Phylogenetic diversity, trichothecene potential, and pathogenicity within *Fusarium sambucinum* species complex

Imane Laraba1,*, Susan P. McCormick1, Martha M. Vaughan1, David M. Geiser2, Kerry O’Donnell1

1 USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research Unit. 1815 N. University, Peoria, IL, United States of America, 2 Department of Plant Pathology and Environmental Microbiology, Pennsylvania State University, University Park, Pennsylvania, PA, United States of America

*Imane.Laraba@usda.gov

Abstract

The *Fusarium sambucinum* species complex (FSAMSC) is one of the most taxonomically challenging groups of fusaria, comprising prominent mycotoxigenic plant pathogens and other species with various lifestyles. Among toxins produced by members of the FSAMSC, trichothecenes pose the most significant threat to public health. Herein a global collection of 171 strains, originating from diverse hosts or substrates, were selected to represent FSAMSC diversity. This strain collection was used to assess their species diversity, evaluate their potential to produce trichothecenes, and cause disease on wheat. Maximum likelihood and Bayesian analyses of a combined 3-gene dataset used to infer evolutionary relationships revealed that the 171 strains originally received as 48 species represent 74 genealogically exclusive phylogenetically distinct species distributed among six strongly supported clades: *Brachygibbosum*, *Graminearum*, *Longipes*, *Novel*, *Sambucinum*, and *Sporotrichioides*. Most of the strains produced trichothecenes in vitro but varied in type, indicating that the six clades correspond to type A, type B, or both types of trichothecene-producing lineages. Furthermore, five strains representing two putative novel species within the *Sambucinum* Clade produced two newly discovered type A trichothecenes, 15-keto NX-3 and 15-keto NX-3. Strains of the two putatively novel species together with members of the *Graminearum* Clade were aggressive toward wheat when tested for pathogenicity on heads of the susceptible cultivar Apogee. In planta, the *Graminearum* Clade strains produced nivalenol or deoxynivalenol and the aggressive *Sambucinum* Clade strains synthesized NX-3 and 15-keto NX-3. Other strains within the *Brachygibbosum*, *Longipes*, *Novel*, *Sambucinum*, and *Sporotrichioides* Clades were nonpathogenic or could infect the inoculated floret without spreading within the head. Moreover, most of these strains did not produce any toxin in the inoculated spikelets. These data highlight aggressiveness toward wheat appears to be influenced by the type of toxin produced and that it is not limited to members of the *Graminearum* Clade.
Introduction

*Fusarium* contains numerous plant pathogenic and toxigenic species that pose a growing threat to a wide range of economically important crops [1]. These species are responsible for a plethora of serious diseases, which result in significant losses in crop yields and quality worldwide. Economic losses are commonly compounded by contamination of food and feed with fusarial toxins that render them unsuitable for consumption by humans and other animals [2,3]. Genealogical Concordance Phylogenetic Species Recognition (GCPSR; [4]) based analyses have resolved *Fusarium* into at least 300 phylogenetically distinct species distributed among 23 monophyletic species complexes and several single-species lineages [1 and references therein,3]. With 41 named species, the *F. sambucinum* species complex (FSAMSC) is among the most species-rich lineages within *Fusarium* [3,5–8]. This species complex comprises some of the most destructive agricultural pathogens, including members of the *F. graminearum* species complex and closely related species (hereafter *Graminearum* Clade; FSAMSC-1 sensu [9]), known to cause Fusarium head blight (FHB) outbreaks on small-grain cereals worldwide [9–12]. FHB epidemics have resulted in multibillion-dollar losses to the North American and global wheat industry due to significant reductions in grain yields and heavy contamination with mycotoxins [13].

Many species in the FSAMSC are known to produce a cocktail of toxic secondary metabolites in plant tissues including trichothecenes, which are among the mycotoxins of greatest concern to food safety and human health [14 and references therein]. Trichothecenes are potent inhibitors of eukaryotic protein synthesis and can cause neurologic, gastrointestinal, immunological, and other health problems [15,16]. Production of certain trichothecenes *in planta* appears to contribute to *Fusarium* pathogenicity on multiple crops where they act as virulence factors that overcome plant defenses and facilitate colonization of host tissues [17–19]. The enzymes required for trichothecene biosynthesis are encoded by TRI genes located at three different loci: a 12 gene core TRI cluster, TRI1—TRI16 2-gene cluster, and TRI101 [20]. Functional analyses of TRI biosynthetic genes indicated that gain, loss, and changes in function of some genes have contributed to trichothecene structural diversity [21–24]. *Fusarium* trichothecenes are classified into two major structural types based on the absence (type A) or the presence (type B) of a carbonyl group (keto) at the C-8 position on the trichothecene ring [20]. Species within the *Graminearum* Clade, for example, have been shown to produce type B trichothecenes with one of three chemical profiles (chemotypes): 3-acetyldeoxynivalenol (3ADON), 15-acetyldeoxynivalenol (15ADON), or nivalenol (NIV) [25,26]. However, a new type A trichothecene analog similar to 3ADON but lacking the C-8 keto group named NX-2 was recently discovered in *F. graminearum* populations from north central U.S. and Canada [9,27,28]. Structural diversity at C-7 and C-8 results from a unique variant of the trichothecene biosynthetic TRI1 gene product, a cytochrome P450 monooxygenase, that catalyzes C-7 but not C-8 hydroxylation [23,28]. Some other species within the FSAMSC are known to produce type A trichothecenes such as diacetoxyscirpenol (DAS), T-2 toxin (T-2), neosolaniol (NEO), and other structurally diverse type A toxins [20,29].

Application of phylogenetic species recognition based on multilocus GCPSR over the past two decades has revolutionized *Fusarium* systematics and changed our understanding of species limits, evolutionary relationships, and toxigenic potential of this agronomically important genus [3,29]. Because *F. graminearum* and some other species within the *Graminearum* Clade associated with cereals have been the main focus of the global scientific community over the past three decades, their ability to induce FHB and Fusarium crown rot (FCR) on cereals and produce toxins are well characterized [11,30 and references therein–32]. However, no comprehensive molecular phylogenetic study of the FSAMSC has been conducted. In addition, little is
known about the potential of many species in this species complex to synthesize trichothecenes and cause FHB and FCR. Given the threat that these species pose to agricultural biosecurity, the present study was initiated to (i) collect and analyze multilocus DNA sequence data to reassess the phylogenetic diversity of 171 strains within the FSAMSC obtained from the Fusarium Research Center (FRC) at Pennsylvania State University and/or the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, U.S.; (ii) test strains for their potential to produce trichothecenes in liquid media and on solid substrates; and (iii) evaluate their ability to induce FHB and synthesize trichothecenes in wheat.

Materials and methods

Strains studied

A global collection of FSAMSC strains (n = 171) selected to represent the phylogenetic diversity of this species complex was included in the present study (Table 1, S1 Table). Strains were selected based on analyses of partial translation elongation factor sequence data. Only one strain of the 23 species in the *Graminearum* Clade was included in the present study because they have been the focus of several recent studies by our research group [6, reviewed in 38]. The strains were recovered from various hosts/substrates and are available from FRC (http://plantpath.psu.edu/facilities/fusarium-research-center) and/or the ARS Culture Collection (https://nrrl.ncaur.usda.gov/) upon request.

Molecular phylogenetic analysis

To obtain mycelium for DNA extraction, strains were grown in 50 mL disposable polypropylene tubes containing 25 mL yeast-malt broth (3 g yeast extract, 3 g malt extract, 5 g peptone, and 20 g dextrose per L; Difco, Detroit, Michigan) on a rotary shaker set at 200 rpm and 25 °C. After 3–4 days mycelia were harvested on #1 Whatman filter paper discs over a Büchner funnel, lyophilized, and ground to a fine powder. Genomic DNA was then extracted following a cetyl trimethyl-ammonium bromide method [62]. For polymerase chain reaction (PCR) amplification, total genomic DNA was diluted 1:50 in sterile deionized water in 96-well plates and stored at -20 °C when not in use. To assess phylogenetic diversity of the 171 strains, portions of DNA-directed RNA polymerase II largest subunit (*RPB1*), RNA polymerase second-largest subunit (*RPB2*), and translation elongation factor (*TEF1*) were amplified and then sequenced as previously described [63]. PCR amplifications were performed using Platinum Taq DNA polymerase (Invitrogen Life Technologies, Carlsbad, California) in an Applied Biosystems ProFlex thermocycler (ABI, Emeryville, California). Amplicons were purified using ExoSAP-IT (ABI) and Sanger sequenced using ABI BigDye v3.1 Terminator reaction mix in a ProFlex thermocycler. Sequencing reactions were then purified using ABI BigDye XTerminator and sequences were obtained using an ABI 3730 48-capillary DNA analyzer. Sequence chromatograms were edited using Sequencher 5.2.4 (Gene Codes Corp., Ann Arbor, Michigan). Sequences were then aligned with MUSCLE [64] in SeaView 4.7 [65] and individual trees were inferred from the *RPB1*, *RPB2*, and *TEF1* data sets using maximum likelihood (ML) as implemented in IQ-TREE 1.6.12 [66]. Sequences of *F. nelsonii* NRRL 13338 from the *F. chlamydosporum* species complex (FCSC) were selected as the outgroup based on more inclusive analyses [67]. Using the Bayesian information criterion scores, ModelFinder [68] identified TIMe+I+G4, TNe+G4, and TIM2e+G4, respectively, as the best-fit models of molecular evolution for the *RPB1*, *RPB2*, and *TEF1* partitions [69]. Clade support assessed by 5000 bootstrap (BS) pseudoreplicates of the data did not reveal any conflict between strongly supported nodes. Therefore, the *RPB1*, *RPB2*, and *TEF1* partitions were analyzed as a combined data set with IQ-TREE. Clade support was also assessed by Bayesian posterior probabilities (BPP).
| Clade* | Species IDb | Strain no. | Geographic origin | Host/substrate | Toxin produced in vitrod | Toxin reportede | Reference |
|--------|------------|------------|-------------------|----------------|-------------------------|----------------|-----------|
| F. brachygibbosum | FRC R-7584 | Ban Tong, Thailand Virgin jungle soil | NEO | FUS-X, 4,15-diANIV | [33,34] |
| F. brachygibbosum | FRC R-7630 | Ban Tong, Thailand Virgin jungle soil | NEO | DAS, NEO |
| F. brachygibbosum | FRC R-7637 | Ban Tong, Thailand Virgin jungle soil | NEO | DAS, NEO |
| F. brachygibbosum | FRC R-8851 | Kolo, Niger Pearl millet | NEO | DAS, NEO |
| F. brachygibbosum | NRRL 20954 | India Sorghum | NEO | – |
| F. brachygibbosum | FRC R-4948 | Iran Citrus root | NEO | BEA, DAS, 8-ANEO | [29] |
| F. sp. nov.-23 | FRC R-7475 | Ban Pha Baen, Thailand Banana Grove soil | NEO | DAS, NEO |
| F. sp. nov.-23 | FRC R-7513 | TX, USA Sorghum soil debris | NEO | – |
| F. sp. nov.-23 | FRC R-7610 | MS, USA Sorghum soil debris | NEO | DAS, NEO |
| F. sp. nov.-23 | FRC R-7688 | AZ, USA Guayule crown | NEO | DAS, NEO |
| F. sp. nov.-23 | FRC R-8117 | Tunis, Tunisia Soil | NEO | DAS, NEO |
| F. sp. nov.-23 | FRC R-8321 | TX, USA Peanut soil debris | NEO | DAS, NEO |
| F. sp. nov.-23 | FRC R-8846 | CA, USA Human nasal sore | NEO | – |
| F. sp. nov.-23 | FRC R-8876 | Beijing, China Rice kernel | NEO | DAS, NEO |
| F. sp. nov.-23 | NRRL 13829 | Japan River sediment | NEO | DAS, NEO |
| F. sp. nov.-23 | NRRL 28448 | Turkey Banana | NEO | DAS, NEO |
| F. sp. nov.-24 | FRC R-9118 | China Soil | NEO | DAS, NEO |
| F. sp. nov.-24 | NRRL 66936 | China Soil | NEO | CAL, DAS, NEO |
| F. sp. nov.-25 | FRC R-8124 | Mahdes, Tunisia Olive Grove soil | NEO | DAS, NEO |
| F. sp. nov.-25 | NRRL 66928 | Mahdes, Tunisia Olive Grove soil | NEO | DAS, NEO |
| F. sp. nov.-25 | NRRL 66931 | Italy Unknown | NEO | DAS, NEO |
| F. sp. nov.-26 | FRC R-8856 | CA, USA Melon root | NEO | – |
| F. sp. nov.-26 | NRRL 66923 | ND, USA Pasture soil debris | NEO | DAS, NEO |
| F. sp. nov.-27 | FRC R-7642 | Puerto Rico Sorghum debris | NEO | DAS, NEO |
| F. sp. nov.-27 | FRC R-8881 | Nigeria Unknown | NEO | DAS, NEO |
| F. sp. nov.-27 | FRC R-9025 | Mashonaland Central, Zimbabwe Millet soil debris | NEO | DAS, NEO |
| F. sp. nov.-27 | KOD 1825 | GA, USA Peanuts | NEO | DAS, NEO |
| F. sp. nov.-27 | NRRL 66930 | IL, USA Corn soil | NEO | DAS, NEO |
| F. sp. nov.-28 | FRC R-9121 | China Soil | NEO | DAS, NEO |
| F. sp. nov.-28 | NRRL 66939 | Anda, China Soybean root | NEO | DAS, NEO |
| F. sp. nov.-29 | FRC R-8779 | GA, USA Sorghum soil debris | NEO | 4,15-diANIV | [34] |
| F. sp. nov.-29 | FRC R-9012 | Zaria, Kaduna, Nigeria Soil | NEO | DAS, NEO |
| F. sp. nov.-29 | KOD 1715 | Ghana Soybean roots | NEO | DAS, NEO |
| F. sp. nov.-29 | KOD 1726 | Ghana Soybean roots | NEO | DAS, NEO |
| F. sp. nov.-29 | NRRL 28725 | Argentina Wheat | NEO | DAS, NEO |
| F. sp. nov.-29 | NRRL 66920 | Ghana Soybean roots | NEO | DAS, NEO |
| F. sp. nov.-29 | NRRL 66922 | Brazil Wheat sub-crown internode | NEO | DAS, NEO |

(Continued)
Table 1. (Continued)

| Clade* | Species IDb | Strain no.c | Geographic origin | Host/substrate | Toxicin produced in vitrod | Toxin reportede | Reference |
|--------|-------------|-------------|-------------------|----------------|---------------------------|----------------|-----------|
| Brachygibbosum | F. sp. nov.-30 | FRC R-8886 | Nigeria | Unknown | – | NR | |
| Brachygibbosum | F. sp. nov.-30 | NRRL 66937 | Pandamatenga, Botswana | Pearl millet | NIV | | |
| Brachygibbosum | F. sp. nov.-31 | NRRL 66919 | Ghana | Soybean roots | DAS, NIV | 4,15-diANIV | [34] |
| Brachygibbosum | F. sp. nov.-32 | FRC R-6436 | Tarlac, Philippines | Grassland soil | – | NR | |
| Brachygibbosum | F. sp. nov.-32 | NRRL 66938 | Nakhon Sawan, Thailand | Corn | DAS | | |
| Brachygibbosum | F. sp. nov.-33 | FRC R-9125 | Australia | Soil | – | NR | |
| Brachygibbosum | F. sp. nov.-33 | NRRL 66933 | NT, Australia | Cultivated soil | DAS, NIV | | |
| Brachygibbosum | F. transvaalense | FRC R-4478 | Australia | Soil | BUT | 4,15-diANIV | [34] |
| Brachygibbosum | F. transvaalense | FRC R-6827 | Zululand, South Africa | Natural vegetation debris | DAS, NIV | | |
| Brachygibbosum | F. transvaalense | FRC R-6855 | Zululand, South Africa | Natural vegetation debris | – | | |
| Brachygibbosum | F. transvaalense | FRC R-7052 | PA, USA | Oat horse feed | – | | |
| Brachygibbosum | F. transvaalense | NRRL 31008 | Australia | Soil | – | | |
| Brachygibbosum | F. transvaalense | NRRL 32021 | Australia | Unknown | – | | |
| Graminearum | F. acaciae-mearnsii | NRRL 26755 | South Africa | Australian acacia | CUL, NIV | DON, NIV, 3ADON, 4-ANIV | [10,35] |
| Graminearum | F. aethiopicum | NRRL 46726 | Amhara region, Ethiopia | Wheat seed | CUL, 15ADON | DON, 15ADON | [36] |
| Graminearum | F. asiaticum | NRRL 13818 | Japan | Barley | – | BUT, DON, NIV, ZEA, 3ADON, 4-ANIV, 4,15-diANIV, 15ADON | [10,29,37] |
| Graminearum | F. austroamericanum | NRRL 28585 | Brazil | Herbaceous vine | NIV | DON, NIV, ZEA, 3ADON, 4-ANIV | [10] |
| Graminearum | F. boothii | NRRL 26916 | South Africa | Corn | CUL, 3ADON | BUT, CUL, DON, ZEA, 15ADON | [10,29,37] |
| Graminearum | F. brasilicum | NRRL 32138 | Pelotas, RS, Brazil | Barley | CAL, CUL, NIV | NIV, 3ADON | [38] |
| Graminearum | F. cerealis | NRRL 13721 | Posnan, Poland | Potato | NIV | NIV, ZEA, 4-ANIV, 4,15-diANIV | [10,29] |
| Graminearum | F. cortaderiae | NRRL 29297 | New Zealand | Toetoe (Cortaderia sp.) | CUL, NIV | NIV, 15ADON | [26,39] |
| Graminearum | F. culmorum | NRRL 25475 | Denmark | Barley kernel | – | BIK, BUT, CUL, NIV, OHCUl, ZEA, 3ADON, 4,15-diANIV, 15ADON | [10,29,32,40] |
| Graminearum | F. dactylidis | NRRL 29380 | USA | Unknown | – | NIV, ZEA | [41] |
| Graminearum | F. gerlachii | NRRL 36905 | MN, USA | Wheat | CUL, NIV | NIV | | |
| Graminearum | F. graminearum | NRRL 31084 | MI, USA | Corn | CUL, 15ADON | BEA, BUT, CUL, DON, NIV, OHCUl, ZEA, 3ADON, 4-ANIV, 15ADON | [10,29,35,42] |
| Graminearum | F. louisianense | NRRL 54197 | LA, USA | Unknown | – | NIV | [43] |
| Graminearum | F. lunulosporum | NRRL 13393 | South Africa | Grapefruit | ZEA | NR | | |
| Graminearum | F. meridionale | NRRL 28436 | New Caledonia | Sweet potato | NIV | NIV, ZEA, 4-ANIV | [10] |
| Graminearum | F. mesoamericanum | NRRL 25797 | Honduras | Banana | – | DON, ZEA | [10] |

(Continued)
| Clade          | Species ID          | Strain no. | Geographic origin       | Host/substrate             | Toxin produced in vitro | Toxin reported | Reference |
|---------------|---------------------|------------|-------------------------|----------------------------|-------------------------|----------------|----------|
| Graminearum   | *F. nepalense*      | NRRL 54222 | Nepal                   | Unknown                    | CUL, 15ADON             | DON, 15ADON    | [43]     |
|               |                     |            |                         |                            |                         |                |          |
| Graminearum   | *F. praegraminearum* | NRRL 39664 | Wellington, New Zealand | Litter in corn field       | CUL, NIV                | CUL, OH, CUL, ZEA, 4-ANIV, 4,15-diANIV | [5]      |
|               |                     |            |                         |                            |                         |                |          |
| Graminearum   | *F. pseudogramearum*| NRRL 28062 | New South Wales, Australia | Barley                    | CUL, 3ADON              | DON, NIV, ZEA, 3ADON | [10,26,39]|
|               |                     |            |                         |                            |                         |                |          |
| Graminearum   | *F. sp. nov.-5*     | NRRL 34461 | Umyaka, South Africa    | Soil                       | CUL, 3ADON              | DON, 3ADON     | [35]     |
|               |                     |            |                         |                            |                         |                |          |
| Graminearum   | *F. subtropicale*   | NRRL 66764 | Paraná, Brazil          | Barley                     | CUL, NIV                | BUT, CUL, FUS C, 4,15-diANIV | [6]      |
|               |                     |            |                         |                            |                         |                |          |
| Graminearum   | *F. ussuriense*     | NRRL 45681 | Primorsky Krai, Russia  | Oat                        | CUL, 3ADON              | DON, 3ADON     | [44]     |
|               |                     |            |                         |                            |                         |                |          |
| Graminearum   | *F. vorosii*        | NRRL 37605 | Hungary                 | Unknown                    | CUL, 15ADON             | DON, NIV, ZEA, 3ADON, 4-ANIV, 15ADON | [35,37] |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. longipes-1*     | FRC R-3460 | Australia               | Soil                       |                         | –              | BEA      | [45]     |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. longipes-1*     | NRRL 13368 | Australia               | Soil                       |                         | –              | DAS      |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. longipes-2*     | FRC R-5122 | New Guinea              | Soil                       |                         | –              | NR       |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. longipes-2*     | FRC R-5127 | New Guinea              | Soil debris                | –                      |                |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. longipes-2*     | NRRL 13374 | New Guinea              | Soil debris                | –                      |                |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. longipes-3*     | NRRL 20723 | United Kingdom          | Soil                       |                         | –              | NR       |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. longipes-4*     | FRC R-7358 | Guanxi, China           | Soil                       | Weedy streamside soil   | –              | NR       |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. longipes-4*     | FRC R-7602 | Thailand                | Soil                       | Pasture soil debris     | –              |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. longipes-4*     | FRC R-7695 | Ban Thain, Thailand     | Soil                       | –                      |                |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. longipes-4*     | FRC R-8789 | Philippines             | Soil                       | –                      |                |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. longipes-4*     | NRRL 13317 | CA, USA                 | Unknown                    | –                      |                |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. longipes-4*     | NRRL 20695 | FL, USA                 | Soil                       | DAS                    |                |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. longipes-4*     | NRRL 39691 | Unknown                 | Unknown                    | –                      |                |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. sp. nov.-18*    | FRC R-6894 | Rockhampton, Australia  | Air plate                  | –                      |                | NR       |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. sp. nov.-18*    | NRRL 6924  | Rockhampton, Australia  | Soil                       | –                      |                |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. sp. nov.-19*    | NRRL 66932 | NT, Australia           | Grassland soil             | –                      |                | NR       |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. sp. nov.-20*    | NRRL 66926 | Urbam, Thailand         | Rice paddy soil            | DAS                    | NR          |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. sp. nov.-21*    | NRRL 66935 | Darwin, Australia       | Soil debris                | DAS                    | NR          |          |
|               |                     |            |                         |                            |                         |                |          |
| Longipes      | *F. sp. nov.-22*    | NRRL 66934 | Niger State, Nigeria    | Sorghum soil debris        | –                      |               | NR       |          |
| Novel         | *F. sp. nov.-6*     | NRRL 28066 | Japan                   | Corn                       | T-2                    | BEA, T-2     | [29]     |
|               |                     |            |                         |                            |                         |                |          |
| Novel         | *F. sp. nov.-6*     | NRRL 54640 | Papua New Guinea        | Sweet potato stem          | NEO                    |              |          |
| Novel         | *F. sp. nov.-6*     | NRRL 54683 | Papua New Guinea        | Sweet potato stem          | DAS, NEO, T-2          |              |          |

(Continued)
| Clade        | Species ID          | Strain no. | Geographic origin          | Host/substrate | Toxin produced in vitro | Toxin reported | Reference |
|-------------|---------------------|------------|---------------------------|----------------|-------------------------|----------------|-----------|
| Novel       | F. sp. nov.-7       | NRRL 46743 | Ethiopia                   | Wheat seed     | NEO, T-2                | T-2            | [34]      |
| Novel       | F. sp. nov.-7       | NRRL 66736 | Ethiopia                   | Soybean roots  | NEO, T-2                |                |           |
| Sambucinum  | F. kyushuense       | NRRL 3509  | Kyushu, Japan              | Wheat seed     | DAS, NIV                | BEA, BUT, diNIV, ENB, ENB₁, FUS-X, NIV, 4,15-diANIV | [29,46–48] |
| Sambucinum  | F. kyushuense       | NRRL 6491  | Kochi, Shikoku, Japan      | Vinyl plate    | DAS, NIV                |                |           |
| Sambucinum  | F. mcaarum          | NRRL 28507 | Panama                     | Banana         | T-2                     | DAS, HT-2, NEO, T-2 | [49]      |
| Sambucinum  | F. poae             | NRRL 25799 | Germany                    | Sweet vernal grass | DAS (Anthoxanthum odoratum) | BEA, BUT, CUL, DAS, ENA₁, ENB, ENB₁, FUS-X, NEO, NIV, SCR, 15-MAS | [48,50] |
| Sambucinum  | F. poae             | NRRL 31280 | Brazil                     | Oat (var. Linhagem 469) | –                        |                |           |
| Sambucinum  | F. poae             | NRRL 36285 | As, Norway                 | Barley         | DAS                     |                |           |
| Sambucinum  | F. robustum         | NRRL 13392 | Argentina                  | Paraná pine (Aruncaria angustifolia) | NEO, T-2 | NR | |
| Sambucinum  | F. sambucinum       | FRC R-4712 | Switzerland                | Potato tuber   | DAS                     | BEA, DAS, ENB, NEO, T-2 | [29,51,52] |
| Sambucinum  | F. sambucinum       | FRC R-738  | NY, USA                    | Potato         | DAS                     |                |           |
| Sambucinum  | F. sambucinum       | NRRL 20666 | United Kingdom             | Potato         | DAS                     |                |           |
| Sambucinum  | F. sambucinum       | NRRL 13394 | New Zealand                | Lupine (Lupinus sp.) | DAS, NEO, T-2 |                |           |
| Sambucinum  | F. sambucinum       | NRRL 20663 | Germany                    | Unknown        | DAS                     |                |           |
| Sambucinum  | F. sambucinum       | NRRL 31964 | New Zealand                | Scotch broom (Cytisus scoparius) | DAS, NEO |                |           |
| Sambucinum  | F. sambucinum       | NRRL 31969 | New Zealand                | Goose (Ulex europaeus) | T-2 |                |           |
| Sambucinum  | F. sp. nov.-17      | NRRL 26795 | USA                        | Soil           | BUT, CAL, DAS           | NR             |           |
| Sambucinum  | F. sp. nov.-10      | FRC R-7403 | South Africa               | Potato         | T-2                     | NEO, T-2       | [53]      |
| Sambucinum  | F. sp. nov.-10      | NRRL 22189 | Brazil                     | Soybean        | NEO, T-2                |                |           |
| Sambucinum  | F. sp. nov.-11      | NRRL 22192 | Indonesia                  | Palm tree      | T-2                     |                | [54]      |
| Sambucinum  | F. sp. nov.-12      | FRC R-642  | Unknown                    | Carnation 'Iroquois' | DAS | NR | |
| Sambucinum  | F. sp. nov.-12      | FRC R-7107 | Transkei, South Africa     | Soil           | DAS                     |                |           |
| Sambucinum  | F. sp. nov.-12      | FRC R-7122 | Transkei, South Africa     | Soil           | DAS, 3OH                |                |           |
| Sambucinum  | F. sp. nov.-12      | FRC R-8227 | Zinggavyi, South Africa    | Debris         | DAS                     |                |           |
| Sambucinum  | F. sp. nov.-12      | NRRL 66929 | Zinggavyi, South Africa    | Debris         | DAS                     |                |           |
| Sambucinum  | F. sp. nov.-13      | NRRL 39635 | Taupo, New Zealand         | Toetoe (Cortaderia sp.) | DAS | NR | |
| Sambucinum  | F. sp. nov.-13      | NRRL 39685 | Gisborne, New Zealand      | Toetoe (Cortaderia sp.) | BUT, CUL, DAS |                |           |
| Sambucinum  | F. sp. nov.-14      | NRRL 36134 | Unknown                    | Unknown        | DAS, NEO, T-2           | NR             |           |
Table 1. (Continued)

| Clade\(^a\) | Species ID\(^b\) | Strain no.\(^c\) | Geographic origin | Host/substrate | Toxin produced in vitro\(^d\) | Toxin reported\(^e\) | Reference |
|------------|-----------------|-----------------|------------------|---------------|-----------------------------|-------------------|-----------|
| **Sambucinum** | F. sp. nov.-15 | FRC R-8154 | Transkei, South Africa | Soil | CUL, 3,7-dioH, 15-decal, 15-keto NX-2/NX-3, 15-OHCUL | NR | |
| **Sambucinum** | F. sp. nov.-15 | FRC R-8203 | Transkei, South Africa | Debris | | | |
| **Sambucinum** | F. sp. nov.-15 | NRRL 66921 | South Africa | Wheat straw | | | |
| **Sambucinum** | F. sp. nov.-16 | FRC R-8136 | Transkei, South Africa | Debris | CUL, 3,7-dioH, 15-decal, 15-keto NX-2/NX-3, 15-OHCUL | NR | |
| **Sambucinum** | F. sp. nov.-16 | NRRL 66927 | South Africa | Natural vegetation soil | | | |
| **Sambucinum** | F. sp. nov.-8 | NRRL 29296 | Auckland, New Zealand | Chayote (Sechium edule) | DAS, NEO, T-2 | NR | |
| **Sambucinum** | F. sp. nov.-9 | NRRL 13465 | Argentina | Tiger pear (Opuntia aurantica) | T-2 | NR | |
| **Sambucinum** | F. sp. nov.-9 | NRRL 66925 | Argentina | Tiger pear (Opuntia aurantica) | DAS, T-2 | | |
| **Sambucinum** | F. venenatum | FRC R-2277 | Australia | Corn | DAS | BEA, BUT, CUL, DAS, SCR, 4-MAS, 15-ASCR | [29,55,56] |
| **Sambucinum** | F. venenatum | FRC R-8978 | Ordu, Turkey | Soil | BUT, DAS | | |
| **Sambucinum** | F. venenatum | NRRL 22196 | Germany | Corn | DAS | | |
| **Sambucinum** | F. venenatum | NRRL 22198 | Finland | Unknown | BUT, DAS, JOH | | |
| **Sambucinum** | F. venenatum | NRRL 25413 | England | Unknown | DAS | | |
| **Sambucinum** | F. venenatum | NRRL 26228 | Austria | Wheat | DAS | | |
| **Sambucinum** | F. venenatum | NRRL 31989 | Australia | Soil | BUT, CAL, DAS | | |
| **Sambucinum** | F. venenatum | NRRL 32015 | Australia | Soil | BUT, CAL, DAS | | |
| **Sporotrichioides** | F. armeniacum | FRC R-6472 | FL, USA | Slash pine seedling | NEO, T-2 | BEA, DAS, HT-2, MAS, NEO, T-2, T-2TE, T-2TR, ZEA | [57] |
| **Sporotrichioides** | F. armeniacum | FRC R-7479 | Australia | Sorghum | DAS, T-2 | | | |
| **Sporotrichioides** | F. armeniacum | FRC R-8519 | GA, USA | Corn soil | DAS, NEO, T-2 | | | |
| **Sporotrichioides** | F. armeniacum | FRC R-8609 | Abetshawe, South Africa | Soil debris | DAS, NEO, T-2 | | | |
| **Sporotrichioides** | F. armeniacum | NRRL 13343 | Unknown | Unknown | DAS, NEO, T-2 | | | |
| **Sporotrichioides** | F. gooolgardii | KOD 1087 | Bungonia, Australia | Grass tree (Xanthorrhoea glauca) | DAS, NEO, T-2 | DAS, NEO, T-2 | [58] |
| **Sporotrichioides** | F. gooolgardii | KOD 1090 | Bungonia, Australia | Grass tree (Xanthorrhoea glauca) | DAS, NEO, T-2 | | | |
| **Sporotrichioides** | F. langsethiae | NRRL 34176 | Unknown | Unknown | CUL, DAS, NEO, T-2 | BEA, CUL, DAS, ENA, ENB, HT-2, iso-NEO, NEO, OHCUL, T-2, T-2TE, T-2TR | [48,59,60] |
| **Sporotrichioides** | F. langsethiae | NRRL 36236 | Unknown | Unknown | CUL, DAS, NEO, T-2 | | | |
| **Sporotrichioides** | F. langsethiae | NRRL 54940 | Norway | Oat | NEO | | | |
| **Sporotrichioides** | F. nodosum | NRRL 13431 | Turkey | Tomato stem | – | NR | | |
| **Sporotrichioides** | F. nodosum | NRRL 36351 | Lisboa, Portugal | Stored peanuts | DAS | | | |
| Cladea | Species IDb | Strain no.c | Geographic origin | Host/substrate | Toxin produced in vitrod | Toxin reportedd | Reference |
|--------|------------|------------|-------------------|---------------|-------------------------|----------------|-----------|
| Sporotrichioides | *F. palustre* | NRRL 43289 | Unknown | Spartina rhizosphere soil | DAS, NEO, T-2 | DAS, NEO, T-2, 8-ANEO | [58] |
| Sporotrichioides | *F. palustre* | NRRL 54056 | CT, USA | Smooth cordgrass (*Spartina alterniflora*) | DAS, T-2 |
| Sporotrichioides | *F. palustre* | NRRL 54058 | CT, USA | Smooth cordgrass (*Spartina alterniflora*) | DAS, T-2 |
| Sporotrichioides | *F. sibiricum* | NRRL 53429 | Vladivostok, Russia | Oat | T-2 | DAS, T-2 | [60] |
| Sporotrichioides | *F. sibiricum* | NRRL 53430 | Chabarovsk, Russia | Oat | DAS, NEO, T-2 |
| Sporotrichioides | *F. sibiricum* | U.S.A. | Smooth cordgrass (*Spartina alterniflora*) | Oat | DAS, T-2 |
| Sporotrichioides | *F. sibiricum* | R-3766 FRC | Australia | Soil | DAS, T-2 |
| Sporotrichioides | *F. sibiricum* | R-5102 FRC | Free State, South Africa | Oat | DAS, NEO, 3OH |
| Sporotrichioides | *F. sibiricum* | R-3090 FRC | South Africa | Oat | NEO, T-2 |
| Sporotrichioides | *F. sibiricum* | R-9068 FRC | Manitoba, Canada | Corn seed | T-2 |
| Sporotrichioides | *F. sibiricum* | 29896 NRRL | Netherlands | Unknown | DAS |
| Sporotrichioides | *F. sibiricum* | 52731 NRRL | Unknown | Unknown | — |
| Sporotrichioides | *F. sibiricum* | 13440 NRRL | WI, USA | Grain elevator | DAS, NEO, T-2 |
| Sporotrichioides | *F. sibiricum* | 25474 NRRL | Germany | Norway spruce (*Picea abies*) seed | DAS, NEO, T-2 |
| Sporotrichioides | *F. sibiricum* | 26923 NRRL | Japan | Peas | DAS, NEO, T-2 |
| Sporotrichioides | *F. sibiricum* | 29131 NRRL | Germany | Oat kernel | DAS, NEO, T-2 |
| Sporotrichioides | *F. sibiricum* | 3299 NRRL | France | Corn | DAS, T-2 |
| Sporotrichioides | *F. sibiricum* | 36295 NRRL | Heverlee, Belgium | Tobacco seedling root | DAS, NEO, T-2 |
| Sporotrichioides | *F. sibiricum* | 52731 NRRL | Unknown | Unknown | DAS, NEO, T-2 |

*aBrachygibbosum, Fusarium brachygibbosum* Clade; *Graminearum, F. graminearum* Clade; *Longipes, F. longipes* Clade; *Novel, Novel Clade; Sambucinum, F. sambucinum* Clade; *Sporotrichioides, F. sporotrichioides* Clade.

*bF. sp. nov.-1 to -33, putatively novel species resolved within the FSAMSC; *F. longipes*-1 to-4, four phylogenetically distinct species within the *F. longipes* Clade previously reported in other studies.*

*cFRC, Fusarium Research Center, The Pennsylvania State University, University Park, Pennsylvania; NRRL, ARS Culture Collection, Peoria, Illinois; KOD, available from Kerry O’Donnell.*

*dToxins were determined via gas chromatography-mass spectrometry. BUT, butenolide; CAL, calonectrin; CUL, culmorin; DAS, diacetoxyscirpenol; NEO, neosolaniol; NIV, nivalenol; T-2, T-2 toxin; ZEA, zearalenone; 3ADON, 3-acetyldeoxynivalenol; 3,7-diOH, 7-hydroxy isotrichodermol; 3OH, isotrichodermol; 15ADON, 15-acetyldeoxynivalenol; 15-decal, 15-decalon ectrin; 15-keto NX-2 and 15-keto NX-3, novel trichothecenes; 15-OHCU L, 15-hydroxy culmorin; SCR, scirpentriol; T-2, T-2 tetraol; T-2TR, T-2 triol; ZEA, zearalenone; 3ADON, 3-acetyldeoxynivalenol; 4-ANIV, 4-acetylnivalenol; 4,15-diANIV, 4,15-diacetylnivalenol; 4-MAS, 4-monoacetoxyscirpenol; 8-ANEO, 8-acetylneosolaniol; 15ADON, 15-acetyldeoxynivalenol; 15-ASCR, 15-acetoxyscirpenol; 15-AT-2TE, 15-acetyl T-2 tetraol; 15-MA S, 15-monoacetoxyscirpenol; NR, no reports.*

References: [58, 60, 42, 48, 51, 61]
Bayesian inference (BI) analyses of the individual partitions and combined 3-locus data set were performed using MrBayes 3.2.7a [70,71] on the CIPRES Scientific Gateway (https://www.phylo.org). Generalized Time Reversible (GTR) with a gamma distribution was selected as the model for nucleotide substitution. Two runs of four MCMC (Markov Chain Monte Carlo) chains were run simultaneously for $10^7$ generations with trees sampled every 1000 generations. For each run, the first 25% trees were discarded as the burn-in phase and BPP were determined from the remaining trees. Lineages that were represented by two or more strains were considered to be phylogenetically distinct if they were supported as genealogically exclusive by a BPP $\geq 0.99$ and ML bootstrap (ML-BS) $\geq 95\%$ from one or more of the individual partitions and/or the combined data set, and none of the individual partitions contradicted their monophyly. The RPB1, RPB2, and TEF1 sequences were deposited in GenBank NCBI (accession numbers MW233055–MW233569).

**Trichothecene production in broth, rice, and corn substrates**

Two liquid culture media were used to test the ability of 171 strains to produce trichothecenes. Strains were initially grown on V8 agar [72] for 7 days at 25 °C. Two 0.5 cm$^2$ mycelial plugs cut from V8 cultures were inoculated into 20 mL of agmatine broth [73] and 20 mL of yeast extract peptone dextrose medium (YEPD: 1 g yeast extract, 1 g peptone, and 50 g dextrose per 1 L distilled water) in 50 mL flasks. Flasks were incubated at 28 °C in the dark on a rotary shaker at 200 rpm. After 7 days the cultures were extracted with 8 mL of ethyl acetate. The extracts were dried under a nitrogen stream in 1-dram vials and then resuspended in 1 mL ethyl acetate. Strains that failed to synthesize trichothecenes in agmatine and YEPD broth, and some isolates of special interest, were cultured on autoclaved rice (4.4 g rice grain + 1.8 mL water). After 15 days incubation in the dark at 25 °C, each rice culture was extracted with 10 mL of ethyl acetate, dried under nitrogen, and then resuspended in 1 mL ethyl acetate. Strains that did not produce detectable levels of trichothecenes in rice cultures were grown on autoclaved cornmeal (4.4 g commercial Quaker® cornmeal + 1.8 mL water) for 15 days in the dark at 25 °C. Toxins were extracted following the protocol described above. Gas chromatography-mass spectrometry (GC-MS) analyses of the ethyl acetate extracts were performed on an Agilent 6890 gas chromatograph fitted with a HP-5MS column (30 m, 0.25 mm, 0.25 μm) coupled to a 5973-mass spectrometer (Agilent, Santa Clara, California). Helium was used as the carrier gas with a 20:1 split ratio and a 20 mL/min split flow. The column was held at 150 °C for 1 min following injection, heated to 280 °C at 30 °C/min where it was held for 7.7 min. Compound identification was based on comparisons of their retention times and mass spectra with a library of mycotoxin standards and a NIST reference library.

**Pathogenicity test on wheat**

Pathogenicity of 75 strains chosen to represent the full range of FSAMSC diversity was tested on the full-dwarf hard red spring wheat cultivar Apogee (*Triticum aestivum*) [74]. This cultivar is characterized by high susceptibility to FHB [75]. Five seeds of Apogee were planted in 2.5 L plastic pots filled with Sun Gro® Horticulture potting mix (Agawam, Massachusetts) supplemented with 2 g of Micromax, and 13 g Osmocote 14-14-14 controlled-release fertilizer (ICL Specialty Fertilizers, Summerville, South Carolina). Pots were arranged randomly and maintained in a controlled growth room under the following conditions: 50–60% relative humidity, 14 h day period, and temperature set at 23 °C day and 20 °C night. Every two weeks each pot was fertilized with 500 mL of a solution containing 0.5 g per L of Peters 20-20-20 (Grace-Sierra Horticultural Products, Milpitas, California). At anthesis, five wheat heads representing biological replicates were inoculated with one of the 75 strains by injecting a spikelet with 10 μL of
0.04% sterile Tween 20 containing 10^5 conidia. The negative control was inoculated with 10 μL of 0.04% sterile Tween 20. *Fusarium graminearum* NRRL 31084 (= PH1), which is a well-characterized FHB pathogen [11], was used as the positive control. The number of symptomatic florets displaying premature whitening or necrosis, typical symptoms of FHB, was scored at 7, 10, 14, 17, and 21 days after inoculation. Isolates that only induced FHB symptoms in the inoculated spikelet were considered as pathogenic. Strains that overcame the plant’s defenses and spread to other florets were scored as aggressive. Disease spread was then assessed by calculating the total percentage of diseased florets 21 days post inoculation.

**Trichothecene production in planta**

To evaluate trichothecene production *in planta*, independent of disease spread, the five wheat heads inoculated with one of the 75 strains were harvested 21 days post inoculation, combined, and frozen at -80 °C. These samples were freeze-dried for 48 hr and then ground to a fine powder using a Geno/Grinder 2010 automated tissue homogenizer (OPS Diagnostics, Lebanon, New Jersey). One g of each sample was extracted with 10 mL of acetonitrile-water (86:14, v/v) by shaking for 15 min. After centrifugation 5 mL of the extract were cleaned using a MycoSep 225 Trich column (Romer Labs, Union, Missouri). Two mL of the purified extract were transferred to a 1-dram vial, dried under a nitrogen stream, and then derivatized with 100 μL of a 100:1 freshly prepared mixture of N-trimethylsilylimidazole/trimethylchlorosilane (Sigma-Aldrich, St. Louis, Missouri). After 20 min, 900 μL of isooctane were added to the vial, followed by 1 mL of water. The samples were vortexed until the top organic layer became clear and then it was analyzed by GC-MS as described above.

**Results**

**Molecular phylogenetic analysis**

Maximum likelihood bootstrap (ML-BS) analyses of the individual RPB1, RPB2, and TEF1 partitions were conducted to assess whether they could be analyzed as a combined 3-gene data set. Because trees inferred from the individual genes did not reveal any conflict between strongly supported nodes (S1–S3 Figs), the data sets were combined and analyzed by Bayesian inference (BI) and ML bootstrapping (ML-BS) to assess species boundaries and to gain insight into evolutionary relationships of the 171 strains. The concatenated 3-gene data set included partial RPB1 (1774 bp alignment, 492 parsimony informative characters = PICs), RPB2 (1786 bp alignment, 489 PICs), and TEF1 (640 bp alignment, 276 PICs) gene sequences. Sequences of NRRL 13338 *F. nelsonii* from the FCSC, which is sister to the FSAMSC, were selected as the outgroup for rooting the phylogeny. BI and ML analyses of the 3-locus data set yielded similar trees and resolved the 171 strains as 74 phylogenetically distinct species (Fig 1). These included 41 named and 33 putatively novel species, which are informally distinguished here as *F. sp. nov.-1 to F. sp. nov.-33* (Table 1, Fig 1). Analyses of the combined data set revealed that the 39 lineages represented by two or more strains were strongly supported as phylogenetically distinct (BPP = 1; ML-BS = 96–100%, Fig 1). These analyses also resolved the FSAMSC as six strongly supported monophyletic clades (BPP = 1; ML-BS = 100%, Table 1, Fig 1) and evolutionary relationships among them (BPP = 1; ML-BS = 89–100%, Fig 1). The analyses provided moderate to strong support for most nodes (BPP = 0.84–1; ML-BS = 80–100%, Fig 1); however, eight internodes in the interior of the ML phylogeny received bootstrap values < 70% (see *Brachygibbosum*, *Graminearum*, and *Sambucinum* Clades). Four of these internodes, however, were strongly supported by the Bayesian posterior probabilities (BPP = 0.99–1, Fig 1). In addition, seven of the nine internodes that received a BPP < 0.7 (see *Brachygibbosum*, *
resolved as 9 phylospecies (BPP = 1; ML-BS = 100%, Fig 1), 5 of which were represented by *F. sambucinum*.

The six multispecies lineages identified within the FSAMSC are (i) the *Sporotrichioides* Clade, which included 32 isolates accounting for 7 described and four novel phylogenetically distinct species closely related to *F. armeniacum* (Fig 1). Each unnamed putatively novel species was represented by two strains from different geographic origins and isolated from cereals or other hosts/substrates (Table 1). Evolutionary relationships among the four putatively novel species and *F. armeniacum* were fully resolved (BPP = 0.99–1; ML-BS = 96–100%, Fig 1); (ii) the *Graminearum* Clade contained one unnamed species (NRRL 34461 *F. sp. nov.-5* and 22 formally described species, each represented by a single strain (Fig 1). NRRL 34461 *F. sp. nov.-5*, recovered from soil in South Africa, formed a strongly supported cluster with *F. acaciae-mearnsii*, *F. vorosii*, and *F. ussuriianum* within what was previously named the *F. graminearum* species complex (BPP = 1; ML-BS = 100%, Fig 1). To facilitate communication, given the recent advances in *Fusarium* systematics, we propose abandoning the informal designation *F. graminearum* species complex and to refer to this group and closely related species as the *Graminearum* Clade, to emphasize its status as a monophyletic multispecies lineage within the FSAMSC. The *Graminearum* and *Sporotrichioides* Clades were strongly supported as sisters (BPP = 1; ML-BS = 99%, Fig 1); (iii) a *Novel* Clade comprised two phylogenetically distinct species, *F. sp. nov.-6* and -7 (BPP = 1; ML-BS = 100%, Fig 1). *Fusarium* sp. nov.-6 is represented by three strains: NRRL 54640 and 54683 from Papua New Guinean sweet potato received as *F. lateritium* and NRRL 28066 isolated from corn in Japan accessioned as *F. graminearum* (S1 Table). *Fusarium* sp. nov.-7 comprised two Ethiopian isolates, NRRL 46743 from wheat seed and NRRL 66736 from soybean plants exhibiting root rot symptoms (S1 Table). BPP and ML bootstrapping resolved the *Novel* Clade as sister to the *Sporotrichioides + Graminearum* Clades (BPP = 1; ML-BS = 89%, Fig 1); (iv) the *Sambucinum* Clade included 42 isolates comprising 6 described and 10 putatively novel phylogenetically distinct species (F. sp. nov.-8 to -17; Fig 1). The latter were represented by 2 or 3 strains except for *F. sp. nov.-8, -11, -14, and -17*, which are single species lineages (Fig 1). Monophyly of the 6 putatively novel and 4 described species represented by 2 or more strains was strongly supported (BPP = 1; ML-BS = 100%, Fig 1). BPP and ML bootstrapping resolved the *Sambucinum* Clade as a basal sister to the *Sporotrichioides, Graminearum*, and *Novel* Clades (BPP = 1; ML-BS = 100%, Fig 1). BI and ML analyses of the 3-locus data set indicated that the three strains received as *F. tumidum* [76] from gorse, lupine, and Scotch broom in New Zealand are *F. sambucinum* (S1 Table, Fig 1); (v) the *Longipes* Clade contained 19 isolates that were fully resolved (BPP = 0.99–1; ML-BS = 89%, Fig 1); (vi) the *Sporotrichioides* Clade contained 23 isolates comprising two species: *Sporotrichioides nelsonii* from its sister group, the *F. olamyliosporum* species complex. The following six clades (from top to bottom defined by thickened internodes) were strongly supported as monophyletic (BPP = 1; ML-BS = 100%): *Sporotrichioides, Graminearum*, *Novel, Sambucinum, Longipes*, and *Brachygibbosum*. Putatively novel species resolved within the FSAMSC are designated *F. sp. nov.-1* to -33. Four phylogenetically distinct species within the *F. Longipes* Clade previously reported in other studies are identified by unique Arabic numbers (1 to 4). Toxins mapped on the phylogeny were determined via gas chromatography-mass spectrometry. BUT, butenolide; CAL, calonectrin; CUL, culmorin; DAS, diacetoxyscirpenol; NEU, neosolaniol; NIV, nivalenol; T-2, T-2 toxin; ZEA, zearalenone; SADON, 3-acetolideoxynivalenol; 15ADON, 15-acetoxynivalenol; 3OH, isotrichodermol; 3,7-diOH, 7-hydroxy isotrichodermol; 15-decal, 15-decalonectrin; 15-keto NX-2 and 15-keto NX-3, novel trichothecenes; 15-OHCU, 15-hydroxy culmorin–, none detected.

https://doi.org/10.1371/journal.pone.0245037.g001

*Graminearum, Longipes, Sambucinum, and Sporotrichioides* Clades) were supported by ML-BS values $\geq 70$–95%.

The six multispecies lineages identified within the FSAMSC are (i) the *Sporotrichioides* Clade, which included 32 isolates accounting for 7 described and four novel phylogenetically distinct species closely related to *F. armeniacum* (Fig 1). Each unnamed putatively novel species was represented by two strains from different geographic origins and isolated from cereals or other hosts/substrates (Table 1). Evolutionary relationships among the four putatively novel species and *F. armeniacum* were fully resolved (BPP = 0.99–1; ML-BS = 96–100%, Fig 1); (ii) the *Graminearum* Clade contained one unnamed species (NRRL 34461 *F. sp. nov.-5*) and 22 formally described species, each represented by a single strain (Fig 1). NRRL 34461 *F. sp. nov.-5*, recovered from soil in South Africa, formed a strongly supported cluster with *F. acaciae-mearnsii*, *F. vorosii*, and *F. ussuriianum* within what was previously named the *F. graminearum* species complex (BPP = 1; ML-BS = 100%, Fig 1). To facilitate communication, given the recent advances in *Fusarium* systematics, we propose abandoning the informal designation *F. graminearum* species complex and to refer to this group and closely related species as the *Graminearum* Clade, to emphasize its status as a monophyletic multispecies lineage within the FSAMSC. The *Graminearum* and *Sporotrichioides* Clades were strongly supported as sisters (BPP = 1; ML-BS = 99%, Fig 1); (iii) a *Novel* Clade comprised two phylogenetically distinct species, *F. sp. nov.-6* and -7 (BPP = 1; ML-BS = 100%, Fig 1). *Fusarium* sp. nov.-6 is represented by three strains: NRRL 54640 and 54683 from Papua New Guinean sweet potato received as *F. lateritium* and NRRL 28066 isolated from corn in Japan accessioned as *F. graminearum* (S1 Table). *Fusarium* sp. nov.-7 comprised two Ethiopian isolates, NRRL 46743 from wheat seed and NRRL 66736 from soybean plants exhibiting root rot symptoms (S1 Table). BPP and ML bootstrapping resolved the *Novel* Clade as sister to the *Sporotrichioides + Graminearum* Clades (BPP = 1; ML-BS = 89%, Fig 1); (iv) the *Sambucinum* Clade included 42 isolates comprising 6 described and 10 putatively novel phylogenetically distinct species (F. sp. nov.-8 to -17; Fig 1). The latter were represented by 2 or 3 strains except for *F. sp. nov.-8, -11, -14, and -17*, which are single species lineages (Fig 1). Monophyly of the 6 putatively novel and 4 described species represented by 2 or more strains was strongly supported (BPP = 1; ML-BS = 100%, Fig 1). BPP and ML bootstrapping resolved the *Sambucinum* Clade as a basal sister to the *Sporotrichioides, Graminearum*, and *Novel* Clades (BPP = 1; ML-BS = 100%, Fig 1). BI and ML analyses of the 3-locus data set indicated that the three strains received as *F. tumidum* [76] from gorse, lupine, and Scotch broom in New Zealand are *F. sambucinum* (S1 Table, Fig 1); (v) the *Longipes* Clade contained 19 isolates that were resolved as 9 phylospecies (BPP = 1; ML-BS = 100%, Fig 1), 5 of which were represented by single strains. Seventeen of the 19 isolates were received as *F. longipes* and these included
representatives of all 9 phylospecies (S1 Table). Following a previously used informal ad hoc nomenclature, four of the species were distinguished as *F. longipes*-1 to -4, and the other 5 as *F. sp. nov.*-18 to -22 (Fig 1). Seven of the 9 species and 15/19 isolates were recovered from soil (Table 1). The *Longipes* Clade was resolved as a basal sister to the *Sporotrichioides, Graminearum, Novel*, and *Sambucinum* Clades (BPP = 0.91; ML-BS = 94%, Fig 1); and (vi) the *Brachygibbosum* Clade, the earliest diverging clade within the FSAMSC, accounted for 50 of the 171 strains and these included 2 described species (i.e., *F. brachygibbosum* and *F. transvaalense*) and 11 putatively novel phylogenetically distinct species distinguished as *F. sp. nov.*-23 to -33 (Fig 1). Representatives of 10/13 species and 28/50 of the *Brachygibbosum* Clade isolates were received as *F. compactum* (S1 Table). Of the 13 species in this clade, NRRL 66919 *F. sp. nov.*-31 from Ghanaian soybean exhibiting root rot was the only species represented by a single strain (Fig 1). Monophyly of the 12 other species was strongly supported (BPP = 1; ML-BS = 95–100%, Fig 1). Evolutionary relationships within this clade were mostly well-resolved by BPP and ML-BS. However, two pairs of putative sisters were only resolved by the Bayesian analysis, and these are *F. transvaalense*- *F. sp. nov.*-31 (BPP = 1; ML-BS = 71%, Fig 1) and *F. sp. nov.*-29– *F. sp. nov.*-30 (BPP = 1; ML-BS = 60%, Fig 1).

**Trichothecene production in broth, rice, and corn substrates**

Structurally diverse toxins were produced by the FSAMSC strains in the current study. Of the 171 strains tested 38 failed to synthesize any toxin in the liquid media and solid substrates we used (S2 Table). The type A trichothecenes DAS, NEO, and T-2 and type B trichothecenes NIV, 3ADON, and 15ADON were among the most prevalent toxins detected. The six clades resolved by BI and ML bootstrap analyses of the 3-locus data set corresponded to six trichothecene-producing lineages represented by strains that synthesized type A trichothecenes, type B, or both types (S2 Table, Fig 1).

**Sporotrichioides Clade.** Within the *Sporotrichioides* Clade, 30/32 strains produced type A trichothecene toxins in vitro. Except for five strains that synthesized DAS, NEO, or T-2 (NRRL 36351 *F. nodosum*, NRRL 29896 *F. sp. nov.*-4, NRRL 54940 *F. langsethiae*, NRRL 53429 *F. sibiricum*, and FRC R-9068 *F. sp. nov.*-3), the other species produced DAS and/or NEO with T-2. Only one strain, FRC R8102 *F. sp. nov.*-1, made the type A trichothecene isotrichodermol (3OH) in agmatine broth. In addition to DAS, NEO, and T-2, the tricyclic sesquiterpene diol culmorin (CUL) was detected in agmatine and YEPD cultures of NRRL 34176 and 36236 *F. langsethiae* (S2 Table, Fig 1).

**Graminearum Clade.** Of the 23 species in this clade, 17 produced type B trichothecenes, and these included 9 that synthesized NIV (*F. acaciae-mearnsii, F. austroamericanum*, *F. brasilicum*, *F. cerealis*, *F. cortaderiae*, *F. gerlachii*, *F. meridionale*, *F. praegraminearum*, and *F. subtropicalic*), 4 made 3ADON (*F. boothii, F. pseudograminearum*, *F. ussurianum*, and NRRL 34461 *F. sp. nov.*-5), and 4 synthesized 15ADON (*F. aethiopicum, F. graminearum, F. nepalense*, and *F. vorosii*). In addition to NIV, NRRL 31238 *F. brasilicum* also produced the type A trichothecene calonectrin (CAL) in agmatine medium. Of the 171 strains included in the present study, NRRL 13393 *F. lunulosporum* was the only strain that made the estrogenic mycotoxin zearalenone (ZEA). Toxins were not detected, however, in individual strains of 5 species (*F. asiaticum, F. culmorum, F. dactylidis, F. louisianense*, and *F. mesoamericanum*) when cultured in liquid media and solid substrates (S2 Table, Fig 1).

**Novel Clade.** Two undescribed species within the *Novel* Clade, *F. sp. nov.*-6 and -7, produced type A trichothecenes. Two isolates of *F. sp. nov.*-6 synthesized T-2 and NEO toxins in liquid media. In addition, NRRL 54683 *F. sp. nov.*-6, and the two isolates of *F. sp. nov.*-7, made DAS + NEO + T-2 and NEO + T-2, respectively, in rice cultures (S2 Table, Fig 1).
**Sambucinum Clade.** Fourteen of the 16 species within *Sambucinum* Clade only produced type A trichothecenes; however, NRRL 6491 and 3509 *F. kyushuense* from Japan synthesized NIV and DAS, which are type B and type A trichothecenes respectively. NRRL 22192 *F. sp. nov.-11* (= BBA 64918), represented by a single strain from an Indonesian palm tree, was the only species within this clade that failed to produce toxins (Fig 1). In addition to *F. kyushuense*, DAS was detected in 9/16 species, T-2 in 7, NEO in 5, CUL in 3, butenolide (BUT) in 3, CAL in 2, and 3OH in 2 (Table 1). Of special note, two putative novel sister species, *F. sp. nov.-15* and -16, which were received as *F. longipes* isolated from South African wheat straw or soil, produced CUL, 7-hydroxy isotrichodermol (3,7-diOH), 15-decalonectrin (15-decal), and two novel type A trichothecenes, 15-keto NX-2 and 15-keto NX-3 in agmatine broth (Fig 2A). The latter two toxins are similar to NX-2 and its deacetylated form NX-3 but differ in that they possess a keto or aldehyde group, respectively, at the C-15 position on the trichothecene ring. When cultured on rice, *F. sp. nov.-15* and -16 produced the same two novel toxins with 15-hydroxy culmorin (15-OHCUL) (Fig 2B).

**Longipes Clade.** Of the 19 isolates comprising 9 phylospecies in the *Longipes* Clade tested, DAS was only produced on rice by one isolate of four different species. The other 15 strains failed to synthesize any toxin in liquid media and on solid substrates (S2 Table, Fig 1).

**Brachygibbosum Clade.** The *Brachygibbosum* Clade was represented by 50 isolates comprising 13 phylogenetically distinct species; however, only *F. brachygibbosum* and *F. transvaalense* possess binomials. Eight of the species synthesized one or more type A trichothecenes (i.e., DAS, NEO, and CAL). Of the 5 remaining species, four produced DAS + NIV, and one only NIV.

In addition to type A and B trichothecenes, several other biologically active secondary metabolites were detected in culture extracts of some isolates in four clades (Table 1, Fig 1). The sesquiterpene CUL was detected in cultures of most strains within the *Graminearum* Clade (14/23), 2 strains in the *Sporotrichioides* Clade, and 6 strains representing 3 novel type A trichothecene-producing species in the *Sambucinum* Clade. In addition, BUT was produced in rice and cornmeal cultures by some isolates of *F. venenatum*, NRRL 39685 *F. sp. nov.-13*, and NRRL 26795 *F. sp. nov.-17* in the *Sambucinum* Clade, and FRC R-4478 *F. transvaalense* in the *Brachygibbosum* Clade (S2 Table, Fig 1).

**Pathogenicity test on wheat**

The pathogenic potential of 75 strains representing the six clades of the FSAMSC (*Brachygibbosum* = 17, *Graminearum* = 12, *Longipes* = 10, *Novel* = 4, *Sambucinum* = 20, and *Sporotrichioides* = 12) was determined by their ability to cause FHB on the highly susceptible wheat cv. Apogee and to spread within the wheat head from a single floret inoculation. Disease spread was evaluated by assessing the percentage of florets exhibiting premature whitening or necrosis at different timepoints, and when the experiment was terminated 21-days post inoculation. Of the 75 strains evaluated, only 17 were nonpathogenic, and heads inoculated with these were asymptomatic and indistinguishable from the negative controls. These nonpathogenic strains were members of the *Brachygibbosum*, *Longipes*, *Novel*, *Sambucinum*, and *Sporotrichioides* Clades. The remaining 58 strains induced FHB symptoms, but the majority (41/58) did not spread beyond the inoculated floret and were classified as nonaggressive (S3 Table). Phenotypic variation in symptoms was noted on wheat heads inoculated with the nonaggressive strains. Some strains produced necrotic lesions or black spots on the inoculated spikelet, while others caused premature bleaching, a typical symptom of FHB. However, bleaching did not spread to neighboring florets. The remaining strains, which belong to the *Graminearum* (n = 12) and *Sambucinum* (n = 5) Clades were able to spread and colonize nearby florets or the
entire wheat head. Some of these strains exhibited levels of aggressiveness like the highly aggressive positive control NRRL 31084 \textit{F. graminearum} (S3 Table, Fig 3). Strains of \textit{F. sp. nov.-15} and -16, the two novel species within the \textit{Sambucinum} Clade that produced 15-keto NX-2 and 15-keto NX-3 along with other toxins in culture, induced bleaching on Apogee and spread, causing considerable disease above and below the inoculation point (S4 Table, Fig 3). Variation in aggressiveness was noted among strains tested within the \textit{Graminearum} Clade (S4 Table, Fig 3). NIV-producing species within this Clade (\textit{F. austroamericanum}, \textit{F. cerealis}, \textit{F. cortaderiae}, \textit{F. meridionale}, and \textit{F. praegraminearum}) displayed moderate (43–64%, Fig 3).
to high (95–100%, Fig 3) aggressiveness toward wheat cv. Apogee (Fig 3). Strains of *F. cortaderiae*, *F. meridionale*, and *F. praegraminearum* infected approximately half of the wheat head by the end of the assay, which is modest compared to the more aggressive species *F. austroamericanum* and *F. cerealis* that were able to spread through the whole head 21 days post inoculation (S4 Table, Fig 3). The 3ADON/15ADON-producing species (*F. graminearum*, *F. pseudograminearum*, *F. vorosii*, *F. boothii*, and NRRL 34461 *F. sp. nov.-5*) as well as *F. asiaticum* and *F. culmorum*, which did not produce toxins in vitro but made NIV and DON, respectively, *in planta* (see below), were able to spread and induce severe head blight symptoms on wheat cv. Apogee where they colonized the entire wheat head 17 to 21 days following single floret inoculation (S4 Table, Fig 3).

### Trichothecene production *in planta*

Analyses of trichothecene production in the inoculated wheat heads showed that two of the 17 nonpathogenic strains (FRC R-738 *F. sambucinum* and FRC R-7107 *F. sp. nov.-12) tested made DAS *in planta*. Similarly, FRC R-8978 *F. venenatum*, which was pathogenic but not aggressive produced DAS, 15-monoacetoxyscirpenol, and scirpentriol. Four nonaggressive strains from the *Sporotrichioides* Clade (NRRL 36295 *F. sporotrichioides*, FRC R-7479 *F. armenniacum*, NRRL 54062 *F. sp. nov.-2*, and NRRL 29296 *F. sp. nov.-4*) synthesized T-2 tetraol 15-acetyl toxin (T-2TE) in the inoculated heads. In addition, NIV was detected in wheat heads inoculated with NRRL 3509 *F. kyushuense* from the *Sambucinum* Clade, and NRRL 66919 *F. sp. nov.-31 and NRRL 66920 *F. sp. nov.-29 from the *Brachygibbosum* Clade (S3 Table). The other nonaggressive strains in the *Brachygibbosum*, *Longipes*, *Novel*, *Sambucinum*, and *Sporotrichioides* Clades failed to produce any toxin in the inoculated heads (S3 Table). By contrast, the five strains representing *F. sp. nov.-15* and -16 from the *Sambucinum* Clade, which synthesized 15-keto NX-2 and 15-keto NX-3 along with other toxins in vitro, made NX-3, 15-keto NX-3, and hydroxy culmorin (OHCUL) *in planta* (S3 Table). Analyses of wheat heads inoculated with members of the *Graminearum* Clade revealed that they were all toxigenic *in planta* (S3 Table). Strains of *F. austroamericanum*, *F. cerealis*, *F. cortaderiae*, *F. meridionale*, *F. praegraminearum*, and *F. asiaticum*, which failed to produce any toxin when tested in vitro, made
NIV in planta. Some of these species also produced CUL and OHCU (S3 Table). Similarly, CUL and OHCU were synthesized by F. sp. nov. -5, F. culmorum, and F. pseudograminearum in the inoculated heads along with deoxynivalenol (DON). The acetyl-ester derivative of DON, 3ADON was also detected in heads inoculated with F. pseudograminearum. DON was also produced in planta by F. boothii, F. graminearum, and F. vorosii.

Discussion

Phylogenetic diversity and trichothecene potential of FSAMSC strains

The present study was initiated to investigate species diversity, trichothecene toxin potential, and pathogenicity on wheat within the FSAMSC. Toward this end, 171 FSAMSC strains were selected from the FRC and NRRL culture collections, based on analyses of partial TEF1 sequence data, to represent the phylogenetic diversity of this species complex. GCPSR-based analysis of combined partial RPB1, RPB2, and TEF1 sequence data, together with prior extensive studies on the Graminearum Clade [5,6, reviewed in 38], resolved the 171 strains as 74 genealogically exclusive phylospecies distributed among six strongly supported trichothecene toxin-producing clades. This finding is consistent with published genomic data that all FSAMSC species examined to date possess intact trichothecene biosynthetic genes [22,77,78]. However, previous phylogenetic studies have found that the evolutionary history of several TRI cluster genes does not mirror the species phylogeny [22,26,78]. As previously reported, results of the current study showed clades within the FSAMSC generally correlate with the type of trichothecene mycotoxin produced [29,49,79,80].

Sporotrichioides Clade. Consistent with what is known about trichothecene production within this clade (Table 1, [29,48,57,58,60]), the 11 species analyzed, including 4 novel undescribed species closely related to F. armeniacum, produced DAS, NEO + T-2, or DAS, NEO, and T-2 type A trichothecenes. Toxin production by the 4 newly discovered phylospecies (F. sp. nov. -1 to -4) and F. nodosum is reported here for the first time. The two strains of F. nodosum studied herein, NRRL 13431 (= FRC T-384) from a tomato stem in Turkey and NRRL 36351 (= CBS 201.63) from stored peanuts in Portugal, were received as F. chlamydosporum. Fusarium nodosum has also been isolated from Arundo donax in France and wheat in Iran [8].

Graminearum Clade. The Graminearum Clade isolates investigated here have been the subject of several detailed phylogenetic studies [6,9,10,38,81]. Nineteen of the 23 species in this clade were discovered in prior GCPSR studies over the past two decades and all have been formally described except for NRRL 34461 F. sp. nov. -5. The latter species was initially reported as F. acaciae-mearnsii [35], but subsequently as a putatively novel sister to F. acaciae-mearnsii [43]. In contrast to F. sp. nov. -5 from soil in South Africa, the few characterized strains of F. acaciae-mearnsii were recovered from Acacia mearnsii and wheat in South Africa and soil in Australia [10,35,82]. Prior to the recent discovery of type A trichothecene NX-2 toxin-producing F. graminearum strains in North America [9,27,28], members of this clade were only known to produce type B trichothecenes. We found that 19/23 strains, each representing a unique phylospecies in this clade, synthesized type B trichothecenes in vitro and/or in planta. However, F. brasilicum made NIV and the type A trichothecene CAL in agmatine broth. Although F. mesoamericanum, F. louisiense, and F. dactylidis failed to synthesize type B trichothecenes here, they are all known to produce them [10,41,43]. Even though NRRL 13393 F. lunulosporum possesses an intact trichothecene toxin gene cluster [77], it failed to synthesize any trichothecene. However, it did produce zearalenone as previously reported [10]. Global surveys have revealed that F. asiaticum, F. culmorum, and F. graminearum are segregating for 15ADON, 3ADON, and NIV worldwide [26,32,82] whereas the available data indicate the 15ADON chemotype is fixed in F. boothii [26,82,83]. Fusarium ussurianum, by contrast,
appears to be fixed for the 3ADON chemotype in the Far East of Russia [44]. Some reports suggest that 3ADON toxin-producing FHB pathogens are more aggressive than those that produce 15ADON on wheat [84]. In North America, an introduced 3ADON population of *F. graminearum* has increased in frequency compared to the native 15ADON population [81,84]. These observations raise concerns about the sustainability of FHB-resistant fungicides and emphasize the need for continued monitoring of their population dynamics.

**Novel Clade.** The current study also resulted in the discovery of a novel type A T-2, DAS, and NEO-producing trichothecene lineage comprising two novel phylogenetically distinct sister species, *F. sp. nov.*-6 and -7. The former species, *F. sp. nov.*-6, has an Asian-Pacific distribution with isolates from sweet potato in Papua New Guinea and corn in Japan (Table 1). The latter species (i.e., *F. sp. nov.*-7) was recovered from soybean roots exhibiting root rot, a symptom of sudden death syndrome [34], and wheat seed in Ethiopia (Table 1).

**Sambucinum Clade.** The ten novel species discovered in this clade were incorrectly identified as 8 different species (S1 Table). Of the 16 Sambucinum Clade species we tested, *F. kyushuense* was the only one capable of producing type B NIV and type A DAS trichothecenes, as previously reported [reviewed in 85,86]. Nivalenol and fusarenon-X were first characterized in this species [46,47]. Consistent with previous reports, *F. musarum*, *F. venenatum*, and *F. sambucinum*, including strains of the latter received as *F. tumidum* [29,49,87], produced the type A trichothecenes T-2, DAS, and NEO. The same toxins were also synthesized by all but one of the 10 novel species. In addition, two strains of *F. venenatum* and NRRL 26795 *F. sp. nov.*-17 (= BBA 70355 received as *F. torulosum* from U.S. soil) synthesized CAL. Production of the type A trichothecene 3OH was also detected in cultures of NRRL 22198 *F. venenatum* and FRC R-7122 *F. sp. nov.*-12. We found that 2/3 strains of *F. poae* synthesized the type A toxin DAS; however, this species has been reported to produce type A and type B trichothecenes, including DAS, NEO, T-2 toxin, and NIV (Table 1, [48]). Proctor et al. [78] discovered that *F. poae* lacks the TRI16 gene, which is required for synthesis of T-2 toxin. Prior to its formal description, the T-2 and HT-2 producer *F. langsethiae* was mistakenly thought to be *F. poae* [59]. These findings may explain the incorrect reports of T-2 toxin production by *F. poae*. *Fusarium venenatum*, the species used to produce Quorn mycoprotein for human consumption, was found to synthesize the select agent DAS when grown in rice culture [29,55]. Consistent with this finding, the isolates of *F. venenatum* analyzed here all produced DAS, but some also made CAL, 3OH, and BUT in agmatine broth and on rice. The Quorn production strain NRRL 26139 (= IMI 145425 = ATCC 20334 = A 3/5; [88]), however, failed to produce DAS and any other toxin in agmatine and rice cultures [McCormick, unpubl, 14,55]. Given that a comparative genomic analysis, which included the Quorn production strain ATCC 20334 revealed it possesses the genes required for type A trichothecene biosynthesis [89], the open question is: Why aren’t they produced in agmatine medium? Agmatine is a strong inducer of TRIS, the gene that encodes the first enzymatic step in the trichothecene biosynthesis pathway [73]. When the Quorn production strain A3/5 (= ATCC 20334) was originally isolated in 1968 [88] it was identified as *F. graminearum* [90]; however, this species was not formally recognized as *F. venenatum* until 1995 [91]. The present study failed to recover additional strains of *F. musarum* [92], the earliest diverging lineage within the Sambucinum Clade. Similarly, *F. robustum* NRRL 13392 recovered from a rotten stem of Paraná pine (*Araucaria angustifolia*) in Argentina [93] is only represented by the ex-type strain.

Global surveys indicated that NX-2 toxin-producing strains of *F. graminearum* are restricted to Canada and the upper Midwest and Northeast U.S., suggesting that this chemotype might be indigenous to this region [27,28,94]. Thus, the discovery of two species within the Sambucinum Clade from South Africa that produced novel type A trichothecenes in vitro similar to NX-2 and NX-3, but with a keto or aldehyde moiety at the C-15 position, and NX-3,
the deacetylated form of NX-2 in planta is one of the most important findings of the present study. This discovery is the first to document that NX-2 toxin-producing fusaria are present outside of North America. Although these species were recovered from soil and debris, future studies are needed to determine whether these novel toxins contaminate food and feed and pose a threat to food safety and human health.

**Longipes Clade.** Previous phylogenetic studies have showed that the morphospecies *F. longipes* encompasses at least four phylospecies [67,95]. Our analyses of 19 strains from the FRC and NRRL culture collections indicate this clade comprises at least 9 phylogenetically distinct species. The fact that only 4/19 strains representing four species produced DAS in rice, but not in agmatine, YEPD, and cornmeal media, suggests the experimental conditions used may have been suboptimal, or these soil inhabiting fusaria may have lost the ability to synthesize trichothecenes. To our knowledge beauvericin production by NRRL 13368 *F. longipes*-1 is the only toxin report from this clade [45]. However, a recent phylogenomic study revealed the presence of an intact TRI cluster in the genome of NRRL 20695 *F. longipes*-4; but trichothecene production by this strain was not investigated [77].

**Brachygibbosum Clade.** Our analysis of 50 strains within this clade resolved 13 phylospecies, including two named (i.e., *F. brachygibbosum* and *F. transvaalense* [7,96]), and 11 unnamed putatively novel phylospecies. Two of the strains, NRRL 13829 *F. sp. nov.-23 (= MRC 2568) from river sediment in Japan and NRRL 31008 *F. transvaalense (= BBA 63772) from soil in Australia were previously reported as *F. cf. compactum* and *F. brachygibbosum*, respectively [67,94]. Although NRRL 13829 was recently reported as *F. brachygibbosum* [7], by including the ex-type strain of *F. brachygibbosum* NRRL 20954 (= BBA 64691) from Indian sorghum [96], the present phylogenetic analysis resolved these two strains as phylogenetically distinct sister taxa. All 13 species produced trichothecenes, and these included NRRL 66937 *F. sp. nov.-30 from pearl millet in Botswana that made the type B toxin NIV, 8 type A producers, including *F. brachygibbosum* NRRL 20954, and four species that produced type A and B trichothecenes, including *F. transvaalense*.

Several strains in each of the six clades failed to produce trichothecenes in vitro under the experimental conditions used. For example, strains of *F. asiaticum*, *F. louisianense*, and *F. dacrylides* tested failed to synthesize any trichothecene in the current study, but they have been shown to synthesize NIV [11,41,43]. Therefore, this failure to produce trichothecenes might be because some strains have lost their toxigenic potential because they have been in culture for decades or the growth conditions we employed were suboptimal for trichothecene production for some strains. The latter hypothesis is supported by studies that found trichothecene production on agmatine was influenced by strain diversity [29,73]. Further research is required to identify optimal nutrient profiling conditions to stimulate trichothecene production in vitro and/or in planta. This is supported by the fact that some strains that failed to produce toxins in agmatine broth, which is a strong inductor of trichothecene synthesis [73], made trichothecenes when grown on rice or cornmeal (S2 Table).

The sesquiterpene diol culmorin was produced by most of the species within the Graminearum Clade, three novel species within the Sambucinum Clade, and by *F. langsethiae* in the Sporotrichioides Clade. Biosynthesis of this toxin requires a terpene synthase (TS) and a cytochrome P450 monoxygenase encoded by CLM1 and CLM2, respectively [97,98]. A comparative phylogenomic analysis revealed that the two genes were conserved and were present in the genomes of TRI cluster-containing species closely related to *F. graminearum* within the FSAMSC [77]. By contrast, one or both genes were absent in the more distantly related species *F. sambucinum*, *F. poae*, *F. sporotrichioides*, *F. longipes*, and *F. langsethiae* [77]. Thus, the fact that CUL was detected in *F. langsethiae* herein and in previous studies [48,59] suggests the genomes should be screened for the presence of these two genes, given that intraspecific
variation in the presence and absence of certain secondary metabolite gene clusters was previously noted [99].

Overall, the BI and ML analyses revealed 101 of the 171 strains included in the present study were received misidentified, in some instances under names that are not accepted today (i.e., *F. trichothecioides* and *F. sulphureum = F. sambucinum*). It is worth mentioning that most of the species within the *Graminearum* Clade (n = 19) were also received misidentified, mostly as *F. graminearum*, prior to GCPSR studies of this clade [10,38]. The present phylogenetic analysis also revealed that close to half of the genealogically exclusive lineages (33/74 = 44.6%) represented novel phylospecies. In addition to the 23 *Graminearum* Clade strains, each representing a unique phylospecies, the other 148 strains were resolved as 19 previously described and 32 novel phylospecies in the following five FSAMSC clades: *Brachygibbosum* (n = 50 strains, 11/13 novel species), *Sambucinum* (n = 42 strains, 10/16 novel species), *Sporotrichioides* (n = 32 strains, 4/11 novel species), *Longipes* (n = 19 strains, 5/9 novel species), and a Novel Clade (n = 5 strains, 2/2 novel species). The current study highlights the importance of multilocus DNA sequence typing (MLST) as an essential tool for accurately identifying fusaria. To illustrate their impact, studies over the past two decades indicate that *Fusarium* comprises several fold more species than included in the traditional morphology-based taxonomic treatments [100]. For example, in a reassessment of fusaria included in Marasas et al. [51], a MLST analysis [29] found that the 17 morphospecies included in this compendium comprised 46 phylospecies, including 10 that were putatively novel.

### Pathogenicity on wheat and trichothecene production in planta

We evaluated pathogenicity, aggressiveness, and trichothecene production of 75 strains representing 57 species spanning six clades of the FSAMSC on wheat (cv. Apogee). Our data showed that only members of the *Graminearum* Clade and two novel species within the *Sambucinum* Clade were aggressive. Strains of the other species were either not pathogenic to wheat or caused head blight symptoms in the inoculated floret without spreading to nearby spikelets. Furthermore, most of these strains failed to produce detectable levels of mycotoxins in the inoculated wheat heads. The failure of these strains to cause FHB and to spread may be because they did not produce trichothecenes, which are required for fungal spread and FHB development in some Triticeae [17,19]. The nonaggressive strains belong to five clades other than *Graminearum* and most were isolated from non-gramineous hosts or other substrates. This finding suggests that the FSAMSC may contain nonpathogenic species that possess an endophytic or saprophytic lifestyle. For example, while *F. poae* and *F. sporotrichioides* are widely recovered from cereals, they are less aggressive secondary invaders of heads infected by the major FHB pathogen *F. graminearum* [101]. The available data also suggest that *F. armeniacum*, *F. brachygibbosum*, *F. longipes*, and *F. venenatum* have evolved principally as common saprophytes of diverse crops, natural grasses, soil, and other substrates [102]. All strains within the *Longipes* Clade and more than half in the *Brachygibbosum* Clade were recovered from soil and debris. By contrast, *Sporotrichioides* and *Sambucinum* Clade strains were recovered from a wide range of hosts including cereals, wild grasses, soil, and debris.

A detailed comparative genomics study of *F. venenatum* and *F. graminearum* revealed a high degree of synteny between the two genomes, including genes known to be involved in virulence [89]. *Fusarium venenatum* may be nonpathogenic to wheat because it is missing two transcription factors previously shown to be required for *F. graminearum* virulence on wheat and five *F. graminearum* specific secondary metabolite gene clusters that are typically upregulated during infection of wheat by *F. graminearum*. The combined loss of these genes, and the toxigenic difference between DON-producing *F. graminearum* and DAS-producing *F.
venenatum, are thought to contribute to the lack of aggressiveness toward wheat in F. venenatum and the divergent lifestyles of these fungi [89]. These findings also highlight the need for further research on the evolution of pathogenesis in other species within the FSAMSC to better understand what factors contribute to FHB pathogen aggressiveness toward cereals.

In that regard, variation in aggressiveness was observed among the Graminearum Clade strains tested in the current study (Fig 3). The NIV-producing species F. australiacanum, F. asiaticum, and F. cerealis were highly aggressive on wheat (cv. Apogee) and spread throughout most of the head at 21-days post inoculation. However, other NIV-producing species were less aggressive (F. cortaderiae, F. meridionale, and F. praegraminearum). Our data are consistent with other reports that evaluated these species on other wheat cultivars including cv. Norm [6,11,41,83]. Even so, toxin analyses of the inoculated wheat heads revealed that these species were all toxigenic and made NIV in planta. Graminearum Clade strains that produced 3ADON or 15ADON in vitro displayed high aggressiveness on wheat and were able to spread throughout the entire wheat heads. Tracking disease progress at different timepoints revealed that these species rapidly spread and colonized the entire heads 17 days post inoculation. Consistent with their ability to spread throughout the wheat head, GC-MS analyses showed they produced DON in planta. Overall, these findings match previous reports that indicate DON functions as a virulence factor allowing the fungus to overcome wheat defenses and spread throughout wheat heads [17,19].

In addition to producing type B trichothecenes, most of the Graminearum Clade strains tested produced CUL and/or its derivative OHCUL along with DON or NIV in planta. Culmorin is considered an “emerging mycotoxin” that is frequently found in naturally contaminated grain and axenic cultures of the most important FHB causal agents where it co-occurs with DON and other trichothecene analogs [103]. However, information on the toxicological relevance of CUL is limited. Nevertheless, a recent study demonstrated that CUL and DON have a synergistic phytotoxic effect on cereals when the concentration of CUL exceeded that of DON [77]. In addition, these authors reported that the severity of FHB induced by F. graminearum on wheat was positively correlated with the sum of CUL and DON. Given that CUL inhibits the activity of UDP-glucosyltransferases [104], their working hypothesis is CUL suppresses glycosylation of DON by serving as an alternate substrate for UDP-glucosyltransferases [77]. While 15 of the 20 Sambucinum Clade isolates tested for pathogenicity on wheat were not aggressive, strains of the novel species F. sp. nov.-15 and -16 induced FHB and showed levels of aggressiveness on cv. Apogee comparable to members of the Graminearum Clade (Fig 3). Mycotoxin analyses revealed that these newly discovered sister species produced the novel toxins 15-keto NX-2 and 15-keto NX-3 with other toxins in vitro and 15-keto NX-3, NX-3, and the sesquiterpene OHCUL in planta. Future research is required to better understand the pathophysiological impact of these novel toxins and to determine whether they function as virulence factors against wheat and other small grain cereals. Results of the pathogenicity assay provide evidence that the potential to induce FHB and aggressiveness are clearly influenced by the type of toxin produced and this appears to be a strain rather than a clade-specific characteristic. In planta toxin data showed that type B trichothecene NIV- and DON-producing strains from the Graminearum Clade and the type A trichothecene NX-3 and 15-keto NX-3 producers from the Sambucinum Clade were aggressive toward wheat. By contrast, NIV producers from the Sambucinum and Brachygibbosum Clades and strains from other clades, which synthesized toxins such as DAS and T-2TE toxin, were unable to spread in the inoculated wheat heads. While the disease assays herein were only conducted on cv. Apogee, selected for its short life cycle that made it feasible to screen such a large strain subset, the data obtained provide robust genotype-pathogenic phenotype correlations between diverse strains within the FSAMSC. Furthermore, these data provide a framework for subsequent investigations on other cultivars.
with different degrees of susceptibility to FHB, which will be needed to further elucidate the role of toxins on pathogen aggressiveness, including the novel trichothecenes.

To summarize, phylogenetic, biochemical, and pathogenicity analyses have provided insights into the phylogenetic diversity, trichothecene toxin potential, and pathogenicity to wheat within the FSAMSC. The robust MLST scheme employed, combined with toxin analyses and pathogenicity assays led to several important discoveries (i) the FSAMSC comprises at least 74 genealogically exclusive phylospecies, 33 of which are novel, distributed among six trichothecene toxin-producing clades; (ii) a Novel type A trichothecene-producing clade comprising two sister species; (iii) two putatively novel species within the Sambucinum Clade from South Africa produced NX-3 and two novel type A trichothecenes similar to NX-2 and NX-3 but with a keto or aldehyde group, respectively, at the C-15 position on the trichothecene ring; and (iv) only members of the Graminearum Clade and F. sp. nov.-15 and -16 within the Sambucinum Clade were aggressive on cv. Apogee, which suggests that aggressiveness is likely determined by the type of toxin produced. These results clearly illustrate that robust molecular systematic data are essential for accurately identifying species within the FSAMSC and they provide a firm foundation for ongoing comparative phylogenomic analyses of these agronomically important fusaria, which are focused on advancing global agricultural biosecurity, plant health and food safety.

Supporting information

S1 Fig. Molecular phylogeny of the Fusarium sambucinum species complex (FSAMSC) inferred from partial RPB1 sequences of 171 isolates using MrBayes 3.2.7a and IQ-TREE 1.6.12. The phylogeny was rooted on a sequence of NRRL 13338 F. nelsonii from the F. chlamydosporum species complex. Support values above or below branches are Bayesian posterior probabilities (BPP)/maximum likelihood bootstrap values (ML-BS). Thickened black internodes identify six strongly supported clades (BPP = 1; ML-BS = 98–100%): Brachygibbosum, Graminearum, Longipes, Novel, Sambucinum, and Sporotrichioidei. The FSAMSC comprises 74 phylogenetic species, including 33 that appear to be novel (i.e., F. sp. nov.-1 to -33). (TIF)

S2 Fig. Molecular phylogeny of the Fusarium sambucinum species complex (FSAMSC) inferred from partial RPB2 sequences of 171 isolates using MrBayes 3.2.7a and IQ-TREE 1.6.12. The phylogeny was rooted on a sequence of NRRL 13338 F. nelsonii from the F. chlamydosporum species complex. Support values above or below branches are Bayesian posterior probabilities (BPP)/maximum likelihood bootstrap values (ML-BS). Six clades of the FSAMSC were strongly supported as monophyletic (BPP = 1; ML-BS = 95–100%): Brachygibbosum, Graminearum, Longipes, Novel, Sambucinum, and Sporotrichioidei. The FSAMSC comprises 74 phylogenetic species, including 33 that appear to be novel (i.e., F. sp. nov.-1 to -33). (TIF)

S3 Fig. Molecular phylogeny of the Fusarium sambucinum species complex (FSAMSC) inferred from partial TEF1 sequences of 171 isolates using MrBayes 3.2.7a and IQ-TREE 1.6.12. The phylogeny was rooted on a sequence of NRRL 13338 F. nelsonii from the F. chlamydosporum species complex. Support values above or below branches are Bayesian posterior probabilities (BPP)/maximum likelihood bootstrap values (ML-BS). Six clades of the FSAMSC were strongly supported as monophyletic (BPP = 1; ML-BS = 95–100%): Brachygibbosum, Graminearum, Longipes, Novel, Sambucinum, and Sporotrichioidei. The FSAMSC comprises 74 phylogenetic species, including 33 that appear to be novel (i.e., F. sp. nov.-1 to -33). (TIF)
S1 Table. Strain histories.
(XLSX)

S2 Table. Toxins produced by *Fusarium sambucinum* species complex strains in liquid media, rice grain, and cornmeal.
(XLSX)

S3 Table. Pathogenicity of *Fusarium sambucinum* species complex strains on cv. Apogee and toxin production *in planta*.
(XLSX)

S4 Table. Spread of Fusarium head blight symptoms on cv. Apogee after inoculation with strains belonging to *Graminearum* and *Sambucinum* Clades.
(XLSX)

**Acknowledgments**

The authors wish to thank Nathan Orwig, Christine Poppe, and Jennifer Teresi for excellent technical assistance in various aspects of this study and Bruce Bugbee for providing the Apogee wheat seed. The mention of company names or trade products does not imply that they are endorsed or recommended by the US Department of Agriculture over other companies or similar products not mentioned. USDA is an equal opportunity provider and employer.

**Author Contributions**

Conceptualization: Imane Laraba, Kerry O'Donnell.

Data curation: Imane Laraba.

Formal analysis: Imane Laraba.

Funding acquisition: David M. Geiser, Kerry O'Donnell.

Investigation: Imane Laraba, Susan P. McCormick.

Methodology: Imane Laraba, Susan P. McCormick, Martha M. Vaughan, Kerry O'Donnell.

Project administration: Kerry O'Donnell.

Resources: Susan P. McCormick, Martha M. Vaughan, David M. Geiser, Kerry O'Donnell.

Software: Imane Laraba.

Validation: Imane Laraba.

Visualization: Imane Laraba, Kerry O'Donnell.

Writing – original draft: Imane Laraba.

Writing – review & editing: Imane Laraba, Susan P. McCormick, Martha M. Vaughan, David M. Geiser, Kerry O'Donnell.

**References**

1. Summerell BA. Resolving *Fusarium*: current status of the genus. Annu Rev Phytopathol. 2019; 57: 323–339. https://doi.org/10.1146/annurev-phyto-082718-100204 PMID: 31226019

2. Geiser DM, Aoki T, Bacon CW, Baker SE, Bhattacharyya MK, Brandt ME, et al. Letter to the Editor: One fungus, one name: defining the genus *Fusarium* in a scientifically robust way that preserves long-standing use. Phytopathology. 2013; 103: 400–408. https://doi.org/10.1094/PHYTO-07-12-0150-LE PMID: 23379853
3. O’Donnell K, Ward TJ, Robert VARG, Crous PW, Geiser DM, Kang S. DNA sequence-based identification of *Fusarium*: current status and future directions. Phytoparasitica. 2015; 43: 583–595.

4. Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, et al. Phylogenetic species recognition and species concepts in fungi. Fungal Genet Biol. 2000; 31: 21–32. https://doi.org/10.1006/fgbi.2000.1228 PMID: 11118132

5. Gräfenhan T, Johnston PR, Vaughan MM, McCormick SP, Proctor RP, Busman M, et al. *Fusarium praeanginae* sp. nov., a novel nivalenol mycotoxin-producing pathogen from New Zealand can induce head blight on wheat. Mycologia. 2016; 108: 1229–1239. https://doi.org/10.3852/16-110 PMID: 27621289

6. Pereira CB, Ward TJ, Tessmann DJ, Del Ponte EM, Laraba I, Vaughan MM, et al. *Fusarium subtrueacrae*, sp. nov., a novel nivalenol mycotoxin-producing species isolated from barley (*Hordeum vulgare*) in Brazil and sister to *F. praeanginae*. Mycologia. 2018; 110: 860–871. https://doi.org/10.1080/00275514.2018.1512296 PMID: 30303468

7. Sandoval-Denis M, Swart WJ, Crous PW. New *Fusarium* species from the Kruger National Park, South Africa. MycoKeys. 2018; 34: 63–92. https://doi.org/10.3897/mycokeys.34.25974 PMID: 29892206

8. Lombard L, van Doorn R, Crous PW. Neotypification of *Fusarium chlamydosporum*—a reappraisal of a clinically important species complex. Fungal Syst Evol. 2019; 4: 183–200. https://doi.org/10.3114/fuse.2019.04.10 PMID: 32467911

9. Kelly A, Proctor RH, Beilzile F, Chulze SN, Clear RM, Cowger C, et al. The geographic distribution and complex evolutionary history of the NX-2 trichothecene chemotype from *Fusarium graminearum*. Fungal Genet Biol. 2016; 95: 39–48. https://doi.org/10.1016/j.fgb.2015.05.016 PMID: 26127017

10. O’Donnell K, Kistler HC, Tacke BK, Casper HH. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proc Natl Acad Sci U S A. 2000; 97: 7905–7910. https://doi.org/10.1073/pnas.130193297 PMID: 11118132

11. Goswami RS, Kistler HC. Pathogenicity and in planta mycotoxin accumulation among members of the *Fusarium graminearum* species complex on wheat and rice. Phytopathology. 2005; 95: 1397–1404. https://doi.org/10.1094/PHYTO-95-1397 PMID: 18943550

12. Kelly AC, Clear RM, O’Donnell K, McCormick S, Turkington TK, Tekauz A, et al. Diversity of Fusarium head blight populations and trichothecene toxin types reveals regional differences in pathogen composition and temporal dynamics. Fungal Genet Biol. 2015; 82: 22–31. https://doi.org/10.1016/j.fgb.2015.05.016 PMID: 26127017

13. Nganje WE, Kaitibie S, Wilson WW, Leistritz FL, Bangsund DA. Economic impacts of Fusarium head blight in wheat and barley: 1993–2001. Agribusiness and Applied Economics Report No. 538. Department of Agribusiness and Applied Economics, North Dakota State University, ND, USA; 2004.

14. Munkvold GP. *Fusarium* species and their associated mycotoxins. In: Moretti A, Susca A, editors. Mycotoxigenic fungi: methods and protocols. New York: Humana Press; 2017. pp. 51–106.

15. Pestka J. Toxicochemical mechanisms and potential health effects of deoxynivalenol and nivalenol. World Mycotoxin J. 2010; 3: 323–347.

16. Wu F, Groopman JD, Pestka JJ. Public health impacts of foodborne mycotoxins. Annu Rev Food Sci Technol. 2014; 5: 351–372. https://doi.org/10.1146/annurev-food-030713-092431 PMID: 24422587

17. Proctor RH, Hohn TM, McCormick SP. Reduced virulence of *Gibberella zeae* caused by disruption of a trichothecene toxin biosynthetic gene. Mol Plant-Microbe Interact. 1995; 8: 593–601. https://doi.org/10.1094/mpmi-8-0593 PMID: 8589414

18. Bai GH, Desjardins AE, Plattner R. Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. Mycopathologica. 2002; 153: 91–98.

19. Jansen C, von Wettstein D, Schäfer W, Kogel K-H, Felk A, Maier FJ. Infection patterns in barley and wheat spikes inoculated with wild-type and trichodiene synthase gene disrupted *Fusarium graminearum*. Proc Natl Acad Sci U S A. 2005; 102: 16892–16897. https://doi.org/10.1073/pnas.0508467102 PMID: 16263921

20. McCormick SP, Stanley AM, Stover NA, Alexander NJ. Trichothecenes: from simple to complex mycotoxins. Toxins. 2011; 3: 802–814. https://doi.org/10.3390/toxins3070802 PMID: 22069741

21. Alexander NJ, McCormick SP, Waalwijk C, van der Lee T, Proctor RH. The genetic basis for 3ADON and 15ADON trichothecene chemotypes in *Fusarium graminearum*. Fungal Genet Biol. 2011; 48: 485–495. https://doi.org/10.1016/j.fgb.2011.01.003 PMID: 21216300

22. Proctor RH, McCormick SP, Kim H-S, Cardoza RE, Stanley AM, Lindo L, et al. (2018) Evolution of structural diversity of trichothecenes, a family of toxins produced by plant pathogenic and
23. Ramdass AC, Villafana RT, Ramperad SN. Comparative sequence analysis of TR11 of Fusarium. Toxins. 2019; 11: 689. https://doi.org/10.3390/toxins11120689 PMID: 31771208

24. Villani A, Proctor RH, Kim H-S, Brown DW, Logrieco AF, Amatulli MT, et al. Variation in secondary metabolite production potential in the Fusarium incarnatum-equiseti species complex revealed by comparative analysis of 13 genomes. BMC Genomics. 2019; 20: 314. https://doi.org/10.1186/s12864-019-5567-7 PMID: 31014248

25. Miller JD, Greenhalgh R, Wang Y, Lu M. Trichotheccene chemotypes of three Fusarium species. Mycologia. 1991; 83: 121–130.

26. Ward TJ, Bielawski JP, Kistler HC, Sullivan E, O’Donnell K. Ancestral polymorphism and adaptive evolution in the trichotheccene mycotoxin gene cluster of phytopathogenic Fusarium. Proc Natl Acad Sci U S A. 2002; 99: 9278–9283. https://doi.org/10.1073/pnas.142307199 PMID: 12080147

27. Liang JM, Xayamongkhon H, Broz K, Dong Y, McCormick SP, Abravama S, et al. Temporal dynamics and population genetic structure of Fusarium graminearum in the upper Midwestern United States. Fungal Genet Biol. 2014; 73: 83–92. https://doi.org/10.1016/j.fgb.2014.10.002 PMID: 25312860

28. Varga E, Wiesenberger G, Hametner C, Ward TJ, Dong Y, Schöfbeck D, et al. New tricks of an old enemy: isolates of Fusarium graminearum produce a type A trichotheccene mycotoxin. Environ Microbiol. 2015; 17: 2588–2600. https://doi.org/10.1111/1462-2920.12718 PMID: 25403493

29. O’Donnell K, McCormick SP, Busman M, Proctor RH, Ward TJ, Doehring G, et al. Marasas et al. 1984 “Toxigenic Fusarium Species: Identity and Mycotoxicology” revisited. Mycologia. 2018; 110: 1058–1080. https://doi.org/10.1080/00275514.2018.1519773 PMID: 30481135

30. Scherm B, Balmas V, Spanu F, Pani G, Delogu G, Pasquali M, et al. Fusarium culmorum: causal agent of foot and root rot and head blight on wheat. Mol Plant Pathol. 2013; 14: 323–341. https://doi.org/10.1111/mpp.12011 PMID: 23279114

31. Vaughan MM, Backhouse D, Ponte EMD. Climate change impacts on the ecology of Fusarium graminearum species complex and susceptibility of wheat to Fusarium head blight: a review. World Mycotoxin J. 2016; 9: 685–700.

32. Laraba I, Boureghda H, Abdallah N, Bouaicha O, Obanor F, Moretti A, et al. Population genetic structure and mycotoxin potential of the wheat crown rot and head blight pathogen Fusarium culmorum in Algeria. Fungal Genet Biol. 2017; 103: 34–41. https://doi.org/10.1016/j.fgb.2017.04.001 PMID: 28392426

33. Tan DC, Flematti GR, Ghisalberti EL, Sivasithamparam K, Chakraborty S, Obanor F, et al. Mycotoxins produced by Fusarium species associated with annual legume pastures and ‘sheep feed refusal disorders’ in Western Australia. Mycotoxin Res. 2011; 27: 123–135. https://doi.org/10.1007/s12550-010-0085-0 PMID: 23605703

34. Hartman GL, McCormick SP, O’Donnell K. Trichotheccene-producing Fusarium species isolated from soybean roots in Ethiopia and Ghana and their pathogenicity on soybean. Plant Dis. 2019; 103: 2070–2075. https://doi.org/10.1094/PDIS-12-18-2286-RE PMID: 31215854

35. Starkey DE, Ward TJ, Aoki T, Gale LR, Kistler HC, Geiser DM, et al. Global molecular surveillance reveals novel Fusarium head blight species and trichotheccene toxin diversity. Fungal Genet Biol. 2007; 44: 1191–1204. https://doi.org/10.1016/j.fgb.2007.03.001 PMID: 17451976

36. O’Donnell K, Ward TJ, Aberra D, Kistler HC, Aoki T, Orwig N, et al. Multilocus genotyping and molecular phylogenetics resolve a novel head blight pathogen within the Fusarium graminearum species complex from Ethiopia. Fungal Genet Biol. 2008; 45: 1514–1522. https://doi.org/10.1016/j.fgb.2008.09.002 PMID: 18824240

37. Lee T, Paek JS, Lee KA, Lee KA, Lee S, Choi J-H, et al. Occurrence of toxigenic Fusarium vorosii among small grain cereals in Korea. Plant Pathol J. 2016; 32: 407–413. https://doi.org/10.5423/PPJ.OA.05.2016.0123 PMID: 27721690

38. Aoki T, Ward TJ, Kistler HC, O’Donnell K. Systematics, phylogeny and trichotheccene mycotoxin potential of Fusarium head blight cereal pathogens. JSM Mycotoxins. 2012; 62: 91–102.

39. Monds RD, Cromey MG, Lauren DR, Di Menna M, Marshall J. Fusarium graminearum, F. cortarudiae and F. pseudograminearum in New Zealand: molecular phylogenetic analysis, mycotoxin chemotypes and co-existence of species. Mycol Res. 2005; 109: 410–420. https://doi.org/10.1017/s0953756204002217 PMID: 15912928

40. Kasitu GC, ApSimon JW, Blackwell BA, Fielder DA, Greenhalgh R, Miller JD. Isolation and characterization of culmorin derivatives produced by Fusarium culmorum CMI 14764. Can J Chem. 1992; 70: 1308–1316.
41. Aoki T, Vaughan MM, McCormick SP, Busman M, Ward TJ, Kelly A, et al. *Fusarium dactylidis* sp. nov., a novel nivalenol toxin-producing species sister to *F. pseudograminearum* isolated from orchard grass (*Dactylis glomerata*) in Oregon and New Zealand. Mycologia. 2015; 107: 409–418. https://doi.org/10.3852/14-213 PMID: 25550300

42. Langseth W, Bernhoff A, Rundberget T, Kosiak B, Gareis M. Mycotoxin production and cytotoxicity of *Fusarium* strains isolated from Norwegian cereals. Mycopathologia. 1999; 144: 103–113.

43. Sarver BAJ, Ward TJ, Gale LR, Broz K, Kistler HC, Aoki T, et al. Novel Fusarium head blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. Fungal Genet Biol. 2011; 48: 1096–1107. https://doi.org/10.1016/j.fgb.2011.09.002 PMID: 22004876

44. Yli-Mattila T, Ward TJ, O'Donnell K, Proctor RH, Burkin AA, Kononenko GP, et al. A novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from the Russian Far East. Mycologia. 2009; 101: 841–852. https://doi.org/10.3852/08-213 PMID: 19927749

45. Logrieco A, Moretti A, Castella G, Kostecki M, Golinski P, Ritieni A, et al. Beauvericin production by *Fusarium sporotrichioides*. Mycopathologia. 1995a; 130: 179–184. https://doi.org/10.1007/BF01103345 PMID: 7566056

46. Tatsuno T, Saito M, Enomoto M, Tsunoda H. Biological and chemical detection of trichothecone mycotoxins of *Fusarium* species. Appl Microbiol. 1973; 125: 699–704. PMID: 6499224

47. Marasas WFO, Nelson PE, Toussoun TA. Toxigenic *Fusarium* species: identity and mycotoxicology. University Park, Pennsylvania: Pennsylvania State University Press; 1984. https://doi.org/10.1002/jc.2910340315 PMID: 6480156

48. Altomare C, Logrieco A, Bottalico A. Clustering of trichothecone-producing *Fusarium* strains determined from 28S ribosomal DNA sequences. Appl Environ Microbiol. 1997; 63: 1843–1846. https://doi.org/10.1128/AEM.63.5.1843-1846.1997 PMID: 9143116

49. Ueno Y, Ueno I, Tatsuno T, Ohkubo K, Tsunoda H. Nivalenol, a toxic principle of *Fusarium graminearum* sp. nov., a novel nivalenol toxin-producing species sister to *F. pseudograminearum* isolated from orchard grass (*Dactylis glomerata*) in Oregon and New Zealand. Mycologia. 2015; 107: 409–418. https://doi.org/10.3852/14-213 PMID: 25550300

50. Ueno Y, Sato N, Ishii K, Sakai K, Tsunoda H. Biological and chemical detection of trichothecone mycotoxins of *Fusarium* species. Appl Microbiol. 1973; 125: 699–704. PMID: 6499224

51. Thrane U, Adler A, Clasen PE, Galvano F, Langseth W, Lew H, et al. Diversity in metabolite production of *Fusarium graminearum* and *Fusarium sporotrichioides*. J Int Food Microbiol. 2004; 95: 257–266. https://doi.org/10.1016/j.ijfoodmicro.2003.12.005 PMID: 15337591

52. Thrane U, Hansen U. Chemical and physiological characteristics of taxa in the *Fusarium sambucinum* species complex. Mycopathologia. 1995; 129: 183–190. https://doi.org/10.1007/BF01103345 PMID: 7566056

53. O’Donnell K, Cigelnik E, Casper H. Molecular phylogenetic, morphological, and mycotoxin data support reidentification of the Quom mycopolysaccharide fungus as *Fusarium venenatum*. Fungal Genet Biol. 1998; 23: 57–67. https://doi.org/10.1006/fgbi.1997.1018 PMID: 9501477

54. Miller JD, MacKenzie S. Secondary metabolites of *Fusarium venenatum* strains with deletions in the Trö5 gene encoding trichodiene synthetase. Mycologia. 2000; 92: 764–771. https://doi.org/10.1002/ijc.1843-1846.1997 PMID: 9143116

55. Miller JD, MacKenzie S. Secondary metabolites of *Fusarium venenatum* strains with deletions in the Trö5 gene encoding trichodiene synthetase. Mycologia. 2000; 92: 764–771. https://doi.org/10.1002/ijc.1843-1846.1997 PMID: 9143116

56. Nichea MJ, Cendoya E, Zachetti VG, Chiacchiera SM, Sulyok M, Kriska R, et al. Mycotoxin profile of *Fusarium armeniacum* isolated from natural grasses intended for cattle feed. World Mycotoxin J. 2005; 28: 451–457.

57. Rocha LO, Laurence MH, McCormick SP, Summerell BA, Liew ECY. Variation in type A trichothecone production and trichothecone biosynthetic genes in *Fusarium goolgardi* from natural ecosystems of Australia. Toxins. 2015; 7: 4577–4594. https://doi.org/10.3390/toxins7114577 PMID: 26556373

58. Torp M, Langseth W. Production of T-2 toxin by a *Fusarium* resembling *Fusarium poae*. Mycopathologia. 1999; 147: 89–96. https://doi.org/10.1023/a:1007060108935 PMID: 10967967

59. Yli-Mattila T, Ward TJ, O’Donnell K, Proctor RH, Burkin AA, Kononenko GP, et al. *Fusarium sibiricum* sp. nov., a novel type A trichothecone-producing *Fusarium* from northern Asia closely related to *F. sporotrichioides* and *F. langsethiae*. J Int Food Microbiol. 2011; 147: 58–68. https://doi.org/10.1016/j. ijfoodmicro.2011.03.007 PMID: 21459470

60. Logrieco A, Chelkowskij B, Bottalico A, Visconti A. Further data on specific trichothecone production by *Fusarium sect. Sporotrichiella* strains. Mycol Res. 1990; 94: 587–589.
62. Gardes M, Bruns TD. ITS primers with enhanced specificity for Basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol. 1993; 2: 113–118. https://doi.org/10.1111/j.1365-294x.1993.tb00005.x PMID: 8180733

63. O’Donnell K, Sutton DA, Rinaldi MG, Gueidan C, Sarver BAJ, Balajee SA, et al. Internet-accessible DNA sequence database for identifying fusaria from human and animal infections. J Clin Microbiol. 2010; 48: 3708–3718. https://doi.org/10.1128/JCM.00989-10 PMID: 20686083

64. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004; 32: 1792–1797. https://doi.org/10.1093/nar/gkh340 PMID: 15034147

65. Gouy M, Guindon S, Gascuel O. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol Biol Evol. 2010; 27: 221–224. https://doi.org/10.1093/molbev/msp259 PMID: 19854763

66. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE : a fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Mol Biol Evol. 2015; 32: 268–274. https://doi.org/10.1093/molbev/msu300 PMID: 25371430

67. O’Donnell K, Rooney AP, Proctor RH, Brown DW, McCormick SP, Ward TJ, et al. Phylogenetic analyses of RPB1 and RPB2 support a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. Fungal Genet Biol. 2013; 52: 20–31. https://doi.org/10.1016/j.fgb.2012.12.004 PMID: 23357352

68. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. 2017; 14: 587–589. https://doi.org/10.1038/nmeth.4285 PMID: 28481363

69. Chernomor O, von Haeseler A, Minh BQ. Terrace aware data structure for phylogenomic inference from supermatrices. Syst Biol. 2016; 65: 997–1008. https://doi.org/10.1093/sysbio/sys037 PMID: 27121966

70. Huelsenbeck JP, Ronquist F. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics. 2001; 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754 PMID: 11524383

71. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 2012; 61: 539–542. https://doi.org/10.1093/sysbio/sys029 PMID: 22357727

72. Stevens RB. Mycology guidebook. Seattle: University of Washington Press; 1974.

73. Gardiner DM, Kazan K, Manners JM. Nutrient profiling reveals potent inducers of trichothecene bio-synthesis in Fusarium graminearum. Fungal Genet Biol. 2009; 46: 604–613. https://doi.org/10.1016/j.fgb.2009.04.004 PMID: 19406250

74. Bugbee B, Koerner G, Albrechtsen R, Dewey W, Clawson S. Registration of USU-Apogee wheat. Crop Sci. 1997; 37: 626. https://doi.org/10.2135/cropsci1997.0011183x003700020053x PMID: 11542531

75. Mackintosh CA, Garvin DF, Radmer LE, Heinen SJ, Mueh Bauer GJ. A model wheat cultivar for transformation to improve resistance to Fusarium head blight. Plant Cell Rep. 2006; 25: 313–319. https://doi.org/10.1007/s00299-005-0059-4 PMID: 16252090

76. Sherbakoff CD. An examination of fusaria in the herbarium of the pathological collections. Bureau of Plant Industry, U.S. Department of Agriculture. Phytopathology. 1928; 18: 148–149.

77. Wirpfler R, McCormick SP, Proctor RH, Teresi JM, Hao G, Ward TJ, et al. Synergistic phytotoxic effects of culmorin and trichothecene mycotoxins. Toxins. 2019; 11: 555. https://doi.org/10.3390/toxins1105055 PMID: 3147160

78. Proctor RH, McCormick SP, Alexander NJ, Desjardins AE. Evidence that a secondary metabolicbiosynthetic gene cluster has grown by gene relocation during evolution of the filamentous fungus Fusarium. Mol Microbiol. 2009; 74: 1128–1142. https://doi.org/10.1111/j.1365-2958.2009.06927.x PMID: 19843228

79. Kristensen R, Torp M, Kosiak B, Holst-Jensen A. Phylogeny and toxigenic potential is correlated in Fusarium species as revealed by partial translation elongation factor 1 alpha gene sequences. Mycol Res. 2005; 109: 173–186. https://doi.org/10.1017/s0953756204002114 PMID: 15839101

80. Watanabe M, Yonezawa T, Sugita-Konishi Y, Kamata Y. Utility of the phylotoxigenic relationships among trichothecene-producing Fusarium species for predicting their mycotoxin-producing potential. Food Addit Contam A. 2013; 30: 1370–1381. https://doi.org/10.1080/19440049.2013.794305 PMID: 23731171

81. Ward TJ, Clear RM, Rooney AP, O’Donnell K, Gaba D, Patrick S, et al. An adaptive evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic Fusarium graminearum in North America. Fungal Genet Biol. 2008; 45: 473–484. https://doi.org/10.1016/j.fgb.2007.10.003 PMID: 18035565
82. Boutigny A-L, Ward TJ, Van Coller GJ, Flett B, Lamprech SC, O’Donnell K, et al. Analysis of the *Fusarium graminearum* species complex from wheat, barley and maize in South Africa provides evidence of species-specific differences in host preference. Fungal Genet Biol. 2011; 48: 914–920. https://doi.org/10.1016/j.fgb.2011.05.005 PMID: 21601644

83. Amarasinge C, Sharanoswki B, Fernando WGD. Molecular phylogenetic relationships, trichothecene chemotype diversity and aggressiveness of strains in a global collection of *Fusarium graminearum* species. Toxins. 2019; 11: 263. https://doi.org/10.3390/toxins11050263.

84. Foroud NA, McCormick SP, MacMillan T, Badea A, Kendra DF, Ellis BE, et al. Greenhouse studies reveal increased aggressiveness of emergent Canadian *Fusarium graminearum* chemotypes in wheat. Plant Dis. 2012; 96: 1271–1279. https://doi.org/10.1094/PDIS-10-11-0863-RE PMID: 30727146

85. Ueno Y, Aikawa Y, Okumura H, Sugiyama Y, Nakamura K, Masuma R, et al. Trichothecenes produced by *Fusarium* species Fn 2B. Mycotoxins. 1997; 45: 25–31.

86. Aoki T, O’Donnell K. *Fusarium kyushuense* sp. nov. from Japan. Mycoscience. 1998; 39: 1–6.

87. Morin L, Gianotti AF, Lauren DR. Trichothecene production and pathogenicity of *Fusarium tumidum*, a candidate bioherbicide for gorse and broom in New Zealand. Mycol Res. 2000; 104: 993–999.

88. Whitaker JA, Johnson RI, Finningan TJ, Avery SV, Dyer PS. The biotechnology of Quom mycoprotein: past, present and future challenges. In: Nevalainen H, editor. Grand challenges in fungal biotechnology. Cham: Springer; 2020. pp. 59–79.

89. King R, Brown NA, Hammond-Kosack KE. Inter-genome comparison of the Quorn fungus *Fusarium venenatum* and the closely related plant infecting pathogen *Fusarium graminearum*. BMC Genomics. 2018; 19: 269. https://doi.org/10.1186/s12864-018-4612-2 PMID: 29673315

90. Trinci APJ. Myco-protein: A twenty-year overnight success story. Mycol Res. 1992; 96: 1–13.

91. Nirenberg HI. Morphological differentiation of *Fusarium sambucinum* Fuckel sensu stricto, *F. torulo- sum* (Bert. & Curt.) Nirenberg comb. nov. and *F. venenatum* Nirenberg sp. nov. Mycopathologia. 1995; 129: 131–141. https://doi.org/10.1007/BF01103337 PMID: 7566048

92. Marasas WFO, Rheeder JP, Logrieco A, Van Wyk APS, Juba JH. *Fusarium nelsonii* and *F. musarum*: two new species in Section *Arthrosporiella* related to *F. camptoceras*. Mycologia. 1998; 90: 505–513.

93. Gerlach VW. *Fusarium robustum* spec. nov., der Erreger einer Stamnifa¨uule an Araucaria angustifolia (Bertol.) O. Kuntze in Argentinien? J phytopathol. 1977; 88: 29–37.

94. Lofgren LA, LeBlanc NR, Cerveto AK, Nachitagil J, LaBine KM, Riddle J, et al. *Fusarium graminearum*: pathogen or endophyte of North American grasses? New Phytol. 2018; 217: 1203–1207. https://doi.org/10.1111/nph.14894 PMID: 29160900

95. Waalwijk C, Taga M, Zheng S-L, O’Donnell K. Karyotype evolution in *Fusarium graminearum* species complex from wheat, barley and maize in South Africa provides evidence of species-specific differences in host preference. Fungal Genet Biol. 2011; 48: 914–920. https://doi.org/10.1016/j.fgb.2011.05.005 PMID: 21601644

96. Baahadoor A, Schneiderman D, Gemmill L, Bosnich W, Blackwell B, Melanson JE, et al. Hydroxylation of longiborneol by a *Clm2*-encoded CYP450 monoxygenase to produce culmorin in *Fusarium graminearum*. J Nat Prod. 2016; 79: 81–88. https://doi.org/10.1021/acs.jnatprod.5b00676 PMID: 26673640

97. Laraba I, Kim S-H, Proctor RH, Busman M, O’Donnell K, Felker FC, et al. *Fusarium xyrophilum*, sp. nov., a member of the *Fusarium fujikuroi* species complex recovered from pseudofollowers on yellow-eyed grass (*Xyris spp.*) from Guyana. Mycologia. 2019; 112: 39–51. https://doi.org/10.1080/00275514.2019.1668991 PMID: 31825746

98. Bahadoor A, Schneidemann D, Gemmill L, Bosnich W, Blackwell B, Melanson JE, et al. Hydroxylation of longiborneol by a *Clm2*-encoded CYP450 monoxygenase to produce culmorin in *Fusarium graminearum*. J Nat Prod. 2016; 79: 81–88. https://doi.org/10.1021/acs.jnatprod.5b00676 PMID: 26673640

99. Summerell BA, Leslie JF, Liew EC, Laurence MH, Bullock S, Petrović T, et al. *Fusarium* species associated with plants in Australia. Fungal Divers. 2010; 46: 1–27.

100. Aoki T, O’Donnell K, Geiser DM. Systematics of key phytopathogenic *Fusarium* species: current status and future challenges. J Gen Plant Pathol. 2014; 80: 189–201.

101. Bottalico A, Perrone G. Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur J Plant Pathol. 2002; 108: 611–624.

102. Summerell BA, Leslie JF, Liew EC, Laurence MH, Bullock S, Petrović T, et al. *Fusarium* species associated with plants in Australia. Fungal Divers. 2010; 46: 1–27.

103. Khaneghah AM, Kamani MH, Fahriy, Coppa CFSC, de Oliveira CAF, Sant’A na AS. Changes in masked forms of deoxynivalenol and their co-occurrence with culmorin in cereal-based products: A systematic review and meta-analysis. Food Chem. 2019; 294: 587–596. https://doi.org/10.1016/j. foodchem.2019.05.034 PMID: 31126504
104. Woelflingseder L, Warth B, Vierheilig I, Schwartz-Zimmermann H, Hametner C, Nagl V, et al. The Fusarium metabolite culmorin suppresses the in vitro glucuronidation of deoxynivalenol. Arch Toxicol. 2019; 93: 1729–1743. https://doi.org/10.1007/s00204-019-02459-w PMID: 31049613