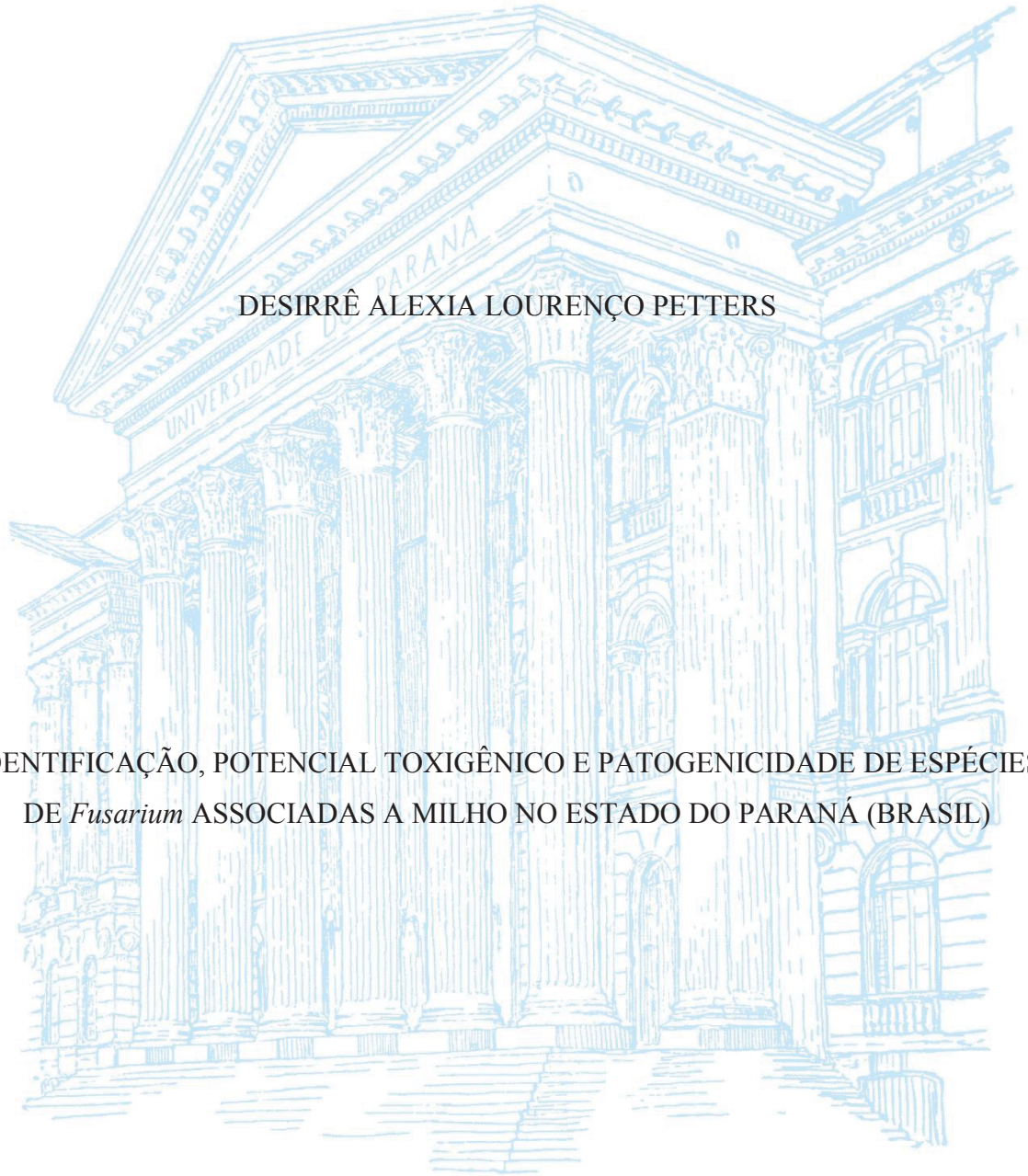
UNIVERSIDADE FEDERAL DO PARANÁ



DESIRRÊ ALEXIA LOURENÇO PETTERS

IDENTIFICAÇÃO, POTENCIAL TOXIGÊNICO E PATOGENICIDADE DE ESPÉCIES DE *Fusarium* ASSOCIADAS A MILHO NO ESTADO DO PARANÁ (BRASIL)

CURITIBA

2018

DESIRRÊ ALEXIA LOURENÇO PETTERS

IDENTIFICAÇÃO, POTENCIAL TOXIGÊNICO E PATOGENICIDADE DE ESPÉCIES DE *Fusarium* ASSOCIADAS A MILHO NO ESTADO DO PARANÁ (BRASIL)

Dissertação apresentada como requisito parcial à obtenção do grau de Mestre em Genética, no curso de Pós-Graduação em Genética, Setor de Ciências Biológicas, Universidade Federal do Paraná

Orientadora: Profª Drª Chirlei Glienke
Co-orientadora: Profª Drª Lygia Vitória Galli-Terasawa

CURITIBA

2018

Universidade Federal do Paraná. Sistema de Bibliotecas.
Biblioteca de Ciências Biológicas.
(Rosilei Vilas Boas – CRB/9-939).

Petters, Desirrê Alexia Lourenço
Identificação, potencial toxigênico e patogenicidade de espécies de *Fusarium* associadas a milho no estado do Paraná (Brasil). / Desirrê Alexia Lourenço Petters. – Curitiba, 2018.
108 f. : il. ; 30cm.

Orientadora: Chirlei Glienke
Coorientadora: Lygia Vitória Galli-Terasawa
Dissertação (Mestrado) – Universidade Federal do Paraná, Setor de Ciências Biológicas. Programa de Pós-Graduação em Genética.

1. *Fusarium*. 2. Fungos toxigênico. 3. Microorganismos patogênicos. 4. Patogênese. 5. Tricotecenos. 6. Fumonisinas. 7. Filogenia. 8. Milho. I. Título II. Glienke, Chirlei. III. Galli-Terasawa, Lygia Vitória. IV. Universidade Federal do Paraná. Setor de Ciências Biológicas. Programa de Pós-Graduação em Genética.

CDD (20. ed.) 632.4

MINISTÉRIO DA EDUCAÇÃO
SETOR CIÊNCIAS BIOLÓGICAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO GENÉTICA

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em GENÉTICA da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **DESIRRÊ ALEXIA LOURENÇO PETTERS** intitulada: **Identificação, Potencial Toxigênico e Patogenicidade de espécies de *Fusarium* associadas a milho no Estado do Paraná (Brasil)**, após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua Aprovação no rito de defesa.

A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

Curitiba, 27 de Março de 2018.

CHIRLEI GLIENKE
Presidente da Banca Examinadora (UFPR)

LUCIMERIS RUARO
Avaliador Externo (UFPR)

DAIANI CRISTINA SAVI
Avaliador Externo (UFPR)

Dedico este trabalho aos isolados de *Fusarium,* que cederam suas vidas à esta pesquisa.

AGRADECIMENTOS

Agradeço a todos que contribuíram de alguma forma, seja direta ou indiretamente, para a realização deste trabalho.

Ao Programa de Pós-Graduação em Genética da Universidade Federal do Paraná e aos professores do Departamento de Genética pela oportunidade de realizar este trabalho e pelas contribuições para minha formação acadêmica, dentro e fora das aulas e do laboratório.

À CAPES pela bolsa concedida durante o mestrado.

À Profª Drª Chirlei Glienke, pela oportunidade de crescimento pessoal e profissional. Pela orientação e confiança no meu potencial, por me desafiar, me incentivar a aprender sempre mais e buscar o meu melhor. Por toda a convivência dentro e fora do laboratório, pela amizade e pelo exemplo tanto como pesquisadora como quanto pessoa.

À profª Drª Lygia Vitória Galli-Terasawa pela orientação, por viabilizar a realização do ensaio em campo. Pela oportunidade de aprender e auxiliar no REMendel. Pelo imenso carinho com que trata seus alunos e que me marcou pelo exemplo de que não só com conhecimento se constrói um bom ambiente acadêmico.

À Profª Drª Vanessa Kava pelas inúmeras oportunidades de aprendizado e crescimento durante a graduação e pela convivência no LabGeM.

À Drª Daiani Savi e ao Prof. Dr. Daniel Bruschi pelas sugestões, conselhos e incentivo como banca de acompanhamento. À Daiani novamente, e à Profª Drª Lucimeris Ruaro pela disponibilidade e pelas contribuições ao avaliar este trabalho.

Ao Dr. Francisco Terasawa Junior representando a empresa Vértika Agropecuária, pelos materiais biológicos e infraestrutura cedidos para o isolamento e realização do teste de patogenicidade em milho, viabilizando etapas importantes deste trabalho.

À Isabelle Pansolin e Letícia Ripka pelo auxílio durante a realização do ensaio em campo.

Ao MSc. Rodrigo Aluizio pelo auxílio nas atividades do laboratório e pelos inúmeros conselhos e sugestões em diversas análises.

Ao Departamento de Bioquímica da Universidade Federal do Paraná, pelo uso da infraestrutura de sequenciamento. Ao Centro de Tecnologias Avançadas em Fluorescência (CTAF) da Universidade Federal do Paraná, CAPES e FINEP, pelo uso da infraestrutura de microscopia.

Aos amigos e colegas da Pós-Graduação em Genética, pela convivência dentro e fora da Universidade.

Aos colegas do LabGeM, pela convivência ao longo destes anos de trabalho. À Raphaely, pela imensa contribuição na bancada de laboratório e pela oportunidade de compartilhar um pouco do que aprendi ao longo do Mestrado. À Dai (mais uma vez!), ao Alan e à Kelly pelos ensinamentos e bons momentos compartilhados.

Aos "Giovanos" amigos que o LabGeM me trouxe, Elvio, Miriam e Sandy. Por todo o companheirismo e pelos momentos de descontração e alegria que me ajudaram a não perder o ânimo e o entusiasmo.

À Juliana, Camila, Carolina, Lucied, Thassi e Luciana, por todo o apoio e pela grande amizade que resiste ao tempo (e obrigações acadêmicas!).

Aos meus pais, Lindamir e Paulo, e o meu irmão Junior, por todo o amor e apoio incondicional, por me darem forças para seguir adiante e acreditarem em mim principalmente nos momentos em que eu não acreditei.

À Vida e à natureza, pela grandiosidade e pelos inúmeros exemplos de transformação, que me mostram que sempre é possível crescer e se reinventar a cada dia.

"O jovem que deseja ser cientista – e à ciência dedicar todo o seu tempo e amor – tem pelo menos três certezas: a de que morrerá um dia (como todo mundo), a de que não ficará rico (como quase todo mundo) e a de que se divertirá muito (como pouca gente). "

NEWTON FREIRE-MAIA

"(...). Em suma, vossa chegada não poderá deixar
De criar uma vida nova, uma vida melhor.
Possa a força do destino conceder-me
O supremo êxtase da alegria terrena,
A meta máxima do êxtase terreno,
Que é de ver, quando da tumba me erguer,
Minha arte florescendo em paz,
Entre os que vierem depois de mim"

GREGOR MENDEL

RESUMO

Espécies de *Fusarium* estão associadas com podridão de espigas e colmos de milho, causando perdas em qualidade e produtividade, além de contaminação com micotoxinas. Para melhorar o manejo de fusarioses e evitar contaminação por micotoxinas, o conhecimento acerca da distribuição de espécies de *Fusarium* é necessário. Sendo assim, este trabalho identificou espécies de *Fusarium* presentes em espigas e colmos de milho sintomáticos no estado do Paraná, Brasil, e avaliou o potencial toxigênico e patogênico destes isolados. Os isolados foram identificados por análises filogenéticas e PCR espécie-específica, testados quanto à presença de genes envolvidos na produção de toxinas por PCR, produção de fumonisinas por ELISA, e patogenicidade em colmos de milho. Os isolados obtidos pertenciam a três complexos de espécies: *F. fujikuroi* (FFSC 79,4%), *F. graminearum* (FGSC 18,1%) e *F. incarnatum-equiseti* (FIESC 2,5%). Além das espécies identificadas *F. verticillioides* (espécie dominante em grãos de milho), *F. fujikuroi, F. tjaetaba, F. napiforme* (FFSC), *F. graminearum, F. meridionale*, as linhagens FIESC 12, 20 e 33, descreve-se neste trabalho a nova espécie *F. awaxy* (dominante em colmos), no complexo FFSC. *F. graminearum* e *F. meridionale* apresentaram os genótipos 15-ADON e NIV, respectivamente, e todos as espécies identificadas no complexo FFSC apresentaram isolados positivos para a presença do gene FUM1, porém a produção de fumonisina foi variável. Quanto à patogenicidade, *F. fujikuroi, F. graminearum, F. meridionale* e *F. verticillioides* causaram podridão de colmo, *F. awaxy* e *F. tjaetaba* demonstraram menor agressividade e *F. napiforme* não causou sintomas. Tais resultados sugerem que a diversidade de *Fusarium* em milho ainda não é suficientemente explorada e estudos mais aprofundados são necessários para caracterizar a relevância das espécies aqui identificadas dentro do contexto deste cultivo economicamente importante.

Palavras-chave: Complexo *Fusarium fujikuroi*. Complexo *Fusarium graminearum*. *Fusarium awaxy*. Patogenicidade. Tricotecenos. Fumonisinas. Filogenia.

ABSTRACT

Fusarium species are associated with maize ears and stalk rot, causing losses to maize crop quality and yield and leading to mycotoxin contamination. In order to improve disease management and reduce contamination, knowledge about *Fusarium* species distribution is required. Therefore, this study identified the *Fusarium* species in symptomatic maize ears and stalks in Paraná state, Brazil and evaluated their toxigenic and pathogenic potential. *Fusarium* isolates were identified through phylogenetic analyses and species-specific PCR, tested for the presence of toxin production genes through PCR, fumonisin production with ELISA, and for pathogenicity in maize stalks. Isolates belonged to three species complexes: *F. fujikuroi* (FFSC 79.4%), *F. graminearum* (FGSC 18.1%) and *F. incarnatum-equiseti* (FIESC 2.5%). Besides the identified species *F. verticillioides* (dominant species in maize kernels), *F. fujikuroi, F. tjaetaba, F. napiforme* (FFSC), *F. graminearum, F. meridionale* (FGSC), FIESC lineages 12, 20 and 33, we described the new species *F. awaxy* (dominant species in maize stalks) in FFSC. *F. graminearum* and *F. meridionale* were of 15—ADON and NIV genotypes, respectively, and all FFSC species identified had positive isolates for FUM1 gene, but fumonisin production was variable. Regarding pathogenicity, *F. fujikuroi, F. graminearum, F. meridionale* and *F. verticillioides* caused stalk rot, *F. awaxy* and *F. tjaetaba* exhibited lower aggressiveness, and *F. napiforme* did not cause symptoms. These findings suggest that *Fusarium* diversity in maize is still underexplored, and further studies are required to characterize the relevance of identified species for this economically important crop.

Keywords: *Fusarium fujikuroi* species complex. *Fusarium graminearum* species complex. *Fusarium awaxy*. Pathogenicity. Trichothecenes. Fumonisins. Phylogeny.

SUMÁRIO

1 INTRODUÇÃO

O milho (*Zea mays* L.) é produzido em quantidade muito superior do que qualquer outro grão (FAOSTAT, 2018), sendo amplamente cultivado ao redor do mundo (CLAYTON et al., 2018). É utilizado como alimento na forma original ou como óleo, amido, cereais matinais e bebidas, na produção de ração animal (DERAL, 2018b) e etanol biocombustível (ECKERT et al., 2018).

O Brasil é terceiro maior produtor de milho do mundo, tendo produzido quase 65 milhões de toneladas em 2016, sendo superado apenas pelos Estados Unidos (que produziu 384 milhões de toneladas em 2016) e a China (que produziu 232 milhões de toneladas) (FAOSTAT, 2018). O milho é o cereal e a terceira *commodity* mais produzida no país, após a cana-de-açúcar (768 milhões de toneladas produzidas em 2016) e a soja (96 milhões de toneladas produzidas em 2016) (FAOSTAT, 2018).

A produção deste grão tem aumentado significativamente no país por meio de mudanças no sistema de cultivo, utilizando genótipos mais produtivos e adaptados à diferentes regiões (incluindo transgênicos e linhagens convencionais), aumento da área de plantio e uso de duas épocas de plantio (COSTA et al., 2010). No entanto, o manejo cultural no milho ainda deve ser bastante aprimorado para obter o aumento de produtividade e rentabilidade que essa cultura, por suas características fisiológicas, é capaz de proporcionar (EMBRAPA, 2010b).

Nos últimos anos, doenças causadas por diferentes patógenos são problemas sérios no cultivo de milho, causando perdas de produtividade e prejuízos aos produtores. A evolução de doenças de milho está relacionada à evolução do sistema de produção desta cultura no país, uma vez que mudanças que ocasionaram o aumento de produtividade também foram responsáveis por aumentar a incidência e severidade das doenças existentes. A expansão das fronteiras agrícolas, ampliação de épocas de plantio, o sistema de plantio direto e ausência de rotação de culturas promovem modificações na dinâmica populacional dos patógenos, de modo que a cada safra surgem novos problemas para a cultura devido à ocorrência de doenças (EMBRAPA, 2010a).

As doenças em milho podem atacar diferentes partes da planta, como folhas, colmos, raízes, espigas, grãos ou até mesmo se propagarem como doenças sistêmicas. Diferentes grupos de patógenos, como vírus, bactérias, fungos e nematoides afligem essa cultura. A importância das doenças pode variar ao longo dos anos e de acordo com a região, devido à influência de condições climáticas, sistema de plantio e cultivares utilizados (EMBRAPA, 2010a).

Como patógenos fúngicos no milho, destacam-se os gêneros *Colletotrichum*, *Fusarium* e *Stenocarpella*, causando principalmente podridões de colmo, que levam a perdas de produtividade e qualidade dos grãos. Tais perdas ocorrem em função ou de morte prematura das plantas jovens antes da fase de enchimento dos grãos, ou pelo tombamento de plantas mais velhas, quando o colmo está apodrecido e não sustenta adequadamente o peso das espigas. Patógenos dos gêneros *Fusarium* e *Stenocarpella* também podem causar podridões de espigas, em que há crescimento fúngico e formação de grãos ardidos (os quais apresentam pelo menos um quarto de sua superfície com descolorações que variam em tonalidades de marrom, roxo e vermelho) (EMBRAPA, 2010a).

Espécies do gênero *Fusarium* são especialmente problemáticas porque, além de causarem podridões de colmo e espigas e gerarem grãos ardidos, são capazes de produzir micotoxinas. Além dos danos físicos nos grãos (como a descoloração e redução no conteúdo de açúcares, proteínas e carboidratos totais), o material torna-se impróprio para consumo animal ou humano, pelo risco de ocorrência de micotoxicoses. Tais doenças podem causar problemas no funcionamento de órgãos vitais e também são relacionadas ao surgimento de tumores malignos (EMBRAPA, 2010a).

As principais micotoxinas encontradas nos grãos de milho produzidas por *Fusarium* são fumonisinas (*Fusarium verticillioides*), zearalenona e tricotecenos (complexo de espécies *Fusarium graminearum*). As toxinas produzidas por espécies do complexo *Fusarium graminearum* estão associadas com hiperestrogenismo, recusa alimentar (LESLIE e SUMMERELL, 2006), síndromes eméticas em animais e humanos (IARC, 1993; LESLIE e SUMMERELL, 2006). Fumonisinas estão associadas com leucoencefalomalácia em equinos, edema pulmonar em suínos, são hepato e nefrotóxicas e possuem potencial carcinogênico (LESLIE e SUMMERELL, 2006). Em humanos, já se sugeriu a associação de fumonisinas com problemas de formação do tubo neural e câncer de esôfago (IARC, 2002).

O manejo da ocorrência de podridões de colmo, espiga e contaminação com micotoxinas é feito através da adoção de várias medidas de maneira integrada, como utilização de cultivares com maior nível de resistência aos patógenos, rotação de culturas, evitar atrasos na colheita e realizar o plantio em condições adequadas, evitando épocas de condições ambientais favoráveis ao desenvolvimento dos patógenos, utilizando sementes sadias e tratadas com fungicidas (EMBRAPA, 2010). Para o controle das podridões em colmo, a utilização de fungicidas não constitui uma alternativa viável, necessitando ainda mais de uma boa estratégia de manejo integrado (COSTA et al., 2017).

Dessa forma, a estratégia de controle baseada no uso de cultivares geneticamente resistentes, é bastante atrativa aos produtores uma vez que seu uso não exige gastos adicionais e não causa impactos negativos ao meio ambiente. Também é compatível com outras medidas de controle, e muitas vezes é o suficiente para controlar a doença sem a utilização de outras estratégias (EMBRAPA, 2010a).

Para a adoção desta estratégia de forma efetiva, e desenvolver cultivares geneticamente resistentes, é necessário conhecer a dinâmica de patógenos de uma região e identificar as espécies presentes. Esta informação é essencial em função da existência de diferenças fisiológicas e de estilo de vida entre as espécies, que devem ser levadas em consideração ao definir a melhor forma de realizar o controle do patógeno. Conhecer as espécies causadoras de doenças em uma região é importante para a busca de possíveis alvos fisiológicos para o controle dos patógenos. Genótipos resistentes podem ser desenvolvidos com múltiplos genes de resistência, dispensando o uso de químicos para protegê-los contra patógenos (DE WIT, 2016).

Nos últimos anos, diversos trabalhos buscam determinar a distribuição de espécies de Fusarium associadas a diferentes cereais economicamente importantes, como trigo (ASTOLFI et al., 2012), arroz (GOMES et al., 2015), cevada (CASTAÑARES et al., 2016) e milho (KUHNEM et al,, 2016) no Sul do Brasil, com o intuito de melhorar o manejo de diversas fusarioses. Especificamente para milho, Kuhnem e colaboradores (2016) descrevem a presença de três espécies do complexo *Fusarium graminearum* (*F. graminearum*, *F. meridionale* e *F. cortaderiae*), sendo *F. meridionale* descrita com a mais encontrada no Paraná, Santa Catarina e Rio Grande do Sul. Entretanto, a distribuição de espécies de *Fusarium* pertencentes a outros complexos (como o complexo *Fusarium fujikuroi*) ainda não está estabelecida para o estado do Paraná.

Sendo assim, este trabalho visa identificar as espécies de *Fusarium* presentes no estado, bem como avaliar a presença de genes envolvidos na produção de toxinas e patogenicidade em híbrido comercial de milho, fornecendo informações epidemiológicas importantes para o manejo de fusarioses.

2 REVISÃO BIBLIOGRÁFICA

2.1 PRODUÇÃO DE MILHO

No Brasil, o uso de duas épocas de plantio é uma característica da produção de milho. A primeira safra compreende os plantios realizados na época tradicional, durante o período chuvoso, que pode variar entre o fim de agosto, na região Sul, até os meses de outubro e novembro da região Centro-Oeste e no Sudeste. Já a segunda safra (safrinha) refere-se ao milho plantado extemporaneamente, em fevereiro ou março, geralmente após o plantio de soja precoce, e é predominante na região Centro-Oeste e nos estados do Paraná, São Paulo e Minas Gerais (EMBRAPA, 2010b).

Segundo a CONAB (2018), na primeira Safra de 2016-2017 o Paraná foi o terceiro maior produtor de milho (~4,7 milhões de toneladas), superado apenas pelo Rio Grande do Sul (~6 milhões de toneladas) e Minas Gerais (~5,8 milhões toneladas). Já para a segunda safra do mesmo período, foi o segundo maior produtor (~13,1 milhões de toneladas), superado pelo Mato Grosso (~28,6 milhões de toneladas). Com relação à produção total de 2016-2017, observa-se que o Paraná foi responsável por quase 19% da produção total do país.

De acordo com o DERAL (2018a), no Paraná, do total de milho produzido na primeira safra 2016/2017, a maior parte está concentrada na região Sul/Sudoeste (responsável por cerca de 80% da produção do Estado) seguida pela região Norte/Noroeste e Oeste/Centro Oeste. Já para a segunda safra, as regiões que mais produzem são aquelas que tradicionalmente cultivam soja durante o verão, destacando-se as regiões Norte/Noroeste e Oeste/Centro Oeste, responsáveis por 95% da produção do estado nesse período.

Nos últimos dez anos, a área de milho plantada durante a primeira safra reduziu mais da metade, mas sem reduções significativas na produção. A redução da área plantada na primeira safra é compensada pelo aumento do rendimento e produtividade das lavouras, e também pelo crescimento da área de plantio da segunda safra (que cresceu mais de 1 milhão de hectares neste período de 10 anos) (DERAL, 2018b). Embora realizados em condições climáticas desfavoráveis, os plantios da segunda safra têm sido melhorados e adaptados a tais condições, o que explica o aumento de rendimento nessa época. (EMBRAPA, 2010b; DERAL, 2014). Desta forma, a importância da segunda safra tem se consolidado, e o volume produzido por ela é responsável por abastecer a maior parte do consumo do estado do Paraná (DERAL, 2014).

2.2 O GÊNERO *Fusarium*

2.2.1 Morfologia, filogenia e identificação

O gênero *Fusarium* foi descrito por Link em 1809 e inclui diversos patógenos de plantas economicamente importantes, patógenos animais e humanos e espécies produtoras de metabólitos secundários, alguns sendo de interesse para propósitos comerciais, e outros associados com doenças em plantas, cânceres e problemas em humanos e animais (LESLIE; SUMMERELL, 2006). O gênero pertence à família Nectriaceae, ordem Hypocreales, classe Sordariomycetes, filo Ascomycota, e compreende cerca de 300 espécies filogeneticamente distintas, das quais muitas ainda não foram descritas formalmente (AOKI et al., 2014).

A principal característica morfológica utilizada para a descrição do gênero era a presença dos macroconídios fusiformes típicos (também descritos como em formato de canoa ou banana) (LESLIE; SUMMERELL, 2006), conforme observado na FIGURA 1. Entretanto, estudos morfológicos e de filogenia molecular demonstraram que essa característica evoluiu de forma convergente em diferentes linhagens de ascomicetos (O'DONNELL et al., 2015), não sendo uma característica sinapomórfica para o gênero.

FIGURA 1 - MACROCONÍDIOS DE *Fusarium tjaetaba*

FONTE: Adaptada de LAURENCE et al., 2015.
NOTA: Barra indicativa de tamanho: 50µm.

Diversos sistemas de classificação de espécies propostos para o gênero *Fusarium* baseavam-se exclusivamente em características morfológicas, como o tamanho (comprimento e largura), número de septos e formato dos macroconídios, formato das células basais nos macroconídios, presença ou ausência de microconídios e seu formato, presença ou ausência de clamidósporos e sua localização e presença ou ausência de escleródios (NELSON et al, 1994).

A avaliação destas características era realizada através do cultivo de isolados em diferentes meios de cultura, geralmente com ênfase em diferenças pequenas e sutis ao invés de enfatizar as similaridades. Entretanto, tais características não são estáveis em diferentes condições de cultivo, e muitas das análises para descrição de espécies eram baseadas em apenas uma ou duas culturas. A avaliação de poucos isolados não revela a gama de variação entre indivíduos que pode ocorrer dentro de uma espécie, sendo necessário muitas vezes trabalhar com um grande número de culturas representativas para determinar a real extensão da variação que pode ocorrer (NELSON et al., 1994), caracterizando as falhas de uma identificação puramente morfológica para este gênero.

Muitas espécies de *Fusarium* ou populações dentro dessas espécies exibem um grande nível de variação de características morfológicas e fisiológicas, e tal variação poderia em parte explicar a habilidade das espécies deste gênero em colonizar nichos distintos nas mais diversas áreas do mundo. Porém, tal variação torna-se um problema no momento de estabelecer um sistema taxonômico confiável e estável para este grupo, que seja amplamente aceito (NELSON et al., 1994). O grande número de diferentes sistemas de classificação propostos, que apresentam diferentes premissas (e. g. separando grupos em diferentes espécies, unindo grupos sob um único nome de espécie) (LESLIE; SUMMERELL, 2006) demonstra a dificuldade de atingir um consenso entre pesquisadores.

Em função desta problemática, alguns trabalhos utilizando sequências de DNA e análises filogenéticas foram desenvolvidos buscando delimitar o gênero *Fusarium* de forma mais precisa, bem como estabelecer sua relação com os outros gêneros da família Nectriaceae (GRÄFENHAN et al., 2011; O'DONNELL et al., 2013; LOMBARD et al., 2015). Devido à baixa correspondência entre uma filogenia robusta baseada em sequências de RPB1/RPB2 (O'DONNELL et al., 2013) e os sistemas de classificação morfológicos anteriormente utilizados, a recomendação atual é pela utilização de sequências de DNA para identificação e delimitação mais precisa de espécies de *Fusarium* (O'DONNELL et al., 2015). Além disso, com a decisão de que o artigo 59 do Código Internacional de Nomenclatura Botânica não seria mais aplicável, fungos pleomórficos que apresentassem diferentes nomes para suas fases sexuada e assexuada deveriam ser conhecidos por apenas um nome, dando prioridade ao nome mais antigo (HAWKSWORTH et al., 2011). Estes dois fatores levantaram diversas questões quanto à maneira mais adequada de delimitar o gênero, levando em consideração tanto aspectos filogenéticos e de nomenclatura, quanto aspectos práticos. Existem diferentes proposições na literatura para resolver este problema.

Uma proposta é de limitar o uso do nome *Fusarium* apenas às linhagens pertencentes ao "clado terminal de *Fusarium*", em que boa parte das espécies possui uma fase sexuada descrita anteriormente sob o nome de *Gibberella*, sendo também o clado em que está localizada a *type species* do gênero, *Fusarium sambucinum*. Sendo assim, seria necessário propor novos gêneros para as espécies descritas como *Fusarium* que não pertencessem a este grupo (GRÄFENHAN et al., 2011). Contrariando esta proposta, outra abordagem propõe a utilização do nome *Fusarium* para designar o grupo que inclui virtualmente todas as espécies de *Fusarium* de importância fitopatológica, micotoxicológica, médica e em pesquisa básica (GEISER et al., 2013).

A segunda proposição se baseia em uma filogenia do gênero elaborada a partir de sequências de RPB1/RPB2 (O'DONNELL et al., 2013) e também em aspectos práticos, como o fato do nome *Fusarium* ser bastante consolidado na literatura (sendo o quarto nome fúngico mais presente em publicações), e a possibilidade de manter espécies que apresentavam diferentes fases sexuadas no sistema de dupla nomenclatura (e. g. *Neocosmospora*, *Albonectria*, Cyanonectria, Cosmospora) sob um mesmo nome (GEISER et al., 2013). Outro ponto que fundamenta esta proposta é a preocupação de que espécies de grande importância fitopatológica e médica, como as do complexo *Fusarium solani*, de fase sexuada *Neocosmospora*, e que não fazem parte do clado terminal de *Fusarium*, fossem agrupadas em um novo gênero e deixassem de ser reconhecidas por um nome há muito tempo utilizado pela comunidade médica e fitopatológica (GEISER et al., 2013).

Uma terceira proposta mais recente surgiu a partir de uma análise mais ampla que as duas anteriores, buscando organizar a filogenia da família Nectriaceae, e não apenas resolver internamente o gênero *Fusarium* e poucos gêneros filogeneticamente próximos. Utilizando sequências de outros loci e diversas linhagens, a proposta de restringir o nome *Fusarium* apenas ao clado *Gibberella* foi retomada e outros gêneros foram propostos para alguns complexos de espécies previamente descritos como *Fusarium* (e. g. *Neocosmospora* para espécies do complexo *Fusarium solani*, *Bisifusarium* para espécies do complexo *Fusarium dimerum*) (LOMBARD et al., 2015). Embora os aspectos práticos desta proposta sejam os mesmos da proposta inicial (GRÄFENHAN et al, 2011), o que provavelmente geraria forte oposição da comunidade fitopatológica, as análises realizadas são muito mais robustas, visto que levaram em conta não apenas o gênero *Fusarium* e gêneros próximos, mas toda a família Nectriaceae. Tais análises demonstram não apenas suporte filogenético para a divisão do gênero *Fusarium* em outros gêneros, como também forneceram descrições detalhadas de características morfológicas diferenciadas para estes grupos, caracterizando o diferencial desta proposta.

Entretanto, independemente da proposta adotada, o ponto comum é que para efetivamente realizar a identificação de espécies de forma correta e confiável, análises filogenéticas ainda são necessárias, utilizando regiões de DNA adequadas para tal finalidade.

Embora a região do espaçador interno transcrito (*internal transcribed spacer*, ITS) seja o marcador de barcoding proposto para fungos (SCHOCH et al., 2012), em função de sua aplicabilidade em diversas situações (STIELOW et al., 2015), sua utilidade é limitada para identificar espécies de *Fusarium*, visto que muitas vezes não é suficiente informativo neste nível taxonômico (O'DONNELL et al., 2015). Outra limitação no uso de ITS para análises filogenéticas e identificação de espécies é a presença de fragmentos de ITS2 parálogos e xenólogos, e altamente divergentes, e que já foram detectados em vários complexos de espécies dentro do gênero (e.g. *Fusarium fujikuroi* species complex, *Fusarium oxysporum* species complex, *Fusarium concolor* species complex) (O'DONNELL et al., 2015).

Em função disso, a sequência mais utilizada para identificação de espécies de Fusarium é um fragmento do fator de alongamento da tradução 1-α (*translation elongation factor*, TEF1), indicado por atingir três critérios importantes para reconhecimento filogenético de espécies no gênero *Fusarium*: a aplicabilidade para todo o gênero, ser informativo a nível de espécie e ser ortólogo para todo o gênero (O'DONNELL et al., 2015).

Sequências de TEF1 podem ser comparadas com sequências disponíveis nos bancos de dados *Fusarium* MLST (O'DONNELL et al., 2010), Fusarium-ID (GEISER et al., 2004) e GenBank utilizando a ferramenta BLAST (JOHNSON et al., 2008), principalmente para identificar isolados no nível de complexo de espécies, como análise preliminar para orientar a análise filogenética a ser executada.

2.2.2 Aspectos fitopatológicos

Doenças causadas por espécies de *Fusarium* em praticamente todas as plantas importantes economicamente causam prejuízos econômicos bilionários à agricultura anualmente (O'DONNELL et al., 2015), por meio de sintomas como murchas, crestamento, podridões e tombamentos (MA et al., 2013). Além disso, diversas espécies do gênero produzem diversos tipos de micotoxinas (e.g. tricotecenos, fumonisinas), os quais contaminam produtos e os tornam inadequados para consumo na alimentação humana ou animal, além de serem importantes fatores de virulência para o processo de infecção nas plantas (MA et al., 2013).

Nas podridões de espiga causadas por espécies de *Fusarium* em milho a infecção pode iniciar pelo topo ou outras partes da espiga, sempre associada a alguma injúria realizada por

insetos ou pássaros. Os grãos infectados apresentam alterações de cor variando de rosa à marrom escuro, estrias de coloração branca no pericarpo e crescimento cotonoso de coloração clara a avermelhada, correspondente ao micélio fúngico (EMBRAPA, 2010a), conforme observado na FIGURA 2.

FIGURA 2 - Podridão de espiga causada por *Fusarium* sp.

FONTE: PIONEER (2016).

Já nas podridões de colmo, o tecido de entrenós inferiores adquire coloração avermelhada, que progride em direção à parte superior da planta (EMBRAPA, 2010a). A casca pode adquirir coloração marrom clara ou escura, pode ocorrer crescimento fúngico na forma de micélio cotonoso branco a rosado, e a coloração da medula pode ser esbranquiçada, rosada ou avermelhada (SILVA et al., 2001), conforme observado na FIGURA 3. Os sintomas tendem a se tornar visíveis após a polinização e aumentam em severidade à medida que a planta entra em senescência (EMBRAPA, 2010a).

FIGURA 3 - Podridão de colmo causada por *Fusarium* sp.

FONTE: IOWA STATE UNIVERSITY (2009).

2.2.3 Aspectos toxigênicos

Espécies do gênero *Fusarium* são conhecidas como produtoras de micotoxinas importantes, envolvidas tanto no desenvolvimento de doenças em plantas, quanto em doenças em animais e humanos (LESLIE; SUMMERELL, 2006). Em grãos de milho, as principais toxinas produzidas por *Fusarium*, e para as quais também existe regulação por meio de legislação específica (BRASIL, 2011) são fumonisinas (complexo de espécies *F. fujikuroi*) e tricotecenos (complexo de espécies *F. graminearum*).

Diferentes tricotecenos podem ser produzidos por espécies de *Fusarium*, como nivalenol, 3-acetil-desoxinivalenol e 15-acetildesoxinivalenol. Identificar o tricoteceno produzido utilizando técnicas químicas pode ser um desafio em amostras grandes, então desenvolveram-se técnicas moleculares para identificar a presença de genes envolvidos na biossíntese de tricotecenos em isolados de *Fusarium* e determinar qual tipo de tricoteceno possivelmente seria produzido (KUHNEM et al., 2015; QUARTA et al., 2006).

Para reações com os primers descritos por Quarta e colaboradores (2006), a diferença no tamanho dos produtos gerados indica o genótipo de tricoteceno do isolado em questão, como produtor de nivalenol (NIV), 3-acetildesoxinivalenol (3-ADON) ou 15-acetildesoxinivalenol (15-ADON).

Tais técnicas moleculares também estão descritas para avaliar a presença de genes envolvidos na biossíntese de fumonisinas no complexo *Fusarium fujikuroi* (BLUHM et al., 2004), em que a presença ou ausência de banda após a reação indica se o isolado apresenta o gene FUM1, envolvido na produção da toxina.

2.3 DIAGNÓSTICO MOLECULAR: PCR ESPÉCIE-ESPECÍFICA

Métodos alternativos ao sequenciamento para o diagnóstico e detecção de fitopatógenos são desenvolvidos com o intuito de identificar espécies rapidamente, com confiabilidade e melhor custo-benefício (ABD-ELMAGID et al., 2013). Diferentes estratégias podem ser empregadas, seja utilizando métodos imunológicos, que dependem do reconhecimento de antígenos específicos presentes nos patógenos, ou métodos baseados em DNA, que reconhecem sequências específicas (MCCARTNEY et al., 2003).

Métodos baseados em DNA tendem a ser altamente específicos, podendo distinguir diferentes espécies ou até mesmo detecção variação dentro de uma mesma espécie. Para os métodos que usam PCR, existe a vantagem da alta sensibilidade, detectando quantias pequenas

de DNA do patógeno, até mesmo a partir de um único esporo (MCCARTNEY et al, 2003). Há primers propostos para diagnóstico de diversos patógenos fúngicos em diferentes cultivos (*e. g. C. abscissum* e *C. gloesporioides* em *Citrus* (SILVA et al., 2016), espécies de *Sclerotinia* em diversas plantas (ABD-ELMAGID et al., 2013). Em *Fusarium*, apesar de existirem vários primers propostos, muitos foram desenvolvidos antes da descrição de diversas espécies e das propostas de reorganização filogenética do gênero (MULÈ et al., 2004; MURILLO et al., 1998; PATIÑO et al., 2004). Muitos deles apresentam amplificações inespecíficas (FARIA et al., 2012), não servindo para um diagnóstico preciso. Sendo assim, para muitas espécies do gênero ainda não existem primers descritos para diagnóstico.

Para o desenho deste tipo de primers normalmente são utilizadas regiões de 17-20 pares de base específicas para as espécies de interesse, e que não apresentem similaridade com sequências de outras espécies (SILVA et al., 2016). Os primers são escolhidos de modo a gerar fragmentos de tamanhos distintos como produtos de PCR, podendo ser diferenciados por eletroforese em gel de agarose ao serem comparados com marcador de peso molecular, permitindo a identificação da espécie.

É importante evitar repetições de um nucleotídeo ou di-nucleotídeos ao longo da sequência e utilizar sequências que apresentem as bases G ou C nas últimas cinco bases da extremidade 3' para promover anelamento específico, além de avaliar os primers com relação a outros parâmetros importantes para uma reação de PCR, como temperatura de *melting* e propensão para a formação de estruturas secundárias. Existem diversos softwares online disponíveis tanto para realizar tais avaliações quanto para levar esses parâmetros em consideração e propor primers automaticamente (PREMIER BIOSOFT, 2018).

Para a temperatura de *melting*, temperaturas na faixa de 52-58°C produzem melhores resultados, e temperaturas acima de 65°C tem tendência a produzir anelamento inespecífico. O conteúdo de bases GC de uma sequência influencia a temperatura de *melting* do primer, e deve estar em cerca de 40-60%. Além disso, é importante que os primers em um par possuam temperaturas de *melting* similares para maximizar o rendimento da reação, pois uma diferença maior que 5°C pode impedir a amplificação (PREMIER BIOSOFT, 2018).

Também é importante avaliar a presença de estruturas secundárias produzidas a partir de interações intra ou intermoleculares, visto que estas afetam o anelamento e consequentemente a amplificação. A estabilidade das estruturas secundárias é geralmente representada por valores de ΔG (Energia Livre de Gibbs), sendo a energia necessária para romper a estrutura. Quanto mais negativos os valores de ΔG, mais estáveis são as estruturas e, portanto, mais vão afetar a performance da reação (PREMIER BIOSOFT, 2018).

O primeiro tipo de estrutura, formado por interações intramoleculares no primer, é o grampo (*hairpin*). Geralmente grampos na extremidade 3' do primer com ΔG de até -2 kcal/mol e grampos no interior do primer com ΔG de até -3 kcal/mol podem ser tolerados (PREMIER BIOSOFT, 2018).

Já para interações envolvendo mais de um primer, o primeiro tipo é o homodímero, quando há homologia do primer com si mesmo, impedindo o anelamento ao DNA alvo e reduzindo o rendimento da reação. Há também a possibilidade dos primers em um par possuírem homologia entre si, gerando heterodímeros. Valores limite de ΔG para homodímeros e heterodímeros na extremidade 3' são de -5 kcal/mol e de -6 kcal/mol para homodímeros e heterodímeros internos (PREMIER BIOSOFT, 2018).

Para primers utilizados em diagnóstico de fitopatógenos, é importante determinar a sensibilidade e especificidade dos primers. A primeira refere-se à quantidade de DNA necessária para que a amplificação ocorra, e pode ser determinada utilizando diferentes diluições de amostra. É influenciada pelas condições da amostra (*e. g.* presença de DNA de outras espécies ou DNA vegetal), sendo necessário determinar a sensibilidade em amostras puras e amostras com DNA vegetal, definindo as condições ideais para cada reação.

Já a especificidade refere-se à amplificação apenas na presença de DNA da espécie de interesse, não ocorrendo amplificações inespecíficas na presença de DNA de outras espécies. Se o primer não for suficientemente específico, não é possível chegar a uma identificação conclusiva. Testes de especificidade normalmente são realizados com outras espécies que são descritas presentes no material vegetal contaminado e com o próprio material sadio (como controle negativo).

3 OBJETIVOS

3.1 OBJETIVO GERAL

O objetivo deste trabalho é avaliar a composição de espécies do gênero *Fusarium* associadas a milho (*Zea mays*) no estado do Paraná – BR, bem como potencial para produção de toxinas, patogenicidade, e desenvolver método molecular para distinção das espécies presentes

3.2 OBJETIVOS ESPECÍFICOS

- Identificar as espécies de isolados provenientes de colmo e espiga de milho no estado do Paraná
- Propor e validar primers para distinguir espécies encontradas por meio de PCR espécie-específica
- Avaliar a presença de genes para produção de toxinas
- Avaliar a patogenicidade de isolados representativos das espécies encontradas

Fusarium species associated with maize ear and stalk rot in Paraná state, Brazil

(submitted to Plant Pathology)

D. A. L. Petters[1], R. S. Almeida[1], F. Terasawa Jr.[2], L. V. G. Terasawa[1], C. Glienke[1*]

[1]Laboratório de Genética de Microrganismos, Departamento de Genética, Universidade Federal do Paraná, UFPR, Curitiba – PR, Brazil

[2]Vértika Agropecuária LTDA, Curitiba – PR, Brazil.

E-mail: ch.glienke@gmail.com

Running-head: *Fusarium* species in maize

Keywords: *Fusarium fujikuroi* species complex, *Fusarium graminearum* species complex, *Fusarium awaxy,* pathogenicity, fumonisin, phylogeny

1 ABSTRACT

Several *Fusarium* species are associated with maize ears and stalk rot, causing losses to maize crop quality and yield and leading to mycotoxin contamination. In order to improve disease management and reduce contamination, knowledge about *Fusarium* species distribution is required. Therefore, this study identified the *Fusarium* species in symptomatic maize ears and stalks in Paraná state, Brazil and evaluated their toxigenic and pathogenic potential. *Fusarium* isolates were identified through phylogenetic analyses and species-specific PCR, tested for the presence of toxin production genes through PCR, fumonisin production with ELISA, and for pathogenicity in maize stalks. The isolates belonged to three species complexes: *F. fujikuroi* (FFSC, 79.4%), *F. graminearum* (FGSC, 18.1%) and *F. incarnatum-equiseti* (FIESC, 2.5%). Besides the identified species *F. verticillioides* (dominant species in maize kernels), *F. fujikuroi*, *F. tjaetaba*, *F. napiforme* (FFSC), *F. graminearum*, *F. meridionale* (FGSC), FIESC

lineages 12, 20 and 33, we described the new species *F. awaxy* (dominant species in maize stalks) in FFSC. *F. graminearum* and *F. meridionale* were of 15—ADON and NIV genotypes, respectively, and all FFSC species identified had positive isolates for FUM1 gene, but fumonisin production was variable. Regarding pathogenicity, *F. fujikuroi*, *F. graminearum*, *F. meridionale* and *F. verticillioides* caused stalk rot, *F. awaxy* and *F. tjaetaba* exhibited lower aggressiveness and *F. napiforme* did not cause symptoms. These findings suggest that *Fusarium* diversity in maize is still underexplored, and further studies are required to characterize the relevance of identified species for this economically important crop.

2 INTRODUCTION

Fusarium genus was described by Link in 1809 and includes important plant, animal, and human pathogens as well as mycotoxin producers (Leslie & Summerell, 2006), considered one of the most renowned fungal genera (Sandoval-Denis *et al.*, 2018). This genus comprises around 300 phylogenetically distinct species, many not yet described (Aoki *et al.*, 2014). More than 1400 *Fusarium* names are listed in Index Fungorum and Mycobank, given the significant changes in generic and species concepts that this genus has experienced (Sandoval-Denis *et al.*, 2018), mainly due to the transition from morphological to molecular taxonomic treatments.

Older taxonomic treatments for *Fusarium* genus were based on morphology, pathogenicity and host ranges, such that the number of recognized species varied between different systems. Many *Fusarium* species exhibit a great level of variation in morphological and physiological characteristics (which could explain this genus ability to colonize distinct niches in different geographical areas across the world), and this variation has led to difficulties in the development of stable and reliable criteria for species recognition in this genus (Nelson *et al.*, 1994).

Recently, with the use of molecular tools, *Fusarium* species were redistributed within the genus in different species complexes (O'Donnell *et al.*, 2000, 2004, 2013), with some species being divided into several others, demonstrating the differences between morphological and molecular taxonomic treatments. Another important change in *Fusarium* taxonomy came with phylogenetic evidence that the long-accepted morphological definition of the genus (including the presence of typical fusiform macroconidia) is polyphyletic (Gräfenhan *et al.*, 2011), suggesting the need to reevaluate this genus and split it into new genera. In a revision of the Nectriaceae family, *Fusarium* genus was confined to species with a *Gibberella* sexual morph, and new genera and combinations were proposed to accommodate species outside this group, such as *Bisifusarium* (previously *F. dimerum* species complex) *Rectifusarium* (formerly *F. ventricosum* species complex) and *Neocosmospora* (previously *F. solani* species complex) (Lombard *et al.*, 2015).

Even with those substantial changes, species complexes retained in *Fusarium* genus (such as *F. graminearum* (FGSC), *F. fujikuroi* (FFSC), *F. incarnatum-equiseti* (FIESC) and *F. oxysporum* (FOSC) species complexes) give *Fusarium* the status of one of the top 10 globally most important genera of plant pathogenic fungi, in terms of scientific and economic importance (Dean *et al.*, 2012). Moreover, most cultivated plants are known to host multiple *Fusarium* species (Leslie & Summerell, 2006), and *Fusarium* diseases cause losses in excess of billions of dollars to agriculture every year (O'Donnell *et al.*, 2015) through symptoms like wilts, rots, dampings, and crests (Ma *et al.*, 2013).

Fusarium is also known to produce mycotoxins associated with plant, animal and human diseases (Leslie & Summerell, 2006) such as fumonisins and trichothecenes. As mycotoxins pose serious risks for animal and human health, maximum tolerated limits for *Fusarium* mycotoxins concentration in feed products have been imposed worldwide (Cheli *et al.*, 2014; Anfossi *et al.*, 2016), including Brazil (ANVISA, 2011). Several mycotoxin detection methods

have been described, either based on chromatographical or immunochemical approaches (Selvaraj *et al.*, 2015; Anfossi *et al.*, 2016). Moreover, genotyping methods targeting genes involved in mycotoxin production have been proposed, so that it is possible to predict the potential for toxin production (Bluhm *et al.*, 2004; Quarta *et al.*, 2006; Ward *et al.*, 2008).

F. fujikuroi, F. graminearum, and *F. incarnatum-equiseti* species complexes members cause destructive diseases and mycotoxin contamination in several economically important cereal crops worldwide (Kvas *et al.*, 2009; Kazan *et al.*, 2012; Villani *et al.*, 2016). In Brazil, one of the greatest cereal-producing countries (FAOSTAT, 2018), species composition and toxigenic potential of *F. graminearum* species complex was described for wheat (Scoz *et al.*, 2009; Del Ponte *et al.*, 2015), rice (Gomes *et al.*, 2015), barley (Astolfi *et al.*, 2011; Castañares *et al.*, 2016) and also for maize (Kuhnem *et al.*, 2016).

Maize is the most-cultivated cereal in Brazil, and Brazil is the largest producer in South America, ranking third in the world (FAOSTAT, 2018). Maize production yields are increasing, due to improved technologies and use of the second harvest of maize in the major production regions of Brazil (Costa *et al.*, 2010). Nevertheless, the presence of *Fusarium* species is still a concern for maize production, mainly due to the difficulties in disease management with available fungicides and the existent mycotoxin limits for maize products in Brazil (Kuhnem *et al.*, 2016). Therefore, information about distribution, toxigenic and pathogenic potential of *Fusarium* in Brazil is required.

For South Brazil, including Paraná state, Kuhnem *et al.* (2016) described the presence of three species from *Fusarium graminearum* species complex in maize (*F. graminearum*, *F. meridionale,* and *F. cortaderiae*), also discussing the toxigenic potential and temperature effects on mycelial growth. However, there is still a lack of information about *Fusarium* species from other important complexes, such as *F. fujikuroi* and *F. incarnatum-equiseti* species complexes. Thus, the aim of this study was to identify *Fusarium* species associated with maize

ear and stalk rot in Paraná state, Brazil, through molecular phylogeny approaches and species-specific PCR, as well as evaluate their pathogenicity in maize stalks and toxigenic potential.

3 MATERIALS AND METHODS

3.1 Sampling, fungal isolation and culture conditions

Samples were obtained during surveys conducted between February and April 2016 and consisted of symptomatic maize ears and stalks collected in different locations in Paraná state, Brazil, from commercial fields near Arapoti, Campo Largo, Castro, Guarapuava and Prudentópolis municipalities (Figure 1).

Maize kernels and stalks were surface disinfected (1 min in 70% ethanol, 3 min in NaOCl, rinsed six times in sterile distilled water), dried in a laminar flow hood, placed on water-agar medium (WA), and incubated for 5 days at 23ºC in the dark. Plates were evaluated daily for fungal growth and *Fusarium*-like isolates were transferred to potato dextrose agar medium (PDA) plates and grown for 7 days at 23ºC in the dark. From these plates, single-spores cultures were obtained by washing the conidia from the mycelium with 0.1% (v/v) Tween 80 solution and spreading 100 µL of a spore suspension (10^3 spores . mL^{-1}) over PDA-medium plates. After incubation at 23ºC for 1 day in the dark, isolated colonies were transferred to synthetic nutrient agar-medium (SNA; Nirenberg, 1976) tubes until further analysis (Leslie & Summerell, 2006). Based on colony morphology and pigmentation on PDA-medium plates, isolates were grouped into species complexes (Leslie & Summerell, 2006).

All isolates were deposited in the Fungal Culture Collection of Laboratório de Genética de Microrganismos (Federal University of Paraná – UFPR, Brazil; www.labgem.ufpr.br) (Table S1).

3.2 DNA extraction

Genomic DNA was extracted from 3-day old cultures grown at 23ºC in the dark over cellophane membranes placed on PDA-medium plates. Mycelium was harvested, ground in liquid nitrogen, and submitted to a phenol-chloroform extraction method (Raeder & Broda, 1985). DNA was resuspended in 50 µL of Tris-HCl, pH 7.6, 10 mM, and treated with 0.5 µL of RNAse A (20 mg . mL^{-1}) (Sigma-Aldrich). For evaluation of quality and concentration, the DNA was submitted to 1% agarose gel electrophoresis using Tris-Acetate-EDTA buffer (TAE), stained with GelRed (Biotium), visualized under UV light, and compared to Lambda DNA/Hind III marker (Invitrogen).

3.3 PCR amplification and sequencing

For *Fusarium fujikuroi* species complex (FFSC) isolates, fragments of the internal transcribed spacer region of the rDNA (ITS) and the translation elongation factor 1-alpha (*EF-1α*), and beta-tubulin (*TUB2)* genes were amplified using, respectively, ITS4/ITS5 (White *et al.*, 1990), EF1/EF2 (O'Donnell *et al.*, 1998) and T1/T22 primers (O'Donnell & Cigelnik, 1997). For *F. graminearum* (FGSC) and *F. incarnatum-equiseti* species complexes isolates, EF728F/EF2 primers (O'Donnell *et al.*, 1998; Carbone & Kohn, 1999) were used instead, as no amplicon was obtained using EF1/EF2 primers pair. Amplification reactions were performed with an Amplitherm Thermal Cycler in a total volume of 12.5 µL, using 50 ng of DNA and 0.2 µM of each forward and reverse primers per reaction and TopTaq PCR Master Mix Kit (Qiagen), following manufacturer's instructions. PCR conditions included an initial denaturation step of 5 min at 94 ºC, 35 cycles of 30 s at 94 ºC, 30 s at 60 ºC and 1 or 2 min at 72º C (for *EF-1α* and *TUB2*, respectively), using a final extension step of 10 min at 72 ºC to conclude the reaction. PCR products were submitted to 1% agarose gel electrophoresis using

TAE buffer, stained with GelRed (Biotium), visualized under UV light and compared to Molecular Marker Ladder 100pb (Axygen) for evaluation of size, quality, and concentration.

For sequencing, amplicons were purified using FastAP and ExoI enzymes (ThermoScientific) and sequenced using the BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems). Sequencing products were purified using Sephadex G-50 (GE Healthcare) in a Multiscreen Column Loader (Millipore). DNA sequences were obtained on an ABI Prism 3500 DNA sequencer (Applied Biosystems) and the electropherograms were examined and manually corrected when necessary using MEGA7 software (Kumar *et al.*, 2016). All sequences generated in this study were deposited in NCBI GenBank (Table S1).

3.4 Phylogenetic analyses

For species complex identification the *EF-1α* nucleotide sequences were compared to NCBI GenBank database using BLAST tool (Johnson *et al.*, 2008), and separate phylogenetic analyses were performed for each complex.

Besides the sequences generated in this study, *EF-1α* sequences from *F. graminearum* (FGSC) and *F. incarnatum-equiseti* (FIESC), *EF-1α* and *TUB2* for *F. fujikuroi* (FFSC) recognized species and lineages were retrieved from NCBI GenBank database and included in the phylogenetic analyses (Table S2). *F. oxysporum* NRRL 22902, *F. pseudograminearum* NRRL 28062 and *F. concolor* NRRL 13459 strains were used as outgroups for FFSC, FGSC and FIESC, respectively. Multiple sequence alignments were obtained with MAFFT v. 7 software (Katoh & Standley, 2013), checked and manually adjusted in MEGA7 software (Kumar *et al.*, 2016) when necessary. Phylogenetic inference was performed under two different algorithms: Maximum Likelihood and Bayesian Inference.

Maximum likelihood (ML) and Bayesian Inference (BI) analyses were performed on CIPRES Science Gateway portal v. 3.3 (Miller *et al.*, 2012) using Garli v. 2.0 (Zwickl, 2006)

and MrBayes v. 3.2.6 (Ronquist *et al.*, 2012), respectively. Evolutionary models were tested with jModelTest v. 2.1.6 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012), and the best-fit model for each data partition was selected according to Akaike criterion. Characteristic of different partitions and evolutionary models used in analyses are summarized in Table 1.

For ML analyses, evolution was simulated until likelihood scores reached convergence. Nonparametric bootstrap analysis was conducted using 1000 pseudoreplicates to generate statistical support for the branches and nodes with zero branch lengths were collapsed. Bootstrap trees were compiled using SumTrees v. 4.3.0 from Dendropy v. 4.3.0 package (Sukumaran & Holder, 2010). Bayesian analyses were conducted using two parallel runs with one cold and three heated chains each, using the number of generations needed to reach split frequencies of less than or equal to 0.01 and a sampling frequency set to every 10,000 generations.

The 50% majority consensus trees (for ML) and posterior probability values (PP, for BI) were calculated after discarding the first 25% of the generated trees as burn-in. Resulting trees were plotted in FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/), and their topology was compared, checking incongruence and conflict between clades with significant ML and BI support before being concatenated in one tree, which was edited with Adobe Illustrator CC 2017.

3.5 Genealogical concordance phylogenetic species recognition (GCPSR)

To assess the recombination level between the new *Fusarium* species proposed here and its closest phylogenetic relatives, a pairwise homoplasy (PHI) test was conducted using the multilocus dataset (EF, TUB). The test was performed using SplitsTree v. 4.14.6 (Huson & Bryant, 2006), and the relationship between the species was visualized building split graphs using both Log-Det transformation and splits decomposition options, as previously described

(Quaedvlieg *et al.*, 2014; Sandoval-Denis *et al.*, 2018). A PHI value below 0.05 ($\Phi w < 0.05$) indicates significant recombination in the dataset.

3.6 Molecular diagnostic

3.6.1 Species-specific PCR for *Fusarium fujikuroi* species complex (FFSC)

To identify non-sequenced isolates through species-specific PCR reactions, specific primers were designed for the *F. fujikuroi* species found, based on *EF-1α* sequence alignments. 17-20bp regions specific to each species were selected to design forward primers, which were tested for performance characteristic such as internal structures, hairpins, self- and hetero-dimers formation using OligoIDTAAnalyzer 3.1 (https://www.idtdna.com/calc/analyzer) (Owczarzy *et al.*, 2008). Melting temperatures were calculated using NEB Tm Calculator (https://tmcalculator.neb.com), in order to select primers compatible with the reverse primer EF2 (O'Donnell *et al.*, 1998). Primer sequences, annealing temperatures and amplicon sizes are summarized in Table 2.

Reactions were performed in a total volume of 12.5 µL, using 0.2 µM of each forward and reverse primers, 1.5 mM MgCl2, 0.2 mM dNTPs, 1X Buffer and 0.5 U Taq Polymerase (Ludwig Biotecnologia), following manufacturer's instructions. PCR conditions included an initial denaturation step of 5 min at 94 ºC, 35 cycles of 30 s at 94 ºC, 30 s at the annealing temperature for the primer (as indicated in Table 2) and 1 min at 72º C, using a final extension step of 10 min at 72 ºC to conclude the reaction. PCR products were submitted to 1% agarose gel electrophoresis using TAE buffer, stained with GelRed (Biotium), visualized under UV light and compared to Ladder 100pb (Axygen) for evaluation of size.

The specificity of the primers was tested in reactions using genomic DNA of the 46 FFSC sequenced isolates (*F. awaxy*: 16 isolates; *F. fujikuroi*: 5 isolates; *F. napiforme*: 3

isolates; *F. tjaetaba*: 3 isolates; *F. verticillioides*: 19 isolates) and 5 sequenced isolates representing strains of FGSC (*F. graminearum* and *F. meridionale)* and FIESC (FIESC 12, FIESC 20-c; FIESC 33*)* found in this work.

To determine the primers sensitivity, the concentration of the genomic DNA from one isolate from each species was measured with NanoDrop 2000 (Thermo Fisher Scientific) and different dilutions with final concentrations of 100 ng, 50 ng, 10 ng, 1 ng, 100 pg, 10 pg, 1 pg, 100 fg, 10 fg and 1 fg per µL were prepared (Costa *et al.* 2017) and used for amplification reactions in the same conditions described above.

After testing specificity and sensitivity of the primers, reactions were performed to identify the remaining FFSC isolates. All reactions included positive (a sequenced isolate for the species being tested) and negative controls (sequenced isolates belonging to the other species of *Fusarium fujikuroi* species complex found in this work), listed in Table 2.

3.6.2 PCR-RFLP for *Fusarium graminearum* species complex (FGSC)

To identify non-sequenced isolates from FGSC, a specific primer for both *F. graminearum* and *F. meridionale* was designed, based on *EF-1α* sequences alignments. A 17bp region was selected to design a forward primer compatible with EF2 primer and tested for performance characteristics and melting temperature as described for FFSC primers above. Primer sequence and annealing temperature are listed in Table 2. For PCR-RFLP, restriction patterns of *F. graminearum* and *F. meridionale* PCR fragments were predicted using NEBCutter online software (http://tools.neb.com/NEBcutter) (Vincze *et al.*, 2003), and predicted sites were compared for choosing a specific restriction site for one of the species so that *Bsa*HI enzyme was chosen.

Reactions were performed in a total volume of 12.5 µL using 0.2 µM of each forward and reverse primers, 1.5 mM MgCl2, 0.2 mM dNTPs, 1X Buffer and 0.5 U Taq Polymerase

(Ludwig Biotecnologia), following manufacturer's instructions. PCR conditions included an initial denaturation step of 5 min at 94 ºC, 35 cycles of 30 s at 94 ºC, 30 s at 52ºC and 1 min at 72º C, using a final extension step of 10 min at 72 ºC to conclude the reaction. PCR products were submitted to 1% agarose gel electrophoresis, stained with GelRed (Biotium), visualized under UV light and compared to Ladder 100pb (Axygen) for evaluation of size. After amplification, the digestion reaction was performed by incubating 10 µL of PCR products (with a concentration around 0.1-0.5 µg. μL^{-1}) with 2.5 U of *BsaHI* enzyme (ThermoFisher Scientific) in a final reaction volume of 15.5 µL at 37ºC for 16 hours, followed by thermal inactivation at 65ºC for 20 minutes. Restricted and non-restricted fragments were submitted to 1% agarose electrophoresis using TAE buffer, stained with GelRed (Biotium), visualized under UV light and compared to Ladder 100pb (Axygen) for evaluation of size and discrimination of species.

Specificity of the primers was tested in reactions using genomic DNA of the 18 FGSC sequenced isolates (*F. graminearum*: 11 isolates; *F. meridionale*: 7 isolates) and 8 sequenced isolates representing strains of FFSC (*F. awaxy*, *F. fujikuroi*, *F. napiforme*, *F. tjaetaba* and *F. verticillioides)* and FIESC (FIESC 12, FIESC 20 and FIESC 33*)* found in this study.

To determine the primers sensitivity, the concentration of the genomic DNA from one isolate from each species was measured with NanoDrop and different dilutions with final concentrations of 100 ng, 50 ng, 10 ng, 1 ng, 100 pg, 10 pg, 1 pg, 100 fg, 10 fg and 1 fg per µL were prepared (Costa *et al.* 2017) and used for amplification reactions in the same conditions described above.

After testing specificity and sensitivity of the primers reactions were performed to identify the remaining FGSC isolates. All PCR reactions included positive (sequenced isolates of *F. graminearum* and *F. meridionale*) and negative controls (sequenced isolates belonging to the species of *Fusarium fujikuroi* species complex found in this work) listed in Table 2, and digestion reactions included the same positive controls of the PCR.

3.7 Taxonomy

Two isolates (LGMF1661 and LGMF1930) from the proposed novel species in *Fusarium fujikuroi* species complex (*Fusarium awaxy*, as indicated by phylogenetic analyses) were selected for morphological characterization.

For macromorphological characterization, colony morphology, pigmentation, odour and growth were evaluated during 7 days on PDA, oatmeal agar (OMA) and SNA media incubated at 24ºC with a 12/12 h cool fluorescent light/dark photoperiod (Sandoval-Denis *et al.*, 2018). Mycelial growth rates were evaluated in PDA and SNA media, incubating cultures in the dark at temperatures ranging from 16-40ºC in 4ºC intervals and measuring radial growth in 8 directions around the colony, in triplicate, to calculate mean values per day.

For micromorphological evaluation, cultures were incubated at 24ºC with a 12/12 h cool fluorescent light/dark photoperiod. Observations included presence and characteristics of sporodochia, sporodochial macroconidia shape, septation and size and presence of chlamydospores in Carnation Leaf Agar (CLA) (Fisher *et al.*, 1982), and microconidial shape, septation, size, mode of formation and disposition in conidiophores, branching patterns (mono or polyphialides) and nature of conidiogenous cells in both CLA and SNA. Structures were examined and documented using water as mounting medium on a Olympus BX51 microscope equipped with a Olympus DP72 camera, using cell^F software. A minimal of 50 measurements per type of structure were made using Fiji software (Schindelin *et al.*, 2012), to calculate mean, minimum and maximum values. For comparison of relevant morphological features, composite photo plates were assembled (Sandoval-Denis *et* al., 2018) in Adobe Photoshop CC 2017.

Descriptions and nomenclature were deposited in Mycobank.

3.8 Fumonisin and trichothecenes PCR

To assess the toxigenic potential of *F. fujikuroi* (FFSC) and *F. graminearum* (FGSC) species complexes isolates, PCR reactions targeting different genes involved in mycotoxins production were performed (fumonisin for FFSC and tricothecenes for FGSC). For FFSC isolates, PCR reactions targeted the FUM1 gene using FUM1F and FUM1R primers (Bluhm *et al.*, 2004), which produce a fragment of 183bp for isolates with FUM1 gene. Amplification reactions were performed in a total volume of 12.5 µL, using using 50 ng of DNA and 0.2 µM of each forward and reverse primers per reaction and TopTaq PCR Master Mix Kit (Qiagen), following manufacturer's instructions. PCR conditions included an initial denaturation step of 5 min at 94 ºC, 35 cycles of 30 s at 94 ºC, 30 s at 60 ºC and 1 min at 72º C, using a final extension step of 10 min at 72 ºC to conclude the reaction. PCR products were submitted to 1% agarose gel electrophoresis using TAE buffer, stained with GelRed (Biotium), visualized under UV light and compared to Ladder 100pb (Axygen) for evaluation of size and presence of the fragment.

For FGSC isolates, reactions targeted *Tri3*, *Tri5* and *Tri7* genes, to identify 3-acetyl-deoxynivalenol (3-ADON) or 15-acetyl-deoxynivalenol (15-ADON), deoxynivalenol (DON), and nivalenol (NIV) producers, respectively, in a multiplex reaction (Quarta *et al.*, 2006). PCR reactions were performed in a total volume of 12.5 µL, using TopTaq PCR Master Mix Kit (Qiagen) and following the annealing temperature and primers concentration described in the multiplex method (Quarta *et al.*, 2006). PCR conditions included an initial denaturation step of 5 min at 94 ºC, 35 cycles of 30 s at 94 ºC, 30 s at 53 ºC and 1 min at 72º C, using a final extension step of 10 min at 72 ºC to conclude the reaction. PCR products were submitted to 1% agarose gel electrophoresis using TAE buffer, stained with GelRed (Biotium), visualized under UV light and compared to Ladder 100pb (Axygen) for evaluation of size and trichothecene

genotype determination. Expected fragment sizes are 625 bp for NIV, 525 bp for DON, 708 bp for 15-ADON and 354 bp for 3-ADON producers (Quarta *et al.*, 2006).

3.9 Fumonisin extraction and quantification

A fumonisin production and quantification assay using maize kernels was performed to confirm FUM1 PCR results, as some *F. fujikuroi* species complex isolates from our study belonged to species not yet evaluated for their toxigenic potential (*F. awaxy* and *F. tjaetaba*). Positive and negative isolates (according to PCR results) from both species were chosen, as well as positive and negative isolates from *F. fujikuroi*, and positive isolates from *F. napiforme* and *F. verticillioides*, species already described as fumonisin producers (Leslie & Summerell, 2006). Isolates chosen are listed in Table 3.

Maize kernels were ground using a Moulinex Super Junior Coffee Grinder (model A505, Moulinex), mixed with distilled water in 1:1 proportion (maize (g) : water (mL)), and autoclaved for 60 min at 121ºC for 2 consecutive days (Vismer *et al.*, 2004; Schoeman *et al.*, 2016). Using the ground maize kernels, maize patties were prepared inside 90-mm polystyrene Petri dishes and inoculated with 1 mL of spore suspensions (10^6 spores . mL^{-1}) (Schoeman *et al.*, 2016). Spores were obtained from 14-day old cultures grown on PDA-medium, by washing with 0.1% (v/v) Tween 80 solution. Three patties were inoculated per isolate, incubated at 25ºC for 14 days, then oven-dried at 60ºC overnight.

Fumonisin quantification was performed through ELISA with AgraQuant® Total Fumonisin Assay (0.25/5.0) (RomerLabs) following manufacturer's instructions. For ELISA sample preparation, the dried patties were pooled and 5 g of pooled sample was mixed with 25 mL methanol/water (70/30, v/v), following 1:5 ratio of sample weight and extraction solution as described by manufacturer. The mixture was vigorously shaken for 3 minutes to extract mycotoxins. The extracts were filtered through Whatman paper filters,had their pH adjusted

(pH between 6 and 8) and were diluted (1:4) in distilled water and submitted to ELISA reactions. The samples and the fumonisin standard solutions had their absorbance measured in Epoch microplate spectrophotometer (Bio Tek, USA) using Gen5 software, and quantification was performed based on the constructed standard curve. Fumonisin detection range of the kit is 0.25-5.0 ppm.

3.10 Pathogenicity tests

Pathogenicity tests were performed with isolates recovered from maize stalks to fulfill Koch's postulates for some of the *Fusarium* species found. One isolate was selected for each species (*F. awaxy*: LGMF1930; *F. fujikuroi*: LGMF1818; *F. graminearum*: LGMF1824; *F. meridionale*: LGMF1853; *F. napiforme*: LGMF1842; *F. tjaetaba*: LGMF1854; *F. verticillioides*: LGMF1961).

Seeds of *Zea mays* hybrid "P2530H" (moderately resistant, Pioneer) were treated with fungicides and insecticides, and planted along a single row divided in blocks of 20 plants, with 0.2m and 0.8m spacing between plants and blocks, respectively. For each species, fifteen healthy plants within a same block were inoculated 90 days after planting, using an adapted cattle injection gun (Höpnner, Model 113) equipped with a hypodermic needle. To prepare the spore suspensions (10^6 spores . mL $^{-1}$), 14-day old cultures grown on PDA-medium were washed with a 0.1% (v/v) Tween 80 solution. Stalks were inoculated at the second internode, using 1 mL of the spore suspension. The 0.1% (v/v) Tween 80 solution was used as negative control.

Symptoms, lesions and disease development were evaluated 70 days after inoculation. Maize stalks were classified as healthy (green and firm stalks, without collapsing when pinched), intermediary (tan and firm stalks, without collapsing when pinched) and rotten (tan to brown stalks, collapsing when pinched). Inoculated fungi were re-isolated from stalks with

lesions and had their identity confirmed through EF-1α sequencing, species-specific PCR, and PCR-RFLP methods described before.

4 RESULTS

4.1 Species complexes composition within Fusarium

A total of 204 *Fusarium* isolates were recovered from symptomatic maize samples, 107 from maize ears (52.5%), and 97 (47.5%) from stalks. Based on colony morphology and pigmentation on PDA-medium, 162 were identified as members of *Fusarium fujikuroi* species complex (FFSC) (79.4%), 37 as *Fusarium graminearum* species complex (FGSC) isolates (18.1%) and 5 as *Fusarium incarnatum-equiseti* (FIESC) species complex (FIESC) members (2.5%). FFSC was the most frequent complex, comprising 85% and 73.2% of ear and stalk collections, respectively. FGSC isolates comprised 15% of ear and 21.6% of stalk collection. FIESC was the least frequent complex, and only recovered from stalks (2.5% of the stalk collection). FFSC isolates were mainly recovered from ears, and on the other hand, most of the FGSC isolates were recovered from stalks (Figure 1).

The generated *EF-1α* sequences were compared to sequences in NCBI Genbank database confirming the previous morphological identification of isolates at complex level, with 46 isolates displaying 98-100% *EF-1α* sequence similarity to FFSC sequences, 18 isolates displaying 98-100% sequence similarity to FGSC sequences and 5 isolates showing 98-100% sequence similarity to FIESC sequences.

4.2 Phylogenetic analysis

For *Fusarium fujikuroi* species complex (FFSC) phylogenetic analysis, the *EF-1α* alignment consisted of 146 sequences from the 75 recognized species and lineages in FFSC, *F. oxysporum* NRRL 22902 as the outgroup taxon and 46 sequenced isolates from our sample (Figure 2). From these 46 isolates, 19 were identified as *F.* v*erticillioides*, 5 as *F. fujikuroi*, 3 as *F. tjaetaba* and 3 as *F. napiforme.* However, 16 isolates did not cluster with any known FFSC species and formed a well-supported group, representing a novel species. This group was confirmed in the multilocus approach using *EF-1α* and *TUB2* sequences as well (data not shown). Moreover, no significant evidence of recombination was observed in the PHI test with the closest phylogenetic relatives, *F. bactridioides*, *F. subglutinans* and *F. temperatum* (Φw = 0.27, Figure S1). Thus, this species is described in our study as *F. awaxy*.

For *Fusarium graminearum* species complex, the *EF*-1*α* alignment consisted of 55 sequences from the 17 recognized species in FGSC, *F. pseudograminearum* as the outgroup taxon and 18 isolates of our sample. From these, 11 were identified as *F. graminearum* and 7 as *F. meridionale* (Figure 3).

Finally, for *Fusarium incarnatum-equiseti* species complex (FIESC) phylogenetic analysis, the alignment comprised 13 sequences from FIESC species and lineages, *F. concolor* as the outgrop taxon and 5 sequences of isolates from our sample (Figure 4). One isolate was identified as a member of FIESC lineage 12, other as a member of FIESC lineage 20. The remaining 3 did not cluster with any other FIESC lineage yet described, suggesting they belong to a new FIESC lineage, regarded here as FIESC lineage 33.

4.3 Species-specific PCR for Fusarium fujikuroi species complex and PCR-RFLP for Fusarium graminearum species complex

Using the *EF-1α* sequences, six primers were designed for species identification. The specificity of these primers was evaluated using 69 isolates previously identified by phylogenetic analysis. All six primers produced amplicons of expected sizes (Table 2) and amplification occurred only in the target species, showing their specificity (Figure 5). In the PCR-RFLP method for FGSC, fragment restriction occurred only for *F. graminearum* isolates, efficiently differentiating *F. graminearum* and *F. meridionale*. Detection limit of the primers was variable, ranging from 50 ng to 1 ng of template DNA per reaction.

4.4 Species composition

From the 162 isolates belonging to FFSC, 46 were identified through phylogenetic analysis (Figure 2) and 116 through species-specific PCR. 83 isolates were identified as *F. verticillioides*, 55 as *F. awaxy*, 12 as *F. fujikuroi*, 9 as *F. tjaetaba* and 3 as *F. napiforme.* From the total of 37 FGSC isolates, 18 were identified through phylogenetic analysis (Figure 3) and 19 through the PCR-RFLP method. From those, 19 isolates were identified as *F. graminearum* and 18 as *F. meridionale*. And finally, all FIESC members were identified through phylogenetic analysis (Figure 4), FIESC lineages 12 and 20 with one isolate each and 3 isolates from a new FIESC lineage (FIESC 33).

F. verticillioides and *F. awaxy*, both species from FFSC, were the dominant species in maize ears (58.9%) and stalks (34%), respectively. For overall sample, *F. verticillioides* was the most frequent (41.7%) species (Figure 1).

4.5 Toxigenic potential

Among the 162 *Fusarium fujikuroi* species complex (FFSC) isolates 101 were positive and 61 were negative for the presence of FUM1 gene, as detected by FUM1 PCR (Table S1). All *F. verticillioides* isolates were positive, on the other hand, both positive and negative isolates were found for *F. awaxy*, *F. fujkuroi, F. napiforme* and *F. tjaetaba*. As expected, FUM1-negative isolates produced no fumonisins within the detection range when inoculated in maize patties. On the other hand, FUM1-positive isolates were variable in the fumonisin production. For *F. awaxy* isolates, fumonisin production was not detected. For *F. fujikuroi*, *F. tjaetaba* and *F. verticillioides,* intermediate (1.1 ppm for *F. fujikuroi* LGMF1818 and 1.6 ppm for *F. tjaetaba* LGMF1723) to high (2.4 and 3.7ppm for *F. verticillioides* isolates) amounts of fumonisin were observed, but some isolates produced low (0.26ppm) or no detectable amounts of fumonisin (Table 3).

Trichothecene genotypes were species dependent for FGSC isolates, as all *F. graminearum* isolates were 15-ADON genotype and all *F. meridionale* isolates were NIV genotype (Table S1). 3-ADON genotype was not present in our sample.

4.6 Taxonomy

Fusarium awaxy Petters & Glienke, sp. nov. – Mycobank 824048, Fig 6.

Etymology: Named after the Tupi-Guarani word for maize, "*awaxy*", referring to isolation substrate (maize ears and stalks) and geographical location (Arapoti and Guarapuava cities, both with names from Tupi-Guarani language).

Description

Colonies on PDA growing in the dark with average radial growth rate of 5.9 mm/d at 24ºC (reaching 74-80mm diameter in 7 d at 24ºC), with abundant aerial mycelium. Colony colour

white, pale pink, pale violet or peach, occasionally becoming dark pink, vinaceous or violet in older cultures. Odour absent.

Microconidia forming abundantly in false heads, arising in monophyalides and polyphialides, oval, 7.8 – 16 µm ($\overline{x}$ = 11.7 µm) long, 2.1 – 5.7 µm ($\overline{x}$ = 4.4 µm) wide, 0 septate. *Chlamydospores* absent. *Sporodochia* tan to cream coloured, formed on the surface of carnation leaves and seldom covered with aerial mycelium, occasionally formed on the surface of CLA or PDA. *Macroconidia* 3 septate, 24.1 – 43.5 µm ($\overline{x}$ = 30.4 µm) long, 3.2 – 5.1 µm ($\overline{x}$ = 4.2 µm) wide, less abundant than microconidia.

Cardinal temperatures for growth: minimum observed of 16ºC, maximum 32ºC, optimal 23-28ºC.

Habitat: Ear and stalk rot of *Zea mays*, seeds of *Sourghum bicolor*

Known geographic distribution: Paraná and Goiás states, Brazil; South Korea, China, South Africa, USA.

Specimens examined:

Brazil, Paraná, Guarapuava, stalk rot of *Zea mays*, March 2016, collected by F. Terasawa, isolated by D. Petters, culture ex-type LGMF1930, NCBI GenBank accession codes MG839004 (*EF-1α*), MG839013 (*TUB2*), MH252922 (*ITS*);

Brazil, Paraná, Arapoti, ear rot of *Zea mays*, February 2016, collected by F. Terasawa, isolated by D. Petters, LGMF1661, NCBI GenBank accession codes MG838954 (*EF-1α*), MG839011 (*TUB2*), MH252921 (*ITS*).

Notes – *F. awaxy* is phylogenetically related to *F. temperatum* and *F. subglutinans*, both species already described causing maize stalk rot (Leslie & Summerell, 2006; Scauflaire *et al.*, 2011). Morphologically *F. temperatum* and *F. subglutinans* are quite similar, producing microconidia in mono and polyphialides arranged in false heads in the aerial mycelium. However, they differ in the degree of septation of the macroconidia, as *F. temperatum* macroconidia are usually 4-

septate and *F. subglutinans* are 3-septate (Scauflaire *et al.*, 2011). There is not a clear morphological delimitation between *F. awaxy* and *F. subglutinans*, but many other species morphologically similar to *F. subglutinans* have been described (*e.g. F. bulbicola*, *F. guttiforme*, *F. sacchari*) and cannot be properly differentiated without the use of molecular information (Leslie & Summerell, 2006). *F. subglutinans* and *F. temperatum* have already been described causing human infections, but *F. awaxy* did not grow over 32ºC, suggesting inability to cause infection in humans. Additionally, both *F. subglutinans* and *F. temperatum* are reported as causal agents of *Fusarium* stalk rot, and in pathogenicity tests *F. awaxy* caused symptoms classified as intermediate, suggesting lower aggressiveness than usually observed for *F. subglutinans* and *F. temperatum.*

4.7 Pathogenicity

Isolates from all species, except *F. napiforme,* caused stalk rot symptoms in P2530H maize hybrid, but at different levels of severity. *F. fujikuroi*, *F. graminearum*, *F. meridionale*, and *F. verticillioides* were the most aggressive, as maize stalks became tan, brown and fragile, crushing when pinched. *F. awaxy* and *F. tjaetaba* were classified as intermediate, as stalks acquired a tan to beige coloration, but maize stalks were still firm, and did not collapse when pinched. Maize plants inoculated with *F. napiforme* were classified as healthy, as stalks were still green and firm. Isolates recovered from lesions had identical *EF-1α* sequences to the inoculated isolates, confirming the identification of the causative agent and fulfilling the Koch's postulates.

5 DISCUSSION

Our study provides information regarding the composition of *Fusarium* species associated with symptomatic maize plants in Paraná state, Brazil, with isolates from three different complexes: *Fusarium fujikuroi* (FFSC), *Fusarium graminearum* (FGSC), *Fusarium incarnatum-equiseti (FIESC).*

For *Fusarium graminearum* species complex (FGSC), both species found, *F. graminearum* and *F. meridionale*, are commonly described causing diseases in several cereal crops, such as maize, barley, rice and wheat, being distributed worldwide (Goswami & Kistler, 2004) and have already been described in maize ears and stalks from South Brazil in a previous study (Kuhnem *et al.*, 2016). Our results for trichothecenes genotyping agree with Kuhnem *et al.* (2016) since *F. graminearum* and *F. meridionale* were also of 15-ADON and NIV genotypes in our sample. However, this previous study (Kuhnem *et al.*, 2016) described *F. meridionale* as the dominant species in overall sample, maize kernels and maize stalks collections, and the vast majority of the maize stalk isolates were identified as *F. meridionale* (97.8%), which differs from the proportions here observed. In our sample *F. graminearum* were not so rare, as they comprised 38.1% of FGSC isolates of our stalk collection. In addition, for our FGSC maize ears collection, *F. graminearum* was the dominant species (68.7%) and for the FGSC overall sample, both species were present in similar proportions (*F. graminearum* comprising 51.3% and *F. meridionale* comprising 48.7%). Differences in these findings may be due to difference in sampling areas, sample sizes, or even due to changes in population composition across time, as species composition can vary between fields in response to environmental factors or even management practices (Del Ponte *et al.*, 2013). Such differences in species composition suggest the relevance of continuous monitoring and evaluation of FGSC populations, to direct disease management and provide knowledge of the biology of such pathogens (Del Ponte *et al.*, 2013) or shifts in population composition. As expected, since *F. graminearum* and *F. meridionale* are

described as maize pathogens, isolates from both species caused maize stalk rot in the pathogenicity test.

For *Fusarium fujikuroi* species complex (FFSC), the tree topology here obtained resembled topologies previously recovered for *EF-1α* region in other studies evaluating FFSC species (O'Donnell *et al.*, 1998, 2000; Herron *et al.*, 2015). This species complex is divided into three large clades (African, Asian and American clades) and in our study, we recovered isolates from all three clades: *F. awaxy* in American, *F. fujikuroi* in Asian, *F. napiforme*, *F. tjaetaba* and *F. verticillioides* in African clade (Figure 2).

F. verticillioides, the most frequent species in maize ears (58.9%) and overall sample (41.7%), was found in all cities except Arapoti and recovered from both ears and stalks. *F. verticillioides* was the second most frequent species in maize stalks (20.6%), and as expected, also caused stalk rot in the pathogenicity test, in a similar level as that observed for *F. graminearum* and *F. meridionale*. Besides being commonly reported associated with maize plants, as a pathogen or endophyte (Leslie & Summerell, 2006), this species is also known as a fumonisin producer, posing serious health risks related to the consumption of contaminated grains. The presence of *F. verticillioides* in the sample, especially in ears, with all the isolates being positive for FUM1 gene, and some with fumonisin production detected through the ELISA assay, reinforces the importance of monitoring mycotoxins in maize, with special attention to fumonisins.

F. fujikuroi reports normally link the species with rice diseases (Leslie & Summerell, 2006), and fewer ones with maize, either causing disease symptoms (Hsuan *et al.*, 2011) or also producing fumonisins in low concentration (Mohammadi *et al.*, 2016). *F. fujikuroi* was frequently supposed to produce fumonisin in low quantities if any, as few reference strains produced fumonisins in low amounts only (Leslie & Summerell, 2006). However, there is evidence to reevaluate the toxigenic potential and threat of *F. fujikuroi,* since isolates recovered

from wine grapes in the United States produced fumonisins in comparable levels to *F. proliferatum* and *F. verticillioides* (Bolton *et al.*, 2016). In our sample, two of the FUM1-positive isolates in PCR produced fumonisins in maize patties, one in low concentration (LGMF1818, 0.26ppm). Finding *F. fujikuroi* isolates, especially in maize ears, suggests such mycotoxins to be possible maize contaminants not yet explored. In order to further characterize this issue more studies are required, either to determine *F. fujikuroi* presence and distribution in maize plants in Brazil, or to verifiy if such mycotoxins are produced in maize grains. In the pathogenicity test *F. fujikuroi* caused stalk rot symptoms similarly to *F. graminearum*, *F. verticillioides* and *F. meridionale*, three species already described as maize pathogens. This suggests *F. fujikuroi* as a possible maize stalk rot causal agent not yet explored.

F. tjaetaba isolates were recovered from Castro and Guarapuava materials, mainly of maize stalks (8 isolates), and only one isolate was found in a maize ear. This species was originally described in Australia in association with *Sorghum interjectum*, a native plant from an isolated environment (Laurence *et al.*, 2016). To this date, there is no information regarding toxigenic potential or pathogenicity for this species, and our study is the first to report it outside of Australia, in association with maize plants. However, we found in GenBank database one EF-1α sequence (KX852321) that matches with *F. tjaetaba* in the BLASTn search and clusters with *F. tjaetaba* type-strain in our phylogenetic analysis. This isolate was misidentified as *Fusarium andiyazi*, and recovered from a symptomatic *Pinus taeda* seedling in São Paulo state, Brazil (Do Carmo, 2017). Finding these isolates in such different plants (*Pinus* and *Zea* in Brasil, *Sorghum* in Australia) suggests that *F. tjaetaba* host range may not be as narrow as previously supposed (Laurence *et al.*, 2016). In addition, *F. tjaetaba* isolates with positive results for FUM1 gene PCR were recovered in our sample, including the isolate found in the maize ear, for which fumonisin production was detected in the ELISA assay (LGMF1723, producing 1.16ppm). This suggests that *F. tjaetaba* may be important not only as pathogen in

Pinus, but also as a fumonisin producer in maize. Regarding pathogenicity, *F. tjaetaba* did not cause stalk rot symptoms in the same level observed for *F. graminearum, F. fujikuroi, F. meridionale* and *F. verticillioides*. This suggests that *F. tjaetaba* is not an important maize stalk rot causative agent, at least for moderately resistant maize hybrids such as P2530H, tested here. Further studies regarding *F. tjaetaba* toxigenic potential, pathogenicity and aggressiveness in other maize hybrids, and geographical distribution are required to determine if this species could represent a threat to maize crops or if its association with maize occurs only in a narrow geographical area in Brazil.

F. napiforme was originally described from cultures isolated from millet and sorghum in Africa (Marasas *et al.*, 1987), and recovered from sorghum grain in Argentina and soil in different locations of Africa and Australia (Leslie & Summerell, 2006). Based on the morphology of the napiform microconidia, *F. napiforme* has already been described in association with maize, being present in symptomatic kernels from Mexico (Morales-Rodríguez *et al.*, 2007) and UK (Basler, 2016), and here we report for the first time the association of *F. napiforme* with maize stalks, as our 3 isolates were recovered from stalks from Castro sample. However, *F. napiforme* did not cause stalk rot symptoms in the pathogenicity test using moderately resistant maize hybrid P2530H. The toxigenic potential of this species has not been properly explored yet, with sporadic reports of few strains capable of producing toxins such as fumonisins, usually in low amounts (Nelson *et al.*, 1992). In our study, we observed 1 isolate positive and 2 isolates negative for FUM1 in PCR analyses, and the only one FUM1-positive isolate produced fumonisin in low amount (0.39ppm). Although we observed no pathogenicity and low fumonisin production in the conditions tested, further studies with other conditions would be necessary in order to evaluate if the *F. napiforme* association with maize represents or not a major threat if compared with other species found, such as *F. verticillioides* and *F. graminearum,* that presented higher agressiveness.

F. awaxy, the new species in American clade of FFSC, is morphologically similar to *F. subglutinans*, another pathogen from maize (Leslie & Summerell, 2006) and the closest phylogenetic relative (Figure 3). Although no significant morphological differences were observed, there is clear phylogenetic delimitation and no evidence of recombination was observed in PHI test, supporting the description of *F. awaxy* as a new species. *F. awaxy* did not grow at temperatures over 32ºC, suggesting the inability to cause infections in humans, a physiological character that contrasts the reports of infections caused by *F. subglutinans* and *F. temperatum*. In the pathogenicity test using a moderately resistant maize hybrid, *F. awaxy* caused stalk rot in similar levels as described for *F. tjaetaba.* For these conditions, *F. awaxy* differs from *F. subglutinans* and *F. temperatum,* which show higheraggressiveness in maize. Moreover, it was previously suggested that additional species remain to be identified and described in *F. subglutinans* sensu lato group (Leslie & Summerell, 2006), being *F. awaxy* one of those. Some EF-1α sequences found in NCBI GenBank matched the sequences generated for our *F. awaxy* isolates, also grouping with them in the phylogenetic analysis. Most of the sequences found in NCBI GenBank were from isolates recovered from maize, in different geographical areas (Africa, Asia, North and South America), and were all misidentified as *F. subglutinans,* probably due to problems in identification methods used (BLASTn based identification, improper phylogenetic analyses which did not include all FFSC species and type-strains). These isolates are here identified as *F. awaxy*, demonstrating that this species has already been recovered from maize before, but not recognized as a distinct species from *F. subglutinans*. Although two *F. awaxy* isolates in our sample were positive for FUM1 gene in PCR, fumonisin production in ELISA assay was not detected for those isolates, suggesting no or low production (below the detection limit of 0.25ppm) in the conditions evaluated here. The toxigenic potential for other mycotoxins already described for *F. subglutinans* sensu lato group, such as moniliformins (Leslie & Summerell, 2006), was not evaluated for our isolates, being

an issue which could be addressed in the future, to characterize these species toxigenic potential and possible risks in maize.

Finally, regarding *Fusarium incarnatum-equiseti* species complex (FIESC), three different lineages were found. We identified one isolate as FIESC 12, a lineage already reported in association with cereals as wheat (O'Donnell *et al.*, 2008) and oat (Villani *et al.*, 2016), and another isolate as FIESC 20, a lineage reported in associations with insects and human infections (O'Donnell *et al.*, 2008). FIESC 33 is proposed here as a new lineage, including three isolates from our sample and one sequence from an isolate recovered from *Trichosanthes dioica,* misidentified as FIESC 23 (Villani *et al.*, 2016). FIESC species comprise isolates usually associated with human and animal infections (O'Donnell *et al.*, 2008) as well as insecticolous fusaria (O'Donnell *et al.*, 2012), but plant pathogenicity, disease epidemiology and mycotoxin production for this complex are not yet clear (Villani *et al.*, 2016). Our study contributes with information regarding species distribution, as we reported for the first time these three lineages associated with maize. As these isolates were all recovered from maize stalks, the relevance of toxin production is reduced, and was not evaluated in our study.

In summary, our study provides relevant information regarding the diversity of *Fusarium* species associated with maize in Brazil, especially for *Fusarium fujikuroi* species complex (FFSC), as in a reduced sample from a small area (Paraná state) several species were found, including a new species and species not yet described in association with maize. These findings demonstrate that *Fusarium* diversity in maize could be still underestimated, and further studies regarding distribution, pathogenicity and toxigenic potential of these species are required to determine their actual impact to maize industry and risks laid upon maize crops and human health.

6 ACKNOWLEDGMENTS

This work corresponds to the first author's master degree research project at the Programa de Pós-Graduação em Genética (Universidade Federal do Paraná), so thanks are given to Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES) for providing a master scholarship to D. A. L. Petters and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for providing a research fellowship to C. Glienke and L. V. G. Terasawa. Authors would like to thank Vértika Agropecuária for providing the maize samples and for the technical and scientific support during the pathogenicity tests. Authors are also grateful for D. P. Bruschi, D. C. Savi, L. Ruaro and R. Aluizio for their suggestions during the research execution and manuscript preparation.

7 FIGURES

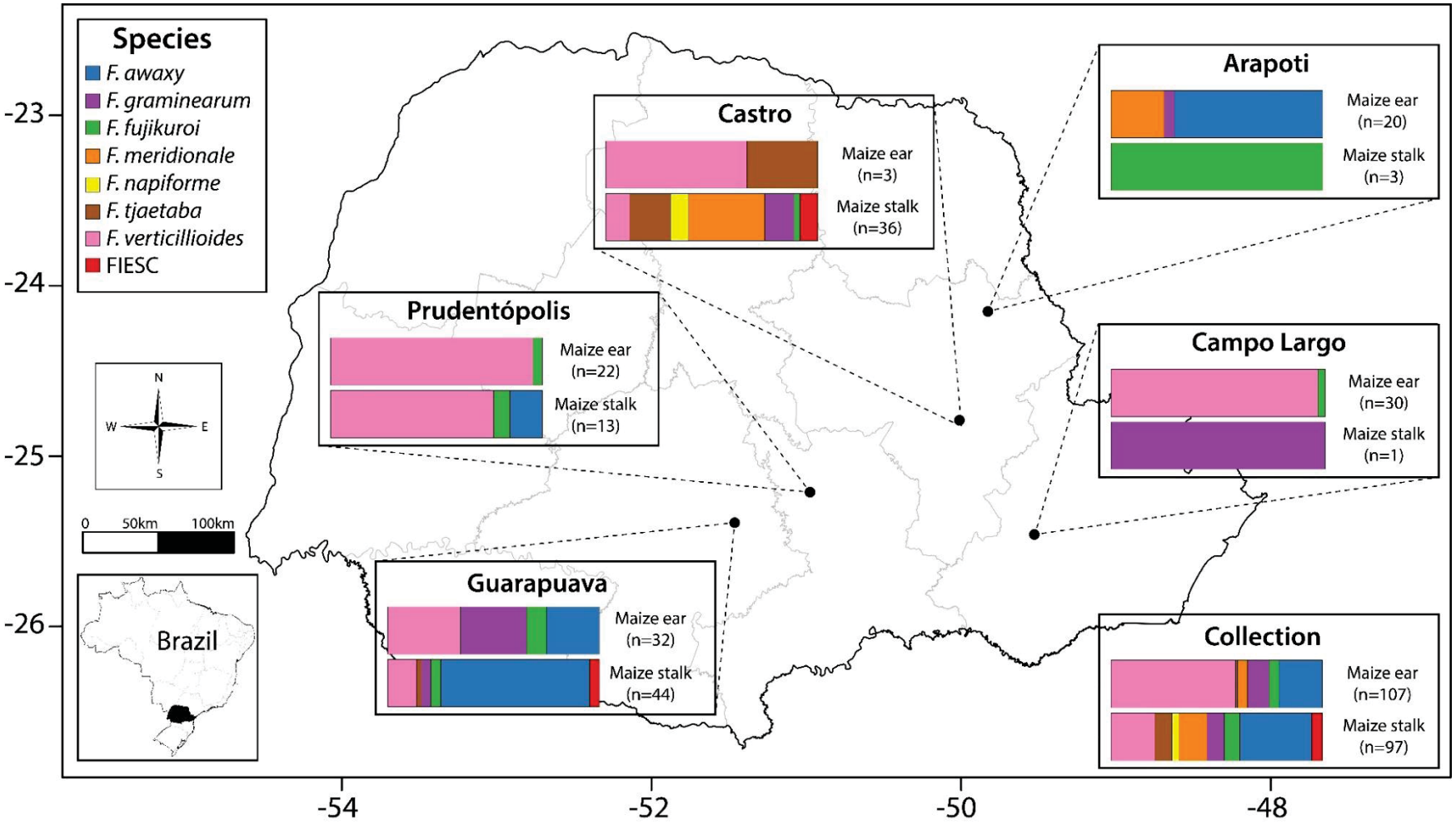


Figure 1 - Municipalities in the Brazilian state of Paraná where maize ears and stalks were collected.

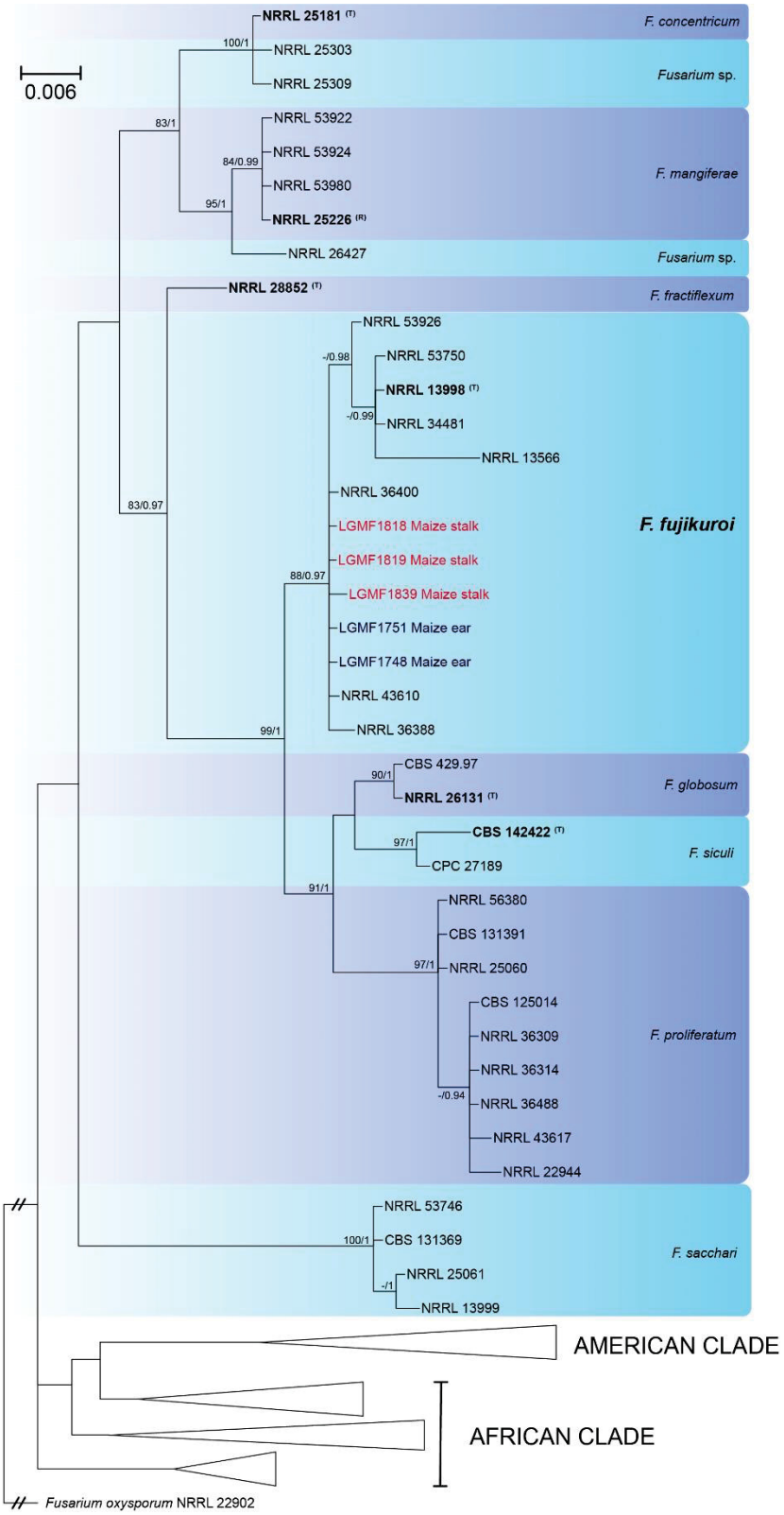


Figure 2 - Bayesian Inference tree based on EF-1α sequences of strains belonging to *Fusarium fujikuroi* species complex. MP and ML bootstrap values above 70% and Bayesian posterior probability values (PP) values above 0.9 indicated to the left of the nodes. Isolates obtained in this study are indicated in blue and red font (for maize ears and stalk isolates, respectively). Type strains included in analysis are indicated in bold and with (T). The tree was rooted to *Fusarium oxysporum* NRRL 22902.

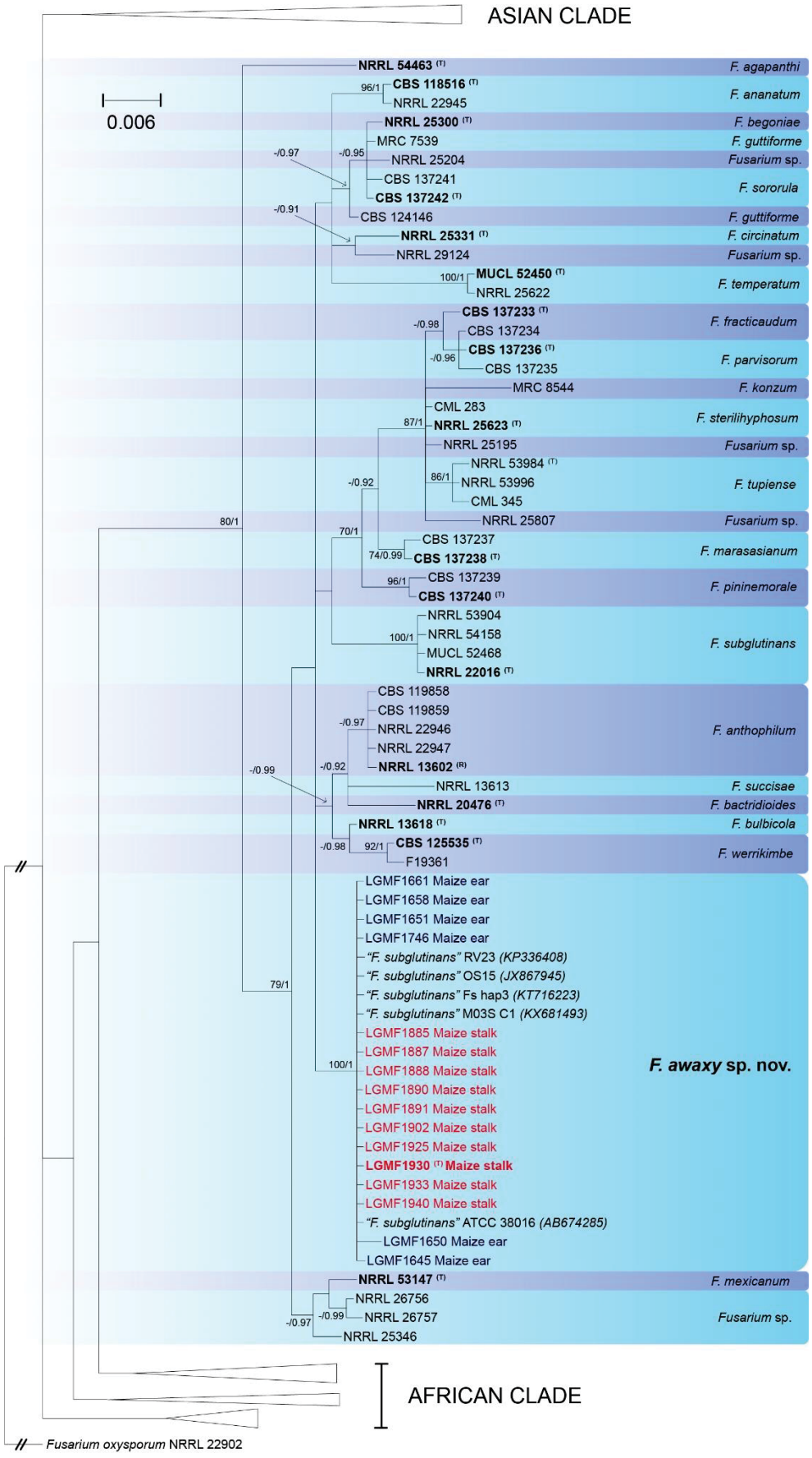


Figure 2 – (continued).

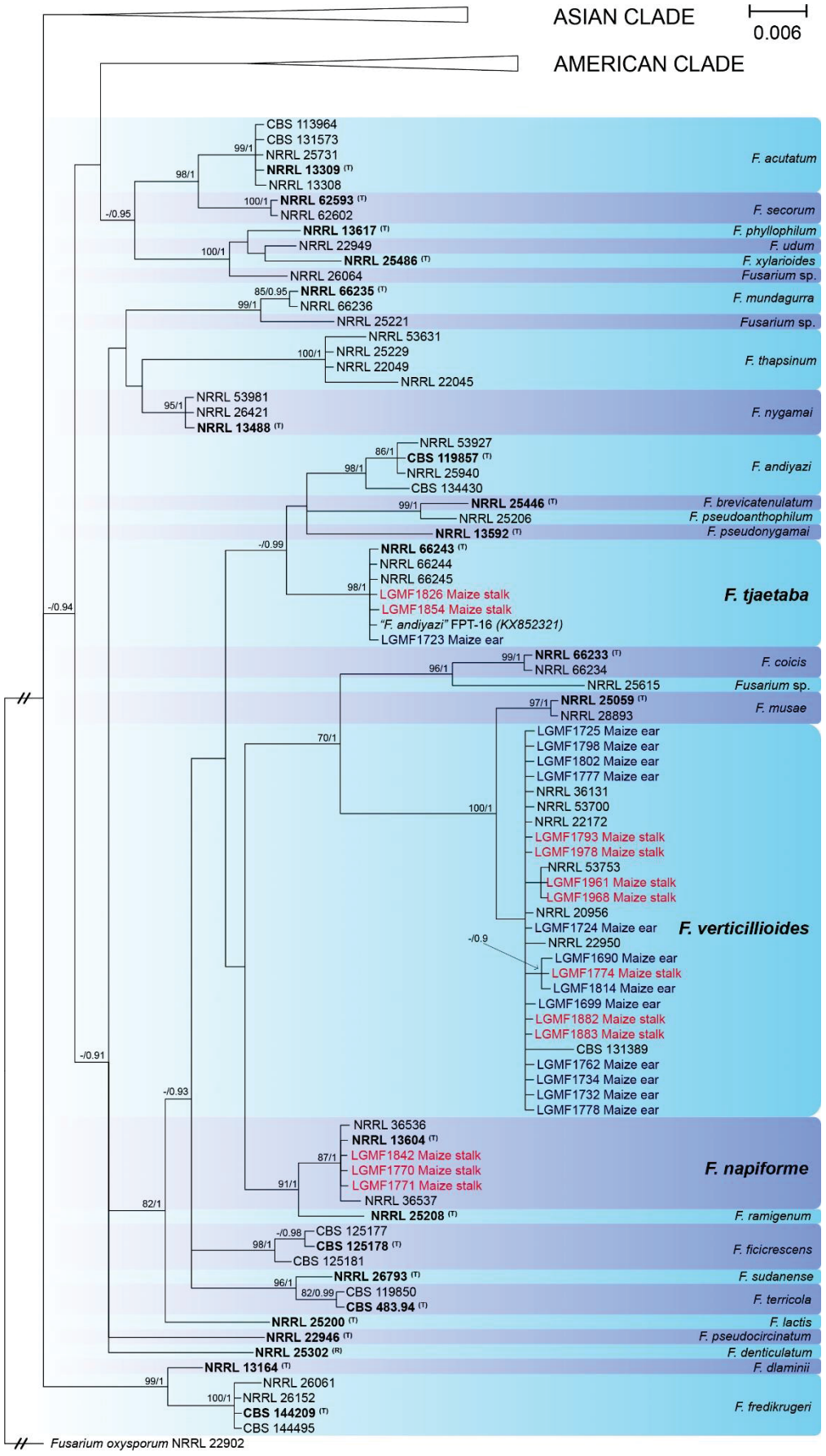


Figure 2 – (continued).

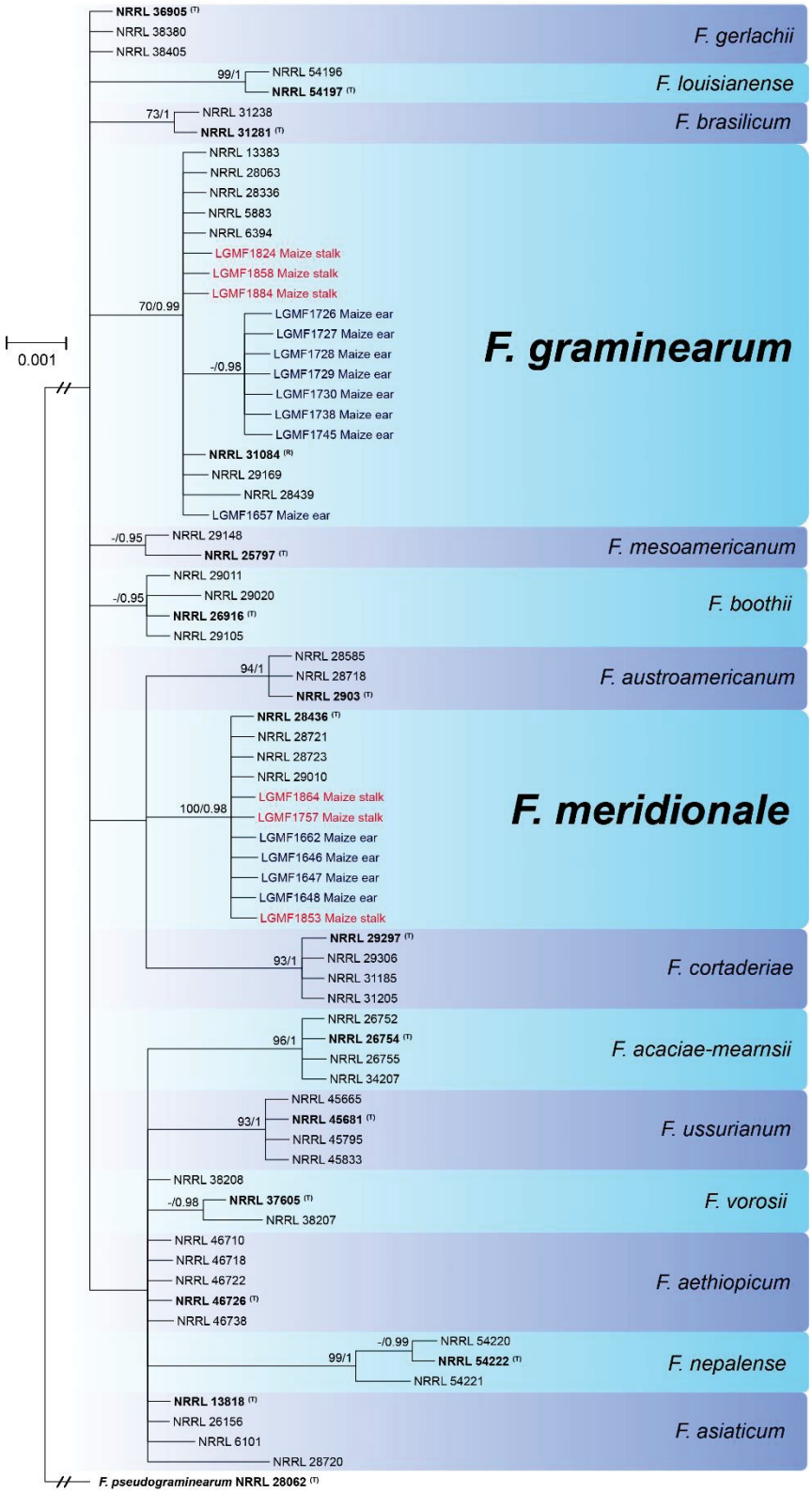


Figure 3 - Bayesian Inference tree based on EF-1α sequences of strains belonging to *Fusarium graminearum* species complex. MP and ML bootstrap values above 70% and Bayesian posterior probability values (PP) values above 0.9 indicated to the left of the nodes. Isolates obtained in this study are indicated in blue and red font (for maize ears and stalk isolates, respectively). Type strains included in analysis are indicated in bold and with (T). The tree was rooted to *Fusarium pseudograminearum* NRRL 28062.

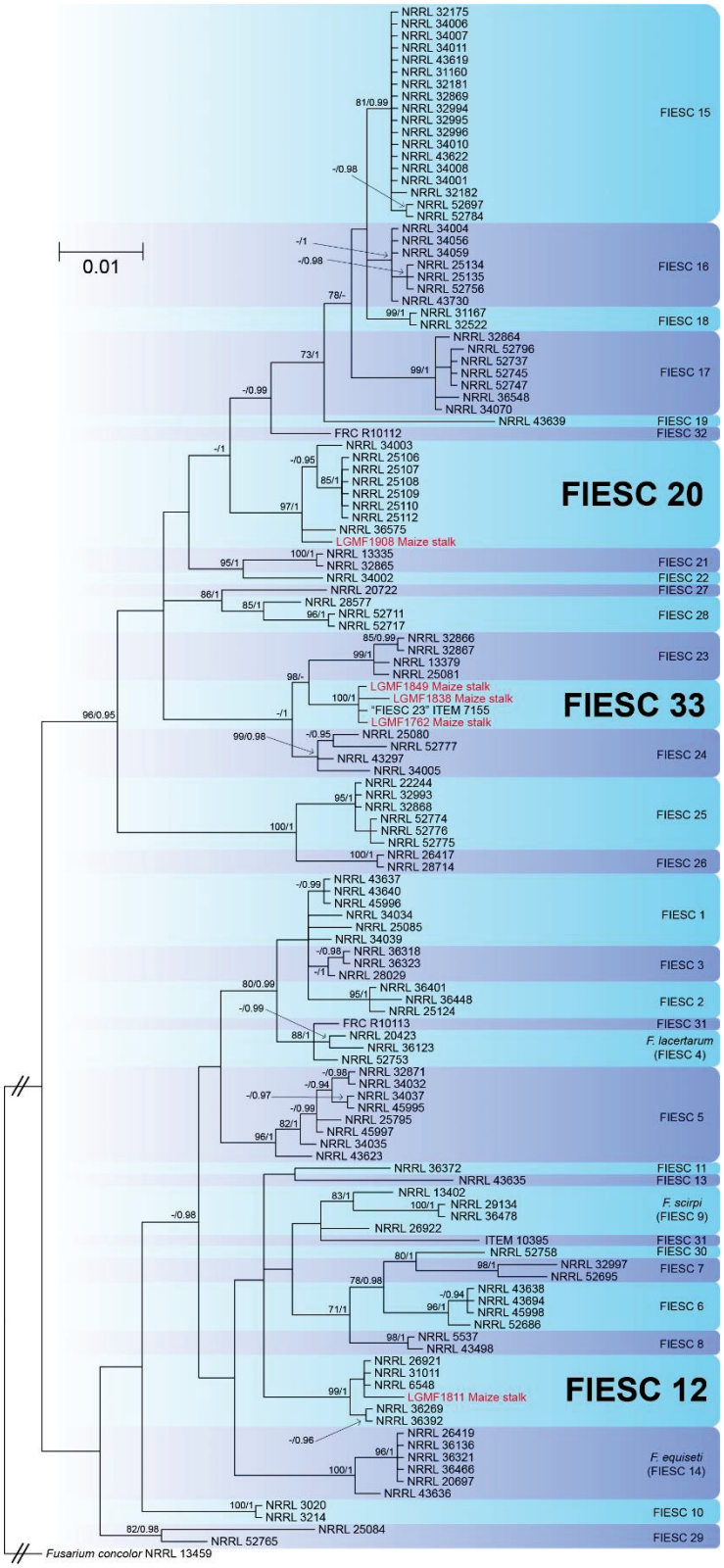


Figure 4 - Bayesian Inference tree based on EF-1α sequences of strains belonging to *Fusarium incarnatum-equiseti* species complex. MP and ML bootstrap values above 70% and Bayesian posterior probability values (PP) values above 0.9 indicated to the left of the nodes. Isolates obtained in this study are indicated in blue and red font (for maize ears and stalk isolates, respectively). Type strains included in analysis are indicated in bold and with $^{(T)}$. The tree was rooted to *Fusarium concolor* NRRL 13459.

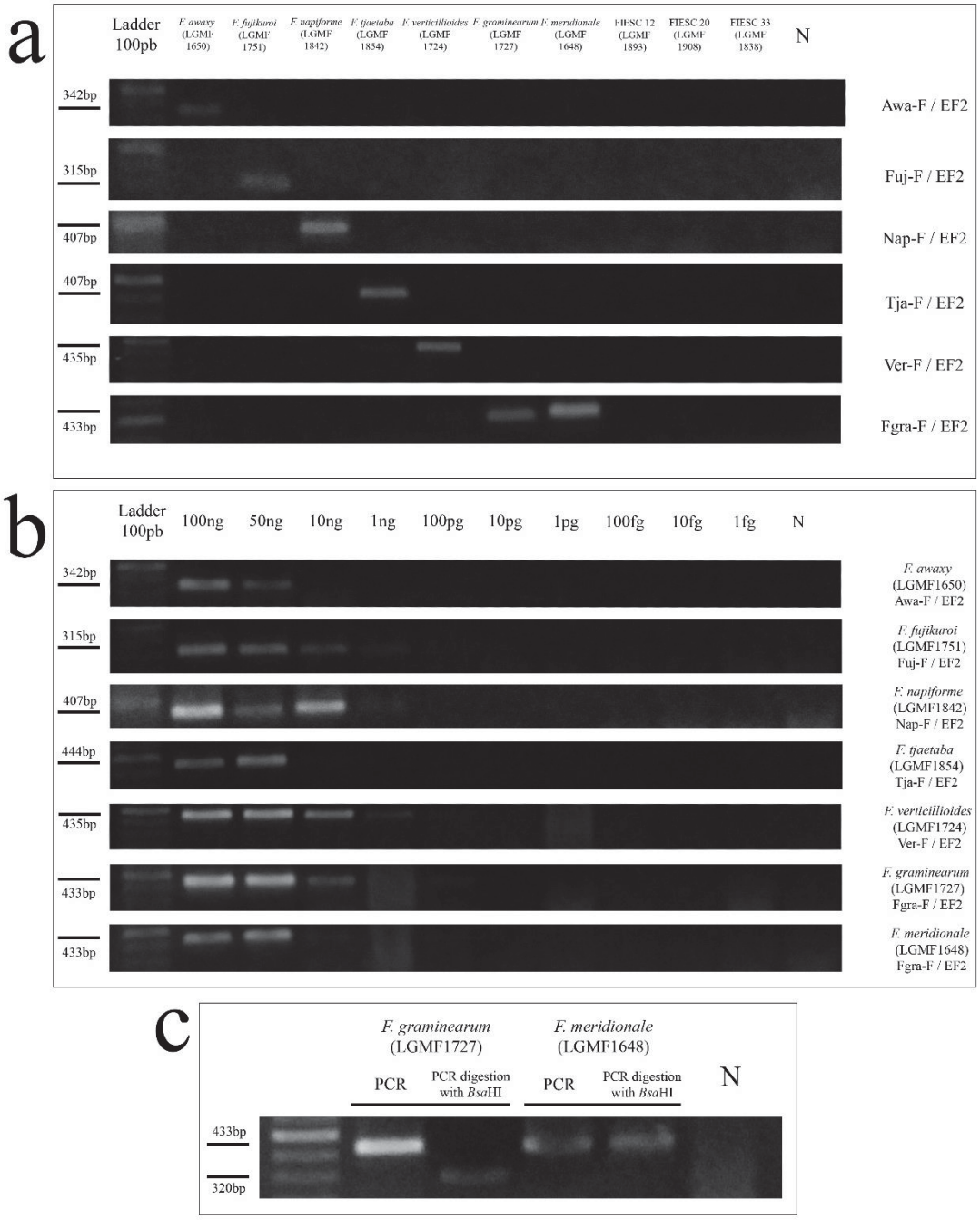


Figure 5 - Species-specific PCR and PCR-RFLP.
a - Specificity test of species-specific and PCR-RFLP primers using genomic DNA from FFSC, FGSC and FIESC species found in this work at the concentration of 50ng. Sidebars at left indicate expected amplicon size for each primer pair. Marker Ladder 100pb (Axygen); N: Negative control (water).
b - Sensitivity test of species-specific and PCR-RFLP primers using different concentrations of genomic DNA from target species, ranging from 100ng to 1fg. Sidebars at left indicate expected amplicon size for each primer pair. Marker Ladder 100pb (Axygen); N: Negative control (water).
c - PCR-RFLP with BsaHI enzyme to differentiate between *F. graminearum* and *F. meridionale.* Sidebars at left indicate expected fragment size. Marker Ladder 100pb (Axygen); N: Negative control (water).

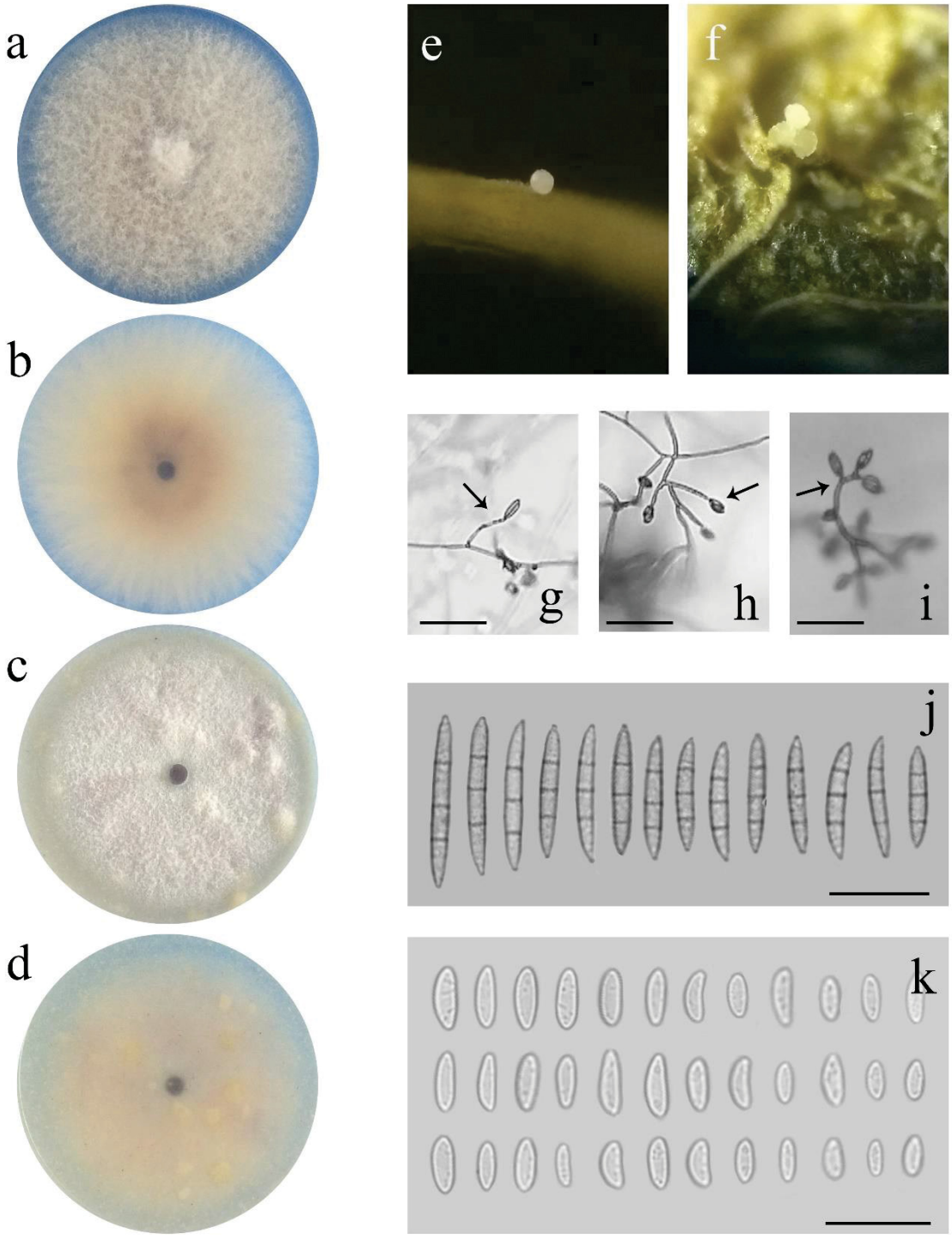


Figure 6. *Fusarium awaxy* LGMF1930.
a-d. Colonies on PDA (a-b) and OMA (c-d) after 7 d at 24ºC in the dark;
e-f. Sporodochia formed on the surface of carnation leaves;
g-i. Aerial conidiophores and conidia. Arrows indicate monophialide (g), false head (h) and polyphialide (i)
j. Sporodochial conidia (macroconidia)
k. Aerial conidia (microconidia)
— Scale bars = 25 µm

8 TABLES

Table 1 – Characteristics of the gene partitions used in phylogenetic analyses

Species complex	Locus[a]	Number of sites				Evolutionary model[b]
		Total	Constant	Variable	Parsimony informative	
Fusarium fujikuroi species complex	*EF-1α*	685	406	262	198	GTR+G+I
	TUB2	589	428	160	101	SYM+G
Fusarium graminearum species complex	*EF-1α*	649	581	67	38	GTR + G
Fusarium incarnatum-equiseti species complex	*EF-1α*	715	437	261	173	GTR+G+I

[a]Locus: *EF-1α*: Translation elongation factor 1-alpha; *TUB2*: Beta-tubulin.

[b]Evolutionary model: G: Gamma distributed rate variation among sites; GTR: Generalised time-reversible; I: Proportion of invariable sites; SYM: Symmetrical model.

Table 2 – Primer names, sequences, annealing temperature and amplicon sizes (when used with EF2 reverse primer) for the five species-specific primers designed to differentiate species within the *Fusarium fujikuroi* species complex and the specific primer for PCR-RFLP method to differentiate species within the *Fusarium graminearum* species complex

Species	Primer name	Primer Sequence (5'-3')	Annealing temperature	Amplicon size (bp) / Size after restriction with BsaHI (bp)	Detection limit	Isolate used as positive control
F. awaxy	Awa-F	CCGCCACTCGAGCGGCGAT	57ºC	342 bp / -	50ng	LGMF 1650
F. fujikuroi	Fuj-F	GCGCGTTTTGCCCTTTCCTG	58ºC	315 bp / -	1ng	LGMF 1751
F. napiforme	Nap-F	CCCACTACCCCGCTTGAGTT	57ºC	407 bp / -	10ng	LGMF 1842
F. tjaetaba	Tja-F	CCTTTGTCCACCGATTTCT	57ºC	444 bp / -	50ng	LGMF 1854
F. verticillioides	Ver-F	TCTTTGCCCATCGATTCCC	57ºC	435 bp / -	1ng	LGMF 1724
F. graminearum	Fgra-F	CCCTCACACGACGACTC	57ºC	433 bp / 320 and 113 bp	10ng	LGMF 1727
F. meridionale	Fgra-F	CCCTCACACGACGACTC	57ºC	433 bp / 432 pb	50ng	LGMF 1648

Table 3 - Species, isolation substrate, geographical origin, PCR and ELISA results of *Fusarium fujikuroi* species complex isolates used for fumonisin production assay.

Species	Isolate	Isolation Substrate	Geographical Origin	PCR[a]	ELISA[b]
F. awaxy	LGMF1645	Ear	Arapoti	-	-
	LGMF1651	Ear	Arapoti	+	-
	LGMF1658	Ear	Arapoti	-	-
	LGMF1661	Ear	Arapoti	-	-
	LGMF1746	Ear	Guarapuava	+	-
F. fujikuroi	LGMF1751	Ear	Guarapuava	-	-
	LGMF1818	Stalk	Arapoti	+	+ (0.26ppm)
	LGMF1819	Stalk	Arapoti	+	+ (1.1ppm)
	LGMF1839	Stalk	Castro	-	-
F. napiforme	LGMF1842	Stalk	Castro	+	+ (0.39ppm)
F. tjaetaba	LGMF1723	Ear	Castro	+	+ (1.16ppm)
	LGMF1826	Stalk	Castro	+	-
	LGMF1854	Stalk	Castro	-	-
F. verticillioides	LGMF1675	Ear	Campo Largo	+	+ (3.7ppm)
	LGMF1724	Ear	Castro	+	+ (2.4ppm)
	LGMF1762	Ear	Guarapuava	+	-
	LGMF1777	Ear	Prudentópolis	+	-
	LGMF1874	Stalk	Guarapuava	+	+ (0.9ppm)
	LGMF1961	Stalk	Prudentópolis	+	-

[a] – Positive (+) or negative (-) according to presence (+) or absence (-) of 183bp fragment in FUM1 PCR.

[b] - Positive (+) when fumonisin concentration between 0.25-5ppm range and negative (-) when below 0.25ppm detection threshold, as defined by manufacturer (Romer Labs).

9 REFERENCES

Anfossi L, Giovannoli C, Baggiani C, 2016. Mycotoxin detection. *Current Opinion in Biotechnology* **37**, 120–126.

Anvisa, 2011. Resolucão RDC 7. Dispõe sobre limites máximos tolerados (LMT) para micotoxinas em alimentos, constante do Anexo desta Resolução. Diário Oficial da União; Poder Executivo, de 18 de fevereiro de 2011. [http://portal.anvisa.gov.br/documents/10181/2968262/RDC_07_2011_COMP.pdf/afe3f054-bc99-4e27-85c4-780b92e2b966]. Accessed 18 July 2018.

Aoki T, O'Donnell K, Geiser DM, 2014. Systematics of key phytopathogenic *Fusarium* species: Current status and future challenges. *Journal of General Plant Pathology* **80**, 189–201.

Astolfi P, dos Santos J, Schneider L *et al.*, 2011. Molecular survey of trichothecene genotypes of *Fusarium graminearum* species complex from barley in Southern Brazil. *International Journal of Food Microbiology* **148**, 197–201.

Basler R, 2016. Diversity of *Fusarium* species isolated from UK forage maize and the population structure of *F. graminearum* from maize and wheat. *PeerJ* **4**, e2143.

Bluhm BH, Cousin MA, Woloshuk CP, 2004. Multiplex real-time PCR detection of fumonisin-producing and trichothecene-producing groups of *Fusarium* species. *Journal of food protection* **67**, 536–43.

Bolton SL, Brannen PM, Glenn AE, 2016. A novel population of *Fusarium fujikuroi* isolated from southeastern U.S. winegrapes reveals the need to re-evaluate the species' fumonisin production. *Toxins* **8**, 1–14.

Carbone I, Kohn LM, 1999. A Method for Designing Primer Sets for Speciation Studies in Filamentous Ascomycetes. *Mycologia* **91**, 553–556.

Castañares E, Dinolfo MI, Del Ponte EM, Pan D, Stenglein SA, 2016. Species composition and genetic structure of *Fusarium graminearum* species complex populations affecting the main barley growing regions of South America. *Plant Pathology* **65**, 930–939.

Cheli F, Battaglia D, Gallo R, Dell'Orto V, 2014. EU legislation on cereal safety: An update with a focus on mycotoxins. *Food Control* **37**, 315–325.

Costa RV da, Silva DD da, Cota L V., Parreira DF, Ferreira A da S, Casela CR, 2010. Incidência de *Colletotrichum graminicola* em colmos de genótipos de milho. *Summa Phytopathologica* **36**, 122–128.

Costa SS, Moreira GM, Pfenning LH, 2017. Development of a PCR protocol for the identification and detection of *Fusarium solani* f. sp. piperis from soil and roots of black pepper (*Piper nigrum*). *Tropical Plant Pathology* **42**, 55–59.

Darriba D, Taboada GL, Doallo R, Posada D, 2012. jModelTest 2 : more models , new heuristics and high- performance computing. *Nature Methods* **9**, 6–9.

Dean R, Van Kan JAL, Pretorius ZA *et al.*, 2012. The Top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology* **13**, 414–430.

Do Carmo ALM, 2017. *Fusarium* em sementes de Pínus no Brasil: distribuição, patogenicidade, identificação de espécies e controle biológico. Curitiba, Brazil: Universidade Federal do Paraná, PhD Thesis.

FAOSTAT, 2018. Agriculture production data. [http://www.fao.org/faostat/en]. Accessed 18 July 2018.

Fisher NL, Burgess W, Toussoun A, Nelson PE, 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology* **72**, 151.

Gomes LB, Ward TJ, Badiale-Furlong E, Del Ponte EM, 2015. Species composition, toxigenic potential and pathogenicity of *Fusarium graminearum* species complex isolates from southern Brazilian rice. *Plant Pathology* **64**, 980–987.

Goswami RS, Kistler HC, 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. *Molecular Plant Pathology* **5**, 515–525.

Gräfenhan T, Schroers HJ, Nirenberg HI, Seifert KA, 2011. An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in *Cosmospora*, *Acremonium*, *Fusarium*, *Stilbella*, and *Volutella*. *Studies in Mycology* **68**, 79–113.

Guindon S, Gascuel O, 2003. A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. *Systematic Biology* **52**, 696–704.

Herron DA, Wingfield MJ, Wingfield BD, Rodas CA, Marincowitz S, Steenkamp ET, 2015. Novel taxa in the *Fusarium fujikuroi* species complex from *Pinus* spp. *Studies in Mycology* **80**, 131–150.

Hsuan HM, Salleh B, Zakaria L, 2011. Molecular identificationn of *Fusarium* species in *Gibberella fujikuroi* species complex from rice, sugarcane and maize from Peninsular Malaysia. *International Journal of Molecular Sciences* **12**, 6722–6732.

Huson DH, Bryant D, 2006. Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* **23**, 254–267.

Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL, 2008. NCBI BLAST: a better web interface. *Nucleic acids research* **36**, 5–9.

Katoh K, Standley DM, 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution* **30**, 772–780.

Kazan K, Gardiner DM, Manners JM, 2012. On the trail of a cereal killer: Recent advances in *Fusarium graminearum* pathogenomics and host resistance. *Molecular Plant Pathology* **13**, 399–413.

Kuhnem PR, Ward TJ, Silva CN *et al.*, 2016. Composition and toxigenic potential of the *Fusarium graminearum* species complex from maize ears, stalks and stubble in Brazil. *Plant Pathology* **65**, 1185–1191.

Kumar S, Stecher G, Tamura K, 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular biology and evolution* **33**, 1870–1874.

Kvas M, Marasas WFO, Wingfield BD, Wingfield MJ, Steenkamp ET, 2009. Diversity and evolution of *Fusarium* species in the *Gibberella fujikuroi* complex. *Fungal Diversity* **34**, 1–21.

Laurence MH, Walsh JL, Shuttleworth LA *et al.*, 2016. Six novel species of *Fusarium* from natural ecosystems in Australia. *Fungal Diversity* **77**, 349–366.

Leslie JF, Summerell BA, 2006. *Fusarium Laboratory Manual*. Blackwell Publishing.

Lombard L, van der Merwe NA, Groenewald JZ, Crous PW, 2015. Generic concepts in Nectriaceae. *Studies in Mycology* **80**, 189–245.

Ma L-J, Geiser DM, Proctor RH *et al.*, 2013. *Fusarium* Pathogenomics. *Annual Review of Microbiology* **67**, 399–416.

Marasas WFO, Rabie CJ, Lübben A, Nelson PE, Toussoun TA, Van Wyk PS, 1987. *Fusarium napiforme*, a new species from millet and sorghum in southern Africa. *Mycologia* **79**, 910–914.

Miller MA, Pfeiffer W, Schwartz T, 2012. The CIPRES science gateway. *Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment on Bridging from the eXtreme to the campus and beyond - XSEDE '12*, 1.

Mohammadi A, Shams-Ghahfarokhi M, Nazarian-Firouzabadi F, Kachuei R, Gholami-Shabani M, Razzaghi-Abyaneh M, 2016. *Giberella fujikuroi* species complex isolated from maize and wheat in Iran: Distribution, molecular identification and fumonisin B1in vitro biosynthesis. *Journal of the Science of Food and Agriculture* **96**, 1333–1340.

Morales-Rodríguez I, De Yañez-Morales MJ, Silva-Rojas H V., García-De-Los-Santos G, Guzmán-De-Peña DA, 2007. Biodiversity of *Fusarium* species in Mexico associated with ear rot in maize, and their identification using a phylogenetic approach.

Mycopathologia **163**, 31–39.

Nelson PE, Dignani MC, Anaissie EJ, 1994. Taxonomy, biology, and clinical aspects of *Fusarium* species. *Clinical microbiology reviews* **7**, 479–504.

Nelson PE, Plattner RD, Shackelford DD, Desjardins AE, 1992. Fumonisin B1 production by *Fusarium* species other than *F. moniliforme* in Section Liseola and by some related species. *Appl Environ Microbiol* **58**, 984–989.

Nirenberg HI, 1976. Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium*-Section Liseola. *Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft.* **169**, 1–117.

O'Donnell K, Cigelnik E, 1997. Two Divergent Intragenomic rDNA ITS2 Types within a Monophyletic Lineage of the Fungus *Fusarium* Are Nonorthologous. *Molecular Phylogenetics and Evolution* **7**, 103–116.

O'Donnell K, Humber RA, Geiser DM *et al.*, 2012. Phylogenetic diversity of insecticolous fusaria inferred from multilocus DNA sequence data and their molecular identification via FUSARIUM-ID and *Fusarium MLST*. *Mycologia* **104**, 427–445.

O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC, 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 2044–2049.

O'Donnell K, Nirenberg H, Aoki T, Cigelnik E, 2000. A multigene phylogeny of the *Gibberella fujikuroi* species complex: detection of additional phylogenetically distinct species. *Mycoscience* **41**, 61–78.

O'Donnell K, Rooney AP, Proctor RH *et al.*, 2013. Phylogenetic analyses of RPB1 and RPB2 support a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. *Fungal Genetics and Biology* **52**, 20–31.

O'Donnell K, Sutton DA, Fothergill A *et al.*, 2008. Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the *Fusarium solani* Species complex. *Journal of Clinical Microbiology* **46**, 2477–2490.

O'Donnell K, Ward TJ, Geiser DM, Kistler HC, Aoki T, 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. *Fungal Genetics and Biology* **41**, 600–623.

O'Donnell K, Ward TJ, Robert VARG, Crous PW, Geiser DM, Kang S, 2015. DNA sequence-based identification of *Fusarium*: Current status and future directions.

Phytoparasitica **43**, 583–595.

Owczarzy R, Tataurov A V., Wu Y *et al.*, 2008. IDT SciTools: a suite for analysis and design of nucleic acid oligomers. *Nucleic acids research* **36**, 163–169.

Del Ponte EM, Spolti P, Ward T *et al.*, 2015. Regional and field-specific factors affect the composition of *Fusarium* head blight pathogens in subtropical no-till wheat agroecosystem of Brazil. *Phytopathology* **105**, 246–254.

Del Ponte EM, Tessmann DJ, Spolti P, Kuhnem PR, da Silva CN, 2013. Species Identification, Genetic Diversity and Phenotypic Variation Studies on the *Fusarium graminearum* Complex Populations from Brazil. In: *Fusarium Head Blight in Latin America*. Dordrecht: Springer Netherlands, 15–29.

Quaedvlieg W, Binder M, Groenewald JZ *et al.*, 2014. Introducing the consolidated species concept to resolve species in the teratosphaeriaceae. *Persoonia: Molecular Phylogeny and Evolution of Fungi* **33**, 1–40.

Quarta A, Mita G, Haidukowski M, Logrieco A, Mulè G, Visconti A, 2006. Multiplex PCR assay for the identification of nivalenol, 3- and 15-acetyl-deoxynivalenol chemotypes in *Fusarium*. *FEMS Microbiology Letters* **259**, 7–13.

Raeder U, Broda P, 1985. Rapid preparation of DNA from filamentous fungi. *Letters in Applied Microbiology* **1**, 17–20.

Ronquist F, Teslenko M, Van Der Mark P *et al.*, 2012. MrBayes 3.2: Efficient Bayesian Phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542.

Sandoval-Denis M, Guarnaccia V, Polizzi G, Crous PW, 2018. Symptomatic Citrus trees reveal a new pathogenic lineage in *Fusarium* and two new *Neocosmospora* species. *Persoonia - Molecular Phylogeny and Evolution of Fungi* **40**, 1–25.

Scauflaire J, Gourgue M, Munaut F, 2011. *Fusarium temperatum* sp. nov. from maize, an emergent species closely related to *Fusarium subglutinans*. *Mycologia* **103**, 586–97.

Schindelin J, Arganda-Carreras I, Frise E *et al.*, 2012. Fiji: An open-source platform for biological-image analysis. *Nature Methods* **9**, 676–682.

Schoeman A, Flett BC, Janse van Rensburg B, 2016. Evaluating three commonly used growth media for assessing fumonisin analogues FB1,FB2and FB3production by nine *Fusarium verticillioides* isolates. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment* **34**, 291–298.

Scoz LB, Astolfi P, Reartes DS, Schmale DG, Moraes MG, Del Ponte EM, 2009. Trichothecene mycotoxin genotypes of *Fusarium graminearum* sensu stricto and

Fusarium meridionale in wheat from southern Brazil. *Plant Pathology* **58**, 344–351.

Selvaraj JN, ZHOU L, WANG Y *et al.*, 2015. Mycotoxin detection - Recent trends at global level. *Journal of Integrative Agriculture* **14**, 2265–2281.

Sukumaran J, Holder MT, 2010. DendroPy: A Python library for phylogenetic computing. *Bioinformatics* **26**, 1569–1571.

Villani A, Moretti A, De Saeger S *et al.*, 2016. A polyphasic approach for characterization of a collection of cereal isolates of the *Fusarium incarnatum-equiseti* species complex. *International Journal of Food Microbiology* **234**, 24–35.

Vincze T, Posfai J, Roberts RJ, 2003. NEBcutter: A program to cleave DNA with restriction enzymes. *Nucleic Acids Research* **31**, 3688–3691.

Vismer HF, Snijman PW, Marasas WFO, van Schalkwyk DJ, 2004. Production of Fumonisins by *Fusarium Verticillioides* Strains on Solid and in a Defined Liquid Medium – Effects of L-Methionine and Inoculum. *Mycopathologia* **158**, 99–106.

Ward TJ, Clear RM, Rooney AP *et al.*, 2008. An adaptive evolutionary shift in *Fusarium* head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium* graminearum in North America. *Fungal Genetics and Biology* **45**, 473–484.

White TJ, Bruns T, Lee S, Taylor J, 1990. AMPLIFICATION AND DIRECT SEQUENCING OF FUNGAL RIBOSOMAL RNA GENES FOR PHYLOGENETICS. In: *PCR Protocols*. 315–322.

Zwickl DJ, 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. *Master Thesis*.

10 SUPPORTING INFORMATION

Table 1 – Isolates from *Zea mays* used in this study, origin, method used for the identification, toxin genotyping and GenBank accession numbers

SPECIES	GEOGRAPHICAL ORIGIN	ISOLATION SUBSTRATE	IDENTIFICATION METHOD	TOXIN[a]	ISOLATE[b]	GENBANK (EF-1α)[c]
Fusarium awaxy	Arapoti - PR	Maize ear	Phylogenetic analysis	FUM1 -	LGMF1645	MG838946
				FUM1 -	LGMF1650	MG838950
				FUM1 -	LGMF1658	MG838953
				FUM1 -	LGMF1661	MG838954
				FUM1 +	LGMF1651	MG838951
			Species-specific PCR	FUM1 -	LGMF1644	-
				FUM1 -	LGMF1649	-
				FUM1 -	LGMF1652	-
				FUM1 -	LGMF1653	-
				FUM1 -	LGMF1654	-
				FUM1 -	LGMF1655	-
				FUM1 -	LGMF1656	-
				FUM1 -	LGMF1659	-
				FUM1 -	LGMF1660	-
	Guarapuava - PR	Maize ear	Phylogenetic analysis	FUM1 +	LGMF1746	MG838970
			Species-specific PCR	FUM1 -	LGMF1722	-
				FUM1 -	LGMF1698	-
				FUM1 -	LGMF1684	-

(continues on next page)

Table S1 – (continued.)

SPECIES	GEOGRAPHICAL ORIGIN	ISOLATION SUBSTRATE	IDENTIFICATION METHOD	TOXIN[a]	ISOLATE[b]	GENBANK (EF-1α)[c]
Fusarium awaxy	Guarapuava - PR	Maize ear	Species-specific PCR	FUM1 -	LGMF1700	-
				FUM1 +	LGMF1713	-
				FUM1 +	LGMF1717	-
				FUM1 +	LGMF1721	-
			Phylogenetic analysis	FUM1 -	LGMF1885	MG838996
				FUM1 -	LGMF1887	MG838997
		Maize stalk	Phylogenetic analysis	FUM1 -	LGMF1888	MG838998
				FUM1 -	LGMF1890	MG838999
				FUM1 -	LGMF1891	MG839000
				FUM1 -	LGMF1902	MG839001
				FUM1 -	LGMF1925	MG839003
				FUM1 -	LGMF1930	MG839004
				FUM1 -	LGMF1933	MG839005
				FUM1 -	LGMF1940	MG839006
			Species-specific PCR	FUM1 -	LGMF1709	-
				FUM1 -	LGMF1807	-
				FUM1 -	LGMF1810	-
				FUM1 -	LGMF1812	-
				FUM1 -	LGMF1813	-
				FUM1 -	LGMF1815	-
				FUM1 -	LGMF1754	-
				FUM1 -	LGMF1756	-
				FUM1 -	LGMF1669	-

(continues on next page)

Table S1 – (continued.)

SPECIES	GEOGRAPHICAL ORIGIN	ISOLATION SUBSTRATE	IDENTIFICATION METHOD	TOXIN[a]	ISOLATE[b]	GENBANK (EF-1α)[c]
Fusarium awaxy	Guarapuava - PR	Maize stalk	Species-specific PCR	FUM1 -	LGMF1670	-
				FUM1 -	LGMF1671	-
				FUM1 -	LGMF1672	-
				FUM1 -	LGMF1758	-
				FUM1 -	LGMF1809	-
				FUM1 -	LGMF1808	-
				FUM1 -	LGMF1805	-
				FUM1 -	LGMF1806	-
				FUM1 -	LGMF1801	-
				FUM1 -	LGMF1803	-
				FUM1 -	LGMF1804	-
				FUM1 +	LGMF1799	-
	Prudentópolis - PR	Maize stalk	Species-specific PCR	FUM1 -	LGMF1768	-
				FUM1 -	LGMF1765	-
Fusarium fujikuroi	Arapoti - PR	Maize stalk	Phylogenetic analysis	FUM1 +	LGMF1818	MG838979
				FUM1 +	LGMF1819	MG838980
			Species-specific PCR	FUM1 +	LGMF1817	-
	Campo Largo - PR	Maize ear	Species-specific PCR	FUM1 +	LGMF1664	-
	Castro - PR	Maize stalk	Phylogenetic analysis	FUM1 -	LGMF1839	MG838985
	Guarapuava - PR	Maize ear	Phylogenetic analysis	FUM1 -	LGMF1751	MG838972
				FUM1 +	LGMF1748	MG838971
			Species-specific PCR	FUM1 -	LGMF1714	-
		Maize stalk	Species-specific PCR	FUM1 -	LGMF1755	-

(continues on next page)

Table S1 – (continued.)

SPECIES	GEOGRAPHICAL ORIGIN	ISOLATION SUBSTRATE	IDENTIFICATION METHOD	TOXIN[a]	ISOLATE[b]	GENBANK (EF-1α)[c]
Fusarium fujikuroi	Guarapuava - PR	Maize stalk	Species-specific PCR	FUM1 +	LGMF1753	-
	Prudentópolis - PR	Maize ear	Species-specific PCR	FUM1 +	LGMF1708	-
		Maize stalk	Species-specific PCR	FUM1 -	LGMF1780	-
Fusarium graminearum	Arapoti - PR	Maize ear	Phylogenetic analysis	15-ADON	LGMF1657	MG838952
	Campo Largo - PR	Maize stalk	Phylogenetic analysis	15-ADON	LGMF1824	MG838982
	Castro - PR	Maize stalk	PCR-RFLP	15-ADON	LGMF1779	-
				15-ADON	LGMF1703	-
				15-ADON	LGMF1791	-
				15-ADON	LGMF1792	-
			Phylogenetic analysis	15-ADON	LGMF1858	MG838990
	Guarapuava - PR	Maize ear	PCR-RFLP	15-ADON	LGMF1704	-
				15-ADON	LGMF1705	-
				15-ADON	LGMF1707	-
			Phylogenetic analysis	15-ADON	LGMF1726	MG838961
				15-ADON	LGMF1727	MG838962
				15-ADON	LGMF1728	MG838963
				15-ADON	LGMF1729	MG838964
				15-ADON	LGMF1730	MG838965
				15-ADON	LGMF1738	MG838968
				15-ADON	LGMF1745	MG838969
		Maize stalk	PCR-RFLP	15-ADON	LGMF1800	-
			Phylogenetic analysis	15-ADON	LGMF1884	MG838995
Fusarium meridionale	Arapoti - PR	Maize ear	PCR-RFLP	NIV	LGMF1643	-

(continues on next page)

Table S1 – (continued.)

SPECIES	GEOGRAPHICAL ORIGIN	ISOLATION SUBSTRATE	IDENTIFICATION METHOD	TOXIN[a]	ISOLATE[b]	GENBANK (EF-1α)[c]
Fusarium meridionale	Arapoti - PR	Maize ear	Phylogenetic analysis	NIV	LGMF1646	MG838947
				NIV	LGMF1647	MG838948
				NIV	LGMF1648	MG838949
				NIV	LGMF1662	MG838955
	Castro - PR	Maize stalk	PCR-RFLP	NIV	LGMF1765	-
				NIV	LGMF1772	-
				NIV	LGMF1773	-
				NIV	LGMF1775	-
				NIV	LGMF1735	-
				NIV	LGMF1782	-
				NIV	LGMF1786	-
				NIV	LGMF1788	-
				NIV	LGMF1789	-
				NIV	LGMF1790	-
			Phylogenetic analysis	NIV	LGMF1757	MH203410
			Phylogenetic analysis	NIV	LGMF1853	MG838988
				NIV	LGMF1864	MG838991
Fusarium napiforme	Castro - PR	Maize stalk	Phylogenetic analysis	FUM1 -	LGMF1770	MH203411
				FUM1 -	LGMF1771	MH203412
				FUM1 +	LGMF1842	MG838986
Fusarium tjaetaba	Castro - PR	Maize ear	Phylogenetic analysis	FUM1 +	LGMF1723	MG838958
		Maize stalk	Phylogenetic analysis	FUM1 +	LGMF1826	MG838983
				FUM1 -	LGMF1854	MG838989

(continues on next page)

Table S1 – (continued.)

SPECIES	GEOGRAPHICAL ORIGIN	ISOLATION SUBSTRATE	IDENTIFICATION METHOD	TOXIN[a]	ISOLATE[b]	GENBANK (EF-1α)[c]
Fusarium tjaetaba	Castro - PR	Maize stalk	Species-specific PCR	FUM1 -	LGMF1759	-
				FUM1 -	LGMF1766	-
				FUM1 -	LGMF1784	-
				FUM1 -	LGMF1785	-
				FUM1 +	LGMF1764	-
	Guarapuava - PR	Maize stalk	Species-specific PCR	FUM1 +	LGMF1797	-
Fusarium verticillioides	Campo Largo - PR	Maize ear	Phylogenetic analysis	FUM1 +	LGMF1690	MG838956
				FUM1 +	LGMF1699	MG838957
			Species-specific PCR	FUM1 +	LGMF1663	-
				FUM1 +	LGMF1665	-
				FUM1 +	LGMF1666	-
				FUM1 +	LGMF1667	-
				FUM1 +	LGMF1668	-
				FUM1 +	LGMF1680	-
				FUM1 +	LGMF1673	-
				FUM1 +	LGMF1674	-
				FUM1 +	LGMF1675	-
				FUM1 +	LGMF1676	-
				FUM1 +	LGMF1677	-
				FUM1 +	LGMF1678	-
				FUM1 +	LGMF1679	-
				FUM1 +	LGMF1682	-
				FUM1 +	LGMF1683	-

(continues on next page)

Table S1 – (continued.)

SPECIES	GEOGRAPHICAL ORIGIN	ISOLATION SUBSTRATE	IDENTIFICATION METHOD	TOXIN[a]	ISOLATE[b]	GENBANK (EF-1α)[c]
Fusarium verticillioides	Campo Largo - PR	Maize ear	Species-specific PCR	FUM1 +	LGMF1685	-
				FUM1 +	LGMF1686	-
				FUM1 +	LGMF1687	-
				FUM1 +	LGMF1688	-
				FUM1 +	LGMF1689	-
				FUM1 +	LGMF1681	-
				FUM1 +	LGMF1691	-
				FUM1 +	LGMF1692	-
				FUM1 +	LGMF1693	-
				FUM1 +	LGMF1694	-
				FUM1 +	LGMF1695	-
				FUM1 +	LGMF1696	-
	Castro - PR	Maize ear	Phylogenetic analysis	FUM1 +	LGMF1724	MG838959
				FUM1 +	LGMF1725	MG838960
		Maize stalk	Phylogenetic analysis	FUM1 +	LGMF1774	MH203413
			Species-specific PCR	FUM1 +	LGMF1760	-
				FUM1 +	LGMF1720	-
				FUM1 +	LGMF1763	-
	Guarapuava - PR	Maize ear	Phylogenetic analysis	FUM1 +	LGMF1732	MG838966
				FUM1 +	LGMF1734	MG838967
				FUM1 +	LGMF1762	MG838973
			Species-specific PCR	FUM1 +	LGMF1710	-
				FUM1 +	LGMF1715	-

(continues on next page)

Table S1 – (continued.)

SPECIES	GEOGRAPHICAL ORIGIN	ISOLATION SUBSTRATE	IDENTIFICATION METHOD	TOXIN[a]	ISOLATE[b]	GENBANK (EF-1α)[c]
Fusarium verticillioides	Guarapuava - PR	Maize ear	Species-specific PCR	FUM1 +	LGMF1716	-
				FUM1 +	LGMF1718	-
				FUM1 +	LGMF1719	-
				FUM1 +	LGMF1697	-
				FUM1 +	LGMF1701	-
				FUM1 +	LGMF1731	-
		Maize stalk	Phylogenetic analysis	FUM1 +	LGMF1874	MG838992
				FUM1 +	LGMF1882	MG838993
				FUM1 +	LGMF1883	MG838994
			Species-specific PCR	FUM1 +	LGMF1793	-
				FUM1 +	LGMF1794	-
				FUM1 +	LGMF1796	-
	Prudentópolis - PR	Maize ear	Phylogenetic analysis	FUM1 +	LGMF1777	MG838974
				FUM1 +	LGMF1778	MG838975
				FUM1 +	LGMF1798	MG838976
				FUM1 +	LGMF1802	MG838977
				FUM1 +	LGMF1814	MG838978
			Species-specific PCR	FUM1 +	LGMF1702	-
				FUM1 +	LGMF1733	-
				FUM1 +	LGMF1736	-
				FUM1 +	LGMF1737	-
				FUM1 +	LGMF1706	-
				FUM1 +	LGMF1739	-

(continues on next page)

Table S1 – (continued.)

SPECIES	GEOGRAPHICAL ORIGIN	ISOLATION SUBSTRATE	IDENTIFICATION METHOD	TOXIN[a]	ISOLATE[b]	GENBANK (EF-1α)[c]
Fusarium verticillioides	Prudentópolis - PR	Maize ear	Species-specific PCR	FUM1 +	LGMF1740	-
				FUM1 +	LGMF1741	-
				FUM1 +	LGMF1742	-
				FUM1 +	LGMF1743	-
				FUM1 +	LGMF1744	-
				FUM1 +	LGMF1711	-
				FUM1 +	LGMF1749	-
				FUM1 +	LGMF1750	-
				FUM1 +	LGMF1712	-
				FUM1 +	LGMF1752	-
	Prudentópolis - PR	Maize stalk	Phylogenetic analysis	FUM1 +	LGMF1961	MG839007
				FUM1 +	LGMF1978	MG839008
				FUM1 +	LGMF1981	MG839009
			Species-specific PCR	FUM1 +	LGMF1787	-
				FUM1 +	LGMF1769	-
				FUM1 +	LGMF1781	-
				FUM1 +	LGMF1783	-
				FUM1 +	LGMF1776	-
				FUM1 +	LGMF1767	-
				FUM1 +	LGMF1747	-
FIESC 12	Guarapuava - PR	Maize stalk	Phylogenetic analysis	-	LGMF1811	MH203414
FIESC 20	Guarapuava - PR	Maize stalk	Phylogenetic analysis	-	LGMF1908	MG839002
FIESC 33	Castro - PR	Maize stalk	Phylogenetic analysis	-	LGMF1762	MH203415

(continues on next page)

Table S1 – (continued.)

SPECIES	GEOGRAPHICAL ORIGIN	ISOLATION SUBSTRATE	IDENTIFICATION METHOD	TOXIN[a]	ISOLATE[b]	GENBANK (EF-1α)[c]
FIESC 33	Castro - PR	Maize stalk	Phylogenetic analysis	-	LGMF1838	MG838984
FIESC 33	Castro - PR	Maize stalk	Phylogenetic analysis	-	LGMF1849	MG838987

[a]Toxin – Positive (FUM1 +) or negative (FUM1 -) isolates according to presence (FUM1+) or absence (FUM1-) of 183bp fragment in FUM1 PCR.
[b]Isolate – LGMF: Collection code for fungal culture collection of Laboratório de Genética de Microrganismos (LabGeM, Federal University of Paraná – UFPR, Brazil; www.labgem.ufpr.br)
[c]GenBank - *EF-1α*: Translation elongation factor 1-alpha;

Table S2 – Host/substrate, geographical origin, collection codes and sequence GenBank accession numbers of *Fusarium* spp. strains from *Fusarium fujikuroi* (FFSC), *Fusarium graminearum* (FGSC) and *Fusarium incarnatum-equiseti* (FIESC) species complexes used for phylogenetic analysis

Species	Host/Substrate	Origin	Collection[a]	*GenBank accession*[b]		References
				EF-1α	*TUB2*	
F. acutatum	Unknown	India	**NRRL 13309**	KR071754	KU603870	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Unknown	India	NRRL 13308	AF160276	U34431	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
	Cajanus cajan	India	NRRL 25731	KR071755	KU603871	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Triticum aestivum	Egypt	CBS 113964	KR071759	KU603876	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)

(continues on next page)

Table S2 – (continued).

Species	Host/Substrate	Origin	Collection[a]	*GenBank accession*[b]		References
				EF-1α	***TUB2***	
F. acutatum	*Triticum* sp.	Iran	CBS 131573	KR071758	KU603872	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
F. agapanthi	*Agapanthus praecox*	Australia	**NRRL 54463**	KU900630	KU900635	(Edwards *et al.*, 2016)
F. ananatum	*Ananas comosus*	South Africa	**CBS 118516**	DQ282167	DQ282174	(Jacobs *et al.*, 2010)
	Ananas comosus	England	NRRL 22945	AF160297	U34420	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
F. andiyazi	*Sorghum bicolor*	South Africa	NRRL 53928	KR071718	KU603866	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Sorghum bicolor	Ethiopia	NRRL 53927	KR071720	KU603868	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Macadamia ternifolia	Cuba	NRRL 25490	KR071719	KU603869	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Human blood	Turkey	CBS 134430	KR071721	KU603867	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
F. anthophilum	*Hippeastrum* sp.	Germany	NRRL 13602	AF160292	U61541	(O'Donnell *et al.*, 1998, 2000b)
	Haemanthus sp.	Germany	NRRL 22947	KR071767	KU603932	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Euphorbia pulcherrima	Germany	NRRL 22943	KR071766	KU603929	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Cymbidium sp.	New Zealand	CBS 119859	KR071765	KU603931	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Triticum sp.	USA	CBS 119858	KR071764	KU603930	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
F. awaxy	*Zea mays*	Brazil	**LGMF1930**	MG839004	MG839013	This study
	Zea mays	Brazil	LGMF1661	MG838954	MG839011	This study
	Zea mays	South Africa	ATCC 38016	AB674285	N/A	(Watanabe *et al.*, 2011)
	Zea mays	Brazil	RV23-2	KP336408	N/A	(Faria *et al.*, 2012)
	Zea mays	South Korea	OS15	JX867945	N/A	(Kim *et al.*, 2012)
	Zea mays	China	Fs_hap3	KT716223	N/A	(Zhang *et al.*, 2016)
	Sorghum bicolor	USA	M03S-C1-1	KX681493	N/A	(Funnell-Harris *et al.*, 2017)

(continues on next page)

Table S2 – (continued).

Species	Host/Substrate	Origin	Collection[a]	GenBank accession[b]		References
				EF-1α	*TUB2*	
F. bactridioides	*Cronartium conigenum*	USA	**NRRL 20476**	AF160290	U34434	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
F. begoniae	*Begonia elatior*	Germany	**NRRL 25300**	AF160293	U61543	(O'Donnell *et al.*, 1998, 2000b)
F. brevicatenulatum	*Striga asiatica*	Madagascar	**NRRL 25446**	AF160265	U61545	(O'Donnell *et al.*, 1998, 2000b)
F. bulbicola	*Nerine bowdenii*	Netherlands	**NRRL 13618**	AF160294	U61546	(O'Donnell *et al.*, 1998, 2000b)
F. circinatum	*Pinus radiata*	USA	**NRRL 25331**	AF160295	U61547	(O'Donnell *et al.*, 1998, 2000b)
F. coicis	*Coix gasteenii*	Australia	**NRRL 66233**	KP083251	N/A	(Laurence *et al.*, 2016)
	Coix gasteenii	Australia	NRRL 66234	KP083252	N/A	(Laurence *et al.*, 2016)
F. concentricum	*Musa sapientum*	Costa Rica	**NRRL 25181**	AF160282	U61548	(O'Donnell *et al.*, 1998, 2000b)
F. denticulatum	*Ipomoea batatas*	USA	NRRL 25302	AF160269	U61550	(O'Donnell *et al.*, 1998, 2000b)
F. dlaminii	*Zea mays* (Field soil)	South Africa	**NRRL 13164**	AF160277	U34430	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
F. ficicrescens	*Ficus carica*	Iran	**CBS 125178**	KP662899	KP662896	(Al-Hatmi *et al.*, 2016c)
	Ficus carica	Iran	CBS 125177	KP662898	KP662895	(Al-Hatmi *et al.*, 2016c)
	Ficus carica	Iran	CBS 125181	KP662900	KP662897	(Al-Hatmi *et al.*, 2016c)
F. fracticaudum	*Pinus maximinoi*	Colombia	**CBS 137233**	KJ541059	KJ541051	(Herron *et al.*, 2015)
	Pinus maximinoi	Colombia	CBS 137234	KJ541058	KJ541048	(Herron *et al.*, 2015)
F. fractiflexum	*Cymbidium* sp.	Japan	**NRRL 28852**	AF160288	AF160315	(O'Donnell *et al.*, 2000b)
F. fredikrugeri	*Melhania acuminata* rhizophere	South Africa	**CBS 144209**	LT996097	LT996117	(Sandoval-Denis *et al.*, 2018b)
	Melhania acuminata rhizophere	South Africa	CBS 144495	LT996096	LT996116	(Sandoval-Denis *et al.*, 2018b)
	Striga hermonthica	Madagascar	NRRL 26061	AF160303	AF160319	(O'Donnell *et al.*, 2000b)
	N/A	Niger	NRRL 26152	AF160306	AF160349	(O'Donnell *et al.*, 2000b)
F. fujikuroi	*Oryza sativa*	Taiwan	**NRRL 13998**	KR071741	KU603884	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Oryza sativa	Taiwan	NRRL 13566	AF160279	U34415	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
	Environmental	France	NRRL 34481	KR071742	KU603888	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)

(continues on next page)

Table S2 – (continued).

Species	Host/Substrate	Origin	Collection[a]	*GenBank accession*[b]		References
				EF-1α	***TUB2***	
F. fujikuroi	*Oryza sativa*	India	NRRL 36400	KR071744	KU603887	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Environmental	Netherlands	NRRL 53750	KR071743	KU603889	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Environmental	USA	NRRL 53926	KR909368	KU603890	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Oryza sativa	Japan	NRRL 36388	KU711678	KU603885	(Al-Hatmi et al., 2016b); (Al-Hatmi et al., unpublished)
	Human skin	USA	NRRL 43610	KU711677	KU603886	(Al-Hatmi *et al.*, 2016b); (Al-Hatmi *et al.*, unpublished)
F. globosum	*Zea mays*	South Africa	**NRRL 26131**	AF160285	KF466439	(O'Donnell *et al.*, 2000b; Proctor *et al.*, 2013)
	Zea mays	South Africa	NRRL 26132	KR909369	N/A	(Al-Hatmi *et al.*, 2016a)
F. guttiforme	*Ananas comosus*	Brazil	**MRC 7539**	DQ282165	N/A	(Jacobs *et al.*, 2010)
	Ananas comosus	Brazil	CBS 124146	DQ282170	N/A	(Jacobs *et al.*, 2010)
F. konzum	*Sorghastrum nuttans*	USA	**KSU 10653**	N/A	AY222294	(Zeller *et al.*, 2003)
	Andropogon gerardii	USA	MRC 8544	EU220235	EU220234	(Kvas *et al.*, 2009)
F. lactis	*Ficus carica*	USA	**NRRL 25200**	AF160272	U61551	(O'Donnell *et al.*, 1998, 2000b)
F. mangiferae	*Mangifera indica*	India	NRRL 25226	AF160281	U61561	(O'Donnell *et al.*, 1998, 2000b)
	Mangifera indica	South Africa	NRRL 53924	KR909363	N/A	(Al-Hatmi *et al.*, 2016a)
	Mangifera indica	Israel	NRRL 53980	KR909361	N/A	(Al-Hatmi *et al.*, 2016a)
	Soil	Australia	NRRL 53922	KR909362	N/A	(Al-Hatmi *et al.*, 2016a)
F. marasasianum	*Pinus patula*	Colombia	**CBS 137238**	KJ541063	KJ541054	(Herron *et al.*, 2015)
	Pinus patula	Colombia	CBS 137237	KJ541062	KJ541052	(Herron *et al.*, 2015)
F. mexicanum	*Mangifera indica*	Mexico	**NRRL 53147**	GU737282	GU737494	(Otero-Colina *et al.*, 2010)
F. mundagurra	Soil	Australia	**NRRL 66235**	KP083256	N/A	(Laurence *et al.*, 2016)
	Soil	Australia	NRRL 66236	KP083255	N/A	(Laurence *et al.*, 2016)
F. musae	*Musa sapientum*	Honduras	**NRRL 25059**	FN552086	FN545368	(Van Hove *et al.*, 2011)
	Musa sapientum	Mexico	NRRL 28893	FN552092	FN545374	(Van Hove *et al.*, 2011)

(continues on next page)

Table S2 – (continued).

Species	Host/Substrate	Origin	Collection[a]	*GenBank accession*[b]		References
				EF-1α	***TUB2***	
F. napiforme	*Pennisetum typhoides*	South Africa	**NRRL 13604**	AF160266	U34428	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
	N/A	N/A	NRRL 36536	KR071715	KU603905	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	N/A	N/A	NRRL 36537	KR071713	KU603904	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
F. nygamai	*Sorghum bicolor*	Australia	**NRRL 13488**	AF160273	U34426	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
	Human blood	Egypt	NRRL 26421	KR071725	KU603865	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Sorghum sp.	Australia	NRRL 53981	KR071723	KU603864	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
F. parvisorum	*Pinus patula*	Colombia	**CBS 137236**	KJ541060	KJ541055	(Herron *et al.*, 2015)
	Pinus patula	Colombia	CBS 137235	KJ541061	KJ541056	(Herron *et al.*, 2015)
F. phyllophilum	*Dracaena deremensis*	Italy	**NRRL 13617**	AF160274	U34432	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
F. pininemorale	*Pinus tecunumanii*	Colombia	**CBS 137240**	KJ541064	KJ541049	(Herron *et al.*, 2015)
	Pinus tecunumanii	Colombia	CBS 137239	KJ541065	KJ541050	(Herron *et al.*, 2015)
F. proliferatum	*Cattleya* sp.	Germany	NRRL 22944	AF160280	U34416	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
	Human eye	Spain	NRRL 53680	KR071731	KU603923	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Human tissue	USA	CBS 125014	KR071738	KU603927	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Human blood	USA	NRRL 43617	KR071739	KU603928	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Triticum sp.	Australia	CBS 131391	KR071732	KU603921	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Ficus carica	USA	NRRL 25060	KR071734	KU603922	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Oryza sativa	Guyana	NRRL 36314	KR071737	KU603924	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)

(continues on next page)

Table S2 – (continued).

Species	Host/Substrate	Origin	Collection[a]	*GenBank accession*[b]		References
				EF-1α	***TUB2***	
F. proliferatum	*Musa* sp.	Netherlands	NRRL 36488	KR071736	KU603925	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Oryza sativa	Japan	NRRL 36309	KR071735	KU603926	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
F. pseudoanthophilum	*Zea mays*	Zimbabwe	NRRL 25206	AF160264	U61553	(O'Donnell *et al.*, 1998, 2000b)
F. pseudocircinatum	*Solanum* sp.	Ghana	**NRRL 22946**	AF160271	U34427	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
F. pseudonygamai	*Pennisetum typhoides*	Nigeria	**NRRL 13592**	AF160263	U34421	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
F. ramigenum	*Ficus carica*	USA	**NRRL 25208**	AF160267	U61554	(O'Donnell *et al.*, 1998, 2000b)
F. sacchari	*Saccharum officinarum*	India	NRRL 13999	AF160278	U34414	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
	Saccharum officinarum	Guyana	NRRL 25061	KR071753	KU603912	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Human eye	India	NRRL 53746	KR071747	KU603911	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Oryza australiensis	Australia	CBS 131369	KR071752	KU603919	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
F. secorum	*Beta vulgaris*	USA	**NRRL 62593**	KJ189225	N/A	(Secor *et al.*, 2014)
	Beta vulgaris	USA	NRRL 62602	KJ189226	N/A	(Secor *et al.*, 2014)
F. siculi	*Citrus sinensis*	Italy	**CBS 142422**	LT746214	LT746346	(Sandoval-Denis *et al.*, 2018a)
	Citrus sinensis	Italy	CPC 27189	LT746215	LT746347	(Sandoval-Denis *et al.*, 2018a)
F. sororula	*Pinus patula*	Colombia	**CBS 137242**	KJ541067	KJ541057	(Herron *et al.*, 2015)
	Pinus patula	Colombia	CBS 137241	KJ541066	KJ541053	(Herron *et al.*, 2015)
F. sterilihyphosum	*Mangifera indica*	South Africa	**NRRL 25623**	AF160300	AF160316	(O'Donnell *et al.*, 2000b)
	Mangifera indica	Brazil	CML 283	DQ452858	DQ445780	(Lima *et al.*, 2012)
F. subglutinans	*Zea mays*	USA	**NRRL 22016**	AF160289	U34417	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
	Zea mays	Belgium	MUCL 52468	HM067691	HM067699	(Scauflaire *et al.*, 2011)

(continues on next page)

Table S2 – (continued).

Species	Host/Substrate	Origin	Collection[a]	*GenBank accession*[b]		References
				EF-1α	***TUB2***	
F. subglutinans	*Zea mays*	South Africa	NRRL 53904	KR071769	KU603892	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Human blood	Italy	NRRL 54158	KR071770	KU603893	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
F. succisae	*Succisa pratensis*	Germany	NRRL 13613	AF160291	U34419	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
F. sudanense	*Striga hermonthica*	Sudan	**NRRL 26793**	KU711697	KU603909	(Moussa *et al.*, 2017)
F. temperatum	*Zea mays*	Belgium	**MUCL 52450**	HM067687	HM067695	(Scauflaire *et al.*, 2011)
F. terrícola	*Zea mays*	South Africa	NRRL 25622	AF160301	AF160317	(O'Donnell *et al.*, 2000b)
	Desert soil	Australia	**CBS 483.94**	KU711698	KU603908	(Moussa *et al.*, 2017)
	Desert soil	Australia	CBS 119850	KU711699	KU603907	(Moussa *et al.*, 2017)
F. thapsinum	*Sorghum bicolor*	South Africa	NRRL 22045	AF160270	U34418	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
	Sorghum sp.	-	NRRL 22049	KR071726	KU603878	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Pennisetum sp.	Yemen	NRRL 53631	KR071729	KU603881	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Human skin	Italy	NRRL 25229	KR071727	KU603883	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
F. tjaetaba	*Sorghum interjectum*	Australia	**NRRL 66243**	KP083263	N/A	(Laurence *et al.*, 2016)
	Sorghum interjectum	Australia	NRRL 66244	KP083264	N/A	(Laurence *et al.*, 2016)
	Sorghum interjectum	Australia	NRRL 66245	KP083265	N/A	(Laurence *et al.*, 2016)
F. tupiense	*Mangifera indica*	Brazil	**NRRL 53984**	DQ452859	DQ445781	(Lima *et al.*, 2012)
	Mangifera indica	Brazil	CML 345	DQ452861	DQ445783	(Lima *et al.*, 2012)
	Mangifera indica	Brazil	NRRL 53996	DQ452860	DQ445782	(Lima *et al.*, 2012)
F. udum	Unknown	Germany	NRRL 22949	AF160275	U34433	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
F. verticillioides	*Zea mays*	Germany	NRRL 22172	AF160262	U34413	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)

(continues on next page)

Table S2 – (continued).

Species	Host/Substrate	Origin	Collection[a]	*GenBank accession*[b]		References
				EF-1α	***TUB2***	
F. verticillioides	*Zea mays*	USA	NRRL 20956	KR071708	KU603854	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Human liver	Germany	NRRL 36131	KR071704	KU603853	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Solanum lycopersicum	-	NRRL 53700	KR071705	KU603855	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Pinus radiata	Spain	NRRL 53753	KR071707	KU603856	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Environmental	Russia	NRRL 22950	KR071703	KU603852	(Al-Hatmi *et al.*, 2016a); (Al-Hatmi *et al.*, unpublished)
	Zea mays	Australia	CBS 131389	KU711695	KU603857	(Al-Hatmi *et al.*, 2016b); (Al-Hatmi *et al.*, unpublished)
F. werrikimbe	*Sorghum leiocladium*	Australia	**CBS 125535**	EF107131	EF107133	(Walsh *et al.*, 2010)
	Sorghum leiocladium	Australia	F19361	EF107132	EF107134	(Walsh *et al.*, 2010)
F. xylarioides	*Coffea* sp.	Ivory Coast	**NRRL 25486**	AY707136	AY707118	(Geiser *et al.*, 2005)
Fusarium sp.	*Sorghum bicolor*	Tanzania	NRRL 26064	AF160302	AF160346	(O'Donnell *et al.*, 2000b)
	Zea mays	Zimbabwe	NRRL 25221	AF160268	U61560	(O'Donnell *et al.*, 1998, 2000b)
	Oryza sativa	Nigeria	NRRL 25615	AF160304	AF160320	(O'Donnell *et al.*, 2000b)
	Soil	Australia	NRRL 25807	AF160305	U61542	(O'Donnell *et al.*, 1998, 2000b)
	Wood	Venezuela	NRRL 25195	AF160298	U61558	(O'Donnell *et al.*, 1998, 2000b)
	Ipomoea batatas	Peru	NRRL 25346	AF160296	U61564	(O'Donnell *et al.*, 1998, 2000b)
	Ornamental grass	South Africa	NRRL 26756	AF160307	AF160322	(O'Donnell *et al.*, 2000b)
	Ornamental reed	South Africa	NRRL 26757	AF160308	AF160323	(O'Donnell *et al.*, 2000b)
	Palm	Venezuela	NRRL 25204	AF160299	U61559	(O'Donnell *et al.*, 1998, 2000b)
	Bidens pilosa	USA	NRRL 29124	AF160311	AF160326	(O'Donnell *et al.*, 2000b)
	Triticum aestivum	Japan	NRRL 25309	AF160284	U61563	(O'Donnell *et al.*, 1998, 2000b)
	Oryza sativa	Japan	NRRL 25303	AF160283	U61562	(O'Donnell *et al.*, 1998, 2000b)
	Soil	Papua New Guinea	NRRL 26427	AF160286	AF160313	(O'Donnell *et al.*, 2000b)
F. acaciae-mearnsii	*Acacia mearnsii*	South Africa	**NRRL 26754**	AF212448		(O'Donnell *et al.*, 2000a)

(continues on next page)

Table S2 – (continued).

Species	Host/Substrate	Origin	Collection[a]	*GenBank accession*[b]		References
				EF-1α	*TUB2*	
F. acaciae-mearnsii	*Acacia mearnsii*	South Africa	NRRL 26752	AF212447		(O'Donnell *et al.*, 2000a)
	Acacia mearnsii	South Africa	NRRL 26755	AF212449		(O'Donnell *et al.*, 2000a)
	Soil	Australia	NRRL 34207	DQ459741		(Starkey *et al.*, 2007)
F. aethiopicum	*Triticum sp.*	Ethiopia	**NRRL 46726**	FJ240298		(O'Donnell *et al.*, 2008)
	Triticum sp.	Ethiopia	NRRL 46710	FJ240295		(O'Donnell *et al.*, 2008)
	Triticum sp.	Ethiopia	NRRL 46718	FJ240296		(O'Donnell *et al.*, 2008)
	Triticum sp.	Ethiopia	NRRL 46722	FJ240297		(O'Donnell *et al.*, 2008)
	Triticum sp.	Ethiopia	NRRL 46738	FJ240299		(O'Donnell *et al.*, 2008)
F. asiaticum	*Hordeum vulgare*	Japan	**NRRL 13818**	AF212451		(O'Donnell *et al.*, 2000a)
	Hordeum vulgare	Japan	NRRL 6101	AF212450		(O'Donnell *et al.*, 2000a)
	Triticum sp.	China	NRRL 26156	AF212452		(O'Donnell *et al.*, 2000a)
F. asiaticum	*Zea mays*	Nepal	NRRL 28720	AF212453		(O'Donnell *et al.*, 2000a)
F. austroamericanum	*Polyporus circinatus*	Brazil	**NRRL 2903**	AF212438		(O'Donnell *et al.*, 2000a)
	Herbaceous vine	Brazil	NRRL 28585	AF212439		(O'Donnell *et al.*, 2000a)
	Zea mays	Brazil	NRRL 28718	AF212440		(O'Donnell *et al.*, 2000a)
F. boothi	*Zea mays*	South Africa	**NRRL 26916**	AF212444		(O'Donnell *et al.*, 2000a)
	-	South Africa	NRRL 29011	AF212445		(O'Donnell *et al.*, 2000a)
	Zea mays	South Africa	NRRL 29020	AF212443		(O'Donnell *et al.*, 2000a)
	Zea mays	Nepal	NRRL 29105	AF212446		(O'Donnell *et al.*, 2000a)
F. brasilicum	*Avena sativa*	Brazil	**NRRL 31281**	AY452964		(O'Donnell *et al.*, 2004)
	Hordeum vulgare	Brazil	NRRL 31238	AY452963		(O'Donnell *et al.*, 2004)
F. cortaderiae	*Cortaderia selloana*	New Zealand	**NRRL 29297**	AY225885		(Ward *et al.*, 2002)
	Zea mays	New Zealand	NRRL 29306	AY225886		(Ward *et al.*, 2002)
	Hordeum vulgare	Brazil	NRRL 31185	AY452962		(O'Donnell *et al.*, 2004)
	Triticum sp.	Brazil	NRRL 31205	AY452960		(O'Donnell *et al.*, 2004)
F. gerlachii	*Triticum aestivum*	USA	**NRRL 36905**	DQ459742		(Starkey *et al.*, 2007)
	Arundo donax	USA	NRRL 38380	DQ459743		(Starkey *et al.*, 2007)

(continues on next page)

Table S2 – (continued).

Species	Host/Substrate	Origin	Collection[a]	*GenBank accession*[b]		References
				EF-1α	***TUB2***	
F. gerlachii	*Triticum sp.*	USA	NRRL 38405	DQ459744		(Starkey *et al.*, 2007)
F. graminearum	*Zea mays*	USA	NRRL 5883	AF212455		(O'Donnell *et al.*, 2000a)
	Millet	Hungary	NRRL 6394	AF212456		(O'Donnell *et al.*, 2000a)
	Zea mays	Iran	NRRL 13383	AF212457		(O'Donnell *et al.*, 2000a)
	Zea mays	USA	NRRL 28063	AF212458		(O'Donnell *et al.*, 2000a)
	Triticum sp.	USA	NRRL 28336	AF212459		(O'Donnell *et al.*, 2000a)
	Rumohra adiantiformis	Netherlands	NRRL 28439	AF212460		(O'Donnell *et al.*, 2000a)
	Triticum sp.	USA	NRRL 29169	AF212461		(O'Donnell *et al.*, 2000a)
	Zea mays	USA	NRRL 31084	AY452957		(O'Donnell *et al.*, 2004)
F. louisianense	*Triticum aestivum*	USA	**NRRL 54197**	KM889633		(Sarver *et al.*, 2011)
	Triticum aestivum	USA	NRRL 54196	KM889632		(Sarver *et al.*, 2011)
F. meridionale	*Ipomoea batatas*	New Caledonia	**NRRL 28436**	AF212435		(O'Donnell *et al.*, 2000a)
	Zea mays	Nepal	NRRL 28721	AF212454		(O'Donnell *et al.*, 2000a)
	Zea mays	Nepal	NRRL 28723	AF212436		(O'Donnell *et al.*, 2000a)
	Soil	South Africa	NRRL 29010	AF212437		(O'Donnell *et al.*, 2000a)
F. mesoamericanum	*Musa sp.*	Honduras	**NRRL 25797**	AF212441		(O'Donnell *et al.*, 2000a)
	Vitis sp.	USA	NRRL 29148	AF212442		(O'Donnell *et al.*, 2000a)
F. nepalense	*Oryza sativa*	Nepal	**NRRL 54222**	KM889631		(Sarver *et al.*, 2011)
	Oryza sativa	Nepal	NRRL 54220	KM889629		(Sarver *et al.*, 2011)
	Oryza sativa	Nepal	NRRL 54221	KM889630		(Sarver *et al.*, 2011)
F. ussurianum	*Avena sativa*	Russia	**NRRL 45681**	FJ240301		(O'Donnell *et al.*, 2008)
	Triticum sp.	Russia	NRRL 45665	FJ240300		(O'Donnell *et al.*, 2008)
	Triticum sp.	Russia	NRRL 45795	FJ240303		(O'Donnell *et al.*, 2008)
	Triticum sp.	Russia	NRRL 45833	FJ240305		(O'Donnell *et al.*, 2008)
F. vorosii	*Triticum sp.*	Hungary	**NRRL 37605**	DQ459745		(Starkey *et al.*, 2007)
	Triticum sp.	Japan	NRRL 38207	DQ459747		(Starkey *et al.*, 2007)
	Triticum sp.	Japan	NRRL 38208	DQ459746		(Starkey *et al.*, 2007)

(continues on next page)

Table S2 – (continued).

Species	Host/Substrate	Origin	Collection[a]	*GenBank accession*[b]		References
				EF-1α	***TUB2***	
FIESC 1-a	Dog	USA	NRRL 43637	GQ505664		(O'Donnell *et al.*, 2009)
	Human sinus	USA	NRRL 45996	GQ505671		(O'Donnell *et al.*, 2009)
	Dog nose	USA	NRRL 43640	GQ505667		(O'Donnell *et al.*, 2009)
FIESC 1-b	Human	USA	NRRL 34039	GQ505639		(O'Donnell *et al.*, 2009)
FIESC 1-c	Human leg	USA	NRRL 34034	GQ505636		(O'Donnell *et al.*, 2009)
FIESC 1-d	[Hemiptera: Coccidae] on stems of *Citrus* sp.	Sri Lanka	NRRL 25085	JF740716		(O'Donnell *et al.*, 2012)
FIESC 2-a	Cotton	Mozambique	NRRL 36401	GQ505651		(O'Donnell *et al.*, 2009)
FIESC 2-b	*Phaseolus vulgaris* (seed)	Sudan	NRRL 36448	GQ505652		(O'Donnell *et al.*, 2009)
FIESC 2-c	[Orthoptera: Acrididae]	Mali	NRRL 25124	JF740749		(O'Donnell *et al.*, 2012)
FIESC 3-a	Cotton	England	NRRL 36323	GQ505648		(O'Donnell *et al.*, 2009)
	Unknown	Unknown	NRRL 36318	GQ505646		(O'Donnell *et al.*, 2009)
FIESC 3-b	Human eye	USA	NRRL 28029	GQ505602		(O'Donnell *et al.*, 2009)
FIESC 4-a, *F. lacertarum*	Lizard skin	India	NRRL 20423	GQ505593		(O'Donnell *et al.*, 2009)
FIESC 4-b	Unknown	Unknown	NRRL 36123	GQ505643		(O'Donnell *et al.*, 2009)
FIESC 4-c, *F. lacertarum*	*Scrobipalpuloides absoluta* (larva) [Lepidoptera: Gelechiidae]	Brazil	NRRL 52753	JF740828		(O'Donnell *et al.*, 2012)
FIESC 5-a	Human abcess	USA	NRRL 32871	GQ505619		(O'Donnell *et al.*, 2009)
	Human abcess	USA	NRRL 34032	GQ505635		(O'Donnell *et al.*, 2009)
FIESC 5-b	Human abcess	USA	NRRL 45995	GQ505670		(O'Donnell *et al.*, 2009)
	Human abcess	USA	NRRL 34037	GQ505638		(O'Donnell *et al.*, 2009)
FIESC 5-c	*Disphyma crassifolium* (seed)	Germany	NRRL 25795	GQ505597		(O'Donnell *et al.*, 2009)
FIESC 5-d	Human sinus	USA	NRRL 34035	GQ505637		(O'Donnell *et al.*, 2009)
FIESC 5-e	Human maxillary sinus	USA	NRRL 43623	GQ505661		(O'Donnell *et al.*, 2009)
FIESC 5-f	Human sinus	USA	NRRL 45997	GQ505672		(O'Donnell *et al.*, 2009)

(continues on next page)

Table S2 – (continued).

Species	Host/Substrate	Origin	Collection[a]	*GenBank accession*[b]		References
				EF-1α	***TUB2***	
FIESC 6-a	Manatee	USA	NRRL 43638	GQ505665		(O'Donnell *et al.*, 2009)
	Human eye	USA	NRRL 43694	GQ505668		(O'Donnell *et al.*, 2009)
FIESC 6-b	Human toe	USA	NRRL 45998	GQ505673		(O'Donnell *et al.*, 2009)
FIESC 6-c	*Zulia colombiana* (adult) [Hemiptera: Cercopidae]	Colombia	NRRL 52686	JF740772		(O'Donnell *et al.*, 2012)
FIESC 7-a	Human toenail	USA	NRRL 32997	GQ505624		(O'Donnell *et al.*, 2009)
FIESC 7-b	*Mahanarva andigena* (adult) [Hemiptera: Cercopidae]	Colombia	NRRL 52695	JF740778		(O'Donnell *et al.*, 2012)
FIESC 8-a	*Festuca* sp.	USA	NRRL 5537	GQ505588		(O'Donnell *et al.*, 2009)
FIESC 8-b	Human eye	USA	NRRL 43498	GQ505658		(O'Donnell *et al.*, 2009)
FIESC 9-a, *F. scirpi*	Pasture soil	Australia	NRRL 29134	GQ505605		(O'Donnell *et al.*, 2009)
	Pasture soil	Australia	NRRL 36478	GQ505654		(O'Donnell *et al.*, 2009)
FIESC 9-b, *F. scirpi*	Pine soil	Australia	NRRL 13402	GQ505592		(O'Donnell *et al.*, 2009)
FIESC 9-c, *F. scirpi*	Soil	France	NRRL 26922	GQ505601		(O'Donnell *et al.*, 2009)
FIESC 10-a	Unknown	Unknown	NRRL 3020	GQ505586		(O'Donnell *et al.*, 2009)
	Unknown	Unknown	NRRL 3214	GQ505587		(O'Donnell *et al.*, 2009)
FIESC 11-a	Air	Netherlands Antilles	NRRL 36372	GQ505649		(O'Donnell *et al.*, 2009)
FIESC 12-a	Wheat	Germany	NRRL 6548	GQ505589		(O'Donnell *et al.*, 2009)
	Wheat	Germany	NRRL 26921	GQ505600		(O'Donnell *et al.*, 2009)
FIESC 12-a	*Thuja* sp.	Germany	NRRL 31011	GQ505606		(O'Donnell *et al.*, 2009)
FIESC 12-b	*Pinus nigra* (seedling)	Croatia	NRRL 36269	GQ505645		(O'Donnell *et al.*, 2009)
FIESC 12-c	Seedling	Germany	NRRL 36392	GQ505650		(O'Donnell *et al.*, 2009)
FIESC 13-a	Horse	USA	NRRL 43635	GQ505662		(O'Donnell *et al.*, 2009)
FIESC 14-a, *F. equiseti*	Soil	Germany	**NRRL 26419**	GQ505599		(O'Donnell *et al.*, 2009)
	Unknown	Unknown	NRRL 36136	GQ505644		(O'Donnell *et al.*, 2009)
	Soil	Netherlands	NRRL 36321	GQ505647		(O'Donnell *et al.*, 2009)
	Potato peel	Denmark	NRRL 36466	GQ505653		(O'Donnell *et al.*, 2009)

(continues on next page)

Table S2 – (continued).

Species	Host/Substrate	Origin	Collection[a]	*GenBank accession*[b]		References
				EF-1α	***TUB2***	
FIESC 14-b, *F. equiseti*	Beet	Chile	NRRL 20697	GQ505594		(O'Donnell *et al.*, 2009)
FIESC 14-c, *F. equiseti*	Dog	USA	NRRL 43636	GQ505663		(O'Donnell *et al.*, 2009)
FIESC 15-a	Human sputum	USA	NRRL 32175	GQ505609		(O'Donnell *et al.*, 2009)
	Human eye	USA	NRRL 34006	GQ505630		(O'Donnell *et al.*, 2009)
	Human sputum	USA	NRRL 34007	GQ505631		(O'Donnell *et al.*, 2009)
	Human sputum	USA	NRRL 34011	GQ505634		(O'Donnell *et al.*, 2009)
	Human finger	USA	NRRL 43619	GQ505659		(O'Donnell *et al.*, 2009)
FIESC 15-b	Human blood	USA	NRRL 32182	GQ505611		(O'Donnell *et al.*, 2009)
FIESC 15-c	Human lung	USA	NRRL 31160	GQ505607		(O'Donnell *et al.*, 2009)
	Human cancer patient	USA	NRRL 32869	GQ505618		(O'Donnell *et al.*, 2009)
	Human blood	USA	NRRL 32181	GQ505610		(O'Donnell *et al.*, 2009)
	Human ethmoid sinus	USA	NRRL 32994	GQ505621		(O'Donnell *et al.*, 2009)
	Human sinus	USA	NRRL 32995	GQ505622		(O'Donnell *et al.*, 2009)
	Human leg wound	USA	NRRL 32996	GQ505623		(O'Donnell *et al.*, 2009)
	Human maxillary sinus	USA	NRRL 34010	GQ505633		(O'Donnell *et al.*, 2009)
	Human lung	USA	NRRL 43622	GQ505660		(O'Donnell *et al.*, 2009)
FIESC 15-d	Human lung	USA	NRRL 34008	GQ505632		(O'Donnell *et al.*, 2009)
FIESC 15-e	Human toe wound	USA	NRRL 34001	GQ505625		(O'Donnell *et al.*, 2009)
FIESC 15-f	*Zulia pubescens* (adult) [Hemiptera: Cercopidae]	Colombia	NRRL 52697	JF740780		(O'Donnell *et al.*, 2012)
	Plutella xylostella (larva) [Lepidoptera: Plutellidae]	Benin	NRRL 52784	JF740852		(O'Donnell *et al.*, 2012)
FIESC 16-a	Human BAL	USA	NRRL 34004	GQ505628		(O'Donnell *et al.*, 2009)
FIESC 16-b	Human bronchial wash	USA	NRRL 34056	GQ505640		(O'Donnell *et al.*, 2009)
FIESC 16-c	Human blood	USA	NRRL 34059	GQ505641		(O'Donnell *et al.*, 2009)
	Contact lenses	USA	NRRL 43730	GQ505669		(O'Donnell *et al.*, 2009)

(continues on next page)

Table S2 – (continued).

Species	Host/Substrate	Origin	Collection[a]	*GenBank accession*[b]		References
				EF-1α	***TUB2***	
FIESC 16-d	*Heteropsylla incisa* [Hemiptera: Psyllidae]	Papua New Guinea	NRRL 25134	JF740755		(O'Donnell *et al.*, 2012)
	Heteropsylla incisa [Hemiptera: Psyllidae]	Papua New Guinea	NRRL 25135	JF740756		(O'Donnell *et al.*, 2012)
FIESC 16-e	*Heteropsylla cubana* [Hemiptera: Psyllidae]	Papua New Guinea	NRRL 52756	JF740831		(O'Donnell *et al.*, 2012)
FIESC 17-a	Human	USA	NRRL 32864	GQ505613		(O'Donnell *et al.*, 2009)
FIESC 17-b	Banana	Congo	NRRL 36548	GQ505655		(O'Donnell *et al.*, 2009)
FIESC 17-c	Tortoise	USA	NRRL 34070	GQ505642		(O'Donnell *et al.*, 2009)
FIESC 17-d	*Aphis fabae* [Hemiptera: Aphididae]	India	NRRL 52737	JF740814		(O'Donnell *et al.*, 2012)
	Aphis fabae [Hemiptera: Aphididae]	India	NRRL 52745	JF740821		(O'Donnell *et al.*, 2012)
	Aphis fabae [Hemiptera: Aphididae]	India	NRRL 52747	JF740823		(O'Donnell *et al.*, 2012)
FIESC 17-e	*Aphis gossypii* [Hemiptera: Aphididae]	India	NRRL 52796	JF740864		(O'Donnell *et al.*, 2012)
FIESC 18-a	Human sputum	USA	NRRL 31167	GQ505608		(O'Donnell *et al.*, 2009)
FIESC 18-b	Human diabetic cellulitis	USA	NRRL 32522	GQ505612		(O'Donnell *et al.*, 2009)
FIESC 19-a	Manatee	USA	NRRL 43639	GQ505666		(O'Donnell *et al.*, 2009)
FIESC 20-a	Human sputum	USA	NRRL 34003	GQ505627		(O'Donnell *et al.*, 2009)
FIESC 20-b	*Juniperus chinensis* (leaf)	Hawaii	NRRL 36575	GQ505656		(O'Donnell *et al.*, 2009)
FIESC 20-c	*Bemisia* (nymph) [Hemiptera: Aleyrodidae]	USA	NRRL 25106	JF740732		(O'Donnell *et al.*, 2012)
	Bemisia (nymph) [Hemiptera: Aleyrodidae]	USA	NRRL 25107	JF740733		(O'Donnell *et al.*, 2012)

(continues on next page)

Table S2 – (continued).

Species	Host/Substrate	Origin	Collection[a]	GenBank accession[b]		References
				EF-1α	*TUB2*	
FIESC 20-c	*Bemisia* (nymph) [Hemiptera: Aleyrodidae]	USA	NRRL 25108	JF740734		(O'Donnell *et al.*, 2012)
	Bemisia (nymph) [Hemiptera: Aleyrodidae]	USA	NRRL 25109	JF740735		(O'Donnell *et al.*, 2012)
	Bemisia (nymph) [Hemiptera: Aleyrodidae]	USA	NRRL 25110	JF740736		(O'Donnell *et al.*, 2012)
	Bemisia (nymph) [Hemiptera: Aleyrodidae]	USA	NRRL 25112	JF740738		(O'Donnell *et al.*, 2012)
FIESC 21-a	Alfafa	Australia	NRRL 13335	GQ505590		(O'Donnell *et al.*, 2009)
FIESC 21-b	Human endocarditis	Brazil	NRRL 32865	GQ505614		(O'Donnell *et al.*, 2009)
FIESC 22-a	Human ethmoid sinus	USA	NRRL 34002	GQ505626		(O'Donnell *et al.*, 2009)
FIESC 23-a	Human cancer patient	USA	NRRL 32866	GQ505615		(O'Donnell *et al.*, 2009)
	Human	USA	NRRL 32867	GQ505616		(O'Donnell *et al.*, 2009)
FIESC 23-b	*Oryza sativa*	India	NRRL 13379	GQ505591		(O'Donnell *et al.*, 2009)
FIESC 23-c	*Adelphocoris* sp. (nymph) [Hemiptera: Miridae]	Italy	NRRL 25081	JF740712		(O'Donnell *et al.*, 2012)
FIESC 24-a	Human intravitreal fluid	USA	NRRL 34005	GQ505629		(O'Donnell *et al.*, 2009)
FIESC 24-b	*Spartina* (rhizomes)	USA	NRRL 43297	GQ505657		(O'Donnell *et al.*, 2009)
FIESC 24-c	*Nilaparvata lugens* [Hemiptera: Delphacidae]	China	NRRL 25080	JF740711		(O'Donnell *et al.*, 2012)
FIESC 24-d	*Eurygaster sp.* [Hemiptera: Scutelleridae]	Syria	NRRL 52777	JF740845		(O'Donnell *et al.*, 2012)
FIESC 25-a	*Oryza sativa*	China	NRRL 22244	GQ505596		(O'Donnell *et al.*, 2009)
FIESC 25-b	Human nasal tissue	USA	NRRL 32993	GQ505620		(O'Donnell *et al.*, 2009)
FIESC 25-c	Human blood	USA	NRRL 32868	GQ505617		(O'Donnell *et al.*, 2009)
FIESC 25-d	*Aphis gossypii* [Hemiptera: Aphididae]	Turkey	NRRL 52774	JF740842		(O'Donnell *et al.*, 2012)

(continues on next page)

Table S2 – (continued).

Species	Host/Substrate	Origin	Collection[a]	*GenBank accession*[b]		References
				EF-1α	***TUB2***	
FIESC 25-e	*Aphis gossypii* [Hemiptera: Aphididae]	Turkey	NRRL 52775	JF740843		(O'Donnell *et al.*, 2012)
FIESC 25-f	*Aphis gossypii* [Hemiptera: Aphididae]	Turkey	NRRL 52776	JF740844		(O'Donnell *et al.*, 2012)
FIESC 26-a	Leaf litter	Cuba	NRRL 26417	GQ505598		(O'Donnell *et al.*, 2009)
FIESC 26-b	*Acacia* sp. (branch)	Costa Rica	NRRL 28714	GQ505604		(O'Donnell *et al.*, 2009)
FIESC 27-a	*Chrysanthemum* sp.	Kenya	NRRL 20722	GQ505595		(O'Donnell *et al.*, 2009)
FIESC 28-a	Grave stone	Romania	NRRL 28577	GQ505603		(O'Donnell *et al.*, 2009)
FIESC 28-b	*Aiolopus longicornis* [Orthoptera: Acrididae]	Ethiopia	NRRL 52711	JF740793		(O'Donnell *et al.*, 2012)
	Aiolopus longicornis [Orthoptera: Acrididae]	Ethiopia	NRRL 52717	JF740799		(O'Donnell *et al.*, 2012)
FIESC 29-a	*Adelphocoris* sp. (nymph) [Hemiptera: Miridae]	Austria	NRRL 25084	JF740715		(O'Donnell *et al.*, 2012)
FIESC 29-b	*Heteropsylla cubana* [Hemiptera: Psyllidae]	Papua New Guinea	NRRL 52765	JF740839		(O'Donnell *et al.*, 2012)
FIESC 30-a	*Prosapia* nr. *bicincta* [Hemiptera: Cercopidae] on *Cynodon*	Costa Rica	NRRL 52758	JF740833		(O'Donnell *et al.*, 2012)
FIESC 31	Wheat	Italy	ITEM 10395	LN901577		(Villani *et al.*, 2016)
FIESC 31-a	Plumbing drain, public park	USA	FRC R10113	JN235494		(Short *et al.*, 2011)
FIESC 32-a	Plumbing drain, public park	USA	FRC R10112	JN235495		(Short *et al.*, 2011)
FIESC 33	*Trichosanthes dioica*	Malawi	ITEM 7155	LN901581		(Villani *et al.*, 2016)
F. concolor	Plant debris	South Africa	NRRL 13459	GQ505674		(O'Donnell *et al.*, 2008)
F. oxysporum	*Pseudotsuga menziesii*	USA	NRRL 22902	AF160312	U34424	(O'Donnell & Cigelnik, 1997; O'Donnell *et al.*, 2000b)
F. pseudograminearum	*Hordeum vulgare*	Australia	NRRL 28062	AF212468		(O'Donnell *et al.*, 2000a)

[a]Collection - Type strains included in analysis are indicated in **bold.** Culture collection abbreviations: ATCC = American Type Culture Collection, Virginia, USA; CML = Coleção Micológica de Lavras, Universidade Federal de Lavras, Minas Gerais, Brazil; CBS = Centraalbureau

voor Schimmelcultures, Utrecht, Netherlands; CPC = Collection of Pedro W. Crous, held at CBS; F = University of Sydney, Sydney, New South Wales, Australia; FRC = Fusarium Research Center, Pennsylvania State University, Pennsylvania, USA; ITEM = ITEM Collection of Institute of Science and Food Protection, Bari, Italy; KSU = Kansas State University, Kansas, USA; LGMF = Laboratório de Genética de Microrganismos, Universidade Federal do Paraná, Paraná, Brazil; MRC = Medical Research Council, Tygerberg, Cape Town, South Africa; MUCL = Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; NRRL= National Center for Agricultural Utilization Research, Peoria, Illinois, USA.

[b]GenBank - *EF-1α*: Translation elongation factor 1-alpha; *TUB2*: Beta-tubulin; N/A: Information or sequence not available

REFERENCES – TABLE S2

Al-Hatmi AMS, Van Den Ende AHGG, Stielow JB *et al.*, 2016a. Evaluation of two novel barcodes for species recognition of opportunistic pathogens in *Fusarium*. *Fungal Biology* **120**, 231–245.

Al-Hatmi AM, Hagen F, Menken SB, Meis JF, De Hoog GS, 2016b. Global molecular epidemiology and genetic diversity of *Fusarium*, a significant emerging group of human opportunists from 1958 to 2015. *Emerging Microbes and Infections* **5**.

Al-Hatmi AMS, Mirabolfathy M, Hagen F *et al.*, 2016c. DNA barcoding, MALDI-TOF, and AFLP data support *Fusarium ficicrescens* as a distinct species within the *Fusarium fujikuroi* species complex. *Fungal Biology* **120**, 265–278.

Edwards J, Auer D, de Alwis S-K *et al.*, 2016. *Fusarium agapanthi* sp. nov., a novel bikaverin and fusarubin-producing leaf and stem spot pathogen of *Agapanthus praecox* (African lily) from Australia and Italy. *Mycologia* **108**, 981–992.

Faria CB, Abe CAL, da Silva CN, Tessmann DJ, Barbosa-Tessmann IP, 2012. New PCR assays for the identification of *Fusarium verticillioides*, *Fusarium subglutinans*, and other species of the *Gibberella fujikuroi* complex. *International Journal of Molecular Sciences* **13**, 115–132.

Funnell-Harris DL, Scully ED, Sattler SE, French RC, O'Neill PM, Pedersen JF, 2017. Differences in *Fusarium* Species in brown midrib Sorghum and in Air Populations in Production Fields. *Phytopathology* **107**, 1353–1363.

Geiser DM, Ivey MLL, Hakiza G, Juba JH, Miller S a, 2005. *Gibberella xylarioides* (anamorph: *Fusarium xylarioides*), a causative agent of coffee wilt disease in Africa, is a previously unrecognized member of the *G. fujikuroi* species complex. *Mycologia* **97**, 191–201.

Herron DA, Wingfield MJ, Wingfield BD, Rodas CA, Marincowitz S, Steenkamp ET, 2015. Novel taxa in the *Fusarium fujikuroi* species complex from *Pinus* spp. *Studies in Mycology* **80**, 131–150.

Van Hove F, Waalwijk C, Logrieco A, Munaut F, Moretti A, 2011. *Gibberella musae* (*Fusarium musae*) sp. nov., a recently discovered species from banana is sister to *F. verticillioides*. *Mycologia* **103**, 570–585.

Jacobs A, Van Wyk PS, Marasas WFO, Wingfield BD, Wingfield MJ, Coutinho TA, 2010. *Fusarium ananatum* sp. nov. in the *Gibberella fujikuroi* species complex from pineapples with fruit rot in South Africa. *Fungal Biology* **114**, 515–527.

Kim JH, Kang MR, Kim HK, Lee SH, Lee T, Yun SH, 2012. Population structure of the *Gibberella fujikuroi* species complex associated with rice and corn in Korea. *Plant Pathology Journal* **28**, 357–363.

Kvas M, Marasas WFO, Wingfield BD, Wingfield MJ, Steenkamp ET, 2009. Diversity and evolution of *Fusarium* species in the *Gibberella fujikuroi* complex. *Fungal Diversity* **34**, 1–21.

Laurence MH, Walsh JL, Shuttleworth LA *et al.*, 2016. Six novel species of *Fusarium* from natural ecosystems in Australia. *Fungal Diversity* **77**, 349–366.

Lima CS, Pfenning LH, Costa SS, Abreu LM, Leslie JF, 2012. *Fusarium tupiense* sp. nov., a member of the *Gibberella fujikuroi* complex that causes mango malformation in Brazil. *Mycologia* **104**, 1408–19.

Moussa TAA, Al-Zahrani HS, Kadasa NMS, Ahmed SA, de Hoog GS, Al-Hatmi AMS, 2017. Two new species of the *Fusarium fujikuroi* species complex isolated from the natural environment. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology* **110**, 819–832.

O'Donnell K, Cigelnik E, 1997. Two Divergent Intragenomic rDNA ITS2 Types within a Monophyletic Lineage of the Fungus *Fusarium* Are Nonorthologous. *Molecular Phylogenetics and Evolution* **7**, 103–116.

O'Donnell KO, Cigelnik E, Nirenberg HI, 1998. Molecular Systematics and Phylogeography of the *Gibberella fujikuroi* Species Complex. *Mycologia* **90**, 465–493.

O'Donnell K, Humber RA, Geiser DM *et al.*, 2012. Phylogenetic diversity of insecticolous fusaria inferred from multilocus DNA sequence data and their molecular identification via FUSARIUM-ID and *Fusarium MLST*. *Mycologia* **104**, 427–445.

O'Donnell K, Kistler HC, Tacke BK, Casper HH, 2000a. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. *Proceedings of the National Academy of Sciences* **97**, 7905–7910.

O'Donnell K, Nirenberg H, Aoki T, Cigelnik E, 2000b. A multigene phylogeny of the *Gibberella fujikuroi* species complex: detection of additional phylogenetically distinct species. *Mycoscience* **41**, 61–78.

O'Donnell K, Sutton DA, Rinaldi MG, Gueidan C, Crous PW, Geiser DM, 2009. Novel multilocus sequence typing scheme reveals high genetic diversity of human pathogenic members of the *Fusarium incarnatum-F. equiseti* and *F. chlamydosporum* species complexes within the United States. *Journal of Clinical Microbiology* **47**, 3851–3861.

O'Donnell K, Ward TJ, Aberra D *et al.*, 2008. Multilocus genotyping and molecular phylogenetics resolve a novel head blight pathogen within the *Fusarium graminearum* species complex from Ethiopia. *Fungal Genetics and Biology* **45**, 1514–1522.

O'Donnell K, Ward TJ, Geiser DM, Kistler HC, Aoki T, 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. *Fungal Genetics and Biology* **41**, 600–623.

Otero-Colina G, Rodríguez-Alvarado G, Fernández-Pavía S *et al.*, 2010. Identification and characterization of a novel etiological agent of mango malformation disease in Mexico, *Fusarium mexicanum* sp. nov. *Phytopathology* **100**, 1176–1184.

Proctor RH, Van Hove F, Susca A *et al.*, 2013. Birth, death and horizontal transfer of the fumonisin biosynthetic gene cluster during the evolutionary diversification of *Fusarium*. *Molecular Microbiology* **90**, 290–306.

Sandoval-Denis M, Guarnaccia V, Polizzi G, Crous PW, 2018a. Symptomatic Citrus trees reveal a new pathogenic lineage in *Fusarium* and two new *Neocosmospora* species. *Persoonia - Molecular Phylogeny and Evolution of Fungi* **40**, 1–25.

Sandoval-Denis M, Swart WJ, Crous PW, 2018b. New *Fusarium* species from the Kruger National Park, South Africa. *MycoKeys* **34**, 63–92.

Sarver BAJ, Ward TJ, Gale LR *et al.*, 2011. Novel *Fusarium* head blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. *Fungal Genetics and Biology* **48**, 1096–1107.

Scauflaire J, Gourgue M, Munaut F, 2011. *Fusarium temperatum* sp. nov. from maize, an emergent species closely related to *Fusarium subglutinans*. *Mycologia* **103**, 586–97.

Secor GA, Rivera-Varas V, Christ DS *et al.*, 2014. Characterization of *Fusarium secorum*, a new species causing *Fusarium* yellowing decline ofsugar beet in north central USA. *Fungal Biology* **118**, 764–775.

Short DPG, O'Donnell K, Zhang N, Juba JH, Geiser DM, 2011. Widespread occurrence of diverse human pathogenic types of the fungus *Fusarium* detected in plumbing drains. *Journal of Clinical Microbiology* **49**, 4264–4272.

Starkey DE, Ward TJ, Aoki T *et al.*, 2007. Global molecular surveillance reveals novel *Fusarium* head blight species and trichothecene toxin diversity. *Fungal Genetics and Biology* **44**, 1191–1204.

Villani A, Moretti A, De Saeger S *et al.*, 2016. A polyphasic approach for characterization of a collection of cereal isolates of the *Fusarium incarnatum-equiseti* species complex. *International Journal of Food Microbiology* **234**, 24–35.

Walsh JL, Laurence MH, Liew ECY *et al.*, 2010. *Fusarium*: Two endophytic novel species from tropical grasses of northern Australia. *Fungal Diversity* **44**, 149–159.

Ward TJ, Bielawski JP, Kistler HC, Sullivan E, O'Donnell K, 2002. Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic Fusarium. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 9278–83.

Watanabe M, Yonezawa T, Lee K *et al.*, 2011. Molecular phylogeny of the higher and lower taxonomy of the *Fusarium* genus and differences in the evolutionary histories of multiple genes. *BMC Evolutionary Biology* **11**, 322.

Zeller K a, Summerell B a, Bullock S, Leslie JF, 2003. *Gibberella konza* (*Fusarium konzum*) sp. nov. from prairie grasses, a new species in the *Gibberella fujikuroi* species complex. *Mycologia* **95**, 943–954.

Zhang H, Brankovics B, Luo W *et al.*, 2016. Crops are a main driver for species diversity and the toxigenic potential of *Fusarium* isolates in maize ears in China. *World Mycotoxin Journal* **9**, 701–715.

CONSIDERAÇÕES FINAIS

A partir de uma amostra reduzida proveniente de uma pequena área geográfica, diferentes espécies de *Fusarium* foram encontradas associadas a espigas e colmos de milhos, algumas delas ainda não descritas associadas a milho (*F. tjaetaba,* FIESC 12, FIESC 20, FIESC 33), outras com relatos esporádicos (*F. fujikuroi, F. napiforme)* e inclusive uma espécie nova, descrita neste trabalho como *F. awaxy.* Tal composição de espécies sugere que a diversidade de *Fusarium* associado a milho ainda é subestimada.

Para todas as espécies isoladas pertencentes ao complexo *Fusarium fujikuroi* foram obtidos isolados positivos para a presença do gene FUM1, envolvido na produção de fumonisinas. Para alguns destes isolados positivos foi detectada a produção em ensaio de ELISA, incluindo isolados obtidos de espigas, o que sugere potenciais riscos associados à ingestão de materiais contaminados pela presença de tais espécies.

Além das espécies já descritas como patogênicas (*F. graminearum, F. meridionale, F. verticillioides)* outra espécie causou sintomas de podridão de colmo em níveis semelhantes (*F. fujikuroi*) e outras causaram sintomas menos severos (*F. awaxy, F. tjaetaba*), sugerindo a possibilidade de associação com o desenvolvimento de podridão de colmo no estado do Paraná. Apenas uma das espécies encontradas (*F. napiforme*) não foi capaz de causar sintomas no híbrido testado.

Como perspectivas, sugerem-se estudos mais amplos de distribuição, patogenicidade, agressividade, produção e detecção de toxinas. Tais estudos permitiriam caracterizar e compreender melhor o papel das espécies encontradas, e estabelecer o seu real impacto para a cultura do milho e para a saúde humana.

REFERÊNCIAS

ABD-ELMAGID, A. et al. Discriminatory simplex and multiplex PCR for four species of the genus *Sclerotinia*. **Journal of Microbiological Methods**, v. 92, p. 293-300, 2013.

AL-HATMI, A. M.; HAGEN, F.; MENKEN, S. B.; MEIS, J. F.; DE HOOG, G. S. Global molecular epidemiology and genetic diversity of *Fusarium*, a significant emerging group of human opportunists from 1958 to 2015. **Emerging Microbes and Infections**, v. 5, n. 12, 2016. Nature Publishing Group. Disponível em: <http://dx.doi.org/10.1038/emi.2016.126>.

AL-HATMI, A. M. S.; MIRABOLFATHY, M.; HAGEN, F.; et al. DNA barcoding, MALDI-TOF, and AFLP data support *Fusarium ficicrescens* as a distinct species within the *Fusarium fujikuroi* species complex. **Fungal Biology**, v. 120, n. 2, p. 265–278, 2016.

AL-HATMI, A. M. S.; VAN DEN ENDE, A. H. G. G.; STIELOW, J. B.; et al. Evaluation of two novel barcodes for species recognition of opportunistic pathogens in *Fusarium*. **Fungal Biology**, v. 120, n. 2, p. 231–245, 2016.

ANFOSSI, L.; GIOVANNOLI, C.; BAGGIANI, C. Mycotoxin detection. **Current Opinion in Biotechnology**, v. 37, p. 120–126, 2016.

AOKI, T.; O'DONNELL, K.; GEISER, D. M. Systematics of key phytopathogenic *Fusarium* species: Current status and future challenges. **Journal of General Plant Pathology**, v. 80, n. 3, p. 189–201, 2014.

ASTOLFI, P.; DOS SANTOS, J.; SCHNEIDER, L.; et al. Molecular survey of trichothecene genotypes of *Fusarium graminearum* species complex from barley in Southern Brazil. **International Journal of Food Microbiology**, v. 148, n. 3, p. 197–201, 2011.

BASLER, R. Diversity of *Fusarium* species isolated from UK forage maize and the population structure of *F. graminearum* from maize and wheat. **PeerJ**, v. 4, p. e2143, 2016.

BLUHM, B. H.; COUSIN, M. A.; WOLOSHUK, C. P. Multiplex real-time PCR detection of fumonisin-producing and trichothecene-producing groups of *Fusarium* species. **Journal of food protection**, v. 67, n. 3, p. 536–43, 2004.

BOLTON, S. L.; BRANNEN, P. M.; GLENN, A. E. A novel population of *Fusarium fujikuroi* isolated from southeastern U.S. winegrapes reveals the need to re-evaluate the species' fumonisin production. **Toxins**, v. 8, n. 9, p. 1–14, 2016.

BRASIL. Agência Nacional de Vigilância Sanitária – ANVISA. Resolução RDC n. 07, de 18 de fevereiro de 2011. Regulamento Técnico sobre limites máximos tolerados (LMT) para micotoxinas em alimentos. **Diário Oficial [da] República Federativa do Brasil**, Brasília, DF, 09 abr. 2011.

CARBONE, I.; KOHN, L. M. A Method for Designing Primer Sets for Speciation Studies in Filamentous Ascomycetes. **Mycologia**, v. 91, n. 3, p. 553–556, 1999.

CASTAÑARES, E.; DINOLFO, M. I.; DEL PONTE, E. M.; PAN, D.; STENGLEIN, S. A. Species composition and genetic structure of *Fusarium graminearum* species complex populations affecting the main barley growing regions of South America. **Plant Pathology**, v. 65, n. 6, p. 930–939, 2016.

CHELI, F.; BATTAGLIA, D.; GALLO, R.; DELL'ORTO, V. EU legislation on cereal safety: An update with a focus on mycotoxins. **Food Control**, v. 37, n. 1, p. 315–325, 2014.

CLAYTON, W.D. et al. **GrassBase - The Online World Grass Flora - *Zea mays*.** Disponível em <https://www.kew.org/data/grasses-db/www/imp10873.htm>. Acesso em 02 abr. 2018

COMPANHIA NACIONAL DE ABASTECIMENTO (CONAB). **Acompanhamento da safra brasileira de grãos**. Brasília, 2018. Disponível em < http://www.conab.gov.br/OlalaCMS/uploads/arquivos/18_03_13_14_15_33_grao_marco_2018.pdf>. Acesso em 02 abr. 2018.

COSTA, S. S.; MOREIRA, G. M.; PFENNING, L. H. Development of a PCR protocol for the identification and detection of *Fusarium solani* f. sp. piperis from soil and roots of black pepper (*Piper nigrum*). **Tropical Plant Pathology**, v. 42, n. 1, p. 55–59, 2017.

COSTA, R. V. DA; SILVA, D. D. DA; COTA, L. V.; et al. Incidência de *Colletotrichum graminicola* em colmos de genótipos de milho. **Summa Phytopathologica**, v. 36, n. 2, p. 122–128, 2010.

COSTA, R. V.; SILVA, D. D.; COTA, L. V.; et al. **Reação de Híbridos de Milho às Podridões da Base do Colmo**. Minas Gerais, 2017. Disponível em <https://ainfo.cnptia.embrapa.br/digital/bitstream/item/171997/1/circ-231.pdf>. Acesso em 23 jul. 2018. Circular técnica.

DARRIBA, D.; TABOADA, G. L.; DOALLO, R.; POSADA, D. jModelTest 2 : more models, new heuristics and high- performance computing. **Nature Methods**, v. 9, n. 8, p. 6–9, 2012.

DEPARTAMENTO DE ECONOMIA RURAL (DERAL), SECRETARIA DA AGRICULTURA E ABASTECIMENTO DO PARANÁ (SEAB). **MILHO – SAFRA 2014/2015**. Paraná, 2014. Disponível em <http://www.agricultura.pr.gov.br/arquivos/File/deral/Prognosticos/milho_2014_15.pdf>. Acesso em 02 abr. 2018. Relatório técnico.

DEPARTAMENTO DE ECONOMIA RURAL (DERAL), SECRETARIA DA AGRICULTURA E ABASTECIMENTO DO PARANÁ (SEAB). **Estimativa de Safra.** Paraná, 2018a. Disponível em <http://www.agricultura.pr.gov.br/arquivos/File/deral/pss.xls>. Acesso em 02 abr. 2018.

DEPARTAMENTO DE ECONOMIA RURAL (DERAL), SECRETARIA DA AGRICULTURA E ABASTECIMENTO DO PARANÁ (SEAB). **MILHO – Análise da Conjuntura**. Paraná, 2018b. Disponível em <http://www.agricultura.pr.gov.br/modules/conteudo/conteudo.php?conteudo=240>. Acesso em 02 abr. 2018. Relatório técnico.

DEAN, R.; VAN KAN, J. A. L.; PRETORIUS, Z. A.; et al. The Top 10 fungal pathogens in molecular plant pathology. **Molecular Plant Pathology**, v. 13, n. 4, p. 414–430, 2012.

DEL PONTE, E. M.; SPOLTI, P.; WARD, T.; et al. Regional and field-specific factors affect the composition of *Fusarium* head blight pathogens in subtropical no-till wheat agroecosystem of Brazil. **Phytopathology**, v. 105, p. 246–254, 2015.

DEL PONTE, E. M. et al. Species Identification, Genetic Diversity and Phenotypic Variation Studies on the *Fusarium graminearum* Complex Populations from Brazil. In: **Fusarium Head Blight in Latin America**. Dordrecht: Springer Netherlands, 2013. p. 15–29.

DE WIT, P. J. G. M. *Cladosporium fulvum* Effectors: Weapons in the Arms Race with Tomato. **Annual Review in Phytopathology**, v. 54, p. 1-23
DO CARMO, A. L. M. ***Fusarium*** **em sementes de Pinus no brasil: distribuição, patogenicidade, identificação de espécies e controle biológico.** 89f. Tese (Doutorado em Agronomia - Produção Vegetal), Universidade Federal do Paraná, Curitiba, 2017.

ECKERT, C.T. et al. Maize ethanol production in Brazil: Characteristics and perspectives. **Renewable and Sustainable Energy Reviews**, v. 82, n. 3, p. 3907-3912, 2018.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. **Cultivo do Milho – Doenças.** 2010a. Disponível em <http://www.cnpms.embrapa.br/publicacoes/milho_6_ed/doencas.htm>. Acesso em 12 set. 2016.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. **Cultivo do Milho – Plantio.** 2010b. Disponível em <http://www.cnpms.embrapa.br/publicacoes/milho_6_ed/plantio.htm>. Acesso em 01 set. 2016.

EDWARDS, J.; AUER, D.; DE ALWIS, S.-K.; et al. *Fusarium agapanthi* sp. nov., a novel bikaverin and fusarubin-producing leaf and stem spot pathogen of *Agapanthus praecox* (African lily) from Australia and Italy. **Mycologia**, v. 108, n. 5, p. 981–992, 2016.

FARIA, C. B.; ABE, C. A. L.; DA SILVA, C. N.; TESSMANN, D. J.; BARBOSA-TESSMANN, I. P. New PCR assays for the identification of *Fusarium verticillioides*, *Fusarium subglutinans*, and other species of the *Gibberella fujikuroi* complex. **International Journal of Molecular Sciences**, v. 13, n. 1, p. 115–132, 2012.

FISHER, N. L.; BURGESS, W.; TOUSSOUN, A.; NELSON, P. E. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. **Phytopathology**, 1982.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO). **FAOSTAT**. Disponível em <http://faostat3.fao.org/>. Acesso em 02 abr. 2018.

FUNNELL-HARRIS, D. L.; SCULLY, E. D.; SATTLER, S. E.; et al. Differences in *Fusarium* Species in brown midrib Sorghum and in Air Populations in Production Fields. **Phytopathology**, v. 107, n. 11, p. 1353–1363, 2017.

GEISER, D. M.; AOKI, T.; BACON, C. W.; et al. One Fungus, One Name: Defining the Genus *Fusarium* in a Scientifically Robust Way That Preserves Longstanding Use. **Phytopathology**, v. 103, n. 5, p. 400–408, 2013.

GEISER, D. M.; GASCO-JIMENEZ, M. DEL M.; KANG, S.; et al. FUSARIUM-ID v. 1.0 : A DNA sequence database for identifying *Fusarium*. **European Journal of Plant Pathology**, v. 110, p. 473–479, 2004.

GEISER, D. M.; IVEY, M. L. L.; HAKIZA, G.; JUBA, J. H.; MILLER, S. A. *Gibberella xylarioides* (anamorph: *Fusarium xylarioides*), a causative agent of coffee wilt disease in Africa, is a previously unrecognized member of the *G. fujikuroi* species complex. **Mycologia**, v. 97, n. 1, p. 191–201, 2005.

GOMES, L. B.; WARD, T. J.; BADIALE-FURLONG, E.; DEL PONTE, E. M. Species composition, toxigenic potential and pathogenicity of *Fusarium graminearum* species complex isolates from southern Brazilian rice. **Plant Pathology**, v. 64, n. 4, p. 980–987, 2015.

GOSWAMI, R. S.; KISTLER, H. C. Heading for disaster: *Fusarium graminearum* on cereal crops. **Molecular Plant Pathology**, v. 5, n. 6, p. 515–525, 2004.

GRÄFENHAN, T.; SCHROERS, H. J.; NIRENBERG, H. I.; SEIFERT, K. A. An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in *Cosmospora*, *Acremonium*, *Fusarium*, *Stilbella*, and *Volutella*. **Studies in Mycology**, v. 68, p. 79–113, 2011.

GUINDON, S.; GASCUEL, O. A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. **Systematic Biology**, v. 52, n. 5, p. 696–704, 2003.

HAWKSWORTH, D. L.; CROUS, P. W.; REDHEAD, S. A. et al. The Amsterdam Declaration on Fungal Nomenclature. **IMA Fungus.** V. 2, n. 1, p. 105-112, 2011.

HERRON, D. A.; WINGFIELD, M. J.; WINGFIELD, B. D.; et al. Novel taxa in the *Fusarium fujikuroi* species complex from *Pinus* spp. **Studies in Mycology**, v. 80, n. 2012, p. 131–150, 2015.

HSUAN, H. M.; SALLEH, B.; ZAKARIA, L. Molecular identificationn of *Fusarium* species in *Gibberella fujikuroi* species complex from rice, sugarcane and maize from Peninsular Malaysia. **International Journal of Molecular Sciences**, v. 12, n. 10, p. 6722–6732, 2011.

HUSON, D. H.; BRYANT, D. Application of phylogenetic networks in evolutionary studies. **Molecular Biology and Evolution**, v. 23, n. 2, p. 254–267, 2006.

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. **Some traditional herb medicines, some mycotoxins, naphtalene and styrene – IARC monographs on the evaluation of carcinogenic risks to humans.** IARCPress, 2002.

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. **Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins – IARC monographs on the evaluation of carcinogenic risks to humans.** IARC, 1993.

IOWA STATE UNIVERSITY. **Stalk and Ear Rots Prevalent in Iowa.** Disponível em <http://crops.extension.iastate.edu/cropnews/2009/10/stalk-and-ear-rots-prevalent-iowa>. Acesso em 23 set. 2016.

JACOBS, A.; VAN WYK, P. S.; MARASAS, W. F. O.; et al. *Fusarium ananatum* sp. nov. in the *Gibberella fujikuroi* species complex from pineapples with fruit rot in South Africa. **Fungal Biology**, v. 114, n. 7, p. 515–527, 2010.

JOHNSON, M.; ZARETSKAYA, I.; RAYTSELIS, Y.; et al. NCBI BLAST: a better web interface. **Nucleic acids research**, v. 36, n. Web Server issue, p. 5–9, 2008.

KATOH, K.; STANDLEY, D. M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. **Molecular Biology and Evolution**, v. 30, n. 4, p. 772–780, 2013.

KAZAN, K.; GARDINER, D. M.; MANNERS, J. M. On the trail of a cereal killer: Recent advances in *Fusarium graminearum* pathogenomics and host resistance. **Molecular Plant Pathology**, v. 13, n. 4, p. 399–413, 2012.

KIM, J. H.; KANG, M. R.; KIM, H. K.; et al. Population structure of the *Gibberella fujikuroi* species complex associated with rice and corn in Korea. **Plant Pathology Journal**, v. 28, n. 4, p. 357–363, 2012.

KÜHNEM, P. R.; SPOLTI, P.; DEL PONTE, E. M.; CUMMINGS, J. A.; BERGSTROM, G. C. Trichothecene Genotype Composition of *Fusarium* graminearum not differentiated among isolates from maize stubble, maize ears, wheat spikes and the atmosphere in New York. **Phytopathology**, v. 105, n. 5, p. 695-699, 2015.

KUHNEM, P. R.; WARD, T. J.; SILVA, C. N.; et al. Composition and toxigenic potential of the *Fusarium graminearum* species complex from maize ears, stalks and stubble in Brazil. **Plant Pathology**, v. 65, n. 7, p. 1185–1191, 2016.

KUMAR, S.; STECHER, G.; TAMURA, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. **Molecular biology and evolution**, v. 33, n. 7, p. 1870–1874, 2016.

KVAS, M.; MARASAS, W. F. O.; WINGFIELD, B. D.; WINGFIELD, M. J.; STEENKAMP, E. T. Diversity and evolution of *Fusarium* species in the *Gibberella fujikuroi* complex. **Fungal Diversity**, v. 34, p. 1–21, 2009.

LAURENCE, M. H.; WALSH, J. L.; SHUTTLEWORTH, L. A.; et al. Six novel species of *Fusarium* from natural ecosystems in Australia. **Fungal Diversity**, v. 77, n. 1, p. 349–366, 2016.

LESLIE, J. F.; SUMMERELL, B. A. **Fusarium Laboratory Manual**. Blackwell Publishing, 2006.

LIMA, C. S.; PFENNING, L. H.; COSTA, S. S.; ABREU, L. M.; LESLIE, J. F. *Fusarium tupiense* sp. nov., a member of the *Gibberella fujikuroi* complex that causes mango malformation in Brazil. **Mycologia**, v. 104, n. 6, p. 1408–19, 2012.

LOMBARD, L.; VAN DER MERWE, N. A.; GROENEWALD, J. Z.; CROUS, P. W. Generic concepts in Nectriaceae. **Studies in Mycology**, v. 80, p. 189–245, 2015.

MA, L.-J.; GEISER, D. M.; PROCTOR, R. H.; et al. *Fusarium* Pathogenomics. **Annual Review of Microbiology**, v. 67, n. 1, p. 399–416, 2013. Disponível em: <http://www.annualreviews.org/doi/10.1146/annurev-micro-092412-155650>.

MARASAS, W. F. O.; RABIE, C. J.; LÜBBEN, A.; et al. *Fusarium napiforme*, a new species from millet and sorghum in southern Africa. **Mycologia**, v. 79, n. 6, p. 910–914, 1987.

MCCARTNEY, H. A. et al. Molecular diagnostics for fungal plant pathogens. **Pest Management Science**, v. 59, n. 2, p. 129–142, 2003.

MILLER, M. A.; PFEIFFER, W.; SCHWARTZ, T. The CIPRES science gateway. **Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment on Bridging from the eXtreme to the campus and beyond - XSEDE '12**, p. 1, 2012.

MOHAMMADI, A.; SHAMS-GHAHFAROKHI, M.; NAZARIAN-FIROUZABADI, F.; et al. *Giberella fujikuroi* species complex isolated from maize and wheat in Iran: Distribution, molecular identification and fumonisin B1 in vitro biosynthesis. **Journal of the Science of Food and Agriculture**, v. 96, n. 4, p. 1333–1340, 2016.

MORALES-RODRÍGUEZ, I.; DE YAÑEZ-MORALES, M. J.; SILVA-ROJAS, H. V.; GARCÍA-DE-LOS-SANTOS, G.; GUZMÁN-DE-PEÑA, D. A. Biodiversity of *Fusarium* species in Mexico associated with ear rot in maize, and their identification using a phylogenetic approach. **Mycopathologia**, v. 163, n. 1, p. 31–39, 2007.

MOUSSA, T. A. A.; AL-ZAHRANI, H. S.; KADASA, N. M. S.; et al. Two new species of the *Fusarium fujikuroi* species complex isolated from the natural environment. **Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology**, v. 110, n. 6, p. 819–832, 2017. Springer International Publishing.

MULÈ, G. et al. A species-specific PCR assay based on the calmodulin partial gene for identification of *Fusarium verticillioides*, *F. proliferatum* and *F. subglutinans*. **European Journal of Plant Pathology**, v. 110, n. 5–6, p. 495–502, 2004.

MURILLO, I.; CAVALLARIN, L.; SAN SEGUNDO, B. The development of a rapid PCR assay for detection of *Fusarium moniliforme*. **European Journal of Plant Pathology**, v. 104, n. 3, p. 301–311, 1998.

NELSON, P. E.; DIGNANI, M. C.; ANAISSIE, E. J. Taxonomy, biology, and clinical aspects of *Fusarium* species. **Clinical microbiology reviews**, v. 7, n. 4, p. 479–504, 1994.

NELSON, P. E.; PLATTNER, R. D.; SHACKELFORD, D. D.; DESJARDINS, A. E. Fumonisin B1 production by *Fusarium* species other than *F. moniliforme* in Section Liseola and by some related species. **Applied Environmental Microbiology**, v. 58, n. 1856, p. 984–989, 1992.

NIRENBERG, H. I. Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium*-Section Liseola. **Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft.**, v. 169, p. 1–117, 1976.

O'DONNELL, K.; CIGELNIK, E. Two Divergent Intragenomic rDNA ITS2 Types within a Monophyletic Lineage of the Fungus *Fusarium* Are Nonorthologous. **Molecular Phylogenetics and Evolution**, v. 7, n. 1, p. 103–116, 1997.

O'DONNELL, K. O.; CIGELNIK, E.; NIRENBERG, H. I. Molecular Systematics and Phylogeography of the *Gibberella fujikuroi* Species Complex. **Mycologia**, v. 90, n. 3, p. 465–493, 1998.

O'DONNELL, K.; HUMBER, R. A.; GEISER, D. M.; et al. Phylogenetic diversity of insecticolous fusaria inferred from multilocus DNA sequence data and their molecular identification via FUSARIUM-ID and *Fusarium MLST*. **Mycologia**, v. 104, n. 2, p. 427–445, 2012.

O'DONNELL, K.; KISTLER, H. C.; CIGELNIK, E.; PLOETZ, R. C. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. **Proceedings of the National Academy of Sciences of the United States of America**, v. 95, n. 5, p. 2044–2049, 1998.

O'DONNELL, K.; KISTLER, H. C.; TACKE, B. K.; CASPER, H. H. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. **Proceedings of the National Academy of Sciences**, v. 97, n. 14, p. 7905–7910, 2000..

O'DONNELL, K.; NIRENBERG, H.; AOKI, T.; CIGELNIK, E. A multigene phylogeny of the *Gibberella fujikuroi* species complex: detection of additional phylogenetically distinct species. **Mycoscience**, v. 41, n. 1, p. 61–78, 2000.

O'DONNELL, K.; SUTTON, D. A.; RINALDI, M. G.; et al. Novel multilocus sequence typing scheme reveals high genetic diversity of human pathogenic members of the *Fusarium incarnatum-F. equiseti* and *F. chlamydosporum* species complexes within the United States. **Journal of Clinical Microbiology**, v. 47, n. 12, p. 3851–3861, 2009.

O'DONNELL, K.; ROONEY, A. P.; PROCTOR, R. H.; et al. Phylogenetic analyses of RPB1 and RPB2 support a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. **Fungal Genetics and Biology**, v. 52, p. 20–31, 2013.

O'DONNELL, K.; SUTTON, D. A.; FOTHERGILL, A.; et al. Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the *Fusarium solani* Species complex. **Journal of Clinical Microbiology**, v. 46, n. 8, p. 2477–2490, 2008.

O'DONNELL, K.; SUTTON, D. A.; RINALDI, M. G.; et al. Internet-accessible DNA sequence database for identifying fusaria from human and animal infections. **Journal of Clinical Microbiology**, v. 48, n. 10, p. 3708–3718, 2010.

O'DONNELL, K.; WARD, T. J.; GEISER, D. M.; KISTLER, H. C.; AOKI, T. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. **Fungal Genetics and Biology**, v. 41, n. 6, p. 600–623, 2004.

O'DONNELL, K.; WARD, T. J.; ABERRA, D.; et al. Multilocus genotyping and molecular phylogenetics resolve a novel head blight pathogen within the *Fusarium graminearum* species complex from Ethiopia. **Fungal Genetics and Biology**, v. 45, n. 11, p. 1514–1522, 2008.

O'DONNELL, K.; WARD, T. J.; ROBERT, V. A. R. G.; et al. DNA sequence-based identification of *Fusarium*: Current status and future directions. **Phytoparasitica**, v. 43, n. 5, p. 583–595, 2015.

OTERO-COLINA, G.; RODRÍGUEZ-ALVARADO, G.; FERNÁNDEZ-PAVÍA, S.; et al. Identification and characterization of a novel etiological agent of mango malformation disease in Mexico, *Fusarium mexicanum* sp. nov. **Phytopathology**, v. 100, n. 2, p. 1176–1184, 2010.

OWCZARZY, R.; TATAUROV, A. V.; WU, Y.; et al. IDT SciTools: a suite for analysis and design of nucleic acid oligomers. **Nucleic acids research**, v. 36, n. Web Server issue, p. 163–169, 2008.

PATIÑO, B. MIRETE, S.; GONZÁLEZ-JÁEN, M. T.; MULÉ, G.; RODRÍGUEZ, M. T.; VÁZQUEZ, C. PCR Detection Assay of Fumonisin-Producing *Fusarium verticillioides* Strains. **Journal of Food Protection**, v. 67, n. 6, p. 1278-1283, 2004.

PIONEER. ***Gibberella* Ear Rot.** 2016. Disponível em <https://www.pioneer.com/home/site/us/agronomy/crop-management/corn-insect-disease/gibberella-ear-rot/>. Acesso em 23 set. 2016.

PREMIER BIOSOFT. **PCR Primer Design Guidelines.** 2018. Disponível em <http://www.premierbiosoft.com/tech_notes/PCR_Primer_Design.html>. Acesso em 10 set. 2017.

PROCTOR, R. H.; VAN HOVE, F.; SUSCA, A.; et al. Birth, death and horizontal transfer of the fumonisin biosynthetic gene cluster during the evolutionary diversification of *Fusarium*. **Molecular Microbiology**, 2013.

QUAEDVLIEG, W.; BINDER, M.; GROENEWALD, J. Z.; et al. Introducing the consolidated species concept to resolve species in the teratosphaeriaceae. **Persoonia: Molecular Phylogeny and Evolution of Fungi**, v. 33, p. 1–40, 2014.

QUARTA, A.; MITA, G.; HAIDUKOWSKI, M.; et al. Multiplex PCR assay for the identification of nivalenol, 3- and 15-acetyl-deoxynivalenol chemotypes in *Fusarium*. **FEMS Microbiology Letters**, v. 259, n. 1, p. 7–13, 2006.

RAEDER, U.; BRODA, P. Rapid preparation of DNA from filamentous fungi. **Letters in Applied Microbiology**, v. 1, n. 1, p. 17–20, 1985.

RONQUIST, F.; TESLENKO, M.; VAN DER MARK, P.; et al. MrBayes 3.2: Efficient Bayesian Phylogenetic inference and model choice across a large model space. **Systematic Biology**, v. 61, n. 3, p. 539–542, 2012.

SANDOVAL-DENIS, M.; GUARNACCIA, V.; POLIZZI, G.; CROUS, P. W. Symptomatic Citrus trees reveal a new pathogenic lineage in *Fusarium* and two new *Neocosmospora* species. **Persoonia - Molecular Phylogeny and Evolution of Fungi**, v. 40, p. 1–25, 2018.

SARVER, B. A. J.; WARD, T. J.; GALE, L. R.; et al. Novel *Fusarium* head blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. **Fungal Genetics and Biology**, v. 48, n. 12, p. 1096–1107, 2011.

SCAUFLAIRE, J.; GOURGUE, M.; MUNAUT, F. *Fusarium temperatum* sp. nov. from maize, an emergent species closely related to *Fusarium subglutinans*. **Mycologia**, v. 103, n. 3, p. 586–97, 2011.

SCHINDELIN, J.; ARGANDA-CARRERAS, I.; FRISE, E.; et al. Fiji: An open-source platform for biological-image analysis. **Nature Methods**, v. 9, n. 7, p. 676–682, 2012.

SCHOCH, C. L. et al. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. **Proceedings of the National Academy of Sciences**, v. 109, n. 16, p. 6241–6246, 2012.

SCHOEMAN, A.; FLETT, B. C.; JANSE VAN RENSBURG, B. Evaluating three commonly used growth media for assessing fumonisin analogues FB1, FB2a nd FB3 production by nine *Fusarium verticillioides* isolates. **Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment**, v. 34, n. 2, p. 291–298, 2016.

SCOZ, L. B.; ASTOLFI, P.; REARTES, D. S.; et al. Trichothecene mycotoxin genotypes of *Fusarium graminearum* sensu stricto and *Fusarium meridionale* in wheat from southern Brazil. **Plant Pathology**, v. 58, n. 2, p. 344–351, 2009.

SECOR, G. A.; RIVERA-VARAS, V.; CHRIST, D. S.; et al. Characterization of *Fusarium secorum*, a new species causing *Fusarium* yellowing decline ofsugar beet in north central USA. **Fungal Biology**, v. 118, n. 9, p. 764–775, 2014.

SELVARAJ, J. N.; ZHOU, L.; WANG, Y.; et al. Mycotoxin detection - Recent trends at global level. **Journal of Integrative Agriculture**, v. 14, n. 11, p. 2265–2281, 2015.

SHORT, D. P. G.; O'DONNELL, K.; ZHANG, N.; JUBA, J. H.; GEISER, D. M. Widespread occurrence of diverse human pathogenic types of the fungus *Fusarium* detected in plumbing drains. **Journal of Clinical Microbiology**, v. 49, n. 12, p. 4264–4272, 2011.

SILVA, H. P.; FANTIN, G. M.; RESENDE, I. C.; PINTO, N. F. J. A.; CARVALHO, R. V. Manejo integrado de doenças na cultura de milho safrinha. In: **SEMINÁRIO NACIONAL DE MILHO SAFRINHA**, v. 6, 2001., p. 113-144.

SILVA, A. O.; SAVI, D. C.; GOMES, F. B.; GOS, F. M. W. R.; SILVA JR, G. J.; GLIENKE, C. Identification of *Colletotrichum* species associated with postbloom fruit drop in Brazil through GAPDH sequencing analysis and multiplex PCR. **European Journal of Plant**

Pathology, 2016.

STARKEY, D. E.; WARD, T. J.; AOKI, T.; et al. Global molecular surveillance reveals novel *Fusarium* head blight species and trichothecene toxin diversity. **Fungal Genetics and Biology**, v. 44, n. 11, p. 1191–1204, 2007.

STIELOW, J. B. et al. One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. **Persoonia - Molecular Phylogeny and Evolution of Fungi**, v. 35, n. 1, p. 242–263, 2015.

SUKUMARAN, J.; HOLDER, M. T. DendroPy: A Python library for phylogenetic computing. **Bioinformatics**, v. 26, n. 12, p. 1569–1571, 2010.

VAN HOVE, F.; WAALWIJK, C.; LOGRIECO, A.; MUNAUT, F.; MORETTI, A. *Gibberella musae* (*Fusarium musae*) sp. nov., a recently discovered species from banana is sister to *F. verticillioides*. **Mycologia**, v. 103, n. 3, p. 570–585, 2011.

VILLANI, A.; MORETTI, A.; DE SAEGER, S.; et al. A polyphasic approach for characterization of a collection of cereal isolates of the *Fusarium incarnatum-equiseti* species complex. **International Journal of Food Microbiology**, v. 234, p. 24–35, 2016.

VINCZE, T.; POSFAI, J.; ROBERTS, R. J. NEBcutter: A program to cleave DNA with restriction enzymes. **Nucleic Acids Research**, v. 31, n. 13, p. 3688–3691, 2003.

VISMER, H. F.; SNIJMAN, P. W.; MARASAS, W. F. O.; VAN SCHALKWYK, D. J. Production of Fumonisins by *Fusarium Verticillioides* Strains on Solid and in a Defined Liquid Medium – Effects of L-Methionine and Inoculum. **Mycopathologia**, v. 158, n. 1, p. 99–106, 2004.

WALSH, J. L.; LAURENCE, M. H.; LIEW, E. C. Y.; et al. *Fusarium*: Two endophytic novel species from tropical grasses of northern Australia. **Fungal Diversity**, v. 44, p. 149–159, 2010.

WARD, T. J.; BIELAWSKI, J. P.; KISTLER, H. C.; SULLIVAN, E.; O'DONNELL, K. Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. **Proceedings of the National Academy of Sciences of the United States of America**, v. 99, n. 14, p. 9278–83, 2002.

WARD, T. J.; CLEAR, R. M.; ROONEY, A. P.; et al. An adaptive evolutionary shift in *Fusarium* head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. **Fungal Genetics and Biology**, v. 45, n. 4, p. 473–484, 2008.

WATANABE, M.; YONEZAWA, T.; LEE, K.; et al. Molecular phylogeny of the higher and lower taxonomy of the *Fusarium* genus and differences in the evolutionary histories of multiple genes. **BMC Evolutionary Biology**, v. 11, n. 1, p. 322, 2011.

ZELLER, K. A; SUMMERELL, B. A; BULLOCK, S.; LESLIE, J. F. *Gibberella konza* (*Fusarium konzum*) sp. nov. from prairie grasses, a new species in the *Gibberella fujikuroi* species complex. **Mycologia**, v. 95, n. 5, p. 943–954, 2003.

ZHANG, H.; BRANKOVICS, B.; LUO, W.; et al. Crops are a main driver for species diversity and the toxigenic potential of *Fusarium* isolates in maize ears in China. **World Mycotoxin Journal**, v. 9, n. 5, p. 701–715, 2016.

ZWICKL, D. J. **Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion**, 115f. Tese (Doctor of Philosophy) – University of Texas at Austin, 2006.