o míldio (*Plasmopara viticola* (Berk. e Curtis) Berl. e De Toni, o oídio (*Uncinula necator* (Schwein.) Burril.), a ferrugem (*Phakopsora euvitis* Ono), o cancro bacteriano (*Xanthomonas campestris* pv.

viticola (Nayudu) Dye), o enrolamento das folhas (*Grapevine leafroll-associated virus* 1-9 - GLRaV 1-9), a malformação infecciosa (*Grapevine fanleaf vírus*) e os nematóides das galhas (*Meloidogyne* spp.) (BARBOSA et al., 2010).

Nos últimos anos, foram observadas plantas de videira apresentando sintomas de declínio, que incluem crescimento inicial e vigor reduzido, demonstrando amarelecimento de folhas e diferentes sintomas na madeira, como necrose setorial e estrias marrons e negras (CORREIA et al., 2013).

3. Declínio da videira

Apesar da importância econômica da uva de mesa para o agronegócio brasileiro e em particular para a pauta de exportações da região Nordeste, os problemas fitossanitários ainda representam fatores limitantes à exploração comercial da videira. Nas últimas décadas, vários estudos sobre doenças e fungos patogênicos associados a declínios de porta-enxertos, plantas jovens e plantas adultas de videira têm sido realizados em nível mundial (AGUSTÍ-BRISACH et al., 2013; AROCA et al., 2010; CORREIA et al., 2013; DÍAZ; LATORRE, 2014; MOHAMMADI et al., 2013; SOFIA et al., 2013; ÚRBEZ-TORRES et al., 2014a; ÚRBEZ-TORRES et al., 2014b). O declínio e a morte de plantas de videira, associados a fungos, representam um problema para a viticultura mundial devido à gravidade dos prejuízos. Esse problema engloba algumas doenças, cuja causa envolve fungos habitantes do solo que possuem uma fase importante de saprofitismo e latência (HALLEEN; CROUS; PETRINI, 2003).

Os sintomas observados incluem pobre crescimento inicial, reduzido vigor demonstrando amarelecimento de folhas, murcha e morte descendente, bem como diferentes sintomas na madeira, como necrose setorial em forma de V e estrias marrons e negras que aparecem como pontos negros necróticos em ramos e troncos seccionados longitudinalmente. Esses sintomas são similares àqueles descritos em outras regiões viticultoras mundiais, os quais têm sido associados com várias doenças de declínio (BERTSCH et al., 2013; BRUEZ et al., 2013; GARCÍA-JIMÉNEZ; RAPOSO; ARMENGOL, 2010; GRAMAJE; ARMENGOL, 2011; HALLEEN; FOURIE; CROUS, 2006; LARIGNON, 2012; LUQUE et al., 2009; MOHAMMADI et al., 2013; MOLLER; KASIMATIS, 1981; MOSTERT et al., 2006a; MUGNAI; GRANITI; SURICO, 1999; ÚRBEZ-TORRES, 2011; ÚRBEZ-TORRES et al., 2014a; ÚRBEZ-TORRES et al., 2014b).

As principais doenças observadas em plantas de videira com declínio em nível mundial são: doença de Petri, pé-preto, esca, morte descendente e eutipiose (GARCÍA-JIMÉNEZ; RAPOSO; ARMENGOL, 2010; LARIGNON, 2012). Dentre estas, a doença de Petri e o pé-preto têm recebido pouca atenção no Brasil.

3.1. Doença de Petri

A doença de Petri foi descoberta no início do século XX, na Sicília, Itália (PETRI, 1912), sendo nos dias de hoje atribuída essencialmente aos fungos *Phaeomoniella chlamydospora* (Gams et al.) Crows e Gams e várias espécies de *Phaeoacremonium* Gams, Crous e Wingf (GROENEWALD et al., 2001; LARIGNON, 2012; MUGNAI; GRANITI; SURICO, 1999; SCHECK et al., 1998).

O gênero *Phaeoacremonium* foi descrito por Crous et al. (1996) e pertence ao filo Ascomycota, classe Sordariomycetes, ordem Diaporthales e família Togniniaceae (MYCOBANK, 2014). O teleomorfo deste gênero é *Togninia* Berl. Morfologicamente, o gênero *Phaeoacremonium* situa-se entre os gêneros *Acremonium* Link. e *Phialophora* Medlar. Quarenta e duas espécies de *Phaeoacremonium* são consideradas válidas (MYCOBANK, 2014), das quais 27 foram constatadas em videira, com destaque para *Pm. aleophilum* Gams et al., a mais comum e amplamente distribuída (MARTÍN; MARTIN, 2013; MUNDY; MANNING, 2010). As espécies de *Phaeoacremonium* têm distribuição mundial e ampla gama de hospedeiros, incluindo plantas lenhosas, larvas de insetos e seres humanos (GUARRO et al., 2003; MOSTERT et al., 2006b).

O uso combinado de técnicas clássicas e moleculares, aplicadas aos estudos taxonômicos do gênero *Phaeoacremonium*, permitiu a reclassificação ou a criação de novas espécies dentro do gênero, uma vez que a distinção entre espécies, apenas por critérios morfológicos, se revelou pouco consistente. Dentre as técnicas moleculares mais usadas, destacam-se PCR-RFLP da região ITS, ou dados de sequenciamento da região ITS do DNA ribossomal, de parte dos genes da β-tubulina, da actina ou da calmodulina (MOSTERT et al., 2006a). Estudos detalhados mostraram que *Pm. chlamydosporum* Gams et al. era morfologicamente e filogeneticamente diferente das outras espécies do mesmo gênero, passando a ser designado como *Phaeomoniella chlamydospora*, formando-se assim um novo gênero, *Phaeomoniella* Crous e Gams (CROUS; GAMS, 2000), que reside no filo Ascomycota, ordem Eurotiomycetes, classe Chaetothyriales e família Herpotrichiellaceae

(MYCOBANK, 2014). Até o momento, o teleomorfo deste gênero não é conhecido (MARTÍN; MIERA; MARTÍN, 2013). Oito espécies de *Phaeomoniella* são consideradas válidas (MYCOBANK, 2014), sendo *Pa. chlamydospora* a mais comum em videira (LARIGNON, 2012; MARTÍN; MARTIN, 2013; MORTON, 2000; MUNDY; MANNING, 2010; ÚRBEZ-TORRES et al., 2014a). Em cultura, o gênero *Phaeomoniella* difere ligeiramente do gênero *Phaeoacremonium*, por ter um crescimento semelhante às leveduras (CROUS; GAMS, 2000).

A doença de Petri ocorre principalmente em videiras jovens e tem causado grandes prejuízos em parreirais recém-implantados. Pode ocasionar um crescimento vegetativo reduzido, bem como a morte de videiras jovens e adultas. Pode-se manifestar através de uma desfoliação repentina, mas, normalmente, começa com um declínio lento que se traduz num crescimento reduzido e vários sintomas (BERTELLI; MUGNAI; SURICO, 1998; MORTON, 2000; MUGNAI; GRANITI; SURICO, 1999; PASCOE; COTTRAL, 2000; SCHECK et al., 1998). Os sintomas externos são crescimento reduzido com entre-nós curtos, folhas, troncos e ramos pequenos e necróticos. Nas folhas observam-se manchas de coloração verde clara ou clorótica, de dimensões irregulares entre as nervuras ou ao longo da margem da folha. As áreas cloróticas inicialmente são pequenas e dispersas pelo limbo, espalhando-se gradualmente e tornando-se parcialmente necróticas. À medida que o tecido clorótico se torna amarelo-acastanhado ou vermelho-acastanhado, as folhas infectadas assumem o padrão chamado ‘listras de tigre’ (CALZARANO; DI MARCO, 2007; DUBOS, 1997; EDWARDS; LAUKART; PASCOE, 2001; MUGNAI; GRANITI; SURICO, 1999). Os sintomas internos podem ser observados quando se fazem cortes transversais ou longitudinais nos troncos e rebentos, observando-se pontuações negras e estrias castanhas ou pretas nos tecidos xilémicos, muitas vezes com a exsudação de uma goma viscosa preta. Por vezes, no lenho dos ramos ou do tronco ocorrem setores necróticos ou com podridão branca, assim como fendilhamentos no ritidoma e no lenho (SURICO; MUGNAI; MARCHI, 2006).

O enegrecimento dos tecidos xilémicos é causado pela formação de tiloses, gomas e acumulação de compostos fenólicos produzidos pelo hospedeiro como resposta de defesa à presença do fungo (DEL RIO et al., 2001; MARTINI et al., 2009; MUGNAI; GRANITI; SURICO, 1999). Estas barreiras impedem o fluxo ascensional de água e sais minerais e conduzem a uma captação de água deficiente, levando ao aparecimento de cloroses nas folhas (EDWARDS et al., 2007).

Nas bagas podem aparecer pontuações negras a que se chama sarampo preto, nome pelo qual chegou a ser conhecida à doença de Petri (VASQUEZ; GUBLER; LEAVITT,

2007). Num estádio mais avançado da doença pode ser observado o dessecamento das bagas (BRUNO; SPARAPANO; GRANITI, 2007). Em plantas afetadas é comum encontrar um sistema radicular pobremente desenvolvido (LARIGNON, 2012). Quando a incidência e a severidade são elevadas, o arranque das mesmas é, normalmente, a única decisão a tomar, uma vez que os custos associados à manutenção dessas videiras doentes se revelam insustentáveis (PINTO, 2010).

A doença de Petri pode causar a apoplexia (morte repentina das plantas), que está relacionada com estações quentes e também com a diminuição da condutividade no xilema e consequentemente a um rápido aumento da concentração e atividade dos compostos tóxicos na parte aérea, quando a taxa de transpiração é elevada (BRUNO; SPARAPANO, 2006; CHICAU, 2006; EDWARDS et al., 2007), podendo começar logo a manifestar-se no primeiro ano depois da plantação (BERTELLI; MUGNAI; SURICO, 1998). A apoplexia pode também estar relacionada à infecção do fungo *Armillaria mellea* (Vahl) Kumm. (CHICAU, 2006).

Os sintomas da doença de Petri podem variar de ano para ano dependendo da idade das videiras e da intensidade do ataque. O tipo de sintoma depende de inúmeros fatores, dos quais se destacam a severidade da doença, a composição da população fúngica presente em cada planta e as condições de estresse a que estas estão sujeitas, sobretudo nos primeiros anos após plantação. Certos sintomas da doença podem ser facilmente confundidos com os causados por outros agentes de natureza biótica e por fatores abióticos, como falta de nutrientes, ataques de vírus, como o do enrolamento foliar, podas em que se deixa carga excessiva nos lançamentos (MUGNAI; GRANITI; SURICO, 1999) ou outras doenças como pé-preto da videira (REGO, 2004). As videiras podem permanecer assintomáticas, ou seja, não demonstram sintomas, pois estes fungos são endófitos capazes de viver no hospedeiro sem que haja sintomas visíveis (SCHECK et al., 1998).

As principais fontes de inóculo dos patógenos causadores da doença de Petri são os materiais de propagação vegetativa infectados, solos infestados e o inóculo aéreo (MOSTERT et al., 2006a). As plantas-mãe de porta-enxertos e de garfos encontram-se frequentemente infectadas, dando origem a estacas portadoras de inóculo (FOURIE; HALLEEN, 2004a; GIMÉNEZ-JAIME et al., 2006; OLIVEIRA; REGO; NASCIMENTO, 2004; WHITEMAN et al., 2007). Os materiais de propagação são contaminados durante as várias operações realizadas nos viveiros, como hidratação, enxertia e calogênese (GRAMAJE; ARMENGOL, 2011; WAITE; MAY; BOSSINGER, 2013; WHITEMAN et al., 2002). A contaminação também pode ocorrer durante o processo de enraizamento em viveiro, através das raízes ou de feridas existentes no porta-enxerto, ou resultantes da poda. No campo, os conídios de *Pa.*

chlamydospora, quando transportados pelo vento, colonizam a videira através das feridas da poda. No entanto, o mesmo não é verificado no caso de *Pm. aleophilum*. Os esporos de *Pa. chlamydospora* podem ser detectados durante todo o ano, ao contrário dos de *Pm. aleophilum*, que são detectados apenas durante o período vegetativo da videira (LARIGNON; DUBOS, 2000).

3.2. Pé-preto

O pé-preto da videira é uma importante doença na maioria dos parreirais e está presente em todas as regiões produtoras de uva no mundo, especialmente em viveiros e parreirais jovens (AGUSTÍ-BRISACH; ARMENGOL, 2013; HALLEEN; FOURIE; CROUS, 2006). Esta doença foi relatada pela primeira vez na França, sob o nome de “gangrena”, em 1961 (GRASSO; MAGNANO DI SANLIO, 1975). Ao longo da última década, a sua incidência tem aumentado significativamente na maioria das áreas de produção da videira em todo o mundo (ALANIZ et al., 2007; HALLEEN; FOURIE; CROUS, 2006; AGUSTÍ-BRISACH; ARMENGOL, 2013), incluindo Europa (ALÁNIZ et al., 2007; REGO et al., 2000), Oriente Médio (MOHAMMADI et al., 2009), Oceania (HALLEEN et al., 2004; WHITELAW-WECKERT et al., 2007), África (HALLEEN et al., 2004), América do Norte e América do Sul (ABREO et al., 2010; AUGER; ESTERIO; PÉREZ, 2007; PETIT et al., 2011; PETIT; GUBLER, 2005; CORREIA et al., 2013; ÚRBEZ-TORRES et al., 2014)

O pé-preto é causado pelos fungos dos gêneros *Campylocarpon*, *Cylindrocarpon*, *Cylindrocladiella* e *Ilyonectria* (AGUSTÍ-BRISACH; ARMENGOL, 2013; CABRAL et al., 2012a, b; CHAVERRI et al., 2011; HALLEEN, et al., 2004, 2006; LOMBARD et al., 2012; SCHROERS et al., 2008). A morfologia das colônias e conídos têm sido amplamente utilizadas para identificar as espécies de *Cylindrocarpon*/*Ilyonectria* (ALANIZ et al., 2007; HALLEEN et al., 2004, 2006; PETIT; GUBLER, 2006; SCHROERS et al., 2008). Alguns caracteres fenotípicos são valiosos para a identificação de algumas espécies (CHAVERRI et al., 2011; HALLEEN et al., 2004; SCHROERS et al., 2008), embora não sejam suficientes para distinguir outras espécies. Além disso, a identificação de *Cylindrocarpon*/*Ilyonectria* baseada apenas na morfologia tornou-se difícil, devido ao grande número de espécies identificadas, incluindo espécies crípticas de videiras e outros hospedeiros (AGUSTÍ-BRISACH; ARMENGOL, 2013).

Espécies de *Campylocarpon*, *Cylindrocarpon*, *Cylindrocladiella* e *Ilyonectria* são geralmente consideradas patógenos e/ou saprófitos de uma ampla gama de hospedeiras angiospermas e gimnospermas e substratos em regiões temperadas, subtropicais e tropicais em todo o mundo (CHAVERRI et al., 2011; LOMBARD et al., 2012; VICTOR et al., 1998). Possuem uma ampla distribuição geográfica, podendo ser encontrados em todos os continentes, desde as florestas tropicais até em solo da tundra Ártica. Geralmente são encontrados desde as camadas mais superficiais do solo até vários centímetros de profundidade, podendo crescer em baixas concentrações de oxigênio (BRAYFORD, 1993).

Embora esses patógenos geralmente se manifestem em videiras maduras, também têm sido frequentemente isolados de plantas em matrizeiros sintomáticos ou assintomáticos, estacas de porta-enxerto enraizadas e videiras jovens enxertadas (AGUSTÍ-BRISACH; ARMENGOL, 2013; AROCA et al., 2006; DUBROVSKY; FABRITIUS, 2007; FOURIE; HALLEEN, 2004a; HALLEEN; CROUS; PETRINI, 2003; HALLEEN, FOURIE; CROUS, 2007; OLIVEIRA; REGO; NASCIMENTO, 2004; RUMBOS; RUMBOU, 2001). Por serem fungos habitantes do solo, a doença frequentemente inicia nos porta-enxertos, que apresentam uma necrose vascular intensa com início na parte basal, e logo toma todo o porta-enxerto. Na maioria das vezes são emitidas novas raízes que, frequentemente, são insuficientes para a sobrevivência da planta, já que o fungo ataca a zona radicular que se torna negra e danificada (GARRIDO; SÔNEGO; GOMES, 2004).

Os sintomas característicos da doença incluem uma redução na biomassa de raízes e pêlos radiculares com lesões escuras, deprimidas e necróticas (AGUSTÍ-BRISACH; ARMENGOL, 2013; ABREO et al., 2010; ALANIZ et al., 2009; HALLEEN; FOURIE; CROUS, 2006; REGO et al., 2000). Na madeira do porta-enxerto são observadas colorações escuras e necroses internas que iniciam desde a base (DUBOS, 2002). Os sintomas externos mostram reduzido vigor com troncos pequenos, entrenós curtos, madeira com amadurecimento desigual, folhagem esparsa e pequenas folhas com clorose e necrose. Os sintomas da doença no campo são frequentemente indistinguíveis dos causados pela doença de Petri (ABREO et al., 2010; ALANIZ et al., 2007, 2009; HALLEEN; FOURIE; CROUS, 2006; REGO et al., 2000; SCHECK et al., 1998). Quando videiras jovens são infectadas, a morte ocorre rapidamente. No entanto, com o passar do tempo, a infecção resulta em um declínio mais gradual e a morte da planta só ocorre depois de um ano (GUBLER et al., 2004).

Resultados de estudos realizados em viveiros comerciais de videira sugerem que as novas plantas são infectadas durante o processo de propagação em viveiros e que até mesmo o material de plantio utilizado no processo de propagação pode ser infectado pelos patógenos. O

solo de viveiros comerciais e de campos de produção constitui outra importante fonte de inóculo. Durante o processo de propagação da videira, no momento do plantio, as extremidades basais susceptíveis (especialmente na área da medula) da maioria das estacas são parcial ou totalmente expostas, e as raízes de calos jovens também podem quebrar durante o processo de plantio, resultando em pequenas feridas suscetíveis à infecção por patógenos habitantes do solo (AGUSTÍ-BRISACH; ARMENGOL, 2013).

A produção de clamidosporos pela maioria das espécies de *Campylocarpon*, *Cylindrocarpon*, *Cylindrocladiella* e *Ilyonectria* permite a sobrevivência por longos períodos no solo (HALLEEN et al., 2004). No entanto, pouca informação é disponível sobre a sobrevivência desses patógenos e o papel de clamidosporos durante infecções subsequentes (HALLEEN; FOURIE; CROUS, 2006).

O risco de ocorrência do pé-preto pode ser aumentado pelo estresse imposto em videiras jovens em viveiros e campos de produção. Fatores ambientais e práticas de manejo do parreiral, incluindo má drenagem, compactação do solo e covas de plantio inadequadas, que causam o pobre desenvolvimento radicular, bem como a má nutrição, poda drástrica de videiras jovens e os efeitos das pragas e patógenos poderiam ser considerados como fatores de estresse. Os processos de propagação no viveiro e estabelecimento do parreiral incluem muitas práticas que causam estresse em videiras jovens. Durante o processo de propagação da videira, as feridas produzidas durante a enxertia, o início do desenvolvimento de raízes e da parte aérea no viveiro, o desenraizamento e a limpeza, o armazenamento a frio e o tempo excessivo em recipientes antes do estabelecimento no parreiral causam estresse nas plantas jovens. Além disso, após o plantio no campo, essas videiras são novamente estressadas pela necessidade de desenvolver as raízes e a parte aérea em um ambiente que é muitas vezes selecionado para limitar o crescimento da parte aérea (PROBST et al., 2012).

3.6. Medidas de controle

O controle do declínio da videira tem sido bastante difícil, isto porque, o arsenito de sódio, o único fungicida eficaz foi proibido por causa seus efeitos cancerígenos em seres humanos e sua elevada toxicidade ao meio ambiente (DECOIN, 2001; LARIGNON et al., 2008). Como consequência da proibição do arsenito de sódio, foi intensificada a incidência do declínio da videira em todas as regiões produtoras de uva no mundo (SURICO; MUGNAI; MARCHI, 2006).

Logo depois dessa proibição, vários métodos de controle, incluindo produtos químicos, agentes de controle biológico, moléculas naturais e métodos de sanitização, têm sido testados contra a doença. Apesar destes esforços, a eficácia de um método único de controle parece ser bastante limitada. Com isso, é necessário utilizar estratégias de controle que possam combinar dois ou mais métodos de controle para reduzir a incidência da doença (BERTSCH et al., 2013).

Os materiais propagativos usados no preparo das mudas em viveiro podem estar contaminados com fungos causadores do declínio (AROCA et al., 2010; DUBROVSKY; FABRITIUS, 2007; FOURIE; HALLEEN, 2004b), motivo pelo qual é essencial a utilização de material vegetal certificado nos viveiros de mudas. Como medida preventiva, o uso de materiais de propagação livres desses fungos seria a medida ideal de controle da doença (WHITEMAN et al., 2007).

O uso do fungicida didecildimetil cloreto de amônio durante o processo de hidratação dos ramos demonstrou ser um tratamento eficaz para prevenir a infecção de *Pa. chlamydospora* e *Pm. aleophilum* (GRAMAJE et al., 2009; FOURIE; HALLEEN, 2006). Rego et al., (2009) imergindo porta-enxertos e ramos em uma suspensão de ciprodinil e fludioxonil conseguiram reduzir a incidência e a severidade do pé-preto.

A erradicação dos fungos causadores de declínio, após a infecção da videira, é muito difícil. O uso da poda curativa, que consiste na eliminação completa das zonas afetadas, é a medida mais efetiva, na qual, é aconselhado aplicar um isolante com ou sem fungicida para evitar uma nova infecção (HERCHE, 2009; MUGNAI; GRANITI; SURICO, 1999). As plantas completamente afetadas, sem possibilidade de serem podadas curativamente, devem ser arrancadas desde a raiz e eliminadas (MUGNAI; GRANITI; SURICO, 1999).

Como as feridas de podas podem constituir uma porta de entrada para os fungos, protegê-las com fungicidas é considerada a medida mais eficaz e viável economicamente para prevenir a infecção da videira pelos patógenos causadores de declínio (BERTSCH et al., 2013; CALZARANO; DIMARCO; CESARI, 2004; CARTER, 1991; PITT et al., 2012; ÚRBEZ-TORRES, 2011). Entre os fungicidas testados destacam-se os princípios ativos carbendazim, procimidone, iprodione, mancozeb, fluazinan, tiofanato-metílico, flusilazole, tebuconazole, piraclostrobina e fosetyl-Al (AMPONSAH et al., 2012; BESTER; CROUS; FOURIE, 2007; DÍAZ; LATORRE, 2013; FOURIE; HALLEEN, 2006; PITT et al., 2012; ROLSHAUSEN et al., 2010).

O uso de agentes biológicos, tais como espécies de *Trichoderma*, para proteger feridas de poda dos fungos têm apresentado excelentes resultados (COMPANT et al., 2013; JOHN et

al., 2005, 2008). No entanto, apesar dos resultados animadores, procedimentos de controle baseados na utilização de *Trichoderma* ainda requerem mais testes em campo para serem avaliados com precisão e eventualmente serem otimizados pela combinação com outras estratégias de manejo (BERTSCH et al., 2013).

Estratégias de controle integrado, que combinam medidas de controle químico, físico e biológico, obtidos até o momento estão focadas na sanitização das plantas durante o período que estão nos viveiros (FOURIE; HALLEEN, 2004b; GRAMAJE; ARMENGOL, 2011; HALLEEN, FOURIE; CROUS, 2007). Em condições de campo, todas as medidas de controle perdem a eficácia. Portanto, muitos fatores contribuem para que não seja possível o controle eficaz do declínio da videira em nível mundial (BERTSCH et al., 2013).

4. Doença de Petri e pé-preto no Brasil

As doenças de Petri e pé-preto vêm se tornando cada vez mais importante na produção de uvas no Brasil nos últimos anos (CAVALCANTI; BUENO; ALMANÇA, 2013). Não há até o presente momento, um resultado de pesquisa que revele precisamente o nível de dano econômico causado pela incidência dessas doenças sobre a viticultura brasileira. Mas, seguidas observações e levantamentos de campo, feitos nos últimos anos, principalmente no Rio Grande do Sul (ALMANÇA et al., 2013), no estado de São Paulo (FERREIRA et al., 2013) e no Nordeste brasileiro (CORREIA, 2014), alertam para o preocupante crescimento da incidência dessas doenças nas áreas de produção de uva.

Os estudos sobre a etiologia dos fungos causadores da doença de Petri e do pé-preto em videira no Brasil são bastante escassos. Em relação à doença de Petri, isolamentos efetuados de plantas sintomáticas no Rio Grande do Sul propiciaram evidências morfológicas para a ocorrência de *Phaeomoniella chlamydospora* (ALMANÇA et al., 2013). Também no Rio Grande do Sul foi isolado um fungo do gênero *Phaeoacremonium* baseado em características morfológicas e identificado apenas em nível de gênero com incidência de 12% das amostras coletadas em plantas de videiras de cultivares americana (GARRIDO; SÔNEGO; GOMES, 2004). Em um trabalho realizado por Correia et al. (2013), foi relatada a primeira ocorrência das espécies *Pa. chlamydospora* e *Pm. aleophilum, Pm. parasiticum*, em videiras no estado de Pernambuco. Em um leventamento de detecção de fungos da madeira da videira no estado de São Paulo também foi constatado a presença de *P. chlamydospora* e *Phaeoacremonium aleophilum* (FERREIRA et al., 2013). Espécies do fungo

Phaeoacremonium também foram relatadas no Brasil causando infecções subcutâneas em humanos (GUARRO et al, 2003).

O pé-preto foi relatado pela primeira vez no Brasil em 1999, no estado do Rio Grande do Sul, e sua identificação foi baseada apenas em características morfológicas do fungo *Cylindrocarpon destructans* (GARRIDO; SONEGO; URBEN, 2004). Em estudos posteriores foram identificados *Cylindrocarpon* sp. e *Cylindrocladium* sp., mas sem a identificação em nível de espécie, com ocorrência de 19% e 6%, respectivamente, no sistema radicular (GARRIDO; SÔNEGO; GOMES, 2004). As espécies fungicas associadas com o pé-preto *Campylocarpon pseudofasciculare e Campylocarpon fasciculare* foram relatadas no Nordeste do Brasil e identificadas através de métodos morfológicos e moleculares, sendo o primeiro relato no Brasil e no continemte americano, respectivamente (CORREIA et al., 2013).

O conhecimento dos diferentes fungos associados ao declínio da videira é fundamental para o sucesso no manejo da doença, aspecto essencial para prolongar a vida econômica dos parreirais (ÚRBEZ-TORRES et al., 2012). Apesar da importância da viticultura para o agronegócio do Nordeste brasileiro e da importância crescente do declínio da videira, não existem informações precisas sobre as espécies fúngicas associadas, bem como sobre a prevalência e a distribuição destas nos pólos de produção.

Os conhecimentos gerados sobre a etiologia e a epidemiologia do declínio da videira são fundamentais para o desenvolvimento de estratégias de ação específicas aos agentes causais, contribuindo para diminuir as perdas de produção e, consequentemente, aumentar a competitividade do agronegócio da viticultura no Nordeste do Brasil.

Diante disso, os objetivos dessa tese foram: a) identificar as espécies de *Phaeoacremonium* e *Campylocarpon* associadas respectivamente à doença de Petri e ao pé-preto no Nordeste brasileiro utilizando características morfológicas e análises filogenéticas; b) investigar a prevalência e a distribuição das espécies de *Phaeoacremonium* e *Campylocarpon* nos pólos de produção de uva no Nordeste brasileiro; e c) avaliar a patogenicidade e a virulência das espécies de *Phaeoacremonium* e *Campylocarpon* em ramos de videira.

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Capítulo II

Species of *Phaeoacremonium* associated with Petri disease of table grape in Northeastern Brazil, with one new species

Submissão: **Fungal Diversity**
Kunming, China
JCR = 5,34

Species of *Phaeoacremonium* associated with Petri disease of table grape in Northeastern Brazil, with a new species

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Abstract This study aims to identify and characterize species of *Phaeoacremonium* associated with Petri disease of table grape in three regions in the Northeastern Brazil. Fungal identifications were made using a combination of morphology together with a phylogenetic analysis based on portions of the β-tubulin (BT) and actin (ACT) genes. Three species of *Phaeoacremonium* (*Pm.*) were identified: *Phaeoacremonium aleophilum, Pm. nordesticola* sp. nov. and *Pm. parasiticum*. *Phaeoacremonium aleophilum* and *Pm. parasiticum* had previously been reported in grapevine. *Phaeoacremonium aleophilum* was the most prevalent species. All species of *Phaeoacremonium* were pathogenic on detached shoots of table grape, with *Pm. aleophilum* being the most virulent.

Key words: *Phaeoacremonium, Vitis vinifera*, *Vitis labrusca*, Trunk disease, Phylogeny, Virulence

Introduction

Table grape (*Vitis* spp.) is an important fresh fruit exported by Brazil. In 2011, 59,400 t of table grapes were exported and accounting for US$ 136 million (FAO 2014). The Northeastern region is responsible for 99% of Brazilian exports of table grapes, where 9,600 ha are cultivated. The São Francisco Valley, located in the semi-arid region of Bahia and Pernambuco States, is the main grapevines growing area in the region, accounting for 98% of the production (Lazzarotto and Fioravanço 2013). In the Siriji Valley, located in the tropical humid region of Pernambuco State, table grapes have been grown for over 40 years with a production intended only for the local market (Araújo and Ramalho 2009). In 2011, a new production pole of table grapes was started in the Baixo Jaguaribe Valley, located in the semi-arid region of Ceará State, but the plants have not yet reached the production stage. In São Francisco and Baixo Jaguaribe Valleys are planted European cultivars (*Vitis vinifera* L.) with rootstock, while in Siriji Valley are planted American cultivars (*Vitis labrusca* L.) without rootstock. Northeastern Brazil is a tropical region, thus the management systems for table grape production are adapted to the specific environmental conditions of a tropical viticulture. In both the dry and wet tropics, the growth and cropping cycle of the vine can be manipulated to extend from 5 to 12 months by a combination of pruning, modifying vine water status and the use of chemical regulators. Thus, it is possible to achieve two and a half to three vegetative cycles per year (Camargo et al. 2008; Possingham 2008; Correia et al. 2013).

A wide range of diseases impact on grapevine production and grapevine trunk diseases are known to occur wherever grapes are grown (Úrbez-Torrez et al. 2014). In this group, the Petri disease is among the most destructive worldwide (Mugnai et al. 1999; Mostert et al. 2006b; Graham et al. 2009; Bertsch et al. 2013; Úrbez-Torrez et al. 2014). Incidence of Petri disease have been worsening in all grape-producing regions since the late 1990s, including Europe (Mugnai et al. 1999), the Near East (Arzanlou et al. 2013), North and South America (Correia et al. 2013; Urbéz-Torrez et al. 2014), Oceania (Graham et al. 2009), and South Africa (Mostert et al. 2006b), and causing significant economic losses due to yield and quality reductions and vineyard replanting (Scheck et al. 1998).

The first report of Petri disease in table grapes in Northeastern of Brazil was in 2013, in the São Francisco and Siriji Valleys (Correia et al. 2013). The symptoms of this disease are characterized by reduced vigor of vine, short internodes, stunted growth, chlorotic and/or wilting leaves, occasional sudden vine collapse, and black streaking in xylem tissues and black spots in shoots and trunk (Scheck et al. 1998; Mugnai et al. 1999; Gramaje and Armengol 2011; Correia et al. 2013; Úrbez-Torres et al. 2014).

Petri disease is caused by a combination of *Phaeomoniella chlamydospora* (Gams, Crous, Wingf. e Mugnai) Crous & W. Gams and several species of *Phaeoacremonium* (*Pm.*) W. Gams, Crous & M.J. Wingf. (Scheck et al. 1998; Mugnai et al. 1999; Groenewald et al. 2001; Gramaje and Armengol 2011; Correia et al. 2013; Úrbez-Torres et al. 2014). The genus *Phaeoacremonium* was established by Crous et al. (1996), having as main morphological characteristics: mycelium branched, with colour relatively pale and relatively deeply brown-coloured species; septate hyphae that occur singly or in bundles; conidiophores long or short and branched or unbranched; conidiogenous cells are phialides which can be either directly from the mycelium or integrated in conidiophores, with three types (I, II and III); conidia occur in slimy heads at the phialide apices, and are hyaline and aseptate, with shape varying from oblong-ellipsoidal to obovate to cylindrical to allantoid to reniform (Mostert et al. 2006a). Species delimitation within *Phaeoacremonium* based solely on cultural and morphological characteristics is challenging, and thus molecular analyses of part of the actin and β-tubulin gene regions have been used routinely for species delineation (Mostert et al. 2006a; Essakhi et al. 2008; Gramaje et al 2009; Gramaje et al. 2014; Úrbez-Torres et al. 2014). To date, 42 species were identified based on morphological along with molecular characters, most linked to *Togninia* teleomorphs (Gramaje et al. 2014; MycoBank 2014; Úrbez-Torres et al. 2014).

Phaeoacremonium species have worldwide distribution and wide host range, including woody plants, insect larvae and humans (Mostert et al., 2006a). Twenty-seven species have been isolated from grapevine (Úrbez-Torres et al. 2014). Of these, *Pm. aleophilum* W. Gams, Crous, M.J. Wingf. & Mugnai appears to be the most widely distributed and the most common in grapevines (Crous et al. 1996; Larignon and Dubos 1997; Mugnai et al 1999; Groenewald et al. 2001; Mostert et al. 2006b; Essakhi et al. 2008; Martín et al. 2014). Other species that have also been isolated in relatively high frequencies from grapevines include *Pm. parasiticum* (Ajello, Georg & C.J.K Wang) W. Gams, Crous & M.J. Wingf. and *Pm. viticola* J. Dupont (Mostert et al. 2006b). In Brazil, three species were reported in grapevine, *Pm. aleophilum*, *Pm. parasiticum* (Correia et al. 2013) and *Pm. angustius* W. Gams, Crous & M.J. Wingf. (Gava et al. 2010).

The increasing economic importance of Petri disease caused by *Phaeoacremonium* in grapevines and the recent discovery of several new species of this fungus associated with other plants (Gramaje et al. 2014) led us to question what species of *Phaeoacremonium* may be associated with table grape Petri disease in São Francisco, Siriji and Baixo Jaguaribe Valleys, Northeastern Brazil. The disease etiology is crucial for epidemiological studies and for a better understanding of the distribution and importance of individual species, as well as finding effective management strategies for each pathogen. Therefore, the objectives of this study were (a) to identify species of *Phaeoacremonium* associated with Petri disease of table grapes in São Francisco, Siriji and Baixo Jaguaribe Valleys, (b) to investigate the distribution of the species in these regions and (c) to evaluate their pathogenicity and virulence in excised green shoots of table grapes.

Materials and methods

Sampling and isolation of fungi

During 2012, samples of table grape plants showing Petri disease symptoms were obtained from 12 vineyards located in the São Francisco, Siriji and Baixo Jaguaribe Valleys (Northeastern Brazil) (Fig. 1). These regions are distant at least 500 km from each other. In each vineyard, 10 grapevines shoots exhibiting Petri disease symptoms were sampled for fungal isolations. Symptomatic wood fragments taken from the margin of internal necroses and brown-black vascular streaking were washed under running tap water, surface-disinfected

for 1 min in a 1.5% sodium hypochlorite solution, and washed twice with sterile distilled water. Small pieces (4–5 mm) of tissue were taken from the margin between necrotic and apparently healthy tissue and plated onto malt extract agar (MEA; Acumedia, Lansing, USA) amended with 0.5 g l^{-1} streptomycin sulfate (MEAS). Plates were incubated at 25 ºC in the dark for 14 to 21 days. Fungal colonies emerging from plant tissue pieces, characterized by having flat slow-growing cultures on MEA and that were morphologically similar to species of *Phaeoacremonium* (Mostert et al. 2006a) were transferred to potato dextrose agar (PDA; Acumedia) plates and incubated at 25 °C in the dark, with observation after 14 to 21 days. Single-spore cultures were obtained using the procedure described by Goh (1999). Isolates were morphologically identified as *Phaeoacremonium* based on typical characteristics of the genus, namely presence of different types of phialides variable in size and shape observed in the aerial mycelium, and either discrete or integrated in conidiophores, and conidia hyaline, sporulation abundant and aseptate (Mostert et al. 2006a). Pure cultures were stored in sterilized water in Eppendorf tubes at 25 °C in the dark and stock cultures were stored in PDA slants at 5 °C in the dark.

DNA isolation, PCR amplification and sequencing

Using a sterile 10 µl pipette tip, a small amount of aerial mycelium was scraped from the surface of a 7 day old culture on PDA at 25 °C and genomic DNA was extracted using the AxyPrep™ Multisource Genomic DNA Miniprep Kit (Axygen Scientific Inc., Union City, USA) following the manufacturer's instructions. DNA was viewed on 0.8 % agarose gels stained with ethidium bromide (0.5 µg ml^{-1}) for 1 min and stored at -20 ºC. Portions of the β-tubulin (BT) and actin (ACT) genes of *Phaeoacremonium* isolates were amplified as described by Mostert et al. (2006a) using primer sets T1 (O'Donnell and Cigelnik 1997) and Bt2b (Glass and Donaldson 1995), and ACT-512F and ACT-783R, (Carbone and Kohn 1999), respectively. Each 50 µl polymerase chain reaction (PCR) mixture included 21 µl of PCR-grade water, 1 µl of DNA template, 1.5 µM of each primer, and 1 µl of PCR Master Mix (2X) (0.05 u $µl^{-1}$ de *Taq* DNA polimerase, reaction buffer, 4 mM MgCl2, 0.4 mM of each dNTP; Thermo Scientific,Waltham, USA). PCR reactions were carried out in a thermal cycler (Biocycler MJ 96; Applied Biosystems, Foster City, USA). The cycling parameters for ACT gene consisted of a denaturation step at 96 ºC for 5 min, followed by 35 cycles at 94 ºC for 30 s, 52 ºC for 30 s, 72 ºC for 80 s and final cycle at 72 ºC for 7 min. The cycling parameters for BT gene were initiated at 96 ºC for 5 min followed by 36 cycles at 94 ºC for 30 s, 58 ºC for 30

s, 72 ºC for 80 s and final cycle at 72 ºC for 7 min (Graham et al. 2009). The PCR amplification products were separated by electrophoresis in 1.5 % agarose gels in 1.0× Tris-acetate acid EDTA (TAE) buffer and were photographed under UV light after staining with ethidium bromide (0.5 μg ml^{-1}) for 1 min. PCR products were purified using the AxyPrep™ PCR Cleanup Kit (Axygen) following the manufacturer's instructions and sequenced in both directions using a ABI 3730 XL DNA Analyzer (Applied Biosystems) at the Macrogen Inc. (Seoul, Korea).

Phylogenetic analysis

Sequences were aligned with ClustalX v. 1.83 (Thompson et al. 1997), using the following parameters: pairwise alignment parameters (gap opening = 10, gap extension = 0.1) and multiple alignment parameters (gap opening = 10, gap extension = 0.2, transition weight = 0.5, delay divergent sequences = 25 %). Alignments were checked and manual adjustments were made where necessary. Phylogenetic information contained in indels (gaps) was incorporated into the phylogenetic analyses using simple indel coding as implemented by GapCoder (Young and Healy 2003). Sequences of *Phaeoacremonium* type strains obtained from GenBank were included in the analyses (Table 1). *Pleurostomophora richardsiae* (Nannf.) (CBS 270.33) was used as outgroup.

Phylogenetic analysis for maximum-parsimony (MP) was performed using PAUP v. 4.0b10 (Swofford 2003). The heuristic search option with 1000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm was used. All characters were unordered and of equal weight and gaps were treated as missing data. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated from 1 000 bootstrap replications (Hillis and Bull 1993). Other measures used were consistency index (CI), retention index (RI) and homoplasy index (HI). Trees were sampled every 1000th generation for a total of 10 000 trees. The first 1 000 trees were discarded as the burn-in phase of each analysis. Posterior probabilities (Rannala and Yang 1996) were determined from a majority-rule consensus tree generated with the remaining 9 000 trees. This analysis was repeated four times starting from different random trees to ensure trees from the same tree space were sampled during each analysis.

Phylogenetic trees were viewed with Treeview (Page 1996). Sequences generated in this study were deposited in GenBank (Table 1). Representative isolates of different

Phaeoacremonium species obtained in this study were deposited in the Culture Collection of Phytopathogenic Fungi “Prof. Maria Menezes” (CMM) at the Universidade Federal Rural de Pernambuco (Recife, Brazil).

Morphological and cultural characterization

Morphological characters used to distinguish *Phaeoacremonium* species included conidiophore morphology, phialide type and shape, size of hyphal warts and conidial size and shape (Mostert et al. 2006a). Colony characters and pigment production on MEA, PDA and oatmeal agar (OA; Difco, Detroit, USA) incubated at 25 °C in the dark were noted after 8 and 16 days. Colony colours were recorded with the colour charts of Rayner (1970). Conidia and other structures were mounted in 100 % lactic acid and digital images recorded with a Leica DFC320 camera on a Leica DMR HC microscope fitted with Nomarski differential interference contrast optics (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK). The length and width of 20 conidiophores, 30 phialides and 50 conidia per isolate were measured with the Leica IM500 measurement module. Mean and standard errors of the measurements, including mean length to width ratio (L/W) of the conidial measurements were calculated.

Isolates were also used to determine the effect of temperature on colony growth of different species. A 5-mm-diameter mycelial plug from the growing margin of a 8-day-old colony was placed in the center of a 90-mm-diameter PDA plate, and three replicates of each isolate were incubated at temperatures ranging from 10 °C to 40 °C in 5 °C intervals in the dark. After 8 and 16-days incubation periods, the colony diameters (mm) were measured in two perpendicular directions. The experiment was done twice. Colony diameters at 16 days were plotted against temperature and a curve was fitted by a cubic polynomial regression ($y=a+bx+cx^2+dx^3$). Optimal temperature was estimated from the regression equation and numeric summary with TableCurve™ 2D v. 5.01 (SYSTAT Software Inc., Chicago, USA). Optimum temperature was defined as the temperature that produced the maximum mycelial growth. The colony diameter data at 25 °C were used to calculate the mycelial growth rate (mm day^{-1}). One-way analyses of variance (ANOVA) were conducted with data obtained from optimum temperature and mycelial growth rate experiments, and means were compared by Fisher’s least significant difference (LSD) test at the 5 % significance level using STATISTIX v. 9.0 (Analytical Software, Tallahassee, USA).

Distribution of *Phaeoacremonium* species

Based on the number of isolates of each *Phaeoacremonium* species recorded, the relative frequency of each species in relation to overall number of isolates and to the total number of isolates within each table grape population was calculated (Zak and Willig 2004).

Pathogenicity and virulence on detached green shoots

Detached green shoots of cultivar Isabel were used to investigate the pathogenicity and virulence of the three *Phaeoacremonium* species found on table grapes in Northeastern Brazil. Asymptomatic branches of plants not sprayed with fungicides were collected in a commercial vineyard in São Vicente Férrer (Siriji Valley). The shoots were immediately placed into large plastic containers filled with sterile water, with the shoots placed over a plastic grid. The plastic containers were partially sealed with plastic bags and transported to Universidade Federal Rural de Pernambuco. The cut ends were dipped in wax and in the centre of each shoot (30 cm long) a superficial wound (~4-mm length, 2-mm deep) was made using a sterilized scalpel. It was inoculated with a mycelial plug (4 mm in diameter) removed from the margin of a 12-day-old PDA culture of each isolate. Non-colonized PDA agar plugs were used as negative controls. The inoculated area was wrapped with Parafilm (Pechiney Co., Chicago, USA) to prevent rapid dehydration. Inoculated shoots were placed in large plastic containers, as described above, and incubated at 25 °C and 12-h photoperiod in a growth chamber. After 23 days, the Parafilm was removed, the shoots were sliced through lengthwise and the internal lesions visually observed. The isolates were considered pathogenic when the lesioned area advanced beyond the 4-mm diameter inoculated area. The virulence of the isolates was evaluated by measurement of the lesion lengths with a digital calliper (Mitutoyo Co., Kanagawa, Japan). The experiment was arranged in a completely randomized design with ten replicates per treatment (isolate) and one shoot per replicate. The experiment was conducted twice. Differences in virulence caused by *Phaeoacremoniuma* species were determined by one-way ANOVA and means were compared by LSD test at the 5 % significance level using STATISTIX v. 9.0 (Analytical Software, Tallahassee, USA).

Results

Twenty-two isolates of *Phaeoacremonium* were obtained from table grape plants showing Petri disease symptoms in Northeastern Brazil. The fungal genus was identified based on the morphological characteristics. The species of these isolates were identified by DNA sequencing and phylogenetic analyses, with further detailed analysis of morphological and cultural characteristics. Finally, the pathogenicity and virulence of the isolates were evaluated in detached green shoots.

DNA sequencing and phylogenetic analyses

The isolates of *Phaeoacremonium* were identified based on MP phylogenetic analysis of the partial β-tubulin (BT) and actin (ACT) genes. The combined BT and ACT data set consisted of 67 taxa, including one outgroup taxa. The alignment contained 933 characters, of which 254 were constant while 674 were variable and parsimony uninformative. A heuristic search of the remaining 134 parsimony-informative characters generated three equally parsimonious trees (Length = 1,389, CI = 0.419; RI = 0.795; RC = 0.359), of which one is shown in Figure 2. Sequences of ex-type isolates of *Phaeoacremonium* species from GenBank were included in the analysis together with isolates obtained in this study (Table 1). The combined dataset resulted in three well supported clades of which two clades corresponded to previously described *Phaeoacremonium* species. The first clade with 15 isolates clustered with *Pm. aleophilum* (STEU 6991 and Y108022z), with 100 % bootstrap support. The second clade with three isolates (CMM 4315, CMM 4320 and CMM 4321) clustered with *Pm. parasiticum* (CBS 101007 and STEU 6993), with 100 % bootstrap support. The third clade with four isolates (CMM 4312, CMM 4313, CMM 4314 and CMM 4344) did not cluster with any known *Phaeoacremonium* species, with 100 % bootstrap support (Fig. 2).

Morphological and cultural characterization

No teleomorph structure was observed during this study. All species showed morphological features typical of the genus *Phaeoacremonium*, namely presence of different types of phialides observed in the aerial mycelium, and either discrete or integrated in conidiophores, and conidia hyaline and aseptate (Mostert et al. 2006a). The morphological characteristics observed in *Pm. aleophilum* were: *colony* colour on MEA honey-brown or beige; *yellow*

pigment not produced on MEA and PDA; *mycelium* texture mostly verruculose; *conidiophore* structure mostly short and usually unbranhed, 18.9–36.5 × 1.9–2.9 μm ($\bar{x}$ = 23.9 ± 1.7 × 2.4 ± 0.4 μm; n = 32); *conidiogenous cells* phialides predominant type II and III; type I phialides cylindrical, 2.5–4.9 × 0.6–1.5 μm ($\bar{x}$ = 4.1 ± 0.7 × 1 ± 0.3 μm; n = 38); type II phialides mostly elongate-ampulliform and attenuated at the base, 6.6–11 × 1.3–2.7 μm ($\bar{x}$ = 9.6 ± 1.7 × 1.8 ± 0.5 μm; n = 66); type III phialides subcylindrical or elongate-ampulliform and attenuated at the base, 11–19.5 × 1.7–2.6 μm ($\bar{x}$ = 14.9 ± 2.1 ×2.1 ± 0.4 μm; n = 52); *conidia* hyaline, mostly oblong-ellipsoida or cylindrical, occasionally reniform, 3.1–7 × 1.3–3.4 μm ($\bar{x}$ = 4.8 ± 0.9 × 2.1 ± 0.3 μm; n = 165), L/W ratio = 2.3. The morphological characteristics observed in *Pm. parasiticum* were: *colony* colour on MEA brown with medium brown center; *yellow pigment* produced on MEA and PDA; *mycelium* texture verrucose; *conidiophore* structure mostly long and branhed, 27.2–56.1 × 2–3.4 μm ($\bar{x}$ = 39.1 ± 8.2 × 2.6 ± 0.5 μm; n = 34); *conidiogenous cells* phialides predominant type III; type I phialides cylindrical, occasionally widened at the base, 5.7–6.4 × 1.1–2.2 μm ($\bar{x}$ = 6.0 ± 0.3 × 1.6 ± 0.5 μm ; n = 32); type II phialides subcylindrical, tapering toward the apex, 12–24.3 × 1.4–2.6 μm ($\bar{x}$ = 17.4 ± 3.0 × 2.1 ± 0.4 μm ; n = 42); type III phialides mostly cylindrical to subulate, 22–40.7 × 2.4–3.6 μm ($\bar{x}$ = 29.9 ± 6.3 × 2.9 ± 0.4 μm ; n = 62); *conidia* hyaline, mostly oblong-ellipsoid, sometimes allantoid to broadly oblong, 2.7–6.3 × 1.3–2.8 μm ($\bar{x}$ = 4 ± 0.7 × 1.8 ± 0.3 μm; n = 134), L/W ratio = 2.2.

All species of *Phaeoacremonium* used in this study grew at temperatures ranging from 10 °C to 35 °C. Only one isolate of *Pm. aleophilum* (CMM 4322) and two isolates of *P. parasiticum* (CMM 4315 and CMM 4321) grew at 40 °C. There were no significant differences (P>0.05) among the *Phaeoacremonium* species in relation to mycelial growth rate (0.22–0.25 mm day^{-1}) and optimum temperature for mycelial growth (29.6–30.1 °C).

Taxonomy

Based on the DNA sequence analyses and morphological characters, one species of *Phaeoacremonium* proved distinct from known species, and is described below.

Phaeoacremonium nordesticola M.A. Silva, M.P.S. Câmara & S.J. Michereff **sp. nov.** MycoBank: xxxxxxx; Figs. 3a–n

Etymology: The name refers to Northeastern Brazilian region, where this species was first found.

Description: colonies on PDA at first hazel (17’’’i) to olivaceous buff (21’’’d), becoming greenish glaucous (23’’’i) and olivaceous (21’’k) at the surface with the reverse side of the colonies olivaceous buff (21’’’d) after 8 days in the dark at 25 °C. Colonies reaching a radius of 16.7 ± 1.7 mm after 8 days in the dark at 25 ºC on MEA. No pigment produced on MEA and PDA. Optimum temperature for mycelial growth: 29.7 ± 0.9 °C. Mycelial growth rate at 25 ºC: 0.22 ± 0.02 mm day^{-1}. *Aerial mycelium* consisting of branched, septate hyphae that occurs singly or in bundles of up to 8; hyphae without warts. *Conidiophores* mostly short, usually unbranched, 18.9–36.5 × 1.90–2.9 μm ($\bar{x}$ = 23.9 ± 1.7 × 2.4 ± 0.4 μm; n = 46). *Conidiogenous cells* phialides predominant type II, terminal or lateral, mostly monophialidic; type I phialides cylindrical, 2.5–4.9 × 0.6–1.5 μm ($\bar{x}$ = 4.0 ± 0.4 × 1.1 ± 0.2 μm; n = 36); type II phialides mostly elongate-ampulliform and attenuated at the base, 6.6–11 × 1.3–2.7 μm ($\bar{x}$ = 9.4 ± 1.0 × 1.9 ± 0.4 μm, *n* = 58); type III phialides cylindrical to subcylindrical, 11–19.5 × 1.6–2.6 μm ($\bar{x}$ = 14.9 ± 21 ×1.9 ± 0.4 μm; n = 42); *Conidia* hyaline, mostly allantoid, few reniform, 2.9–7.5 × 1.1–2.4 μm ($\bar{x}$ = 4.2 ± 0.9 ×1.7 ± 0.3 μm; n = 178), L/W ratio = 2.6.

Sexual morph: not produced in culture.

Substrate: *Vitis vinifera*

Known Distribution: Brazil (Ceará, Pernambuco).

Holotype: Brazil, Pernambuco, Petrolina, isolated from *Vitis vinifera* tree, 2012, coll. M.A. Silva (holotype living culture CMM 4312; ex-type in URM xxxxxxx; isotype in MFLU and ex-type culture in MFLUCC xxxxxxx).

Additional cultures examined: Brazil, Pernambuco, Petrolina, isolated from *Vitis vinifera* trees, 2012, coll. M.A. Silva, CMM 4313 and CMM 4314; Brazil, Ceará, Russas, isolated from *Vitis vinifera* tree, 2012, coll. M.A. Silva, CMM 4334.

Notes – Phylogenetically *Pm. nordesticola* is closely related to *Pm. luteum* D. Gramaje, T.I. Burgess & J. Armengol, but cultures of *Pm. luteum* produced yellow pigment on MEA, PDA and OA, which did not occur with *Pm. nordesticola*. The mycelial growth of *Pm. nordesticola* in the dark at 25 °C after 8 days ($\bar{x}$ = 16.7 mm) was higher that of *Pm. luteum* (4.5-5 mm). Phialides type I (4–6 × 1.5–2 μm), type II (10.5–17 × 2–3.5 μm) and type III (20–30 × 2–3 μm), and conidia (4–6 × 2–3 μm; $\bar{x}$ = 5 × 2.5 μm) of *Pm. luteum* are longer and wider than those of *Pm. nordesticola* (type I phialides: 2.5–4.9 × 0.6–1.5 μm; type II phialides: 6.6–11 × 1.3–2.7 μm; type III phialides: 11–19.5 × 1.6–2.6 μm; conidia: 2.9–7.5 × 1.1–2.4 μm, $\bar{x}$ = 4.23 ×1.65 μm). The conidia L/W ratio of *Pm. luteum* (2.2) is smaller that of *Pm. nordesticola* (2.6). *Phaeoacremonium nordesticola* differs from its closest phylogenetic neighbor, *Pm. luteum*, by unique fixed alleles in one loci based on alignments of the separate loci deposited

in TreeBase as study S16135: alignments and β-tubulin positions 118 (T), 242 (G), 361 (C), 508 (A) and Actin positions 2 (A), 13 (C), 59 (C), 102 (A), 745 (T) and 259 (A).

Distribution of *Phaeoacremonium* species

Phaeoacremonium aleophilum was the predominant species isolated from table grape trees (68.2 %), followed by *Pm. nordesticola* (18.2 %) and *Pm. parasiticum* (13.6 %). The distribution of *Phaeoacremonium* species differed between the three regions of the Northeastern Brazil. Only in São Francisco Valley the three *Phaeoacremonium* species were found. In Siriji Valley was found *Pm. aleophilum* and *Pm. parasiticum* (Fig. 4). In Baixo Jaguaribe Valley was obtained a single isolate and identified as *Pm. nordesticola*.

Pathogenicity and virulence in detached green shoots

All isolates of *Phaeoacremonium* species were pathogenic to detached green shoots of grapevine, resulting in visible lesions 23 days after inoculation. The symptoms observed both on the surface and internally were necrotic lesions brown dark which extended upward and downward from the point of inoculation. There were significant ($P \leq 0.05$) differences in virulence among the species. *Phaeoacremonium aleophilum* was the most virulent, causing the largest lesion (10.9 ± 1.1 mm), while *Pm. nordesticola* was the less virulent, causing the smaller lesion (8.6 ± 0.6 mm). *Phaeoacremonium parasiticum* showed intermediate virulence, causing lesion of 9.5 ± 0.9 mm.

Discussion

Table grape trunk diseases and the associated pathogens have been little studied in Brazil. This study constitutes the first attempt to assess the diversity of *Phaeoacremonium* species on table grapes showing Petri disease symptoms in Brazil. Species identification was based on morphological characters and analysis of partial sequences of actin and β-tubulin genes. Three species were identified, namely *Pm. aleophilum*, *Pm. parasiticum*, and new species *Pm. nordesticola*. The colony characteristics and conidiophores, phialides and conidia dimensions of first two species obtained in this study were similar to those previously described in the literature (Mostert et al. 2006a).

Since 1996, when the genus *Phaeoacremonium* was first identified (Crous et al. 1996), 27 species of *Phaeoacremonium* had been isolated from grapevines and identified based on their cultural, morphological, and molecular characters (Crous et al. 1996; Dupont et al. 2000; Groenewald et al. 2001; Mostert et al. 2005; Mostert et al. 2006b; Essakhi et al. 2008; Graham et al 2009; Gramaje et al. 2009; Úrbez-Torres et al. 2014). Interestingly, the majority of these species (21 of 27) have been identified within the last 8 years, which may be a response to a significant upsurge in the number of field surveys conducted since the early 2000s spurred by increases in Petri disease incidences in grape growing regions worldwide. Discovery of a novel species in the present study increases to 28 and 43, respectively, the total number of *Phaeoacremonium* species occurring in grapevine and in this genus.

Phaeoacremonium nordesticola is recognized as a new species in the genus *Phaeoacremonium*, closely related to *Pm. luteum*. However, six nucleotides in the actin gene and four nucleotides in the β-tubulin gene distinguish *Pm. nordesticola* from *Pm. luteum*. Considering the phylogenetic data, the table grape isolates of *Pm. nordesticola* formed a clade strongly supported in the maximum-parsimony analysis (100%). *Phaeoacremonium nordesticola* can also be distinguished from *Pm. luteum* based on colony characteristics, mycelial growth and phialides dimensions described for this species (Gramaje et al. 2014). *Phaeoacremonium nordesticola* not produced yellow pigment on MEA, PDA or OA, which occurs with from *Pm. luteum*. Mycelial growth of *Pm. nordesticola* was higher that of *Pm. luteum*. Phialides type I, II and III of *Pm. luteum* are longer and wider than those of *Pm. nordesticola*. The conidia L/W ratio of *Pm. luteum* is smaller that of *Pm. nordesticola*.

In this work, *Pm. aleophilum* was the most frequently isolated species associated with Petri disease of table grape, and also the most widespread species in vineyards of Northeastern Brazil. This species had been reported in table grapes in Northeastern Brazil (Correia et al. 2013), being recognized as the most common species on grapevines worldwide (Mostert *et al.* 2006b; Essakhi *et al.* 2008; Martín et al. 2014; Úrbez-Torres et al. 2014). Besides Brazil, *Pm. aleophilum* was also registered in Algeria (Berraf-Tebbal et al. 2011), Argentina (Dupont et al. 2002), Australia (Pascoe and Cottral 2000), Austria (Reisenzein et al. 2000), Chile (Auger et al. 2005), France (Larignon and Dubos 1997), Germany (Fischer and Kassemeyer, 2003), Iran (Arzanlou et al. 2013), Italy (Mugnai et al. 1996), New Zealand (Mundy and Manning 2010), Serbia (Crous et al. 1996), South Africa (Crous et al. 1996; Groenewald *et al.*, 2001), Spain (Armengol et al. 2001), Turkey (Ari 2000), USA (Schek et al. 1998; Groenewald et al. 2001) and Uruguay (Abreo et al. 2011). This species has been isolated from other host plants than grapevine, including *Actinidia chinensis* Planch. (Crous

and Gams 2000), *Malus domestica* Bork. (Cloete et al. 2011), *Olea europea* L. (Crous and Gams 2000), *Phoenix dactylifera* L. (Mohammadi 2014), *Prunus armeniaca* L. (Damm et al. 2008), *Prunus persica* (L.) Batsch. (Damm et al. 2008), *Prunus salicina* Lindl. (Damm et al. 2008), *Prunus pennsylvanica* L. (Hausner et al. 1992), *Pyrus communis* L. (Cloete et al. 2011) and *Salix* sp. (Hausner et al. 1992).

The other two species registered in this work, *Pm. parasiticum* and *Pm. nordesticola*, were isolated with similar frequency (18.2 %) from table grapes with Petri disease symptoms. *Phaeoacremonium parasiticum* is isolated from grapevines in relatively high frequencies (Dupont et al. 2002; Mostert et al. 2006b) and had been reported in table grapes in Northeastern Brazil (Correia et al. 2013). Besides Brazil, *Pm. parasiticum* was also registered in Algeria (Berraf-Tebbal et al. 2011), Argentina (Dupont et al. 2002), Australia (Mostert et al. 2005), Chile (Auger et al. 2005), Italy (Essakhi et al. 2008), Iran **(**Mohammadi et al. 2013), Peru (Romero-Rivas et al. 2009), South Africa (Crous et al. 1996), Spain (Aroca et al. 2006), Turkey (Dupont et al. 2000) and USA (Rolshausen et al. 2010). It is also found on other woody hosts as an endophyte or as agent of plant disease, including *A. chinensis* (Di Marco et al. 2004), *Aquilaria agallocha* Lamk. (Mostert et al. 2006b), *Cupressus* sp. (Mostert et al. 2006b), *Nectandra* sp. (Hawksworth et al. 1976), *O. europea* (Nigro et al. 2013), *P. dactylifera* (Hawksworth et al. 1976), *P. armeniaca* (Hawksworth et al. 1976), *Prunus avium* L. (Rumbos 1986), *Quercus virginiana* Mill. (Halliwell 1966) and *Santalum album* L. (Gramaje et al. 2014). *Phaeoacremonium parasiticum* have also been associated with human infections, often causing phaeohyphomycosis (lumps of fungal growth under the skin) (Crous et al., 1996; Mostert et al. 2005; Mostert et al. 2006a). Species of *Phaeoacremonium* are opportunistic pathogens needing a subcutaneous traumatic inoculation or predisposed host to be able to infect and cause disease (Mostert et al. 2006b).

Regarding cultural characteristics, the optimum temperature for mycelial growth for *Phaeoacremonium* species from table grape varied between 29.6 and 30.1 °C, and some isolates of *Pm. aleophilum* and *Pm. parasiticum* grew at 40 °C, corroborating with information from the literature (Mostert et al. 2006a). The growth of *Pm. parasiticum* at 10 °C observed in this study contrast to information that the minimum temperature for this species is 15 °C (Mostert et al. 2006a). As can be observed, cultural characteristics may vary among isolates of the same species and therefore are of limited value in the determination of species.

Although many new *Phaeoacremonium* species have been identified from Petri disease infected plants, there is a lack of evidence for pathogenicity in most studies (Dupont et al. 2000; Groenewald et al. 2001; Mostert et al. 2006b ; Mostert et al. 2006b; Essakhi et al.

2008; Gramaje et al. 2009), making it difficult to evaluate whether the majority of *Phaeoacremonium* species are involved in disease development. All isolates of *Phaeoacremonium* species in this study were able to infect, colonize and produce lesions in detached grapevine shoots, confirming their status as Petri disease pathogens, including the new species *Pm. nordesticola*. Differences in virulence among *Phaeoacremonium* species inoculated in grapevine, as observed in this study, have been reported previously (Mostert et al. 2006b; Halleen et al 2007; Aroca and Raposo 2009; Úrbez-Torres et al. 2014).

This paper reports three species of the genus *Phaeoacremonium* associated with Petri disease of table grape in Northeastern Brazil. All the species found in Northeastern Brazil have potential to cause disease to table grapes, but *Pm. aleophilum* was the most virulent species. Studies are needed on the epidemiology and impact on table grape production together with information referring to ecology, distribution, host range and fungicide sensitivity of all species of *Phaeoacremonium* found in this study.

The results of this study will certainly be crucial to a better formulation of Petri disease control strategies and genetic improvement programs in tropical viticulture.

Acknowledgments This work was financed by Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco - FACEPE (APQ 137-5.01/12) and by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (Project 203/2009 - International Cooperation CAPES-Brazil/DGU-Spain). M. P. S. Câmara, and S. J. Michereff also acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq research fellowship.

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Table 1 Isolates of *Phaeoacremonium* species used in this study

Taxon	Culture accession no.[a]	Host	Location	Collector	GenBank accession no.[b]	
					ACT	BT
Phaeoacremonium aleophilum	Y108022z	*Vitis vinifera*	Spain	L. Martin	JF275893	JF275879
Pm. aleophilum	STEU 6991	*V. vinifera*	South Africa	L. Mostert	JQ038921	JQ038910
Pm. alvesii	CBS 110034	*Homo sapiens*	Brazil	L. Mostert	AY579234	AY579301
Pm. americanum	ICMP 17421	*V. vinifera*	New Zealand	B.S. Weir	EU595463	EU596526
Pm. amstelodamense	CBS 110627	*H. sapiens*	The Netherlands	J. Bruins	AY579228	AY579295
Pm. amygdalium	Psp4	*Prunus dulcis*	Spain	D. Gramaje	JN191304	JN191308
Pm. angustius	CBS 114992	*V. vinifera*	USA	P. Laringon	DQ173127	DQ173104
Pm. argentinum	CBS 77783	*Soil*	Argentina	A. Martinez	DQ173135	DQ173108
Pm. australiense	CBS 113589	*V. vinifera*	Australia	T. Knaggs	AY579229	AY579296
Pm. austroafricanum	CBS 112949	*V. vinifera*	South Africa	L. Mostert	DQ173122	DQ173099
Pm. cinereum	Pm5	*V. vinifera*	Iran	H. Mohammadi	FJ517153	FJ517161
Pm. croatiense	113Pal	*V. vinifera*	Croatia	B. Cvjetković	EU863514	EU863482
Pm. fuscum	STEU 5969	*Prunus salicina*	South Africa	U. Damm	EU128141	EU128098
Pm. globosum	ICMP 16988	*Vitis* sp.	New Zealnd	U. Damm	EU595466	EU596525
Pm. griseorubrum	CBS 111657	*H. sapiens*	USA	D. Sutton	AY579227	AY579294
Pm. hispanicum	Pm8	*V. vinifera*	Spain	D. Gramaje	FJ517156	FJ517164
Pm. hungaricum	90Pal	*V. vinifera*	hungary	S. Essakhi	EU863515	EU863483
Pm. inflatipes	CBS 39171	*Quercus virginiana*	USA	R.S. Halliwell	AY579259	AF246805
Pm. iranianum	CBS 101357	*Actinidia chinensis*	Italy	F. Calzarano	DQ173120	DQ173097

Pm. krajdenii	CBS 110118	*V. vinifera*	South Africa	G. van Coller	AY579261	AY579324
Pm. luteum	A33	*Santalum album*	Australia	D. Gramaje	KJ533542	KJ533540
Pm. luteum	A34	*S. album*	Australia	D. Gramaje	KJ533543	KJ533541
Pm. mortoniae	CBS 110212	*Fraxinus pensylvanica*	USA	T.E. Hinds	DQ173136	DQ173109
Pm. noveazealandiae	CBS 114512	*Desmoschoenus spiralis*	New Zealnd	J.R. George	DQ173141	DQ173112
Pm. occidentale	ICMP 17037	*Vitis* sp.	New Zealnd	B.S. Weir	EU595460	EU596524
Pm. pallidum	STEU 6104	*Prunus armeniaca*	South Africa	U. Damm	EU128144	EU128103
Pm. parasiticum	STEU 6993	*P. armeniaca*	South Africa	Unknown	JQ038927	JQ038916
Pm. parasiticum	CBS 101007	*A. chinensis*	Italy	L. Calzarano	AY579252	AF246804
Pm. prunicola	STEU 5967	*P. salicina*	South Africa	U. Damm	EU128137	EU128095
Pm. roseum	PARC 281	*V. vinifera*	Canada	J.R. Urbez-Torres	KF764507	KF764659
Pm. rubrigenum	CBS 112046	*H. sapiens*	USA	G. Conover	AY579239	AY579305
Pm. santali	CBS 137498	*S. album*	Australia	D. Gramaje	KJ533539	KJ533535
Pm. scolyti	DUCC 407	Bark beetles	South Korea	M.W. Hyun	KC166686	KC166684
Pm. sicilianum	48Pal	*V. vinifera*	Italy	S. Essakhi	EU863520	EU863488
Pm. sphinctrophorum	CBS 69488	*H. sapiens*	USA	A.A. Padhye	DQ173143	DQ173114
Pm. subulatum	CBS 113584	*V. vinifera*	South Africa	L. Mostert	AY579231	AY579298
Pm. tardicrescens	CBS 110573	*H. sapiens*	USA	Levi	AY579233	AY579300
Pm. theobromathis	CBS 111586	*Theobroma gileri*	Ecuador	H.C. Evans	DQ173132	DQ173106
Pm. tuscanum	1Pal	*V. vinifera*	italy	S. Essakhi	EU863490	EU863458
Pm. vezenuelense	CBS 65185	*H. sapiens*	Venzuela	M.B. de Albornoz	AY579256	AY579320
Pm. viticola	Y271031d	*V. vinifera*	Spain	L. Martin	HQ700719	HQ700718
Togninia africana	STEU 6177	*P. armeniaca*	South Africa	U. Damm	EU128142	EU128100
T. griseoolivacea	STEU 5966	*P. armeniaca*	South Africa	U. Damm	EU128139	EU128097

T. vibratilis	CBS 117115	*Fagus sylvatica*	France	J. Fournier	DQ649064	DQ649063
Pleurostomophora richardsiae	CBS 27033	-	Sweden	E. Melin	AY579271	AY579334
Pm. aleophilum	CMM 4322	*Vitis labrusca*	Brazil	M.A. Silva	**This study**	**This study**
Pm. aleophilum	CMM 4325	*V. labrusca*	Brazil	M.A. Silva	**This study**	**This study**
Pm. aleophilum	CMM 4332	*V. labrusca*	Brazil	M.A. Silva	**This study**	**This study**
Pm. aleophilum	CMM 4328	*V. labrusca*	Brazil	M.A. Silva	**This study**	**This study**
Pm. aleophilum	CMM 4333	*V. labrusca*	Brazil	M.A. Silva	**This study**	**This study**
Pm. aleophilum	CMM 4326	*V. labrusca*	Brazil	M.A. Silva	**This study**	**This study**
Pm. aleophilum	CMM 4319	*V. vinifera*	Brazil	M.A. Silva	**This study**	**This study**
Pm. aleophilum	CMM 4318	*V. vinifera*	Brazil	M.A. Silva	**This study**	**This study**
Pm. aleophilum	CMM 4323	*V. labrusca*	Brazil	M.A. Silva	**This study**	**This study**
Pm. aleophilum	CMM 4329	*V. labrusca*	Brazil	M.A. Silva	**This study**	**This study**
Pm. aleophilum	CMM 4331	*V. labrusca*	Brazil	M.A. Silva	**This study**	**This study**
Pm. aleophilum	CMM 4327	*V. labrusca*	Brazil	M.A. Silva	**This study**	**This study**
Pm. aleophilum	CMM 4317	*V. vinifera*	Brazil	M.A. Silva	**This study**	**This study**
Pm. aleophilum	CMM 4316	*V. vinifera*	Brazil	M.A. Silva	**This study**	**This study**
Pm. aleophilum	CMM 4324	*V. labrusca*	Brazil	M.A. Silva	**This study**	**This study**
Pm. parasiticum	CMM 4315	*V. vinifera*	Brazil	M.A. Silva	**This study**	**This study**
Pm. parasiticum	CMM 4321	*V. vinifera*	Brazil	M.A. Silva	**This study**	**This study**
Pm. parasiticum	CMM 4320	*V. vinifera*	Brazil	M.A. Silva	**This study**	**This study**
Pm. nordesticola	CMM 4313	*V. vinifera*	Brazil	M.A. Silva	**This study**	**This study**
Pm. nordesticola	CMM 4312	*V. vinifera*	Brazil	M.A. Silva	**This study**	**This study**
Pm. nordesticola	CMM 4314	*V. vinifera*	Brazil	M.A. Silva	**This study**	**This study**
Pm. nordesticola	CMM 4334	*V. vinifera*	Brazil	M.A. Silva	**This study**	**This study**

[a] *CBS* Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; *CMM* Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes", Universidade Federal Rural de Pernambuco, Recife, Brazil; *DUCC* Dankook University Culture Colletion, Cheonan, Korea; *ICMP* International Collection of Microorganisms from Plants, Lincoln, New Zealand; *PARC* Culture Collection of the Pacific Agri-Food Research Centre, Summerland, Canada; *STEU* Culture Collection of the Department of Plant Pathology, University of Stellenbosch, Stellenbosch, South Africa

[b] Sequence numbers in ***bold*** were obtained in the present study

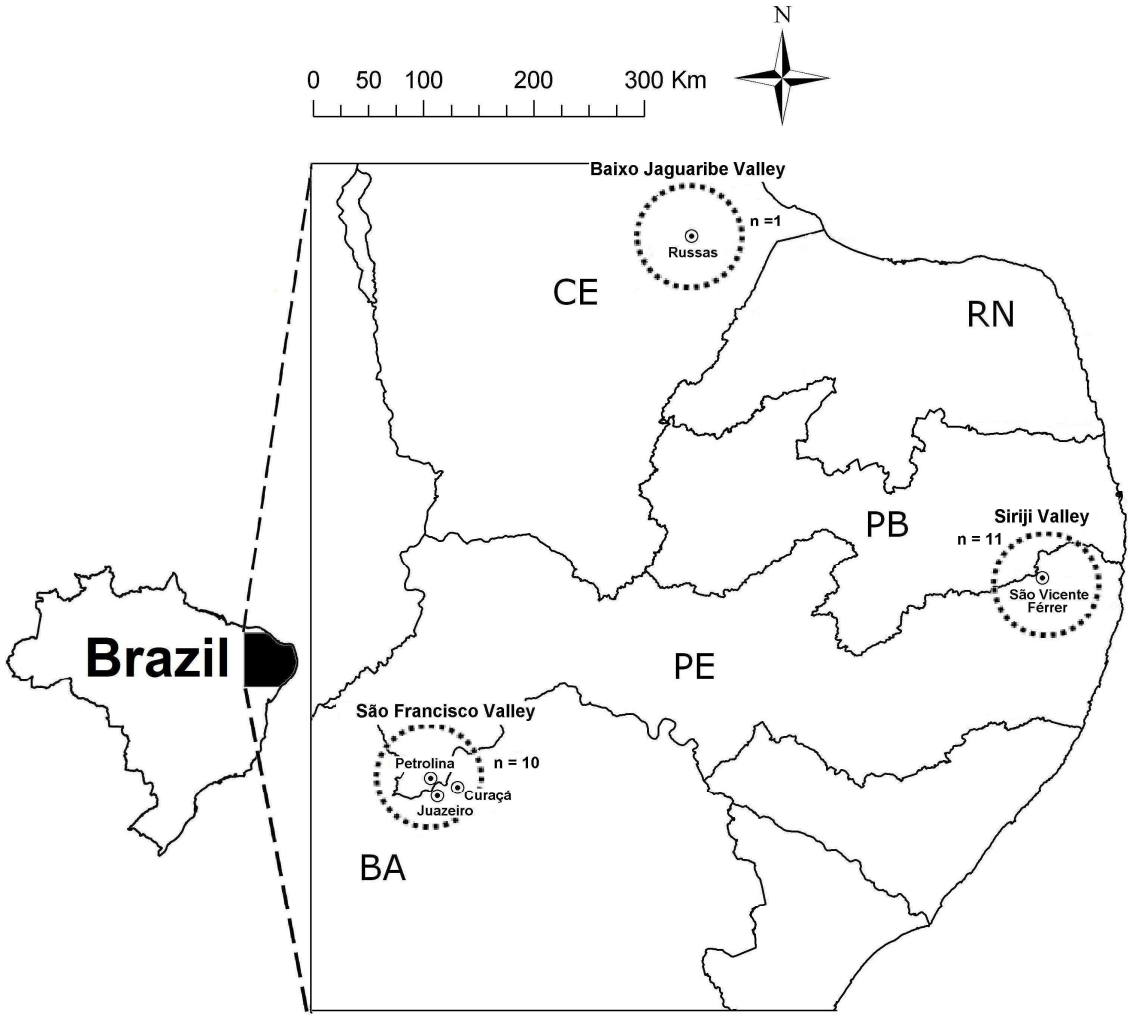

Fig. 1 Collection sites of *Phaeoacremonium* isolates from Northeastern Brazil vineyards, located in the states of Bahia (BA), Pernambuco (PE) and Ceará (CE). The names next to the dots correspond to the cities corresponding to the sampled vineyard. Dotted semicircles represent the regions defined for this study; n = number of isolates in each region

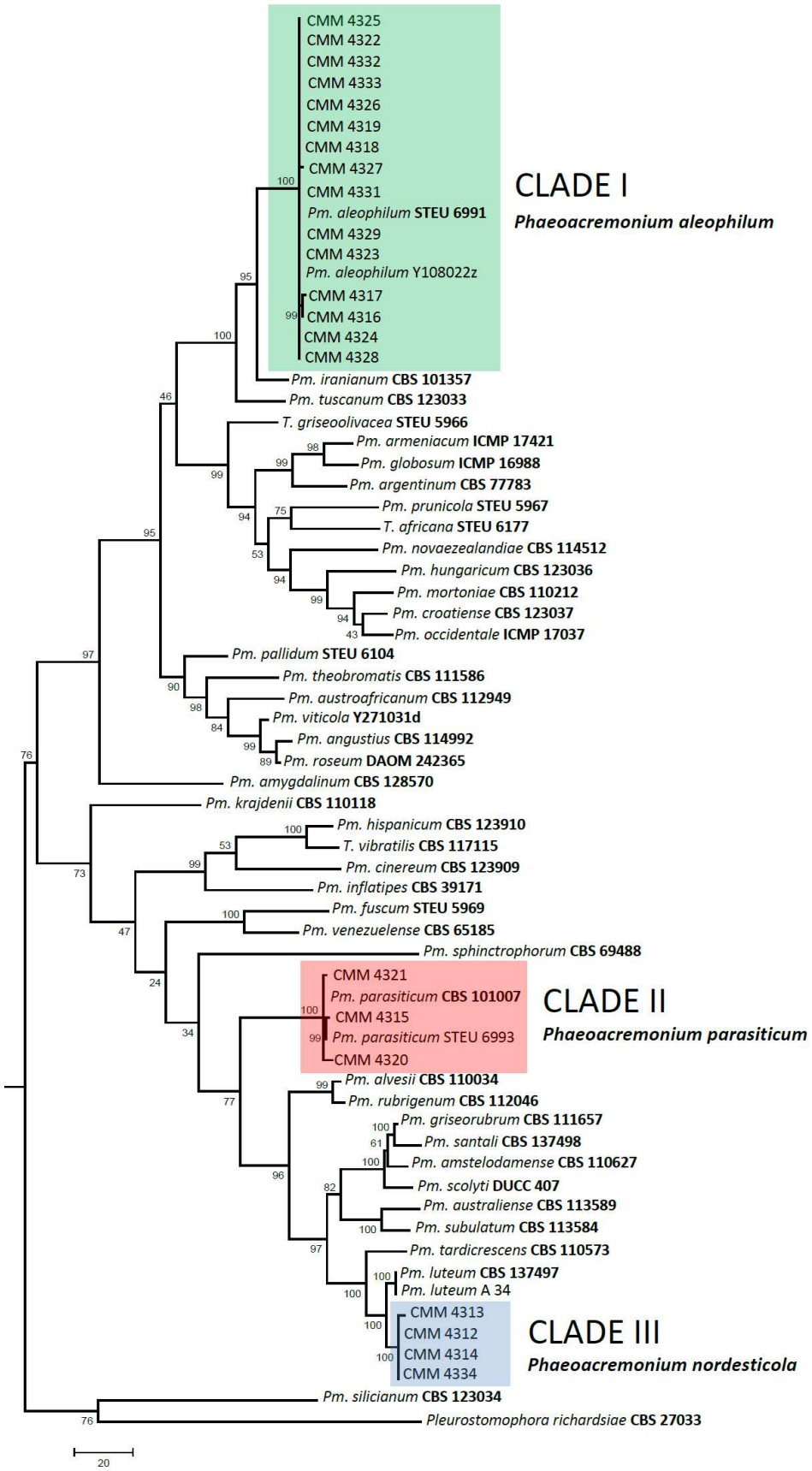

Fig. 2 One of three most parsimonious trees (Length = 1,389, CI = 0.419; RI = 0.795; RC = 0.359) obtained from combined actin (ACT) and β-tubulin (BT) sequence data from *Phaeoacremonium* isolates. Maximum parsimony bootstrap support values from 1000 replications. Ex-type isolates are in *bold*

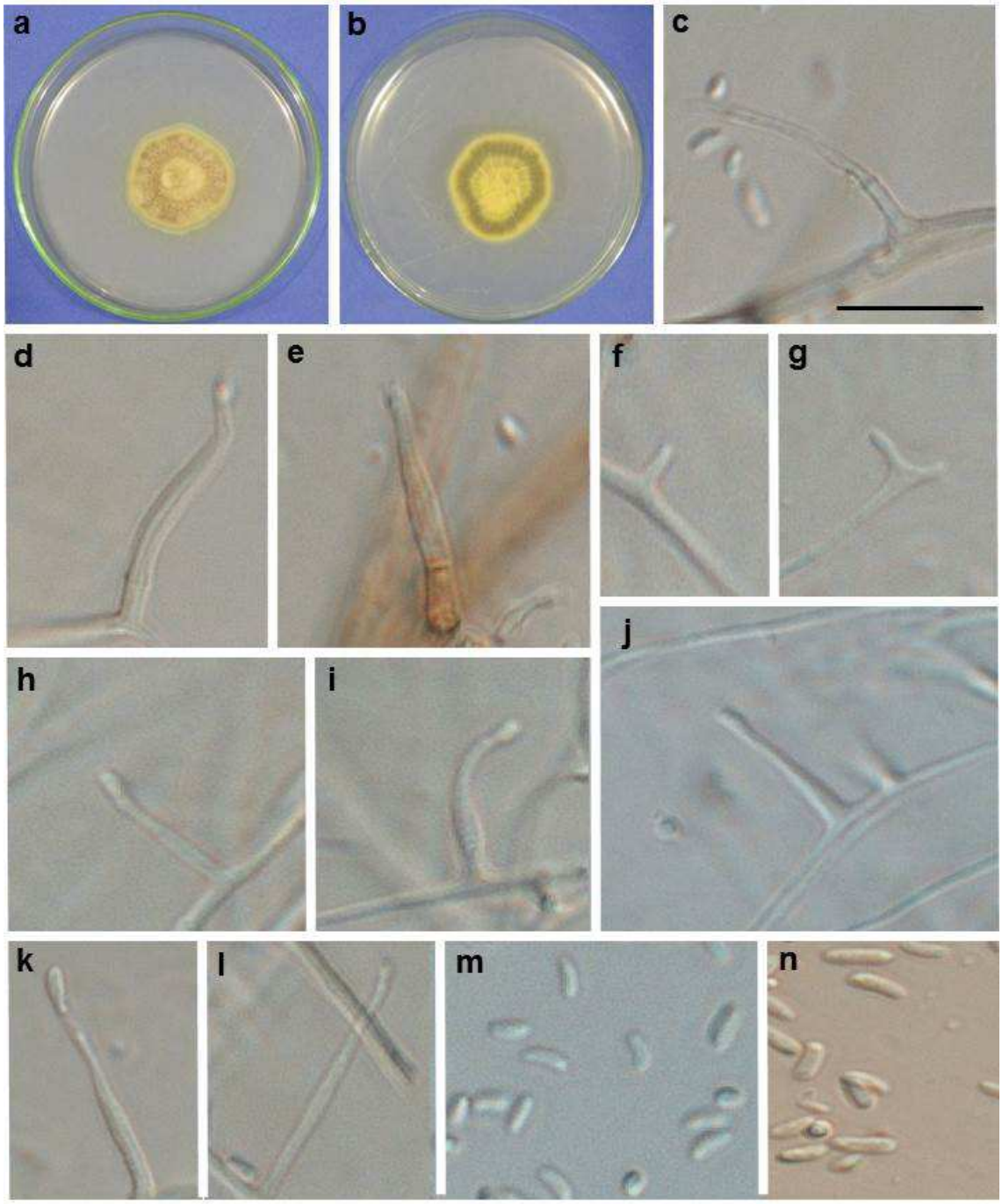

Fig. 3. *Phaeoacremonium nordesticola* holotype (culture CMM 4312). **a** Sixteen-day-old colony incubated at 25 ºC on PDA in the dark and, **b** Colony reverse (**b**). **c–e** Conidiophores. **f–g** Phialides type I. **h–j** Phialides type II. **k–l** phialides type III. **m**-**n** Conidia. Scale bars: **c–n** = 10 μm

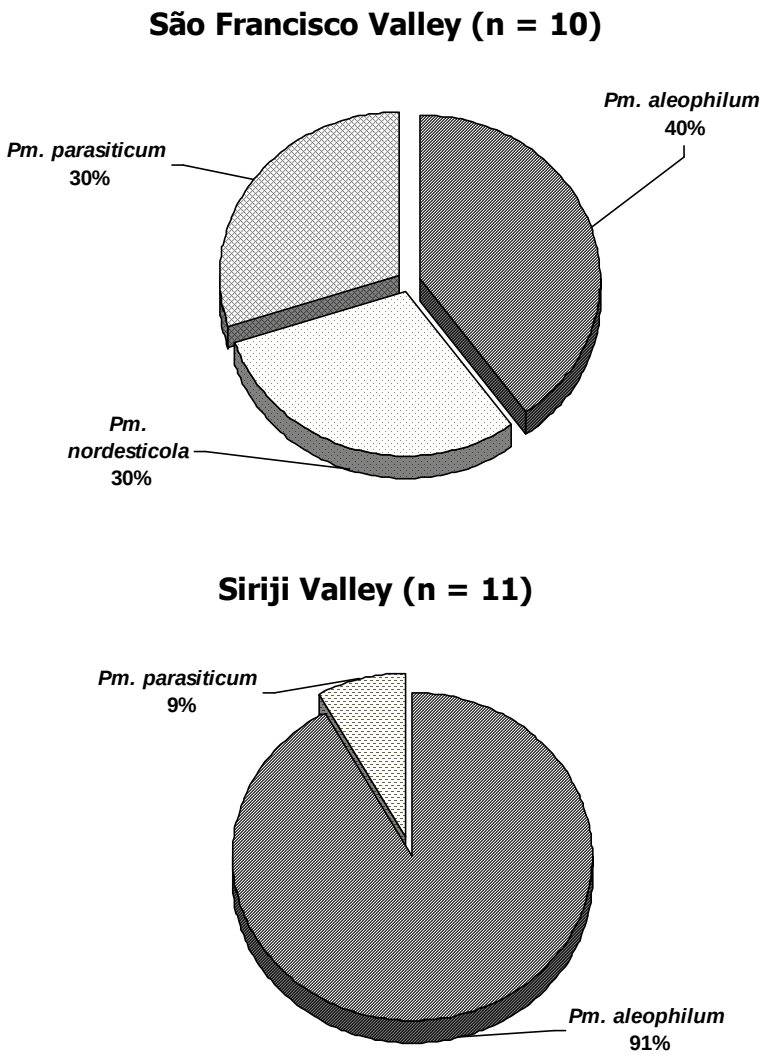

Fig. 4 Frequency (%) of *Phaeoacremonium* species associated with Petri disease of table grape in São Francisco and Siriji Valleys, Northeastern Brazil

Capítulo III

Phylogeny, distribution and pathogenicity of *Campylocarpon* species associated with black foot disease of table grape in the main Brazilian exporting region

Submissão: **Plant Pathology**
Londres, Inglaterra
JCR = 2,32

Phylogeny, distribution and pathogenicity of *Campylocarpon* species associated with black foot disease of table grape in the main Brazilian exporting region

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Abstract

Black foot is an important disease of grapevines in most of the wine-producing countries of the world. This study aims to identify and characterize species of *Campylocarpon* (*Campyl.*) associated with black foot disease of table grape in the São Francisco Valley, the main Brazilian exporting region. Thirty-eight *Campylocarpon* isolates were obtained from table grape plants showing black foot disease symptoms in 12 vineyards from five localities (Casa Nova, Curaçá, Juazeiro, Lagoa Grande and Petrolina) in the São Francisco Valley. Fungal identifications were made using a combination of morphology together with a phylogenetic analysis based on internal transcribed spacer (ITS) region of rDNA. Three species of *Campylocarpon* were identified: *Campyl. fasciculare*, *Campyl. pseudofasciculare* and *Campyl. semiaridus* sp. nov. The first two species had been reported in table grapes in Northeastern Brazil, but not in the São Francisco Valley. *Campylocarpon pseudofasciculare* was the most prevalent species. All species of *Campylocarpon* were pathogenic on detached shoots of table grape, but there were no differences in virulence among the species.

Keywords: *Vitis vinifera*, trunk disease, phylogeny, virulence

Introduction

Northeastern region is responsible for 99% of Brazilian exports of table grapes (*Vitis vinifera* L.), where 9,600 ha are cultivated. The São Francisco Valley, located in the semi-arid region of Bahia and Pernambuco states, is the main table grape growing area in the region, accounting for 98 % of the production (Lazzarotto & Fioravanço, 2013). In 2011, 59,400 t of table grapes were exported and accounting for US$ 136 million (FAO, 2014).

In the last years, a decline of table grape plants has been noticed in young vineyards of the Northeastern Brazil, and was reported the occurrence of black foot disease (Correia et al., 2013). This disease was first reported in Brazil in 1999, in vineyards of the state of Rio Grande do Sul (Southern region), under subtropical climate (Garrido et al., 2004). Although the black foot disease is known to occur in grapevines since 1961, when was registered in France (Grasso & Magnano Di San Lio, 1975), it has become a worsening problem in both nurseries and young commercial vineyards since the early 1990s (Gramaje & Armengol, 2011). Currently, black foot disease of grapevines is a serious disease in most wine and grape-

producing regions of the world, particularly in nurseries and young vineyards, responsible for the decline and death of young vines, and loss of productivity (Agustí-Brisach & Armengol, 2013).

Black foot primarily affects young grapevines (up to 8 years old), and symptoms of the disease are characterized by sunken necrotic root lesions with a reduction in root biomass and root hairs. Internally, necrotic tissue and plugged xylem vessels with black inclusion and/or tyloses can be observed in cross-section at the basal end of the trunk. Aboveground, vines can show stunted growth characterized by short shoot internodes and small chlorotic leaves, which lead to eventual death of the vines (Halleen et al., 2006; Alaniz et al., 2007; Agustí-Brisach & Armengol, 2013).

Black foot disease is caused by several fungal species in the genera *Campylocarpon* Halleen, Schroers & Crous, *Cylindrocarpon* Wollenw., *Cylindrocladiella* Boesew. and *Ilyonectria* P. Chaverri & C. Salgado (Agustí-Brisach & Armengol, 2013; Úrbez-Torres et al., 2014). Among these genera, *Campylocarpon* is one of the least studied in grapevine worldwide. The genus *Campylocarpon* was established by Halleen et al. (2004), having as main morphological characteristics: colony morphology on potato dextrose agar (PDA) presented abundant aerial mycelium, covering the whole or sectors of the colony, white to off-white or slightly brownish, thickly cottony to felty, intermingled with or giving rise to erect white or brown hyphal strands; microconidia are absent; conidiophores appear arising laterally from single or fasciculate aerial hyphae or from creeping substrate hyphae, singly or in loose or dense aggregates; macroconidia mostly curved, hyaline, with up to 6- septate, apical cell obtuse, basal cell obtuse or with inconspicuous hilum; chlamydospores rare or absent (Halleen et al., 2004). Species delimitation within *Campylocarpon* based solely on cultural and morphological characteristics can lead to misidentification, and thus molecular analyses of internal transcribed spacer (ITS) rDNA region have been used routinely for species delineation (Abreo et al., 2010). Based on morphological and molecular studies, only two species were included in the genus, without known teleomorph, *Campylocarpon fasciculare* Schroers, Halleen & Crous and *Campyl. pseudofasciculare* Halleen, Schroers & Crous (Halleen et al., 2004; Chaverri et al., 2011; MycoBank, 2014). These species are similar in several morphological characteristics, but differ regarding the presence of chlamydospores. In *Campyl. fasciculare* the chlamydospores are absent, while in *Campyl. pseudofasciculare* the chlamydospores are sparse, typically in clusters of 3–5, intercalary or borne on short side-branches, round to somewhat angular (Halleen et al., 2004).

Campylocarpon species have the grapevine as single host and were reported causing black foot disease in some countries (Farr & Rossman, 2014).The species are known to be saprobes in soil, which can occur on dead plant substrata, or act as pathogens of plants infecting roots and stems of hosts through wounds and/or natural openings. Furthermore, the production of chlamydospores by *Campyl. pseudofasciculare* may allow his survival for extended periods in soil (Agustí-Brisach & Armengol, 2013).

A field survey conducted throughout grape-growing areas of Northeastern Brazil in 2010, with few samples collected, revealed that *Campyl. fasciculare* and *Campyl. pseudofasciculare* occur in black foot symptomatic table grapes in Assú Valley, a new area of production located in Rio Grande do Norte state, but not in the São Francisco Valley, the main Brazilian table grape exporting region (Correia et al., 2013). Since large investments in vineyard establishment and maintenance were done in São Francisco Valley, and decline of young plants has been noticed, is fundamental to perform a more extensive sampling of black foot symptomatic plants and characterize the *Campylocapon* species associated with this disease. Therefore, the objective of this study were (a) to identify the *Campylocapon* species associated with black foot disease of table grapes in the main Brazilian table grape exporting region, (b) to investigate the prevalence and distribution of the species in the region and (c) to evaluate their pathogenicity and virulence in excised green shoots of table grape.

Materials and methods

Sampling and isolation of fungi

During 2012, samples of table grape plants showing black foot disease symptoms were taken from 12 vineyards located in the São Francisco Valley, Northeastern Brazil. These vineyards represented five table grape populations (Casa Nova, Curaçá, Juazeiro, Lagoa Grande and Petrolina) according to their geographical origin (Fig. 1). In each vineyard, 10 grapevines showing external symptoms of general decline without specific darkening of their xylem, associated with reduced root biomass and root hairs with sunken and necrotic root lesions, were sampled for fungal isolations. Segments of symptomatic tissues from trunk or roots were surface-disinfected by immersion in ethanol 70 % for 1 min, NaOCl 1.5 % for 2 min and washed twice in sterile distilled water. Small pieces (4–5 mm) of tissue were taken from the margin between necrotic and apparently healthy tissue and plated onto malt extract agar

(MEA) (Acumedia, Lansing, USA) amended with 0.5 g l^{-1} streptomycin sulfate (MEAS). Plates were incubated at 25 ºC in the dark until fungal colonies were observed. Colonies with white to off-white or slightly brownish cottony to felty aerial mycelium were individually transferred to potato dextrose agar (PDA) (Acumedia) plates and incubated at 25 °C in the dark. Afterwards, single-spore cultures were obtained using the procedure described by Goh (1999). Pure culture colonies were used to conduct a preliminary morphological identification of the different fungi isolated from symptomatic table grape tissue. Isolates were morphologically identified as *Campylocarpon* based on typical characteristics of the genus, namely abundant curved macroconidia, with up to 6-septate, and microconidia absent (Halleen et al., 2004). Pure cultures were stored in sterilized water in Eppendorf tubes at 25 °C in the dark and stock cultures were stored in PDA slants at 5 °C in the dark.

DNA isolation, PCR amplification and sequencing

Using a sterile 10 µl pipette tip, a small amount of aerial mycelium was scraped from the surface of a 7 day old culture on PDA at 25 °C and genomic DNA was extracted using the AxyPrep™ Multisource Genomic DNA Miniprep Kit (Axygen Scientific Inc., Union City, USA) following the manufacturer's instructions. DNA was viewed on 0.8 % agarose gels stained with ethidium bromide (0.5 µg ml^{-1}) for 1 min and stored at -20 ºC. Oligonucleotide primers ITS1 and ITS4 (White *et al*., 1990) were used to amplify the internal transcribed spacer (ITS) region of rDNA of *Campylocarpon* isolates, as described by Halleen et al. (2004). Each 50 µl polymerase chain reaction (PCR) mixture included 21 µl of PCR-grade water, 1 µl of DNA template, 1.5 µM of each primer, and 1 µl of PCR Master Mix (2X) (0.05 u $µl^{-1}$ de *Taq* DNA polimerase, reaction buffer, 4 mM MgCl2, 0.4 mM of each dNTP; Thermo Scientific,Waltham, USA). PCR reactions were carried out in a thermal cycler (Biocycler MJ 96; Applied Biosystems, Foster City, USA). The cycling parameters consisted of a denaturation step at 94 ºC for 5 min, followed by 35 cycles at 94 ºC for 1 min, 57 ºC for 1 min, 72 ºC for 1 min and final cycle at 72 ºC for 10 min. The PCR amplification products were separated by electrophoresis in 1.5 % agarose gels in 1.0x Tris-acetate acid EDTA (TAE) buffer and were photographed under UV light after staining with ethidium bromide (0.5 µg ml^{-1}) for 1 min. PCR products were purified using the AxyPrep™ PCR Cleanup Kit (Axygen) following the manufacturer's instructions and sequenced in both directions using a ABI 3730 XL DNA Analyzer (Applied Biosystems) at the Macrogen Inc. (Seoul, Korea).

Phylogenetic analyses

Sequences were aligned with ClustalX v. 1.83 (Thompson *et al*., 1997), using the following parameters: pairwise alignment parameters (gap opening = 10, gap extension = 0.1) and multiple alignment parameters (gap opening = 10, gap extension = 0.2, transition weight = 0.5, delay divergent sequences = 25%). Alignments were checked and manual adjustments were made where necessary. Phylogenetic information contained in indels (gaps) was incorporated into the phylogenetic analyses using simple indel coding as implemented by GapCoder (Young & Healy, 2003). Sequences of *Campylocarpon* type strains obtained from GenBank were included in the analyses (Table 1). *Neonectria rugulosa* (Pat. & Gaillard) Mantiri & Samuels (TPPH 32) was used as outgroup.

Phylogenetic analysis for Maximum-parsimony (MP) was performed using PAUP v. 4.0b10 (Swofford 2003). The heuristic search option with 1000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm was used. All characters were unordered and of equal weight and gaps were treated as missing data. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated from 1 000 bootstrap replications (Hillis & Bull, 1993). Other measures used were consistency index (CI), retention index (RI) and homoplasy index (HI). Trees were sampled every 1000th generation for a total of 10 000 trees. The first 1 000 trees were discarded as the burn-in phase of each analysis. Posterior probabilities (Rannala & Yang, 1996) were determined from a majority-rule consensus tree generated with the remaining 9 000 trees. This analysis was repeated four times starting from different random trees to ensure trees from the same tree space were sampled during each analysis.

Phylogenetic trees were viewed with Treeview (Page, 1996). Sequences generated in this study were deposited in GenBank (Table 1). Representative isolates of different *Campylocarpon* species obtained in this study were deposited in the Culture Collection of Phytopathogenic Fungi “Prof. Maria Menezes” (CMM) at the Universidade Federal Rural de Pernambuco (Recife, Brazil).

Morphological and cultural characterization

Morphological characters used to distinguish *Campylocarpon* species included conidiophore morphology and size, conidial shape and size, and presence or absence of chlamydospore

(Halleen et al., 2004). Colony characters PDA and oatmeal agar (OA) (Difco, Detroit, USA) incubated at 25 °C in the dark were noted after 8 and 10 days. Colony colours were recorded with the colour charts of Rayner (1970). Conidiophore, conidia and other structures were mounted in 100 % lactic acid and digital images recorded with a Leica DFC320 camera on a Leica DMR HC microscope fitted with Nomarski differential interference contrast optics (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK). The length and width of 20 conidiophores, 50 conidia and 10 chlamydospores (if present) per isolate were measured with the Leica IM500 measurement module. Mean and standard errors of the measurements were calculated.

Isolates were also used to determine the effect of temperature on colony growth of different species. A 4-mm-diameter mycelial plug from the growing margin of a 8-day-old colony was placed in the center of a 90-mm-diameter PDA plate, and three replicates of each isolate were incubated at temperatures ranging from 10 °C to 40 °C in 5 °C intervals in the dark. After 8-days incubation period, the colony diameter (mm) was measured in two perpendicular directions. The experiment was done twice. Colony diameters at 8 days were plotted against temperature and a curve was fitted by a cubic polynomial regression ($y=a+bx+cx^2+dx^3$). Optimal temperature was estimated from the regression equation and numeric summary with TableCurve™ 2D v. 5.01 (SYSTAT Software Inc., Chicago, USA). Optimum temperature was defined as the temperature that produced the maximum mycelial growth. The colony diameter data at 30 °C were used to calculate the mycelial growth rate (mm day^{-1}). One-way analyses of variance (ANOVA) were conducted with data obtained from optimum temperature and mycelial growth rate experiments, and means were compared by Fisher's least significant difference (LSD) test at the 5 % significance level using STATISTIX v. 9.0 (Analytical Software, Tallahassee, USA).

Distribution of *Campylocarpon* species

Based on the number of isolates of each *Campylocarpon* species recorded, the relative frequency of each species in relation to overall number of isolates and to the total number of isolates within each table grape population was calculated (Zak & Willig, 2004).

Pathogenicity and virulence on detached green shoots

Detached green shoots of cultivar Isabel were used to investigate the pathogenicity and virulence of the three *Campylocarpon* species found on table grapes in São Francisco Valley. Asymptomatic branches of plants not sprayed with fungicides were collected in a commercial vineyard in São Vicente Férrer (Siriji Valley). The shoots were immediately placed into large plastic containers filled with sterile water, with the shoots placed over a plastic grid. The plastic containers were partially sealed with plastic bags and transported to Universidade Federal Rural de Pernambuco. The cut ends were dipped in wax and in the centre of each shoot (30 cm long) a superficial wound (~4-mm length, 2-mm deep) was made using a sterilized scalpel. It was inoculated with a mycelial plug (4 mm in diameter) removed from the margin of a 12-day-old PDA culture of each isolate. Non-colonized PDA agar plugs were used as negative controls. The inoculated area was wrapped with Parafilm (Pechiney Co., Chicago, USA) to prevent rapid dehydration. Inoculated shoots were placed in large plastic containers, as described above, and incubated at 25 °C and 12-h photoperiod in a growth chamber. After 23 days, the Parafilm was removed, the shoots were sliced through lengthwise and the internal lesions visually observed. The isolates were considered pathogenic when the lesioned area advanced beyond the 4-mm diameter inoculated area. The virulence of the isolates was evaluated by measurement of the lesion lengths with a digital calliper (Mitutoyo Co., Kanagawa, Japan). The experiment was arranged in a completely randomized design with ten replicates per treatment (isolate) and one shoot per replicate. The experiment was conducted twice. Differences in virulence caused by *Campylocarpon* species were determined by one-way ANOVA and means were compared by LSD test at the 5 % significance level using STATISTIX v. 9.0 (Analytical Software, Tallahassee, USA).

Results

Thirty-eight isolates of *Campylocarpon* were obtained from table grape plants showing black foot disease symptoms in São Francisco Valley, Northeastern Brazil. The fungal genus was identified based on the morphological characteristics. The species of these isolates were identified by DNA sequencing and phylogenetic analyses, with further detailed analysis of morphological and cultural characteristics. Finally, the pathogenicity and virulence of the isolates were evaluated in detached green shoots.

DNA sequencing and phylogenetic analyses

The isolates of *Campylocarpon* were identified based on MP phylogenetic analysis of the ITS region of rDNA. The ITS data set consists of 42 taxa, including one outgroup. The alignment contained 500 characters, of which 358 were constant while 142 were variable and parsimony uninformative. The Maximum-parsimony evolutionary history generated 10 equally most parsimonious trees (Length = 85 steps, CI = 0964, RI = 0.995, RC = 0.984), of which one is shown in Figure 2. Sequences of ex-type isolates of *Campylocarpon* species from GenBank were included in the analysis together with isolates obtained in this study (Table 1). The dataset resulted in three clades, which two clades corresponded to previously described *Campylocarpon* species. The first clade with 27 isolates clustered with *Campy. pseudofasciculare* (CBS 112592 and CBS 112679), with 99 % bootstrap support. The second clade with four isolates (CMM 4268, CMM 4269, CMM 4270 and CMM 4276) did not cluster with any known *Campylocarpon* species, with 97 % bootstrap. The third clade with seven isolates (CMM 4271, CMM 4272, CMM 4273, CMM 4274, CMM 4277, CMM 4278 and CMM 4279) clustered with *Campy. fasciculare* (CBS 113559), with 37 % bootstrap support (Fig. 2).

Morphological and cultural characterization

No teleomorph structure was observed during this study and all species showed morphological features typical of the genus *Campylocarpon* (Halleen et al., 2004). The morphological characteristics observed in *Campy. fasciculare* were: *colonies* on PDA and OA brown to chocolate-brown on top and chocolate-brown to dark brown or, in some sectors, camel, in reverse; reaching 45–50 mm diameter after 8 days at 30 ºC on PDA in the dark; mycelial growth rate 5.9 ± 0.2 mm day^{-1} at 30 ºC on PDA in the dark. *Aerial mycelium* on PDA and OA abundantly formed, covering the whole of colony or sectors thereof, white, thick, cottony to felty, intermingled with or giving rise to erect, white or brown hyphal strands. *Conidiophores* initially simple, consisting of single phialides, or phialides in whorls of up to three members, that are formed on short supporting cells situated laterally on hyphae of the aerial mycelium; later arranged in dense fascicles, arising laterally from unpigmented hyphae of the aerial mycelium, hyphal cells supporting the conidiophore fascicles 18.6–35.4 × 6.6–14.7 μm ($\bar{x}$ = 30.7 ± 3.9 × 7.8 ± 0.6 μm; n = 32). *Macroconidia* mostly 3–4-septate, also 1-, 2-, and 5-septate, cylindrical, slightly to moderately curved, sometimes somewhat more

strongly tapering at the base; when 3-septate 32.9–44.3 × 5.8–8.9 µm ($\bar{x}$ = 41.3 ± 0.9 × 7.8 ± 0.3 µm; n = 118). *Microconidia* not observed. *Chlamydospores* not observed. *Cardinal temperatures for growth*: minimum temperature 15 °C; optimum temperature 29.3 ± 1.1 °C, maximum temperature 35 °C. The morphological characteristics observed in *Campy. pseudofasciculare* were: *colonies* on PDA and OA brown to chocolate-brown on top and chocolate-brown to dark brown in reverse; reaching 48–60 mm diameter after 8 days at 30 ºC on PDA in the dark; mycelial growth rate 6.8 ± 0.2 mm day^{-1} at 30 ºC on PDA in the dark. *Aerial mycelium* on PDA and OA abundant, covering the whole or sectors of the colony, white to off-white or slightly brownish, thickly cottony to felty, intermingled with or giving rise to erect white or brown hyphal strands. *Conidiophores* rarely single, consisting of single phialides, or phialides in whorls of up to 3 members, that are formed on short supporting that develop laterally on hyphae or hyphal strands, basal cells of conidiophores 13.4–18.3 × 5.4–6.8 µm ($\bar{x}$ = 15.7 ± 1.1 × 6.3 ± 0.4 µm; n = 35). *Macroconidia* mostly 3–5-septate, also 2-, and 6-septate, cylindrical, slightly to moderately curved, typically somewhat more curved at the tip than at the base; when 3-septate 29.4–40.4 × 6.5–9.5 µm ($\bar{x}$ = 33.2 ± 0.9 × 7.5 ± 0.3 µm; n = 122). *Chlamydospores* sparse, typically in clusters of 3–5, intercalary or borne on short side branches, round to somewhat angular, 8.3–10.8 × 7.2–8.3 µm ($\bar{x}$ = 9.7 ± 0.5 × 7.6 ± 0.6 µm; n = 22). *Cardinal temperatures for growth*: minimum temperature 15 °C; optimum temperature 28.9 ± 1.2 °C, maximum temperature 35 °C.

All species of *Campylocarpon* used in this study grew at temperatures ranging from 15 °C to 35 °C, and none isolated grew at 10 °C at 40 °C. *Campylocarpon semiaridus* had the lowest mycelial growth rate (4.4 mm day^{-1}), differing significantly (P≤0.05) from *Campy. fasciculare* (6.1 mm day^{-1}) and *Campy. pseudofasciculare* (6.8 mm day^{-1}), that do not differ each other. There were no significant differences (P≤0.05) among the *Campylocarpon* in relation optimum temperature for mycelial growth (28.9–30 °C).

Taxonomy

Based on the DNA sequence analyses and morphological characters, one species of *Campylocarpon* proved distinct from known species, and is described below.

Campylocarpon semiaridus M.A. Silva, M.P.S. Câmara & S.J. Michereff **sp. nov.**
MycoBank: xxxxxxx; Figs. 3a–j
Etymology: The name refers to semiarid Brazilian region, where this species was first found.

Description:

Colonies on PDA and OA sienna (13i) on top and chreous (13'b) to dark brink (7''k) in reverse; reaching 47-56 mm diameter after 8 days at 30 ºC on PDA in the dark; mycelial growth rate 4.3 ± 0.2 mm day^{-1} at 30 ºC on PDA in the dark. *Aerial mycelium* on PDA and OA abundant, covering the whole or sectors of the colony, thickly cottony to felty, septate hyphae brownish that occurs singly or in bundles of up to 10; hyphae without warts. *Conidiophores* initially simple, consisting of single phialides, unbranched or sparsely branched, that are formed on short supporting that develop laterally on hyphae or hyphal strands, basal cells of conidiophores 18.7–41.5 × 3.4–7.0 μm ($\bar{x}$ = 31.6 ± 2.1 × 4.6 ± 0.8 μm; n = 35). *Macroconidia* mostly 3–4-septate, also 1-, 2-, and 5-septate, cylindrical, slightly to moderately curved, with minutely tapering, obtuse ends, sometimes somewhat more strongly tapering at the base; when 3-septate 22.5–38.8 × 5.5–7.6 μm ($\bar{x}$ = 30.1 ± 0.8 × 6.6 ± 0.2 μm; n = 112). *Microconidia* not observed. *Chlamydospores* sparse, typically in clusters of 3–5, intercalary or borne on short side branches, round to somewhat angular, 7.6–9.8 × 7.1–8.2 μm ($\bar{x}$ = 8.4 ± 0.3 × 7.4 ± 0.5 μm; n = 43). *Cardinal temperatures for growth*: minimum temperature 15 °C; optimum temperature 30 ± 0.8 °C, maximum temperature 35 °C.

Sexual morph: not produced in culture.

Substrate: *Vitis vinifera*

Known Distribution: Brazil (Bahia, Pernambuco).

Holotype: Brazil, Pernambuco, Petrolina, isolated from *Vitis vinifera* tree, 2012, coll. M.A. Silva (holotype living culture CMM 4270; ex-type in URM xxxxxxx; isotype in MFLU and ex-type culture in MFLUCC xxxxxxx).

Additional cultures examined: Brazil, Pernambuco, Petrolina, isolated from *Vitis vinifera* trees, 2012, coll. M.A. Silva, CMM 4268 and CMM 4269; Brazil, Bahia, Casa Nova, isolated from *Vitis vinifera* tree, 2012, coll. M.A. Silva, CMM 4276.

Notes – Phylogenetically *Campy. semiaridus* is closely related to *Campy. fasciculare*, but cultures of *Campy. semiaridus* produced chlamydospores, which did not occur with *Campy. fasciculare*. *Campylocarpon semiaridus* differs from its closest phylogenetic neighbor, *Campy. fasciculare*, by unique fixed alleles in one loci based on alignments of the separate loci deposited in TreeBase as study S16143: alignments ITS positions 65 (G), 146 (T), 384 (C), 402 (A) and 449 (A).

Distribution of *Campylocarpon* species

Campylocarpon pseudofasciculare the species was isolated more frequently in Petrolina (51 %) followed by Lagoa Grande (19 %), Juazeiro (15 %) and Curaçá (15 %). *Campylocarpon fasciculare* the species was predominantly isolated more often in Petrolina (86%) followed by Curaçá (15 %). *Campylocarpon semiaridus* the species was isolated in cities of Petrolina (75 %) and Casa Nova (25 %). The distribution of species *Campylocarpum* differ among the five regions producing table grapes of the São Francisco. Only in the population of Petrolina all *Campylocarpon* species were found (Fig. 4)

Pathogenicity and virulence in detached green shoots

All isolates of *Campylocarpon* species were pathogenic to detached green shoots of grapevine, resulting in visible lesions 23 days after inoculation. The symptoms observed both on the surface and internally were necrotic lesions brown dark which extended upward and downward from the point of inoculation. There were no significant differences in virulence among the *Campylocarpon* species and lesion lengths induced in table grape shoots ranged from 12.4 mm to 13.2 mm.

Discussion

The disease etiology is crucial for epidemiological studies and for a better understanding of the distribution and importance of individual species, as well as finding effective management strategies to each pathogen. Black foot disease of table grape and the associated pathogens have been little studied in Brazil and worldwide. This study constitutes the first attempt to assess the diversity of *Campylocarpons* species on table grapes showing black foot disease symptoms in the São Francisco Valley, the main Brazilian exporting region. Species identity was based on morphological characters and analysis of the ITS region of rDNA. Three species were identified, namely *Campy. fasciculare*, *Campy. pseudofasciculare*, and new species *Campy. semiaridus*. The colony characteristics, as well as the characteristics and dimensions of conidiophores, conidia and chlamydospores of *Campy. fasciculare* and *Campy. pseudofasciculare* obtained in this study were similar to those previously described in the literature (Halleen et al., 2004).

Since 2004, when the genus *Campylocarpon* was established (Halleen et al., 2004), the only two described species are *Campyl. fasciculare* and *Campyl. pseudofasciculare*, both having grapevine as single host (Farr & Rossman, 2014). *Campylocarpon semiaridus* is recognized as a new species in the genus *Campylocarpon*, closely related to *Campyl. fasciculare*. However, five nucleotides in the ITS region distinguish *Campyl. semiaridus* from *Campyl. fasciculare*. Considering the phylogenetic data, the table grape isolates of *Campyl. semiaridus* formed a clade strongly supported in the Maximum-parsimony analysis (99 %). *Campylocarpon semiaridus* can also be distinguished from *Campyl. fasciculare* based on chlamydospores production, since the first species produces this structure while the second does not.

The species of *Campylocarpon* identified in this work were isolated from plants showing symptoms of black foot disease include a reduction in root biomass and root hairs with sunken and necrotic root lesions. However, *Campylocarpon* species can be found in asymptomatic nursery plants (Halleen et al., 2006; Gramaje & Armengol, 2011).

Campylocarpon pseudofasciculare was the most frequently isolated species associated with black foot disease of table grape, and also the most widespread species in vineyards of São Francisco Valley. *Campylocarpon fasciculare* was isolated with smallest frequency from table grapes with black foot disease symptoms that *Campyl. pseudofasciculare*, but higher than that recorded for *Campyl. semiaridus*. The first two species had been reported in table grapes in Northeastern Brazil, but not in the São Francisco Valley (Correia et al., 2013). Besides Brazil, *Campyl. fasciculare* was reported in South Africa (Halleen et al., 2004) and Spain (Alaniz et al., 2011), while *Campyl. pseudofasciculare* was reported in South Africa (Halleen et al., 2004), Uruguay (Abreo et al., 2010) and Perú (Álvarez et al., 2012).

Regarding cultural characteristics, the optimum temperature for mycelial growth for *Campylocarpon* species from table grape varied between 28.9 and 30 °C, corroborating with information from the literature (Halleen et al., 2004; Halleen et al., 2006; Agustí-Brisach & Armengol, 2013). The no growth of *Campylocarpon* species at 10 °C observed in this study contrast to information that the minimum temperature for this species is 10 °C (Halleen et al., 2004).

The production of chlamydospores by *Campyl. semiaridus* may allow them to survive for extended periods in soil, as found for *Campyl. pseudofasciculare* (Halleen et al., 2004). However, very little information is currently available regarding the survival of these pathogens, and the role of chlamydospores during subsequent infections (Halleen *et al.*, 2006; Agustí-Brisach & Armengol, 2013).

All isolates of *Campylocarpon* species in this study were able to infect, colonize and produce lesions in detached grapevine shoots, confirming their status as black foot disease pathogens, including the new species *Campyl. semiaridus*. No differences in virulence among *Campylocarpon* species inoculated in grapevine, as observed in this study, have been reported previously by Halleen et al. (2004).

In the current study, three of the four vineyards containing *Campy. semiaridus* were established for less than five years on noncultivated lands or at sites previously planted with grapevines. The black foot inoculum may have pre-existed in the soil previously planted with grapevines, but it is improbable that high inoculum levels of *Campy. semiaridus* were present in the noncultivated soils. Studies conducted during the past decade have documented that grapevine transplants can become infected by black foot pathogens during propagation at nurseries before establishment in vineyards (Gramaje & Armengol, 2011). However, practices can be implemented at nurseries to control black foot infections, including hot water treatments, fungicide baths, and soil amendments with biological agents such as *Trichoderma* spp. and/or mycorrhizal fungi (Halleen et al., 2006; Gramaje & Armengol, 2011; Agustí-Brisach & Armengol, 2013).

In São Francisco Valley are planted more than 9,500 hectares of vineyards using transplants produced in different localities. Consequently, it is recommended that the transplants be acquired from registered nurseries that produce disease-free plants and practice strategies to avoid and control black foot contamination.

This paper reports three species of the genus *Campylocarpon* associated with back foot disease of table grape in São Francisco Valle and all the species have potential to cause disease to table grapes. The results contribute towards relevant information regarding the identification of *Campylocarpon* species associated with black foot symptoms of grapevines in Brazil and is the first step towards a better understanding and management of the disease. Studies are needed on the epidemiology and impact on table grape production together with information referring to ecology, distribution, and fungicide sensitivity of all species of *Campylocarpon* found in this study. Further studies to determine the quality and infection status of the planting material being currently used in São Francisco Valley should be performed.

Acknowledgments

This work was financed by Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco - FACEPE (APQ 137-5.01/12) and by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (Project 203/2009 - International Cooperation CAPES-Brazil/DGU-Spain). M. P. S. Câmara, and S. J. Michereff also acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq research fellowship.

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Table 1. Isolates of *Campylocarpon* species used in this study

Taxon	Culture accession no.[a]	Host	Location	Collector	GenBank ITS accession no.[b]
Campylocarpon fasciculare	CBS 112613	*Vitis vinifera*	South Africa	-	AY677301
Campy. pseudofasciculare	CBS 112679	*V. vinifera*	South Africa	-	AY677306
Campy. pseudofasciculare	CBS 112592	*V. vinifera*	South Africa	-	AY677305
Campy. pseudofasciculare	CMM 4289	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4288	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4307	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4301	*V vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4297	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4286	*V vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4291	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4302	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4308	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4303	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4287	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4284	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4290	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4306	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4285	*V. vinifera*	Brazil	M.A. Silva	**This study**

Campy. pseudofasciculare	CMM 4305	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4296	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4304	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4280	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4283	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4299	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4282	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4281	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4298	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4292	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4294	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. pseudofasciculare	CMM 4300	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. fasciculare	CMM 4271	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. fasciculare	CMM 4272	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. fasciculare	CMM 4274	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. fasciculare	CMM 4278	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. fasciculare	CMM 4277	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. fasciculare	CMM 4279	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. fasciculare	CMM 4273	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campy. fasciculare	CMM 4275	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campylocarpon semiaridus	CMM 4276	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campylocarpon semiaridus	CMM 4270	*V. vinifera*	Brazil	M.A. Silva	**This study**

Campylocarpon semiaridus	CMM 4269	*V. vinifera*	Brazil	M.A. Silva	**This study**
Campylocarpon semiaridus	CMM 4268	*V. vinifera*	Brazil	M.A. Silva	**This study**
Neonectria rugulosa	TPPH 32	*Myrica rubra*	Japan	Y. Hirooka	AB233176

[a] *CBS* Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; *CMM* Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes", Universidade Federal Rural de Pernambuco, Recife, Brazil; *TPPH* Tokyo University of Agriculture, Laboratory of Tropical Plant Plantation, Tokyo, Japan.

[b] Sequence numbers in *bold* were obtained in the present study

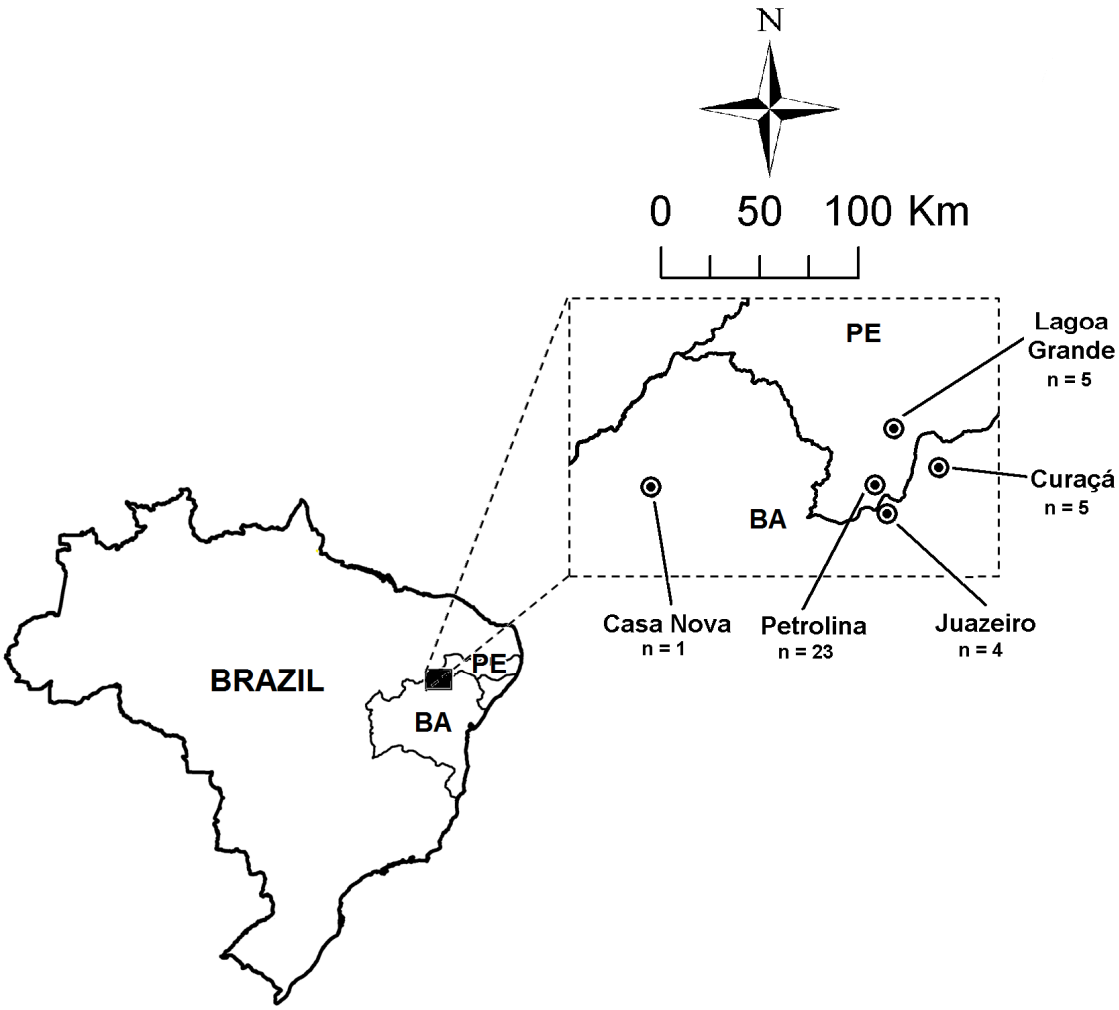

Figure 1. Collection sites of *Campylocarpon* isolates from São Francisco Valley vineyards in Northeastern Brazil, located in the states of Bahia (BA) and Pernambuco (PE). The names next to the dots indicate to the localities corresponding to the sampled vineyard. n = number of isolates in each locality

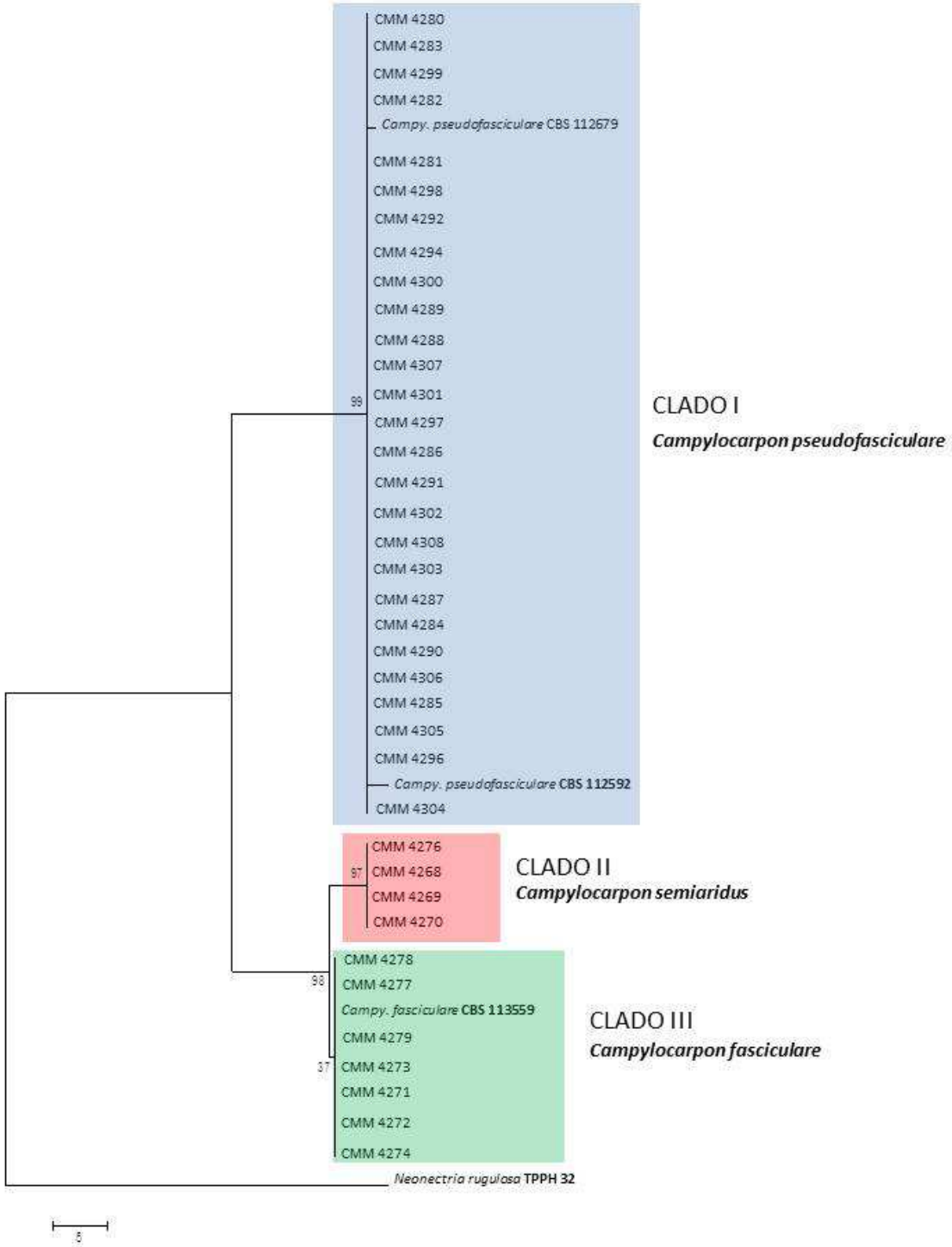

Figure 2. One of 10 most parsimonious trees (length=93; CI=0.875; RI=0.983; HI=0.940) obtained from ITS sequence data from *Campylocarpon* isolates. Maximum parsimony bootstrap support values from 1000 replications.

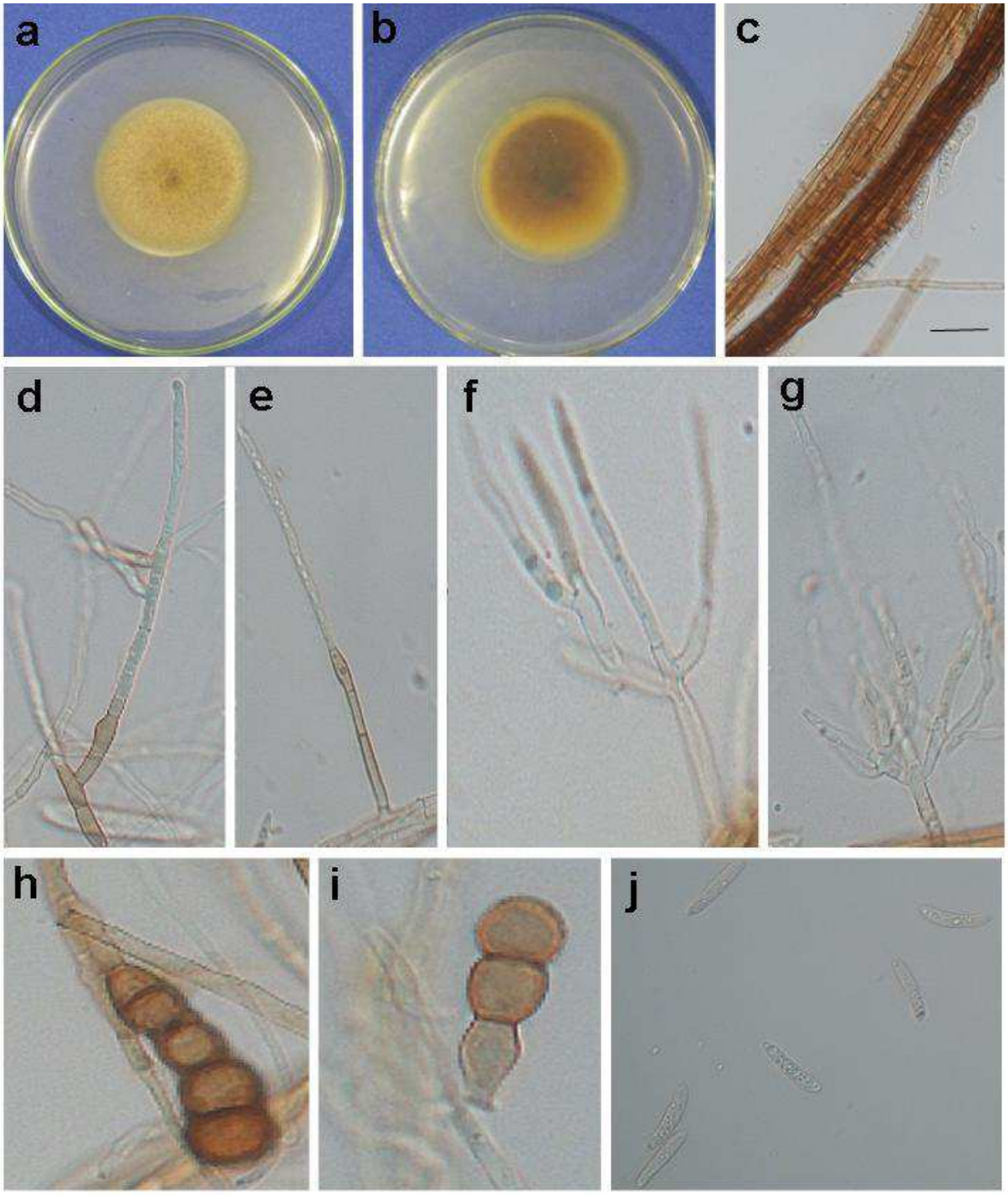

Figure 3. *Campylocarpon semiaridus* holotype (culture CMM 4270). **a** Eight-day-old colony incubated at 25 ºC on PDA in the dark and, **b** Colony reverse. **c** Aerial structures on PDA, with Brownish hyphal strand formed in the aerial mycelium. **d–e** Simple, unbranched, septate conidiophore. **f–g** Branched conidiophores. **h–i** chlamydospores. **j** Macroconidia. Scale bars: **c–i** = 10 μm.

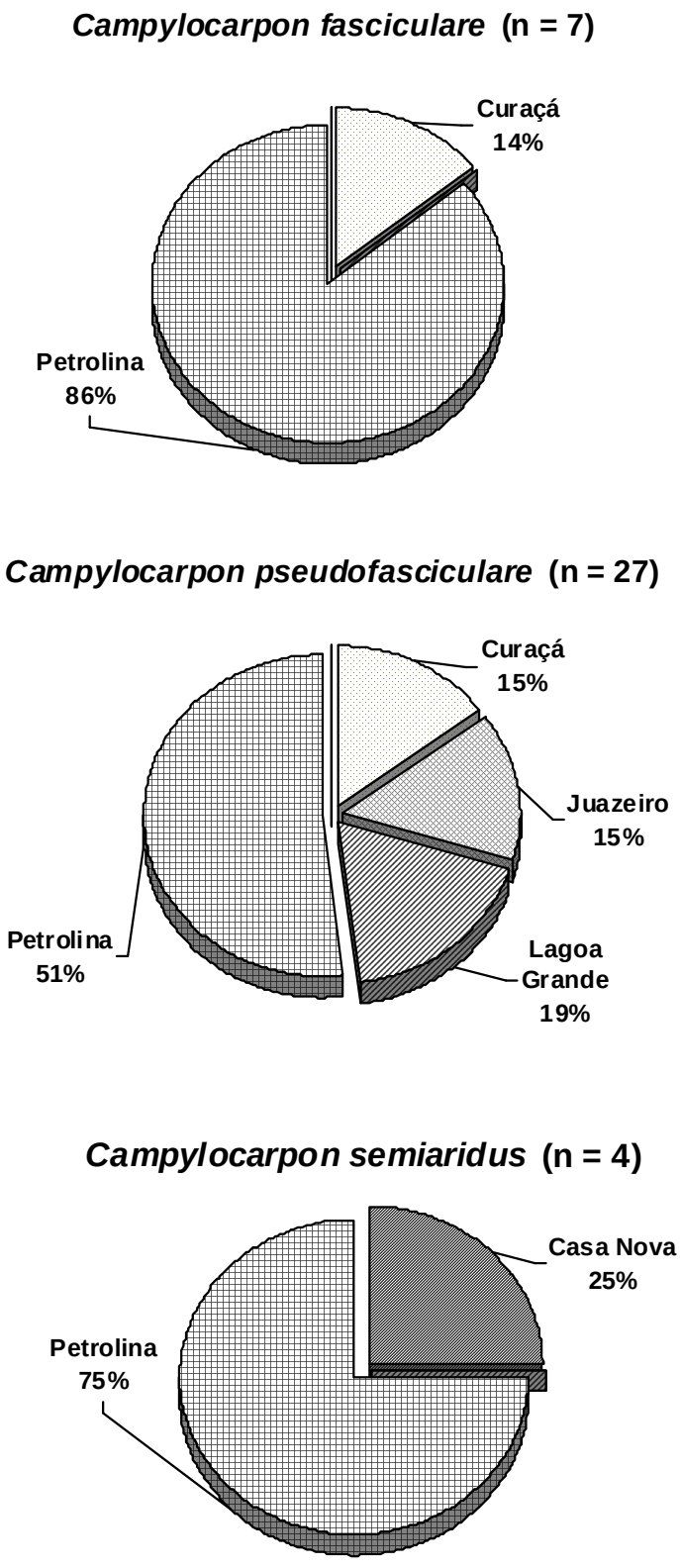

Figure 4. Frequency (%) of *Campylocarpon* species associated with black foot disease of table grape in five localities (Casa Nova, Curaçá, Juazeiro, Lagoa Grande and Petrolina) of the São Francisco Valley, Northeastern Brazil.

Conclusões Gerais

CONCLUSÕES GERAIS

- Três espécies de *Phaeoacremonium* (*Pm.*) estão associadas à doença de Petri da videira no Nordeste Brasileiro, sendo duas previamente descritas como *Pm. aleophilum* e *Pm. parasiticum*, e uma nova espécie denominada *Pm. nordesticola* sp. nov.;

- *Phaeoacremonium aleophilum* e a espécie mais prevalente e virulenta associada à doença de Petri no Nordeste brasileiro;

- Três espécies de *Campylocarpon* (*Campyl.*) estão associadas ao pé-preto da videira no vale do São Francisco, sendo duas previamente descritas como *Campyl. fasciculare* e *Campyl. pseudofasciculare*, e uma nova espécie denominada *Campyl. semiaridus* sp. nov.

- *Campylocarpon pseudofasciculare* e a espécie mais prevalente associada ao pé-preto da videira no vale do São Francisco;

- Todas as espécies de *Phaeoacremonium* e *Campylocarpon* relatadas no Nordeste brasileiro são patogênicas à videira.