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Marasas et al. 1984 “Toxigenic *Fusarium* Species: Identity and Mycotoxicology” revisited

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ABSTRACT

This study was conducted to determine the species identity and mycotoxin potential of 158 *Fusarium* strains originally archived in the South African Medical Research Council's Mycotoxigenic Fungal Collection (MRC) that were reported to comprise 17 morphologically distinct species in the classic 1984 compilation by Marasas et al., *Toxigenic Fusarium Species: Identity and Mycotoxicology*. Maximum likelihood and maximum parsimony molecular phylogenetic analyses of single and multilocus DNA sequence data indicated that the strains represented 46 genealogically exclusive phylogenetically distinct species distributed among eight species complexes. Moreover, the phylogenetic data revealed that 80/158 strains were received under a name that is not accepted today (ex *F. moniliforme*) or classified under a different species name. In addition, gas chromatography–mass spectrometry (GC-MS) and/or high-performance liquid chromatography–mass spectrometry (HPLC-MS)-based mycotoxin analyses were conducted to determine which toxins the strains could produce in liquid and/or solid cultures. All of the trichothecene toxin-producing fusaria were nested within the *F. sambucinum* (FSAMSC) or *F. incarnatum-equiseti* (FIESC) species complexes. Consistent with this finding, GC-MS analyses detected trichothecenes in agmatine-containing broth or rice culture extracts of all 13 FSAMSC and 10/12 FIESC species tested. Species in six and seven of the eight species complexes were able to produce moniliformin and beauvericin, respectively, whereas B-type fumonisins were only detected in extracts of cracked maize kernel cultures of three species in the *F. fujikuroi* (FFSC) species complex.

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trichothecene; zearalenone

INTRODUCTION

Fusarium is widely recognized today as one of the world's most economically destructive and taxonomically challenging genera of mycotoxigenic plant pathogens (Geiser et al. 2013). This is reflected, in part, by the numerous efforts to catalog its taxonomic diversity over the past century. These efforts have yielded vastly different estimates that range from 9 to 75 species based on morphological species recognition (MSR) (Wollenweber and Reinking 1935; Booth 1971; Gerlach and Nirenberg 1982; Nelson et al. 1983; Leslie and Summerell 2006). The *Fusarium* taxonomic landscape has changed dramatically over the past two decades largely due to the introduction of phylogenetic species recognition based on multilocus genealogical concordance and nondiscordance (O'Donnell et al. 2015). Genealogical concordance phylogenetic species recognition (GCPSR; see Sarver et al. 2011) is a method that identifies shared partitions

among multiple gene genealogies as landmarks for species boundaries (Taylor et al. 2000). GCPSR-based molecular phylogenetic studies to date conservatively indicate that *Fusarium* comprises at least 300 phylogenetically distinct species based on ongoing surveys of fusaria housed in the Agricultural Research Service (ARS) Culture Collection (NRRL; Peoria, Illinois), the *Fusarium* Research Center (FRC; Pennsylvania State University, University Park, Pennsylvania), and the Westerdijk Fungal Biodiversity Institute (formerly the CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands). Close to half of these phylotypes do not appear to have been formally described and lack Latin binomials (Aoki et al. 2014).

Over the past half century, natural product chemists and mycotoxicologists have created a vast body of literature characterizing the plethora of toxic secondary metabolites produced by fusaria (e.g., fumonisins, moniliformin, trichothecenes, and zearalenone, just to

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 Supplemental data for this article can be accessed on the publisher's Web site.

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mention a few). Given the significant threat that these toxins pose to agricultural biosecurity, food safety, and plant and animal health (Wu et al. 2014), several compilations have attempted to catalog the diversity of toxigenic fusaria and the toxins they produce (Marasas et al. 1984; Desjardins 2006; Munkvold 2017). However, toxigenic fusaria identified using only morphological data should be interpreted with caution because GCPSR studies over the past two decades have revealed that the vast majority of *Fusarium* species cannot be distinguished using only MSR (O'Donnell et al. 2015 and references therein). DNA sequence-based phylogenetic analyses have demonstrated that morphologically defined species frequently comprise multiple genealogically exclusive species-level lineages (ex *F. graminearum* sensu lato comprises 16 phylogenetic species; Sarver et al. 2011), which may differ in pathogenicity, mycotoxin production, and host range (Boutigny et al. 2011; Kuhnem et al. 2016; Lee et al. 2016). A number of factors, including the lack of clear morphological characters separating species, have led to the creation of MSR-based taxonomic systems that poorly reflect *Fusarium* species diversity (Booth 1971; Gerlach and Nirenberg 1982; Nelson et al. 1983; Leslie and Summerell 2006). This confusion between differing taxonomic systems has resulted in the widespread misapplication of species names to toxigenic and pathogenic isolates in the scientific literature and in DNA/protein databases (e.g., GenBank, National Center for Biotechnology Information [NCBI]; O'Donnell et al. 2015). The toxigenic *Fusarium* compendium by Marasas et al. (1984) is unique because they personally tested over half of the strains for toxins and/or toxigenicity, and because the strains reported in this compendium were archived in the South African Medical Research Council (MRC) and FRC Culture Collections. Given the preeminent position that this 1984 treatise occupies in the mycotoxicology literature, and the transformative impact GCPSR-based studies have had on *Fusarium* systematics subsequent to its publication, the present research was initiated to (i) collect and analyze multilocus DNA sequence data to reassess the species identity and phylogenetic diversity of 158 MRC strains; and (ii) test the MRC strains for mycotoxin production in liquid media and/or rice or cracked maize kernel cultures using gas chromatography–mass spectrometry (GC-MS) and/or high-performance liquid chromatography–mass spectrometry (HPLC-MS).

MATERIALS AND METHODS

***Fusarium* isolates studied.**—The 158 MRC strains included in the present study (TABLE 1), which were

chosen to represent the phylogenetic diversity of toxigenic *Fusarium* species reported in Marasas et al. (1984), are currently accessioned in the Agricultural Research Council Collection, South Africa (<http://www.arc.agric.za/arc-ppri>), and the Fusarium Research Center (<http://plantpath.psu.edu/facilities/fusarium-research-center>), Pennsylvania State University, where they are available for distribution. The MRC acronym is used herein as reported in Marasas et al. (1984) to avoid unnecessary confusion. In addition to the 30 Agricultural Research Service Culture Collection (NRRL; Peoria, Illinois) strains included in this study as a reference (SUPPLEMENTARY TABLE 1), close to 80% of the MRC strains were archived in the NRRL prior to the inception of this study (TABLE 1). All of the NRRL strains are available for distribution upon request (<https://nrml.ncaur.usda.gov/>).

Molecular phylogenetic analysis.—To obtain total genomic DNA for polymerase chain reaction (PCR), the strains were grown in 50 mL disposable polypropylene centrifuge tubes containing 20 mL of yeast malt broth (20 g dextrose, 5 g peptone, 3 g yeast extract, and 3 g malt extract per L water; Difco, Detroit, Michigan) for 3–5 d on a rotary shaker set at 200 rpm and 25 °C. Mycelium was collected on Whatman no. 1 filter paper discs over a Büchner funnel, freeze-dried overnight, and then DNA was extracted from approximately 100 mg of pulverized mycelium using a cetyltrimethylammonium bromide (Sigma-Aldrich, St. Louis, Missouri) protocol (Gardes and Bruns 1993). Genomic DNA stocks were diluted 1:50 in sterile MilliQ water in 96-well plates for PCR. Portions of DNA-directed RNA polymerase second largest subunit (*RPB2*) and translation elongation factor (*TEF1*) were PCR-amplified and sequenced as previously described (O'Donnell et al. 2010) to identify the MRC strains to species (TABLE 1). Three-locus haplotypes within the *Fusarium incarnatum-equiseti* (FIESC), *F. chlamydosporum* (FCSC), and *F. solani* (FSSC) species complexes were determined by sequencing the internal transcribed spacer + large subunit (ITS+LSU) rDNA in addition to *RPB2* and *TEF1* (O'Donnell et al. 2008, 2009b). Two-locus sequence types (STs) within the *F. oxysporum* species complex (FOSC) were identified by sequencing the entire nuclear ribosomal intergenic spacer region (IGS rDNA) together with the intron-rich 5'-end of *TEF1* (O'Donnell et al. 2009a). All PCR reactions were conducted using Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, California) in an Applied Biosystems (ABI) 9700 or ProFlex thermocycler (Emeryville, California) following published protocols (O'Donnell et al. 2010). After Sanger sequences were

Table 1. Strain histories.

MRC no./FRC no. ^a	NRL no. ^b	MLST ID ^c	Marasas et al. (1984)	Species complex	Toxin ^d	Marasas et al. ^e	Geographic origin	Host/substrate
35/T-428	13825 = 13713	<i>F. sp. nov.-6</i> [FCSC 5-a]	<i>F. chlamydosporum</i>	<i>chlamydosporum</i>	BEA	MON	South Africa	Millet
42/M-1253	13574	<i>F. verticillioide</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	FB, MBO	—	South Africa	Corn
43/T-424	3299	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, BUT, T-2	T-2+	France	Corn
69/M-1324	—	<i>F. pseudonygamai</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, MON	MON	Unknown	Unknown
115/M-811	13827 = 13601	<i>F. sp. nov.-8</i> [FSC]	<i>F. subglutinans</i>	<i>fujikuroi</i>	BIK, MON	MON	South Africa	Corn cobs
117/T-227	13636	<i>F. sp. nov.-6</i> [FCSC 5-b]	<i>F. chlamydosporum</i>	<i>chlamydosporum</i>	BUT, MON	—	South Africa	Bean hay
121/R-4057	29009	<i>F. boothii</i>	<i>F. graminearum</i>	<i>sambucinum</i>	BUT, CUL, 15ADON	DON, ZEA	Zambia	Corn
134/M-1351	13599	<i>F. temperatum</i>	<i>F. subglutinans</i>	<i>fujikuroi</i>	—	—	Zambia	Corn
137/M-1352	13581	<i>F. verticillioide</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, FB	—	Zambia	Corn
460/R-4053	29008	<i>F. boothii</i>	<i>F. graminearum</i>	<i>sambucinum</i>	15ADON	—	South Africa	Corn
514/R-5389	13449 = 20654	<i>F. sambucinum</i>	<i>F. sambucinum</i>	<i>sambucinum</i>	BEA, DAS	DON, ZEA	Iran	Potato
515/M-1166	13592	<i>F. pseudonygamai</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, MBO, MON	DAS+	South Africa	Millet
548/M-1353	13580	<i>F. verticillioide</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, FB, MBO	—	South Africa	Corn
602/M-1167	13576	<i>F. verticillioide</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, FB, MBO	—	South Africa	Corn
756/M-1168	13291	<i>F. temperatum</i>	<i>F. subglutinans</i>	<i>fujikuroi</i>	BEA, BIK, MON	MON	South Africa	Corn
826/M-1325	20960 = 13447	<i>F. verticillioide</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, FB	—	South Africa	Corn
846/R-5390	13450	<i>F. sambucinum</i>	<i>F. sambucinum</i>	<i>sambucinum</i>	BEA, DAS	DAS	Iran	Potato
929/M-1036	13577	<i>F. verticillioide</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK	—	South Africa	Corn chaff
930/M-1037	13575	<i>F. verticillioide</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, FB	—	South Africa	Ground corn
939/R-5452	32055	<i>F. culmorum</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, 4,15-dIANIV	—	Australia	Corn
940/M-1354	13600	<i>F. bulbicola</i>	<i>F. subglutinans</i>	<i>sambucinum</i>	MBO	—	Germany	<i>Haemanthus</i>
941/M-1355	13602	<i>F. anthophilum</i>	<i>F. anthophilum</i>	<i>fujikuroi</i>	BIK, MON	—	Germany	<i>Hippeastrum</i>
1369/R-5145	29368	<i>F. culmorum</i>	<i>F. culmorum</i>	<i>sambucinum</i>	3ADON	—	Finland	Wheat
1371/R-5146	34636	<i>F. culmorum</i>	<i>F. culmorum</i>	<i>sambucinum</i>	CUL, 3ADON	ZEA	Finland	Wheat
1411/M-1033	31551	<i>F. thapsinum</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, MON	MON	Georgia, USA	Corn
1412/M-1034	6022	<i>F. pseudonygamai</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, MON	MON	South Africa	Millet
1413/R-4606	54760	<i>F. avenaceum</i>	<i>F. avenaceum</i>	<i>tricinum</i>	ENB, MON	—	Unknown	Unknown
1439/M-1118	13582	<i>F. verticillioide</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, FB	—	Hungary	Poultry feed
1445/R-5169	54140	<i>F. sp. [FIESC 25-g]</i>	<i>F. incarnatum</i>	<i>incarnatum-equiseti</i>	DAS	T-2	India	Sorghum grain
1605/T-403	—	<i>F. poae</i>	<i>F. poae</i>	<i>sambucinum</i>	DAS	T-2	Wisconsin, USA	Corn
1606/T-625	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, BUT, T-2	T-2	Unknown	Unknown
1620/T-405	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, T-2	T-2	Minnesota, USA	Corn
1621/R-5250	31537	<i>F. graminearum</i>	<i>F. graminearum</i>	<i>sambucinum</i>	15ADON	ZEA	Minnesota, USA	Corn
1623/R-5251	34641	<i>F. culmorum</i>	<i>F. culmorum</i>	<i>sambucinum</i>	BUT, CUL, ZEA, 3ADON	DON, ZEA	Washington, USA	Unknown
1642/R-4485	54132	<i>F. sp. [FIESC 29-a]</i>	<i>F. semitectum</i>	<i>incarnatum-equiseti</i>	DAS	DAS+	Unknown	Millet
1646/R-4838	31531 = 6392	<i>F. graminearum</i>	<i>F. graminearum</i>	<i>sambucinum</i>	BUT, CUL, 15ADON	DON, ZEA	Hungary	Wheat
1692/T-347A	3511	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	T-2	—	Unknown	Wheat
1693/R-5316	5864	<i>F. graminearum</i>	<i>F. graminearum</i>	<i>sambucinum</i>	15ADON	NIV	Minnesota, USA	Corn
1694/O-1011	1943	FOSC ST-1	<i>F. oxysporum</i>	<i>oxysporum</i>	BEA, BIK, MON	—	Unknown	Human
1704/T-345A	3510	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, BUT, T-2	T-2+	USSR	Millet
1705/T-344	3249	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, T-2	T-2	Illinois, USA	Festuca
1708/T-288	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, BUT, T-2	T-2	Unknown	Unknown
1767/T-346A	3509	<i>F. kyushuense</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, 4,15-dIANIV	NIV+	Japan	Wheat seed
1768/T-423	3299	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BUT, T-2	T-2	USA	Corn
1781/R-5317	5908	<i>F. graminearum</i>	<i>F. graminearum</i>	<i>sambucinum</i>	BEA, CUL, 15ADON	T-2	Missouri, USA	Fescue hay
1782/R-5318	31542	<i>F. graminearum</i>	<i>F. graminearum</i>	<i>sambucinum</i>	ZEA, 15ADON	DON	Indiana, USA	Fescue hay
1783/R-5319	6227	<i>F. armeniacum</i>	<i>F. armeniacum</i>	<i>sambucinum</i>	T-2	T-2	North Carolina, USA	Raw cotton
1784/M-1138	6322	<i>F. fujikuroi</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BEA, BIK, FB, MON	MON	Ohio, USA	Corn
1785/R-5320	5883	<i>F. graminearum</i>	<i>F. graminearum</i>	<i>sambucinum</i>	CUL, ZEA, 15ADON	DON+	Unknown	Unknown
1786/T-423	3287	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, BUT, T-2	T-2+	Canada	Unknown
1787/R-5321	29372 = 3288	<i>F. culmorum</i>	<i>F. culmorum</i>	<i>sambucinum</i>	3ADON	DON, ZEA	Unknown	Unknown
1888/R-4608	54756	<i>F. avenaceum</i>	<i>F. avenaceum</i>	<i>tricinum</i>	ENB, MON	—	Unknown	Unknown
1889/R-4607	29364	<i>F. culmorum</i>	<i>F. culmorum</i>	<i>sambucinum</i>	3ADON	ADON+	Germany	Barley grain

(Continued)

Table 1. (Continued).

MRC no./FRC no. ^a	NRRL no. ^b	MLST ID ^c	Marasas et al. (1984)	Species complex	Toxin ^d	Marasas et al. ^e	Geographic origin	Host/substrate
1890/R-5391	29373	<i>F. culmorum</i>	<i>F. culmorum</i>	<i>sambucinum</i>	3ADON	ADON+	Germany	Barley
1891/R-4482	54131	<i>F. equiseti</i> [FIESC 14-a]	<i>F. equiseti</i>	<i>incanatum-equiseti</i>	DAS, 4,15-diANIV	—	Germany	Barley
1892/R-4783	54138	<i>F. equiseti</i> [FIESC 14-d]	<i>F. equiseti</i>	<i>incanatum-equiseti</i>	—	—	Germany	Barley grain
1893/T-247	13637	<i>F. poae</i>	<i>F. poae</i>	<i>sambucinum</i>	BEA, BUT, DAS	—	Unknown	Unknown
1895/T-246	13638	<i>F. tritinctum</i>	<i>F. tritinctum</i>	<i>tritinctum</i>	ENB, MON	—	Unknown	Unknown
1902/T-432	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BUT, T-2	T-2	South Dakota, USA	Corn
1903/R-5455	13451	<i>F. sambucinum</i>	<i>F. sambucinum</i>	<i>sambucinum</i>	BEA, DAS	DAS+	Minnesota, USA	Corn
1904/R-2121	34300 = 13333	<i>F. gramineum</i>	<i>F. gramineum</i>	<i>sambucinum</i>	BEA, 15ADON	ZE+	Minnesota, USA	Corn
1959/R-5466	13555	<i>F. armeniacum</i>	<i>F. armeniacum</i>	<i>sambucinum</i>	T-2	T-2+	Japan	Barley grain
1961/R-5468	54133	<i>F. sp.</i> [FIESC 1-e]	<i>F. equiseti</i>	<i>incanatum-equiseti</i>	DAS, 4,15-diANIV	NIV+	Japan	Barley grain
1962/T-436	25348	<i>F. kyushuense</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	NA	T-2+	Japan	Wheat
1963/R-5469	13818 = 31547	<i>F. asiaticum</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BUT, 4,15-diANIV*	T-2+	Japan	Barley grain
1976/R-5470	13819	<i>F. asiaticum</i>	<i>F. gramineum</i>	<i>sambucinum</i>	4,15-diANIV*	NIV+	Japan	Barley grain
2017/R-5701	13554	<i>F. armeniacum</i>	<i>F. acuminatum</i>	<i>sambucinum</i>	DAS	T-2	Georgia, USA	C. <i>paspali</i> sclerotia
2031/T-245	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BUT, T-2	T-2+	Hungary	Millet
2033/T-248	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	—	T-2+	Unknown	Unknown
2065/R-5798	31549 = 34303	<i>F. gramineum</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	3ADON, ZEA	DON	Italy	Unknown
2066/O-1042	38288 = 13817	FOSC ST-30	<i>F. gramineum</i>	<i>oxysporum</i>	BEA, BIK, ENB	FA	Unknown	Date palm
2079/M-1225	6442	<i>F. verticilloides</i>	<i>F. oxysporum</i>	<i>fujikuroi</i>	BIK, FB	—	Egypt	Corn
2181/T-503	13714	<i>F. poae</i>	<i>F. poae</i>	<i>sambucinum</i>	BUT, DAS	DAS	Canada	Overwintered wheat
2182/T-504	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BUT, T-2	T-2	Unknown	Unknown
2183/T-505	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BUT, T-2	T-2	Unknown	Unknown
2184/T-506	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	T-2	T-2+	Unknown	Unknown
2186/T-507	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BUT, T-2	T-2+	Unknown	Unknown
2187/R-6354	13454 = 29419	<i>F. cerealis</i>	<i>F. sambucinum</i>	<i>sambucinum</i>	4,15-diANIV	ZE+	Canada	Corn
2188/T-508	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BUT, T-2	T-2	Unknown	Unknown
2189/T-509	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BUT, T-2	T-2+	Unknown	Unknown
2190/R-6379	13553	<i>F. armeniacum</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BUT, T-2	T-2+	Unknown	Unknown
2193/R-6380	13455 = 13708	<i>F. sambucinum</i>	<i>F. acuminatum</i>	<i>sambucinum</i>	BUT, T-2	T-2+	Canada	Tomato
2195/R-6381	—	<i>F. avenaceum</i>	<i>F. acuminatum</i>	<i>sambucinum</i>	BEA, T-2	—	Germany	Potato
2197/R-6382	29375	<i>F. culmorum</i>	<i>F. avenaceum</i>	<i>tritinctum</i>	ENB, MON	—	South Africa	Potato
2198/S-679	—	<i>F. culmorum</i>	<i>F. culmorum</i>	<i>sambucinum</i>	3ADON	—	Germany	Potato
2199/O-1071	—	<i>F. sp.</i> [FOSC 21-c]	<i>F. solani</i>	<i>solani</i>	NA	—	Germany	Potato
2228/M-1245	28056	FOSC ST-51	<i>F. oxysporum</i>	<i>oxysporum</i>	BEA, BIK	—	Egypt	Potato
2231/R-6053	6442	<i>F. verticilloides</i>	<i>F. oxysporum</i>	<i>fujikuroi</i>	FB	—	Unknown	Corn
2232/R-6054	3020	<i>F. sp.</i> [FIESC 10-b]	<i>F. equiseti</i>	<i>incanatum-equiseti</i>	DAS, MBO, MON	DAS+	Unknown	Unknown
2233/R-6055	3214	<i>F. sp.</i> [FIESC 10-a]	<i>F. equiseti</i>	<i>incanatum-equiseti</i>	NA	DAS+	Unknown	Unknown
2234/R-6056	6450	<i>F. gramineum</i>	<i>F. equiseti</i>	<i>sambucinum</i>	BUT, CUL, ZEA, 15ADON	DON	Austria	Corn
2316/M-1247	6452	<i>F. gramineum</i>	<i>F. gramineum</i>	<i>sambucinum</i>	BUT, CUL, ZEA, 15ADON	DON	Austria	Corn
2317/M-1248	6396	<i>F. verticilloides</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, FB	—	Arkansas, USA	Poultry feed
2320/R-6112	13452 = 20681	<i>F. sambucinum</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, FB	—	Arkansas, USA	Poultry feed
2322/M-1250	2284	<i>F. fujikuroi</i>	<i>F. sambucinum</i>	<i>sambucinum</i>	DAS	DAS+	Unknown	Unknown
2323/T-494	3197	<i>F. sporotrichioides</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK	FA	Japan	Rice
2324/M-1243	13598	<i>F. proliferatum</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	T-2	T-2+	Unknown	Unknown
2325/O-1055	28055	FOSC ST-219	<i>F. proliferatum</i>	<i>fujikuroi</i>	BIK, FB	—	Alabama, USA	Cotton boll
2326/M-1246	13579	<i>F. verticilloides</i>	<i>F. oxysporum</i>	<i>oxysporum</i>	BEA, BIK, MON	—	Alabama, USA	Carrot
2327/M-1251	13578	<i>F. verticilloides</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, FB	—	Tennessee, USA	Sweet potato
2330/R-6137	—	<i>F. equiseti</i> [FIESC 14-e]	<i>F. moniliforme</i>	<i>fujikuroi</i>	FA, 4,15-diANIV	—	China	Corn meal
2386/M-1267	13597	<i>F. fujikuroi</i>	<i>F. equiseti</i>	<i>incanatum-equiseti</i>	BIK, FB	—	Arkansas, USA	Barley
2387/M-1268	13593	<i>F. fujikuroi</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BEA, BIK, MON	FA	Japan	Rice
2388/M-1269	13595	<i>F. fujikuroi</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK	FA	Japan	Rice
2390/M-1271	13594	<i>F. fujikuroi</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BEA	FA	Unknown	Unknown
2394/R-6239	13453	<i>F. venenatum</i>	<i>F. sambucinum</i>	<i>sambucinum</i>	BEA, BUT, DAS	DAS	Unknown	Unknown
2433/R-6324	3211	<i>F. sp.</i> [FIESC 5-g]	<i>F. equiseti</i>	<i>incanatum-equiseti</i>	NA	DAS+	Unknown	Unknown
2435/R-6336	3212	<i>F. sp. nov.-3</i> [FIESC 34-a]	<i>F. equiseti</i>	<i>incanatum-equiseti</i>	—	DAS+	Unknown	Unknown
2476/T-563	—	<i>F. kyushuense</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, 4,15-diANIV	NIV+	Japan	Wheat
2478/T-567	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	T-2	T-2+	Japan	Unknown

(Continued)

Table 1. (Continued).

MRC no./FRC no. ^a	NRRL no. ^b	MLST ID ^c	Marasas et al. (1984)	Species complex	Toxin ^d	Marasas et al. ^e	Geographic origin	Host/substrate
2483/R-6325	5537	<i>F. sp.</i> [FIESC 8-a]	<i>F. equiseti</i>	<i>incarnatum-equiseti</i>	DAS	T-2	Missouri, USA	Fescue hay
2486/T-502	6358	<i>F. sp. nov.-10</i> [FSAMSC]	<i>F. chlamydosporum</i>	<i>sambucinum</i>	8-ANEO	ANEO	Georgia, USA	Peanuts
2523/T-570	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, BUT, T-2	T-2	Italy	Unknown
2531/T-565	6491	<i>F. kyushuense</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, 4,15-dIANIV	NIV+	Japan	Vinyl plate
2532/R-6750	—	<i>F. sp. nov.-9</i> [FTSC 11]	<i>F. avenaceum</i>	<i>tricinatum</i>	MON	—	Japan	Soybean
2533/T-564	—	<i>F. kyushuense</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, BUT, 4,15-dIANIV	NIV+	Japan	Barley
2534/T-566	—	<i>F. kyushuense</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	4,15-dIANIV	diANIV	Japan	Unknown
2535/M-1327	13584	<i>F. proliferatum</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	NA	—	Japan	River sediment
2536/O-1166	28057	FOSC ST-220	<i>F. oxysporum</i>	<i>oxysporum</i>	BEA, BIK	ZEA	Japan	Unknown
2557/T-568	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, T-2	T-2+	Japan	Unknown
2558/R-6765	54135	<i>F. sp. nov.-4</i> [FIESC 35-a]	<i>F. equiseti</i>	<i>incarnatum-equiseti</i>	DAS	DAS	Japan	Soybean
2559/R-6766	54137	<i>F. sp. nov.-4</i> [FIESC 35-b]	<i>F. equiseti</i>	<i>incarnatum-equiseti</i>	DAS	DAS	Japan	Soybean
2560/R-6767	28066 = 13852	<i>F. sp. nov.-1</i> [FSAMSC]	<i>F. gramineum</i>	<i>sambucinum</i>	BEA, T-2	T-2+	Japan	Corn
2561/R-6768	54136	<i>F. sp. nov.-4</i> [FIESC 35-c]	<i>F. equiseti</i>	<i>incarnatum-equiseti</i>	DAS	15ADON*	Japan	Soybean
2563/R-6777	13820	<i>F. gramineum</i>	<i>F. gramineum</i>	<i>sambucinum</i>	BEA, BIK	T-2, DAS	Japan	Unknown
2564/O-1173	28058	<i>F. commune</i>	<i>F. oxysporum</i>	<i>nisikadoi</i>	—	—	Japan	River sediment
2565/S-735	22854	<i>F. solani</i> [FSSC 5-pp]	<i>F. solani</i>	<i>solani</i>	—	—	Japan	River sediment
2566/O-1174	13816	<i>F. commune</i>	<i>F. oxysporum</i>	<i>nisikadoi</i>	BEA, BIK, MON	ZEA	Japan	Unknown
2568/R-6784	13829	<i>F. sp. nov.-2</i> [FSAMSC]	<i>F. equiseti</i>	<i>sambucinum</i>	BEA, DAS	DAS+	Japan	River sediment
2580/R-6795	29151	<i>F. gramineum</i>	<i>F. gramineum</i>	<i>sambucinum</i>	3ADON*	T-2, DAS+	Japan	Soybean hulls
2583/R-6798	31567	<i>F. gramineum</i>	<i>F. gramineum</i>	<i>sambucinum</i>	BEA, 15ADON	ZEA	Japan	Soybean hulls
2609/R-6605	54134	<i>F. lacertarum</i> [FIESC 4-d]	<i>F. equiseti</i>	<i>incarnatum-equiseti</i>	ZEA, 4,15-dIANIV	—	India	Rice straw
2610/R-6606	54143	<i>F. sp.</i> [FIESC 25-a]	<i>F. semitectum</i>	<i>incarnatum-equiseti</i>	DAS	—	India	Rice straw
2627/M-1294	13588	<i>F. subglutinans</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	—	—	lowa, USA	Corn
2628/M-1295	13586	<i>F. sp. nov.-7</i> [FFSC]	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, MBO, MON	—	lowa, USA	Corn
2629/M-1296	13587	<i>F. verticilloides</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, FB, MBO	—	lowa, USA	Corn
2630/M-1297	13589	<i>F. verticilloides</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	—	—	lowa, USA	Corn
2631/M-1298	13590	<i>F. verticilloides</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	—	—	lowa, USA	Corn
2632/M-1299	13591	<i>F. verticilloides</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BIK, FB	—	lowa, USA	Corn
2633/M-1293	13585	<i>F. proliferatum</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BEA, BIK, FB, MBO, MON	—	India	Wheat
2634/S-693	22855	<i>F. solani</i> [FSSC 5-g]	<i>F. solani</i>	<i>solani</i>	—	—	India	Wheat
2635/S-694	22856	<i>F. solani</i> [FSSC 5-g]	<i>F. solani</i>	<i>solani</i>	—	—	India	Wheat
2636/R-6520	54142	<i>F. sp. nov.-5</i> [FIESC 36-a]	<i>F. semitectum</i>	<i>incarnatum-equiseti</i>	BEA, BUT, 4,15-dIANIV	—	India	Wheat
2637/R-6521	54139	<i>F. sp.</i> [FIESC 7-c]	<i>F. equiseti</i>	<i>incarnatum-equiseti</i>	—	—	India	Wheat
2677/M-1300	13583	<i>F. proliferatum</i>	<i>F. moniliforme</i>	<i>fujikuroi</i>	BEA, BIK	—	Finland	Poultry feed
2728/T-592	A-27954	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, BUT, T-2	T-2	Canada	Horse feed
2803/S-787	22857	<i>F. falciforme</i> [FSSC 3+4-iii]	<i>F. solani</i>	<i>solani</i>	—	—	India	Corn
2804/R-6983	—	<i>F. sp. nov.-5</i> [FIESC 36-b]	<i>F. semitectum</i>	<i>incarnatum-equiseti</i>	BEA	—	India	Corn
2805/S-788	22858	<i>F. solani</i> [FSSC 5-g]	<i>F. solani</i>	<i>solani</i>	BEA	—	Unknown	Unknown
2806/R-6984	54145	<i>F. sp. nov.-5</i> [FIESC 36-a]	<i>F. semitectum</i>	<i>incarnatum-equiseti</i>	BEA, 4,15-dIANIV	—	India	Wheat
2910/T-290	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, BUT, T-2	T-2	Unknown	Unknown
2911/T-306	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, BUT, T-2	T-2	Unknown	Unknown
2912/T-336	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, BUT, T-2	T-2	Unknown	Unknown
2913/T-340	13427	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	T-2	T-2	England	Unknown
2914/T-341	—	<i>F. sporotrichioides</i>	<i>F. sporotrichioides</i>	<i>sambucinum</i>	BEA, BUT, T-2	T-2	Unknown	Vinyl plate
2940/R-4613	31523	<i>F. gramineum</i>	<i>F. gramineum</i>	<i>sambucinum</i>	15ADON*	ZEA	Hungary	Feed
3081/R-4610	31522	<i>F. gramineum</i>	<i>F. gramineum</i>	<i>sambucinum</i>	3ADON, BUT, CUL	ZEA	Germany	Feed
3082/R-4611	31615	<i>F. gramineum</i>	<i>F. gramineum</i>	<i>sambucinum</i>	3ADON*	ZEA	Germany	Feed

^aMRC, former South African Medical Research Council Collection currently housed at the Agricultural Research Council, Pretoria, South Africa; FRC, Fusarium Research Center, The Pennsylvania State University, University Park, Pennsylvania.

^b NRRL, ARS Culture Collection, Peoria, Illinois.

^c Phylospores in the Fusarium chlamydosporum [FCS], *F. fujikuroi* [FFSC], *F. incarnatum-equiseti* [FIESC], *F. sambucinum* [FSAMSC], *F. solani* [FSSC] and *F. tricinatum* [FTSC] are in parentheses. Species are identified by Arabic numerals and haplotypes within species by lowercase roman letters. ST, sequence types in the *F. oxysporum* complex [FOSC]. Ten putatively novel phylospores are reported as *F. sp. nov.* 1-10.

^d 3ADON, 3-acetyldeoxynivalenol; 15ADON, 15-acetyldeoxynivalenol; 4, 15-dIANIV, 4, 15-diacetylneovalenol; 8-ANEO, 8-acetylneovalenol; BEA, beauvericin; BIK, bikaverin; BUT, butenolide; CUL, culmorin; DAS, diacetoxyscirpinol; ENB, enniatin B; FA, fusaric acid; FB, B fumonisins; MBO, 8-O-methylbostrychoidin; MON, moniliformin; NIV, nivalenol; T-2, T-2 toxin; ZEA, zearalenone; —, toxin not detected; NA, not tested; *, species and trichothecene chemotype determined by SNP-based Lumines assay (Ward et al. 2008). Bikaverin, bostrychoidin, fumonisins fusaric acid and zearalenone are polyketides; beauvericin and enniatins are nonribosomal peptides; culmorin and trichothecenes are terpenoids; butenolide is thought to be derived from glutamic acid, and the biogenic origin of moniliformin is not known.

^e Key toxins reported on in Marasas et al. (1984). +, indicates that additional toxins were reported on in Marasas et al. (1984).

generated using BigDye 3.1 Terminator reaction mix (ABI, Emeryville, California) in a 9700 or ProFlex thermocycler, they were purified using a BigDye XTerminator kit before running them on an ABI 3730 genetic analyzer. Sequencher 5.2.4 (Gene Codes, Ann Arbor, Michigan) was used to edit the sequence chromatograms prior to exporting the data as NEXUS files. NEXUS files were analyzed with Collapse 1.2 to identify unique haplotypes (Posada 2006). In addition to using Sanger sequences to identify the MRC strains, a multilocus single-nucleotide polymorphism (SNP)-based assay was run to identify trichothecene toxin-producing fusaria within *F. sambucinum* species complex lineage 1 (FSAMSC-1; Kelly et al. 2016), formerly referred to as the B-clade, and to predict their chemotype as previously described (Ward et al. 2008; Aoki et al. 2015, TABLE 1).

Maximum parsimony (MP) and maximum likelihood (ML) phylogenetic analyses were conducted, respectively, with PAUP* 4.0b10 (Swofford 2003) and GARLI 2.01 (Zwickl 2006). Unweighted MP analyses were conducted using the heuristic search option with 1000 random sequence addition replicates, MULPARS on, and the tree bisection-reconnection (TBR) branch-swapping algorithm. ML analyses were run on the CIPRES Science Gateway TeraGrid (<https://www.phylo.org/>) using the GTR+I+Γ model of molecular evolution. Clade support was assessed by 1000 ML and MP bootstrap pseudoreplicates of the data. Bootstrapping did not reveal any significant conflicts between the individual partitions, so they were analyzed as a combined data set. Ten putatively novel phylospecies (i.e., *F. sp. nov.*-1 through *F. sp. nov.*-10), which included representatives of five different species complexes, were highly divergent from their closest relatives in the multilocus molecular phylogenetic analyses presented here and in more inclusive analyses (data not shown). DNA sequences were deposited in GenBank under accession numbers MH582073–MH582472, and the NEXUS files and most-parsimonious trees were deposited in TreeBASE (accession numbers S22999 and tree numbers Tr113002–Tr113007).

Toxin production in broth and solid rice grain cultures.—Two types of liquid culture media were used to evaluate trichothecene production by strains comprising the FIESC, FCSC, and *F. sambucinum* species complex (FSAMSC; TABLE 1, SUPPLEMENTARY TABLE 2). Each strain was initially grown on V8 juice agar plates (20% V8 juice, 0.3% CaCO₃, 2% agar; Stevens 1974). Twenty mL of agmatine medium (30 g sucrose, 1.14 g agmatine, 1 g KH₂PO₄, 0.5 g MgSO₄·7H₂O, 0.5 g KCl, 10 mg FeSO₄·7H₂O) and 200 µL of trace element solution (per 100 mL: 5 g citric acid, 5 g ZnSO₄·7H₂O, 0.25 g

CuSO₄·5H₂O, 50 mg MnSO₄·H₂O, 50 mg H₃BO₃, 50 mg NaMoO₄·2H₂O) per 1 L distilled water (Gardiner et al. 2009) or 20 mL of yeast extract peptone dextrose (YEPD) medium (50 g dextrose, 1 g peptone, 1 g yeast extract) per 1 L distilled water in 50-mL Erlenmeyer flasks was inoculated with two 0.5-cm² plugs cut from V8 plates. The cultures were grown at 28 C on a rotary shaker at 200 rpm in the dark. After 7 d, each culture was transferred to a 50-mL conical tube and extracted with 8 mL ethyl acetate with shaking for 30 min. The mixture was separated with centrifugation at 3000 rpm, after which the top ethyl acetate layer was transferred to a 1-dram vial and dried under a nitrogen stream. The residue was then resuspended in 1 mL ethyl acetate for GC-MS.

Strains that failed to produce detectable levels of trichothecenes in liquid culture were grown on autoclaved rice (4.4 g + 1.8 mL water) in 6-dram vials at 25 C in the dark for 1 wk, after which the cultures were extracted with 10 mL ethyl acetate for 30 min with shaking. Each extract was transferred to a 1-dram vial and dried under nitrogen with heat, and the dried extracts were resuspended in 1 mL ethyl acetate and analyzed for trichothecenes with GC-MS.

GC-MS analyses were performed with an Agilent 5873 chromatograph (Wilmington, Delaware) fitted with a HP-5MS column (Wilmington, Delaware) and products detected with a mass spectrometer with an electron impact source. Samples were injected at 150 C, the temperature was held for 1 min and then the column was heated at 30 C/min to 280 C and then held for 7.7 min. Individual peaks in chromatograms were examined for trichothecenes. Under these conditions, 15-acetyldeoxynivalenol (15ADON) is detected at 7.1 min, diacetoxyscirpenol (DAS) at 7.3 min, 4,15-diacetylnivalenol (4,15-diANIV) at 8.1 min, and T-2 toxin at 10.5 min. Trichothecenes were identified based on retention time and comparison of ion fragmentation patterns with a library prepared with purified standards.

Toxin production on cracked maize kernel substrates.—All of the MRC strains were screened for production of other mycotoxins (e.g., fumonisin, moniliformin, beauvericin) by inoculating them on cracked maize kernels (25 g + 11 mL water) as previously described (Aoki et al. 2015; TABLE 1). After 14 d incubation in the dark, 10 g of each culture was extracted with 20 mL 86:14 (v/v) acetonitrile:water for 30 min with shaking. After the extracts were clarified via filtration, they were analyzed by high-performance liquid chromatography–mass spectrometry (HPLC-MS), using a Dionex Ultimate U3000 liquid chromatograph (Phenomenex, Torrance, California) coupled to a

QTRAP 3200 tandem mass spectrometer (AB SCIEX, Thornhill, Ontario, Canada) with atmospheric pressure or electrospray chemical ionization. A 0.6 mL/min reverse-phase gradient (40%–95%) flow between acetonitrile and water over 10 min was used to elute fungal metabolites. HPLC-MS analyses were conducted in positive and negative ionization modes to detect mycotoxins. HPLC-MS and HPLC-MS/MS comparisons of ion mass, elution time, and ion fragmentation with purified standards were used to identify the metabolites (Busman et al. 2012; Busman 2017). For LC-MS analysis, the number and diversity of compounds detected was limited by the availability of analytical standards. For GC-MS analysis, we used analytical standards of butenolides, culmorin, and trichothecenes to confirm their production, but we also employed a mass spectrometry reference library (Savard and Blackwell 2001) for untargeted analysis.

RESULTS

Identity of toxigenic MRC fusaria inferred from phylogenetic analysis of partial RPB2 sequence data.—Maximum likelihood (ML) and maximum parsimony (MP) analyses of a portion of *RPB2* (1839 bp alignment, 650 parsimony-informative characters [PICs]) were conducted to obtain an initial estimate of evolutionary relationships and species status of 158 MRC strains based on the demonstrated utility of this locus for phylogenetic inference within *Fusarium* (FIG. 1, TABLE 1). Sequences of three species in the FFSC were selected as the outgroup based on more inclusive analyses (O'Donnell et al. 2013). The MRC strains were received as 17 morphospecies, but ML and MP analyses of the partial *RPB2* data resolved them as 46 phylogenetically distinct species distributed among eight species complexes. Twelve of the 17 morphospecies harbored two or more phylopecies, and these included nine different taxa received as *F. equiseti*, seven as *F. moniliforme*, four as *F. graminearum*, and three as *F. solani* (FIG. 1). Names could not be applied to 18 of the phylopecies, and these included 10 putatively novel species not previously noted in published phylogenetic analyses (i.e., *F. sp. nov.-1* to *-10*; TABLE 1). The vast majority of the MRC strains (138/158 = 87.3%) belonged to the FSAMSC, FIESC, or FFSC. The 79 isolates within the FSAMSC accounted for half of the MRC strains and 14 phylopecies, followed by the FFSC with 39 strains and 11 species, and FIESC with 20 strains and 12 species. The 20 remaining MRC strains included representatives of five species complexes and accounted for 9 of the 46 phylopecies (TABLE 1). Three species were disproportionately represented among the MRC strain set, and these included *F.*

sporotrichioides (n = 28), *F. verticillioides* (n = 18), and *F. graminearum* (n = 16). With the exception of the FFSC, which received lower bootstrap scores, ML and MP bootstrapping provided strong support (93%–100%) for the monophyly of the seven other species complexes. Evolutionary relationships among the species complexes were mostly unresolved by the *RPB2* data, as reflected by poor bootstrap support along the backbone of the phylogeny (FIG. 1). The *F. oxysporum* species complex (FOSC), however, was strongly supported as sister to the FFSC + *F. commune*. The latter species, which was received as *F. oxysporum* MRC 2564 and 2566 from river sediment in Japan, was the sole representative of the *F. nisikadoi* species complex (FNSC) sampled in the present study.

Phylogenetic diversity and mycotoxin potential of MRC strains within the FSAMSC, FIESC, and FCSC.—

A two-locus data set comprising partial *TEF1* (686 bp alignment, 184 PICs) and *RPB2* (1856 bp alignment, 325 PICs) sequences was constructed to further investigate evolutionary relationships and species limits of 79 MRC strains within the FSAMSC and assess the potential of these strains to produce mycotoxins in vitro (FIG. 2). Sequences of MRC 2568 *F. sp. nov.-2* received as *F. equiseti* from river sediment in Japan and MRC 2486 received as *F. chlamydosporum* from peanuts in Georgia were used to root the phylogeny based on the results presented in FIG. 1, which indicated that these putatively novel 8-acetylneosolaniol- and diacetoxyscirpenol (DAS)-producing species likely represented the earliest diverging lineage sampled within the FSAMSC (TABLE 1). ML and MP bootstrap analyses of the two-locus data set provided strong support (100%) for four trichothecene toxin-producing clades that generally correlated with toxin type. The trichothecenes produced by *Fusarium* are divided into two broad categories based on the presence (type B trichothecenes) or absence (type A trichothecenes) of a keto group at the C-8 position of the trichothecene ring (McCormick et al. 2011). Of the trichothecenes detected in the current study, DAS, T-2 toxin, and 8-acetylneosolaniol are type A trichothecenes, whereas 15ADON and 4,15-diANIV are type B trichothecenes. Within the FSAMSC, these clades were represented by (i) a type A+B trichothecene lineage that included DAS-producing *F. sambucinum*, *F. venenatum* (received as *F. sambucinum*), and *F. poae* and 4,15-diANIV-producing *F. kyushuense* received as atypical *F. sporotrichioides*; (ii) the FSAMSC-1 lineage included 3-acetyldeoxynivalenol (3ADON)- or 15-acetyldeoxynivalenol (15ADON)-producing *F. graminearum*, 15ADON-producing *F.*

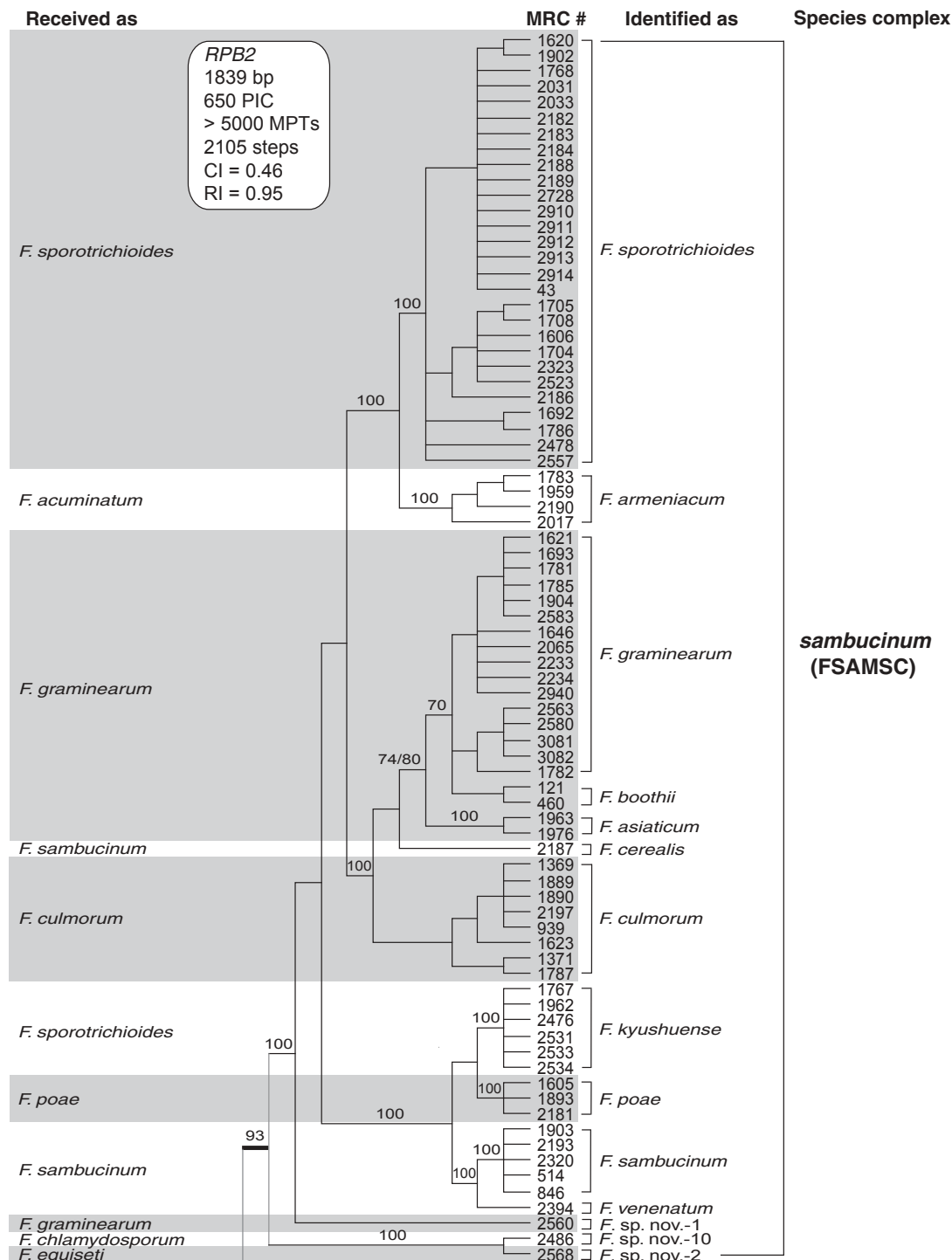


Figure 1. a, b. One of >5000 most-parsimonious cladograms inferred from aligned partial *RPB2* sequences from 158 toxigenic MRC *Fusarium* strains. Strain histories are provided in TABLE 1 and as an excel file (SUPPLEMENTARY TABLE 4). The cladogram was rooted on sequences of six members of the *F. solani* species complex based on more inclusive analyses (O'Donnell et al. 2013). The 17 morphospecies listed under "Received as" were identified using morphological criteria (Marasas et al. 1984). Phylogenetic species listed under "Identified as" were determined here based on phylogenetic analysis of DNA sequence data from portions of two to three loci (see MATERIALS AND METHODS). Thickened black internodes identify eight monophyletic species complexes. Published typing schemes were used to identify two-locus sequence types (STs) within the *F. oxysporum* species complex (FOSC) and three-locus species haplotypes within the *F. incarnatum-Equiseti* (FIESC), *F. chlamydosporum* (FCSC), and *F. solani* (FSSC) species complexes. Species and haplotypes in the latter three species complexes are identified, respectively, by Arabic numerals and lowercase roman letters. Maximum likelihood (ML) bootstrap values are indicated above nodes based on 1000 pseudoreplicates of the data. Maximum parsimony (MP) bootstrap values are only indicated if they differed by $\geq 5\%$ from the ML bootstrap value (ML/MP). Ten putatively novel phylogenetically distinct species are listed as *F. sp. nov.-1* to *-10*. CI = consistency index; MPTs = most-parsimonious trees; PIC = parsimony-informative character; RI = retention index.

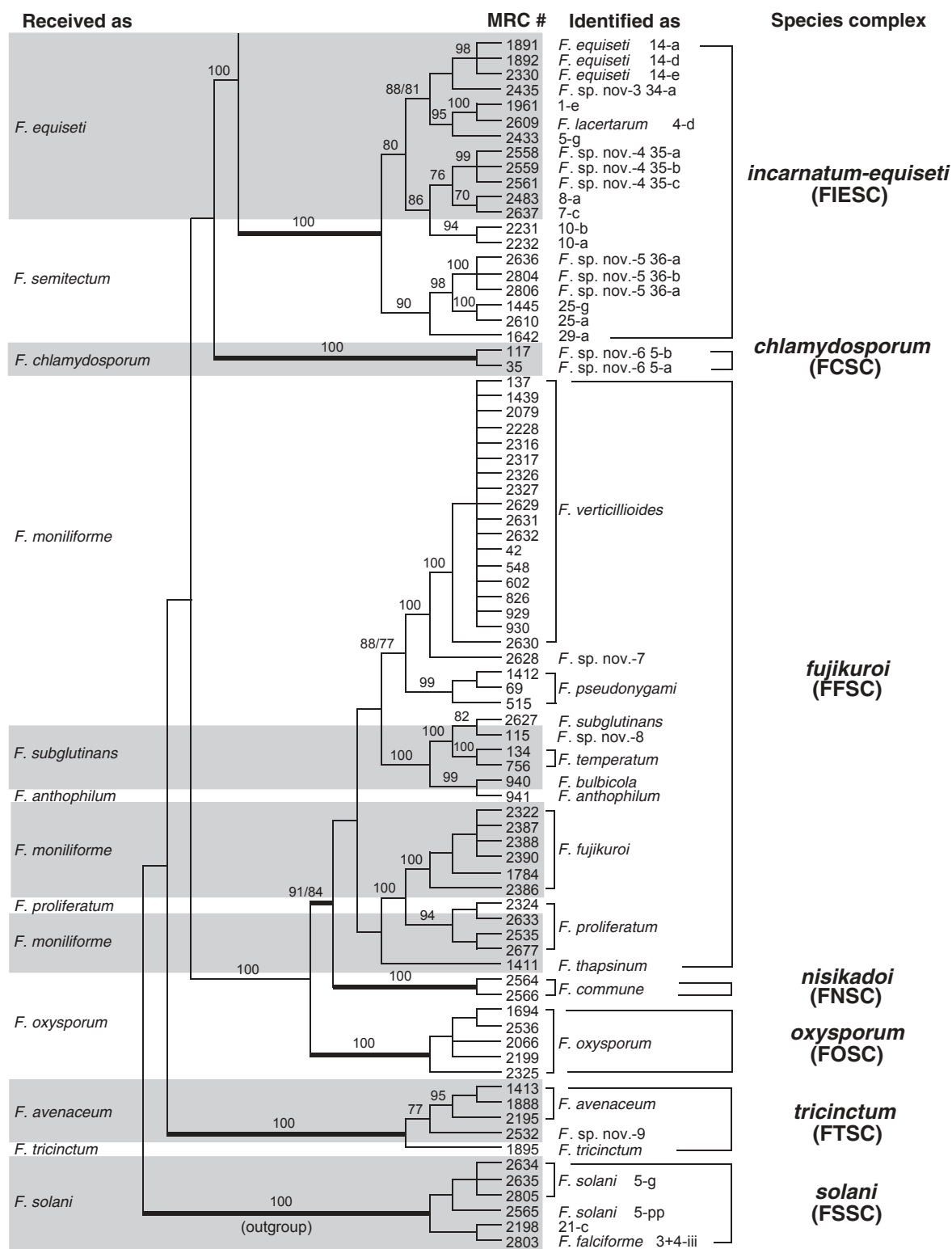


Figure 1. (Continued).

boothii received as *F. graminearum* from corn in South Africa (MRC 460) and Zambia (MRC 121), 4,15-diANIV-producing *F. asiaticum* received as *F. graminearum* from Japanese barley grain (MRC 1963 and 1976), 3ADON- or 4,15-diANIV-producing *F. culmorum*, and 4,15-diANIV-producing *F. cerealis*; (iii) a type A trichothecene lineage that included *F. sporotrichioides* and *F. armeniacum*; and (iv) a type A trichothecene lineage that included MRC 2568 *F. sp. nov.-2* and MRC 2486 *F. sp. nov.-10*, which were received as *F. equiseti* and *F. chlamydosporum*, respectively (TABLE 1). In addition, MRC 2560 *F. sp. nov.-1*, received as *F. graminearum* from Japanese corn, was resolved as a putatively novel T-2 toxin-producing type A trichothecene lineage whose phyletic relations remain to be determined. With the exception of MRC 2017 *F. armeniacum* received as *F. acuminatum*, which produced DAS, the three other MRC strains of this species, together with 28 strains of *F. sporotrichioides*, produced T-2 toxin. The putative trichothecene chemotype of six strains in FSAMSC-1 that failed to produce toxins in agmatine or YEPD broth, or rice kernel cultures, was determined using a SNP-based assay (identified by an asterisk in FIG. 2 and TABLE 1). Support for the monophyly of the trichothecene toxin-producing clades within the FSAMSC received strong ML and MP bootstrap support (FIG. 1), but the relationships of these clades were mostly unresolved by the two-locus data set (FIG. 2).

A three-locus typing scheme, which included portions of *TEF1* (711 bp alignment, 158 PICs), *RPB2* (1766 bp alignment, 268 PICs), and the ITS+LSU rDNA (1149 bp alignment, 45 PICs) identified the 20 MRC strains nested in the FIESC as 12 phylogenetically distinct species, including three that appear to be novel (FIESC 34, 35, and 36; FIG. 3, TABLE 1). Sequences of *F. sp. nov.-6* from South African millet (MRC 35) and bean hay (MRC 117) appear to represent two haplotypes of a novel species in the *F. chlamydosporum* species complex informally designated FCSC 5, and they were used to root the FIESC phylogeny based on more inclusive analyses (O'Donnell et al. 2013). MP and ML analyses of the FIESC data set provided 100% bootstrap support for monophyly of the 10 species represented by two or more strains, and relationships among most of the species were resolved with moderate to high clade support (FIG. 3). However, monophyly of the Equiseti clade was weakly supported by bootstrapping (i.e., 70%). The MRC strains received as *F. equiseti* were resolved as nine phylogenetically distinct species, including two that appear to be putatively novel (i.e., MRC 2435 *F. sp. nov.-3* = FIESC 34, and MRC 2558, 2559, and 2561 *F. sp. nov.-4* = FIESC 35 from soybean

in Japan). Similarly, the five MRC strains received as *F. semitectum* appeared to comprise three phylopecies. One of these, represented by MRC 2636, 2804, and 2806 *F. sp. nov.-5* = FIESC 36 from Indian wheat and corn, appears to be novel. Seven of the species within the Equiseti clade and all three species within the Incarnatum clade produced the A type DAS or B type 4,15-diANIV trichothecene when tested for toxin production in agmatine broth (FIG. 3). Trichothecenes were not detected in agmatine broth or rice cultures of the outgroup strains MRC 35 FCSC 5-a and MRC 117 FCSC 5-b. Beauvericin was detected in culture extracts of the former and moniliformin and butenolide in the latter strain (FIG. 3, TABLE 1).

Several other toxins and biologically active metabolites were detected in culture extracts of the FSAMSC, FIESC, and FCSC strains (TABLE 1, SUPPLEMENTARY TABLES 2–3). Butenolide was produced by 11 species, including 9 species in the FSAMSC, MRC 2636 FIESC 36-a, and MRC 117 FCSC 5-b. The antifungal sesquiterpene culmorin was detected in some strains of *F. graminearum*, *F. boothii*, and *F. culmorum*. Lastly, 9/14 species in the FSAMSC and FCSC 5-a and 5-b produced the antimicrobial griseoxanthone C (SUPPLEMENTARY TABLE 2); beauvericin was also produced by 8/14 species in the FSAMSC and by at least one representative of the FIESC, FCSC, FFSC, FNESC, and FOSC (TABLE 1).

Phylogenetic diversity and mycotoxin potential of MRC strains within the FFSC, FNESC, and FOSC.

A two-locus data set that included partial *TEF1* (694 bp alignment, 126 PICs) and *RPB2* (1852 bp alignment, 211 PICs) sequences was constructed to (i) assess species boundaries within and evolutionary relationships among the FFSC, FNESC, and FOSC; and (ii) map mycotoxins produced in cracked maize kernel cultures on the molecular phylogeny (FIG. 4). The phylogeny was rooted on sequences of the five FOSC strains received as *F. oxysporum* based on more inclusive analyses (FIG. 1 and O'Donnell et al. 2013). ML and MP bootstrap analyses of the two-locus data set provided strong support for most internodes in the phylogeny but failed to resolve relationships among the African, American, and Asian clades of the FFSC (FIG. 4). Bootstrapping also failed to support MRC 1411 *F. thapsinum* from corn from Georgia as a member of the African clade where it was previously reported to be nested (O'Donnell et al. 1998b). Phylogenetic analyses resolved the 39 MRC strains within the FFSC as 11 phylogenetically distinct species (TABLE 1), including the following two putatively novel species: MRC 2628

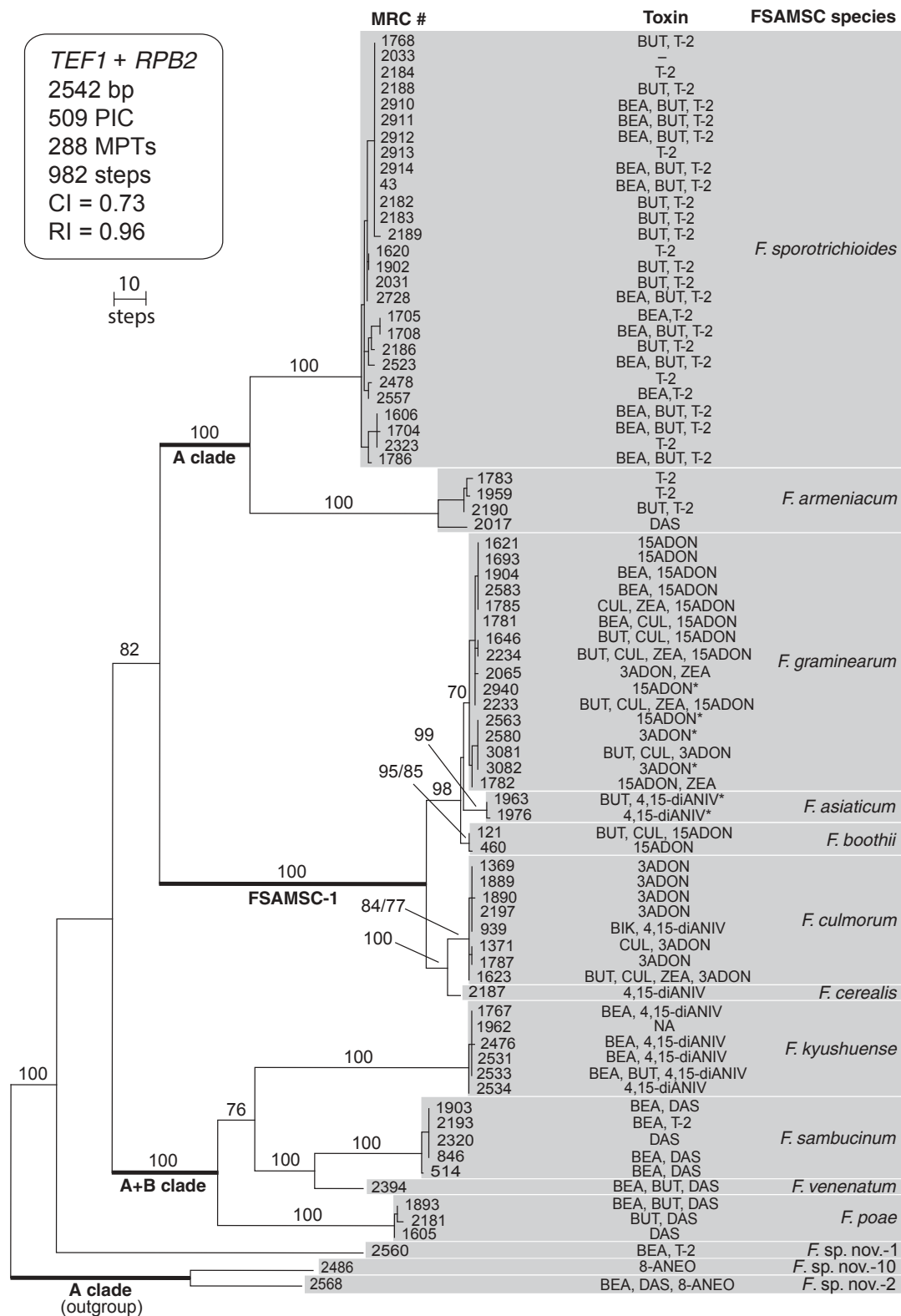


Figure 2. One of 288 most-parsimonious phylograms inferred from aligned partial *TEF1* + *RPB2* sequences from 79 MRC strains representing 14 species in the *Fusarium sambucinum* species complex (FSAMSC). The phylogram was rooted on sequences of MRC 2568 *F. sp. nov.-2* and MRC 2486 *F. sp. nov.-10* based on the analysis presented in FIG. 1. Maximum likelihood (ML) bootstrap values are indicated above nodes based on 1000 pseudoreplicates of the data. Maximum parsimony (MP) bootstrap values are only indicated if they differed by $\geq 5\%$ from the ML bootstrap value (ML/MP). Phylogenetic metrics include CI, consistency index; MPTs, most-parsimonious trees; PIC, parsimony-informative character; and RI, retention index. The primary toxins produced were determined via GC-MS and HPLC-MS, except for six strains identified by an asterisk within FSAMSC-1. The predicted trichothecene toxin chemotype of these six strains was determined using a published SNP-based multilocus genotyping assay (Ward et al. 2008). A clade, type A trichothecene toxin producers; A +B clade, type A and B trichothecene toxin producers. The primary toxins produced include BEA, beauvericin; BIK, bilaverin; BUT, butenolide; CUL, culmorin; DAS, diacetoxyscirpenol; T-2, T-2 toxin; ZEA, zearalenone; 4,15-diANIV, 4,15-diacetylinalenol; 15ADON, 15-acetyldeoxynivalenol; 3ADON, 3-acetyldeoxynivalenol; and 8-ANEO, 8-acetylneosalinol. NA, strain not tested; –, none detected.

F. sp. nov.-7 from corn in Iowa and MRC 115 *F. sp. nov.-8* from a cotton boll in Alabama. Analyses of the MRC data set resolved these species as sister to *F. verticillioides* and *F. subglutinans* (FIG. 4), respectively, but more inclusive analyses resolved MRC 2628 *F. sp. nov.-7* and *F. andiyazi* as sisters (O'Donnell, unpubl.). Only 2 of the 39 FFSC strains, MRC 941 *F. anthophilum* from *Hippeastrum* sp. from Germany and MRC 2324 *F. proliferatum* from a cotton boll in Alabama, were received correctly identified under names accepted today. The 33 strains received as *F. moniliforme* represented seven different phylospecies (i.e., *F. fujikuroi*, *F. proliferatum*, *F. pseudonygamai*, *F. subglutinans*, *F. thapsinum*, *F. verticillioides*, and *F. sp. nov.-7*). The phylogenetic results revealed that the name *F. subglutinans* was applied to three MRC strains that were identified herein as *F. temperatum* (MRC 134 from corn from Zambia), *F. bulbicola* (MRC 940 from *Haemanthus* sp. from Germany), and *F. sp. nov.-8* (MRC 115 from corn from South Africa). Similarly, MRC strains 2564 and 2566, received as *F. oxysporum* from river sediment in Japan, were identified as *F. commune*, which is nested within the FNSC (FIG. 4). Lastly, a two-locus typing scheme (*TEF1* [634 bp alignment] + IGS rDNA [2220 bp alignment]) indicated that the five MRC strains received as *F. oxysporum* each represented a unique sequence type (ST), including the novel ST 257 represented by MRC 2536 from Japan (FIG. 4).

HPLC-MS analyses of cracked maize culture extracts revealed that fumonisins (FB = FB₁ + FB₂ + FB₃) at ≥0.2 µg/mL or 1 ppm were only produced by three species within the FFSC; these included 2/6 *F. fujikuroi*, 2/4 *F. proliferatum*, and 15/18 *F. verticillioides* MRC strains tested (FIG. 4, SUPPLEMENTARY TABLE 3). Eight of the FFSC strains, including *F. bulbicola*, *F. proliferatum*, *F. pseudonygamai*, *F. verticillioides*, and *F. sp. nov.-7*, produced significant levels of the cytotoxic polyketide 8-O-methylbostrycoidin at ≥1.0 µg/mL. In addition, several representatives of the FFSC, FNSC, and FOSC were able to produce the polar mycotoxin moniliformin, the cyclic hexadepsipeptide beauvericin, and the reddish pigment bikaverin on cracked maize cultures. Moniliformin at ≥1.0 µg/mL was detected in 8/11 species in the FFSC, *F. commune*, and *F. oxysporum*, but not in *F. bulbicola*, *F. verticillioides*, and *F. subglutinans* (FIG. 4, SUPPLEMENTARY TABLE 3). The highest moniliformin production was detected in *F. pseudonygamai* where it ranged from 71.55 to 106.5 µg/mL, followed by MRC 2628 *F. sp. nov.-7* at 75.1 µg/mL. Beauvericin was detected in culture extracts of three species within the FFSC (*F. fujikuroi*, *F. proliferatum*, and *F.*

temperatum), *F. commune* in the FNSC, and the five FOSC strains, where production up to 80.1 µg/mL was observed in MRC 2199 FOSC ST-51. Bikaverin was detected in 31/36 FFSC, 2/2 FNSC, and 5/5 FOSC strains tested and in 11/12 species, but not in MRC 2627 *F. subglutinans*. Over 100 µg/mL bikaverin was produced by 7/11 species in the FFSC and all five FOSC strains; moreover, the following three strains produced over 200 µg/mL bikaverin: MRC 2199 FOSC ST-51, 2066 FOSC ST-30, and 2677 *F. proliferatum*. None of the FFSC, FNSC, and FOSC strains tested produced detectable levels of trichothecenes, and when these strains were tested for enniatin B, it was only detected in maize kernel cultures of MRC 2066 FOSC ST-30 (SUPPLEMENTARY TABLE 3).

Phylogenetic diversity and mycotoxin potential of MRC strains within the FTSC and FSSC.

—A two-locus data set that included portions of *TEF1* (671 bp alignment, 54 PICs) and *RPB2* (1738 bp alignment, 127 PICs), to which 18 NRRL reference strains were added, was constructed to identify five MRC strains nested in the FTSC. ML and MP bootstrapping yielded a nearly fully resolved phylogeny, except that the relationship between *F. avenaceum* and a putatively novel species represented by MRC 2532 *F. sp. nov.-9* from Japanese soybean was unresolved (FIG. 5). The molecular phylogenetic results support the identification of MRC strains 1413, 1888, and 2195 as *F. avenaceum* and MRC 1895 as *F. tricinctum*. Moniliformin + enniatin B, or moniliformin, were the only mycotoxins detected in culture extracts of the five FTSC strains. Moniliformin at ≥1.0 µg/mL was detected in cracked maize cultures of six species within the FTSC, ranging from 1.5 µg/mL in MRC 2532 *F. sp. nov.-9* to moderately high levels in the three strains of *F. avenaceum* (22.9–58.6 µg/mL). Enniatin B was produced by the latter three strains and *F. tricinctum*, with the highest levels observed in MRC 1413 *F. avenaceum* (49.8 µg/mL) and reference strains NRRL 45994 *Fusarium* sp. FTSC-2 (77.2 µg/mL) and NRRL 52727 *Fusarium* sp. FTSC-5 (58.1 µg/mL) (SUPPLEMENTARY TABLE 3). Of the strains included in the present study, enniatin B was only detected in members of the FTSC.

A multilocus typing scheme that employed portions of *TEF1* (693 bp alignment, 9 PICs), *RPB2* (1664 bp alignment, 46 PICs), and ITS+LSU rDNA (1013 bp alignment, 24 PICs) was used to determine species identity of six MRC strain nested in the FSSC (SUPPLEMENTARY FIG. 1). When sequences of these six strains were added to a more inclusive data set (O'Donnell et al. 2008) and analyzed with Collapse

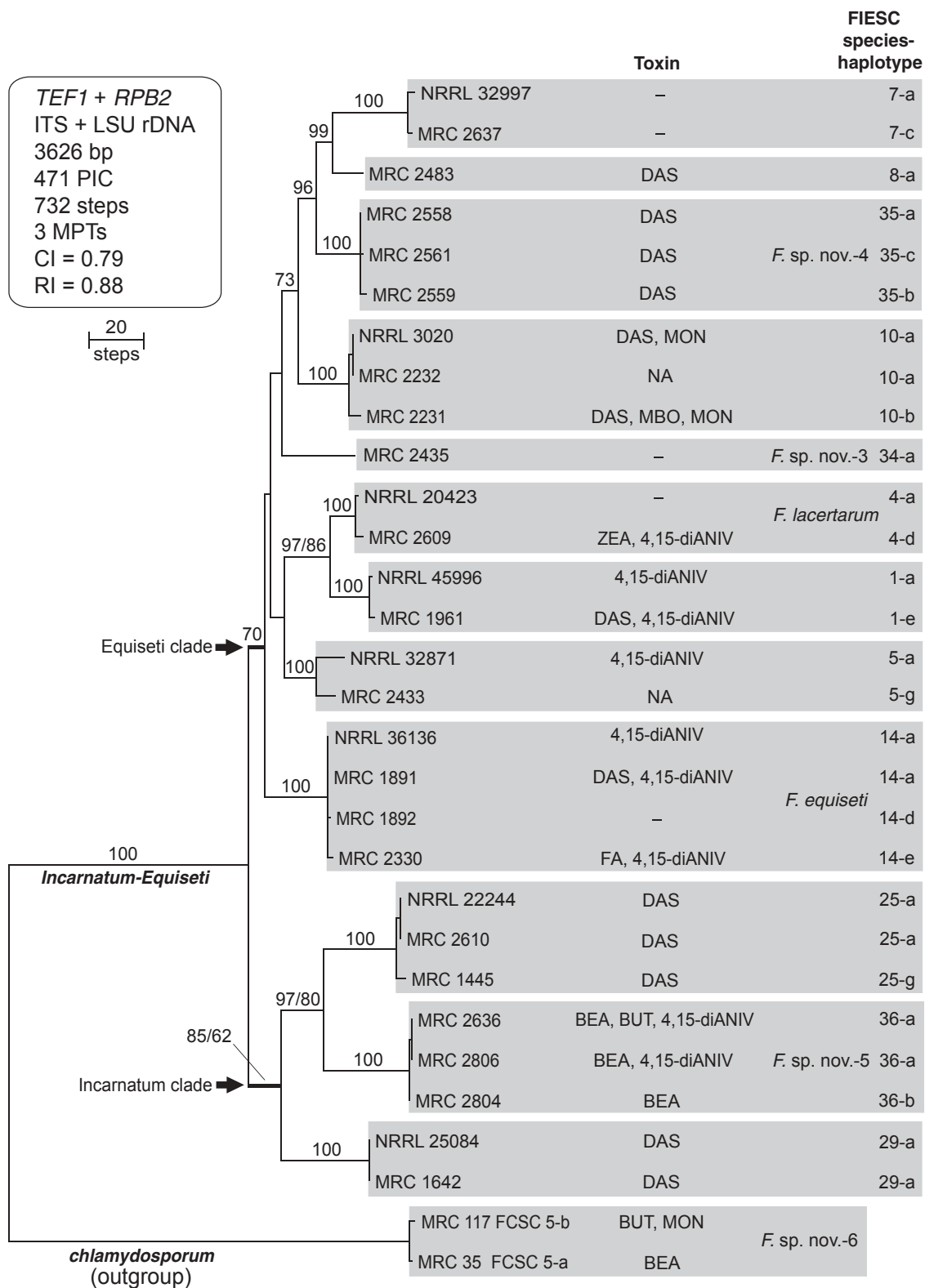


Figure 3. Molecular phylogeny of 28 *Fusarium Incarnatum-Equiseti* clade (FIESC) strains, including eight reference isolates from the ARS Culture Collection (NRRL) used to identify MRC strains to species. The phylogeny represents one of three most-parsimonious phylograms inferred from portions of three loci used to determine species haplotypes within this species complex (O'Donnell et al. 2009b). Sequences of two MRC strains representing the putatively novel species FCSC 5 within the *F. chlamydosporum* species complex were used to root the phylogram. Maximum likelihood (ML) bootstrap values are indicated above nodes based on 1000 pseudoreplicates of the data. Maximum parsimony (MP) bootstrap values are only indicated if they differed by $\geq 5\%$ from the ML bootstrap value (ML/MP). Thickened internodes are used to identify a basal split between members of the Equiseti and Incarnatum clades. Phylogenetic metrics include CI, consistency index; MPTs, most-parsimonious trees; PIC, parsimony-informative character; and RI, retention index. DAS or 4,15-diANIV were detected in 20 of FIESC strains analyzed by GC-MS. The primary toxins produced include BEA, beauvericin; BUT, butenolide; DAS, diacetoxyscirpenol; FA, fusaric acid; MBO, 8-*O*-methylbostrycoidin; MON, moniliformin; T-2, T-2 toxin; ZEA, zearalenone; and 4,15-diANIV, 4,15-diacetylinalenol. NA, not tested; –, none detected.

1.2 (Posada 2006), four of the FSSC strains were identified as *F. solani* (FSSC 5 haplotypes g and pp), MRC 2803 from corn in India was typed as *F. falciforme* FSSC 3+4-iii, and MRC 2198 from potato grown in Germany was identified as an unnamed phylopecies designated *F. sp.* FSSC 21-c. Of the five FSSC strains tested, beauvericin was the only mycotoxin detected, but only in a culture extract of MRC 2805 *F. solani* FSSC 5-g (SUPPLEMENTARY TABLE 3).

DISCUSSION

Multilocus molecular systematic studies over the past two decades collectively indicate that *Fusarium* comprises at least an order of magnitude more species than included in the Nelson et al. (1983) taxonomic treatment of the genus (Aoki et al. 2014). Given that this taxonomy was adopted in the companion compendium *Toxigenic Fusarium Species* (Marasas et al. 1984), the present study was initiated to reassess species identity and toxin potential of strains included in this compilation. Although published over three decades ago in a premolecular era, where species identity was based entirely on morphological species recognition, this treatise remains a standard reference for mycotoxigenic fusaria today. The present study highlights the importance of archiving key strains in publically accessible microbial culture collections such as the MRC, FRC, or NRRL (Kang et al. 2006). Access to these invaluable genetic resources allowed us to critically evaluate the wealth of knowledge within this compendium, thereby advancing our understanding of mycotoxigenic fusaria and their toxin potential.

Herein, species identity of 158 MRC strains was determined via genealogical concordance phylogenetic species recognition employing multilocus DNA sequence data (GCPSR; Taylor et al. 2000). Tentative identification of the strains and species complex membership was inferred phylogenetically from partial *RPB2* sequence data, which resolves at or near the species level, and they have the added advantage that they can be easily aligned across the breadth of the genus (O'Donnell et al. 2013). Once species complex membership was determined, published clade-specific multilocus typing schemes were employed to identify species/haplotypes within the FIESC and FCSC (O'Donnell et al. 2009b), FSSC (O'Donnell et al. 2008), and STs within the FOSC (O'Donnell et al. 2009a). GCPSR-based analyses of partial *TEF1* + *RPB2* sequence data, as previously demonstrated (O'Donnell et al. 2012), reliably resolved species identity of the 158 MRC strains employing current molecular techniques.

Important findings regarding the phylogenetic diversity of the MRC fusaria we typed include the following: (i) the 17 morphospecies received from MRC were determined to comprise 46 phylospecies, including 10 putatively novel taxa distributed among five species complexes (FIG. 1, TABLE 1); (ii) 12 of the 17 morphospecies harbored two or more cryptic species, with MRC strains received as *F. equiseti*, *F. moniliforme*, *F. graminearum*, and *F. solani* comprising 9, 7, 4, and 3 phylospecies, respectively; (iii) 138/158 (87.3%) of the strains were nested within the FSAMSC, FFSC, or FIESC; and (iv) half of the strains (79/158 = 50%) were identified as trichothecene toxin-producing members of the FSAMSC. Taking advantage of the discovery that agmatine is a strong inducer of *TRI5* expression and trichothecene biosynthesis in *F. graminearum* and other fusaria (Gardiner et al. 2009; Pasquali et al. 2016), we demonstrated experimentally that the majority of the FSAMSC and FIESC strains could produce trichothecenes in broth containing this amine, including strains that have been in culture for over five decades (Aoki and O'Donnell 1998). We also ran a validated SNP-based genotyping assay (Ward et al. 2008) to predict trichothecene toxin potential of six strains within FSAMSC-1 that failed to produce trichothecenes in agmatine broth and rice kernal cultures. Our results, together with those of Gardiner et al. (2009), suggest that nutrient profiling offers considerable promise for discovering strong inducers of other mycotoxin biosynthetic pathways within *Fusarium*. Amylopectin, for example, was reported to be a strong inducer of fumonisins (Bluhm and Woloshuk 2005). Thus, experiments are needed to determine whether this carbon source is a strong inducer of expression of the fumonisin gene *FUM1* and fumonisin biosynthesis in *F. verticillioides* and related fusaria that possess a functional fumonisin gene cluster (Proctor et al. 2013).

Mycotoxin production profiles reported here are consistent with published genomic data. For example, trichothecene production was only detected in members of FSAMSC and FIESC, and published genome sequences of members of these two complexes have the trichothecene biosynthetic gene cluster, but sequences of members of other species complexes do not have the cluster (Gardiner et al. 2012; Ma et al. 2013; Moolhuijzen et al. 2013; King et al. 2015, 2018; Urban et al. 2016; Vanheule et al. 2016). As noted in the present study, the presence of a mycotoxin biosynthetic gene cluster does not guarantee that a strain will produce the corresponding mycotoxin under laboratory conditions (Niehaus et al. 2017).

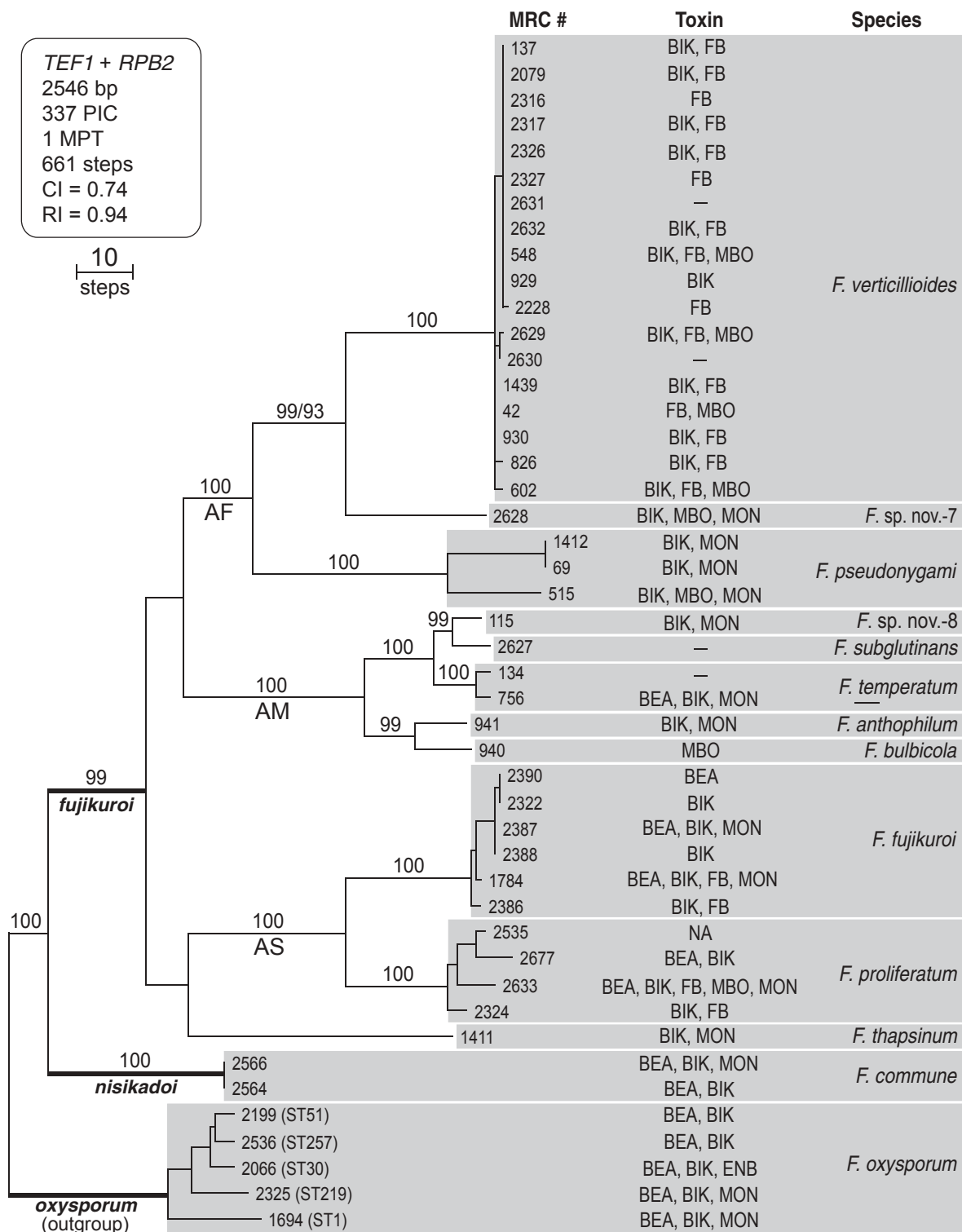


Figure 4. Single most-parsimonious phylogram inferred from aligned partial *TEF1* + *RPB2* sequences of toxigenic fusaria representing the *Fusarium fujikuroi* (FFSC), *F. nisikadoi* (FNSC), and *F. oxysporum* (FOSC) species complexes. Thickened internodes identify these three clades. The species-rich FFSC is represented by 39 MRC strains comprising 11 phylogenetic species, including two that appear to be novel (i.e., *F. sp. nov.-7* and *-8*). Sequences were rooted on five FOSC MRC strains based on the analysis presented in FIG. 1. Maximum likelihood (ML) bootstrap values are indicated above nodes based on 1000 pseudoreplicates of the data. Maximum parsimony (MP) bootstrap values are only indicated if they differed by $\geq 5\%$ from the ML bootstrap value (ML/MP). A two-locus typing scheme employing partial *TEF1* and complete IGS rDNA sequence data (O'Donnell et al. 2009a) was used to determine sequence type (ST) within the FOSC. Subclades of the FFSC: AF, African; AM, American; AS, Asian. Phylogenetic metrics include CI, consistency index; MPT, most-parsimonious tree; PIC, parsimony-informative character; and RI, retention index. The primary toxins produced include BEA, beauvericin; BIK, bikaverin; BUT, butenolide; ENB, enniatin B; FB, fumonisins FB₁ + FB₂ + FB₃; MBO, 8-O-methylbostrycoidin; and MON, moniliformin. NA, not tested; —, none detected.

Phylogenetic diversity and mycotoxin potential of MRC strains within the FSAMSC, FIESC, and FCSC.—

Although the evolutionary history of a number of genes within the trichothecene gene cluster does not track with the species phylogeny inferred from housekeeping genes (Proctor et al. 2009, 2013), results of the present study and others (Ward et al. 2002; Kristensen et al. 2005; O'Donnell et al. 2013; Watanabe et al. 2013) demonstrate that subclades within the FSAMSC generally correlate with trichothecene type. The MRC strains included representatives of 4/5 subclades reported in O'Donnell et al. (2013). MRC 2568 *F. sp. nov.-2* and 2468 *F. sp. nov.-10* were the sole members of a type A clade in the aforementioned study that included NRRL 13829 *F. cf. compactum*. The fifth FSAMSC subclade, the type B *F. longipes* clade, was not represented among the MRC strains. Further study is required to determine whether MRC 2560 *F. sp. nov.-1*, which was received as T-2 toxin-producing *F. sambucinum* from Japanese corn, represents a novel type A subclade within the FSAMSC.

Eighteen of the 26 phylopecies represented by the MRC strains in the FIESC + FSAMSC were discovered after Marasas et al. (1984) was published, but only 5 of these have been described formally. *Fusarium kyushuense* (Aoki and O'Donnell 1998), the species from which nivalenol, diANIV, and fusarenon-X were first isolated and characterized (reviewed in Ueno et al. 1997), was received from MRC as atypical *F. sporotrichioides*. The Quorn mycoprotein-producing species (O'Donnell et al. 1998a), originally reported as *F. graminearum* (Trinci 1994), was segregated from *F. sambucinum* as *F. venenatum* (Nirenberg 1995; Thrane and Hansen 1995). Of the 16 phylopecies discovered within *F. graminearum* sensu lato, 15 have been described formally (Sarver et al. 2011), including *F. boothii* and *F. asiaticum* (O'Donnell et al. 2004), and *F. acuminatum* subsp. *armeniicum* was elevated to species status based on molecular data that indicated that it and subsp. *acuminatum* were distantly related (Burgess and Summerell 2000). The conflation of the latter two species has contributed to erroneous reports that *F. acuminatum* produces trichothecenes (Marasas et al. 1984; Desjardins 2006). However, there are no authentic reports to date that *F. acuminatum* and other species within the FTSC produce trichothecenes.

Most of the authentic MRC strains of *F. graminearum* produced the type B trichothecene 15ADON, the predominant chemotype in North America (Ward et al. 2008; Kelly et al. 2015), Europe (Tóth et al. 2005), Asia (Zhang et al. 2012), South Africa (Boutigny et al. 2011), and South America (Castañares et al. 2016). Two

strains of 4,15-diANIV-producing *F. asiaticum* from Japan, two of 15ADON *F. boothii* from Africa, and a novel T-2 toxin-producing species represented by MRC 2560 *Fusarium sp. nov.-1* from Japanese corn were received as *F. graminearum*. Although a small population of *F. graminearum* that produces the type A trichothecene NX-2 was recently discovered and characterized in North America (Liang et al. 2014; Kelly et al. 2016), the report in Marasas et al. (1984) that MRC 2580 produces T-2 and DAS appears to be erroneous given that the results of the SNP-based assay predicts that it might produce 3ADON (TABLE 1). Global surveys have revealed that *F. graminearum* and *F. asiaticum* are segregating for 15ADON, 3ADON, and NIV worldwide (Ward et al. 2002; Yang et al. 2008; Boutigny et al. 2011), whereas the 15ADON chemotype appears to be fixed in *F. boothii* (Ward et al. 2002; Boutigny et al. 2011). As reported in a global population genetic analysis of *F. culmorum* (Laraba et al. 2017), MRC strains of this pathogen were segregating for 3ADON and NIV, but 3ADON appears to represent the predominant chemotype worldwide.

The FIESC phylogeny that resolved the 20 MRC strains as 12 phylopecies revealed that the Marasas et al. (1984) morphological concepts of *F. semitectum* and *F. equiseti* were overly broad. Interestingly, the three phylopecies within the Incarnatum clade were received as *F. semitectum* and the nine species within the Equiseti clade as *F. equiseti*, presumably because Nelson et al. (1983) distinguished these two broadly defined morphospecies by the production of polyphialides in the former and only monophialides in the latter. These species were classified in the morphologically defined sections *Arthrosporiella* and *Gibbosum*, respectively, by Nelson et al. (1983), but both sections are nonmonophyletic (O'Donnell et al. 2013). Multilocus molecular phylogenetic surveys of medically important fusaria (O'Donnell et al. 2009b), Sardinian soils (Balmas et al. 2010), insecticolous fusaria (O'Donnell et al. 2012), cereals from Italy (Villani et al. 2016), and the MRC strain collection have revealed that the FIESC is one of the most species-rich lineages within *Fusarium*. Currently, Latin binomials can be applied with confidence to only 3/36 phylospecies, and these are *F. equiseti* FIESC 14, *F. lacertarum* FIESC 4, and *F. sciripi* FIESC 9. The former two species were represented among the 20 FIESC strains from MRC, but an ad hoc nomenclature for species and multilocus haplotypes (O'Donnell et al. 2009b) was used to accurately report on the 10 unnamed phylospecies received from MRC. We determined the identity and mycotoxin potential of several of the phylospecies implicated in mycotoxicoses of

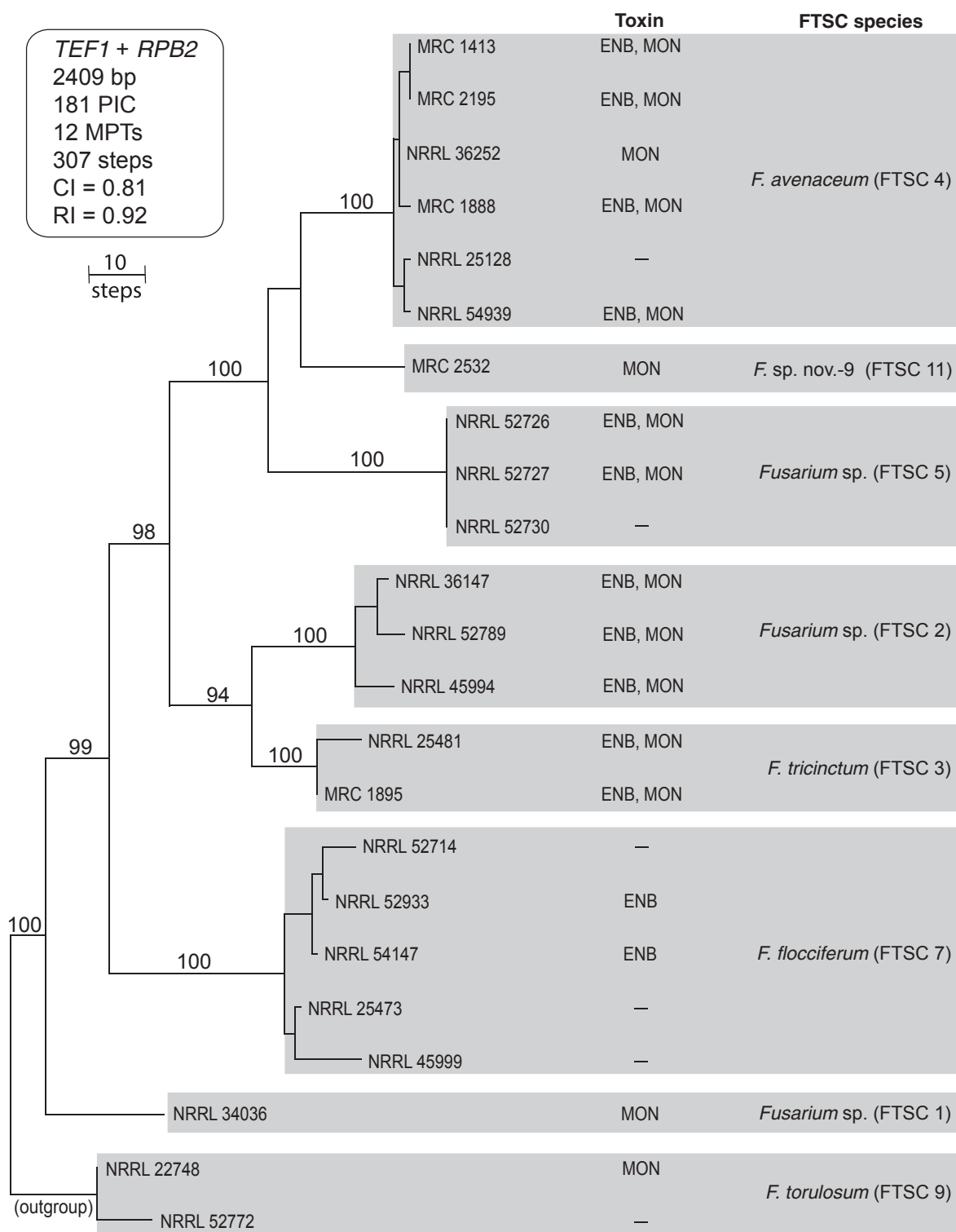


Figure 5. One of 12 most-parsimonious phylograms inferred from aligned partial *TEF1* + *RPB2* sequences from 23 strains (18 NRRL reference and 5 MRC strains) representing eight species within the *Fusarium tricinctum* species complex (FTSC). Each species is designated with FTSC followed by a unique Arabic number because Latin binomials could not be applied to four of the phylopecies. The MRC strains were identified as *F. avenaceum* (FTSC 4), *F. tricinctum* (FTSC 3), and a putatively novel species (*F. sp. nov.-9* = FTSC 11) represented by MRC 2532 received identified as *F. avenaceum*. Sequences of *F. torulosum* were used to root the phylogeny based on more inclusive analyses (O'Donnell unpubl.). Maximum likelihood (ML) bootstrap values are indicated above nodes based on 1000 pseudoreplicates of the data. Phylogenetic metrics include CI, consistency index; MPT, most-parsimonious tree; PIC, parsimony-informative character; and RI, retention index. The primary toxins produced include ENB, enniatin B; and MON, moniliformin. NA, not tested; —, none detected.

animals that Marasas et al. (1984) reported as *F. equiseti* or *F. semitectum*. *Fusarium lacertarum* FIESC 4 and FIESC 25 were isolated from toxic rice straw linked to Degnala disease of cattle and buffaloes in India, FIESC 29 was isolated from moldy corn thought to be responsible for the death of dairy cattle in Minnesota, and FIESC 35 was associated with bean hulls poisoning of horses in Japan. Type A (DAS) or type B (4,15-diANIV) trichothecenes may have been responsible for the toxicoses, which were produced in agmatine broth by these species. We observed, as did Villani et al. (2016), that trichothecene profiles were variable in several FIESC phylopecies where type A or B or both types were produced. Strains of *F. equiseti* FIESC 14 and FIESC 1, for example, produced 4,15-diANIV or 4,15-diANIV + DAS. Although not reported in Marasas et al. (1984), we discovered that MRC 2609 *F. lacertarum* FIESC 4-d produced the estrogenic toxin zearalenone and MRC 2636 FIESC 36-a produced butenolide. Given the huge gulf that currently exists between morphological and phylogenetic species recognition in the FIESC, and the likelihood that many novel phylopecies will be discovered in future plant pathogen and mycotoxin surveys, multilocus sequence typing has become an essential tool for accurately reporting on the FIESC and their mycotoxin potential.

Although a preliminary study revealed that the morphospecies *Fusarium chlamydosporum* comprised four medically and agriculturally important phylopecies (O'Donnell et al. 2009b), more extensive investigations are needed to elucidate FCSC species diversity and their mycotoxin potential. Of the MRC strains included in Marasas et al. (1984) as *F. chlamydosporum*, only MRC 2486 (= NRRL 6358) from peanuts from Georgia was reported to produce the trichothecene neosoloniol monoacetate (Lansden et al. 1978). However, MRC 2486 appears to represent the undescribed species *F. sp. nov.-10* in the FSAMSC, which is closely related to MRC 2568 *F. sp. nov.-2* from river sediment in Japan. As reported in Marasas et al. (1984), we found that MRC 117 (*F. sp. nov.-6* = FSCS 5) can produce moniliformin, and we extended their results by discovering that MRC 117 and MRC 35 produce butenolide and beauvericin, respectively. The present study appears to represent the first report of butenolide production by FCSC 5, *F. boothii*, *F. asiaticum*, *F. kyushuense*, and FIESC 36.

Phylogenetic diversity and mycotoxin potential of MRC strains within the FFSC, FNCS, and FOSC.—

With over 50 phylospecies distributed among three biogeographically structured clades, the FFSC is one

of the most species-rich and taxonomically challenging groups within *Fusarium* (O'Donnell et al. 2015). The perceived limitations of morphological species recognition within this clade was reflected by our discovery that only 2/39 MRC strains were received using names in current use. This discrepancy is due in part to the overly broad morphological concepts of the four FFSC species recognized by Marasas et al. (1984) and subsequent abandonment of the name *F. moniliforme* because it was being applied to an indeterminate number of species within the FFSC (Seifert et al. 2003). In addition to *F. verticillioides*, with which it is sometimes mistakenly thought to be equivalent, we determined that Marasas et al. (1984) applied this name to six other phylospecies, including *F. fujikuroi*, *F. proliferatum*, *F. subglutinans*, and MRC 2628 *Fusarium sp. nov.-7* from Iowa corn together with two species described after Marasas et al. (1984) was published, *F. thapsinum* (Klittich et al. 1997) and *F. pseudonygamai* (Nirenberg and O'Donnell 1998). In addition to the plethora of economically important plant diseases that members of the FFSC cause, the carcinogenic foodborne fumonisins were first discovered and characterized in *F. verticillioides* by South African scientists in 1988 (reviewed in Marasas 2001; Rheeder et al. 2002). Subsequently, fumonisins were demonstrated experimentally to cause equine leukoencephalomalacia (Marasas et al. 1988a) and they have been linked epidemiologically to high levels of human esophageal cancer in the Transkei region of South Africa (Rheeder et al. 1992).

Our results match those of prior studies (Rheeder et al. 2002; Proctor et al. 2004) that showed that fumonisin B analogs were produced by *F. verticillioides*, *F. proliferatum*, and *F. fujikuroi*, but not by the eight other FFSC species received from MRC. Two strains of *F. oxysporum* from Korea have been shown to produce FC fumonisin analogs (Seo et al. 1996), but they were not detected in cracked maize extracts of the five MRC strains of this species. We observed, as noted by Munkvold (2017), that moniliformin was produced by some of the FOSC strains and most of the FFSC, but not by *F. verticillioides* and *F. subglutinans*. Two strains of *F. pseudonygamai* produced the most moniliformin on cracked maize cultures, including MRC 1412, which was initially reported as *F. moniliforme* (Marasas et al. 1984) and subsequently reidentified incorrectly as *F. nygamai* (Marasas et al. 1988b). The two strains of *F. commune* (Skovgaard et al. 2003) that were reported as *F. oxysporum* in Marasas et al. (1984) were not tested for mycotoxins by them; however, our analyses established that this FNCS species can produce moniliformin, bikaverin, and beauvericin. Marasas et al. (1984)

did not analyze strains for the latter two toxins, but *F. subglutinans* MRC 2627 was the only species among the FFSC, FNCS, and FOSC strains we tested that failed to produce the cytotoxic red pigment bikaverin (Wiemann et al. 2009). Our results match reports that the nonribosomal cyclic hexadepsipeptide beauvericin is produced by members of the FFSC and FOSC (Logrieco et al. 1998), but this toxin was only detected in some of the strains of *F. oxysporum*, *F. temperatum*, *F. fujikuroi*, and *F. proliferatum*. Although beauvericin and enniatin are structurally related (Logrieco et al. 2002; Zhang et al. 2013), MRC 2066 *F. oxysporum* was the only strain among the FOSC, FFSC, and FNCS tested that produced enniatin B. With the recent development of a HPLC-MS method for detecting 8-O-methylbostrycoidin (Busman 2017), we detected this toxic pigment in *F. verticillioides*, the species in which it was first reported (Steyn et al. 1979), together with *F. bulbicola*, *F. proliferatum*, *F. pseudonygamai*, and *F. sp. nov.-7* in the FFSC, and MRC 2231 FIESC 10-b.

Phylogenetic diversity and mycotoxin potential of MRC strains within the FTSC and FSSC.

Nelson et al. (1983) classified *F. avenaceum* and *F. tricinctum* within sections *Roseum* and *Sporotrichiella*, respectively, but because these subgeneric groups are artificial, we prefer to refer to the clade in which these species are nested as the FTSC. The three phylopecies within the FTSC and FSSC were represented by five and six MRC strains, respectively. Mycotoxin profiles of the three FTSC species from MRC matched those reported for *F. avenaceum* (Logrieco et al. 2002; Lysøe et al. 2014) in that moniliformin was detected in cracked maize cultures of all three and enniatin B in *F. avenaceum* and *F. sp. nov.-9* FTSC 11. In contrast to the FTSC, where species diversity is poorly understood, numerous studies of medically and agronomically important strains indicate that the FSSC comprises over 50 phylopecies distributed among three clades (O'Donnell 2000; O'Donnell et al. 2008; Nalim et al. 2011; Aoki et al. 2014). These species are typically reported incorrectly as *F. solani*, but this name should only be applied to phylospecies FSSC 5 (Schroers et al. 2016). Based on published reports, Marasas et al. (1984) indicated that the six FSSC strains from MRC produced metabolites that were toxic to animals, but none of these were characterized. We tested these strains and the only toxin detected was beauvericin in MRC 2805 from wheat in India.

Concluding remarks.—Our current understanding of *Fusarium* species diversity and mycotoxin chemistry

has seen significant advances since the publication of Marasas et al. (1984), including the discovery of many toxigenic and phytopathogenic fusaria via multilocus molecular phylogenetics, development of more sensitive GS-MS and HPLC-MS techniques for detecting and characterizing mycotoxins (Jestoi 2008; McCormick et al. 2011; Busman et al. 2012; Busman 2017), nutrient profiling to identify compounds that stimulate toxin production (Gardiner et al. 2009), and comparative phylogenomics of fusaria to predict their mycotoxin potential from secondary metabolite gene clusters (Edwards et al. 2016; Kim et al. 2017). To illustrate the impact of molecular systematics, only 13 fusaria were described in as many years (i.e., 1985–1997) following the publication of Marasas et al. (1984), whereas 127 species, or 6.35/y on average, were described during the molecular systematic era (1998–present), which spans the past two decades.

Results of the present study have important implications for advancing our understanding of mycotoxigenic fusaria. First, most fusaria can only be accurately identified using multilocus DNA sequence data. To illustrate this point, our results revealed that the 17 morphospecies of Marasas et al. (1984) comprised 46 phylogenetically distinct species, including many that are morphologically cryptic (Aoki et al. 2014). By extension, reports of mycotoxin production generally should be viewed as unconfirmed unless the strains were identified molecularly. Secondly, as evidenced by the survey of the strain set presented here, key strains should be archived in publically accessible culture collections (e.g., CBS-KNAW, FRC, NRRL) so that the results can be verified and extended (Kang et al. 2006). Also, the DNA sequence data generated herein were deposited in NCBI GenBank and *Fusarium* MLST (<http://www.westerdijknstitute.nl/Fusarium/>) to facilitate strain identification via the Internet (O'Donnell et al. 2015). In that regard, because evolutionary relationships and species limits are not fully resolved in most clades, including the important toxin-producing FSAMSC and FFSC lineages, the genomes of phylogenetically diverse fusaria will be mined to identify additional phylogenetically informative marker loci. Lastly, ongoing research among our research groups is actively employing a three-prong approach that includes GCPSR-based research to discover novel toxigenic and phytopathogenic fusaria, mining the genomes of these novel species to predict mycotoxin potential from intact gene clusters, and then testing them experimentally for toxin production in vitro and/or in vivo via GC-MS and/or HPLC-MS. The overarching goal of this research is to develop a knowledge base directed at minimizing the threat that fusaria and

their toxins pose to plant, human, and animal health, and food safety.

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