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# *Gibberella musae* (*Fusarium musae*) sp. nov., a recently discovered species from banana is sister to *F. verticillioides*

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***Abstract:*** Several strains of *Fusarium* isolated from banana were identified previously as *F. verticillioides* (Sacc.) Nirenberg but described as unable to produce fumonisin. Here we report biochemical and morphological evidence, as well as multilocus phylogenetic analyses based on elongation factor (EF-1α), calmodulin, β-tubulin, and the second largest subunit of RNA polymerase II (*RPB2*) sequences, indicating that these isolates represent a unique lineage in the *Gibberella fujikuroi* species complex related to but distinct from *F. verticillioides*. Together with previous results of molecular studies, as well as with results of metabolite analyses, crossing experiments, pathogenicity tests and morphological characterization, these new data indicate that these strains isolated from banana represent a new species, *Gibberella musae* Van Hove et al. sp. nov. (anamorph: *Fusarium musae* Van Hove et al. sp. nov.), which is described herein.

***Key words:*** β-tubulin, calmodulin, elongation factor EF-1α, excision, FGC, fumonisin, *Gibberella fujikuroi* complex, multilocus phylogeny, *RPB2*

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## INTRODUCTION

The *Gibberella fujikuroi* species complex (GFSC) historically encompassed *Fusarium* species of section *Liseola*. Section *Liseola* was erected to cover *Fusarium* species that produce whitish to purplish colonies on PDA and aerial and sporodochial conidia but lack chlamydospores. Up to the end of the 1980s the number of species within this section varied from one to 10, depending on the morphological characters used for species delimitation (Booth 1971, Gerlach and Nirenberg 1982, Nelson et al. 1983, Snyder and Hansen 1945, Wollenweber and Reinking 1935). Since then other species concepts based on chemical, biological, pathogenic or molecular data led to great modifications in the species delimitation within the GFSC (Kvas et al. 2009). In the past two decades 10 species have been described based on the biological species concept and referred to as mating populations A–J. More recently multilocus phylogenetic analyses confirmed all these biological species but also revealed that the GFSC was composed of at least 40 additional phylogenetically distinct species, covering all species of section *Liseola* and some species previously included in sections *Dlaminia*, *Discolor*, *Lateritium* and *Elegans* (Kvas et al. 2009, Nirenberg and O'Donnell 1998, O'Donnell et al. 2000). Many members of the GFSC are important plant pathogens. Most of them show narrow host specificity (i.e. *F. fujikuroi* Nirenberg on rice; *F. thapsinum* Klittich, J.F. Leslie, P.E. Nelson & Marasas on sorghum; *F. circinatum* Nirenberg & O'Donnell : Britz T.A. Cout. M.J. Wingf. & Marasas on pine; *F. konzum* Zeller, Summerell & J.F. Leslie on prairie grasses; *F. xylarioides* Steyaert on coffee tree) while *F. verticillioides*, *F. proliferatum* (Matsush.) Nirenberg and *F. nygamai* L.W. Burgess & Trimboli show much less specificity. A factor worthy of consideration for species such as *F. verticillioides* and *F. proliferatum* is that, like *F. nygamai* (Leslie et al. 2005b), their wide host ranges reflect yet undiscovered cryptic species boundaries within the currently applied species concept. In this paper we described a group of isolates from banana previously identified as *F. verticillioides* as *Gibberella musae* sp. nov. (anamorph: *F. musae*), hereinafter referred to as *Fm.*

*F. verticillioides* (teleomorph *G. moniliformis* Wineland) frequently is associated with maize worldwide where it causes ear rot and produces fumonisin mycotoxins. Nevertheless *F. verticillioides* strains also

were identified from other plant hosts, including teosinte, millet, sorghum, tallgrass and banana (Desjardins et al. 2000, Hirata et al. 2001, Jimenez et al. 1997, 1993; Leslie et al. 2005b, 2004a) and occasionally is isolated from human infections (O'Donnell et al. 2007). Although several *Fusarium* strains recovered from banana were identified as *F. verticillioides* in these studies, a number of differences have been highlighted between some of these strains isolated from banana (*Fm*) and typical *F. verticillioides* strains primarily originating from maize (*Fv*).

Concerning pathogenicity, *Fm* strains induced spots on wounded and nonwounded parts of inoculated healthy green banana fruits. In contrast, symptoms developed only occasionally on wounded pods and leaves of soybean and on corn whereas no symptoms were observed after inoculation of nonwounded parts of soybean or corn or after the potting soil of corn or soybean plants was inoculated with a conidial suspension of the *Fm* strain NRRL 28893 (Hirata et al. 2001). Moreover significant differences in pathogenicity of *Fm* and *Fv* strains on banana fruits have been observed, *Fm* strains having a greater ability to cause infection than *Fv* strains (Moretti et al. 2004).

Subtle morphological differences have been noted that distinguish *Fm* strains from typical *F. verticillioides*. *F. musae* isolates were found to produce slightly smaller non-septate microconidia than *Fv* strains. In addition *Fm* strains formed septate microconidia twice as frequently as *Fv* strains (Hirata et al. 2001).

*Fm* strains are cross fertile but produce perithecia that are significantly smaller than *Fv* crosses. Although *Fm* and *Fv* strains show interfertility, the resulting perithecia developed more slowly and were significantly larger than perithecia from *Fv* crosses (Moretti et al. 2004). Nevertheless the same degree of fertility was observed when comparing *Fm* × *Fm*, *Fv* × *Fv* and *Fm* × *Fv* crosses (Moretti pers comm, Moretti et al. 2004). These authors also showed that similarly to *Fv*, *Fm* strains were unfertile when crossed with tester strains of the other *G. fujikuroi* mating populations.

Concerning secondary metabolism, *Fm* strains typically produce moniliformin (MON) and do not produce fumonisins (FB), in contrast to most *Fv* strains that do produce FB but fail to produce MON or were misidentified as MON producers (Jimenez et al. 1997, Klittich et al. 1997, Leslie et al. 1996, Leslie and Summerell 2006, Moretti et al. 2004, Schütt et al. 1998). It has been established that *Fm* strains are unable to produce fumonisins because they lack most of the gene cluster that encodes the enzymes necessary for fumonisin biosynthesis (Glenn et al. 2008, Proctor et al. 2003, Van Hove et al. 2006). Moreover *Fv* strains are highly tolerant to 2-benzoxazolinone (BOA), an antimicrobial compound from maize, due to the active transformation of this compound to a non-toxic metabolite, while *Fm* strains are sensitive to BOA and unable to metabolize it (Glenn 2006, Glenn et al. 2001).

At the molecular level *Fm* and *Fv* strain populations differ by numerous traits. Restriction fragment length polymorphism analysis of the intergeneric spacer (IGS) of the ribosomal RNA gene repeat showed that *Fm* strains segregate from other *Fv* strains as well as other species of the GFSC (Llorens et al. 2006, Patiño et al. 2006). With two sets of primers based on the IGS sequence a PCR assay has been developed for the general detection of all *Fv* and *Fm* strains with one set of primers, and the specific detection of *Fv* strains only that putatively produce fumonisins, with the other set (Patiño et al. 2004). Individual and combined phylogenetic analyses of the EF-1α and the mitochondrial small subunit (mtSSU) rDNA partial sequences, and also of the IGS region and the EF-1 α partial sequence, clustered strains of both *Fm* and *Fv* strain populations into two well supported groups (Hirata et al. 2001, Mirete et al. 2004). It has been shown with AFLP analysis that strains of these two groups have only 51% AFLP band identity (Moretti et al. 2004).

Together these differences strongly suggested that *Fm* strains are a distinct population within a *F. verticillioides* complex and that *Fv* and *Fm* represent related cryptic species. The objective of this study therefore was to accumulate additional biochemical, morphological and molecular evidence to clarify the taxonomical position of this *Fm* population. Here we describe *Gibberella musae* Van Hove et al. sp. nov. (anamorph: *Fusarium musae* Van Hove et al. sp. nov.) corresponding to this newly recognized species.

## MATERIALS AND METHODS

*Strains and culture conditions.*—The 22 *Fusarium* isolates in this study are listed (TABLE I). Strains were obtained from the Fungal Genetics Stock Center (FGSC; School of Biological Sciences, University of Missouri, Kansas City), the Agri-Food Toxigenic Fungi Culture Collection (ITEM; Institute of Sciences of Food Production, Bari, Italy), the Medical Research Council collection (MRC; Tygerberg, South Africa), the Mycothèque de l'Université catholique de Louvain (MUCL; Université catholique de Louvain, Louvain-la-Neuve, Belgium) and the Northern Regional Research Laboratory collection (NRRL; NCAUR, Peoria, Illinois). Eleven *Fv* strains originated from maize or banana, including both *F. verticillioides* tester strains (FGSC 7600 and FGSC 7603 in boldface). Eleven representative *Fm* strains were isolated from banana. Sequence data from a 12th *Fv* strain (NRRL 22172) from O'Donnell et al. (1998a,

TABLE I. *Fusarium* strains used in this study

| | Haplotype[a] | Host | Origin | MAT[b] | *FUM*[c] | $FB_1$; $FB_2$[d] | MON[e] | BOA[f] | β-tubulin | Calmodulin | EF-1α | *RPB2* | FGC/ΔFGC[g] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *F. verticillioides* *(Fv)* strain number | | | | | | | | | | | | | |
| **MUCL 43478, FGSC 7600, FRC M-3125** | haplotype-1 | *Zea mays* | California, USA | *MAT1-1* | +[h,i] | 638; 216[i] 57; 8.1[k] 6160; 1845[m] | −[m] | +[i,q] | FN545356 | FN552052 | FN552074 | FN552096 | AF155773[h] |
| MUCL 51064 | haplotype-2 | *Zea mays* | Philippines | *MAT1-2* | +[i] | 137; 15[n] | nt | +[i] | FN545357 | FN552053 | FN552075 | FN552097 | |
| ITEM 2537, JFL A03823 | haplotype-3 | *Musa* sp. | Turkey | *MAT1-1* | +[i] | nt | nt | +[i,q] | FN545358 | FN552054 | FN552076 | FN552098 | |
| MUCL 42991, FGSC 7598, FRC M-3120 | haplotype-3 | *Zea mays* | USA | *MAT1-2* | +[i,j] | 650; 171[i] 1640; 330[m] | −[m] | +[i,q] | FN545359 | FN552055 | FN552077 | FN552099 | |
| **MUCL 43479, FGSC 7603, FRC M-3703** | haplotype-3 | *Zea mays* | Indiana, USA | *MAT1-2* | +[i,k] | 150; 26[i] 673; 212[k] 1045; 240[m] 3622; 986[p] | −[m] | +[i,q] | FN545360 | FN552056 | FN552078 | FN552100 | |
| MUCL 49894 | haplotype-3 | *Zea mays* | Philippines | *MAT1-2* | +[i] | 101; 27[n] | nt | +[i] | FN545361 | FN552057 | FN552079 | FN552101 | |
| MUCL 51637 | haplotype-4 | *Musa* sp. | Congo (RDC) | *MAT1-2* | +[i] | nt | nt | +[i] | FN545362 | FN552058 | FN552080 | FN552102 | |
| ITEM 2541, JFL A04426 | haplotype-5 | *Musa* sp. | Thailand | *MAT1-2* | +[i] | 75; 5[m] | +[m] | +[i,q] | FN545363 | FN552059 | FN552081 | FN552103 | |
| MUCL 51366 | haplotype-6 | *Musa* sp. | Philippines | *MAT1-2* | +[i] | 3420; 369[i] | nt | +[i] | FN545364 | FN552060 | FN552082 | FN552104 | |
| MUCL 42990, MRC 826, FRC M-1325 | haplotype-7 | *Zea mays* | South-Africa | *MAT1-1* | +[i,k] | 364; 164[k] 2170; 670[m] | −[m] | +[i,q] | FN545365 | FN552061 | FN552083 | FN552105 | |
| MUCL 42883, FGSC 7606, FRC M-5500 | haplotype-8 | *Zea mays* | Kathmandu, Nepal | *MAT1-2* | +[i,k] | nd; nd[k] trace; nd[m] | +[m] | +[i,q] | FN545366 | FN552062 | FN552084 | FN552106 | |
| NRRL 22172, CBS 734.97 | haplotype-9 | *Zea mays* | Germany | nt | nt | nt | nt | nt | U34438 | AF158315 | AF160262 | EF470122 | |
| *F. musae* sp. nov. *(Fm)* strain number | | | | | | | | | | | | | |
| MUCL 31965 | haplotype-1 | *Musa* sp. | Nicaragua | *MAT1-2* | −[l] | nd; nd[i] | nt | −[i] | FN545367 | FN552063 | FN552085 | FN552107 | FN543478 |
| [T] NRRL 25059, CBS 624.87, MUCL 52574 | haplotype-1 | *Musa* sp. | Honduras | *MAT1-2* | −[l] | nd; nd[i,k] | nt | −[i,q,r] | FN545368 | FN552064 | FN552086 | FN552108 | EF653133[k] |
| ITEM 1143 | haplotype-2 | *Musa* sp. | Ecuador | *MAT1-2* | −[l] | nd; nd[i,o,p] | +[o] | −[i] | FN545369 | FN552065 | FN552087 | FN552109 | FN545135 |
| ITEM 1245 | haplotype-2 | *Musa* sp. | Canary Islands | *MAT1-2* | −[l] | nd; nd[i,o,p] | +[o] | −[i] | FN545370 | FN552066 | FN552088 | FN552110 | FN545137 |
| **MUCL 51371** | haplotype-2 | *Musa* sp. | Philippines | *MAT1-2* | −[i] | nd; nd[i] | nt | −[i] | FN545371 | FN552067 | FN552089 | FN552111 | FN545139 |

TABLE I. Continued

| | Haplotype[a] | Host | Origin | MAT[b] | *FUM*[c] | $FB_1$; $FB_2$[d] | MON[e] | BOA[f] | β-tubulin | Calmodulin | EF-1α | *RPB2* | FGC/ΔFGC[g] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ITEM 1250 | haplotype-3 | *Musa* sp. | Canary Islands | *MAT1-1* | −[i] | nd; nd[i,o,p] | +[o] | −[i] | FN545372 | FN552068 | FN552090 | FN552112 | FN545138 |
| NRRL 25673 | haplotype-3 | *Musa* sp. | Guatemala | *MAT1-1* | −[l] | nt | nt | −[i,r] | FN545373 | FN552069 | FN552091 | FN552113 | FN545131 |
| NRRL 28893 | haplotype-4 | *Musa* sp. | Mexico | *MAT1-2* | −[l] | nt | nt | −[i,r] | FN545374 | FN552070 | FN552092 | FN552114 | FN545132 |
| **ITEM 1121, MUCL 52573** | haplotype-5 | *Musa* sp. | Panama | *MAT1-1* | −[i] | nd; nd[i,o] | +[o] | −[i] | FN545375 | FN552071 | FN552093 | FN552115 | FN545133 |
| ITEM 1142 | haplotype-6 | *Musa* sp. | Ecuador | *MAT1-1* | −[l] | nd; nd[i,o,p] | +[o] | −[i] | FN545376 | FN552072 | FN552094 | FN552116 | FN545134 |
| ITEM 1149 | haplotype-6 | *Musa* sp. | Panama | *MAT1-1* | −[l] | nd; nd[i,o,p] | +[o] | −[i] | FN545377 | FN552073 | FN552095 | FN552117 | FN545136 |

Strain number: Tester reference strains are in boldface and the *F. musae* type strain is designed by [T]. **CBS**, CentraalBureau voor Schimmelcultures, Utrecht, the Netherlands; **FGSC**, Fungal Genetics Stock Center, School of Biological Sciences, University of Missouri, Kansas City; **FRC**, *Fusarium* Research Center, Pennsylvania State University, University Park; **ITEM**, Agri-Food Toxigenic Fungi Culture Collection, Institute of Sciences of Food Production, Bari, Italy; **JFL**, J.F. Leslie, Department of Plant Pathology, Kansas State University, Manhattan; **MRC**, Medical Research Council, Tygerberg, South Africa; **MUCL**, Mycothèque de l'Université catholique de Louvain, Université catholique de Louvain, Louvain-la-Neuve, Belgium; **NRRL**, Northern Regional Research Laboratory, NCAUR, Peoria, Illinois.

[a] Strains belonging to one haplotype code have unique identical combined EF-1α, calmodulin, β-tubulin and *RPB2* sequences.

[b] PCR determination of mating type with gxp8 and gxp13 primers for *MAT1-1* and Gfmat2d and gxp21 for *MAT1-2*.

[c] PCR amplification of *FUM3*, *FUM6*, *FUM7* and/or *FUM8* partial sequences. + = amplification. − = no amplification.

[d] Fumonisin $B_1$ and $B_2$ production in μg/g. trace: 1–2 μg/g, according to Leslie et al. 1996 (usually indicates a high background level in uninoculated control grain). nd: not detected.

[e] Moniliformin production. + = production. − = no production.

[f] 2-benzoxazolinone (BOA) tolerance determined by culture on PDA medium amended with 1 mg/mL BOA. +, growth. −, no growth.

[g] FGC = Fumonisin gene cluster sequence, and ΔFGC = excised Fumonisin Gene Cluster flanking region sequence.

[h] Proctor et al. 2003, [i] this study, [j] Proctor et al. 2004, [k] Glenn et al. 2008, [l] Van Hove et al. 2006, [m] Leslie et al. 1996, [n] Cumagun et al. 2009, [o] Moretti et al. 2004, [p] Mirete et al. 2004, [q] Glenn et al. 2001, [r] Glenn 2006.

nt: not tested.

2000, 2007) also were included in the phylogenetic analysis (TABLE I).

Strains were grown on potato dextrose agar (PDA; Scharlab S.L., Barcelona, Spain) 7 d at 25 C in the dark to observe colony morphology (color, mycelial characteristics, presence/absence of sporodochia, sclerotia and pseudochlamydospores). Growth rates were examined at 25 C as described by Hirata (Hirata et al. 2001). Conidiogenesis and conidial characteristics, as well as the presence of sporodochia, sclerotia and pseudochlamydospores, were recorded from strains grown on Spezieller Nährstoffarmer agar (SNA) (Leslie and Summerell 2006) 7 d at 25 C in the dark. The shape, length and width of 50 randomly selected conidia were recorded with an Olympus BX 52 phase contrast microscope under 1000× magnification.

Crossing experiments were performed as described by Klittich and Leslie (1988). Reciprocal crosses were executed with all the *Fm* strains, as well as between the *Fm* strains and the *Fv* standard tester strains FGSC 7600 (*MAT1-1*) and FGSC 7603 (*MAT1-2*) (Fungal Genetics Stock Center, Missouri, USA). A cross was scored fertile when a cirrhus of ascospores emerged from a mature perithecium within 2–4 wk after combined growth of both strains.

For DNA extraction strains were grown in 50 mL 2% (wt/vol) malt extract (Duchefa, Haarlem, the Netherlands) medium 10 d at 25 C in the dark on an orbital shaker at 90 rpm (Edmund Bühler, Johanna Otto GmbH, Hechingen, Germany). Fresh mycelia were collected by vacuum filtration through No. 1 Whatman filter paper (Whatman Biosystems Ltd, Maidstone, UK).

*Fumonisin analysis and 2-benzoxazolinone tolerance determination.*—The method used to test the capacity of strains to produce fumonisins was adapted from Thiel et al. (1991) with a number of modifications. Erlenmeyer flasks (250 mL) were filled with 20 g maize kernels, 10 mL distilled water and autoclaved twice for 15 min at 121 C. After cooling each flask was inoculated with a 10 mL suspension of $2 \times 10^4$ conidia in a 0.9% peptone solution. Flask cultures were incubated in the dark 14 d at 25 C. During the first 3 d flasks were shaken once daily to distribute the inoculum more evenly. After 14 d the colonized maize kernels were transferred into plastic bags with 150 mL methanol : water (70 : 30, v : v) and ground 2 min at 230 rpm in a stomacher (Led Techno, Heusden-Zolder, Belgium). The extraction mixtures were centrifuged 10 min at 3000 × *g* in Falcon tubes, and the pellets were discarded. Supernatants were retained for fumonisin detection and quantification with methods adapted from Shephard et al. (1990) and Sydenham et al. (1996). A 10 mL sample of each supernatant was purified on a SAX SPE-column (500 mg, Varian, Sint-Katelijne-Waver, Belgium). The fumonisins were eluted with 10 mL methanol : acetic acid (99 : 1, v : v) and dried in a SC1100 SpeedVac system (Thermo Electron Corp., Brussels, Belgium). Samples were redissolved in 1 mL methanol and filtered through a 0.45 µm Acrodisc® filter before analysis. Aliquots (100 µL) of each redissolved sample were derivatized with 300 µL o-phthaldialdehyde (OPA, Fluka Biochemika, Buchs, Switzerland), and 40 µL were injected on the HPLC column. The limits of detection (LOD, signal/noise = 3) for fumonisin $B_1$ ($FB_1$) and fumonisin $B_2$ ($FB_2$) were respectively 0.029 and 0.062 µg/g, and the limit of quantification (LOQ, signal/noise = 10) was 0.096 µg/g for $FB_1$ and 0.208 µg/g for $FB_2$. 2-benzoxazolinone (BOA) tolerance was determined by measuring the colony diameter of strains on PDA medium amended with 1 mg/mL BOA (Sigma-Aldrich, Bornem, Belgium) as described by Glenn et al. (2001).

*Molecular analysis.*—DNA was extracted and purified from fresh mycelia with the Invisorb Spin Plant Minikit (Invitek Gmbh, Berlin, Germany). Based on the *F. verticillioides* fumonisin gene cluster (FGC) sequence from EMBL (AF155773) as template, primers fvh55 and fvh59 (TABLE II) were designed with *Primer3* software (Rozen and Skaletsky 2000) to amplify and sequence the flanking regions of the FGC excision site (ΔFGC) within the genome of all the *F. musae* strains in this study (TABLE I). The fvh59 and fvh55 primers were designed in the second exon of the first FGC gene (*FUM21*) and downstream the stop codon of the last FGC gene (*FUM19*) gene respectively (FIG. 1). The size of the expected ΔFGC amplified fragment was 1178 bp. Primers pairs fvh9/fvh10, fvh3/fvh4, fvh5/fvh6 and fvh7/fvh8 (TABLE II) were designed similarly to amplify internal sequences of the *FUM3*, *FUM6*, *FUM7* and *FUM8* genes respectively in all *F. verticillioides* strains having an intact FGC.

Two external primers, *RPB2*-1F and *RPB2*-1R, and two internal primers, *RPB2*-2F and *RPB2*-2R (TABLE II), were designed based on the gene sequence of the *RPB2* homolog in the *F. verticillioides* genome sequence (Broad Institute; http://www.broad.mit.edu). These primers were used to amplify and sequence a portion of the *RPB2* gene from *F. musae* and *F. verticillioides*. Primers used for the amplification and sequencing of EF-1α, calmodulin, β-tubulin, *MAT1-1* and *MAT1-2* sequences are provided (TABLE II).

PCR reaction mixtures (total volume of 50 µL) contained 2 µL DNA (10 ng/µL) in a 1× PCR buffer, 2.5 mM $MgCl_2$ (Fermentas GmbH, St Leon-Rot, Germany), 0.25 mM concentrations of each dNTP, 0.1 µM concentrations of each primer, and 1U Taq DNA Polymerase (Invitrogen Life Technologies, Carlsbad, California). All PCR reactions were performed in a Biometra TGradient thermal cycler (Biometra GmbH, Goettingen, Germany) with cycling conditions as follows: initial denaturation at 95 C for 3 min, followed by 30 cycles of 94 C denaturation 1 min, 60 C annealing 30 s and 72 C elongation 1 min. A final extension was performed at 72 C for 10 min. Amplification of the *RPB2* gene was done for 39 cycles under comparable conditions, except that the extension was prolonged to 2 min. PCR products were purified before sequencing with the MSB Spin PCRapace kit (Invitek Gmbh, Berlin, Germany).

Sequencing reactions were performed with the Dye Terminator Cycle Sequencing Quick Start kit (Beckman Coulter, Fullerton, California) and analyzed on a Beckman Coulter CEQ™ 2000XL DNA Analysis System. Sequences were edited with Sequencher 4.0.5 (Gene Codes Corp., Ann Arbor, Michigan) and were deposited in the EMBL database (TABLE I).

TABLE II. Primers used for PCR amplification (in boldface) and sequencing

| Primers | Sequence | References |
|---|---|---|
| β-tubulin | | |
| **T1** | 5′-AACATGCGTGAGATTGTAAGT-3′ | O'Donnell and Cigelnik 1997 |
| **T22** | 5′-TCTGGATGTTGTTGGGAATCC-3′ | O'Donnell and Cigelnik 1997 |
| T21 | 5′-GGTTTGCCAGAAAGCAGCACC-3′ | O'Donnell and Cigelnik 1997 |
| T121 | 5′-CCACCTGTCTCCGTTTCCCCG-3′ | O'Donnell and Cigelnik 1997 |
| calmodulin | | |
| **CL1** | 5′-GAGTTCAAGGAGGCCTTCTC-3′ | O'Donnell et al. 2000 |
| **CL2A** | 5′-TTTTTGCATCATGAGTTGGAC-3′ | O'Donnell et al. 2000 |
| CL11 | 5′-ACCATGATGGCGCGCAAG-3′ | O'Donnell et al. 2000 |
| CL22 | 5′-TCCTTCATCTTGCGCGCC-3′ | O'Donnell et al. 2000 |
| elongation factor | | |
| **EF1** | 5′-ATGGGTAAGGA(A/G)GACAAGAC-3′ | O'Donnell et al. 1998b |
| **EF2** | 5′-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3′ | O'Donnell et al. 1998b |
| EF11 | 5′-GTGGGGCATTTACCCCGCC-3′ | O'Donnell et al. 1998b |
| EF21 | 5′-GAGTGGCGGGGTAAATGCC-3′ | O'Donnell et al. 1998b |
| EF22 | 5′-AGGAACCCTTACCGAGCTC-3′ | O'Donnell et al. 1998b |
| RPB2 | | |
| **RPB2-1F** | 5′-GGGGTGATCAGAAGAAGGC-3′ | this study |
| **RPB2-1R** | 5′-CGTGAATCTTGTCGTCCACC-3′ | this study |
| RPB2-2F | 5′-CCACCAGGAGGATGACTATGA-3′ | this study |
| RPB2-2R | 5′-GAGAAGCGAACTTGTCACCA-3′ | this study |
| *MAT1-1* | | |
| Gxp1 | 5′-ATCGCTTGCCCTTTGATGAA-3′ | Lepoint 2006 |
| Gxp2 | 5′-CACTCTCTACAACTTCCACAT-3′ | Lepoint 2006 |
| **Gxp8** | 5′-GAACGACTTCCTCTACCATAC-3′ | Lepoint 2006 |
| Gxp10 | 5′-TCTCCACGCTCAGAAATTCT-3′ | Lepoint 2006 |
| **Gxp13** | 5′-TCGCCGRTCAGGTATCATTTCA-3′ | Lepoint 2006 |
| *MAT1-2* | | |
| Gfmat2c | 5′-AGCGTCATTATTCGATCAAG-3′ | Steenkamp et al. 2000 |
| **Gfmat2d** | 5′-CTACGTTGAGAGCTGTACAG-3′ | Steenkamp et al. 2000 |
| **Gxp21** | 5′-CTCCTCCTCACAACACCAAA-3′ | Lepoint 2006 |
| ΔFGC | | |
| **fvh55** | 5′-CGCTGCTGTGTGTGGTAACT-3′ | this study |
| **fvh59** | 5′-AGCTTGTCAACCCAGCAGAT-3′ | this study |
| *FUM3* | | |
| **fvh9** | 5′-AACTTCCACGGCATGAAGAC-3′ | this study |
| **fvh10** | 5′-AAGGAAATTGGCGCACATAG-3′ | this study |
| *FUM6* | | |
| **fvh3** | 5′-CGCTCTGTGTCCACTTCAAA-3′ | this study |
| **fvh4** | 5′-CCACCACCGTATCGACTTCT-3′ | this study |
| *FUM7* | | |
| **fvh5** | 5′-ACCAGTTCCACCAAGCAAAC-3′ | this study |
| **fvh6** | 5′-TGCAACTTGTCTCTCCATGC-3′ | this study |
| *FUM8* | | |
| **fvh7** | 5′-GTCGCCGGTCACTATTTTGT-3′ | this study |
| **fvh8** | 5′-CACAAAGCTTGCCTTTCTCC-3′ | this study |

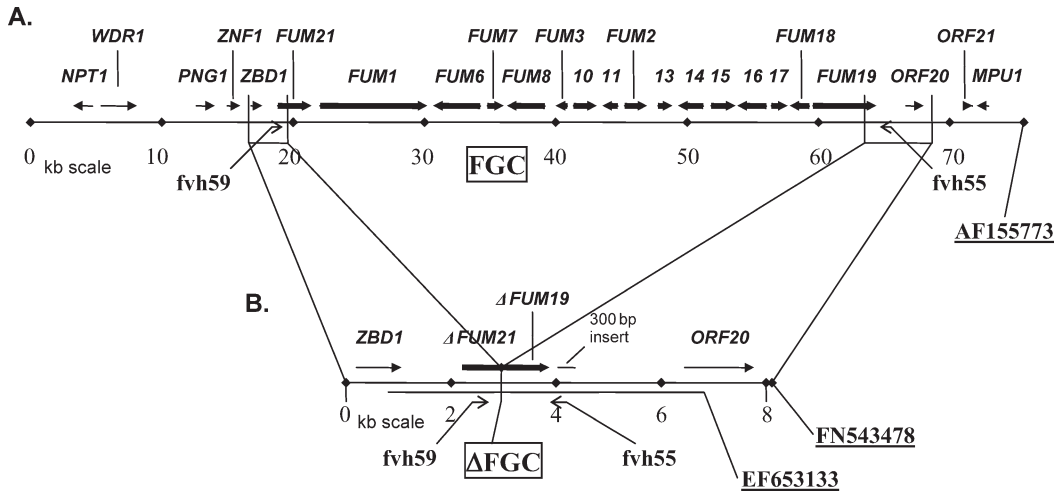


FIG. 1. Schematic illustration of the fumonisin gene cluster (FGC). A. The genome region (EMBL AF155773) from *Fusarium verticillioides* strain M-3125 (= MUCL 43478, FGSC 7600) isolated from maize. B. FGC-excised genome regions from *F. musae* strains MUCL 31965 (EMBL FN543478) and NRRL 25059 (EF653133) isolated from banana.

*Phylogeny.*—In addition to EF-1α, β-tubulin, calmodulin and *RPB2* partial gene sequences obtained in this study for *F. verticillioides* and *F. musae* strains (TABLE I), corresponding sequences for other species of the GFSC were from O'Donnell et al. (1998a, 2000, 2007), except for *RPB2* sequences that were kindly provided by Kerry O'Donnell, U.S. Department of Agriculture, Peoria, Illinois. The sequences were aligned initially with Clustal X software (Thompson et al. 1997) and manually edited. The alignment was deposited in TreeBASE (http://www.treebase.org/treebase/index.html). Phylogenetic analyses were performed with PAUP* 4.0b10 (Swofford 2003) on the individual and combined datasets with the heuristic search option with 1000 random addition sequences with MULPARS on and tree bisection-reconnection (TBR) branch swapping. Models of evolution used in the parsimony inferences were estimated with the Akaike information criterion (AIC) as implemented in Modeltest 3.7 (Posada and Crandall 1998). The best-fit model having the higher likelihood score was chosen for subsequent analyses. Phylogenetically informative indels were coded as a single event (i.e. fifth state). The gene trees were rooted by the outgroup method with sequences of a putative sister group, *F. oxysporum* species complex isolate NRRL 22902 and *F. inflexum* isolate NRRL 22902, following O'Donnell (1998a). Stability of clades was assessed by 1000 parsimony bootstrap replications, using heuristic searches with 1000 replicates of random sequence addition implemented with PAUP*.

## RESULTS

*Strains collected.*—In a preliminary survey of *Fusarium* isolates from banana cultivated in Africa and Asia (data not shown) we identified two *Fv* and one *Fm* strains among 40 *Fusarium* isolates from the Philippines and three *Fv* strains but none *Fm* strain among 20 isolates from the Democratic Republic of the Congo (RDC). The *Fm* strain (MUCL 51371) and one *Fv* strain (MUCL 51366) isolated on the ''Latundan'' banana variety from the Philippines and one *Fv* strain (MUCL 51637) of the RDC were used together with other representative *Fv* and *Fm* strains available in culture collections (TABLE I).

*Mycotoxin production and BOA tolerance.*—HPLC analyses performed on extracts of *Fv* and *Fm* strains cultivated on maize kernels (TABLE I) showed that all *Fv* strains were fumonisin producers, except strain MUCL 42883 that was previously shown to harbor a naturally mutated *FUM1* gene (Desjardins et al. 1996). In contrast, none of the *Fm* strains produced detectable $FB_1$ or $FB_2$. Data from the literature on moniliformin (MON) production by *Fv* and *Fm* strains (Leslie et al. 1996, Moretti et al. 2004) were included (TABLE I). Four of the six *Fv* strains did not produce MON, while all of the six tested *Fm* strains were MON producers (Leslie et al. 1996, Moretti et al. 2004). BOA tolerance determination experiments revealed that all *Fv* strains were able to grow in the presence of BOA while *Fm* strains did not grow at all (TABLE I).

*Fumonisin gene cluster excision.*—The presence of the FGC in the genome of all *Fv* strains was confirmed by PCR amplification (TABLE I) and sequencing (data not shown) of partial sequences of four *FUM* genes, *FUM3*, *FUM6*, *FUM7* and *FUM8*. The excision of the FGC from the genome of all the *F. musae* (*Fm*) strains was confirmed by PCR amplification and sequencing (TABLE I) of the flanking regions of the FGC excision site (ΔFGC). A corresponding ~ 8.1 kb sequence from one *Fm* strain (MUCL 31965) was deposited in

EMBL (FN543478, FIG. 1B). This ~ 8.1 kb sequence shows 100% identity to the ~ 6.0 kb sequence (EF653133, FIG. 1B) from another *Fm* strain (NRRL 25059) deposited by Glenn and collaborators (Glenn et al. 2008), except that the ~ 8.1 kb sequence extends 841 and 1301 bp from 5′ and 3′ ends of the ~ 6.0 kb sequence, respectively (FIG. 1B). The fvh59 and fvh55 primers amplified the expected PCR fragment of 1178 bp in all *Fm* strains. The sequences of these fragments correspond to 127 bp upstream and 1049 bp downstream of the ΔFGC (FIG. 1B, TABLE II). The high similarity at the nucleotide level (94–99%) of the flanking regions of the ΔFGC from strain MUCL 31695 (FN543478, FIG. 1B) with homologous portions of the full FGC sequence from the *Fv* M-3125 strain (AF155773, FIG. 1A), including *ZBD1*, *ORF21* and remnant portions of *FUM21* and *FUM19*, confirmed that these sequences represent the same locus. The variations observed were mainly point mutations and small insertion/deletion events (1–9 bases), except for one major excision (44 kb) and one large insertion (300 bp). Schematically, based on the AF155773 and FN543478 sequences, the 44 kb excised region extends 756 bp after the start codon, within the third exon of the first FGC gene (*FUM21*) to 900 bp before the stop codon, at the 3′ end of the third intron of the last FGC gene (*FUM19*) (FIG. 1). The 300 bp insertion is characterized by the presence of +/− 300 nucleotides in the intergenic region between the 3′ remnant portion of *FUM19* and *ORF20* within the genome of the *Fm* MUCL 31965 strain (FIG. 1B). This insert is flanked by two direct repeats of 14 bases, indicating that it could be the result of recombination or transposition. Indeed similar sequences (31–296 nucleotides, SUPPLEMENTARY TABLE I) are found at 54 other locations in the *F. verticillioides* M-3125 strain genome (Broad Institute, http://www.broad.mit.edu). Because the inserted sequence does not share any similarities with known amino acid or nucleotide sequences of enzymes including transposases or with other known sequences from the EMBL database we were not able to identify the origin of this event or to establish that it could be directly related to the FGC excision.

*Molecular phylogeny.*—Phylogenetic analyses were conducted on partial sequences of four protein-encoding nuclear genes (EF-1α, β-tubulin, calmodulin and *RPB2*), individually and combined, of *Fv* and *Fm* strains together with those of representative strains of species from the GFSC. *F. verticillioides* and *F. musae* strains sharing identical EF-1α, β-tubulin, calmodulin and *RPB2* combined sequences have been grouped respectively in nine and six haplotypes (TABLE I). Alignment of EF-1α, β-tubulin, calmodulin and *RPB2* partial sequences included respectively 693, 1190, 687 and 1769 nucleotide positions, totalling 4339 aligned nucleotide positions. There were 3306 constant characters, 415 parsimony uninformative variable characters and 618 parsimony informative characters. Of these 618 parsimony informative characters, 148 were from EF-1α, 150 from β-tubulin, 86 from calmodulin and 234 from *RPB2*. Maximum parsimony heuristic analysis of the combined dataset yielded 104 most parsimonious trees (MPTs, length = 1990 steps, CI = 0.619, RI = 0.808) in which the *Fm* and *Fv* clades are strictly conserved, as revealed by the strict-rule consensus analysis (data not shown). The EF-1α, β-tubulin, calmodulin and *RPB2* individual (data not shown) and combined MPTs (FIG. 2) are topologically concordant with those from O'Donnell et al. (1998a). The *Fm* and *Fv* haplotypes are located within the African clade of the GFSC, and the monophyletic origin of the corresponding *Fm* and *Fv* clades is strongly supported within the combined gene tree, with 98% and 99% maximum parsimony bootstrap value respectively (FIG. 2). On one hand the maximum parsimony bootstrap values for the *F. musae* clades in EF-1α, calmodulin and *RPB2* individual genealogies are respectively 95%, 65% and 51%, while they are 95% and 89% for the *F. verticillioides* clades in EF-1α and β-tubulin genealogies. On the other hand *F. musae* is not monophyletic in the β-tubulin genealogy and *F. verticillioides* is not monophyletic in calmodulin and *RPB2* individual genealogies but it is important to note that the topology of these genealogies are not contradicting the monophyly of these two species at the same level of support. Both clades are closely related because together they form a strongly supported monophyletic group in the combined and individual analyses (100% bootstrap) within the African clade of the GFSC.

## TAXONOMY AND MORPHOLOGY

**Gibberella musae** Van Hove, Waalwijk, Munaut, Logrieco and Moretti sp. nov. FIG. 3A–D
MycoBank MB519034

Perithecia superficialia, livida, 250–420 μm alta × 220–260 μm lata. Asci fusiformes, dehiscentes 55–65 μm alti × 8–10 μm lati, octospori. Ascosporae exudate in cirrhis, laeves, hyalinae, ellipsoidae vel obovoidae, 1-septatae, ad septum leviter constrictae, 10–22 × 4–9 μm. Anamorphosis: *Fusarium musae* Van Hove, Waalwijk, Munaut, Logrieco et Moretti sp. nov. Species heterothallica.

*Holotype for* Gibberella musae. From laboratory cross ITEM 1121 (= MUCL 52573) × NRRL 25059 (= MUCL 52574) on carrot agar (MUCL 52575, dry carrot agar culture).

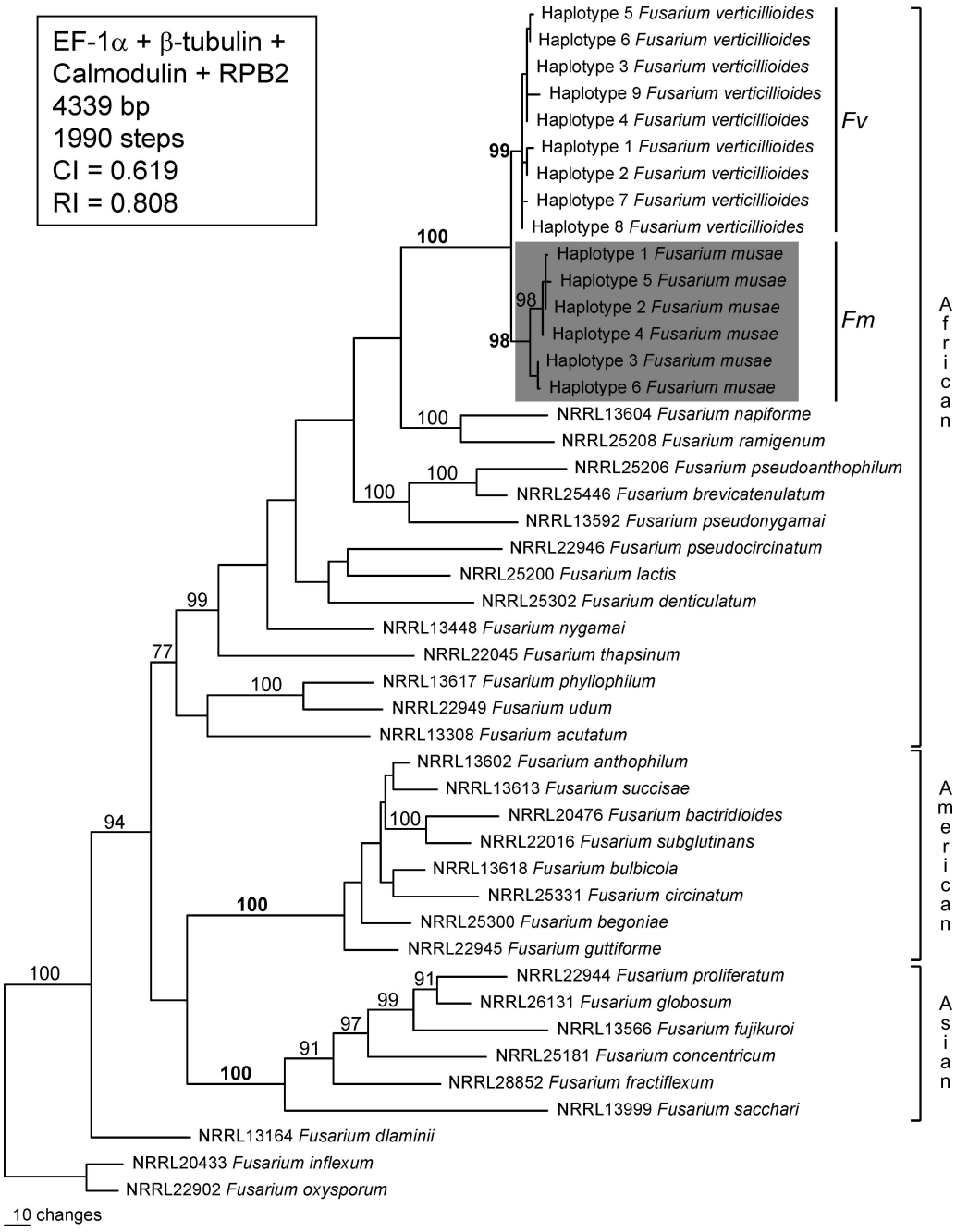


FIG. 2. One of 104 MPTs inferred from the combined translation elongation factor EF-1α, β-tubulin, calmodulin and *RPB2* gene partial sequences rooted by the outgroup method. Bootstrap intervals higher than 75% are indicated. Biogeographic subclades defined by O'Donnell et al. (1998a) are indicated. *Fm* and *Fv* clades are strictly conserved in the 104 MPTs, as revealed by the strict-rule consensus analysis (data not shown).

*Teleomorph.* Perithecia superficial, solitary to aggregated in groups and seated on a minute stromatic base, obovoidal, and warty; 250–420 μm × 220–260 μm diam (TABLE III); blue-black (FIG. 3A–B). Asci fusiform, dehiscent, 55–65 μm long × 8–10 μm wide, eight-spored (FIG. 3C). Ascospores exuded in cirrhi, ellipsoidal to obovoid, one-septate and slightly constricted at the septum, 10–22 × 4–9 μm (FIG. 3D, TABLE III). Heterothallic. Strains ITEM 1121 (= MUCL 52573) and MUCL 51371 were chosen as respective MAT-1 and MAT-2 reference strains for mating.

*Etymology.* The epithet is derived from the fact that the only known host plant for this species is *Musa sapientum* L.

*Known distribution.* Central America, Mexico, Ecuador, Canary Islands and the Philippines.

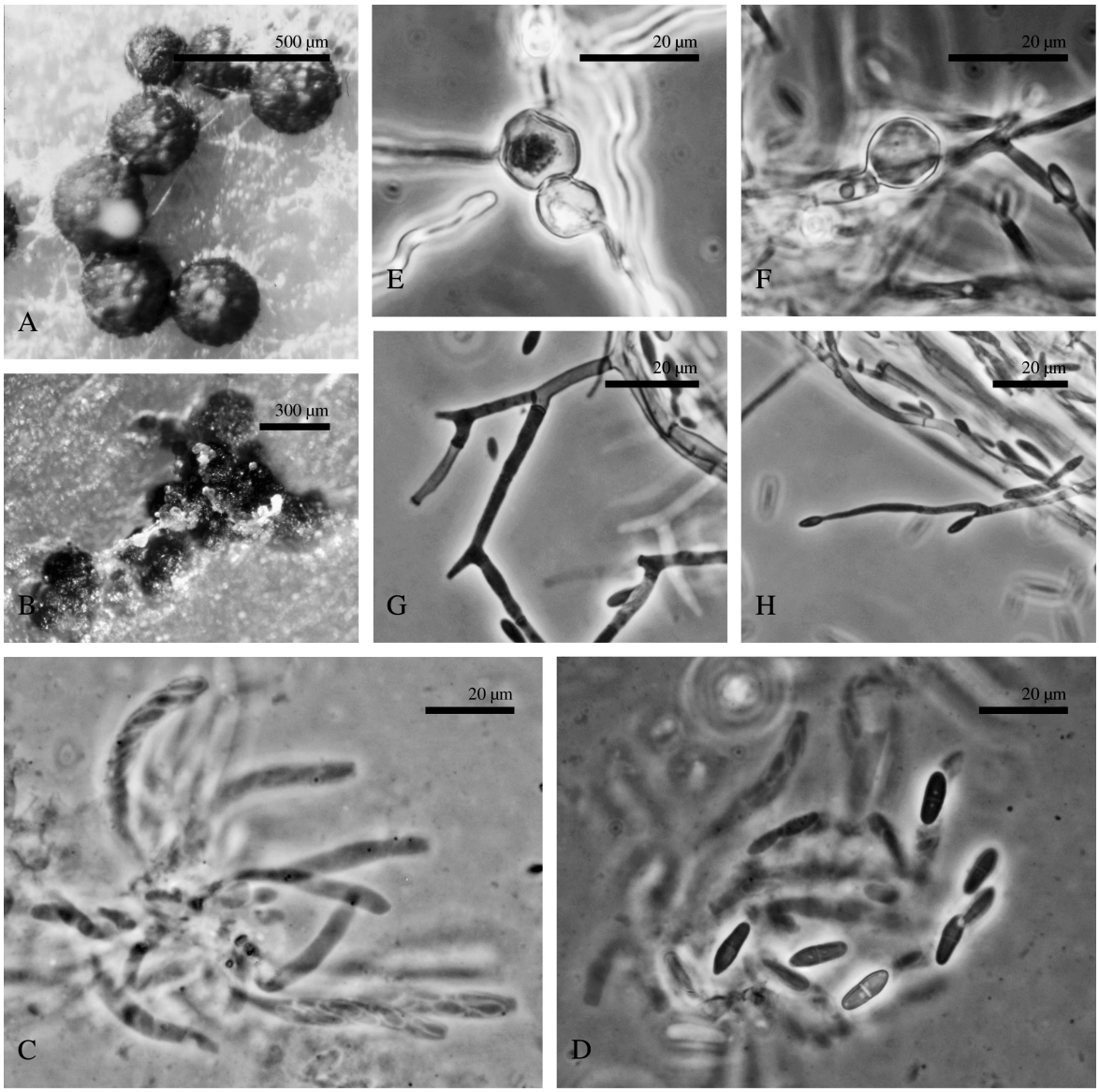


FIG. 3. *Gibberella musae*. A, B. perithecia. C. asci. D. ascospores. E, F. *Fusarium musae* pseudochlamydospores produced respectively at 25 C on SNA (MUCL 31965) and PDA (NRRL 25059). G, H. Intercalary conidiophores from *F. musae* MUCL 31965 and ITEM 1143 cultivated on SNA at 25 C.

**Fusarium musae** Van Hove, Waalwijk, Munaut, Logrieco and Moretti sp. nov. FIG. 3E–H
MycoBank MB519035

Coloniae in agaro PDA apud 25 C 3.7 mm per diem crescunt, post dies septem 54.9 mm diam. Mycelium aerium album deinde violaceum. Coloniae supra et infra rubrae. Sporodochia nulla. Sclerotia nulla. Rare pseudochlamydosporae terminales, hyalinae, solitariae, globosae, 12–14 µm diam. In agaro SNA conidiophora simplicia vel ramosa, cellulae conidiogenae monophialides, 11–60 × 2–4 µm longae. Constantia producentes rare aut non unmquam presentia conidiophora, quae possunt producere nova monophialida in sub-apicale positura precedentium monophialidorum, intercalaribus phialidibus fiantibus. Microconidia abudantia, in catenis et capitulis falsis, hyalina, clavata vel ellipsoidea saepe ad basim truncata, non septata rare 1-septata, 5–17.5 × 1.5–4 µm. Sporodochia nulla. Macroconidia nulla. Rare pseudochlamydosporae terminales, hyalinae, solitariae, globosae, 12–14 µm diam.

Teleomorphosis: *Gibberella musae*.

*Holotype for* Fusarium musae. HONDURAS. From fruit of *Musa* sp. (MUCL 52574, dry SNA culture; ex MUCL 52574 = NRRL 25059 = CBS 624.87).

*Anamorph*. Colonies on potato dextrose agar (PDA) growing 3.7 mm/d at 25 C, attaining 54.9 mm diam after 7 d; aerial mycelium white to vinaceous. Colonies from above and below reddish. Pigmentation uniform or variegated. Sporodochia absent. Sclerotia absent.

TABLE III. Morphological differences of the *Gibberella* species from the *Gibberella fujikuroi* species complex

| | *Perithecia* | | *Ascospore* | |
|---|---|---|---|---|
| *Gibberella* spp. | Diameter (μm) min–max (mean) | Maturation time (d) min–max (mean) | Length (μm) min–max (mean) | Width (μm) min–max (mean) |
| *G. circinata* | 288–358 (337)[a] | | 11.1–14.0 (12.6)[f] | 4.7–5.5 (5.1)[f] |
| *G. fujikuroi* | 140–300 (231)[b] | | 10–20 (12.5)[b] | 4–7 (4.7)[b] |
| | | | 7.0–17.0 (12.8)[g] | 3.2–6.3 (4.7)[g] |
| *G. intermedia* | 280–520 (389)[b] | | 10–21 (14.6)[b] | 4–7 (4.8)[b] |
| *G. konza* | 360–780 (628)[c] | | 12–18 (15.2)[c] | 4–7 (5.9)[c] |
| *G. moniliformis* | 291–305 (298)[d] | 15–19 (16)[d] | 12–23 (17.5)[b] | 4–6 (4.8)[b] |
| | 228–460 (321)[b] | | 14.5–21.0 (17.7)[g] | 3.4–5.6 (4.6)[g] |
| *G. musae* | 220–260 (240)[d] | 18–21 (19)[d] | 10–22[h] | 4–9[h] |
| *G. nygamai* | 184–238 (209)[e] | | 8.5–20 (13.9)[e] | 4–8 (5.3)[e] |
| *G. sacchari* | 240–420 (307)[b] | | 16–32 (22.4)[b] | 4–8 (5.6)[b] |
| *G. thapsina* | 216–229 (220)[d] | | | |

[a] Britz et al. 2002, [b] Kuhlman 1982, [c] Zeller et al. 2003, [d] Moretti et al. 2004, [e] Klaasen and Nelson 1996, [f] Nirenberg and O'Donnell 1998, [g] Nirenberg 1976, [h] this study.

Rare hyaline pseudochlamydospores single and globose, at the ends of hyphae, 12–14 μm diam (FIG. 3F). On SNA conidiophores unbranched or branched, bearing monophialidic conidiogenous cells 11–60 × 2–4 μm long (FIG. 3G–H). Sympodially proliferating conidiophores occasionally are present, which may form additional new monophialides on the subapical position of the previous monophialides, resulting in intercalary phialides (FIG. 3G–H). Microconidia abundant, borne in chains or false heads, hyaline, claviform or ellipsoidal, often truncated, aseptate or rarely one-septate 5–17.5 × 1.5–4 μm. Sporodochia absent. Macroconidia absent. Rare hyaline pseudochlamydospores single and globose, at the end of hyphae, 12–14 μm diam (FIG. 3E).

Delimitation of this species is strongly supported based on the exclusivity criterion under genealogical concordance phylogenetic species recognition (GCPSR) (Taylor et al. 2000) in which reciprocal monophyly received strong MP (most parsimonious) bootstrap support from analyses of EF-1α, α-tubulin, calmodulin and *RPB2* combined sequences. *F. musae* formed a robust clade with *F. verticillioides* in the multilocus phylogeny.

*Known distribution.* Central America, Mexico, Ecuador, Canary Islands and the Philippines.

*Habitat.* Fruits of banana.

*Etymology.* Refers to teleomorph.

*Isolates studied.* MUCL 31965, Nicaragua, *Musa* sp. fruit; MUCL 51371, Philippines, *Musa* sp. fruit; NRRL 25059 = CBS 624.87 = MUCL 52574 (EX HOLOTYPE), Honduras, *Musa* sp. fruit; NRRL 25673, Guatemala, *Musa* sp. fruit; NRRL 28893, Mexico, *Musa* sp. fruit; ITEM 1121, Panama, *Musa* sp. fruit; ITEM 1142, Ecuador, *Musa* sp. fruit; ITEM 1143, Ecuador, *Musa* sp. fruit; ITEM 1149, Panama, *Musa* sp. fruit; ITEM 1245, Canary Islands, *Musa* sp. fruit; ITEM 1250, Canary Islands, *Musa* sp. fruit.

## DISCUSSION

The *Gibberella fujikuroi* species complex (GFSC) refers to *Fusarium* species with similar and overlapping morphological traits that complicate their differentiation (Kvas et al. 2009). The main objective of this study was to investigate the taxonomic status of a *Fusarium* strain population isolated from banana that initially were identified as *F. verticillioides*. To reach this objective a polyphasic evaluation of published and new biochemical, morphological and molecular data was performed on *F. verticillioides* sensu stricto (*Fv*) and the distinct strains isolated from banana (*Fm*). Based on the results of this evaluation, the *Fm* population has been identified as a new species, *Gibberella musae* Van Hove et al. sp. nov. (anamorph: *Fusarium musae* Van Hove et al. sp. nov.).

Morphologically *F. musae* shares a number of characteristics with species of the GFSC, especially with *F. verticillioides*, particularly the presence of hyaline clavate/ellipsoid microconidia formed in long chains and/or false heads on monophialides. Nevertheless *F. musae* can be differentiated from *F. verticillioides* on the basis of the production of pseudochlamydospores and characteristics of microconidia and phialides. The rare pseudochlamydospores from *F. musae* observed on PDA and SNA (FIG. 3E, F) are distinguishable from true chlamydospores because they have no surface ornamentation and they are not thick- or double-walled (Leslie and Summerell 2006). These pseudochlamydospores differ also from ''swollen hyphal cells'' because they do not have transverse septa (Klaasen and Nelson 1998). Pseudochlamydospores produced by *F. musae* are similar to those produced by *F. andiyazi* Marasas, Rheeder, Lampr., K.A. Zeller & J.F. Leslie (Marasas

et al. 2001), but further study is required to compare their structures in more detail. Although *F. musae* shares common morphological characters with *F. andiyazi* (e.g. long chain on monophialides, pseudochlamydospores), phylogenetic analyses including EF-1α and β-tubulin gene sequences from *F. andiyazi* available on EMBL and *Fusarium* ID databases demonstrate that *F. musae* is clearly distinct from *F. andiyazi* (data not shown). Moreover *F. musae* differs also from *F. andiyazi* because the latter is not able to produce moniliformin (Leslie et al. 2005). *F. musae* strains produce slightly smaller nonseptate microconidia than *F. verticillioides* strains and form septate microconidia twice as frequently (Hirata et al. 2001). Regarding phialides, this species also can be differentiated from *F. verticillioides* because, in addition to typical monophialides, it produces intercalary phialides as the results of sympodially proliferating monophialidic conidiophores. In comparison with other *Gibberella* species of the GFSC (TABLE III), the perithecia of *G. musae* appeared significantly smaller (220–260 μm diam, mean 240 μm diam) than perithecia of its sister species, *G. moniliformis* (291–305 μm, mean 298 μm) (Kuhlman 1982, Moretti et al. 2004), and also of more distantly related species, *G. circinata* Nirenberg & O'Donnell : Britz, Coutinho, Wingfield & Marasas, *G. intermedia* (Kuhlman) Samuels, Nirenberg & Seifert, *G. konza* Zeller, Summerell & Leslie and *G. sacchari* Summerell & Leslie (Britz et al. 2002, Kuhlman 1982, Zeller et al. 2003). On the other hand the perithecia diameter of *G. musae* was slightly larger than *G. nygamai* Klaasen & Nelson perithecia (Klaasen and Nelson 1996). Although *G. musae* perithecia was of the similar size as perithecia of *G. fujikuroi* (Sawada) Ito in Ito & K. Kimura (140–300 μm, mean 231 μm) and *G. thapsina* Klittich, Leslie, Nelson & Marasas (216–229 μm, mean 220 μm) (Kuhlman 1982, Moretti et al. 2004), these three species are more distantly related. *G. musae* produced ascospores of similar length but slightly wider than its sister species *G. moniliformis* (TABLE III) (Kuhlman 1982, Nirenberg 1976).

Phylogenetic analyses based on EF-1α, mtSSU and IGS sequences showed that *Fm* and *Fv* strains clustered into two well supported groups (Hirata et al. 2001, Mirete et al. 2004). In the present study results of a multilocus (EF-1 α, β-tubulin, calmodulin and *RPB2*) phylogenetic analysis for species of the GFSC clearly resolved the 11 *F. musae* isolates as a novel phylogenetically distinct species that is sister of *F. verticillioides*. Indeed the analysis of (i) the combined phylogenetic tree topology demonstrates the monophyletic origin of *F. musae* (98% bootstrap) along with *F. verticillioides* (99% bootstrap) and (ii) the single-locus phylogenetic tree topologies satisfy the genealogical concordance and nondiscordance criteria (Dettman et al. 2003). The *F. musae* clade is present in three of the four single-locus phylogeny analyses, as revealed by a majority-rule consensus tree (data not shown), and the *F. musae* clade is well supported by high bootstrap value (95%) in the EF-1α single-locus phylogeny analyses but is not contradicted in the other (β-tubulin, calmodulin and *RPB2*) single-locus phylogeny analyses at the same level of support. Together both sister species form a well supported monophyletic group (100% bootstrap) within the GFSC African clade described by O'Donnell et al. (1998a). Using reciprocal monophyly as the exclusivity criterion under GCPSR (Taylor et al. 2000), our multilocus phylogenetic results strongly support the recognition of these *F. musae* isolates as a novel species within the GFSC.

Both mating types are present in *F. musae* isolates sampled and strains of opposite mating type show some degree of cross fertility (Mirete et al. 2004, Moretti et al. 2004). Perithecia obtained from these crosses were significantly smaller than those produced by *F. verticillioides* strains. *F. musae* strains also showed some degree of fertility with *F. verticillioides* tester strains but they were infertile with tester strains of other *Fusarium* mating populations of the GFSC (Moretti et al. 2004). However two anomalous traits of the fertile crosses of *F. musae* isolates with *F. verticillioides* tester strains are clear indications of genetic differences between both species. First anomalous trait is the significant differences in maturation time and size of perithecia (Moretti et al. 2004). The second trait is the occurrence of spontaneous conidiation mutations resulting in aconidial mutants among random ascospore progeny from crosses involving one *Fv* strain (MRC 826) and one *Fm* strain (NRRL 25059) (Glenn et al. 2004). Moreover examples of interfertility between different biological species of the GFSC, including *F. fujikuroi* and *F. proliferatum* (Leslie et al. 2004b) and *F. circinatum* and *F. subglutinans* (Wollenw. & Reinking) P.E. Nelson, Toussoun & Marasas (Desjardins et al. 2000, Steenkamp et al. 2001), illustrate that a degree of sexual compatibility may exist between closely related phylogenetically and morphologically diagnosable species (Kvas et al. 2009, Steenkamp et al. 2002). Despite the degree of fertility observed between *F. musae* and *F. verticillioides*, a wealth of phylogenetic, biochemical and morphological characteristics support its distinction as a separate species. This is another example of the limitation of mating tests as primary or unique tool for species recognition that highlights the importance of using polyphasic methods for accurate description and/or identification.

Our results and those from previous studies show that *F. musae* and *F. verticillioides* also can be distinguished based on the production of fumonisin and moniliformin mycotoxins (TABLE I). Indeed, even though production of moniliformin by *F. verticillioides* strains has been reported, the strains that produced it were few and they produced only trace amounts of the toxin (Leslie and Summerell 2006). Moreover moniliformin-producing strains often were misidentified as *F. verticillioides*, being instead *F. thapsinum* or *F. fujikuroi*, clearly distinct species of the GFSC (Jimenez et al. 1997, Klittich et al. 1997, Leslie et al. 1996, Moretti et al. 2004, Schütt et al. 1998). In contrast to *F. verticillioides*, *F. musae* strains produce moniliformin but not fumonisins. Moreover we have shown here that *F. musae* isolates have lost their fumonisin-production capacity because of the nearly complete excision (FIG. 1) of the FGC, which is intact in all *F. verticillioides* strains analyzed. Indeed the presence of remnant portions of the first (*FUM21*) and last (*FUM19*) *FUM* genes in tandem within *F. musae* genomes demonstrated clearly that the FGC was present but had been excised in this species (FIG. 1). These results complemented our data in Van Hove et al. (2006) and confirmed similar results published independently by Glenn et al. (2008) with another set of *Fm* strains. The comparison of the excision site and ΔFGC flanking region sequences of all the *F. musae* strains demonstrated clearly that one excision took place once in a common ancestor on the *F. musae* lineage because the excision site is identical and flanking sequences are highly similar (> 99%) in all these strains. With transformation-mediated complementation Glenn and collaborators transformed the *Fm* strain NRRL 25059 with the full FGC from the *Fv* strain M-3125 via two overlapping cosmids. Transformants receiving both cosmids then were able to produce fumonisins (Glenn et al. 2008). Moreover in the same paper they reported data showing that fumonisin production by *F. verticillioides* is required for development of foliar disease symptoms on maize seedlings, thus demonstrating that fumonisins can significantly affect maize-*F. verticillioides* interactions (Glenn et al. 2008). Our results and those of Glenn (2006) and Glenn et al.(2001) on BOA tolerance (TABLE I) and metabolization revealed that *F. musae* strains are sensitive to and unable to metabolize BOA, contrary to *F. verticillioides* strains. Given that (i) fumonisins and BOA appear to be important factors involved in the interactions of maize with *Fusarium* and (ii) multiple studies have shown that *F. musae* strains are pathogenic on banana but not on maize, while *F. verticillioides* strains respond inversely (Glenn et al. 2008, Hirata et al. 2001, Moretti et al. 2004), our data are consistent with the hypothesis that loss of the FGC along with changes in BOA metabolism and tolerance have roles in the differences in host specificity that occurred in speciation that separated *F. musae* from *F. verticillioides* (Moretti et al. 2004).

All *F. musae* strains initially were isolated on banana fruits cultivated in the Neotropics (Guatemala, Honduras, Nicaragua, Panama, Mexico, Ecuador) as well as in the Canary Islands (Hirata et al. 2001, Jimenez et al. 1997, Mirete et al. 2004, Moretti et al. 2004). However in a preliminary survey of *Fusarium* from banana we identified one *F. musae* strain (MUCL 51371) among 40 *Fusarium* isolates from the Philippines but none among 20 isolates from the Democratic Republic of the Congo (RDC). Although this survey was not sufficiently representative to ensure that *F. musae* is not present in mainland Africa, the presence of *F. musae* in the Philippines and Canary Islands indicated that this species is not geographically restricted to the Central American region as was reported by Mirete and collaborators (Mirete et al. 2004). It is worthwhile noting that the ΔFGC flanking sequence amplified with the fvh59 and fvh55 primers (TABLE I) is identical for all *F. musae* isolates except the Philippines isolate (MUCL 51371), which differs by six single mutations and two deletions. Although this observation alone indicates that the Asian *F. musae* strain could be distinct, multilocus genotyping revealed that MUCL 51371 belongs with two other isolates to haplotype 2 (TABLE I), which is clearly nested within the *F. musae* clade (FIG. 2). From a biogeographical point of view there are some inconsistencies between the *F. musae* evolutionary origin within the GFSC African clade and the Asian origin of *Musa* spp., which is its apparent current preferential host. It seems likely that *F. musae* evolved in Africa on some unknown host or hosts and then moved onto banana once the pathogen was brought in contact with this host after it was introduced in Africa from the Indo-Malaya region. Moreover because it has been stated that bananas from West Africa were planted by the Portuguese on the Canary Islands (by early 1500s) and from there to other Neotropical countries (Simmonds 1966) it is consistent with the detection of *F. musae* on banana in these regions. However this hypothesis could not fully explain the identification of one *F. musae* strain (MUCL 51371) isolated on a banana fruit of the ''Latundan'' variety from the Philippines. In this respect a larger number of Asian (and possibly African) *F. musae* strains isolated from banana or other hosts are needed to better understand the evolution of their biogeographic distribution and the origin of their current host preference.

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