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# Global molecular surveillance reveals novel *Fusarium* head blight species and trichothecene toxin diversity

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**Abstract**

To expand our knowledge of *Fusarium* head blight (FHB) pathogen and trichothecene toxin diversity, a global collection of 2100 isolates was screened for novel genetic variation, resulting in the identification of 16 phylogenetically divergent FHB isolates. The affinities and taxonomic status of these novel isolates were evaluated via phylogenetic analyses of multilocus DNA sequence data (13 genes; 16.3 kb/strain) together with analyses of their morphology, pathogenicity to wheat, and trichothecene toxin potential. Based on the results of these analyses, we formally describe two novel species (*Fusarium vorosii* and *Fusarium gerlachii*) within the *Fusarium graminearum* species complex (*Fg* complex), and provide the first published report of *Fg* complex isolates with either a nivalenol or 3-acetyldeoxynivalenol chemotype within the U.S. In addition, we describe a highly divergent population of *F. graminearum* from the Gulf Coast of the U.S., and divergent isolates of *F. acaciae-mearnsii* from Australia and South Africa.
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## 1. Introduction

*Fusarium* head blight (FHB) is one of the most economically important diseases of wheat, barley, rice and other small grain cereals worldwide. Within the last 15 years FHB reached epidemic levels in North America, resulting in U.S. wheat and barley losses of nearly $ 3 billion dollars (Windels, 2000). Recent outbreaks in Asia, Canada, Europe, and South America suggest that FHB is a growing threat to the world's grain supply (reviewed in Goswami and Kistler, 2004). Infection of cereals by FHB pathogens lowers grain yield and quality, and FHB-infected grain is often contaminated with trichothecene mycotoxins and estrogenic compounds (Kim et al., 2005). Trichothecenes inhibit protein synthesis (McLaughlin et al., 1977; Ueno et al., 1973), they are powerful modulators of human immune function (Pestka and Smolinski, 2005), and they have been implicated in a number of human and animal

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mycotoxicoses (Peraica et al., 1999; Ueno and Ishii, 1985). Trichothecenes also are acutely phytotoxic and act as virulence factors on sensitive host plants (Jansen et al., 2005; Maier et al., 2006). In addition, the three strain-specific profiles of B-trichothecenes (chemotypes) most frequently associated with FHB (Miller et al., 1991) appear to have been maintained through multiple speciation events by balancing selection, indicating that chemotype differences are adaptive (Ward et al., 2002).

Although a number of fusaria can cause FHB, the primary etiological agents of this disease belong to the *Fusarium graminearum* species complex of B-trichothecene toxin producers, hereafter referred to as the *Fg* complex (O'Donnell et al., 2000). Until recently, members of this species-rich complex were viewed as a single panmictic species (*F. graminearum*; teleomorph *Gibberella zeae*) worldwide (Gerlach and Nirenberg, 1982; Nelson et al., 1983). However, employing genealogical concordance phylogenetic species recognition (GCPSR; Taylor et al., 2000) as an objective criterion for defining species limits, phylogenetic analyses of DNA sequences from portions of 11 single-copy nuclear genes totaling 13.6 kb provided strong evidence that this morphologically defined species comprises at least nine phylogenetically distinct and biogeographically structured species (O'Donnell et al., 2000, 2004). Therefore, species rank was formally proposed for the eight previously unnamed species, with *F. graminearum sensu stricto* retained for the species most commonly associated with FHB worldwide (O'Donnell et al., 2004).

The objective of the present study was to expand our current understanding of FHB pathogen and trichothecene toxin diversity worldwide. A genetic screen for novel phylogenetic variation among 2100 FHB isolates from five continents identified 16 phylogenetically divergent isolates, which were evaluated by GCPSR using DNA sequences from 13 nuclear genes. These novel isolates were characterized further by determining their trichothecene chemotypes, morphological phenotypes, and their ability to induce FHB on wheat in greenhouse pathogenicity experiments. Integrated analyses of these data led to the following discoveries: (1) two novel species of FHB pathogens; (2) a highly divergent population of *F. graminearum* currently only known from the Gulf Coast of the U.S.; (3) *Fg* complex isolates from the U.S. with either nivalenol (NIV) or 3-acetyldeoxynivalenol (3ADON) chemotypes; and (4) phylogenetically divergent isolates of *F. acaciae-mearnsii* from Australia and South Africa.

## 2. Materials and methods

### 2.1. Strains, DNA amplification, and sequencing

A panel of 2100 FHB isolates was screened for novel phylogenetic variation based on partial translation elongation factor-1α (*EF-1α*) and reductase (*RED*) sequences collected as described previously (O'Donnell et al., 2004). We elected to use partial DNA sequence data from the *EF*-1α and *RED* genes based on the results of previous analyses which indicated that a combined analysis of these loci resolved all of the described B clade species as monophyletic (O'Donnell et al., 2000, 2004). This panel included isolates identified as genetically novel in preliminary analyses using independent molecular markers (Suga, unpublished; Tóth et al., 2005). A total of 16 phylogenetically divergent isolates were selected for further study (see Table 1 for histories). DNA sequences for these isolates have been deposited in GenBank under Accession numbers DQ441531–DQ441588, DQ452399–DQ452415, and DQ459630–DQ459873. Histories and DNA sequences (Accession numbers AF212435–AF212825 and AY102567–AY102605) for reference isolates have been reported previously (O'Donnell et al., 2000, 2004; Ward et al., 2002). Culture manipulation, DNA isolation, PCR amplification, and DNA sequencing were performed as described previously (O'Donnell et al., 2004). In addition to sequencing the 11 genes utilized in published analyses (O'Donnell et al., 2004), partial sequences from two additional regions, the alpha-tubulin (α-*tub*) gene and the nuclear ribosomal internal transcribed spacer (ITS) regions together with domains D1 and D2 at the 5′ end of the nuclear large ribosomal subunit (LSU) RNA gene (rDNA), were obtained for this study. The 13-gene dataset was analyzed as eight individual loci and as a combined dataset (Tables 2 and 3). The four adjacent mating-type (MAT) genes (*MAT1-1-3*, *MAT1-1-2*, *MAT1-1-1*, and *MAT1-2-1*), plus the corresponding intergenic regions (A, B, and C), were analyzed as a single locus. Similarly, the contiguous phosphate permease (*PHO*), trichothecene 3-*O*-acetyltransferase (*Tri101*; Kimura et al., 1998) and ammonia ligase (*URA*) genes were also treated as a single locus (*URA–Tri101–PHO*). The six other genes were analyzed separately.

A 1.7-kb portion of the α-tub gene was amplified in a 40-μL PCR mix containing a final concentration of 0.2 mM dNTPs, 2 mM $MgSO_4$, and 0.8 U Platinum *Taq* (Invitrogen Life Technologies, Carlsbad, CA) containing 10 pmol of the following PCR primers: atuA1 (5′-CATCTGCAACACTG CGTGARG-3′) and atuA2 (5′- CTCAGCCTCCAARTCR TCYTC-3′). The α-*tub* gene was amplified in an Applied Biosystems (ABI, Foster City, CA) 9700 thermocycler, using the following cycling parameters: 94 °C 90 s, 40 cycles of 94 °C 30 s, 52 °C 30 s, 68 °C 2 min, followed by 68 °C for 5 min and a 4 °C soak. Sequence obtained with the PCR primers was used to design the following forward and reverse internal sequencing primers: atuA11 (5′-CACKCCGACTG CTCCTTCATG-3′), and atuA21 (5′-CGGCAGATGTCG TAGATGG-3′). The ITS + LSU rDNA locus was amplified as a single 1.2 kb fragment using PCR primers ITS5 and NL4, and sequenced as described previously (O'Donnell et al., 1998; White et al., 1990).

### 2.2. Phylogenetic analysis

Phylogenetic analyses were conducted using PAUP* v. 4.0b10 (Swofford, 2002) to characterize the genetic diversity

Table 1
Strains used in this study

| Taxon | NRRL# | Equivalent strain number[a] | Host/substrate | Geographic Origin |
|---|---|---|---|---|
| *F. acaciae-mearnsii* | 34197 | FRC R-4339 | Soil | Queensland, Australia |
| *F. acaciae-mearnsii* | 34461 | FRC R-8601*i* | Soil | Umyaka, South Africa |
| *F. gerlachii* | 36905 **T**[b] | LRG 00-551 | Wheat head | Minnesota, USA |
| *F. gerlachii* | 38380 | LRG 02-225 | *Arundo donax* | Wisconsin, USA |
| *F. gerlachii* | 38405 | LRG 3ND7-17 | Wheat head | North Dakota, USA |
| *F. graminearum* | 28439 | FRC R-6238 | *Rumohra adiantiformis* | Florida, USA |
| *F. graminearum* | 29149 | FRC R-6237 | *Rumohra adiantiformis* | Florida, USA |
| *F. graminearum* | 38369 | LRG 03-65 | Wheat head | Louisiana, USA |
| *F. graminearum* | 38371 | LRG 03-126 | Wheat head | Louisiana, USA |
| *F. graminearum* | 38381 | LRG 03-54 | Wheat head | Louisiana, USA |
| *F. graminearum* | 38383 | LRG 03-132 | Wheat head | Louisiana, USA |
| *F. graminearum* | 38393 | LRG 03-33 | Wheat head | Louisiana, USA |
| *F. graminearum* | 38395 | LRG 03-46 | Wheat head | Louisiana, USA |
| *F. vorosii* | 37605 **T** | Tóth FgHF012 | Wheat head | Ipolydamásd, Hungary |
| *F. vorosii* | 38207 | Suga 0301112 | Wheat head | Hokkaido, Japan |
| *F. vorosii* | 38208 | Suga 0301831 | Wheat head | Hokkaido, Japan |

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[b] T, Ex-holotype strain.

Table 2
Location and protein designation for individual gene partitions

| Chromosome[a] | Gene (abbreviation) | Position[b]/Scaffold | Protein designation |
|---|---|---|---|
| 1 | α-Tubulin (α-*tub*) | 56,237–57,922/1 | FG00639.1 |
| 2 | Elongation factor 1α (*EF-1α*) | 131,683–132,325/5 | FG08811.1 |
| 2 | Histone H3 (*HIS*) | 316,602–317,050/2 | FG04290.1 |
| 2 | Mating-type locus (MAT) | 144,244–150-208/5 | FG08890.1-93.1 |
| 2 | Reductase (*RED*) | 13,517–14,507/2 | FG03224.1 |
| 4 | ITS/28S rDNA | N/A[c] | N/A |
| 4 | β-Tubulin (β-*tub*) | 51,456-52,787/6 | FG09530.1 |
| 4 | Phosphate permease (*PHO*) | 11,994-12,960/4 | FG07894.1 |
| 4 | Trichothecene 3-*O*-acetyltransferase (*Tri101*) | 15,122–16,457/4 | FG07896.1 |
| 4 | Ammonium ligase (*URA*) | 17,275–17,831 & 18,471–19,812/4 | Not annotated |

[a] A detailed genetic and physical map of *F. graminearum* PH-1 = NRRL 31084 has been published (Gale et al., 2005).
[b] Corresponding nucleotide positions in the whole genome sequence of *F. graminearum* strain PH-1 = NRRL 31084 (http://www.broad.mit.edu/annotation/fungi/fusarium/).
[c] Although the ITS/28S rDNA repeat is in the excluded reads of the whole genome sequence of *F. graminearum*, cytological analysis determined that it is telomeric on chromosome 4 (Gale et al., 2005).

and evolutionary relationships of the novel isolates. Maximum parsimony (MP) searches were conducted using 100 random sequence addition replicates and the tree bisection–reconnection (TBR) method of branch swapping. Ambiguously aligned nucleotide positions ($N = 391$) were excluded from all analyses and four parsimony-informative gaps within MAT were coded as single characters (Table 3). Neighbor-joining (NJ) analyses were conducted using the Kimura 2-parameter model (Kimura, 1980) as implemented in PAUP* (Swofford, 2002). Non-parametric bootstrap percentages (BP), using 1000 pseudoreplicates of the dataset, were used to assess relative support for internal nodes and clade stability under both parsimony and distance frameworks.

### 2.3. Pathogenicity experiments and mycotoxin analyses

Pathogenicity experiments were conducted on wheat cultivar Norm as described previously (Goswami and Kistler, 2005), and as described for NRRL 37605 (= FgHF012; Tóth et al., 2005), to determine the aggressiveness of the novel isolates to wheat. Norm is a cultivar of hard red spring wheat that has been a widely planted in Minnesota and North Dakota and is considered very susceptible to FHB. Norm was chosen because of its susceptibility and, since FHB race specialization has never been found, a single cultivar inoculation is considered an adequate assay of strain aggressiveness. All strains were point inoculated with a level of inoculum ($10^4$ macroconidia per plant) that was capable of detecting a full range of pathogenic response from very high to low levels of disease among *Fg* complex strains (Goswami and Kistler, 2005). All strains tested were compared to the control *F. graminearum* strain NRRL 31084, which has a moderately high level of aggressiveness. The correlation between trichothecene chemotype, toxin levels and aggressiveness on wheat among FHB isolates has been described in a previous publication (Goswami and Kistler, 2005).

Table 3
Tree statistics for each partition[a]

| | # Characters[b] | # Haplotypes | # MPTs | MPT length | CI | RI | PIC/bp (%) | Bootstrap support (%)[c] *F. vorosii* | Bootstrap support (%)[c] *F. gerlachii* | # *Fg* species supported as monophyletic[c] |
|---|---|---|---|---|---|---|---|---|---|---|
| ITS/28S rDNA | 1133 | 20 | 2 | 23 | 0.957 | 0.923 | 0.53 | <70 | <70 | 0 |
| β-Tubulin | 1257 | 37 | 60 | 105 | 0.771 | 0.761 | 3.26 | 95 | <70 | 2 |
| Histone H3 | 449 | 26 | 18 | 68 | 0.853 | 0.833 | 5.34 | 70 | <70 | 4 |
| *EF-1α* | 648 | 35 | >20,000 | 86 | 0.907 | 0.927 | 7.09 | <70 | <70 | 4 |
| Reductase | 1154 | 41 | 36 | 258 | 0.868 | 0.888 | 10.65 | 90 | 70 | 6 |
| α-Tubulin | 1,686 | 31 | 10 | 110 | 0.945 | 0.927 | 2.61 | 99 | 87 | 8 |
| MAT | 6,189 | 55 | 48 | 846 | 0.849 | 0.936 | 8.32 | 100 | 72 | 11 |
| *URA–Tri101–PHO*[d] | 4124 | 48 | 456 | 649 | 0.829 | 0.91 | 8.22 | 100 | 79 | 11 |
| Combined | 16,330 | 58 | 72 | 2361 | 0.792 | 0.901 | 7.17 | 100 | 100 | 11 |

[a] *Abbreviations used:* MPTs, most-parsimonous trees; CI, consistency index; RI, retention index; PIC, parsimony informative character (*i.e.*, shared derived character).
[b] The combined dataset consisted of 16,717 bp of which 391 were excluded as ambiguously aligned as follows: 297 from MAT, 13 from reductase and 81 from the phosphate permase segment of *URA–Tri101–PHO*. In addition, four indels in MAT were coded as parsimony informative.
[c] Based on maximum parsimony bootstrap support (see Section 2).
[d] Ammonium ligase (*URA*), trichothecene 3-*O*-acetyltransferase (*Tri101*), and phosphate permase (*PHO*) are contiguous in the genome on chromosome 4 (Kimura et al., 1998; Gale et al., 2005).

Trichothecene toxin chemotype potential *in planta* was determined as previously described (O'Donnell et al., 2004; Ward et al., 2002). Isolates were assigned to their respective trichothecene toxin chemotype groups based on metabolite profiles (Rodrigues-Fo et al., 2002).

Trichothecene chemotypes were also determined with a multiplex version of the chemotype-specific PCR test validated previously (Ward et al., 2002). Two sets of primers specific to individual chemotypes were designed from the trichothecene 15-*O*-acetyltransferase (*TRI3*) and trichothecene efflux pump (*TRI12*) genes (Ward et al., 2002). The *TRI3* multiplex included a primer common to all chemotypes, 3CON (5′-TGGCAAAGACTGGTTCAC-3′), and three chemotype-specific primers: 3NA (5′-GTGCACAGAATA TACGAGC-3′), 3D15A (5′-ACTGACCCAAGCTGCCAT C-3′), and 3D3A (5′-CGCATTGGCTAACACATG-3′). This multiplex produced amplicons of approximately 840-, 610-, and 243-bp with isolates that had NIV, 15ADON, and 3ADON chemotypes respectively. The *TRI12* multiplex similarly included a primer common to all chemotypes, 12CON (5′-CATGAGCATGGTGATGTC-3′), and three chemotype-specific primers: 12NF (5′-TCTCCTCGTTGTATCTG G-3′), 12-15F (5′-TACAGCGGTCGCAACTTC-3′), and 12-3F (5′-CTTTGGCAAGCCCGTGCA-3′). This multiplex produced amplicons of approximately 840-, 670-, and 410-bp with isolates that had NIV, 15ADON, and 3ADON chemotypes, respectively. Both multiplex reactions were performed in 10-μl volumes with 1× GeneAmp PCR buffer (Applied Biosystems, Foster City, California), 2 mM $MgCl_2$, 0.2 mM concentrations of each deoxynucleoside triphosphate, 0.2 μM concentrations of each primer, 0.5 U of Ampli*Taq* DNA Polymerase (Applied Biosystems), and ~100 ng of genomic DNA. PCR consisted of an initial denaturation of 2 min at 94 °C, followed by 25 cycles of 30 s at 94 °C, 30 s at 52 °C, and 1 min at 72 °C. Amplification products were resolved on 1.5% (wt/vol) agarose gels and scored by size in comparison to a 100-bp DNA size ladder (Invitrogen Life Technologies, Carlsbad, CA).

### 2.4. Phenotypic analyses

Isolates were compared phenotypically as previously described (Aoki and O'Donnell, 1999; O'Donnell et al., 2004) to determine whether they could be distinguished using morphological data. Colony morphology, color and odor of the strains were examined using cultures grown on PDA (Difco, Detroit, MI) in 9 cm plastic Petri dishes in the dark at 25 °C. Microscopic examination was conducted from cultures grown on SNA (Nirenberg, 1990) at 25 °C under continuous black light up to 5 days to induce constant conidiogenesis. Fifty 5-septate conidia were randomly selected from each culture and their length, width, and widest position were measured. A dried culture of the two new species described in the present study has been deposited as the holotype in the U.S. National Fungus Collection (BPI), USDA/ARS, Beltsville, MD, USA. Cultures of each species included in this study are available from the ARS Culture Collection

(NRRL), the Fusarium Research Center (FRC), and the Centraalbureau voor Schimmelcultures (CBS).

## 3. Results

### 3.1. Phylogenetic analysis

Sequence data from the *RED* (1154 bp) and *EF-1α* (648 bp) genes was obtained from a global collection of 2100 B-trichothecene toxin-producing FHB pathogens. Maximum parsimony analyses of these sequences (data not shown), and those from representatives of all previously described B-FHB clade species, identified 16 isolates that represented novel phylogenetic diversity within the *Fg* complex (Table 1). Hypotheses of species limits for these isolates were tested by phylogenetic analyses (Fig. 1) of the eight individual data partitions and the combined 13-gene dataset (16.3 kb/strain) (Table 2). Tree statistics from analyses of the eight individual data partitions contained within the 13-gene dataset are summarized in Table 3. Based on these phylogenetic and phenotypic analyses, we formally describe two novel species within the *Fg* complex (*Fusarium vorosii* and *Fusarium gerlachii*) that fulfill the requirements for species recognition under GCPSR.

### 3.2. Fusarium vorosii

The first isolate of this species was collected in 2002 from head blight of wheat in Hungary (NRRL 37605 = FgHF012) and identified as novel using randomly amplified polymorphic DNA (RAPDs), IGS-RFLP, and partial *RED* gene sequence data (Tóth et al., 2005). In a genetic screen using partial *EF-1α* and *RED* sequences, we identified two isolates of this species from blighted wheat collected in 2003 from Hokkaido, Japan (NRRL 38207 = Suga 0301112, NRRL 38208 = Suga 0301831; Table 1). These three isolates formed a strongly supported (BP = 100%) monophyletic sister group to *Fusarium asiaticum* (BP = 97%) in the combined multilocus phylogeny (Fig. 1). In addition, the monophyly of this novel group of isolates was strongly supported (BP ⩾ 70%) in individual genealogies from six of the eight loci (Table 3). Although relationships in the *EF-1α* and rDNA data partitions were unresolved, they did not contradict the monophyly of this species relative to other members of the *Fg* complex. Taken together, these results indicate genetic isolation from all previously described species consistent with an advanced state of speciation, thereby fulfilling the criteria for species recognition under GCPSR. On this basis, we recognize NRRL 37605, 38207, and 38208 as representatives of the novel species, *F. vorosii*. All three strains of *F. vorosii* induced head blight of wheat cultivar Norm in greenhouse pathogenicity tests (Table 4). In addition, analyses of mycotoxin production *in planta* demonstrated that these isolates make 15-acetyldeoxynivalenol as the primary acetyl-ester derivative of deoxyvnivalenol (15ADON chemotype; Table 4).

### 3.3. Fusarium gerlachii

A second group of isolates was obtained from wheat exhibiting head blight symptoms (NRRL 36905 = LRG 00-551 and NRRL 38405 = LRG 3ND7-17) and an asymptomatic giant reed, *Arundo donax*, (NRRL 38380 = LRG 02-225) in the upper Midwest of the U.S. in 2000, 2003, and 2002, respectively. These isolates formed a well-supported (BP = 100%) monophyletic sister group to *F. graminearum* in the multilocus phylogeny (Fig. 1), and phylogenetic trees inferred from the individual data partitions were congruent with the monophyly of this group. Four of the individual data partitions provided strong support (BP ⩾ 70%) for reciprocal monophyly between this group and previously described members of the *Fg* complex, whereas relationships within the other partitions were unresolved (Table 3). Overall, these analyses strongly suggest an extended history of genetic isolation between this lineage and other members of the *Fg* complex, consistent with an advanced state of speciation. On this basis, we recognize NRRL 36905, 38380, and 38405 as representatives of a second novel species within the *Fg* complex, *F. gerlachii*. Strains of *F. gerlachii* induced head blight on wheat cultivar Norm in greenhouse pathogenicity tests (Table 4) and produced nivalenol and acetylated derivatives (NIV chemotype) *in planta* (Table 4).

### 3.4. Fusarium graminearum

Eight novel isolates (NRRL 28439, 29149, 38369, 38371, 38381, 38383, 38393, and 38395; Table 1) formed a well-supported monophyletic sister group (BP = 91%) to *F. graminearum* (BP = 100%) in the multilocus phylogeny (Fig. 1). The monophyly of this novel group of isolates (BP = 99%) and the sister group relationship to *F. graminearum* (BP = 99%) was also supported in the genealogy resolved by MAT and three additional loci. However, phylogenies inferred from β-tubulin, *RED*, and *URA–Tri101–PHO* were discordant with the hypothesized monophyly of this group. With the available data, it is impossible to ascertain whether the shared DNA polymorphism observed at these three loci is due to contemporary gene flow between these isolates and ones previously identified as *F. graminearum*, or whether the shared polymorphism reflects a relatively recent speciation event that cannot be identified by GCPSR. Given our conservative application of GCPSR, we recognize these isolates as part of a genetically diverse *F. graminearum* clade, with the provision that the basis of the genealogical discord needs to be investigated more fully to evaluate the question of species boundaries. NRRL 28439 and 29149 were isolated in 1981 from leatherleaf fern (*Rumohra adiantiformis*) grown commercially in Florida, whereas the eight remaining strains were isolated from blighted wheat in Louisiana in 2003. The three isolates tested (NRRL 28439, 29149, and 38371) induced head blight on wheat cultivar Norm in a greenhouse pathogenicity experiment (Table 4). Results of the

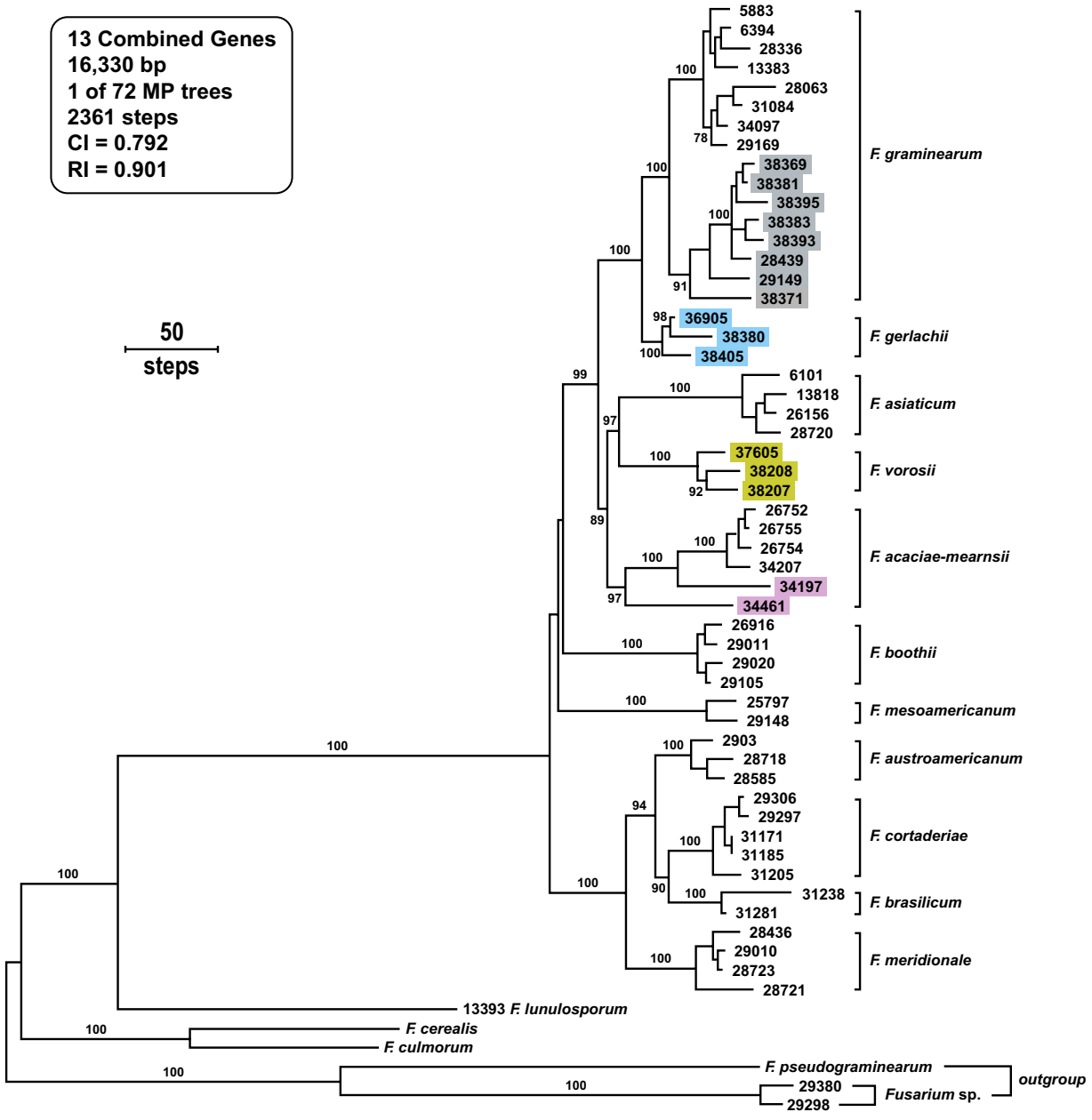


Fig. 1. One of 72 most-parsimonious phylograms inferred from the combined dataset of 16.3 kb from eight loci comprising portions of 13 genes. Sequences of *Fusarium pseudograminearum* and *Fusarium* sp. NRRL 29380 and 29298 were used to root the tree. Branches that received $\geqslant 70\%$ bootstrap values are indicated. Our biogeographic hypothesis suggests that the basal most species lineages within the *F. graminearum* species complex are endemic to the Southern Hemisphere while the derived phylogeographic lineages evolved within the Northern Hemisphere. Several species, however, are now found in both hemispheres (*i.e.*, *F. graminearum*, *F. asiaticum*, *F. boothii*, *F. meridionale*). Color coding is used to identify novel genetic variation reported is this study, including the two newly discovered species, *F. gerlachii* and *F. vorosii*, formally described herein, a divergent population of *F. graminearum* from the Gulf Coast of the U.S., and genetically divergent strains of *F. acaciae-mearnsii*.

multiplex PCR assay for trichothecene chemotype demonstrated that all three B-trichothecene chemotypes (*i.e.*, 15ADON +DON, 3ADON+DON, and NIV) were segregating within this group of isolates (Table 4).

### 3.5. *Fusarium acaciae-mearnsii*

Two isolates (NRRL 34197 and 34461) identified in analyses of *RED* and *EF-1α* gene sequences were strongly supported as divergent members of the *F. acaciae-mearnsii* clade (BP = 97%) in multilocus analyses (Fig. 1). Phylogenetic trees reconstructed from three loci (MAT, *URA–Tri101–PHO*, and *EF-1α*) were consistent in supporting a sister group relationship (BP $\geqslant$ 70%) between NRRL 34197 and *F. acaciae-mearnsii.* NRRL 34461 was strongly supported as the sister to NRRL 34197 plus *F. acaciae-mearnsii* in MAT analyses (BP = 100%), but unresolved in the remaining data partitions. Overall, these

Table 4
Mycotoxin potential and pathogenicity of *Fusarium graminearum* clade species

| Taxon | NRRL# | Chemotype[a] | Trichothecene concentration[b] | | | | Pathogenicity |
|---|---|---|---|---|---|---|---|
| | | | NIV | DON | 3ADON | 15ADON | |
| *F. acaciae-mearnsii* | 34197 | 3ADON | nd | 961 | 42.5 | 1.9 | 4.3 ± 1.9[c] |
| *F. acaciae-mearnsii* | 34461 | 3ADON | nd | 1262 | 53 | 4.1 | 5.4 ± 3.0 |
| *F. gerlachii* | 36905 | NIV | 182 | nd | nd | nd | 6.4 ± 2.2[c] |
| *F. gerlachii* | 38380 | NIV | 89.7 | nd | nd | nd | 7.3 ± 2.2 |
| *F. gerlachii* | 38405 | NIV | 115 | nd | nd | nd | 6.7 ± 1.9[c] |
| *F. graminearum* | 28439 | NIV | 50.5 | nd | nd | nd | 4.3 ± 2.7[c] |
| *F. graminearum* | 29149 | NIV | • | • | • | • | • |
| *F. graminearum* | 38369 | 3ADON | • | • | • | • | • |
| *F. graminearum* | 38371 | NIV | • | • | • | • | • |
| *F. graminearum* | 38381 | 3ADON | • | • | • | • | • |
| *F. graminearum* | 38383 | NIV | • | • | • | • | • |
| *F. graminearum* | 38393 | 15ADON | • | • | • | • | • |
| *F. graminearum* | 38395 | 3ADON | • | • | • | • | • |
| *F. vorosii* | 37605 | 15ADON | nd | 308 | 6.8 | 83.5 | 8.8 ± 0.9 |
| *F. vorosii* | 38207 | 15ADON | nd | 250 | 5 | 49.3 | 8.7 ± 1.2 |
| *F. vorosii* | 38208 | 15ADON | nd | 195 | 3.6 | 36.7 | 5.9 ± 0.5[c] |

•not tested.
[a] 3ADON, 3-acetyldeoxynivalenol + deoxynivalenol; 15ADON, 15-acetyldeoxynivalenol + deoxynivalenol; NIV, nivalenol + acetylated derivatives (Miller et al., 1991; Ward et al., 2002).
[b] Mean trichothecene content (ppm) from four replications. nd ≤ 1 ppm.
[c] Mean significantly less than control inoculated with NRRL 31084 ($p < 0.05$).

results are inconclusive regarding the genetic isolation of NRRL 34197, 34461, and *F. acaciae-mearnsii*. Given the ambiguous results, additional sampling of variation from this group is needed to assess species limits. Therefore, we suggest a conservative approach to GCPSR, in which these two newly studied isolates are provisionally resolved as part of a genetically diverse *F. acaciae-mearnsii* clade. Both strains were isolated from soil samples, NRRL 34197 from Australia in 1977 and NRRL 34461 from South Africa in 1987 (Table 1). Both strains induced head blight of wheat cultivar Norm in a greenhouse pathogenicity experiment (Table 4), and mycotoxin analyses confirmed that these isolates belong to the 3ADON chemotype (Table 4).

### 3.6. Novel structural variation within MAT1-1-2

Four putative introns ranging in size from 42 to 52 bp have been predicted in the *MAT1-1-2* gene of *F. graminearum* (http://www.broad.mit.edu/annotation/fungi/fusarium; Yun et al., 2000), and in all previously described members of the *Fg* complex (O'Donnell et al., 2004). The four introns postulated for *F. graminearum* contain the standard GT/AG splice donor/acceptor site. However, as annotated the second intron for *F. graminearum MAT1-1-2* requires the use of a non-canonical AT donor site for every other species within the *Fg* complex (Fig. 2). Although non-canonical donor/acceptor sites are utilized in *Fusarium* (Rep et al., 2006), AT donor sites have not been identified in *Fusarium* and appear to be exceptionally rare in other organisms (Burset et al., 2000; reviewed for mammalian genomes). In addition, the putative second intron lacks standard branch site sequences typical of introns in filamentous fungi (Rep et al., 2006). Moreover, the putative intron does not disrupt the open reading frame of *MAT1-1-2* and *in silico* translation of this putative intron results in the addition of 14 amino acids within the *MAT1-1-2* protein in every species within the *Fg* complex (Fig. 2). On this basis, we conclude that *MAT1-1-2* is probably annotated incorrectly, and likely contains four exons encoding a protein of 477 amino acids. Sequence analysis also identified a unique 27-bp insertion in the *MAT1-1-2* gene in the three isolates of *F. vorosii* (Fig. 2). Translation of this sequence suggests that it may code for a *MAT1-1-2* protein with nine additional amino acids between nucleotide positions 5774 and 5775, (Yun et al., 2000; GenBank Accession numberAF318048).

### 3.7. Phenotypic analysis and taxonomy

No obvious differences were observed in colony characteristics of the two newly discovered members of the *Fg* complex when compared with related B-clade species on PDA in complete darkness at 25 °C. Phenotypes of the new species, including conidial sizes and morphology, and little if any chlamydospore production, matched published morphological descriptions of *F. graminearum* (Booth, 1971; Gerlach and Nirenberg, 1982; Nelson et al., 1983; Wollenweber, 1931; Wollenweber and Reinking, 1935). Sizes (length and width) of 5-septate conidia were compared among the different members of the *Fg* complex and with representative strains of *Fusarium cerealis*, *Fusarium culmorum*, *Fusarium lunulosporum* and *Fusarium pseudograminearum*. Based on average width of conidia, the two new *Fg* complex species could be distinguished from one another: *F. gerlachii* (mean width of 4.5–5 μm) and *F. vorosii* (more than 5 μm) (Table 5,

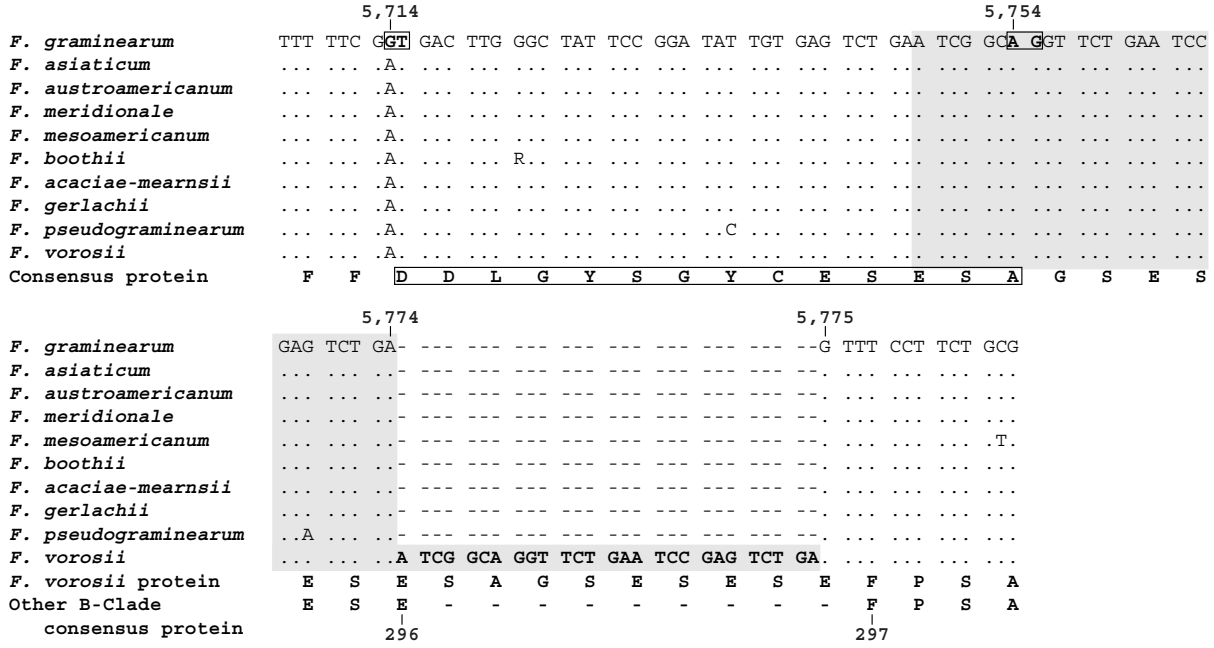


Fig. 2. Novel coding sequence within the *MAT1-1-2* gene of B-clade fusaria. Aligned nucleotide and amino acid sequences showing the presence of a canonical GT/AG splice donor/acceptor site in *F. graminearum* (boxed) between putative intron 2. Numbering of the nucleotide (GenBank accession AF318048) and amino acid (GenBank accession AAG42811) sequence follows that published previously (Yun et al., 2000). Intron 2, however, appears to be an artifact because all other species contain an extremely rare non-canonical AT/AG splice site, and *in silico* translation predicts this sequence codes for a *MAT1-1-2* protein with 14 additional amino acids (boxed). In addition, note that the 27 bp indel in strains of *F. vorosii* may code for a *MAT1-1-2* protein with nine additional amino acids. The indel is a direct repeat of upstream sequence highlighted in grey.

and Figs. 3 and 4). Among members of the *Fg* complex, and the four related B-clade species, *F. gerlachii* grouped with five species, *i.e.*, *F. acaciae-mearnsii, F. asiaticum*, *F. graminearum*, *Fusarium cortaderiae* and *Fusarium brasilicum*, based on conidial width. *Fusarium vorosii*, in contrast, grouped with *F. cerealis* and *F. culmorum* in producing broader conidia (Fig. 4). The widest position of 5-septate conidia in individual strains was also studied. Strains of *F. vorosii* produced 5-septate conidia most frequently widest above the mid-region, whereas conidia of *F. gerlachii* were typically widest at the mid-region. Morphological features that help differentiate the two new species include the frequent formation of conidia with a narrow apical beak in strains of *F. gerlachii*, and typically straight conidia in strains of *F. vorosii*. When the most frequent conidial morphology of each species was considered (O'Donnell et al., 2004), five individual species and three species groups could be distinguished within the *Fg* complex based on a combination of the following characters (Table 5, Fig. 4): conidial width, longitudinal axis of conidia, presence or absence of a narrow apical conidial beak, morphological symmetry of the upper and lower halves of conidia, and the most frequent widest position of conidia. However, because only a limited number of strains were available in the present study, infraspecific phenotypic variation of the two new species still needs to be evaluated based on future collections. Moreover, overlapping ranges of the morphological features indicate that morphological species recognition is either difficult or impossible for most of members of the *Fg* complex.

In the present study, eight novel isolates of the divergent U.S. Gulf Coast population of *F. graminearum* (NRRL 28439, 29149, 38369, 38371, 38381, 38383, 38393, and 38395; Table 1), and *F. acaciae-mearnsii* (NRRL 34197 and NRRL 34461) were also compared phenotypically. The two Gulf Coast strains of *F. graminearum* from *R. adiantiformis* (NRRL 28439 and NRRL 29149) were unique morphologically in that they formed conidia with a narrow apical beak (Table 5). Also, the most phylogenetically divergent strain of *F. acaciae-mearnsii* NRRL 34461 was unique in that it formed asymmetric conidia that are widest above mid-region, suggesting an intermediate morphology with that of *F. graminearum*. In addition to the morphological descriptions provided for *F. gerlachii* and *F. vorosii* in Appendix A, these two new species are also diagnosed on the basis of genealogical exclusivity and concordance among genealogies inferred from multiple loci.

## 4. Discussion

### 4.1. Phylogenetic analysis

The present study adds to our growing knowledge of cryptic speciation within the *F. graminearum* species complex of head blight pathogens (O'Donnell et al., 2004). On the basis of genealogical concordance phylogenetic species recognition (GCPSR) we report the discovery and formal description of two cryptic head blight species, *F. vorosii* from Japan and Hungary (Tóth et al., 2005) and *F. gerlachii*

Table 5
Conidial morphology characteristic of B trichothecene toxin-producing clade fusaria[a]

| Species | Width of 5-septate conidia (average value in μm) | Longitudinal axis of conidia | Narrow apical beak (±) | Upper and lower half of conidia | Widest region of conidia |
|---|---|---|---|---|---|
| *F. lumulosporum* | <4.5 | Curved | + | Symmetric | Mid-region |
| *F. austroamericanum* | <4.5 | Typically straight | ± | Asymmetric | Mid-region |
| *F. boothii* | <4.5 | Gradually curved | + | Mostly symmetric | Mid-region |
| *F. meridionale* | <4.5 | Gradually curved | + | Mostly symmetric | Mid-region |
| *F. mesoamericanum* | 4–4.5 | Typically straight | − | Asymmetric | Above Mid-region |
| *F. pseudograminearum* | 4–4.5 | Curved | + | Symmetric | Mid-region |
| *F. acaciae-mearnsii* | 4.5–5 | Gradually curved | + | Asymmetric[b] | Above/below mid-region[b] |
| *F. asiaticum* | 4.5–5 | Gradually curved | − | Asymmetric | Above mid-region |
| *F. graminearum* | 4.5–5 | Gradually curved | ±[c] | Asymmetric | Above mid-region |
| *F. brasilicum* | 4.5–5 | Straight or gradually curved | + | Asymmetric | Below mid-region |
| *F. cortaderiae* | 4.5–5 | Straight or gradually curved | + | Asymmetric | Below mid-region |
| *F. gerlachii* | 4.5–5 | gradually curved | + | Asymmetric | Mid-region |
| *F. vorosii* | >5 | Straight or gradually curved | − | Asymmetric | Above mid-region |
| *F. cerealis* | >5 | Curved | + | Symmetric | Mid-region |
| *F. culmorum* | >5 | Curved | − | Symmetric | Mid-region |

[a] When using the combined conidial characters, the following five species and three species groups could be distinguished within the *Fg* clade: *F. austroamericanum*, *F. mesoamericanum*, *F. acaciae-mearnsii*, *F. gerlachii*, *F. vorosii*, *F. meridionale* + *F. boothii*, *F. cortaderiae* + *F. brasilicum*, and *F. asiaticum* + *F. graminearum*.
[b] A single strain of *F. acaciae-mearnsii* strain, NRRL 34461 was unique in that it formed asymmetric conidia that are widest above the mid-region.
[c] Strains of the divergent Gulf Coast population of *F. graminearum* were unique in that they produced conidia with a narrow apical beak.

from the upper Midwest of the U.S. *F. vorosii* and *F. gerlachii* meet the requirements for species recognition under the highly conservative genealogical non-discordance criterion for GCPSR (Dettman et al., 2003a), in which a clade is recognized as a species when supported as reciprocally monophyletic by bootstrap analyses in some or all independent gene genealogies, and when monophyly was not contradicted by bootstrap analyses in any other data partition. While this operational criterion for GCPSR may be overly conservative (Pringle et al., 2005) because not all gene trees can be expected to accurately reflect evolutionary history (see Koufopanou et al., 1997; and Ward et al., 2002 for examples), species recognition under this operational criterion indicates an extended history of genetic isolation.

By way of contrast, based on the level of shared DNA polymorphism in three of the eight allelic genealogies, the available data suggests that the divergent U.S. Gulf Coast isolates and *F. graminearum* may be conspecific. The divergent Gulf Coast population of *F. graminearum* was originally detected by DNA sequence analyses of isolates from leatherleaf fern (*R. adiantiformis*) grown commercially in Florida (O'Donnell et al., 2000, 2004), and subsequently as rare NIV and 3ADON-producing wheat head blight isolates in Louisiana (Table 4), using a multiplex assay for trichothecene chemotype (Ward et al., 2002). We interpret similar evidence of potential gene flow to characterize NRRL 34197 (= FRC R-4339) from North Queensland, Australia and NRRL 34461 (= FRC R-8601) from South Africa as genetically divergent members of *F. acaciae-mearnsii*. Although GCPSR based on a majority-rule criterion of genealogical exclusivity has previously been adopted (Baum and Shaw, 1995; Pringle et al., 2005), the ambiguous or conflicting results provided by the current data limit inferences regarding species limits for these two groups of isolates. Therefore, we conservatively recognize these isolates as part of genetically diverse *F. graminearum* and *F. acaciae-mearnsii* clades. However, additional sampling and molecular evolutionary analyses will be required to investigate further species limits and the basis for genealogical discord for *F. acaciae-mearnsii* and the Gulf-Coast population of *F. graminearum*.

Results of the present study extend our knowledge of the biogeography and toxin potential of the *Fg* complex (Tables 1 and 4). Our working hypothesis is that *F. gerlachii*, and the divergent population of *F. graminearum*, may be endemic to the upper Midwest and the Gulf Coast of the U.S., respectively. While neither presently cause significant levels of FHB within the U.S., the fact that *F. gerlachii* produces nivalenol, and members of the Gulf Coast population are segregating for all three trichothecene chemotypes (Table 4), is of notable concern because at present, *F. graminearum* isolates with a 15ADON chemotype represent the predominant cause of FHB in North America (Miller et al., 1991). Nivalenol has been shown to be considerably more toxic to humans than 15-acetyldeoxynivalenol (Mirocha et al., 1985; Ueno and Ishii,

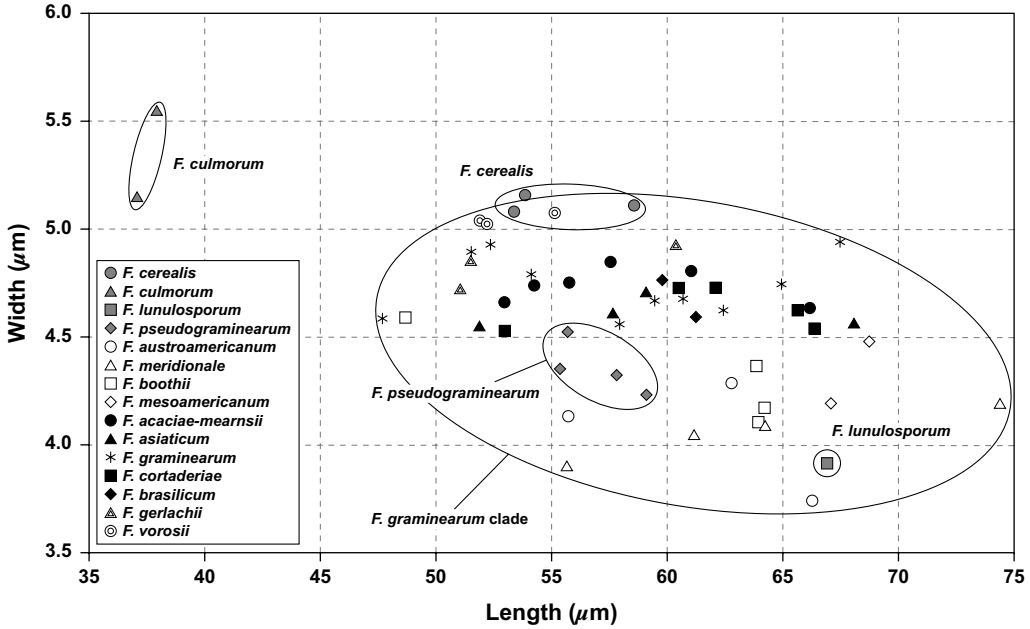


Fig. 3. Length and width of 5-septate conidia of the two newly discovered species cultured under continuous black light together with the previously described species within the *Fg* complex and representative strains of *F. cerealis*, *F. culmorum*, *F. lunulosporum* and *F. pseudograminearum*. The two new species are represented by symbols with double-lines. The species described previously can be divided into two groups based on average width of conidia, represented by white and black symbols. The two new species, *F. gerlachii* and *F. vorosii*, form conidia that are broader than other species within the *Fg* complex. Conidial sizes of *F. vorosii* isolates are similar to *F. cerealis.* However, conidia of *F. vorosii* are morphologically distinct in that they are typically straight, asymmetric in the upper and lower halves, and typically widest above the mid-region. Overlapping average values of conidial length and width for individual strains show this character cannot be used for species recognition.

1985). In addition, trichothecene chemotype variation has been maintained by selection and appears to have adaptive potential for FHB pathogens (Ward et al., 2002).

Each of the two newly described species contained individuals that, in growth chamber tests, caused head blight disease on wheat indistinguishable from disease caused by an aggressive *F. graminearum* strain from the U.S. (Table 4). Variation in aggressiveness was noted among individual strains within each species, consistent with variation noted for other species of the *Fg* complex (Goswami and Kistler, 2005). However, the three strains of each newly discovered species were found to represent only a single chemotype: NIV for the three *F. gerlachii* strains and 15ADON for the *F. vorosii* strains. As additional isolates are examined, we anticipate that other chemotypes may be found for both new species. Despite their ability to cause disease in greenhouse experiments, the potential for either species to cause significant levels of FHB in the field is unknown.

Hierarchical relationships of species within the *Fg* complex inferred from the multilocus phylogeny (Fig. 1) clearly reflect the genealogical descent of their genomes in genetic and reproductive isolation of one another on an evolutionary timescale (Avise and Wollenberg, 1997). While some level of cross fertility between several species within the *Fg* complex has been reported from laboratory experiments (Bowden and Leslie, 1999), including what we interpret as a *F. asiaticum* × *F. graminearum* interspecific cross used for a genetic map (Jurgenson et al., 2002), any interpretation of these results in the absence of a robust phylogenetic framework is problematic, for a number of reasons. Sexual interfertility is a symplesiomorphic or shared ancestral character and therefore cannot be used as a sole criterion for inferring species boundaries (Rosen, 1979), as evidenced by the discovery that two phylogenetically distinct sister species of *Neurospora* are not isolated reproductively (Dettman et al., 2003b). Secondly, meaningful evaluation of biological species limits via mating experiments requires determination of the fitness and fertility of progeny. Even where the production of fully fit and fertile hybrids can be demonstrated (Arnold, 1997), the frequency of gene flow between natural populations must be sufficient to oppose genetic drift in order to have a significant impact on genetic isolation. In the case of FHB pathogens, these factors have not (Bowden and Leslie, 1999), or can not be evaluated rigorously via laboratory mating experiments, which cannot be extrapolated fully to a natural setting.

By way of contrast, GCPSR has proven to be a pragmatic tool for assessing 'biological' species limits because concordance of multiple gene genealogies provides a means for evaluating the significance of gene flow between groups on an evolutionary timescale (see Taylor et al., 2000 for a more thorough discussion of GCPSR). In addition, GCPSR provides the requisite phylogenetic framework for interpretation of additional comparative studies, including the proper interpretation of sexual crosses conducted in a laboratory as representing intraspecific crosses (between members of the same biological species) or interspecific hybridization. The most detailed

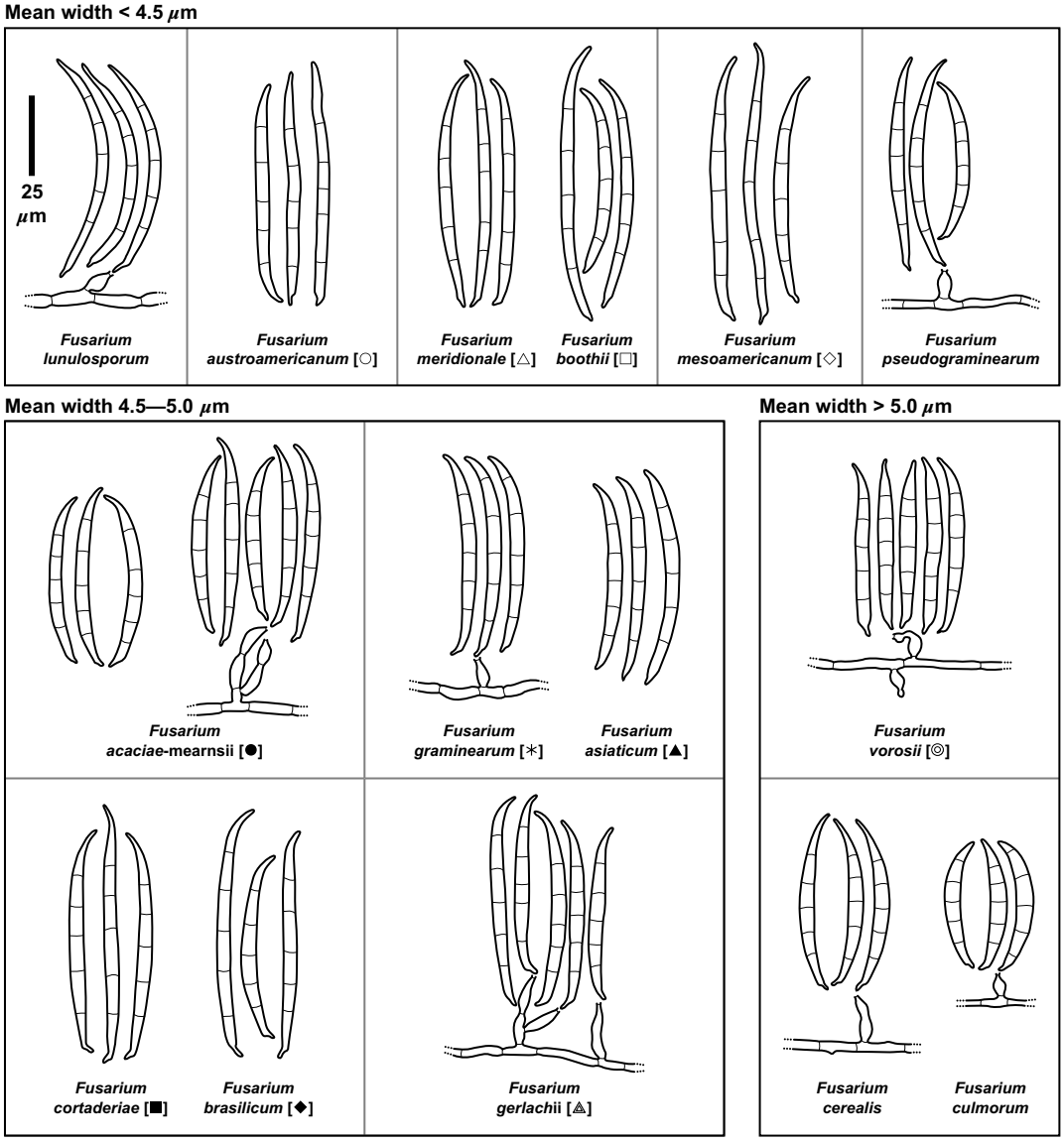


Fig. 4. Conidial morphology most frequently observed for members of the *Fg* complex and four related B-trichothecene clade species, *F. cerealis*, *F. culmorum*, *F. lunulosporum* and *F. pseudograminearum*. Three groups of species can be delimited according to mean width of conidia (indicated by solid black lines). Morphologically distinct species within each of these three groups are separated by a grey line. Symbols in parentheses for species within the *Fg* complex correspond to those in Fig. 3. For the two new species within the *Fg* complex, five typical 5-septate conidia produced from each strain cultured on SNA under black light at 25 °C are illustrated. For the other species, three conidia are drawn from ex-holotype strains, except for *F. graminearum* (NRRL 31084), *F. cerealis* (NRRL 13721) and *F. culmorum* (NRRL 3288). Five 5-septate conidia from a morphologically divergent strain of *F. acaciae-mearnsii*, NRRL 34461 (right) are also illustrated. The following five species and three species groups can be distinguished using these conidial characters within the *Fg* complex: *F. austroamericanum*, *F. mesoamericanum*, *F. acaciae-mearnsii*, *F. gerlachii*, *F. vorosii*, *F. meridionale* + *F. boothii*, *F. cortaderiae* + *F. brasilicum*, and *F. asiaticum* + *F. graminearum*.

genetic analysis of the progeny of an interspecific cross (Jurgenson et al., 2002) reported a number of anomalies that were not observed in the intraspecific cross of *F. graminearum* NRRL 31084 × *F. graminearum* NRRL 34097 used to construct a detailed genetic map (Gale et al., 2005). Hallmarks of the interspecific cross include pronounced segregation distortion in five of the nine linkage groups, chromosomal inversions in two linkage groups, and recombination suppression in four linkage groups (Jurgenson et al., 2002; Gale et al., 2005).

### 4.2. Phenotypic analysis

Two novel species were identified within the *Fg* complex in the present study using GCPSR. The *a posteriori* phenotypic analysis also revealed that the new species within the *Fg* complex could be diagnosed by a combination of conidial characters. Based on morphological analyses, five individual species and three species groups could be distinguished within the *Fg* complex (Table 5). However, due to the limited number of strains currently available for study, we anticipate

that the full range of their infraspecific phenotypic variation is yet to be discovered. Overlapping ranges of morphological features in some species, as seen in *F. graminearum* and *F. acaciae-mearnsii*, also indicate that morphological species recognition within the *Fg* complex will be difficult or impossible. These facts suggest that strong stabilizing selection may be acting on the conidial phenotypes and/or insufficient evolutionary time has transpired for each species to have become differentiated morphologically. Given the ever increasing number of cryptic species being discovered by GCPSR of importance to agriculture and medicine, there is a compelling practical reason and growing trend to use molecular phylogenetic data in species descriptions (Couch and Kohn, 2002; Fisher et al., 2002; O'Donnell et al., 2004), thereby insuring that species names are validly published according to the International Code of Botanical Nomenclature (Greuter et al., 2000).

### 4.3. Conclusions

The results of several recent FHB pathogen surveys suggest that the relatively recent globalization of trade in horticultural and agricultural plants has resulted in the inadvertent introduction of foreign FHB pathogens with novel trichothecene toxin chemotypes into heretofore non-endemic areas (Lee et al., 2004; Martinelli et al., 2004; Monds et al., 2005; O'Donnell et al., 2000, 2004; Tóth et al., 2005), thereby contributing to the rapid reemergence of this economically devastating disease worldwide. Added to this is the concern that these anthropogenic activities may bring together closely related, previously allopatric FHB species that could give rise to novel pathogens following interspecific hybridization (Brasier, 2001; Schardl and Craven, 2003). The present, as well as our prior GCPSR studies (O'Donnell et al., 2000, 2004; Ward et al., 2002), were initiated to provide plant disease management, plant breeders and quarantine officials with a detailed understanding of FHB pathogen species limits, chemotype diversity, host range and their global geographic distribution. Given the morphological crypsis of many FHB species (O'Donnell et al., 2004), the robust multilocus phylogenetic framework and GenBank accessible (http://www.ncbi.nlm.nih.gov/) DNA sequence database developed in the present study should greatly facilitate the development of molecular diagnostic tools for global surveillance programs directed at minimizing the introduction of foreign FHB species with novel toxin potential into non-endemic cereal growing areas. In addition, the molecular evolutionary framework and epidemiological data developed herein provide a unique baseline for monitoring changes in the population dynamics of these cereal pathogens worldwide.

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## Appendix A. Species descriptions

*Fusarium gerlachii* T. Aoki, Starkey, Gale, Kistler, O'Donnell, sp. nov.

Anamorphus ut in speciebus *Fusario graminearum*-cladus in morphologia similis, sed distinguibilis characteribus conidiis. Conidia sporodochialia in SNA sub illuminationem nigram formata, (1-)3-5(-7)-septata; 5-septata 37–51.0–60.4–72.5 × 4–4.71–4.91–6 μm, gradatim curvata, plerumque medio lattissima, cellula apicali plerumque anguste rostrata.

*Fusarium gerlachii* is morphologically similar to *F. graminearum* including colony characters on PDA, but has slightly different conidial features from it and other species within the *Fg* clade (Figs. 3, 4, and Table 4). Five-septate sporodochial conidia formed by *F.gerlachii* on SNA under black light are 4.5–5 μm wide on average (size ranges of total and average values: 37–51.0–60.4–72.5 × 4–4.71–4.91–6 μm; ex type: 50.5–60.4–72.5 × 4–4.91–6 μm), gradually curved and often widest at the mid-region, and frequently with a narrow beak at the apex (Aoki and O'Donnell, 1999; O'Donnell et al., 2004). Also this species can be diagnosed because it fulfills the criterion of genealogical concordance phylogenetic species recognition based on strong monophyly bootstrap support from analyses of reductase, α-tubulin, MAT, *URA–Tri101–PHO*, and the combined partition (Table 3; Dettman et al., 2003a), and because of its strongly supported, reciprocally monophyletic sister-group relationship to *F. graminearum* in the multilocus phylogeny (Fig. 1).

Distribution: USA

HOLOTYPUS: BPI 871657, a dried culture, isolated from a collection of diseased spring wheat heads (*Triticum aestivum* L.) in a commercial field, Climax, Polk County, MN, U.S.A., Liane Gale, 25 July 2000, deposited in the herbarium of BPI, U. S. A. Ex holotype culture: NRRL 36905 = LRG 00-551. Other strains examined: NRRL 38380 = LRG 02-224, NRRL 38405 = LRG 3ND7-17.

Etymology: *gerlachii*; after the German fusariologist, Wolfgang Gerlach.

*Fusarium vorosii* B. Tóth, Varga, Starkey, O'Donnell, Suga, T. Aoki, sp. nov.

Anamorphus ut in speciebus *Fusario graminearum*-cladus in morphologia similis, sed distinguibilis characteribus conidiis. Conidia sporodochialia in SNA sub illuminationem nigram formata, (1-)3-5(-7)-septata; 5-septata 42–51.9–55.1–70 × 4.5–5.02–5.08–6 μm, typice recta, nonnumquam gradatim curvata, in dimidio superiore plerumque leviter expansa.

*Fusarium vorosii* is morphologically similar to *F. graminearum* including colony characters on PDA, but has slightly different conidial features from it and other species within the *Fg* clade (Figs. 3, 4, and Table 4). Five-septate sporodochial conidia formed by *F. vorosii* on SNA under black light are more than 5 μm wide on average (size ranges of total and average values: 42–51.9–55.1–70 × 4.5–5.02–5.08–6 μm; ex type: 47.5–55.1–70 × 4.5–5.08–6 μm), typically straight but sometimes gradually curved and frequently widest above the mid-region (Aoki and O'Donnell, 1999; O'Donnell et al., 2004). In addition, this species can be diagnosed because it fulfills the criterion of genealogical concordance phylogenetic species recognition based on strong monophyly bootstrap support from analyses of β-tubulin, histone H3, reductase, α-tubulin, MAT, *URA–Tri101–PHO*, and the combined partition (Table 3; Dettman et al., 2003a), and because of its strongly supported, reciprocally monophyletic sister-group relationship to *F. asiaticum* in the multilocus phylogeny (Fig. 1).

Distribution: Hungary and Japan

HOLOTYPUS: BPI 871658, a dried culture, isolated from a spikelet of *T. aestivum* L. (wheat), Ipolydamásd, Pest County, Hungary, G. Giczey and L. Hornok, December 2002, deposited in the herbarium of BPI, U. S. A. Ex holotype culture: NRRL 37605 = TÓTH FgHF012. Other strains examined: NRRL 38207 = Suga 0301112, NRRL 38208 = Suga 0301831.

Etymology: *vorosii*; after the Hungarian plant pathologist, József Vörös.

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