Supplementary Table S1. The isolate name, initial BLASTN hit from the ITS sequence, sequencing technology used to generate genomes, and final species designation inferred from phylogenies in the main text of the 12 *Fusarium graminearum* species complex isolates collected from the 2022 outbreak in Ethiopia. Two isolates, ET-2022-49 and ET-2022-52 were sequenced with both Nanopore and Illumina technologies and assembled with a hybrid approach. ET-2022-52 is formally described in the main text as a novel species, *Fusarium kistleri*.

Isolate Name	BLAST Top Hit	Sequenced	Species
ET-2022-3	*F. graminearum*	Nanopore	*F. graminearum*
ET-2022-15	*F. graminearum*	Nanopore	*F. boothii*
ET-2022-17	*F. graminearum*	Nanopore	*F. graminearum*
ET-2022-21	*F. graminearum*	Nanopore	*F. graminearum*
ET-2022-30	*F. boothii*	Illumina	*F. boothii*
ET-2022-33	*F. boothii*	Illumina	*F. graminearum*
ET-2022-34	*F. boothii*	Illumina	*F. boothii*
ET-2022-35	*F. boothii*	Illumina	*F. boothii*
ET-2022-41	*F. boothii*	Illumina	*F. boothii*
ET-2022-49	*F. graminearum*	Nanopore; Illumina	*F. aethiopicum*
ET-2022-52	*F. graminearum*	Nanopore; Illumina	*F. kistleri*
ET-2022-53	*F. graminearum*	Illumina	*F. aethiopicum*

Supplementary Table S2. Genome quality and completeness for Nanopore and Illumina genomes. The completeness of assemblies was assessed with BUSCO (Benchmarking Universal Single-Copy Orthologs) (v. 5.4.7) using the sordariomycetes_odb10 databases. QUAST (v. 4.3) was run against the NCBI reference genome assembly of *F. graminearum* PH-1 (GCA_000240135.3) to determine genome quality.

Isolate Name	Sequenced	BUSCO	# Contigs	N50[1]	L50[2]	Average Coverage	Assembly Size (Mb)
ET-2022-3	Nanopore	93.0%	1,226	74,583	145	20x	35.45
ET-2022-15	Nanopore	97.8%	207	419,790	24	37x	36.67
ET-2022-17	Nanopore	98.4%	44	2,302,193	6	36x	36.88
ET-2022-21	Nanopore	98.3%	84	1,164,824	10	41x	36.95
ET-2022-30	Illumina	98.3%	240	1,585,114	9	48x	38.13
ET-2022-33	Illumina	98.3%	151	2,263,181	6	57x	36.89
ET-2022-34	Illumina	98.3%	139	1,556,940	8	47x	38.16
ET-2022-35	Illumina	98.3%	158	1,556,976	8	51x	38.15
ET-2022-41	Illumina	98.3%	63	2,292,517	6	44x	36.62
	Nanopore	98.1%	64	1,764,069	8	41x	37.02
ET-2022-49	Illumina	98.4%	47	2,232,397	6	34x	37.03
	Hybrid	98.3%	28	3,679,250	4	37x	37.06
	Nanopore	97.2%	421	255,351	47	23x	36.97
ET-2022-52	Illumina	98.4%	188	1,263,452	10	52x	37.11
	Hybrid	98.3%	67	2,635,639	5	30x	37.24
ET-2022-53	Illumina	98.4%	76	1,510,722	8	53x	37.17

[1] N50 is the length for which the collection of all contigs of that length or longer cover at least half of the assembly.
[2] L50 is the number of contigs equal to or longer than N50 (in other words, the minimal number of contigs that cover half the assembly).

Supplementary Table S3. Genome accession and species names of the 79 publicly available genomes of *Fusarium graminearum* species complex isolates obtained from NCBI and used in the phylogeny depicted in Figure 4.

Genome Accession	Species Names
GCA_012600195.1	*Fusarium_cerealis*
GCA_013184295.1	*Fusarium_brasilicum*
GCA_013184305.1	*Fusarium_cortaderiae*
GCA_013364965.1	*Fusarium_austroamericanum*
GCA_013618305.1	*Fusarium_asiaticum*
GCA_013618375.1	*Fusarium_culmorum*
GCA_014822065.1	*Fusarium_acaciae-mearnsii*
GCA_016952355.1	*Fusarium_culmorum*
GCA_001717825.1	*Fusarium_meridionale*
GCA_001717845.1	*Fusarium_asiaticum*
GCA_001717855.1	*Fusarium_meridionale*
GCA_017656575.1	*Fusarium_vorosii*
GCA_017656585.1	*Fusarium_ussurianum*
GCA_017656615.1	*Fusarium_vorosii*
GCA_017656675.1	*Fusarium_nepalense*
GCA_017656685.1	*Fusarium_ussurianum*
GCA_017656695.1	*Fusarium_ussurianum*
GCA_017656735.1	*Fusarium_nepalense*
GCA_017656745.1	*Fusarium_mesoamericanum*
GCA_017656775.1	*Fusarium_louisianense*
GCA_017656785.1	*Fusarium_meridionale*
GCA_017656825.1	*Fusarium_louisianense*
GCA_017656835.1	*Fusarium_gerlachii*
GCA_017656845.1	*Fusarium_gerlachii*
GCA_017656885.1	*Fusarium_cortaderiae*
GCA_017656915.1	*Fusarium_cortaderiae*
GCA_017656935.1	*Fusarium_cortaderiae*
GCA_017656945.1	*Fusarium_boothii*
GCA_017656955.1	*Fusarium_brasilicum*
GCA_017656985.1	*Fusarium_boothii*
GCA_017656995.1	*Fusarium_boothii*
GCA_017657025.1	*Fusarium_austroamericanum*
GCA_017657035.1	*Fusarium_austroamericanum*
GCA_017657045.1	*Fusarium_aethiopicum*
GCA_017657095.1	*Fusarium_asiaticum*
GCA_017657105.1	*Fusarium_acaciae-mearnsii*
GCA_017657115.1	*Fusarium_acaciae-mearnsii*
GCA_017657135.1	*Fusarium_acaciae-mearnsii*
GCA_018219515.1	*Fusarium_graminearum*
GCA_018345515.1	*Fusarium_graminearum*
GCA_018345745.1	*Fusarium_graminearum*
GCA_018346015.1	*Fusarium_graminearum*
GCA_018346165.1	*Fusarium_graminearum*
GCA_018346265.1	*Fusarium_graminearum*
GCA_018346495.1	*Fusarium_graminearum*
GCA_018346565.1	*Fusarium_graminearum*
GCA_019055205.1	*Fusarium_cerealis*
GCA_019055245.1	*Fusarium_culmorum*

GCA_002093855.1	*Fusarium_praegraminearum*
GCA_020991245.1	*Fusarium_graminearum*
GCA_023242275.1	*Fusarium_graminearum*
GCA_000240135.3	*Fusarium_graminearum*
GCA_025258505.1	*Fusarium_asiaticum*
GCA_025427435.1	*Fusarium_asiaticum*
GCA_025427455.1	*Fusarium_asiaticum*
GCA_025427465.1	*Fusarium_asiaticum*
GCA_025427475.1	*Fusarium_asiaticum*
GCA_025427855.1	*Fusarium_asiaticum*
GCA_025427995.1	*Fusarium_asiaticum*
GCA_025428395.1	*Fusarium_asiaticum*
GCA_032355295.1	*Fusarium_meridionale*
GCA_003670145.1	*Fusarium_subtropicale*
GCA_037074405.1	*Fusarium_graminearum*
GCA_037074415.1	*Fusarium_graminearum*
GCA_037179535.1	*Fusarium_vorosii*
GCA_037179565.1	*Fusarium_vorosii*
GCA_037213105.1	*Fusarium_graminearum*
GCA_037255895.1	*Fusarium_graminearum*
GCA_000966645.2	*Fusarium_graminearum*
GCA_900073075.1	*Fusarium_graminearum*
GCA_000599445.1	*Fusarium_graminearum*
GCA_000974265.2	*Fusarium_pseudograminearum*
GCA_000303195.2	*Fusarium_pseudograminearum*
GCA_009617505.1	*Fusarium_austroamericanum*
GCA_009617525.1	*Fusarium_austroamericanum*
GCA_009617495.1	*Fusarium_cortaderiae*
GCA_009617515.1	*Fusarium_meridionale*
GCA_900044135.1	*Fusarium_graminearum*
GCA_001703955.2	*Fusarium_pseudograminearum*

Supplementary Table S4. Disease severity (0 - 5) ± standard error of infected wheat heads and the corresponding species and sample sizes.

Species	Disease Severity	N
FGSC	3.3 ± 0.3	12
Epicoccum species	3.6 ± 0.2	29
Alternaria species	3.6 ± 0.5	10
Fusarium species	4.2 ± 0.3	10

Supplementary Table S5. Deoxynivalenol (DON) and nivalenol (NIV) values reported as parts per million (ppm) for wheat flour ground from wheat heads collected in Ethiopia in 2022 predominately in the Oromia region across 10 locations[*].

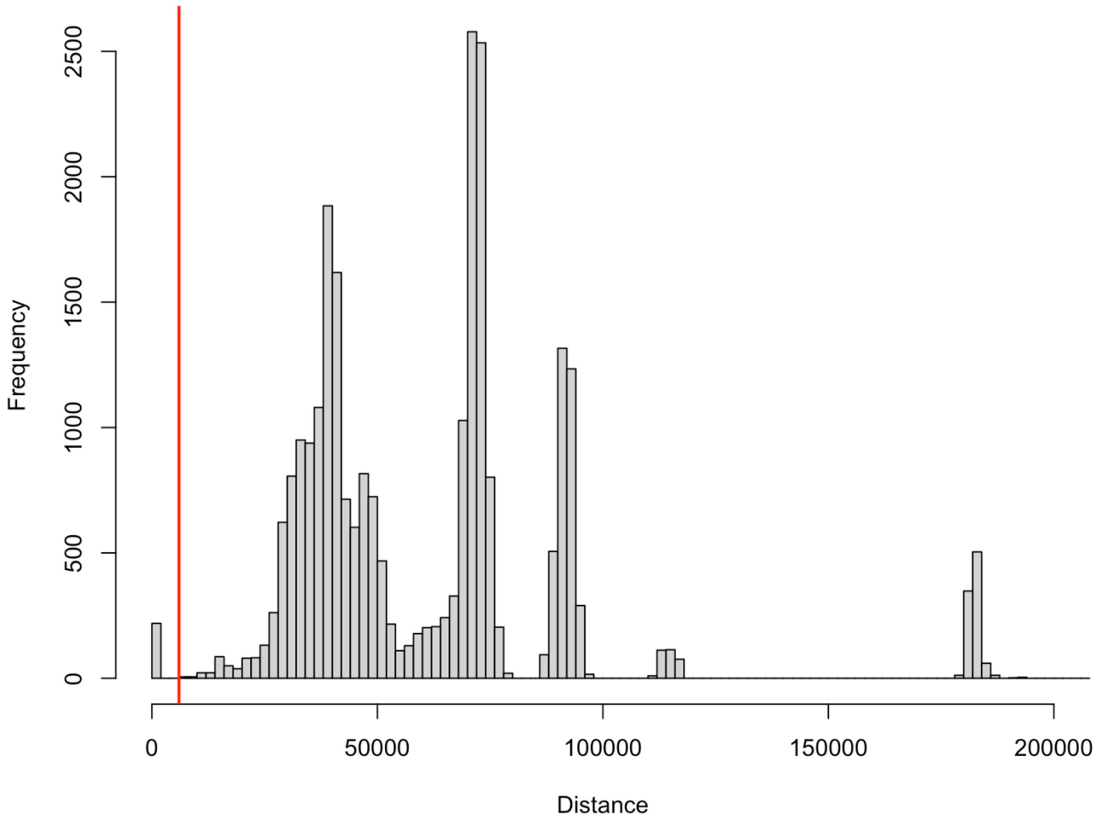
Sample Number	DON (ppm)[1]	NIV (ppm)[1]
KLB113	nd	0.57
KLB114	0.19	0.15
KLB115	nd	0.27
KLB116	0.23	nd
KLB117	0.99	0.08
KLB118	nd	nd
KLB119	nd	nd
KLB120	nd	nd
KLB121	nd	nd
KLB122	nd	nd
KLB123	0.77	1.2
KLB124	nd	nd
KLB125	nd	nd
KLB126	1.9	nd
KLB127	nd	nd
KLB128	1.1	0.74
KLB129	nd	nd
KLB130	1.3	0.24
KLB131	1.0	nd
KLB132	nd	nd
KLB133	0.42	nd
KLB134	0.31	nd
KLB135	5.4	0.71
KLB136	4.9	0.52
KLB137	0.29	nd
KLB138	0.32	nd
KLB139	0.34	0.17

[1] No detectable (nd) levels reported for mycotoxins that are 0.00 ppm to 0.05 ppm.
[*] There were no detectable levels of the mycotoxin NX2.

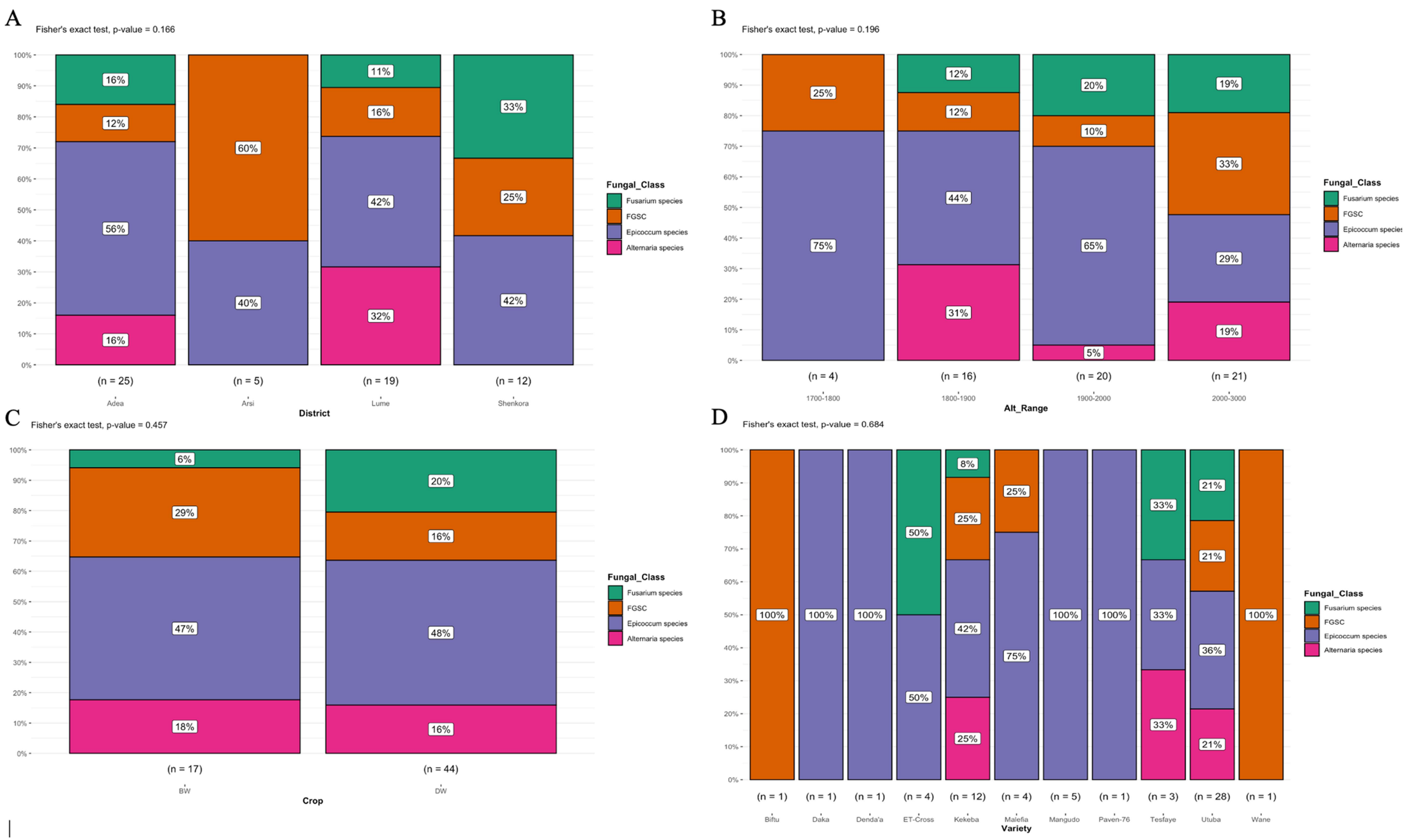
Supplementary Table S6. Total trichothecene contamination (ppm) ± standard error for wheat flour ground from wheat heads based on district and altitude of collection and the corresponding fusarium head blight severity ranking.

		Total Trichothecene Contamination (ppm)	N
District	Adea	0.4 ± 0.2	12
	Tiyo	2.1 ± 1.1	6
	Lume	0.9 ± 0.3	7
	Minjar Shenkora	0.0 ± 0.0	1
Altitude Range (m.a.s.l.)[1]	1800-1900	0.5 ± 0.3	6
	1900-2000	0.4 ± 0.2	10
	2000-3000	1.7 ± 0.7	10
Fusarium Head Blight Severity Rating (0-5)	2	1.1 ± 0.8	2
	3	0.8 ± 0.3	9
	4	2.1 ± 1.2	6
	5	0.3 ± 0.1	9

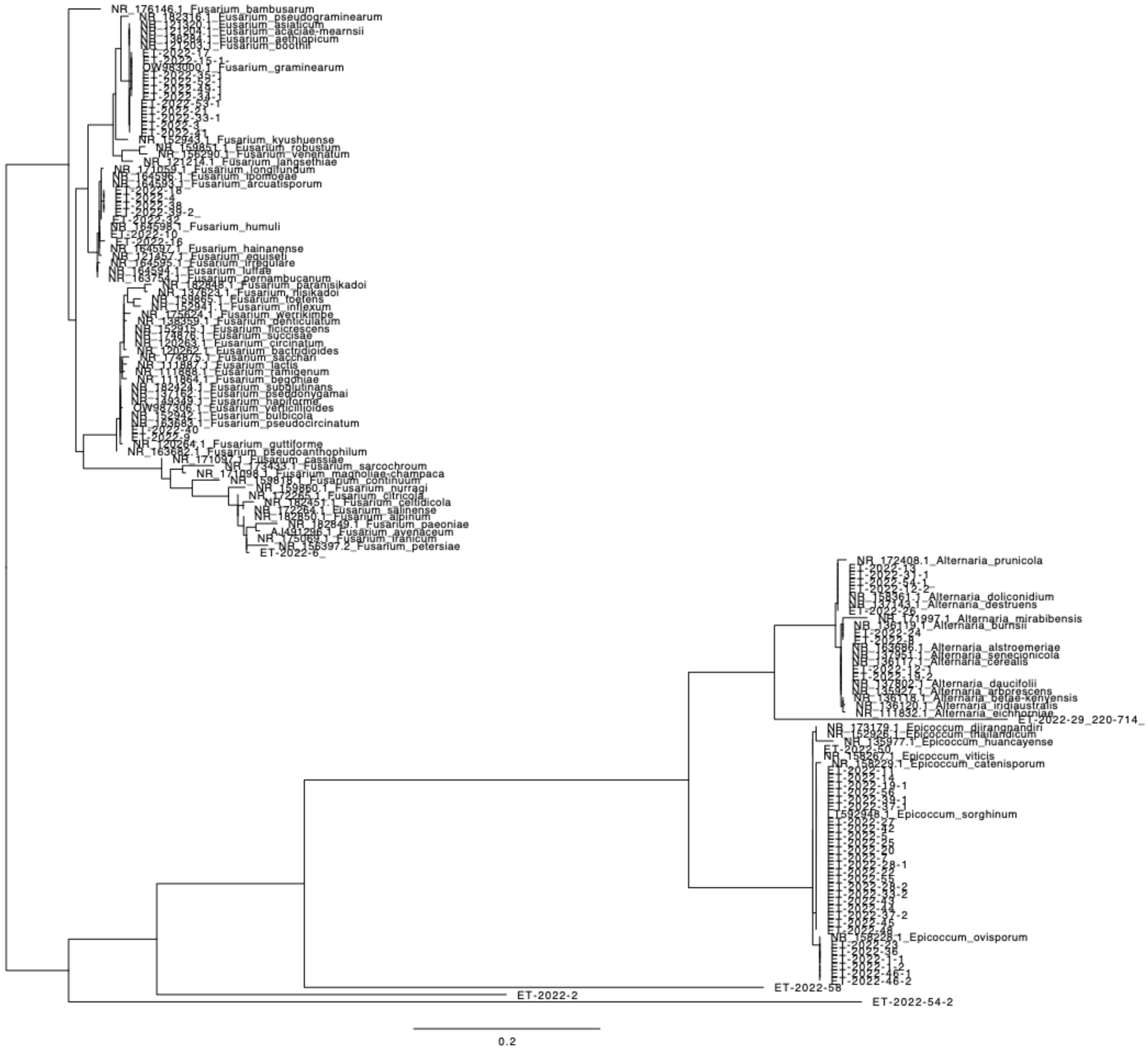
[1] Meters above mean sea level (m.a.s.l.).



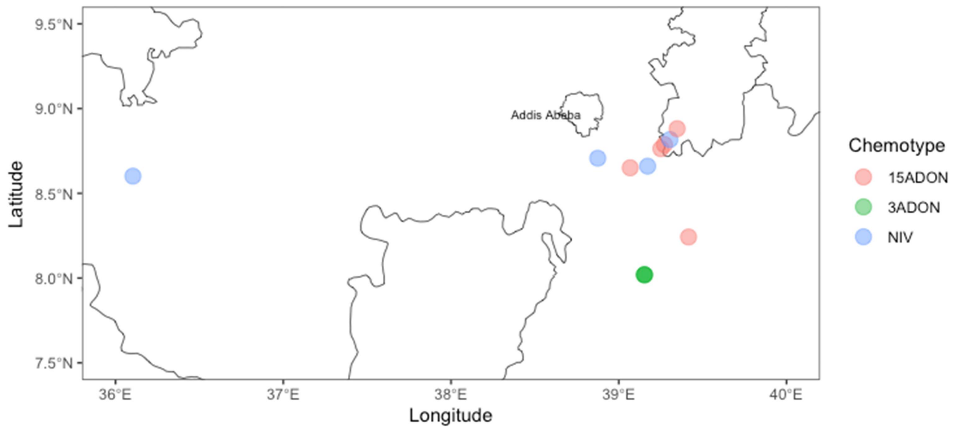
Supplementary Figure S1. A histogram of pairwise genetic distances between isolates as inferred from distance matrix constructed from 1,268,021 SNPs using plink. Based on the distribution of distances, a peak corresponding to the most closely related isolates was identified and a distance value cutoff was selected to separate isolates wit this relationship from those with more genetic distance. All associations between isolates with a distance less than the cutoff, were defined as clones. The x-axis has been truncated to 200,000 to remove comparisons to distantly related species that were irrelevant for this selection. The selected cutoff is marked with a red vertical line.



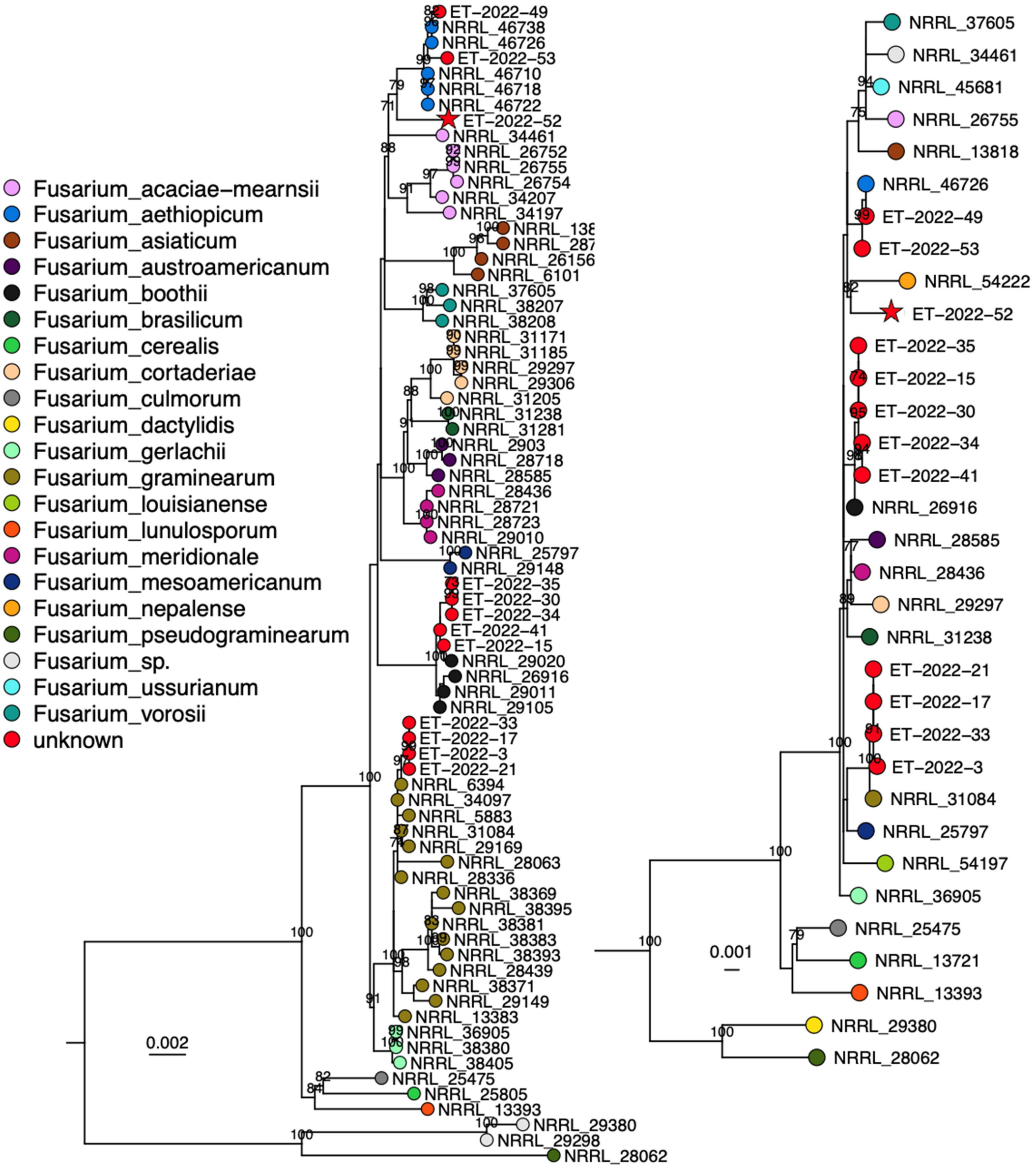
Supplementary Figure S2. Mosaic plots depicting the distribution genera of isolates collected from infected wheat as grouped by (A) district; (B) altitude, (C) crop, and (D) wheat variety. The p-values reported are based on Fisher's exact test. The isolates of *Aspergillus*, *Exserohilum*, and *Penicillium* were removed from this analysis as only one sample was collected for each genus, resulting in a total of 61 isolates represented in the graphs.



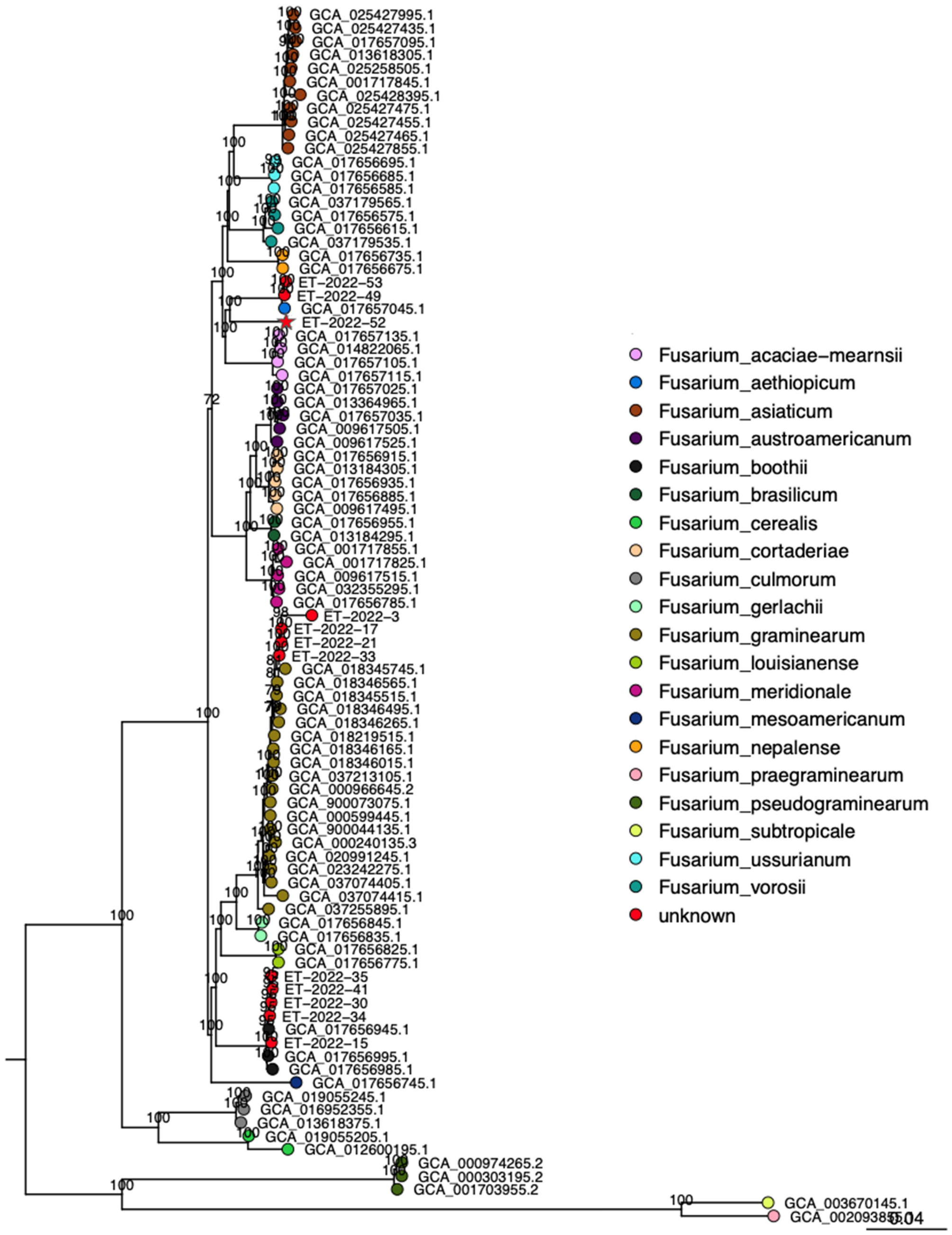
Supplementary Figure S3. Maximum likelihood tree based on ITS sequences of all 64 Ethiopian isolates (ET-2022) and publicly available Sordariomycetes from NCBI. The tree is rooted at the common ancestor of Sordariomycetes. Isolates collected in this study are designated with names beginning with "ET". All publicly available data are labeled with the NCBI ITS accession number followed by the corresponding species.



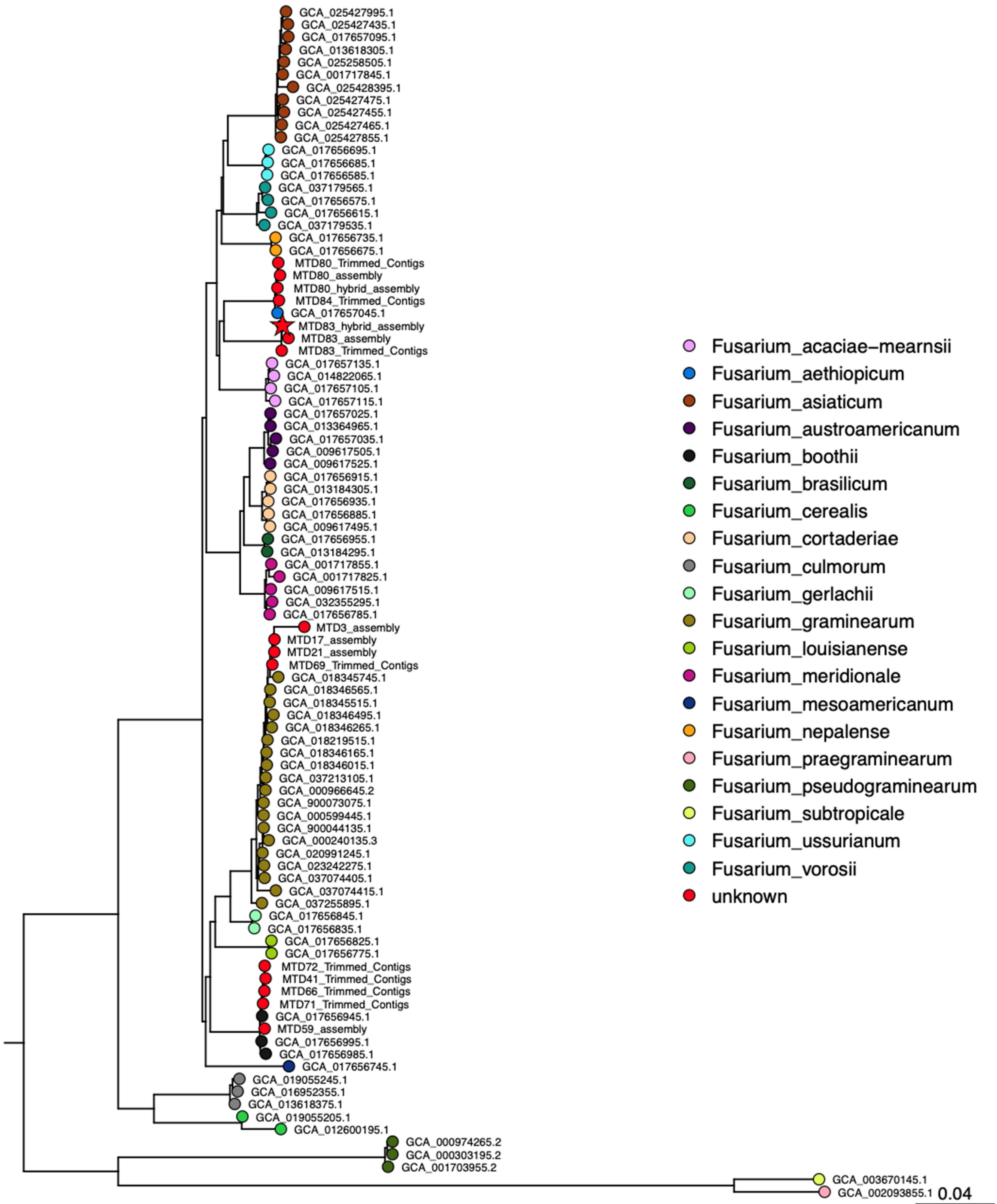
Supplementary Figure S4. Location of FGSC isolates on a zoomed in Ethiopian map (full map found in Figure 1) and color coded by chemotype. Five isolates were classified as 15-acetyl,deoxynivalenol (15ADON), three as 3-acetyl,deoxynivalenol (3ADON) (all within the same location), and four as nivalenol (NIV).



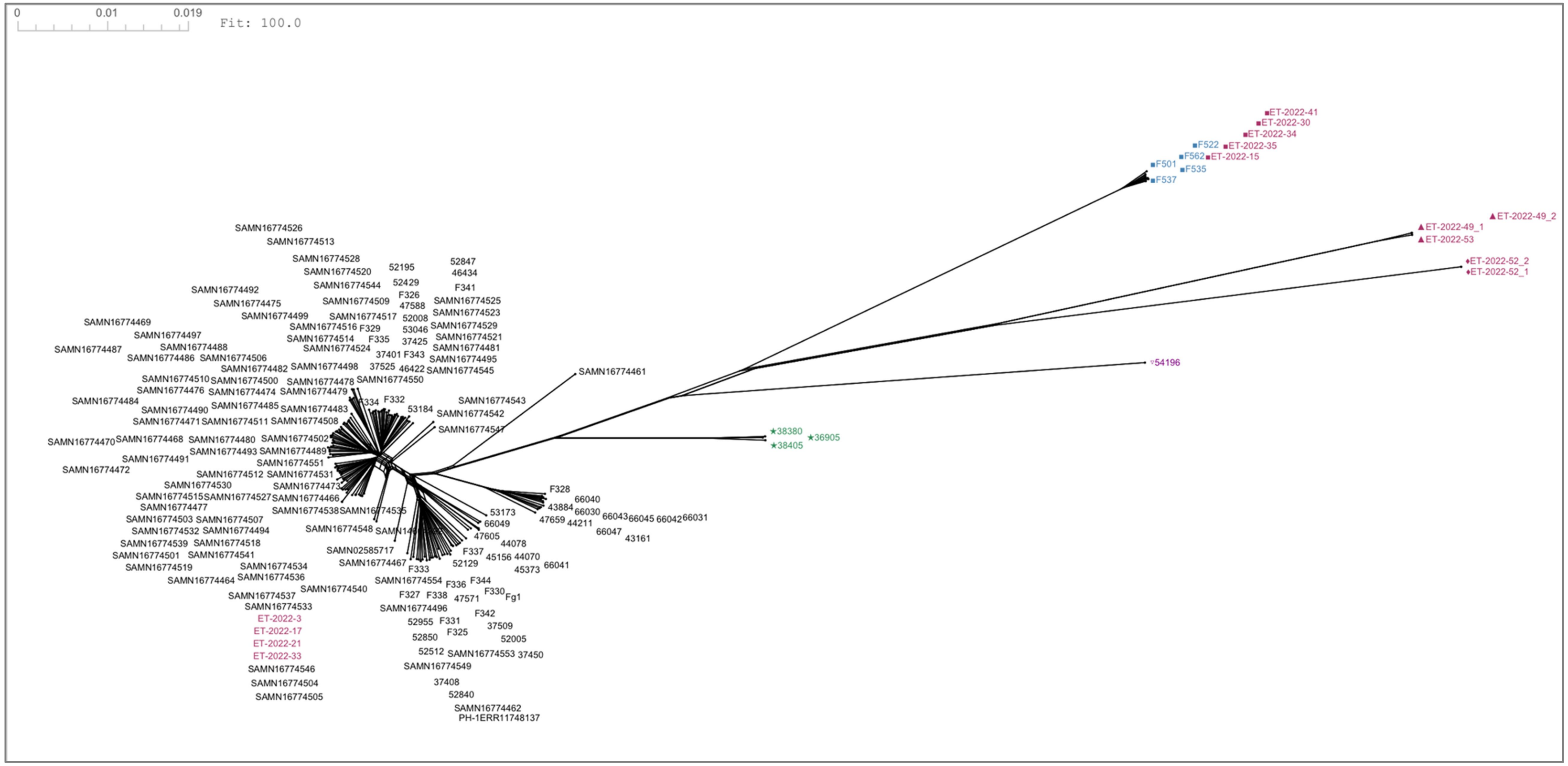
Supplementary Figure S5. Two maximum likelihood trees of the Fusarium graminearum species complex inferred through a partition model of concatenated data from O'Donnell et al. (2008) (left) and Laraba et al. (2021) (right) respectively. The O'Donnell et al. tree was created using five housekeeping genes: translation elongation factor (TEF1), histone H3, internal transcribed spaces 28S ribosomal RNA (ITS28S), alpha-tubulin (atub), and reductase gene (rg). The Laraba et al. tree was created using three housekeeping genes: RNA polymerase II largest subunit (RPB1), RNA polymerase II second largest subunit (RPB2), and TEF1. In both cases, genes corresponding to these sequences were also extracted from whole-genome assemblies of Ethiopian isolates generated in this study and used to incorporate our isolates into the respective phylogenies. All Ethiopian isolates have names beginning with "ET" and are marked with red tips that indicate an 'unknown' species, however, ET-2022-52 (marked with a star tip) was the only isolate that could not be placed into a species based on phylogenetic relationships evident in the trees. We describe ET-2022-52 as a novel species *Fusarium kistleri* in the main text. Both trees are rooted based on the known divergences as depicted in their respective manuscripts. Branches that received > 70% support from ultra fast bootstrapping performed in IQtree are labeled. Tips labeled '*Fusarium* species' reflect designations given in the original datasets.



Supplementary Figure S6. A maximum likelihood tree depicts the genetic relationships between 12 FGSC isolates (indicated with red tips) from the 2022 outbreak and 79 publicly available genomes. This figure is identical to the phylogeny presented in Figure 4, but has branches that received > 70% support from ultra fast bootstrapping performed in IQtree labeled Relationships were inferred using a partition model of 2,580 concatenated benchmarking single copy orthologs (BUSCOs) that were present in all isolates. Isolates from this study are labeled beginning with “ET” while all public data are named after their NCBI genomic accessions. A single isolate ET-2022-52 is marked with a star in the phylogeny to indicate that it is described as a novel species, *Fusarium kistleri*, in the main text.



Supplementary Figure S7. A maximum likelihood tree based on the 79 publicly available genomes of FGSC isolates from NCBI. This figure is nearly identical to that presented in Figure 4, but contains Nanopore, Illumina, and Hybrid assemblies for MTD83 (ET-2022-52) and MTD80 (ET-2022-49). The hybrid assembly of MTD83 is marked with a star in the phylogeny to indicate that it is described as a novel species, *Fusarium kistleri*, in the main text. The tree was constructed based on **all** the 2,580 BUSCOs. Isolates from this study are labeled beginning with "MTD" while all public data are named after their NCBI genomic accessions.



Supplementary Figure S8. Neighbor-net network depicting the genetic relationships of *Fusarium graminearum* species complex isolates from this study contextualized in public data from Kulik et al. (2022) and Kelly and Ward (2018). The network was created using SplitsTree (v. 6.1.16) with 1,268,021 SNPs from 176 isolates. The *Fusarium graminearum* species complex isolates from Ethiopia are color coded in deep pink. *F. graminearum* isolates are labeled in black, *F. gerlachii* are labeled in sea green with a star, *F. louisianense* are labeled in purple with an upside-down triangle, *F. boothii* are labeled in blue with a squared, *F. aethiopicum* are triangled, and *F. unknown* is diamonded. Isolates ET-2022-49 and ET-2022-52 are both presented twice (labeled 1 and 2) to reflect two distinct Illumina and nanopore datasets corresponding to these isolates. Importantly, both datasets suggest identical phylogenetic placement.