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A novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from the Russian Far East

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Abstract: We investigated *Fusarium graminearum* complex (*Fg* complex) species diversity and toxin potential in European and Asian regions of the Russian Federation and adjoining regions northwest to Finland and south near Harbin, Heilongjiang Province, China, to expand our knowledge of the host range and geographic distribution of these economically devastating cereal head blight pathogens. Results of a recently described multilocus genotyping (MLGT) assay revealed that *F. graminearum* was the sole *Fg* complex pathogen in northern Europe and the predominant one in Asia (90.5%). Even though isolates of *F. graminearum* were segregating for 3-acetyldeoxynivalenol (3ADON) and 15-acetyldeoxynivalenol (15ADON) chemotype in nearly equal frequencies in the regions sampled on both continents, significant differences in the geographic distribution of isolates producing these acetyl ester derivatives of deoxynivalenol (DON) were observed in Europe. While 93.5% of the isolates in southern Russia (n = 43 of 46) possessed the 15ADON chemotype, isolates of *F. graminearum* recovered in Finland and northwestern Russia (n = 40) were exclusively 3ADON producers. Based on results of the MLGT assay, species identity of 10 genetically novel *Fg* complex isolates from the Russian Far East was investigated further via molecular phylogenetic analyses of multilocus DNA sequence data. Results of these analyses resolved these isolates as a phylogenetically distinct, reciprocally monophyletic sister lineage of *F. asiaticum*, which together with *F. vorosii* form a newly discovered Asian clade within the *Fg* complex. Because this novel lineage fulfills the highly conservative criterion of genealogical exclusivity under phylogenetic species recognition it is formally described herein as *F. ussurianum*. In addition to morphologically characterizing isolates of *F. ussurianum*, experiments were conducted to assess pathogenicity to wheat and trichothecene toxin potential in planta.

Key words: deoxynivalenol, genotyping, mycotoxin, nivalenol, phylogenetics, trichothecene

INTRODUCTION

Fusarium head blight (FHB) or scab of small grain cereals re-emerged over the past two decades to become one of the most economically devastating plant diseases throughout the world (Goswami and Kistler 2004). FHB is caused by a complex of species, many of which contaminate grain with estrogenic and trichothecene mycotoxins, resulting in substantial losses due to reduction in yield and price-discounted toxin-contaminated grain. Mycotoxin-contaminated grain poses a significant threat to global food safety because these toxins have been linked to mycotoxicoses of humans and livestock (Peraica et al 1999). Trichothecenes are an additional concern to human and animal health because they inhibit eukaryotic protein synthesis (Ueno et al 1973) and alter immune function (Pestka and Smolinski 2005). In addition some acutely phytotoxic trichothecenes function as virulence factors on sensitive cereal hosts (Jansen et al 2005). Remarkably three strain-specific B-type trichothecene chemotypes, nivalenol (NIV), 3-acetyldeoxynivalenol (3ADON) and 15-acetyldeoxynivalenol (15ADON), have been maintained by strong balancing selection within the B trichothecene toxin-

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producing clade (Ward et al 2002, hereafter referred to as the B-clade), where trichothecene chemotype polymorphism has been demonstrated in more than half the species.

In response to the growing threat to the world's food supply prompted by recent outbreaks and epidemics of FHB, pathogen surveys have been launched in Europe (Waalwijk et al 2003; Gagkaeva and Yli-Mattila 2004; Jennings et al 2004a, b; Láday et al 2004; Yli-Mattila et al 2004; Tóth et al 2005; Qu et al 2008), Asia (Gale et al 2002, Lee et al 2004, Ji et al 2007, Qu et al 2007, Zhang et al 2007, Suga et al 2008, Yang et al 2008) and North America (Gale et al 2007, Ward et al 2008). They have revealed that *F. graminearum* and other members of the *Fg* complex are the primary etiological agents of FHB worldwide. Although the morphospecies *F. graminearum* was thought until recently to represent a single panmictic species, phylogenetic species recognition, with genealogical concordance (GCPSR, Taylor et al 2000), has provided strong molecular evolutionary genetic evidence that *F. graminearum sensu lato* comprises as least 13 biogeographically structured and phylogenetically distinct species (O'Donnell et al 2000, 2004, 2008; Starkey et al 2007).

Given the significant limitations of morphological species recognition within *Fusarium* in general and within the *Fg* complex in particular (O'Donnell et al 2008), the primary focus of the present study was to establish a baseline of *Fg* complex species and trichothecene chemotype diversity within European and Asian regions of the Russian Federation and adjoining regions in Finland and China employing a recently described microsphere-based multilocus genotyping assay (MLGT, Ward et al 2008). Results of this assay, together with multilocus molecular phylogenetic analyses, identified a novel phylogenetically distinct species within the Russian Far East, which satisfied the highly conservative requirements of GCPSR using reciprocal monophyly as the exclusivity criterion. Herein this B-FHB pathogen is formally described as a novel species within a newly discovered Asian clade within the *Fg* complex, supported by phenotypic and multilocus molecular phylogenetic data. In addition experiments were conducted to assess whether this novel pathogen could induce FHB on wheat and produce trichothecenes in planta.

MATERIALS AND METHODS

Strains and multilocus genotyping.—Histories for the 223 *Fg* complex isolates included in this study are provided (SUPPLEMENTAL TABLE I). All isolates were single-spored and identified morphologically as *Fusarium graminearum* (Nirenberg 1981, Aoki and O'Donnell 1999). Isolates were subjected to a multilocus genotyping assay (MLGT) for species determination and trichothecene chemotype prediction with a Luminex 100 flow cytometer as described by Ward et al (2008). Most isolates were obtained from FHB-contaminated wheat (n = 155), barley (n = 50) and oat (n = 13) seed collected 1986–2006 in Finland and European Russia (n = 96), and Asian Russia and near Harbin in northeastern China (n = 127).

Protocols for assessing morphological phenotype have been described elsewhere in detail (Aoki and O'Donnell 1999, O'Donnell et al 2004). A dried culture of the new species (NRRL 45681 = TG-2662/0) has been deposited in the U.S. National Fungus Collection (BPI), USDA/ARS, Beltsville, Maryland) as the holotype (BPI 878845). Cultures are available from the ARS Culture Collection (NRRL) and the Centraalbureau voor Schimmelcultures (CBS) Fungal Diversity Center.

Phylogenetic analysis of species boundaries.—Results of the MLGT assay indicated that 10 isolates identified morphologically as *F. graminearum* from the Russian Far East were members of the *Fg* complex but represented novel genetic variation unaccounted for by the species probes. Six of these isolates were collected near Ussuriysk (NRRL 45676, 45684, 45693, 45697, 45833 all ex wheat and NRRL 45681 ex oat), two in Kamen-Ribolov (NRRL 45792 and 45795 both ex wheat) and two in the Jewish Autonomous Region (JAR, NRRL 45665 ex wheat and 45667 ex barley). Phylogenetic analyses of partial *EF-1α* and 3-*O*-acetyltransferase (*Tri101*) sequences of these 10 isolates, combined with 63 sequences representing all known B-clade species (O'Donnell et al 2008) and the previously described Gulf Coast population of *F. graminearum* (Starkey et al 2007), indicated that these 10 isolates might represent a novel *Fg* complex species. To fully assess their evolutionary relationships and species identity four isolates from the putatively novel set of 10 isolates were selected for multilocus DNA sequencing (13 genes totaling 16.3 kb), employing reciprocal monophyly as the exclusivity criterion under GCPSR (Taylor et al 2000).

Maximum parsimony (MP) and maximum likelihood (ML) analyses of the individual and combined partitions were conducted respectively in PAUP* 4.0b10 (Swofford 2002) and GARLI (Zwickl 2006) as described by O'Donnell et al (2008). Results of these analyses demonstrated that the four isolates represent a novel phylogenetically distinct species within the *Fg* complex. Multilocus DNA sequence data have been deposited in GenBank under accession numbers FJ240230-FJ240349 and TreeBASE as SN4208. Other sequences included in the present study were deposited in GenBank (O'Donnell et al 2000, 2004, 2008; Starkey et al 2007, Ward et al 2002).

Pathogenicity, mycotoxin production and phenotypic analyses.—Pathogenicity experiments conducted to determine whether isolates of the new species could induce FHB symptoms on wheat followed published protocols (Goswami and Kistler 2005, Starkey et al 2007), using the highly susceptible hard red spring wheat cultivar Norm as host. Ten spikes from separate plants were inoculated per replication, and three replications were conducted for each

strain. The fifth, fully formed spikelet from the base of each flower was inoculated by introducing 10 000 spores in 10 μL water. After inoculation plants were placed in a humidity chamber for 72 h and transferred to a growth cabinet at 20 C. Pathogenicity was assessed by the number of symptomatic plant spikelets (i.e. showing necrotic lesions and/or blighting) 14 d after inoculation. In all pathogenicity experiments *Fusarium graminearum* strain NRRL 31084 (= PH-1) and *F. verticillioides* NRRL 20956 were used respectively as positive and negative controls for induction of FHB symptoms and mycotoxin production in planta. Analyses of trichothecene toxin production in planta have been described by Ward et al (2002) and O'Donnell et al (2004).

RESULTS

Molecular surveillance.—A MLGT assay described by Ward et al (2008) was used to investigate FHB species and trichothecene chemotype diversity among 223 *Fg* complex isolates collected 1986–2006 in Finland and European Russia (n = 96), as well as the Russian Far East and near Harbin, Heilongjiang Province, China (n = 127) (FIG. 1, SUPPLEMENTAL TABLE I). Results of the MLGT assay indicated that *F. graminearum* was the sole *Fg* complex species in northern Europe and the predominant one in Asia (90.5%). Although 15ADON and 3ADON chemotype frequencies for *F. graminearum* were nearly identical on both continents, 15ADON-producing isolates of *F. graminearum* were represented at a slightly higher frequency in Europe (54.2%) and Asia (53.9%) than 3ADON-producing isolates of this species. NIV-producing isolates of *F. graminearum* were not recovered in this survey. In addition the two isolates of *F. vorosii* and 10 isolates of the novel species within the *Fg* complex from the Russian Far East represented respectively the 15ADON and 3ADON chemotype. Some significant (Fisher's exact test; $P < 0.001$) regional differences were observed in chemotype frequencies in Europe. All isolates of *F. graminearum* (n = 15) from Finland and from St Petersburg and Kaliningrad in northwestern Russia (n = 25) were 3ADON, for example, whereas 93.5% (n = 43 of 46) of *F. graminearum* from North Ossetia-Alania and Krasnodar in southern Russia possessed the 15ADON chemotype, suggesting that different *F. graminearum* populations might predominate in these regions.

Fusarium graminearum isolate NRRL 45659, collected in 2002 from blighted wheat in the JAR in the Russian Far East, produced discordant results with the MLGT probes that target trichothecene chemotype-specific variation in the *TRI3* and *TRI12* biosynthetic genes near either end of the gene cluster (TRI-cluster; Ward et al 2002, 2008). This isolate yielded a positive signal with the 15ADON-specific probe

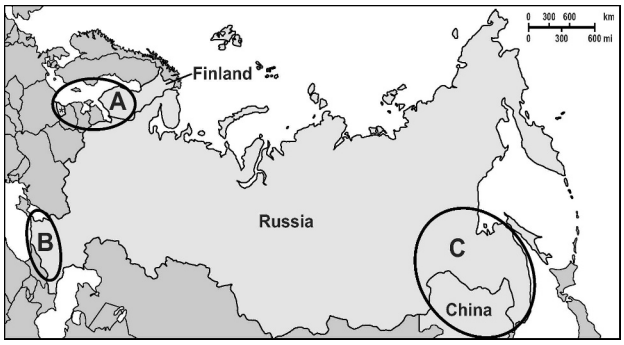

FIG. 1. The major regions of sample collection in Finland and northwestern Russia (A), southern Russia (B) and the Russian Far East and China (C). The noncontiguous region of Russia, which includes the Kaliningrad sampling area is marked with an asterisk to the far left in A. In addition to the 213 isolates collected from these major sampling areas, we also examined 10 isolates from a region of central Russia near the Ukrainian border.

targeting *TRI3*, which is essential for C-15-acetylation, and the 3ADON-specific probe targeting *TRI12*, a MFS transporter. Because the MLGT probe data suggested interchemotype recombination involving the genes that determine the trichothecene chemotype, genes on both ends of the gene cluster, including *TRI3* and *TRI12*, were sequenced in NRRL 45659 and analyzed phylogenetically. Results of these analyses (not shown) revealed that *TRI3*, *TRI7* and *TRI8* on the left side of the TRI-cluster possess 15ADON clade-specific alleles whereas 3ADON clade-specific alleles were possessed by *TRI11* and *TRI12* on the right side of the cluster. Because *TRI3* and *TRI8*, a C-3 deacetylase, play an essential role in the production of 15ADON (Kimura et al 2003) the recombinant isolate NRRL 45659 was predicted to synthesize 15ADON in planta. As predicted *F. graminearum* NRRL 45659 produced 15ADON as the primary acetyl ester derivative of deoxynivalenol in planta when spray-inoculated on heads of the highly susceptible hard red spring wheat cultivar Norm.

Phylogenetic analyses.—Results of the MLGT screen for *Fg* complex species diversity identified 10 isolates collected 2002–2006 in Kamen-Ribolov, the JAR and near Ussuriysk in eastern Russia, which were novel in that they gave positive signals for the MLGT B-clade and *Fg* complex probes but yielded negative results for all *Fg* complex species probes (Ward et al 2008). Species identity of these 10 isolates was evaluated further by maximum parsimony (MP) analyses of partial 3-*O*-acetyltransferase (*Tri101*) and translation elongation factor (*EF-1α*) gene sequences. Results of the MP analyses indicated that these 10 isolates might

TABLE I. Tree statistics and summary of phylogenetic analyses[a]

Locus[b]	Number characters	Number MPT	MPT length	CI	RI	Syn	Aut	Bootstrap support (%) *F. ussurianum*	Number of *Fg* species supported as monophyletic[c]
α-Tubulin (1)	1686	129	119	0.94	0.97	71	41	95	8
β-Tubulin (4)	1262	>20 000	112	0.85	0.79	53	31	92	5
EF-1α (2)	648	>20 000	89	0.91	0.97	58	20	95	6
Histone H3 (2)	449	18	72	0.80	0.95	41	17	81	5
MAT (2)	5910	46	972	0.84	0.94	524	246	100	11
URA-Tri101-PHO (4)	4124	2400	706	0.80	0.91	379	160	100	11
ITS-28S rDNA (4)	1133	2	23	0.96	0.97	8	14	<70	0
Reductase (2)	1141	8287	266	0.87	0.91	140	75	92	10
Combined	16 353	252	2501	0.79	0.91	1274	604	100	13

[a] Abbreviations used: MPT, most parsimonous trees; CI, consistency index; RI, retention index; Syn, synapomorphy or parsimony informative character; Aut, autapomorphy, uniquely derived character or parsimony uninformative character.

[b] The number in parenthesis indicates the chromosome location in the whole genome sequence of NRRL 31084 = PH-1 *F. graminearum* (Cumo et al 2007).

[c] MP bootstrap support ≥ 70%.

represent a phylogenetically distinct species within the *Fg* complex. To rigorously assess their species status and evolutionary relationships a 13-gene dataset (16.3 kb/isolate) was constructed for four isolates of the putatively new *Fg* complex species. This set included two isolates collected in 2002 (NRRL 45665 ex wheat seed in JAR and NRRL 45681 ex oat seed near Ussuriysk) and two isolates collected in 2006 (NRRL 45795 ex wheat seed in Kamen-Ribolov and NRRL 45833 ex root rot of wheat near Ussuriysk). The eight individual data partitions and combined 13-gene dataset were analyzed phylogenetically with MP and ML (TABLE I). In the combined multilocus phylogeny (FIG. 2), four 15ADON-producing isolates of *F. vorosii*, formed a strongly supported (MP/ML bootstrap = 100%) sister group of a clade (MP and ML bootstrap = 86% and 89% respectively) containing *F. asiaticum* (MP/ML bootstrap = 100%) and a sister lineage containing the four novel *Fg* complex isolates collected in the Russian Far East (MP/ML bootstrap = 100%). Moreover the genealogical exclusivity (monophyly) of the lineage containing the four isolates was strongly supported (MP bootstrap > 80%) in genealogies inferred from seven of the eight individual partitions (TABLE I). Although evolutionary relationships within the nuclear rDNA partition were unresolved, analyses of this partition did not contradict the monophyly of this novel species relative to other members of the *Fg* complex. Results of the phylogenetic analyses show that this novel taxon fulfills GCPSR's highly conservative exclusivity criterion of reciprocal monophyly (Taylor et al 2000), thereby providing strong support for the formal recognition of these isolates as a novel species within the *Fg* complex. Because the reciprocal monophyly observed in the individual gene partitions is indicative of genetic isolation on an evolutionary timescale, this novel species is formally described herein as *Fusarium ussurianum*. In addition the MP analyses also confirmed the MLGT identification of NRRL 45800 ex head blight of barley from Kamen-Ribolov collected in 2005 and NRRL 45790 ex diseased wheat near Ussuriysk collected in 2006 as *F. vorosii*, the recently described sister species of *F. asiaticum* (Starkey et al 2007). We did not include sequences of NRRL 45790 *F. vorosii* in the combined analysis of the 13-gene dataset because 220 bp at the 5′ end of the β-tubulin partition was missing.

The four isolates of *F. ussurianum* included in a pathogenicity experiment induced head blight on the highly susceptible hard red spring wheat cultivar Norm (TABLE II). Of the isolates tested for pathogenicity only NRRL 45681 *F. ussurianum* was significantly less aggressive on wheat than the positive control strain NRRL 31084 (PH-1) *F. graminearum* from USA. In addition trichothecene metabolite profiles obtained from isolates of *F. ussurianum* demonstrated that 3ADON was the primary acetyl ester derivative of deoxynivalenol elaborated in planta, as predicted by results of the MLGT assay. The least aggressive isolate of *F. ussurianum*, NRRL 45681, also produced the lowest levels of DON and 3ADON in planta (TABLE II, Goswami and Kistler 2005).

The MLGT assay for species identification and prediction of trichothecene chemotype (Ward et al 2008, O'Donnell et al 2008) was updated by incorporating two novel probes for identification of *F. ussurianum* strains. The probes, ATu(25), 5′-CTTTTCAATTACTTCAAATCTTCAGATGTAGCTG-

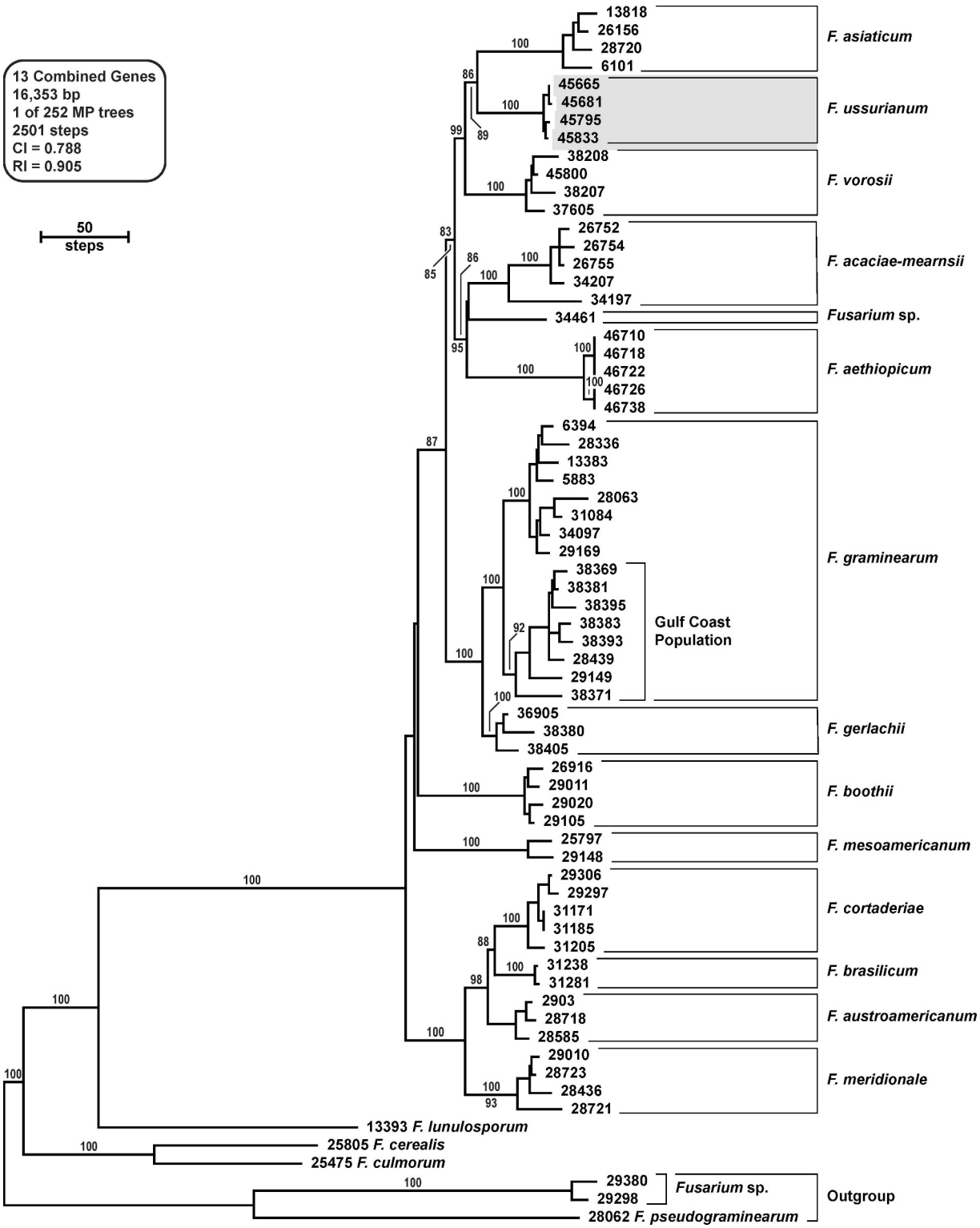

FIG. 2. Multilocus phylogeny of species within the B-type trichothecene toxin-producing clade, including members of the *Fusarium graminearum* species complex. One of 252 most parsimonious phylograms inferred from the combined dataset of 16.3 kb from eight loci comprising portions of 13 nuclear genes. Sequences of *Fusarium pseudograminearum* NRRL 28062 and *Fusarium* sp. 29380 and 29298 were used to root the phylogeny. Maximum parsimony (MP) bootstrap values ≥ 80% are indicated above branches based on 1000 replicates of the data. Maximum likelihood (ML) bootstrap values ≥ 80% are indicated below branches if they differed by ≥ 5% from the MP bootstrap value, except for the node supporting *F. asiaticum* and *F. ussurianum* as sister taxa. A genetically distinct Gulf Coast population of *F. graminearum* is indicated.

TABLE II. Disease severity and trichothecene accumulation in wheat inoculated with *F. ussurianum*

Species	Strain	Disease[b]	Mean trichothecene concentration (ppm)[a]		
			DON	15ADON	3ADON
F. graminearum	31084	9.9	281.0	31.0	4.9
F. ussurianum	45665	9.9	641.7	3.1	68.2
F. ussurianum	45681	5.1[c]	537.2	2.2	49.1
F. ussurianum	45795	9.9	631.9	2.2	60.0
F. ussurianum	45833	9.7	805.4	2.9	84.0
F. verticillioides	20956	0[d]	nd	nd	nd

[a] Mean concentration of trichothecene in inoculated wheat spikelets, 14 d after inoculation (dai). nd = not detected. Nivalenol was detected at no more than trace amounts (<2 ppm) for all samples.

[b] Mean number of symptomatic spikelets, 14 dia.

[c] Disease index significantly less than *F. graminearum* strain 31084 ($P < 0.05$), 14 dai.

[d] Disease not detected on intact plants, 14 dai. Slight necrotic flecking (ca. 1 mm^2) was noted internally on some plants upon dissection of inoculated spikelets.

GTGGTGAT-3′ and REDu(27), 5′-CTTTTCAAATCAATACTCAACTTTCATCACGTGTCAACCAGC-3′ were developed from variation identified respectively in the *TRI101* and reductase genes. Each probe was appended with a unique sequence tag (underlined) used to sort extension products via hybridization with fluorescent microspheres (Luminex Corp.) as described by Ward et al (2008). The expanded MLGT assay was run against an isolate panel consisting of the 10 *F. ussurianum* isolates and 57 isolates representing

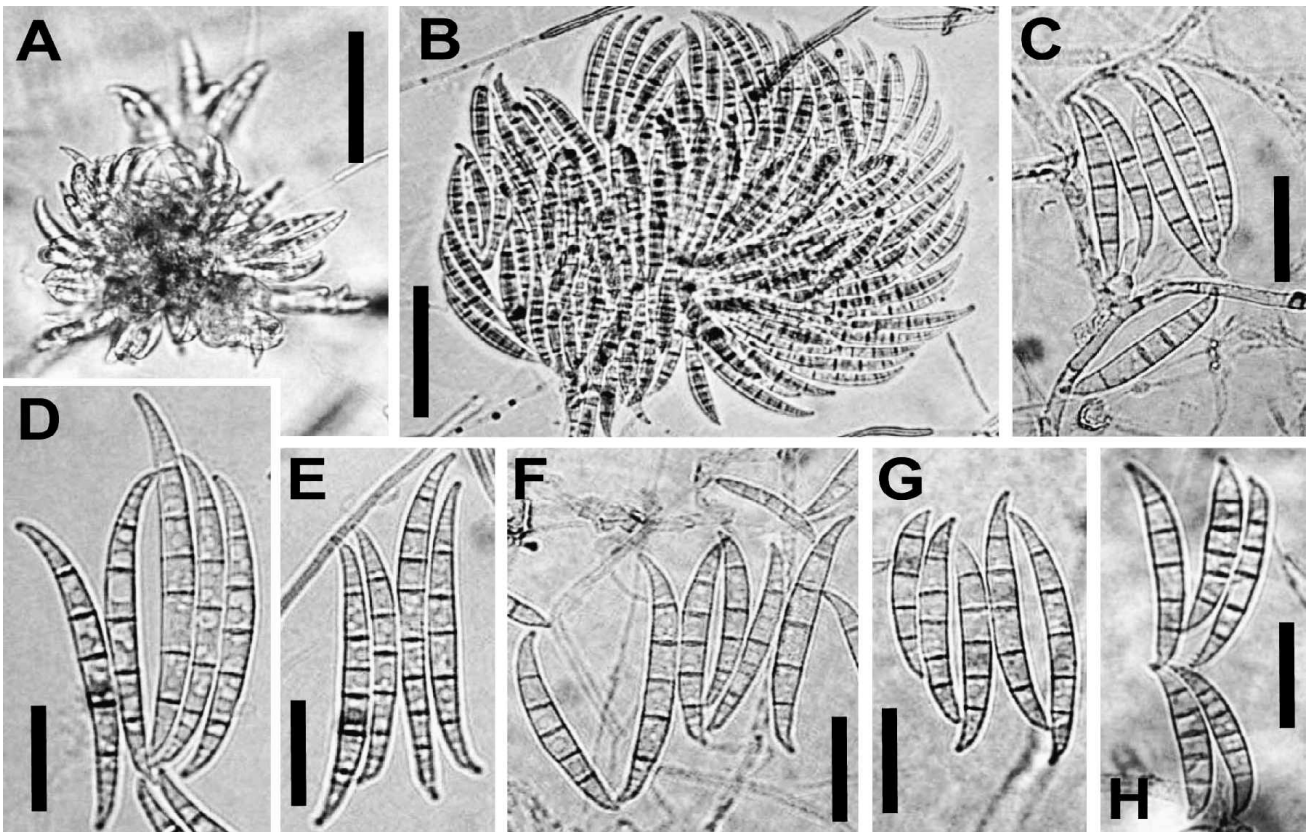

FIG. 3. Conidial morphology of *Fusarium ussurianum* formed on SNA under black light. A–B. Sporodochial conidia produced in and on the surface of agar (NRRL 45681 ex-holotype). C. Conidia formed from phialides on a short lateral branch of a running hypha. D–H. Sporodochial conidia formed by different strains of *F. ussurianum*. D–E. NRRL 45681 (ex-holotype). F–G. NRRL 45833. H. NRRL 45795. Bars: A–B = 50 μm. C–H = 20 μm.

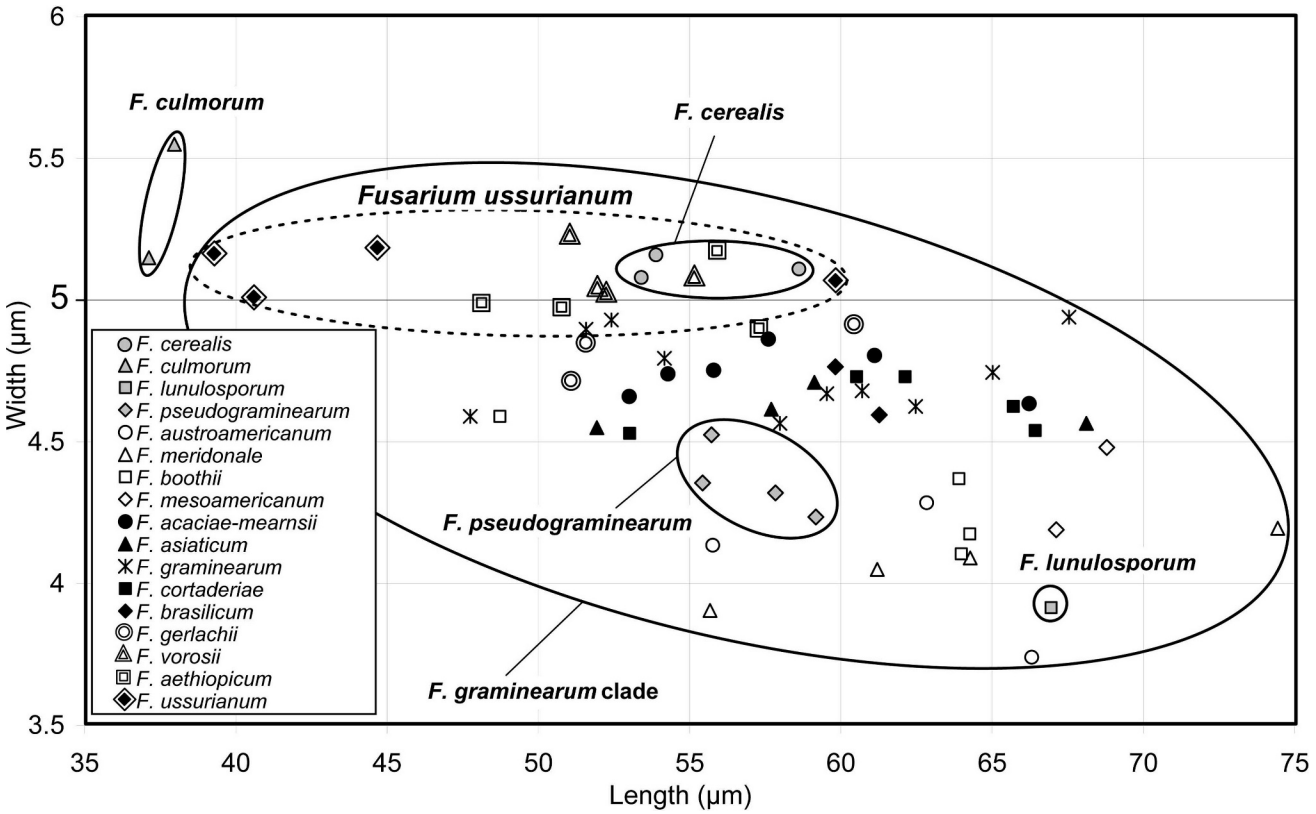

FIG. 4. Width and length of 5-septate conidia of B-clade species cultured under continuous black light. Isolates of *F. ussurianum* are indicated by a solid diamond with a white border. Note that the dotted oval circumscribing the four isolates of this species is used to graphically illustrate conidial dimensions cannot be used to differentiate this species.

all other species within the B-trichothecene lineage of FHB pathogens used in the design and validation of previous versions of the MLGT assay (Ward et al 2008, O'Donnell et al 2008). Index of discrimination values (Ward et al 2008) for ATu(25) and REDu(27) were 7.0 and 9.1 respectively, meaning that fluorescence intensity values for these probes were at least sevenfold higher among isolates of *F. ussurianum* than the highest values observed among all other B-clade species.

TAXONOMY

Fusarium ussurianum T. Aoki, Gagkaeva, Yli-Mattila, Kistler, O'Donnell, sp. nov.

MycoBank MB 512612 FIGS. 1–5

Coloniae obscuritate 20 C in agaro PDA dicto 2.8–10.1 mm in dies crescent, rubrum, brunneo-rubrum, griseo-rubrum, rubro-album, griseo-brunneum vel album; coloniae reversum intense rubrum vel rubro-brunneum. Mycelium aerium in agaro PDA plerumque copiosum, laxe vel dense floccosum, album vel rubro-album vel griseo-brunneum. Chlamydosporae et sclerotia absentes. Sporulatio in agaro SNA dicto sub illuminationem nigram fere praecox et copiosa, obscuritate retardata et pauca, Conidiophora vel ex hyphis oriunda vel in sporodochiis in superficie substrati aggregata; verticillata vel simplicia. Phialides subulatae, ampulliformes vel subcylindricae, monophialidicae. Conidia monomorphica, typice falcata et curvata, dorsiventralia, plerumque latissima in medio aliquanto superiore, utrinque aequaliter angustatae, nonnumquam fere cylindrica et leniter curvata, cellulae apicali arcuatae, cellulae basilari pediformi, (1–)3–5(–7)-septata; conidia 5-septata sub illuminationem nigram 30–39.3–59.8–75.5 × 4–5–5.2–6 µm.

Colonies in darkness at 20 C on PDA show average growth of 2.8–10.1 mm per d. Colony color on PDA red, pastel-red, brownish-red, grayish-red, reddish-white, grayish-brown, brownish-yellow to white; reverse pigmentation red, deep-red, reddish-brown, brownish-red, brownish-violet, ruby, reddish-white to white. Colony margin entire to undulate. Odor absent or moldy. Aerial mycelium on PDA generally abundant, some sparsely developed, loose to densely floccose, white, reddish-white, brownish-yellow, brownish-orange to grayish-brown. Hyphae on SNA 1.5–7 µm wide. Chlamydospores and sclerotia absent but some globose hyphal swelling sometimes present, intercalary or occasionally terminal. Sporulation on SNA under black light quick and abundant, starting within a few days from conidiophores formed directly on hyphae or aggregated in sporodochia on the agar

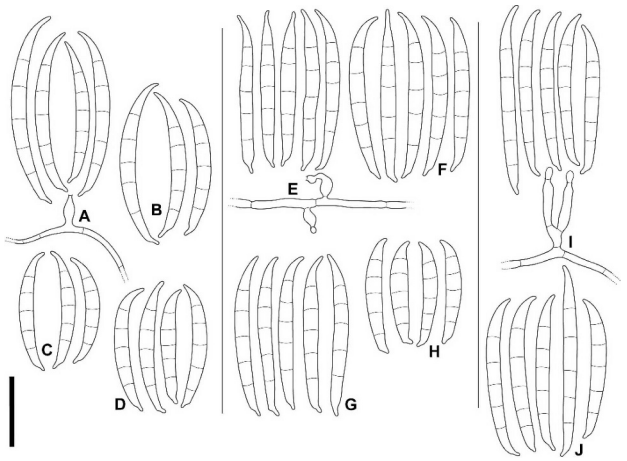

FIG. 5. Morphological comparison of 5-septate conidia formed by isolates of *Fusarium ussurianum*, *F. vorosii* and *F. aethiopicum* on SNA under black light. These three species produce conidia that are wider than 5 μm on average. A–D. *Fusarium ussurianum*. A. NRRL 45681 (ex-holotype). B. NRRL 45665. C. NRRL 45795. D. NRRL 45833. E–H. *Fusarium vorosii*. E. NRRL 37065 (ex-holotype). F. NRRL 38207. G. NRRL 38208. H. NRRL 45800. I–J. *Fusarium aethiopicum*. I. NRRL 46726 (ex-holotype). J. NRRL 46738. A, E, I. conidia formed on phialides. Conidia of *F. ussurianum* are typically curved and the upper and lower halves are nearly symmetrical. Conidia of *F. vorosii* and *F. aethiopicum* in contrast are typically straight and asymmetrical. Bar = 25 μm.

surface; in darkness sporulation retarded and sparse; sporodochia sparse or abundant. Conidiophores branched verticillately or unbranched, forming monophialides on the apices or abbreviated as single monophialides formed on running hyphae. Phialides simple, subulate, ampulliform to subcylindrical, sometimes doliiform, monophialidic. Conidia of a single type, typically falcate and curved, dorsiventral, most frequently widest slightly above the mid-region of their length, mostly tapering and curving equally toward both ends, with an arcuate apical cell and a distinct basal foot cell, forming symmetrical upper and lower halves, (1–)3–5(–7)-septate. In some strains conidia were observed that were almost cylindrical and gently curved. Under black light, 5-septate conidia: 30–75.5 × 4–6 μm in total range, 39.3–59.8 × 5.0–5.2 μm on average, (ex-type strain: 45.5–59.8–75.5 × 4–5.1–6 μm).

HOLOTYPUS: BPI 878845, a dried culture, isolated from an oat seed, *Avena sativa* L. in a field near Ussuriysk, Primorsky krai, Russian Federation, in 2002, deposited in herbarium BPI (US National Fungus Collection, Beltsville, Maryland). Ex holotype culture: NRRL 45681 = TG-2662/0 = CBS 123752.

Other strains examined. NRRL 45665 = TG-2981/3 = CBS 123751 ex wheat seed in JAR, Russian Federation, in 2002; NRRL 45795 = TG-65202 = CBS 123753 ex wheat seed in Kamen-Ribolov, Russian Federation, in 2006; and NRRL 45833 = TG-k.3–6 = CBS 123754 ex rot of wheat (root rot) in an agricultural field near Ussuriysk, Russian Federation, in 2006.

Etymology. The epithet *ussurianum* refers to the type locality near Ussuriysk in Primorsky krai in the Far East of the Russian Federation.

Distribution. Kamen-Ribolov and near Ussuriysk in Primorsky krai, and the Jewish Autonomous Region in the Far East of the Russian Federation.

Note. Five-septate conidia formed by *F. ussurianum* on SNA under black light are slightly wider than 5 μm on average. The measurements are similar to *F. vorosii* and *F. aethiopicum*, both of which are members of the *Fg* species complex (FIGS. 4–5, O'Donnell et al 2008). However *F. vorosii* and *F. aethiopicum* produce mostly straight conidia, which are asymmetrical in that they are typically widest above the mid-region. By comparison conidia produced by *F. ussurianum* are typically curved and nearly symmetrical (FIGS. 3–5). *Fusarium ussurianum* is most similar morphologically to *F. cerealis* (Cooke) Sacc. (= *F. crookewellense* Burgess et al) because both form nearly symmetrical conidia tapering and curving equally toward both ends (Burgess et al 1982, Nirenberg 1990). *Fusarium cerealis* however produces conidia that are widest at the mid-region and forms chlamydospores abundantly (Burgess et al 1982, Aoki and O'Donnell 1999, O'Donnell et al 2004). In strains of *F. ussurianum* by way of contrast conidia are widest slightly above the mid-region and chlamydospores were never observed. Delimitation of *F. ussurianum* is strongly supported with the exclusivity criterion under GCPSR in that it received strong MP and MP monophyly bootstrap support from analyses of α-tubulin, β-tubulin, *EF-1a*, HIS, MAT, reductase, *URA-Tri101-PHO* and the combined partition (TABLE I). *Fusarium ussurianum* and *F. asiaticum* are strongly supported as sisters in the multilocus phylogeny (FIG. 2) and together with *F. vorosii* appear to represent an Asian clade within the *Fg* species complex.

DISCUSSION

The primary objective of the present study was to elucidate *Fg* complex species and trichothecene toxin chemotype diversity in regions of the Russian Federation in Europe and Asia where recent outbreaks of scab have occurred. Isolates from adjoining regions to the northwest in Finland and to the southeast near Harbin, China, also were included in this survey to obtain a more comprehensive estimate of the geographic distribution, genetic diversity and

toxin potential of these pathogens for subsequent molecular surveillance of FHB in these regions. Consistent with the findings of Waalwijk et al (2003) and other surveys conducted over the past half decade (Gagkaeva and Yli-Mattila 2004; Jennings et al 2004a, b; Láday et al 2004; Tóth et al 2005; Yli-Mattila et al 2004; Yli-Mattila et al 2008; Ji et al 2007; Zhang et al 2007; Qu et al 2008) results of the present MLGT assay (Ward et al 2008) clearly show that *F. graminearum sensu stricto* is the dominant *Fg* complex pathogen of wheat and barley in the Russian Federation and in Heilongjiang Province of China where we sampled.

Although DON-producing isolates of *F. graminearum* were reported to comprise more than 70% of *Fg* complex species recovered from diseased corn 1999–2001 in the northern province of Gangwon, South Korea (Lee et al 2004), isolates of *F. asiaticum* (formerly known as *Fg* complex lineage 6 *sensu* O'Donnell et al 2000), producing mostly NIV (i.e. 97%), accounted for all FHB isolates from rice in the southern provinces of South Korea. Similar dramatic differences in the geographic distribution, host preference and frequency of *Fg* complex species and chemotypes were reported by Gale et al (2002), Zhang et al (2007) and Yang et al (2008) in China and by Suga et al (2008) in Japan. Results of these surveys show that Chinese populations of *F. graminearum* on wheat and barley produce 15ADON predominately or exclusively, in contrast to populations of *F. asiaticum*, which predominately produce 3ADON or NIV, depending on their geographic origin. Similarly in Japan (Suga et al 2008) isolates of *F. asiaticum* and *F. graminearum* respectively produce predominately NIV and 3ADON.

While there is abundant evidence that infraspecific out crossing occurs in both species (Gale et al 2002, 2007; Ward et al 2008), no interspecific hybrids were detected in regions of China (Zhang et al 2007) and Japan (Suga et al 2008) where these species were sympatric. Although one interspecific hybrid between *F. asiaticum* and *F. meridionale* has been documented in Nepal under natural field conditions (O'Donnell et al 2000, 2004) the reciprocally monophyletic phylogenetically distinct species lineages recovered in the individual and combined multilocus phylogenies clearly indicate that the forces of genetic drift have played a paramount role in the evolutionary diversification of the *Fg* complex (O'Donnell et al 2008).

Results of our MLGT assay indicate that *F. graminearum* is segregating for 3ADON and 15ADON in nearly equal proportions in European and Asian Russia and that NIV-producing isolates of this species were either absent or present in too few numbers to be detected in the regions sampled. In contrast *F. graminearum* is segregating for all three chemotypes in northwestern Europe (Qu et al 2008), including the UK where 15ADON-producing isolates of *F. graminearum* represented the predominate chemotype on wheat 1997–2002 (Jennings et al 2004b). Nearly identical results were obtained from surveys of FHB on wheat 2000–2001 in the Netherlands where DON- and NIV-producing *F. graminearum* appears to be displacing *F. culmorum* (Waalwijk et al 2003). While significant differences in the geographic distribution of *F. graminearum* chemotypes were observed in European Russia in the present study, no such differences were observed in a survey of FHB in England and Wales (Jennings et al 2004b). Population studies need to be conducted to determine whether the spatial differences in trichothecene chemotype we observed within *F. graminearum* in Finland and the European region of the Russian Federation are associated with population structure (Ward et al 2008).

Surveys of FHB within North America have shown that 3ADON-producing *F. graminearum* appears to be replacing the resident 15ADON population in the upper Midwest of USA (Gale et al 2007) and Canada (Ward et al 2008). Remarkably isolates from the two 3ADON populations in Canada have significantly higher growth rates and fecundity, and they produced significantly more trichothecene toxins than isolates from the 15ADON population they are displacing (Ward et al 2008). Because the more toxigenic 3ADON population in eastern Canada appears to have been introduced from outside North America, similar population level studies are urgently needed to identify the source population so that it can be carefully monitored.

Results of the present study add to our rapidly expanding knowledge of *Fg* complex species and chemotype diversity in Asia based on recent surveys in China, South Korea and Japan. These studies have documented a dramatic longitudinal *Fg* complex species cline, with *F. asiaticum* predominating on wheat and barley in the warmer and wetter regions in southern China (Gale et al 2002; Ji et al 2007; Qu et al 2007, 2008; Zhang et al 2007; Yang et al 2008) and Japan (Suga et al 2008) and on rice in southern provinces of South Korea (Lee et al 2004). Although *F. asiaticum* is segregating for all three chemotypes in these countries 3ADON is the predominant chemotype on wheat in southern China (Zhang et al 2007), while depending on the geographic origin, NIV- or DON-producing isolates predominant on barley in southern China (Yang et al 2008). In contrast NIV-producing isolates of *F. asiaticum* are dominant on wheat and barley in southern Japan (Suga et al 2008)

and on rice in the southern provinces of South Korea (Lee et al 2004). Of note, no isolates of *F. asiaticum* were recovered in the current survey of the Russian Federation and near the northern city of Harbin, Heilongjiang Province, China. However because NIV- and DON-producing isolates of *F. asiaticum* have been reported to cause FHB on wheat in low numbers in Harbin (Ji et al 2007) more extensive studies are needed to determine whether the range of this species extends into the Russian Federation.

The discovery of *F. ussurianum* and *F. vorosii* ex wheat in the Russian Far East, and isolates of *F. vorosii* in the same latitude across the Sea of Japan in Hokkaido (Starkey et al 2007, Suga et al 2008) suggests that these two species might define the northern limits of the longitudinal *Fg* complex species cline in continental Asia. In contrast to *F. asiaticum* and *F. graminearum*, which are the only two species within the *Fg* complex known to be segregating for all three chemotypes (Ward et al 2002), *F. vorosii* and *F. ussurianum* appear to be fixed respectively for 15ADON and 3ADON chemotype based on limited sampling. This finding highlights the need for additional surveys of FHB pathogens in northern Asia to more fully assess their genetic diversity, geographic distribution and mycotoxin potential.

The utility of the high-throughput MLGT screen for B-clade species and trichothecene chemotype determination we employed in the present study is reflected in the initial detection of isolates of *F. ussurianum* as genetically novel. Isolates of this species were positive for both *Fg* complex probes; however they were negative for all *Fg* complex species probes (Ward et al 2008). Prompted by the MLGT results, we conducted multilocus molecular phylogenetic analyses, which strongly supported species recognition for *F. ussurianum*, using the highly conservative exclusivity criterion of reciprocal monophyly and genealogical nondiscordance under GCPSR (Taylor et al 2000) as applied to other members of the *Fg* complex (O'Donnell et al 2000, 2004, 2008; Starkey et al 2007; Ward et al 2002). The discovery of *F. ussurianum* adds to our growing knowledge of cryptic speciation, sister group relationships and global biogeography of the *Fg* complex. Before the discovery of this species *F. vorosii* and *F. asiaticum* were identified as sister taxa (Starkey et al 2007). However phylogenetic analyses of the combined 13-gene dataset clearly indicate that *F. ussurianum* and *F. asiaticum* are sisters and that these two species together with *F. vorosii* form a strongly supported clade that appears to be endemic to Asia. Under this biogeographic hypothesis the first isolate of *F. vorosii*, which was discovered on blighted wheat in Hungary (Tóth et al 2005), appears to have been introduced to Europe from northern Asia.

Similarly the discovery of nonindigenous FHB pathogens in China (Yang et al 2008, *F. meridionale*), South Korea (Lee et al 2004, *F. meridionale* and *F. boothii*), Nepal (O'Donnell et al 2000, 2004; *F. meridionale* and *F. boothii*), Europe (Láday et al 2004, *F. boothii*) and elsewhere (O'Donnell et al 2004) underscores the enormous challenge that globalization of world trade in agricultural commodities poses to plant disease specialists and quarantine officials charged with preventing the global transposition of foreign FHB pathogens into nonindigenous areas. This challenge is exacerbated by the fact that morphological species recognition cannot resolve several of the phylogenetically distinct species within the *Fg* complex, thereby requiring that quarantine officials employ molecular diagnostic tools, such as the MLGT assay, in their active surveillance programs. Toward this end the MLGT assay was updated in the present study to include two novel probes for *F. ussurianum*. Knowledge gained from pathogen surveys, such as the one described herein, provide a baseline for monitoring changes in FHB pathogen diversity and mycotoxin potential over time, both of which are critical to the ultimate control and elimination of this economically devastating disease.

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