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Phylogenetically Diverse *Fusarium* Species Associated with Sorghum (*Sorghum Bicolor* L. Moench) and Finger Millet (*Eleusine Coracana* L. Garten) Grains from Ethiopia

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Abstract: *Fusarium* is one of the most diverse fungal genera affecting several crops around the world. This study describes the phylogeny of *Fusarium* species associated with grains of sorghum and finger millet from different parts of Ethiopia. Forty-two sorghum and 34 finger millet grain samples were mycologically analysed. All of the sorghum and more than 40% of the finger millet grain samples were contaminated by the *Fusarium* species. The *Fusarium* load was higher in sorghum grains than that in finger millet grains. In addition, 67 test isolates were phylogenetically analysed using *EF-1α* and β-tubulin gene primers. Results revealed the presence of eight phylogenetic placements within the genus *Fusarium*, where 22 of the isolates showed a close phylogenetic relation to the F. incarnatum–equiseti species complex. Nevertheless, they possess a distinct shape of apical cells of macroconidia, justifying the presence of new species within the *Fusarium* genus. The new species was the most dominant, represented by 33% of the test isolates. The current work can be seen as an important addition to the knowledge of the biodiversity of fungal species that exists within the *Fusarium* genus. It also reports a previously unknown *Fusarium* species that needs to be investigated further for toxin production potential.

Keywords: beta-tubulin gene; DNA sequence; elongation factor gene (EF1-α); species diversity

1. Introduction

The genus *Fusarium* is one of the most diverse fungal pathogens of plants and animals, including human beings. Fungi that belong to this genus are considered to be among the most harmful pathogens of cultural plants all over the world [1]. In addition to causing substantial yield and quality reduction on crop plants [2,3], members of this genus are also known as producers of important mycotoxins that affect the health of human beings and animals alike [4–6]. *Fusarium* species are widely distributed across the world, inhabiting the majority of bioclimatic regions and ecosystems [7,8]. The ability of *Fusarium* spp. to survive under diverse environmental conditions and their potential to infect a wide array of plants, both in natural and managed ecosystems, have contributed a great deal to attaining an immense diversity within the genus. As a result, *Fusarium* is known as the single most important toxigenic fungi with a confusing and unstable taxonomic history [9]. Many studies that aim to characterize

fungal population structures, including that of the genus *Fusarium*, largely depended on conserved homologues genes [10]. Nowadays, there is an increased interest to utilize multilocus approaches to assess population structures of fungi, including that of *Fusarium* spp. [11–14].Since the early report in 1935 [15], much research has been conducted to resolve the diversity of the genus *Fusarium*, including phylogenetic relationships between and within species. On the other hand, research on *Fusarium* spp. from sorghum has been given only peripheral importance until recently [16], while those from millets are largely ignored. As a result, the diversity and population structure of *Fusarium* spp. associated with sorghum and finger millets is poorly understood. Yet the two crops are important parts of production systems and daily diet for millions of people around the world, especially in the resource-poor drylands of tropical Africa and Asia.

In Ethiopia alone, 4,219,257.2 metric tons of sorghum was harvested from about 1,854,710.93 hectares of land, making it the fourth and third important cereal in total production and area coverage, respectively, [17]. On the other hand, 940,246.3 metric tons of finger millet grains were harvested from 465,508 hectares of land, and this made the crop the sixth most important cereal in Ethiopia [17]. All the sorghum and finger millets are produced, stored, and sold by subsistence farmers.

Toxigenic fungi and associated mycotoxins are reported from sorghum and finger millet grains in Ethiopia by some (rather limited) previous works [18–22]. However, the identity and diversity of *Fusarium* spp. infecting sorghum and finger millet grains are not yet ascertained. Hence, the current work was conducted with the objective to unravel the genetic diversity of the genus *Fusarium*, associated with the two crops in Ethiopia. Results of this work would help to better understand the phylogeny and evolutionary relationship of members of this important pathogenic and toxigenic fungus, as the country is one of the major centers of origin and diversity for the two crops.

2. Materials and Methods

2.1. Isolate Collection

A total of 76 (42 sorghum and 34 finger millet) grain samples that were meant for household consumption and sale on the local markets were collected from farmers' stores in five districts of Northwest, Central, and South Ethiopia (Table 1). Sample collection districts are known producers of the two crops but they were largely ignored by previous studies in the field of toxigenic fungi. Samples (1 kg each) were properly labeled with the name of the location and GPS coordinates, and stored at 4 °C until isolation.

Table 1. Geographic origin of *Fusarium* isolates.

Geographic Location	No. of Samples Collected		Altitude (m)	Latitude	Longitude
	Sorghum	Finger Millet			
South	16	5	1297–1590	5°19′–5°42′	37°22′–37°27′
Central	6	9	1846–1915	7°18′–7°24′	38°38′–38°40′
Northwest	20	20	1054–1465	11°04′–11°19′	36°20′–36°25′
Total	42	34			

2.2. Isolation, Identification, and Storage of the Isolates

Grain samples were surface-sterilized using 1% sodium hypochlorite (NaOCl) solution for 90 s, and rinsed three times in sterile, distilled water. The surface-sterilized grains were placed on potato dextrose agar (PDA) and incubated at 25 °C under continuous fluorescent light for 10 days. After 10 days of incubation, sporulation was observed in the PDA plates. Isolates were tentatively identified as *fusaria*, based on pigmentation and conidial shape [23]. For confirmation, each isolate was transferred to Spezieller Nährstoffarmer Agar (SNA) plates, incubated under 12 h light–dark cycles with UV and daylight color fluorescent lights at 25 °C, and the shapes of macroconidia were assessed after 15 days. Pure cultures of each *Fusarium* isolate were maintained on PDA and stored at 4 °C as stock cultures.

2.3. Grain Contamination by Fusarium Species

The number of colony-forming units (CFU) of *Fusarium* species per gram of grain was determined as follows [19]: Ten grams of each sample was surface-sterilized by a quick rinse in 70% isopropanol and soaked for 1–2 min in 1% sodium hypochlorite solution. The samples were ground in 90 mL of saline solution (composed of 0.58 g NH_2PO_4, 4 g NaCl, and 2.6 g Na_2HPO_4 in 1 L distilled water) with an ultraturrax for 30 s, and 2 mL of the resulting suspension was pipetted into 14.5 cm sterile petri plates in three replications. About 50 mL of autoclaved *Fusarium* selective medium, Nash and Snyder medium (15 g Peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 750 mg PCNB (Pentachloronitrobenzene), and 20 g agar to 1 L distilled water) [23], was poured into each of the petri plate. The plates were incubated at 25 ± 2 °C for 7 days and colony-forming units were counted. In the end, colony-forming units (CFU) were calculated per g of seed.

2.4. Molecular Characterization

2.4.1. DNA Extraction

Each single spore isolate was grown on PDA for seven days under dark conditions at room temperature. Approximately 100 mg of fresh mycelium per isolate was crushed in liquid nitrogen, using a mortar and pestle. The fine powder of mycelium was transferred to a 2 mL microcentrifuge tube and genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA), following the manufacturer's instructions. The quality of the extracted DNA was controlled on 0.8% agarose gel electrophoresis and the DNA was stored at −20 °C.

2.4.2. DNA Sequencing and Species Identification

The elongation factor 1-alpha gene of 67 *Fusarium* isolates was partially sequenced using the EF-1α gene primers [24] (Table 2). The PCR conditions were as follows: Initial denaturation at 94 °C for 5 min, 39 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and primer extension at 72 °C for 45 s and 72 °C for 7 min.

Table 2. Nucleotide sequences of primers used in this study.

Primer	Gene	Sequences	Reference
EF-728F	EF-1α	5′-CATCGAGAAGTTCGAGAAGG-3′	24
EF-986R	EF-1α	5′-TACTTGAAGGAACCCTTACC-3′	24
BT3	ß-tubulin	5′-CGTCTAGAGGTACCCATACCGGCA-3′	25
BT5	ß-tubulin	5′-GCTCTAGACTGCTTTCTGGCAGACC-3′	25

The PCR amplification was carried out in 25 µL reaction volumes mix, containing 2 µL dNTP (2.5 mM), 2.5 µL of 10 × PCR buffer, 0.125 µL of Taq DNA polymerase (5 U/µL), 1 µL of each of the forward and reverse primers (25 pM each), and 4 µL of template DNA. The resulting PCR products were checked on 0.8% agarose gel to assess product quality and estimate the concentration.

The PCR amplicons were sent to GATC Biotecth AG (Konstanz, Germany) for purification of PCR products and DNA sequencing, using the same primers used for PCR amplification. Both the forward and reverse strands were sequenced. The resulting sequences of EF-1α were BLAST searched with the NCBI nucleotide database [25] for molecular species identification.

Furthermore, 50 isolates representing the various EF-groups were sequenced using the ß-tubulin gene primer [26]. PCR conditions for the ß-tubulin gene were set as follows: Initial denaturation at 94 °C for 5 min, followed by 39 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and primer extension at 72 °C for 1 min and 72 °C for 7 min. PCR amplifications, DNA sequencing, and BLAST searching of sequences in the GenBank data base were carried out following the methods described above (for EF-1α). Sequences representing the various phylogenetic groups have been deposited in the GenBank (Table 3).

Table 3. GenBank accession numbers of *Fusarium* species test isolates, representing the different phylogenetic groups.

Isolate	Host	Gene Bank Accession Number		Isolate	Host	Gene Bank Accession Number	
		EF-1α	ß-tubulin			EF-1α	ß-tubulin
2	F. millet	MH765585	MH748458	63	Sorghum	MH765627	NA
6	F. millet	MH765586	MH748461	64	Sorghum	MH765628	MH748496
9	F. millet	MH765587	MH748463	65	F. millet	MH765629	MH748497
10	Sorghum	MH765588	MH748464	68	Sorghum	MH784424	MH748500
12	Sorghum	MH765589	NA	69	Sorghum	MH784425	MH748501
13	Sorghum	MH765590	NA	70	Sorghum	MH784426	MH748502
15	F. millet	MH765592	NA	71	Sorghum	MH784427	MH748503
16	Sorghum	MH765593	NA	72	Sorghum	MH784428	MH748504
17	Sorghum	MH765594	NA	73	Sorghum	MH784429	MH748505
18	F. millet	MH765595	MH748465	74	Sorghum	MH784430	MH748506
19	F. millet	MH765596	MH748466	76	Sorghum	MH784431	MH748507
20	Sorghum	MH765597	MH748467	77	Sorghum	MH784432	MH748508
21	F. millet	MH765598	MH748468	78	Sorghum	MH784433	MH748509
25	Sorghum	MH765600	MH748469	83	Sorghum	MH784434	MH748514
26	F. millet	MH765601	MH748470	87	F. millet	MH784435	MH748517
27	F. millet	MH765602	MH748471	90	Sorghum	MH784436	MH748518
29	F. millet	MH765603	MH748472	91	Sorghum	MH765634	NA
30	Sorghum	MH784416	MH748473	92	Sorghum	MH765635	NA
31	Sorghum	MH784417	MH748474	95	Sorghum	MH765636	MH748520
33	F. millet	MH765605	MH748475	96	Sorghum	MH784437	MH748521
34	F. millet	MH765606	MH748476				
35	F. millet	MH784418	MH748477				
36	F. millet	MH765607	MH748478				
37	F. millet	MH765608	MH748479				
38	Sorghum	MH765609	MH748480				
39	Sorghum	MH765610	NA				
40	F. millet	MH765611	NA				
41	Sorghum	MH765612	MH748481				
42	Sorghum	MH765613	MH748482				
43	F. millet	MH765614	MH748483				
44	F. millet	MH765615	MH748484				
45	F. millet	MH765616	MH748485				
46	Sorghum	MH765617	NA				
47	Sorghum	MH784419	MH748486				
48	F. millet	MH765618	MH748487				
49	Sorghum	MH784420	MH748488				
51	Sorghum	MH784421	MH748490				
52	Sorghum	MH765619	MH748491				
53	Sorghum	MH765620	MH748492				
54	Sorghum	MH784422	MH748493				
55	Sorghum	MH784423	MH748494				
56	Sorghum	MH765621	NA				
57	Sorghum	MH765622	NA				
58	Sorghum	MH765623	NA				
60	Sorghum	MH765624	NA				
61	Sorghum	MH765625	NA				
62	Sorghum	MH765626	NA				

F. millet: Finger millet; NA: Not amplified by the β-tubulin gene primers.

2.4.3. Phylogenetic Analysis

DNA sequences from the present study were aligned with reference sequences obtained from GenBank, using the multiple alignment program, Clustal W [27], and the resulting alignment was manually edited.

Phylogenetic analyses were carried out using the Maximum Likelihood method of the MEGA program version 7.0 [28] on the individual sequences of EF-1α and ß-tubulin genes. The same method was also used to assess the molecular phylogeny of the genus *Fusarium* in previous studies, e.g., [29]. For constructing the trees, sequences of the corresponding genes from the database were retrieved

for as many reference strains as possible. Then, the reference strains that were distant from our test isolates in terms of the phylogenetic resemblance (based on sequence similarity) were excluded and closely related reference strains were included. The closest phylogenetic neighbours to the entire test isolates were retrieved from the NCBI database for the phylogenetic analysis. Statistical significance of the obtained tree was determined by running 5000 bootstrap replications.

3. Results and Discussion

3.1. Grain Contamination by Fusarium Species

All the sorghum grain samples were contaminated with *Fusarium* species, while the contamination frequency of finger millet grain samples varied from 44.4% in Central Ethiopia to 60% in the South region. Marked variations were also evident in terms of CFU among the samples (Table 4).

Table 4. Contamination of sorghum and finger millet grains with *Fusarium* species.

Region	CFU/g Seed of Positive Samples					
	Sorghum			Finger millet		
	Range	Mean	Median	Range	Mean	Median
South	250–753	528.4	524.5	100–442	285	312
Central	241–558	352.7	306	230–500	370	375
Northwest	612–1085	843.4	865	200–780	473.3	450

Current results also revealed the contamination of sorghum and finger millet grains by other fungi, i.e., *Aspegillus*, *Alternaria*, *Penicillium*, *Rhizopus*, and *Epicochum* species, which is in line with previous findings in which cereal grains were found to be contaminated by a multitude of fungi [30,31]. On average, *Fusarium* load was the highest (843 CFU/g) in sorghum grains from Northwest Ethiopia, followed by sorghum grains from South and finger millet grains from Northwest Ethiopia. This could have resulted from the more humid and warm weather conditions in the region, which favor fungal growth and grain contamination. Finger millet grains from Southern Ethiopia had the lowest average *Fusarium* load as compared to the other samples. These results are in agreement with those of previous reports, e.g., [32], in which sorghum was reported to be more likely to be contaminated by fungi than millets and maize. A study in the United States has also reported a mild contamination of another millet type (pearl millet, *Pennisetum glaucum* (L.) R. Br.), [33], suggesting the possibility that these small cereals might be less prone to fungal contamination as compared to other cereals. We have also seen that wheat grains are least contaminated by toxigenic fungi and associated mycotoxins as compared to sorghum and finger millet grains.However, the exact mechanisms of resistance in these crops should be investigated further.

3.2. Morphological Identification

All the *Fusarium* isolates included in the current study fulfilled the morphological characteristics of the genus *Fusarium* [23]. Based on conidial shape, 41 *Fusarium* isolates analysed in the current study were categorised as members of the species complex *Fusarium fujikuroi*, while 23 isolates were grouped within the Fusarium incarnatum–equiseti species complex and three within the *Fusarium oxysporium* species complex.

Although cultural/morphological characterizations provide a basis for both inter- and intra-species diversity studies, they may be unstable, highly influenced by the growth environments, and rather change with the age of the colonies [34–36]. *Fusarium* species delimitations by morphological features alone have also been hampered by the limited variability within such markers [37]. As a result, such taxonomic features need to be supplemented with other characters like molecular markers as reported in previous works, e.g., [38].

3.3. Phylogenetic Inference

Sequencing of the elongation factor 1-alpha (EF-1α) gene was used for species identification and results were supplemented with the ß-tubulin gene sequencing. EF-1α has been recommended as a primary marker for the identification of *Fusarium* isolates to a species level [9,38].

PCR amplification of the EF-1α gene was positive for all the 67 *Fusarium* isolates, while only 50 of the isolates had positive PCR amplification for the ß-tubulin gene primers used in the current experiment. Seventeen isolates, all of which were identified as *F. andiyazi* (based on EF-1 gene sequence analysis), were not amplified by the ß-tubulin gene primer set implemented for others. This might be because of the differences in the of ß-tubulin gene within the genome of *Fusarium* species. To better understand the relationship between the species, phylogenetic trees were constructed for each locus separately [38]. Different genes within the genus *Fusarium* may have different evolutionary histories [29]. As a result, separate analysis of each gene would be appropriate.

Based on sequencing of the EF-1α of 67 isolates, the *fusaria* were categorized into eight species, distributed across five main and 13 sub-clusters (Figure 1). The ß-tubulin gene tree produced three main clusters with seven sub-clusters. Nevertheless, grouping of the *Fusarium* isolates by both the EF-1α and ß-tubulin genes in the current experiment showed a very high level of concordance. Furthermore, main and sub clusters of both trees had high bootstrap values (>70%), confirming the highly significant association between isolates within each group/sub-group. The recovery of diverse *Fusarium* species in the current experiment could be attributed to the existence of various host genotypes and widely ranging environmental conditions in sorghum- and finger millet-producing regions of the country. Such diversity should be considered in future breeding programs to achieve effective and sustainable fungal and mycotoxin management strategy.

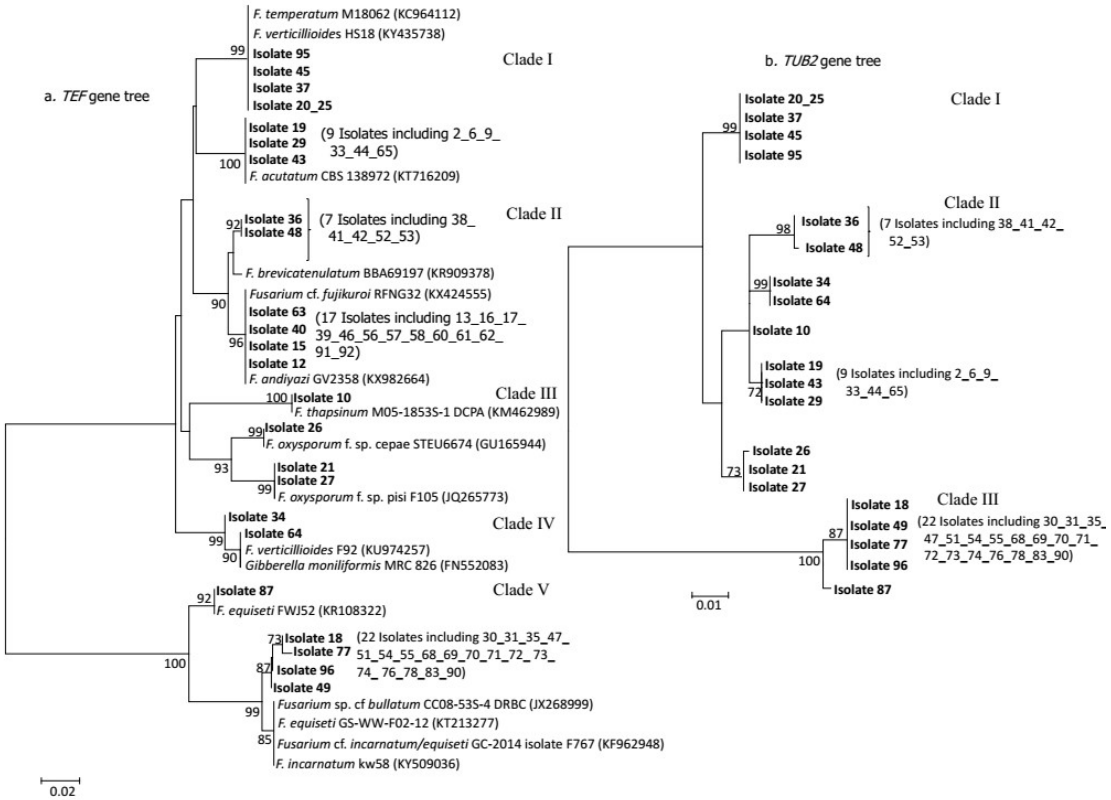

Figure 1. Maximum Likelihood phylogenetic tree showing the diversity of *Fusarium* isolates associated with sorghum and finger millet grains from Ethiopia. (Closely related *Fusarium* species from the NCBI GenBank sequences were included on the EF-1α gene tree for comparison purposes). Bootstrap values (5000 replications) are shown on the branches.

Cluster I of the EF-1α gene tree contained a total of 14 isolates in two sub-groups. The first sub-group had five isolates that clustered together with *F. verticillioides*. These isolates had 100% sequence similarity with that of *F. verticillioides* isolates deposited in the GenBank. *F. verticillioides* is a cosmopolitan pathogenic fungus infecting maize stalks and grains worldwide [23]. The fungus was identified as the most dominant species associated with maize grains from Ethiopia [39]. Its identification from sorghum (three isolates) and finger millet (two isolates) grains in the present study may reveal a wider host range of the species or its variant/s. The remaining nine isolates that make up 13% of the total *Fusarium* isolates included in the present study occupied the second sub-group of main group I, along with *F. acutatum* on the EF-1α gene tree, and the fourth sub-group of main group II on the ß-tubulin gene tree. All of these nine isolates were isolated from finger millet grains from Northwest Ethiopia and they had 100% EF-1α sequence similarity with several isolates of *F. acutatum* in the NCBI GenBank. *F. acutatum* has been earlier described [40]. In a later study, the same species was recovered from sesame seeds in Egypt [41]. However, the present study could be the first to report this species from finger millet grains in Ethiopia.

Cluster II of the EF-1α gene tree consisted of 24 isolates belonging to *F. brevicatenulatum* and *F.andiyazi*. This cluster was further divided into two sub-clusters. The first sub-cluster is made of seven isolates (10% of all the isolates in the present study) that formed the same clade, thus showing 100% sequence similarity with *F. brevicatenulatum*. *F. brevicatenulatum* was first described in 1998 [42] and it was recovered from the parasitic weed (*Striga asiatica*) from Madagascar and from millets in east Africa [23,43]. In our study, isolates that belong to this species were recovered from grains of sorghum and finger millet collected from south and northwest Ethiopia. The same isolates occupied the first sub-cluster of main cluster II of the ß-tubulin gene tree.

The second sub-cluster of cluster II on the EF-1α gene tree was made of 17 (25%) of the total *Fusarium* isolates from the present study. They were identified as *F. andiyazi*, a species that was first described in 2001 [37]. This species was subsequently reported to be present in different parts of the world, including Australia, Ethiopia, Nigeria, South Africa, and the United States [16,23,37,44]. Nevertheless, except for the initial report, no further work has been done on this particular species in Ethiopia to the best of our knowledge. As a result, the diversity of this pathogen remains largely unknown to date. The *Fusarium* isolates that clustered with *F. andiyazi* formed the second sub-group of main group II on the EF-1α gene tree with a bootstrap value of 96%. However, none of these isolates were amplified by ß-tubulin gene primers (both forward and reverse), though we exhaustively tried different PCR conditions to amplify the target gene.

Cluster III of the EF-1α gene tree is made of four isolates, distributed along three sub-clusters. Sub-cluster I consists of one isolate, along with an isolate of *Fusarium thapsinum* with 100% bootstrap. This isolate also formed its own independent sub-cluster within cluster II of the ß-tubulin gene tree. Based on the EF-1α gene, sub-clusters II and III had one and two isolates, respectively, which were grouped along with *F. oxysporium* with high bootstrap value (93%). These three isolates formed the fifth sub-cluster of cluster II of the ß-tubulin gene with high bootstrap value (73%). All the four *Fusarium* test isolates within cluster III had 100% sequence similarity with isolates of the respective species in the GenBank. *Fusarium thapsinum* was first described in 1997 [45] and was associated with sorghum [41,46,47]. The isolate of *F. thapsinum* in our study was recovered from sorghum grains from South Ethiopia. On the other hand, *F. oxysporium* is generally believed to be a vascular wilt pathogen, commonly occurring within the soils, roots, and stems of diverse plants. However, isolates representing this species were recovered from finger millet grains in the present study and from maize, sorghum, and lentil seed from Egypt [41]. Two *Fusarium* isolates (34 and 64) formed cluster IV of the EF-1α gene tree and they were grouped with isolates of *F. verticillioides*. Both isolates also had 100% EF-1α sequence similarity with that of several isolates of *F. verticillioides* in the GenBank. On the ß-tubulin gene tree, these two isolates formed their own sub-cluster within the main cluster of other isolates from the *F. fujikuroi* species complex.

Cluster V has 23 isolates that belong to the *F. incarnatum–equiseti* species complex in three sub-clusters. This group was clearly separated from the rest with 100% bootstrap value. The first sub-cluster contains a single isolate, which had 100% EF-1α sequence similarity with that of *F. equiseti*. This isolate also clustered with an isolate of the same species from the NCBI data base. The remaining two sub-clusters are made of 22 isolates that are clustered along with various strains of the *F. incarnatum–equiseti* species complex. These isolates had 98–99% sequence similarity with various known and unknown species of the species complex like *F. equiseti*, *Fusarium* sp. (FIESC), *Fusarium* cf. *incarnatum/equiseti*, *Fusarium* sp. cf *bullatum*, and *F. incarnatum*. Accordingly, the isolates were identified as members of the *F. incarnatum–equiseti* species complex. However, these isolates exhibited certain morphological distinctions from those of the other members of the species complex (*F. incarnatum* and *F. equiseti*). As a result, they were suggested to represent a novel species (*Fusarium* sp. nov) within the complex. With 22 (33% of the total) isolates, the novel species was the single most dominant *Fusarium* species of all in the present study. They were recovered from sorghum (20 isolates) and finger millet (two isolates) grains from the south and northwest Ethiopia. Isolates belonging to this species complex were identified from various crops across a range of environmental conditions [48]. Similar isolates were also obtained from stalks of sorghum, grown in South Ethiopia in an earlier report [49], suggesting a wider geographic distribution and a lack of specificity in host colonization. On the ß-tubulin gene tree, isolates of the novel species also clustered together with 87% bootstrap value and were clearly separated (100% bootstrap) from all other isolates, except one that belonged to *F. equiseti*. Thus, both the EF-1α and ß-tubulin gene trees were in agreement that all the novel isolates belonged to the same species/group, regardless of their hosts and geographic origin. These isolates also lacked phenotypic variations, in terms of conidial shape, growth rate, and pigmentation (data not shown). Such results further strengthen the possibility that they all belong to a single species. Farmers in different regions of Ethiopia are engaged in formal and informal seed exchanges, and these could have led to the spread of the novel isolates across geographic regions.

Results of the current work reveal the phylogeny of *Fusarium* species associated with sorghum and finger millet grains in Ethiopia, a country considered to be one of the major centers of origin and diversity for the two crops. Current results serve as important additions to the existing knowledge on the genetic diversity of the genus *Fusarium*. Many of the isolates recovered in the current work are in clades with species known to produce mycotoxins. Therefore, further research should be conducted to assess the contamination of grains by mycotoxins and determine whether the isolates are toxigenic. Species description within the genus *Fusarium* is best accomplished by combining morphological and biological traits in addition to phylogenetic studies based on DNA sequences [50]. Future research on morpho-genetic and biological description should be undertaken to assign a taxonomic placement for isolates belonging to the novel group detected in the current work. Such studies should include the sequencing of multiple genes [43,51], studies on sexuality and fertility of the isolates [52], pathogenicity, and morpho-genetic divergence within the species. The recovery of novel isolates from grains in the current experiment and stalks in a previous work [49] may indicate that the fungus could over-season in both parts of the host plants. As a result, use of healthy seeds and removal of crop residues should be considered as important parts of managing the fungus in agricultural fields.

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