

Short title: Insecticolous fusaria

Phylogenetic diversity of insecticolous fusaria inferred from multilocus DNA sequence data and their molecular identification via FUSARIUM-ID and *Fusarium MLST*

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Abstract: We constructed several multilocus DNA sequence datasets to assess the phylogenetic diversity of insecticolous fusaria, especially focusing on those housed at the Agricultural Research Service Collection of Entomopathogenic Fungi (ARSEF), and to aid molecular identifications of unknowns via the FUSARIUM-ID and *Fusarium MLST* online databases and analysis packages. Analyses of a 190-taxon, two-locus dataset, which included 159 isolates from insects, indicated that: (i) insect-associated fusaria were nested within 10 species complexes spanning the phylogenetic breadth of *Fusarium*, (ii) novel, putatively unnamed insecticolous species were nested within 8/10 species complexes and (iii) Latin binomials could be applied with confidence to only 18/58 phylogenetically distinct fusaria associated with pest insects. Phylogenetic analyses of an 82-taxon, three-locus dataset nearly fully resolved evolutionary relationships among the 10 clades containing insecticolous fusaria. Multilocus typing of isolates within four species complexes identified surprisingly high genetic diversity in that 63/65 of the fusaria typed represented newly discovered haplotypes. The DNA sequence data, together with corrected ABI sequence chromatograms and alignments, have been uploaded to the following websites dedicated to identifying fusaria: FUSARIUM-ID (<http://isolate.fusariumdb.org>) at

Pennsylvania State University's Department of Plant Pathology and *Fusarium MLST* (<http://www.cbs.knaw.nl/fusarium>) at the Centraalbureau voor Schimmelcultures (CBS-KNAW) Fungal Biodiversity Center.

Key words: Ascomycota, DNA sequence, *EF-1 α* , GCPSR, *RPB1*, *RPB2*, species limits

INTRODUCTION

Efforts to use species of *Fusarium* for the biological control of agriculturally important pest insects and as novel sources of insecticidal compounds (Claydon and Grove 1984, Claydon et al. 1977, Gupta et al. 1991, Strongman et al. 1988) have been limited in part due to the legitimate concern of inadvertently releasing phytopathogens and their toxins into the environment (Teetor-Barsch and Roberts 1983). This concern is well founded because morphological species recognition (MSR) has given rise to several conflicting taxonomic schemes that all greatly underestimate species diversity within the genus (Gerlach and Nirenberg 1982, Leslie and Summerell 2006, Nelson et al. 1983). The significant limitations of MSR within *Fusarium* have been documented in several multilocus molecular phylogenetic studies (see O'Donnell et al. 2010), which clearly demonstrated that the majority of fusaria cannot be identified to species with morphology alone.

Fusaria have been reported from phylogenetically diverse insect pests (Bai and Chen 1991, Claydon and Grove 1984, Teetor-Barsch and Roberts 1983), with the largest collection of these being housed at the Agricultural Research Service Collection of Entomopathogenic Fungi (ARSEF) at Cornell University, Ithaca, New York (<http://www.ars.usda.gov/Main/docs.htm?docid=12125>). Because MSR cannot be used to identify most fusaria to species the majority of the ARSEF fusaria are listed in the catalog as *Fusarium* sp. Fortunately multilocus molecular phylogenetic studies have identified several

informative loci that resolve relationships between taxa at or near the species rank within *Fusarium* (Geiser et al. 2004, 2005; O'Donnell 2000; O'Donnell et al. 1998a, b, 2000a, b, 2010), employing genealogical concordance phylogenetic species recognition (i.e. GCPSR; Dettman et al. 2003, Taylor et al. 2000). More generally GCPSR-based studies have consistently discovered high cryptic speciation within diverse agriculturally and medically important fungi (reviewed in Taylor et al. 2000, 2006), including the model entomopathogens *Beauveria* (Rehner and Buckley 2005, Rehner et al. 2011) and *Metarhizium* (Bischoff et al. 2009).

Therefore the primary objectives of the present study were: (i) to determine the diversity of insecticolous fusaria and their relationships to animal and/or plant pathogenic species using multilocus molecular phylogenetics, (ii) to increase the utility of insecticolous fusaria in ARSEF and several other culture collections and concomitantly minimize the risk of inadvertently employing phytopathogens as biological control agents of insect and mite (Acari) pests by providing robust marker sequences for identifying these fusaria to species and (iii) to make these data available to the global scientific community via two web servers dedicated to DNA sequence-based identification of fusaria. To accomplish these objectives the following three multilocus DNA sequence datasets were constructed and analyzed phylogenetically: (i) a 190-taxon, two-locus dataset used to identify 159 insecticolous, three acaricolous, one nematogenous and 27 other fusaria to species rank, (ii) an 82-taxon, three-locus dataset used to develop a robust hypothesis of evolutionary relationships among clades containing insecticolous fusaria and (iii) published multilocus sequence typing (MLST) schemes were expanded to distinguish species/haplotypes within four clades containing insecticolous fusaria (O'Donnell et al. 2008, 2009b, 2010). The resulting DNA sequence data, including corrected chromatograms and alignments, have been uploaded to the FUSARIUM-ID database (<http://isolate.fusariumdb.org>)

at Pennsylvania State University and the *Fusarium* MLST database

(<http://www.cbs.knaw.nl/fusarium>) at the Centraalbureau voor Schimmelcultures (CBS-KNAW)

Biodiversity Center.

MATERIALS AND METHODS

Fungal isolates.—Strain histories of the 190 isolates included in this study are provided (TABLE I). Of the 168 isolates obtained from ARSEF, 140 were isolated mostly from diverse insects but also from two Acari and a nematode; the remaining 28 isolates were from soil or the source was unknown. Twelve additional insecticolous isolates were obtained from other culture collections (i.e. CBS, FRC, ICMP, IMI, MUCL; TABLE I), and one insecticolous isolate was provided by Gary J. Samuels (ARS-USDA, BARC-W). With the exception of NRRL 52709 (ARSEF 6448) and NRRL 52754 (ARSEF 3464), which represented unidentified putative Hypocrealean taxa whose sequences were used for rooting the phylogeny, the remaining 188 isolates studied were nested within *Fusarium* (sensu Gerlach and Nirenberg 1982). Isolates used in this study are available on request from ARSEF (<http://www.ars.usda.gov/Main/docs.htm?docid=12125>), the respective culture collections are provided herein (TABLE I) or at the ARS Culture Collection (NRRL, <http://nrml.ncaur.usda.gov/TheCollection/index/html>), National Center for Agricultural Utilization Research, Peoria, Illinois.

Molecular biology.—Isolates were cultured in 100 mL yeast-malt broth (20 g dextrose, 5 g peptone, 3 g malt extract, 3 g yeast extract per liter; Difco, Detroit, Michigan) in 300 mL Erlenmeyer flasks 2–4 d at 100 rpm on a rotary shaker. Mycelium was harvested over a Büchner funnel, freeze dried overnight and total genomic DNA was extracted from approximately 50–100 mg pulverized mycelium with a cetyl trimethyl-ammonium bromide (CTAB; Sigma-Aldrich, St Louis, Missouri) protocol (O'Donnell et al. 1998a). Portions of translation elongation factor 1-alpha (*EF-1 α*) and RNA polymerase II second largest subunit (*RPB2*) were selected to identify all 190 isolates to species and/or species complex based on published analyses (Geiser et al. 2004; O'Donnell et al. 1998b, 2007, 2008, 2009a, b, 2010). All PCR and sequencing primers used in this study are provided herein (SUPPLEMENTARY TABLE I). Published MLST schemes were used to identify species/haplotypes in four species complexes as follows. In addition to *EF-1 α* and *RPB2* gene sequences, the ITS rDNA region and domains D1 and D2 of the nuclear large-subunit (LSU) rDNA were sequenced in members of the solani and tricinctum species complexes to place them in three-locus typing schemes for identifying species/haplotypes (O'Donnell et al. 2008, 2009b). For members of the

incarnatum-equiseti and chlamydosporum species complexes portions of *EF-1 α* , *RPB2*, ITS + LSU rDNA and a portion of the calmodulin gene (*CAM*) were sequenced as described by O'Donnell et al. (2009b). Based on analyses of the 190-taxon, two-locus dataset, a separate 82-taxon, three-locus dataset was constructed to infer evolutionary relationships within *Fusarium*. The 82-taxon, three-locus dataset consisted of partial *EF-1 α* , *RPB1* and *RPB2* gene sequences, based on their demonstrated phylogenetic informativeness within the genus (O'Donnell et al. 2010). PCR reactions were conducted with Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, California), using published cycling parameters (O'Donnell et al. 1998a, 2008). Amplicons were purified with Montage₉₆ 96-well filter plates (Millipore Corp., Billerica, Massachusetts) and cycle sequenced with ABI BigDye Terminator 3.1 as described by O'Donnell et al. (1998a). All sequencing reaction mixtures were purified with ABI XTerminator and were run on an ABI 3730 automated 48-capillary sequencer.

DNA sequence alignment.—ABI chromatograms were edited with Sequencher 4.9 (Gene Codes Corp., Ann Arbor, Michigan), after which sequences from each of the four genes were separately exported as NEXUS files. Sequences from the *RPB1* and *RPB2* partitions were aligned manually with TextPad 5.1.0 for Windows (Helios Software Solutions, Longridge, UK). The MAFFT alignment program (<http://align.bmr.kyushu-u.ac.jp/mafft/software>) was used to align sequences from the *EF-1 α* and ITS + LSU rDNA partitions, after which minor manual adjustments were conducted to improve the alignments with TextPad. Nucleotide sequence alignments also were translated in silico into proteins with MacClade (Maddison and Maddison 2000) to search for potential sequencing errors in the coding sequence and to identify amino acid residues associated with unique indels.

Phylogenetic analyses.—Before conducting phylogenetic analyses 560 ambiguously aligned, mostly intronic positions were excluded from the 190-taxon, two-locus dataset (SUPPLEMENTARY TABLE II, FIG. 1). Similarly 487 nucleotide positions in the 82-taxon, three-locus dataset were excluded from maximum parsimony (MP) and maximum likelihood (ML) analyses as ambiguously aligned (SUPPLEMENTARY TABLE III, FIG. 2). Phylogenetic frameworks using these two datasets were constructed respectively with MP employing PAUP* 4.0b.10 (Swofford 2002) as described by O'Donnell et al. (2010) and by ML implemented in GARLI (Zwickl 2006). MP clade support of the 190-taxon, two-locus dataset was assessed by 1000 bootstrapped pseudoreplicates of the data, using 10 random addition sequences per pseudoreplicate. Selection of models of nucleotide substitution for the GARLI ML analyses, implementing the Akaike information criterion (AIC), was determined with Modeltest 3.7 (Posada and Crandall 1998). For the ML analysis the three-gene dataset was divided into six partitions and these and their

substitution models are: *EF-1 α* exon 1st, 2nd and 3rd positions (GTR+I+G), *EF-1 α* introns (TIM+I+G), *RPB1* exons 1st and 2nd positions (GTR+I+G), *RPB1* exon 3rd positions (TVM+I+G), *RPB2* exon 1st and 2nd positions (GTR+I+G), and *RPB2* 3rd positions (TIM+I+G). Two replicate ML searches (Felsenstein and Churchill 1996) and 800 ML bootstrap (ML BS) replicates were conducted with GARLI 0951 (Zwickl 2006). COLLAPSE 1.1 (http://inbio.byu.edu/Faculty/kac/crandall_lab/Computer.html) was used to identify multilocus haplotypes within four species complexes (see SUPPLEMENTARY TABLE V).

Nucleotide sequence accession numbers.—DNA sequence data reported in this study were deposited in GenBank (<http://www.ncbi.nlm.nih.gov/>) under accession numbers JF740690–JF741204 and TreeBASE (<http://www.treebase.org/treebase-web/home.html>) as Tr44942 and Tr44943. In addition all sequences, corrected ABI sequence chromatograms and alignments also were deposited in the FUSARIUM-ID database (<http://isolate.fusariumdb.org>) at the Pennsylvania State University and the *Fusarium* MLST database (<http://www.cbs.knaw.nl/fusarium>) at the Centraalbureau voor Schimmelcultures (CBS-KNAW) Fungal Biodiversity Center

RESULTS

This study was conducted to expand existing web multilocus DNA sequence databases to aid accurate identification of insecticolous and other fusaria, to elucidate their phylogenetic diversity so as to increase their use as biocontrol agents of agriculturally important pest insects and to increase our understanding of their host range and geographic distribution. To accomplish these objectives we constructed and analyzed the following three multilocus DNA sequence datasets: (i) a 190-taxon, two-locus dataset comprising partial *EF-1 α* and *RPB2* gene sequences to identify the 188 fusaria included in this study to species and species complex, (ii) an 82-taxon, three-locus dataset comprising partial *EF-1 α* , *RPB1* and *RPB2* gene sequences to identify the phylogenetic spectrum of species complexes (i.e. clades) containing insect-associated fusaria and to develop a robust hypothesis of their evolutionary relationships and (iii) existing MLST schemes were expanded to determine multilocus haplotypes of insecticolous fusaria within the *Fusarium* *solani*, *tricinctum*, *chlamydosporum* and *incarnatum-equiseti* species complexes

(O'Donnell et al. 2008, 2009b, 2010). Throughout this paper we opted to refer to each species complex by name, rather than by an abbreviation, as done by O'Donnell et al. (2010), to increase their familiarity and usage within the phytopathological and mycological communities.

Phylogenetic analysis of 190-taxon, two-locus dataset.—Portions of the nuclear genes *EF-1 α* (1158 bp alignment) and *RPB2* (1817 bp alignment) were selected for molecular phylogenetic identification of all 190 isolates included in this study based on the proven utility of these loci for resolving at or near the species rank within *Fusarium* (see Geiser et al. 2004, O'Donnell et al. 2010, Gräfenhan et al. 2011). As a result two web databases dedicated to identifying fusaria are becoming heavily populated with these sequences (Geiser et al. 2004, Park et al. 2011). Both genes were successfully amplified and sequenced from all 190 isolates, except the *RPB2* 5f2 \times 7cr and 7cf \times 11ar regions (hereafter referred to as *RPB2* 5-7 and 7-11 regions respectively) could not be obtained for separate sets of eight isolates. Therefore the latter sequences were coded as missing data for the MP and ML analyses. Before conducting maximum parsimony (MP) analyses 512 and 48 ambiguously aligned, indel-containing, predominately intronic nucleotide positions were excluded respectively from the *EF-1 α* and *RPB2* partitions. The combined dataset totaled 2417 bp of aligned positions, 866 (35.9%) of which were parsimony informative (SUPPLEMENTARY TABLE II). Two isolates, NRRL 52709 (ARSEF 6448) from a tef grasshopper (*Aiolopus longicornis*, Orthoptera: Acrididae) from Ethiopia and NRRL 52754 (ARSEF 3464) from a sugar beet root aphid (*Pemphigus betae*, Hemiptera: Aphididae) from Arizona, were received from ARSEF as putative microconidial fusaria. However they possessed highly divergent sequences that showed 80% or less identity to fusaria in GenBank, FUSARIUM-ID and *Fusarium* MLST. BLAST queries of GenBank indicated that they likely were nested within the *Hypocreales*, but neither could be identified to genus rank. Preliminary

MP analyses conducted with PAUP* (Swofford 2002), including outgroup sequences of *Nectria cinnabarina* and *Hypocrea lutea* (Schoch et al. 2009), indicated that sequences of the two aforementioned ARSEF isolates could be used as outgroups to root the molecular phylogeny (FIG. 1). With the exception of 27 isolates from soil or unknown sources (identified by gray highlighting in FIG. 1), the remaining isolates were recovered from diverse insects, two Acari and a nematode (TABLE I). MP bootstrapping revealed that 184 of the 188 isolates comprising the ingroup were nested within 10 species complexes identified as monophyletic (O'Donnell et al. 2010, Schroers et al. 2009). Nine of these clades received moderate to strong bootstrap support (BS = 77–100%); the fujikuroi species complex was the only lineage that was not supported by bootstrapping. Four isolates representing *F. redolens*, *F. commune* and a novel unnamed *Fusarium* sp. NRRL 52700 = ARSEF 6460 from an aerial spittlebug (*Mahanarva andigena*, Hemiptera: Cercopidae) from Colombia were not placed in informally named species complexes because they represented single-species lineages.

The coccophilum species complex (section Coccophilum sensu Booth 1971) was strongly supported (BS = 100%) as the earliest diverging lineage of insecticolous fusaria (FIG. 1). Bootstrap analyses of the individual and combined partitions provided support for the recognition of three phylogenetically distinct species within the *F. larvarum* clade, as well as four genealogical exclusive species within the *F. coccidicola* and *F. coccophilum* clades (FIG. 1). The 11 species within the coccophilum complex included in this study were collected mostly in tropical and subtropical regions and are associated predominately with scale insects; however one unnamed species (*F. sp. 2*) from Japan (NRRL 22102 = CBS 169.30) within the *F. larvarum* clade was reportedly isolated from aphids (TABLE I). The known disjunct distribution of species within the coccophilum (*F. sp. 1* = Florida and Puerto Rico, *F. sp. 2* = Brazil, *F. sp. 3* = New

Zealand and Indonesia, *F. sp. 4* = Iran, Italy and Washington, D.C.), *coccidicola* (*F. sp. 1* = Iran, *F. sp. 2* = Papua New Guinea, *F. sp. 3* = Thailand, *F. sp. 4* = Honduras), and *larvarum* (*F. sp. 1* = New Zealand, *F. sp. 2* = Japan, *F. sp. 3* = Iran) clades suggests widespread allopatric speciation within the *coccophilum* species complex.

The *solani* species complex was resolved as the next lineage to diverge within *Fusarium* and sister to the remaining ingroup taxa comprising the *Gibberella* clade in this dataset. The *solani* complex was represented by seven insecticolous and two acaricolous species together, with one novel species (FSSC 37) from diseased cocoa pods from Papua New Guinea and two isolates of *F. falciforme* (FSSC 3 + 4) from soil. In addition to being associated with diverse insects in three orders, the following two species within the *solani* complex were the only acaricolous isolates included in this study. FSSC 25-b (NRRL 25101 = ARSEF 3296) was isolated from a tick (*Boophilus sp.*, Acari: Ixodidae) on a cow in Mexico and FSSC 2-gg and 2-hh (NRRL 52704 = ARSEF 6572 and NRRL 52715 = ARSEF 6443) were recovered from two-spotted spider mites (*Tetranychus urticae*, Acari: Tetranychidae) in Virginia.

The *Gibberella* clade comprised all the remaining ingroup taxa and was represented by eight of the informally named species complexes and 89.4% (168/188) of the fusaria genotyped. Although evolutionary relationships among species complexes within the *Gibberella* clade were poorly resolved by the two-locus dataset, four nodes along the backbone of the phylogeny received modest bootstrap support (BS = 70–86%). In addition the lateritium species complex was strongly supported as the earliest diverging lineage within the *Gibberella* clade (BS = 100%); however this complex does not appear to represent the basal-most lineage based on analyses that included a broader sampling of non-insecticolous fusaria (O'Donnell unpubl). The four most species-rich insect-associated lineages within the *Gibberella* clade included the

incarnatum-equiseti complex (15 species from insects, 31 isolates), the fujikuroi complex (8/9 species from insects, 43 isolates), the tricinctum complex (5/7 species from insects, 12 isolates), and the oxysporum complex (17/22 strains from insects, 15 two-locus haplotypes). The two-locus dataset for this limited taxon sampling poorly resolved the monophyly of the fujikuroi species complex, as well as the genealogical exclusivity of its African (Af) and Asian (As) subclades. Only three insecticolous species within the *Gibberella* clade were represented by 10 or more isolates, and two of these were nested within the fujikuroi complex. These included *F. proliferatum* (n = 15) and *F. sacchari* (n = 10). Reflecting its known cosmopolitan distribution (Leslie and Summerell 2006), *F. proliferatum* was isolated on several continents in both hemispheres from insects representing three orders and nine families. Eight of the 10 isolates of the putatively Asian endemic *F. sacchari* were recovered from diverse insect hosts in India, while two isolates were from spotted sugarcane borers (*Chilo sacchariphagus*, Lepidoptera: Pyralidae) introduced into Mozambique, presumably from the lepidopteran's native range in southeastern Asia. An unnamed *Fusarium* sp. within the concolor species complex, represented by 13 isolates, was the only other species represented by 10 or more isolates in the present study. This unnamed *Fusarium* sp. was isolated from diverse hosts, including gypsy moths (*Lymantria dispar*, Lepidoptera: Lymantriidae) in five states in eastern USA, hemlock woolly adelgid (*Adelges tsugae*, Hemiptera: Adelgidae) in Massachusetts (NRRL 52779 = ARSEF 5803 and NRRL 52780 = ARSEF 5825) and an apple maggot (*Cydia pomonella*, Lepidoptera: Tortricidae) in France (NRRL 25121 = ARSEF 3976). Because the isolate from France is genetically divergent further sampling is needed to assess whether it represents a phylogenetically distinct species. Isolates of the unnamed *Fusarium* sp. from gypsy moths within USA were reported as *F. polyphialidicum* and *F. sambucinum* (Hajek et al. 1993). The single strain of *F. concolor*

typed was isolated from a nematode (Nemata: Secernentea) in Hawaii. In summary, phylogenetic analyses of the 190-taxon, two-locus dataset identified 58 insecticolous, two acaricolous and one nematogenous species of *Fusarium*.

Phylogenetic analysis of 82-taxon, three-locus dataset.—A three-locus dataset, comprising portions of the nuclear genes *EF-1 α* (1146 bp alignment), *RPB1* (1596 bp alignment) and *RPB2* (1848 bp alignment), was constructed to develop a robust hypothesis of evolutionary relationships among clades containing insecticolous fusaria. The 82-taxon dataset included 67 isolates from insects, one isolate from a two-spotted spider mite (NRRL 52715 = FSSC 2-hh), one from a nematode (NRRL 52927 = ARSEF 3042 *F. concolor*) and 12 ARSEF strains from other sources (identified by gray highlighting in FIG. 2). The combined dataset comprised 4103 nucleotide positions of which 1631 (39.8%, SUPPLEMENTARY TABLE III) were phylogenetically informative. The three genes were amplified and sequenced in all 82 isolates, except that the *RPB2* 5-7 region is missing from one isolate and the *RPB2* 7-11 region could not be obtained for four isolates. The latter sequences were coded as missing data for the MP and ML analyses. Alignment of the *RPB2* 5-7 coding region required the insertion of four indels 3–39 bp long due to the presence of 1–13 additional codons within the ingroup sequences. Alignment of the *RPB2* 5–7 sequence of NRRL 52709 identified a 6 bp insertion (coding for alanine and aspartic acid) and two separate single codon deletions that were unique to this isolate. In addition a 3 bp indel was inserted in the *RPB2* 7-11 region to accommodate for a unique valine codon within NRRL 52709. In addition, due to the presence of unique insertions within the *RPB1* sequences of *Fusarium* sp. 1 and 2 within the *F. larvarum* clade, alignment gaps needed to be inserted in all other sequences.

Phylogenetic frameworks were constructed with ML in GARLI (Zwickl 2006) and MP in PAUP* (Swofford 2002) after 48 and 439 ambiguously aligned intron nucleotide positions were excluded respectively from the *RPB2* and *EF-1 α* partitions. ML and MP analyses recovered trees that were highly concordant topologically (FIG. 2, only the ML tree is shown). Evolutionary relationships among the 10 informally named species complexes were nearly fully resolved by ML bootstrap analysis of the concatenated dataset (SUPPLEMENTARY TABLE IV, BS = 98–100%), as was the backbone of the phylogeny (BS = 97–100%). Bootstrapping of the three-locus dataset provided strong support for several relationships among the species complexes not recovered in the two-locus phylogeny, including *incarnatum-equiseti* as a sister to *chlamydosporum* + *sambucinum* and *F. commune* as a sister to NRRL 52700 *Fusarium* sp. + *fujikuroi*. Bootstrap analyses of the individual and combined partitions provided strong support for the recognition of three phylogenetically distinct species within the *F. larvarum* clade and four within the *F. coccophilum* and *F. coccidicola* clades (SUPPLEMENTARY TABLE VI, FIG. 2). Although monophyly of the *fujikuroi* complex was strongly supported by MP and ML bootstrap analyses of the combined dataset (BS = 98/100% respectively, SUPPLEMENTARY TABLE IV), the genealogical exclusivity of this complex was not supported by separate analyses of each of the individual partitions. The combined three-locus dataset also provided strong support for the monophyly of the Asian and African subclades within the *fujikuroi* complex (BS = 99-100%), as reported by O'Donnell et al. (1998a, 2000b). Last, an unnamed *Fusarium* sp. (NRRL 52700 = ARSEF 6460) isolated from the Colombian spittlebug (*Mahanarva andigena*, Hemiptera: Cercopidae) was strongly supported (BS = 99%) as sister to the *fujikuroi* complex (FIG. 2).

Species and multilocus haplotypes within the Fusarium solani, tricinctum, chlamydosporum and incarnatum-equiseti species complexes.—Isolates within four species complexes were genotyped

using published three- or four-locus typing schemes to assess whether they represented novel species and haplotypes (O'Donnell et al. 2008, 2009b). Members of the solani and tricinctum species complexes were typed with a three-locus typing scheme consisting of partial *EF-1 α* , ITS + LSU rDNA and *RPB2* gene sequences (SUPPLEMENTARY TABLE V). Of the 20 solani complex isolates typed, 11 species and 20 three-locus haplotypes were identified; all were nested within Clade 3 as defined by O'Donnell 2000). Two of the 11 species were novel: FSSC 37 (NRRL 25137 = ARSEF 2313, NRRL 25138 = ARSEF 2314) from diseased cocoa pods from Papua New Guinea and FSSC 38 (NRRL 52781 = ARSEF 5875, NRRL 52782 = ARSEF 5878, NRRL 52783 = ARSEF 5879) from coffee borer beetles (*Hypothenemus hampei*, Coleoptera: Scolytidae) from Benin and Uganda (TABLE I). In addition 18/20 solani haplotypes were novel. The two previously identified haplotypes were both nested within phylogenetic species FSSC 5 and included 5-d (NRRL 52798 = ARSEF 7382) from a sugar beet root maggot (*Tetanops myopaeformis*, Diptera: Ulidiidae) from North Dakota and 5-m (NRRL 25083 = ARSEF 1522) from a common housefly (*Musca domestica*, Diptera: Muscidae) from France. Both haplotypes were recovered from human ocular mycoses within USA (O'Donnell et al. 2008). MLST indicated the 12 tricinctum complex isolates represented seven species, several of which appear to be novel, and nine unique haplotypes. Of the five species within the tricinctum complex recovered from insects, FTSC 9-a *F. torulosum* (NRRL 52772 = ARSEF 5560) from a greater wax moth (*Galleria mellonella*, Lepidoptera: Pyralidae) from Norway was the only isolate for which a Latin binomial could be applied.

The four-locus typing scheme for members of the chlamydosporum and incarnatum-equiseti species complexes included partial sequences from the *EF-1 α* , ITS + LSU rDNA, *RPB2* and calmodulin nuclear genes (SUPPLEMENTARY TABLE V). The concatenated datasets for the

chlamydosporum and incarnatum-equiseti complexes respectively comprised 4384 and 4366 nucleotide characters. The chlamydosporum complex was represented by two isolates, each representing novel haplotypes of phylogenetic species FCSC 1: 1-n (NRRL 52702 = ARSEF 6590) from an unknown source and 1-o (NRRL 52797 = ARSEF 7381) from chili thrips larva (*Scirtothrips dorsalis*, Thysanoptera: Thripidae) from India. The incarnatum-equiseti complex was identified as the most species-rich clade, containing insecticolous fusaria with 15 species, 31 isolates and 21 haplotypes, the latter of which were all novel. Although members of incarnatum-equiseti complex were recovered from insects representing three orders, 26/31 were isolated from Hemipterans comprising eight families (TABLE I). Two novel species within the incarnatum-equiseti complex were discovered: FIESC 29, represented by haplotype 29-a (NRRL 25084 = ARSEF 1641) from a hemipterian nymph (*Adelphocoris* sp., Hemiptera: Miridae) from Austria and 29-b (NRRL 52765 = ARSEF 2304) from a *Leucaena* psyllid (*Heteropsylla incisa*, Hemiptera: Lymantriidae) from Papua New Guinea; FIESC 30-a (NRRL 52758 = ARSEF 4714) from a spittlebug (*Prosapia* nr. *bicincta*, Hemiptera: Cercopidae) from Costa Rica was the other novel species discovered within this complex. Of the 65 isolates subjected to the MLST typing schemes, those sharing the same multilocus haplotype were recovered from the same host and geographic location, except for two isolates of FIESC 15-f (TABLE I). One of these (NRRL 52697 = ARSEF 6576) was isolated from an adult spittlebug (*Zulia pubescens*, Hemiptera: Cercopidae) in Colombia; the other (NRRL 52784 = ARSEF 5881) was recovered from a diamondback moth larva (*Plutella xylostella*, Lepidoptera: Plutellidae) in Benin.

DISCUSSION

The present study was conducted to assess species diversity and evolutionary relationships of insecticolous fusaria within the ARSEF culture collection and to aid molecular identification of

isolates whose taxonomic status is unknown (Geiser et al. 2004, Park et al. 2011). These objectives were directed at increasing their potential for the biocontrol of insect pests and decreasing the risk of employing a mycotoxigenic phytopathogen as a biocontrol agent. To this end cultures of 159 insecticolous, three acaricolous, one nematogenous and 27 fusaria from other hosts/substrates were analyzed with multilocus DNA sequence data and GCPSR (Taylor et al. 2000). Major findings of the present study include: (i) insect-associated fusaria were nested within 10 species complexes, (ii) novel, putatively unnamed insecticolous species were discovered within 8/10 species complexes, (iii) Latin binomials could be applied with confidence to only 18/58 phylogenetically distinct, insecticolous fusaria and (iv) MLST-based analyses of fusaria within four species complexes (i.e. *solani*, *tricinctum*, *chlamydosporum*, *incarnatum-equiseti*) identified surprisingly high genetic diversity: 63/65 isolates genotyped represented newly discovered haplotypes. Similarly high novel genetic diversity was discovered in a survey of Sardinian soils (Balmas et al. 2010) and human pathogens in northern and central Italy (Migheli et al. 2010) using the aforementioned MLST schemes.

In addition to accessing the DNA sequence data from this study via GenBank (<http://www.ncbi.nlm.nih.gov/>) and the alignments from TreeBASE (<http://www.treebase.org/treebase-web/home.html>), the multilocus DNA sequence data, alignments and corrected ABI sequence chromatograms have been integrated into the FUSARIUM-ID (<http://isolate.fusariumdb.org>) and *Fusarium MLST* (<http://www.cbs.knaw.nl/fusarium>) online databases. In addition to being able to use a sequence from various loci as a BLAST query of these databases, both sites provide a number of unique visualization and analytical tools (O'Donnell et al. 2010, Park et al. 2011, <http://www.cbs.knaw.nl/fungi/BioloMICS.aspx?searchopt=4>). Because MSR currently can be

used to distinguish approximately one-quarter of the insecticolous fusaria the web databases provide the only means by which the majority of these isolates can be reliably identified to species. Detailed discussions on how to use the databases and interpret the results have been published (Geiser et al. 2004, O'Donnell et al. 2010, Park et al. 2011), and web guides to FUSARIUM-ID (<http://isolate.fusariumdb.org/guide.php>) and *Fusarium MLST* (<http://www.cbs.knaw.nl/Fusarium/>) also are available at their respective websites. Confidence on the phylogenetic placement of taxa whose taxonomic status is unknown, as noted by Bruns et al. (1998), can be assessed independently by sequencing more than one locus and by strong bootstrap support for the isolate nesting within a phylogenetically distinct species. Users are strongly encouraged to download one or more of the corrected ABI chromatograms from each of the loci sampled to use as a reference when checking their own sequence data for errors. In this regard the 5' and 3' ends of each sequence chromatogram especially need to be carefully edited because they typically contain most of the sequencing errors, but any other differences between overlapping sequences also need to be reconciled. In addition, translating the coding sequence in silico with a program such as MacClade (Maddison and Maddison 2000) provides a quick means for identifying sequencing errors that result in frame shifts or stop codons because the errors will be readily visible within the affected codons upon generation of the deduced amino acid sequence.

Although surveys of insecticolous fusaria have been conducted with MSR (Teeter-Barsch and Roberts 1983, Bai and Chen 1991), the present study represents the first detailed assessment of their phylogenetic diversity. As documented for human/animal pathogenic fusaria (O'Donnell et al. 2010), MP analysis of the two-locus dataset (FIG. 1) and ML analysis of the three-locus (FIG. 2) dataset recovered topologically concordant phylogenies that were nearly fully resolved

by bootstrapping. The loci we employed have been used extensively to reconstruct higher level relationships within the Fungi (Hofstetter et al. 2007, James et al. 2006, Liu and Hall 2004, Lutzoni et al. 2004, Reeb et al. 2004, Schoch et al. 2009) as well as for resolving species limits within the Basidiomycota (e.g. Matheny 2005, Frøslev et al. 2005) and Ascomycota (e.g. Bischoff et al. 2009; O'Donnell et al. 2007, 2008, 2009b; Rehner and Buckley 2005). While partial sequences of *EF-1 α* have proven to be extraordinarily useful for resolving species boundaries in *Fusarium* (Geiser et al. 2004, 2005; O'Donnell et al. 2000a, b, 2004), most of the intronic sequences are too divergent to align beyond the species complex, mainly due to the presence of large phylogenetically informative introns that comprise more than one-third of the amplicon. For this reason *RPB1* and *RPB2* nucleotide sequences are more informative for genus-wide phylogenetics within *Fusarium* (sensu Gerlach and Nirenberg 1982) and they are easy to align because the region sampled is entirely protein coding except for a 42 bp intron near the 3' end of the *RPB2* 5-7 region. Not surprisingly the highly resolved three-locus phylogeny reported here was topologically concordant with one inferred with the same loci for medically important fusaria (O'Donnell et al. 2010). It is important to note that several single or multiple species lineages, basal to and nested within the *Gibberella* clade, were not sampled in either study. Thus the basal-most lineages within *Fusarium* and the *Gibberella* clade remain to be determined. However the discovery of NRRL 52700 *Fusarium* sp. (ARSEF 6460) from the South American hemipteran *Mahanarva andigena*, which is endemic to Colombia, is noteworthy because it appears to be the sister to the fujikuroi species complex. This novel finding together with phylogenetic support for the American clade representing the basal-most divergence within this complex (O'Donnell et al. 1998a, 2000b) may indicate a South American evolutionary origin of this clade. Failure of the individual gene partitions to support the monophyly of the fujikuroi

species complex can be explained in part by the exclusion of the ambiguously aligned introns within *EF-1 α* , which contain approximately 80% of the synapomorphies within this gene fragment. In addition we found that only 5–7% of the nucleotide positions within the *RPB1* and *RPB2* genes were synapomorphic within the fujikuroi complex. Thus of the genes sampled to date only the partial beta tubulin gene sequences provide strong support the monophyly of the fujikuroi species complex and its three geographically structured clades (O'Donnell et al. 2000b). Results of the present study suggest the spectrum of insecticolous and human pathogenic fusaria may differ in several important respects. First, no member of the *Fusarium dimerum* species complex has been reported to be insecticolous; however four species within this complex have been recovered from opportunistic infections of humans (Schroers et al. 1999). Conversely no representatives of the lateritium and concolor species complexes have been shown to cause mycotic infections in humans or other vertebrates; however our GCPSR analyses identified one insecticolous species within the lateritium complex and 11 biogeographically structured scale insect- or aphid-associated fusaria within the coccophilum complex. The *Fusarium*-scale insect relationship has been characterized as parasitic (Booth 1971, Gerlach and Nirenberg 1982) and appears to have been derived monophyletically based on the available data. However co-evolutionary studies are needed to assess whether this association is mutualistic (Feldman et al. 2008, Mueller et al. 1998). Detailed studies of these associations should yield exciting discoveries such as the one elucidating a mutualistic symbiosis involving *F. ambrosium* and ambrosia beetles in Sri Lanka (Gadd and Loos 1947). As the epithet suggests, this species appears to be farmed in galleries of ambrosia beetles (*Euwallacea* spp., Coleoptera: Scolytidae; Baker and Norris 1968). Studies by Kok et al. (1970), using a chemically defined medium, established that ergosterol produced by the fungus is essential for pupation of the larvae.

Fusarium ambrosium was not represented among the ARSEF fusaria; however our studies show that it is nested within Clade 3 of the solani species complex (O'Donnell 2000, O'Donnell et al. 2008).

Unfortunately Latin binomials can be applied with confidence to only 18/58 of the insecticolous fusaria and correspondingly only 22/69 species associated with mycotic infections of humans and other animals (O'Donnell et al. 2010). The results of both studies demonstrate human and insecticolous fusaria are phylogenetically diverse in that they are nested respectively within nine and 10 species complexes spanning the breadth of *Fusarium*. Although the majority of fusaria included in the present study have not been reported to cause infections in humans, 25/67 species have, and most of these are nested within the following three species complexes: incarnatum-equiseti (n = 10), solani (n = 6) and fujikuroi (n = 4). Due to intensive systematic and molecular phylogenetic study within the fujikuroi complex (Nirenberg and O'Donnell 1998, O'Donnell et al. 1998a), Latin binomials can be applied with confidence to 8/9 fusaria within this clade, including four species that also are known to cause mycotic infections (i.e. *F. verticillioides*, *F. proliferatum*, *F. nygamai*, *F. sacchari*). However we were able to apply names with confidence to only 1/15 species within the incarnatum-equiseti complex, 2/11 species within the solani complex (O'Donnell et al. 2008, Zhang et al. 2006) and 2/7 species within the tricinctum complex (O'Donnell et al. 2009b). For this reason the species/haplotype nomenclature developed for the contact lens solution-associated *Fusarium* keratitis outbreak investigation within the U.S. in 2006 (Chang et al. 2006) and extended in subsequent studies on medically (O'Donnell et al. 2007, 2008, 2009a, b, 2010; Migheli et al. 2010) and ecologically important fusaria (Balmas et al. 2010) provide the only means presently by which species identity and diversity within most of the species complexes can be accurately determined and

communicated within the scientific community. The molecular phylogenetic identifications provided in the present and a previous study (O'Donnell et al. 1998a) should enhance the utility of the ARSEF collection, given that approximately 80% of the fusaria in the catalog (<http://www.ars.usda.gov/Main/docs.htm?docid=12125>) were listed as *Fusarium* sp. at the onset of this study. Our analyses also serve to highlight the difficulty of applying MSR within *Fusarium*. Although the gypsy moth pathogens were reported as *F. polyphialidicum* and *F. sambucinum* by Hajek et al. (1993, see TABLE I), our data clearly show they belong to an unnamed species within the concolor species complex. The difficulty of identifying *Fusarium* species with MSR is further illustrated by *F. polyphialidicum* (Marasas et al. 1986), given that our molecular phylogenetic data, including partial *EF-1 α* , beta tubulin, *RPB1* and *RPB2* gene sequences (O'Donnell unpubl), clearly show is a later synonym of *F. concolor* (Reinking 1934).

The FUSARIUM-ID (<http://isolate.fusariumdb.org>) and *Fusarium MLST* (<http://www.cbs.knaw.nl/fusarium>) databases will be regularly updated with phylogenetically informative sequences and corrected chromatograms generated from voucher cultures. As a result the expanding databases should let users identify fusaria with greater precision and place them within a robust phylogenetic framework. GCPSR-based studies on *Fusarium*, including the present one, have consistently identified novel species, which suggests many fusaria remain to be discovered (Geiser et al. 2005, O'Donnell et al. 2010).

To ensure that the archived phylogenetic data is used maximally visualization and data analysis tools integrated in FUSARIUM-ID and *Fusarium MLST* were modeled in part after the *Phytophthora* Database (Park et al. 2008, <http://www.phytophthoradb.org/>) and TrichoKEY (Druzhinina et al. 2005, <http://www.isth.info/tools/molkey/index.php>). The latter two websites are dedicated respectively to the identification of species of *Phytophthora* and *Trichoderma*. To

promote further study by the scientific community all fusarial isolates listed on the FUSARIUM-ID and *Fusarium MLST* websites are available from publically accessible culture collections (see TABLE I). In summary, results of the present study provide the first phylogenetic estimate of insecticolous fusarial diversity and help set the stage for future studies focused on determining whether any entomopathogenic species might be developed for the safe and effective biological control of insect pests.

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LEGENDS

FIG. 1. Phylogram inferred from partial *EF-1 α* and *RPB2* gene sequences for 159 insecticolous, three acaricolous, one nematogenous and 27 other fusaria. Sequences of NRRL 52709 (ARSEF 6448) and NRRL 52754 (ARSEF 3464) were used to root the maximum parsimony (MP) phylogeny by the outgroup method. Gray highlighting is used to denote 27 fusaria from soil or unknown sources. The phylogram was inferred from 2417 nucleotide characters of which 863 were parsimony informative (PIC). Numbers above internodes represent MP bootstrap support based on 1000 pseudoreplicates of the data. With the exception of four isolates, which represent three single species lineages, all of the ingroup were placed in one of 10 species complexes identified in the right margin. The root of each species complex is identified by a bold internode. Note that monophyly of the fujikuroi complex and sister group relationship of its African (Af) and Asian (As) subclades was not supported by this dataset. Species and multilocus-locus haplotypes within the solani, chlamydosporum, incarnatum-equiseti and tricinctum complexes were determined using three- and four-locus typing schemes (O'Donnell et al. 2008, 2009; SUPPLEMENTARY TABLE V) in which species and haplotypes were identified respectively by Arabic numbers and lowercase Roman letters. In addition Arabic numbers identify phylogenetically distinct species within the *F. larvarum* (1–3), *F. coccophilum* (1–4) and *F. coccidicola* (1–4) clades of the coccophilum species complex. Note that 168/188 (89.4%) of the fusaria are nested with the *Gibberella* clade.

FIG. 2. Best maximum likelihood tree inferred from the combined three-locus dataset for 67 insecticolous, one acaricolous (NRRL 52715 FSSC 2-hh = ARSEF 6443), one nematogenous (NRRL 52927 = ARSEF 3042 *F. concolor*) and 11 fusaria from other sources (identified by gray highlighting). Sequences of NRRL 52709 (ARSEF 6448) and NRRL 52754 (ARSEF 3464) were used as outgroups to root the phylogeny. ML bootstrap values based on 800 pseudoreplicates of the data are indicated above internodes. Bold internodes identify the root of each species complex. Of the 10 species complexes represented, the eight most derived are nested within the *Gibberella* clade. In contrast to the two-locus phylogeny (FIG. 1), the fujikuroi complex and its African (Af) and Asian (As) subclades were strongly supported as monophyletic. Species within the solani, tricinctum, incarnatum-equiseti and chlamydosporum complexes are identified by Arabic numbers and haplotypes by lowercase Roman letters, employing published multilocus typing schemes (O'Donnell et al. 2008, 2009b; SUPPLEMENTARY TABLE V). In addition Arabic numbers identify phylogenetically distinct species within the *F. larvarum*, *F. coccophilum* and *F. coccidicola* clades of the coccophilum complex.

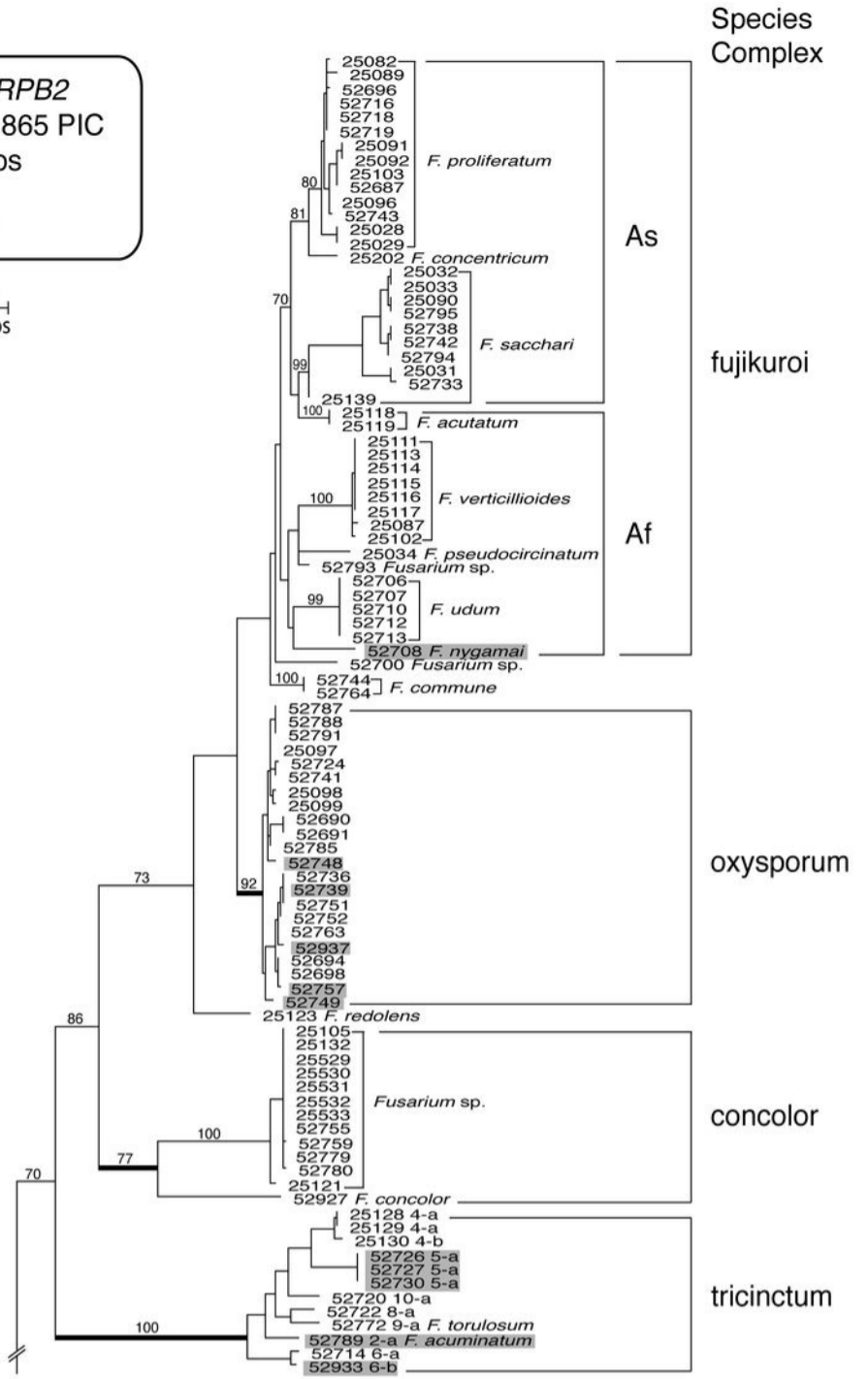
FOOTNOTES

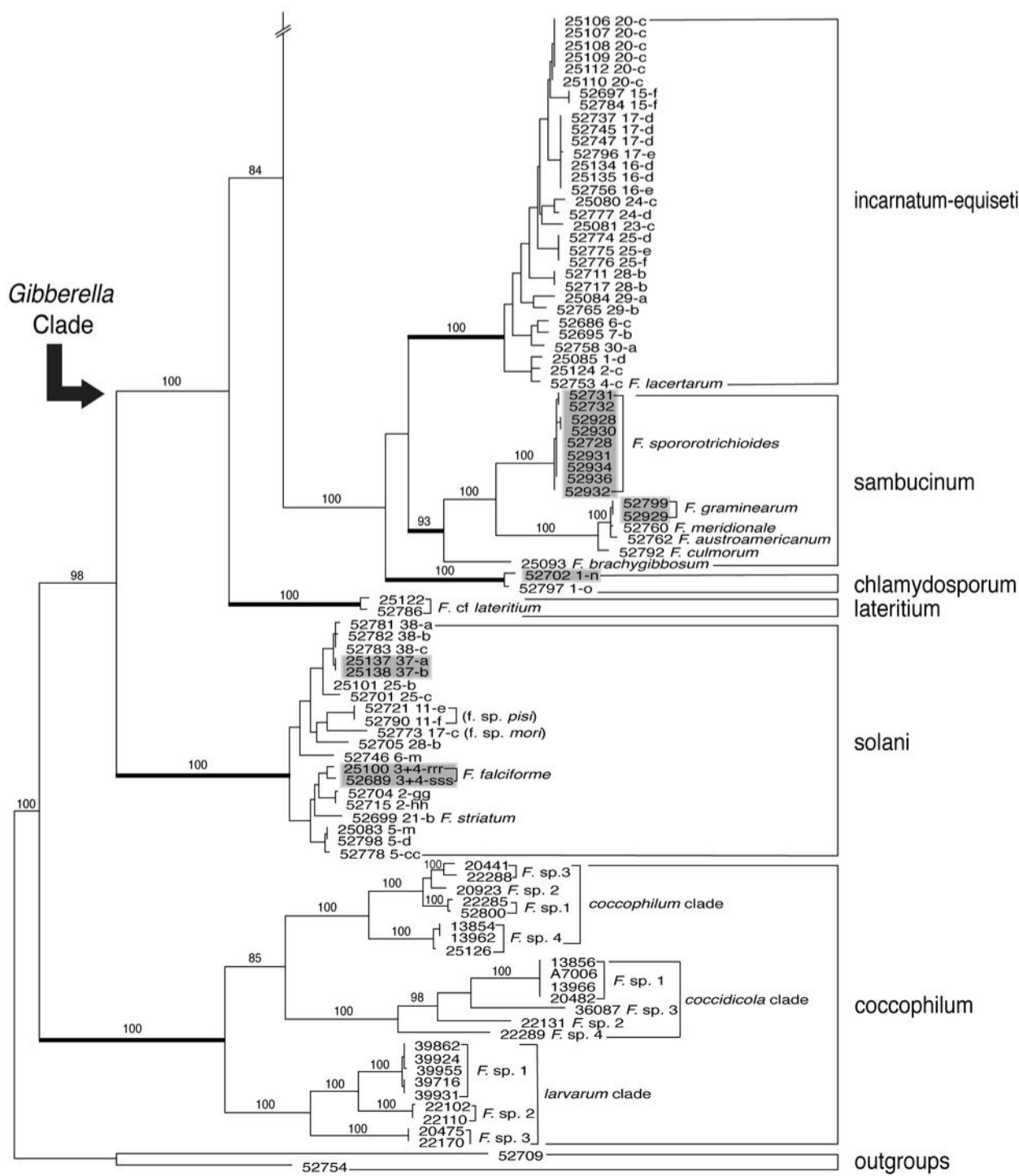
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EF-1a + RPB2
 2415 bp, 865 PIC
 4342 steps
 CI = 0.36
 RI = 0.92

20
 steps





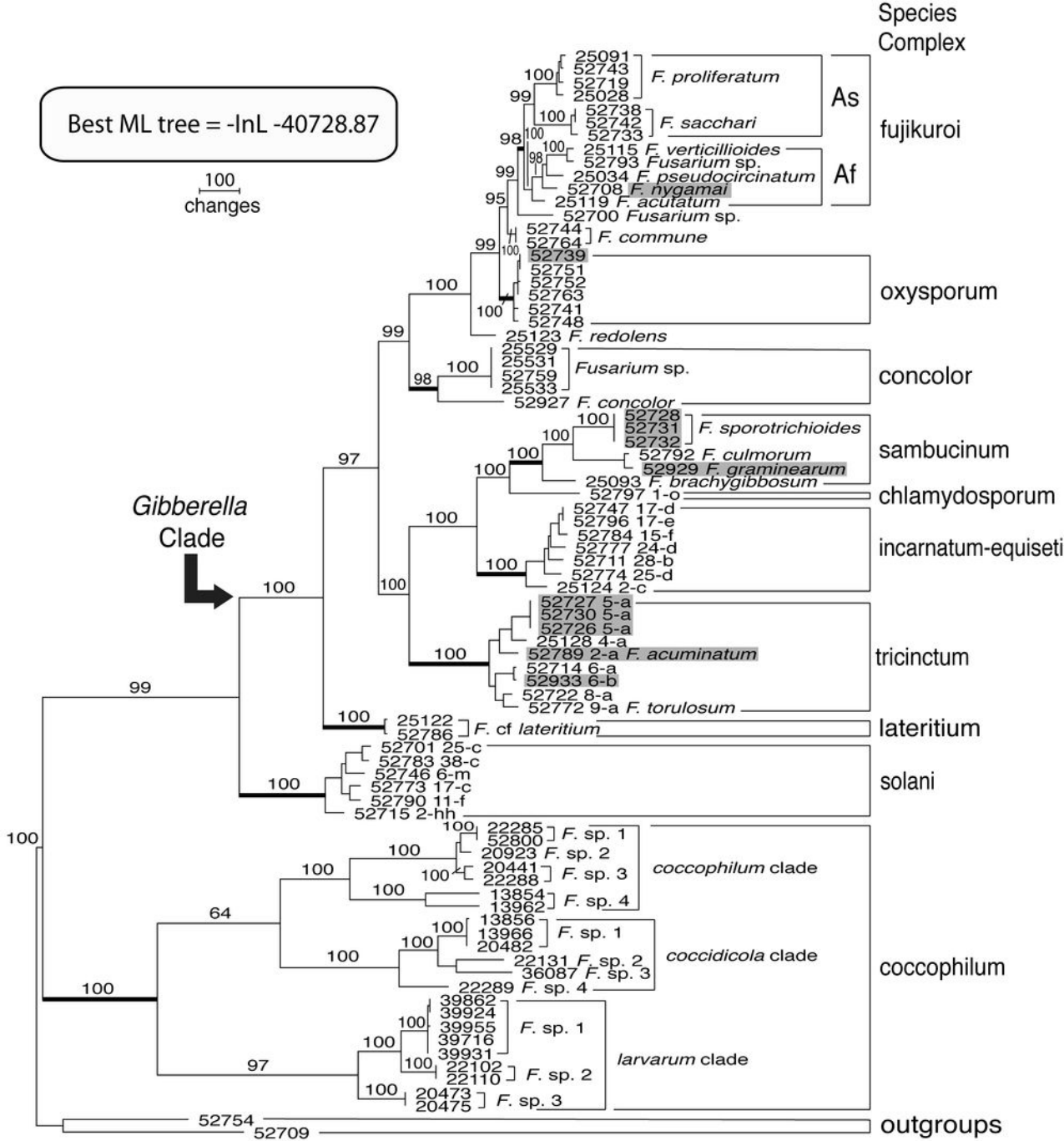


TABLE I. Isolates subjected to MLST

NRRL number ^a	Equivalent # ^b	Species complex	MLST ID ^c	Host/substrate	Geographic origin
13854	FRC E-94	coccophilum	<i>F. sp. 4 (coccophilum clade)</i>	Scale insect	Iran
13856	FRC E-99	coccophilum	<i>F. sp. 1 (coccidicola clade)</i>	<i>Quadraspidotus perniciosus</i> [Hemiptera, Coccidae]	Iran
13962	CBS 310.34	coccophilum	<i>F. sp. 4 (coccophilum clade)</i>	Scale insect on <i>Laurus nobilis</i>	Italy
13966	CBS 735.79	coccophilum	<i>F. sp. 1 (coccidicola clade)</i>	<i>Quadraspidotus perniciosus</i> [Hemiptera, Coccidae]	Iran
20441	IMI 143094A	coccophilum	<i>F. sp. 3 (coccophilum clade)</i>	Scale insect on <i>Coffea arabica</i>	Papua New Guinea
20475	CBS 638.76	coccophilum	<i>F. sp. 3 (larvarum clade)</i>	<i>Quadraspidotus perniciosus</i> [Hemiptera, Coccidae]	Iran
20482	MUCL 19031	coccophilum	<i>F. sp. 1 (coccidicola clade)</i>	<i>Quadraspidotus perniciosus</i> [Hemiptera, Coccidae]	Iran
20923	GJS 91-51	coccophilum	<i>F. sp. 2 (coccophilum clade)</i>	Scale insect	Brazil
22102	CBS 169.30	coccophilum	<i>F. sp. 2 (larvarum clade)</i>	Aphids on <i>Pyrus communis</i>	Japan
22110	CBS 158.57	coccophilum	<i>F. sp. 2 (larvarum clade)</i>	Scale insect on <i>Citrus maxima</i>	Unknown
22170	BBA 62460	coccophilum	<i>F. sp. 3 (larvarum clade)</i>	<i>Quadraspidotus perniciosus</i> [Hemiptera, Coccidae]	Iran
22131	IMI 264358	coccophilum	<i>F. sp. 2 (coccidicola clade)</i>	Scale insect on <i>Citrus sp.</i>	New Guinea
22285	CBS 498.88	coccophilum	<i>F. sp. 1 (coccophilum clade)</i>	Scale insect	USA-FL
22288	CBS 499.88	coccophilum	<i>F. sp. 3 (coccophilum clade)</i>	Scale insect	Indonesia

			clade)		
22289	CBS 119.31	coccophilum	<i>F. sp. 4 (coccidicola clade)</i>	Scale insect on <i>Citrus</i> sp.	Honduras
25028	ARSEF 2074	fujikuroi	<i>F. proliferatum</i>	<i>Melanaspis glomerata</i> [Hemiptera: Diaspididae]	India
25029	ARSEF 3223	fujikuroi	<i>F. proliferatum</i>	<i>Nilaparvata lugens</i> [Hemiptera: Delphacidae]	India
25031	ARSEF 2141	fujikuroi	<i>F. sacchari</i>	<i>Scirpophaga excerptalis</i> [Lepidoptera: Pyralidae]	India
25032	ARSEF 2143	fujikuroi	<i>F. sacchari</i>	<i>Pyrilla perpusilla</i> [Hemiptera: Lophopidae]	India
25033	ARSEF 2145	fujikuroi	<i>F. sacchari</i>	<i>Pulvinaria elongata</i> [Hemiptera: Coccidae]	India
25034	ARSEF 2301	fujikuroi	<i>F. pseudocircinatum</i>	<i>Heteropsylla incisa</i> [Hemiptera: Psyllidae]	Papua New Guinea
25080	ARSEF 687	incarnatum-equiseti	FIESC 24-c	<i>Nilaparvata lugens</i> [Hemiptera: Delphacidae]	China
25081	ARSEF 1307	incarnatum-equiseti	FIESC 23-c	<i>Adelphocoris</i> sp. nymph [Hemiptera: Miridae]	Italy
25082	ARSEF 1310	fujikuroi	<i>F. proliferatum</i>	<i>Sitona discoideus</i> adult [Coleoptera: Curculionidae]	Morocco
25083	ARSEF 1522	solani	FSSC 5-m	<i>Musca domestica</i> pupa [Diptera: Muscidae]	France
25084	ARSEF 1641	incarnatum-equiseti	FIESC 29-a	<i>Adelphocoris</i> sp. nymph [Hemiptera: Miridae]	Austria
25085	ARSEF 1828	incarnatum-equiseti	FIESC 1-d	[Hemiptera: Coccidae] on stems of young <i>Citrus</i> sp.	Sri Lanka
25087	ARSEF 2252	fujikuroi	<i>F. verticillioides</i>	Adult [Diptera: Bibionidae] on <i>Solidago</i>	France
25089	ARSEF 2337	fujikuroi	<i>F. proliferatum</i>	<i>Rhopalosiphum padi</i> [Hemiptera: Aphididae]	USA-ID
25090	ARSEF 2418	fujikuroi	<i>F. sacchari</i>	<i>Emmalocera depressella</i> [Lepidoptera: Phycitinae]	India
25091	ARSEF 2568	fujikuroi	<i>F. proliferatum</i>	<i>Chalcodermus aeneus</i> larva [Coleoptera: Curculionidae]	USA-GA
25092	ARSEF 2569	fujikuroi	<i>F. proliferatum</i>	<i>Chalcodermus aeneus</i> larva [Coleoptera: Curculionidae]	USA-GA
25093	ARSEF 2595	sambucinum	<i>F. brachygibbosum</i>	<i>Hyblaea puer</i> on teak [Lepidoptera: Hyblaeidae]	India

25096	ARSEF 2776	fujikuroi	<i>F. proliferatum</i>	<i>Plutella xylostella</i> [Lepidoptera: Plutellidae]	Philippines
25097	ARSEF 3030	oxysporum	nd	<i>Anthonomus musculus</i> adult [Coleoptera: Curculionidae]	USA-NJ
25098	ARSEF 3031	oxysporum	nd	<i>Anthonomus musculus</i> adult [Coleoptera: Curculionidae]	USA-NJ
25099	ARSEF 3032	oxysporum	nd	<i>Anthonomus musculus</i> adult [Coleoptera: Curculionidae]	USA-NJ
25100	ARSEF 3051	solani	FSSC 3+4-rrr	Soil	Mali
25101	ARSEF 3296	solani	FSSC 25-b	<i>Boophilus</i> sp. [Acari: Ixodidae] on cow	Mexico
25102	ARSEF 3300	fujikuroi	<i>F. verticillioides</i>	<i>Spodoptera frugiperda</i> [Lepidoptera: Noctuidae]	Mexico
25103	ARSEF 3345	fujikuroi	<i>F. proliferatum</i>	<i>Aeneolamia postica</i> [Hemiptera: Cercopidae]	Costa Rica
25105	ARSEF 3532	concolor	<i>Fusarium</i> sp.	<i>Lymantria dispar</i> [Lepidoptera: Lymantriidae]	USA-MD
25106	ARSEF 3668	incarnatum-equiseti	FIESC 20-c	<i>Bemisia</i> nymph [Hemiptera: Aleyrodidae]	USA-CA
25107	ARSEF 3669	incarnatum-equiseti	FIESC 20-c	<i>Bemisia</i> nymph [Hemiptera: Aleyrodidae]	USA-CA
25108	ARSEF 3670	incarnatum-equiseti	FIESC 20-c	<i>Bemisia</i> nymph [Hemiptera: Aleyrodidae]	USA-CA
25109	ARSEF 3671	incarnatum-equiseti	FIESC 20-c	<i>Bemisia</i> nymph [Hemiptera: Aleyrodidae]	USA-CA
25110	ARSEF 3672	incarnatum-equiseti	FIESC 20-c	<i>Bemisia</i> nymph [Hemiptera: Aleyrodidae]	USA-CA
25111	ARSEF 3673	fujikuroi	<i>F. verticillioides</i>	<i>Bemisia</i> nymph [Hemiptera: Aleyrodidae]	USA-CA
25112	ARSEF 3674	incarnatum-equiseti	FIESC 20-c	<i>Bemisia</i> nymph [Hemiptera: Aleyrodidae]	USA-CA
25113	ARSEF 3675	fujikuroi	<i>F. verticillioides</i>	<i>Bemisia</i> nymph [Hemiptera: Aleyrodidae]	USA-CA
25114	ARSEF 3676	fujikuroi	<i>F. verticillioides</i>	<i>Bemisia</i> nymph [Hemiptera: Aleyrodidae]	USA-CA
25115	ARSEF 3677	fujikuroi	<i>F. verticillioides</i>	<i>Bemisia</i> nymph [Hemiptera: Aleyrodidae]	USA-CA
25116	ARSEF 3678	fujikuroi	<i>F. verticillioides</i>	<i>Bemisia</i> nymph [Hemiptera: Aleyrodidae]	USA-CA

25117	ARSEF 3679	fujikuroi	<i>F. verticillioides</i>	<i>Bemisia</i> nymph [Hemiptera: Aleyrodidae]	USA-CA
25118	ARSEF 3704	fujikuroi	<i>F. acutatum</i>	Adult [Hemiptera: Aphididae]	Pakistan
25119	ARSEF 3707	fujikuroi	<i>F. acutatum</i>	Adult [Hemiptera: Aphididae]	Pakistan
25121	ARSEF 3976	concolor	<i>Fusarium</i> sp.	<i>Cydia pomonella</i> larva [Lepidoptera: Tortricidae]	France
25122	ARSEF 4002	lateratum	<i>F. cf. lateritium</i>	[Hemiptera: Aleyrodidae] on <i>Citrus</i> sp.	Brazil
25123	ARSEF 4011	not designated	<i>F. redolens</i>	<i>Lilioceris lili</i> [Coleoptera: Chrysomelidae]	Denmark
25124	ARSEF 3218	incarnatum-equiseti	FIESC 2-c	[Orthoptera: Acrididae]	Mali
25126	ARSEF 821	coccophilum	<i>F. sp. 4 (coccophilum clade)</i>	<i>Melanaspis obscura</i> [Hemiptera: Diaspididae]	USA-Wash-DC
25128	ARSEF 1331	tricinctum	FTSC 4-a	Adult [Hymenoptera: Ichneumonidae]	Poland
25129	ARSEF 1416	tricinctum	FTSC 4-a	Adult [Hymenoptera: Ichneumonidae]	Poland
25130	ARSEF 2059	tricinctum	FTSC 4-b	<i>Lymantria dispar</i> egg mass [Lepidoptera: Lymantriidae]	USA-OR
25132	ARSEF 3483	concolor	<i>Fusarium</i> sp.	<i>Lymantria dispar</i> [Lepidoptera: Lymantriidae]	USA-VA
25134	ARSEF 2299	incarnatum-equiseti	FIESC 16-d	<i>Heteropsylla incisa</i> [Hemiptera: Psyllidae]	Papua New Guinea
25135	ARSEF 2300	incarnatum-equiseti	FIESC 16-d	<i>Heteropsylla incisa</i> [Hemiptera: Psyllidae]	Papua New Guinea
25137	ARSEF 2313	solani	FSSC 37-a	Diseased cocoa pods	Papua New Guinea
25138	ARSEF 2314	solani	FSSC 37-b	Diseased cocoa pods	Papua New Guinea
25139	ARSEF 2142	fujikuroi	<i>F. sacchari</i>	<i>Chilo sacchariphagus indicus</i> [Lepidoptera: Pyralidae]	India
25202	ARSEF 2053	fujikuroi	<i>F. concentricum</i>	<i>Nilaparvata lugens</i> [Hemiptera: Delphacidae] on rice	Korea
25529	ARSEF 3692	concolor	<i>Fusarium</i> sp.	<i>Lymantria dispar</i> [Lepidoptera: Lymantriidae]	USA-NY
25530	ARSEF 3693	concolor	<i>Fusarium</i> sp.	<i>Lymantria dispar</i> [Lepidoptera: Lymantriidae]	USA-NY

25531	ARSEF 3694	concolor	<i>Fusarium</i> sp.	<i>Lymantria dispar</i> [Lepidoptera: Lymantriidae]	USA-MA
25532	ARSEF 3695	concolor	<i>Fusarium</i> sp.	<i>Lymantria dispar</i> [Lepidoptera: Lymantriidae]	USA-VA
25533	ARSEF 3696	concolor	<i>Fusarium</i> sp.	<i>Lymantria dispar</i> [Lepidoptera: Lymantriidae]	USA-MD
36087	CBS 101065	coccophilum	<i>F.</i> sp. 3 (<i>coccidicola</i> clade)	Scale insect	Thailand
39716	ICMP 5616	coccophilum	<i>F.</i> sp. 1 (<i>larvarum</i> clade)	Scale insect	New Zealand
39862	ICMP 10612	coccophilum	<i>F.</i> sp. 1 (<i>larvarum</i> clade)	<i>Hemiberlesia lataniae</i> scale insect	New Zealand
39924	ICMP 11180	coccophilum	<i>F.</i> sp. 1 (<i>larvarum</i> clade)	Scale insect	New Zealand
39931	ICMP 11182	coccophilum	<i>F.</i> sp. 1 (<i>larvarum</i> clade)	Scale insect	New Zealand
39955	ICMP 11181	coccophilum	<i>F.</i> sp. 1 (<i>larvarum</i> clade)	Scale insect	New Zealand
52686	ARSEF 6348	incarnatum-equiseti	FIESC 6-c	<i>Zulia colombiana</i> adult [Hemiptera: Cercopidae]	Colombia
52687	ARSEF 6320	fujikuroi	<i>F. proliferatum</i>	<i>Zulia colombiana</i> adult [Hemiptera: Cercopidae]	Colombia
52689	ARSEF 4863	solani	FSSC 3+4-sss	Eggplant soil	Taiwan
52690	ARSEF 6355	oxysporum	nd	<i>Zulia colombiana</i> adult [Hemiptera: Cercopidae]	Colombia
52691	ARSEF 6353	oxysporum	nd	<i>Zulia colombiana</i> adult [Hemiptera: Cercopidae]	Colombia
52694	ARSEF 6375	oxysporum	nd	<i>Zulia pubescens</i> adult [Hemiptera: Cercopidae]	Colombia
52695	ARSEF 6464	incarnatum-equiseti	FIESC 7-b	<i>Mahanarva andigena</i> adult [Hemiptera: Cercopidae]	Colombia
52696	ARSEF 6649	fujikuroi	<i>F. proliferatum</i>	<i>Spodoptera litura</i> larva [Lepidoptera: Noctuidae]	India
52697	ARSEF 6576	incarnatum-equiseti	FIESC 15-f	<i>Zulia pubescens</i> adult [Hemiptera: Cercopidae]	Colombia
52698	ARSEF 6465	oxysporum	nd	<i>Zulia carbonaria</i> adult [Hemiptera: Cercopidae]	Colombia
52699	ARSEF 6461	solani	FSSC 21-b (<i>F. striatum</i>)	<i>Mahanarva andigena</i> adult [Hemiptera: Cercopidae]	Colombia
52700	ARSEF 6460	not designated	<i>Fusarium</i> sp.	<i>Mahanarva andigena</i> adult [Hemiptera: Cercopidae]	Colombia

52701	ARSEF 6602	solani	FSSC 25-c	<i>Hypothenemus hampei</i> [Coleoptera: Scolytidae]	Colombia
52702	ARSEF 6590	chlamydosporum	FCSC 1-n	Unknown	Unknown
52704	ARSEF 6572	solani	FSSC 2-gg	<i>Tetranychus urticae</i> [Acari: Tetranychidae]	USA-VA
52705	ARSEF 6587	solani	FSSC 28-b	Unidentified insect	Unknown
52706	ARSEF 6455	fujikuroi	<i>F. udum</i>	<i>Aiolopus longicornis</i> [Orthoptera: Acrididae]	Ethiopia
52707	ARSEF 6447	fujikuroi	<i>F. udum</i>	<i>Aiolopus longicornis</i> [Orthoptera: Acrididae]	Ethiopia
52708	ARSEF 6453	fujikuroi	<i>F. nygamai</i>	Unknown	Ethiopia
52709	ARSEF 6448	outgroup	Unknown	<i>Aiolopus longicornis</i> [Orthoptera: Acrididae]	Ethiopia
52710	ARSEF 6452	fujikuroi	<i>F. udum</i>	<i>Aiolopus longicornis</i> [Orthoptera: Acrididae]	Ethiopia
52711	ARSEF 6450	incarnatum-equiseti	FIESC 28-b	<i>Aiolopus longicornis</i> [Orthoptera: Acrididae]	Ethiopia
52712	ARSEF 6451	fujikuroi	<i>F. udum</i>	<i>Aiolopus longicornis</i> [Orthoptera: Acrididae]	Ethiopia
52713	ARSEF 6446	fujikuroi	<i>F. udum</i>	<i>Aiolopus longicornis</i> [Orthoptera: Acrididae]	Ethiopia
52714	ARSEF 6428	tricinctum	FTSC 6-a	<i>Eurygaster</i> sp. [Hemiptera: Scutelleridae]	Turkey
52715	ARSEF 6443	solani	FSSC 2-hh	<i>Tetranychus urticae</i> [Acari: Tetranychidae]	USA-VA
52716	ARSEF 6409	fujikuroi	<i>F. proliferatum</i>	<i>Eurygaster</i> sp. [Hemiptera: Scutelleridae]	Turkey
52717	ARSEF 6445	incarnatum-equiseti	FIESC 28-b	<i>Aiolopus longicornis</i> [Orthoptera: Acrididae]	Ethiopia
52718	ARSEF 6411	fujikuroi	<i>F. proliferatum</i>	<i>Dolycorus</i> sp. [Hemiptera: Pentatomidae]	Turkey
52719	ARSEF 6425	fujikuroi	<i>F. proliferatum</i>	<i>Eurygaster</i> sp. [Hemiptera: Scutelleridae]	Turkey
52720	ARSEF 6410	tricinctum	FTSC 10-a	<i>Eurygaster</i> sp. [Hemiptera: Scutelleridae]	Turkey
52721	ARSEF 6403	solani	FSSC 11-e (f. sp. <i>pisi</i>)	<i>Eurygaster</i> sp. [Hemiptera: Scutelleridae]	Turkey
52722	ARSEF 6401	tricinctum	FTSC 8-a	<i>Eurygaster</i> sp. [Hemiptera: Scutelleridae]	Turkey

52724	ARSEF 4963	oxysporum	nd	<i>Meloidogyne hapla</i> egg mass [Tylenchida: Heteroderidae]	USA-NY
52726	ARSEF 8299	tricinctum	FTSC 5-a	Unknown	Turkey
52727	ARSEF 8300	tricinctum	FTSC 5-a	Unknown	Turkey
52728	ARSEF 8304	sambucinum	<i>F. sporotrichioides</i>	Unknown	Turkey
52730	ARSEF 8302	tricinctum	FTSC 5-a	Unknown	Turkey
52731	ARSEF 8306	sambucinum	<i>F. sporotrichioides</i>	Unknown	Turkey
52732	ARSEF 8305	sambucinum	<i>F. sporotrichioides</i>	Unknown	Turkey
52733	ARSEF 8283	fujikuroi	<i>F. sacchari</i>	<i>Sogatella furcifera</i> [Hemiptera: Delphacidae]	India
52736	ARSEF 8290	oxysporum	nd	Soil	India
52737	ARSEF 8287	incarnatum-equiseti	FIESC 17-d	<i>Aphis fabae</i> [Hemiptera: Aphididae]	India
52738	ARSEF 8285	fujikuroi	<i>F. sacchari</i>	<i>Sogatella furcifera</i> [Hemiptera: Delphacidae]	India
52739	ARSEF 8288	oxysporum	nd	Soil	India
52741	ARSEF 8266	oxysporum	nd	<i>Conotrachelus nenuphar</i> [Coleoptera: Curculionidae]	USA-MI
52742	ARSEF 8282	fujikuroi	<i>F. sacchari</i>	<i>Sogatella furcifera</i> [Hemiptera: Delphacidae]	India
52743	ARSEF 8267	fujikuroi	<i>F. proliferatum</i>	<i>Conotrachelus nenuphar</i> [Coleoptera: Curculionidae]	USA-MI
52744	ARSEF 8281	not designated	<i>F. commune</i>	<i>Tetrix granulata</i> [Orthoptera: Tetrigidae]	India
52745	ARSEF 8277	incarnatum-equiseti	FIESC 17-d	<i>Aphis fabae</i> [Hemiptera: Aphididae]	India
52746	ARSEF 8279	solani	FSSC 6-m	<i>Ceresa bubalus</i> [Hemiptera: Membracida]	India
52747	ARSEF 8278	incarnatum-equiseti	FIESC 17-d	<i>Aphis fabae</i> [Hemiptera: Aphididae]	India
52748	ARSEF 7473	oxysporum	nd	<i>Triatoma infestans</i> [Hemiptera: Reduviidae]	Argentina
52749	ARSEF 6622	oxysporum	nd	Unknown	Unknown

52751	ARSEF 4964	oxysporum	nd		<i>Meloidogyne hapla</i> egg mass [Tylenchida: Heteroderidae]	USA-NY
52752	ARSEF 4956	oxysporum	nd		<i>Meloidogyne hapla</i> egg mass [Tylenchida: Heteroderidae]	USA-NY
52753	ARSEF 5219	incarnatum-equiseti	FIESC 4-c (<i>F. lacertarum</i>)		<i>Scrobipalpuloides absoluta</i> larva [Lepidoptera: Gelechiidae]	Brazil
52754	ARSEF 3464	outgroup	Unknown		<i>Pemphigus betae</i> [Hemiptera: Aphididae]	USA-AZ
52755	ARSEF 3483	concolor	<i>Fusarium</i> sp.		<i>Lymantria dispar</i> [Lepidoptera: Lymantriidae]	USA-VA
52756	ARSEF 2303	incarnatum-equiseti	FIESC 16-e		<i>Heteropsylla cubana</i> [Hemiptera: Psyllidae]	Papua New Guinea
52757	ARSEF 4864	oxysporum	nd		Eggplant soil	Taiwan
52758	ARSEF 4714	incarnatum-equiseti	FIESC 30-a		<i>Prosapia</i> nr. <i>bicincta</i> [Hemiptera: Cercopidae] on <i>Cynodon</i>	Costa Rica
52759	ARSEF 3916	concolor	<i>Fusarium</i> sp.		<i>Lymantria dispar</i> [Lepidoptera: Lymantriidae]	USA-PA
52760	ARSEF 4715	sambucinum	<i>F. meridionale</i>		<i>Prosapia</i> nr. <i>bicincta</i> [Hemiptera: Cercopidae] on <i>Cynodon</i>	Costa Rica
52762	ARSEF 4713	sambucinum	<i>F. austroamericanum</i>		<i>Prosapia</i> nr. <i>bicincta</i> [Hemiptera: Cercopidae] on <i>Cynodon</i>	Costa Rica
52763	ARSEF 4962	oxysporum	nd		<i>Meloidogyne hapla</i> egg mass [Tylenchida: Heteroderidae]	USA-NY
52764	ARSEF 4825	oxysporum	<i>F. commune</i>		<i>Otiorhynchus ligustici</i> [Coleoptera: Curculionidae]	USA-NY
52765	ARSEF 2304	incarnatum-equiseti	FIESC 29-b		<i>Heteropsylla cubana</i> [Hemiptera: Psyllidae]	Papua New Guinea
52772	ARSEF 5560	tricinctum	FTSC 9-a (<i>F. torulosum</i>)		<i>Galleria mellonella</i> larva [Lepidoptera: Pyralidae]	Norway
52773	ARSEF 5680	solani	FSSC 17-c (f. sp. <i>mori</i>)		<i>Eurygaster</i> sp. [Hemiptera: Scutelleridae]	Turkey
52774	ARSEF 5726	incarnatum-equiseti	FIESC 25-d		<i>Aphis gossypii</i> [Hemiptera: Aphididae]	Turkey
52775	ARSEF 5727	incarnatum-equiseti	FIESC 25-e		<i>Aphis gossypii</i> [Hemiptera: Aphididae]	Turkey

52776	ARSEF 5728	incarnatum-equiseti	FIESC 25-f	<i>Aphis gossypii</i> [Hemiptera: Aphididae]	Turkey
52777	ARSEF 5755	incarnatum-equiseti	FIESC 24-d	<i>Eurygaster</i> sp. [Hemiptera: Scutelleridae]	Syria
52778	ARSEF 5757	solani	FSSC 5-cc	<i>Eurygaster</i> sp. [Hemiptera: Scutelleridae]	Syria
52779	ARSEF 5803	concolor	<i>Fusarium</i> sp.	<i>Adelges tsugae</i> [Hemiptera: Adelgidae]	USA-MA
52780	ARSEF 5825	concolor	<i>Fusarium</i> sp.	<i>Adelges tsugae</i> [Hemiptera: Adelgidae]	USA-MA
52781	ARSEF 5875	solani	FSSC 38-a	<i>Hypothenemus hampei</i> adult [Coleoptera: Scolytidae]	Benin
52782	ARSEF 5878	solani	FSSC 38-b	<i>Hypothenemus hampei</i> adult [Coleoptera: Scolytidae]	Benin
52783	ARSEF 5879	solani	FSSC 38-c	<i>Hypothenemus hampei</i> adult [Coleoptera: Scolytidae]	Uganda
52784	ARSEF 5881	incarnatum-equiseti	FIESC 15-f	<i>Plutella xylostella</i> larva [Lepidoptera: Plutellidae]	Benin
52785	ARSEF 6102	oxysporum	nd	<i>Orachrysops subravus</i> [Lepidoptera: Lycaenidae]	South Africa
52786	ARSEF 6225	lateritium	<i>F. cf. lateritium</i>	<i>Lopholeucaspis japonica</i> [Hemiptera: Diaspididae]	Rep. of Georgia
52787	ARSEF 6394	oxysporum	nd	<i>Eurygaster</i> sp. [Hemiptera: Scutelleridae]	Turkey
52788	ARSEF 6395	oxysporum	nd	<i>Eurygaster</i> sp. [Hemiptera: Scutelleridae]	Turkey
52789	ARSEF 4863	tricinctum	FTSC 2-a (<i>F. acuminatum</i>)	Eggplant soil	Taiwan
52790	ARSEF 6397	solani	FSSC 11-f (f. sp. <i>pisi</i>)	<i>Eurygaster</i> sp. [Hemiptera: Scutelleridae]	Turkey
52791	ARSEF 6398	oxysporum	nd	<i>Eurygaster</i> sp. [Hemiptera: Scutelleridae]	Turkey
52792	ARSEF 6400	sambucinum	<i>F. culmorum</i>	<i>Eurygaster</i> sp. [Hemiptera: Scutelleridae]	Turkey
52793	ARSEF 6928	fujikuroi	<i>Fusarium</i> sp.	<i>Eurygaster</i> sp. [Hemiptera: Scutelleridae]	Turkey
52794	ARSEF 7045	fujikuroi	<i>F. sacchari</i>	<i>Chilo sacchariphagus</i> [Lepidoptera: Pyralidae]	Mozambique
52795	ARSEF 7046	fujikuroi	<i>F. sacchari</i>	<i>Chilo sacchariphagus</i> [Lepidoptera: Pyralidae]	Mozambique
52796	ARSEF 7233	incarnatum-equiseti	FIESC 17-e	<i>Aphis gossypii</i> [Hemiptera: Aphididae]	India

52797	ARSEF 7381	chlamydosporum	FCSC 1-o	<i>Scirtothrips dorsalis</i> larva [Thysanoptera: Thripidae]	India
52798	ARSEF 7382	solani	FSSC 5-d	<i>Tetanops myopaeformis</i> pupa [Diptera: Ulidiidae]	USA-ND
52799	ARSEF 8303	sambucinum	<i>F. graminearum</i>	Unknown	Turkey
52800	ARSEF 7409	coccophilum	<i>F. sp. 1 (coccophilum clade)</i>	[Hemiptera: Coccoidea] on <i>Citrus</i> sp.	USA-PR
52927	ARSEF 3042	concolor	<i>F. concolor</i>	[Nemata: Secernentea]	USA-HI
52928	ARSEF 8643	sambucinum	<i>F. sporotrichioides</i>	Unknown	Turkey
52929	ARSEF 8644	sambucinum	<i>F. graminearum</i>	Unknown	Turkey
52930	ARSEF 8645	sambucinum	<i>F. sporotrichioides</i>	Unknown	Turkey
52931	ARSEF 8646	sambucinum	<i>F. sporotrichioides</i>	Unknown	Turkey
52932	ARSEF 8647	sambucinum	<i>F. sporotrichioides</i>	Unknown	Turkey
52933	ARSEF 8648	tricinctum	FTSC 6-b	Unknown	Turkey
52934	ARSEF 8649	sambucinum	<i>F. sporotrichioides</i>	Unknown	Turkey
52936	ARSEF 8651	sambucinum	<i>F. sporotrichioides</i>	Unknown	Turkey
52937	ARSEF 8652	oxysporum	nd	Unknown	Turkey
A7006	none	coccophilum	<i>F. sp. 1 (coccidicola clade)</i>	Scale insect	USA?

^a NRRL, ARS Culture Collection, Peoria, IL.

^b ARSEF, ARS Collection of Entomopathogenic Fungal Cultures, Ithaca, NY; CBS-KNAW, Centraalbureau voor Schimmelcultures—Fungal Biodiversity Center, Utrecht, Netherlands; FRC, Fusarium Research Center, The Pennsylvania State University, State College, PA; GJS, Gary J. Samuels, ARS-USDA, Beltsville, MD; ICMP, International Collection of Micro-organisms from Plants, Auckland, New Zealand; IMI, CABI Biosciences, Egham, Surrey, England; MUCL, Mycotheque de l'Universite catholique de Louvain, Louvain-la-Neuve, Belgium.

^c Identification based on phylogenetic analysis of 2-locus (i.e., *EF-1 α* and *RPB2*) and 3-locus (i.e., *EF-1 α* , *RPB1* and *RPB2*) datasets. Species/haplotype designations for members of the chlamydosporum, incarnatum-equiseti, solani and tricinctum species complexes follow published conventions (O'Donnell et al. 2008, 2009b, 2010). nd, haplotype not determined. Arabic numerals are used to identify phylogenetically distinct species within the *Fusarium coccophilum* (1-4), *F. coccidicola* (1-4) and *F. larvarum* (1-3) clades.

