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# Characterization of the Coffee Wilt Pathogen in Uganda



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## Abstract

Sixteen isolates of *Fusarium* sp. were isolated from robusta coffee trees in Uganda with symptoms of vascular wilt and characterized based on morphology and partial DNA sequences of translation elongation factor 1-alpha (ef) and beta-tubulin (bena). *Fusarium* sp. isolates from robusta and arabica coffee trees in Zimbabwe, New Caledonia and Ethiopia were also characterized. Phylogenetic analyses indicated that these isolates represent a new species lineage in the African clade of the *Gibberella fujikuroi* species complex (GFC). The species most commonly associated with this disease, *Fusarium xylarioides*, is not a member of GFC, suggesting that recent outbreaks of vascular wilt in Uganda may have been caused by a new pathogen.

## Introduction

The economy of Uganda is based on agriculture, with more than 90% of exports being agricultural products (6, 8, 12). Despite continuous price fluctuations in the world coffee markets coffee cultivation has remained a viable means of livelihood for the people of Uganda. Coffee provides employment for over 2.5 million people who are involved in its cultivation, processing, and trading (14). An estimated 270,000 ha of Uganda's farmland were dedicated to robusta coffee production prior to the appearance of the wilt disease in the early 1990's (14).

Two species of coffee are cultivated in Uganda: *Coffea canephora* Pierre (robusta) and *C. arabica* L. (arabica), with robusta making up 90% of production (14). Until the appearance of coffee wilt disease (CWD) in 1993, robusta coffee was subject only to minor diseases (4). Coffee wilt disease is a vascular wilt caused by *Fusarium xylarioides* Steyaert (*Gibberella xylarioides* Heim and Sacc.). The disease attacks plants at all stages of growth and all infected plants are killed (Fig. 1). Symptoms include wilting, defoliation and blue-black discoloration of the vascular tissue. Coffee berries on diseased plants ripen prematurely and can have brown sunken lesions at the stalk end. Perithecia form readily in the cracks of the stem bark.

Coffee wilt has spread to all robusta growing districts in Uganda, killing an estimated 14 million trees (14). Currently, the only methods of control are improved crop management strategies together with sanitary measures. However these methods do not offer effective disease control and are expensive and impractical to implement. Host resistance offers the best prospect for control and demands very little effort from the farmers. Diversity among isolates of *Fusarium* spp. collected from diseased plants from different locations in Uganda is still largely unknown. The objective of this research was to identify and study the genetic diversity of the coffee wilt pathogen in order to provide information for the breeding of resistant varieties.



Fig. 1. Robusta tree showing CWD symptoms.

## Materials & Methods

### Sample collection and pathogen isolation.

Tissue showing typical CWD symptoms was collected from farmers' fields in nine districts within Uganda (Table 1). Marginal samples of infected tissue were split length wise, surface sterilized in 2% (v/v) Jik, placed onto water agar and incubated at 25°C under fluorescent light for 4 days.

**Table 1.** Isolates collected from stem tissue of diseased *Coffea canephora* Pierre trees in various districts in Uganda. Isolates from Uganda were deposited into the collection of the Fusarium Research Center under the accession numbers listed below.

Isolate Number	Accession Number	Geographic Region (District)
A1	L-0387	Mayuge
A2	L-0388	Mayuge
B1	L-0389	jinja
B2	L-0390	jinja
C1	L-0391	Kamuli
C2	L-0392	Kamuli
D1	L-0393	Iganga
D2	L-0394	Iganga
E1	L-0395	Hoima
E2	L-0396	Hoima
E3	L-0397	Hoima
F	L-0398	Mubende
H	L-0399	Mukono
K	L-0400	Bushenyi
G1	L-0410	Masindi
G2	L-0411	Masindi

### Morphology and colony growth of *Fusarium* isolates.

Cultures were derived from single macroconidia and stored on synthetic low-nutrient agar (SNA) slants at 4°C or lyophilized as described by Nelson et al. (7). To study morphological characteristics the isolates were grown on PDA and SNA at 25°C with a 12 hr

photoperiod. To compare mycelial growth rates, 2 mm plugs were centrally placed on petri dishes of PDA and SNA. Four replicates of each isolate were prepared. Two perpendicular measurements of the diameter of the cultures were taken starting after the fifth day of growth and continuing every 2 days until day 14. The experiment was repeated once and data were analyzed by analysis of variance (ANOVA) using SAS statistical software. To determine macro- and microconidia size, 20 spores (stained with lactophenol cotton blue) for each isolate were measured by viewing them with a compound microscope. Data were analyzed using GLM of SAS and the means separated using LSD.

### Pathogenicity tests.

Five clones of robusta coffee at the 7-month old stage were inoculated by soaking the roots in 500 ml of spore suspension ( $1.3 \times 10^5$  conidia/ml) for 2 hours. Control plants were soaked in sterile distilled water. All were placed under polythene cover to provide high humidity for 24 hours and then placed at random in a screen house. Plants were rated for latent periods (number of days from inoculation to first symptoms) and incidence 90 days after inoculation. Data were analyzed using GLM of SAS and the means separated using LSD.

### DNA extraction, PCR and nucleotide sequencing.

DNA was extracted from mycelium using the methods described by Lee and Taylor (5). PCR amplification and sequencing of the  $\beta$ -tubulin (bena) gene region was performed using primers benat1 and benat22 (9). The translation elongation factor 1- $\alpha$  (tef) gene was also amplified and sequenced using primers ef1 and ef2 (10). Sequences were generated at the Plant-Microbe Genomics Facility, OSU. Base calling and sequence quality were determined using the software Phred (2,3). Sequencher was used to assemble, edit and generate high quality sequences.

### Phylogenetic analysis

BLAST (1) was used to perform similarity searches comparing CWD sequences with those in the GenBank database and local sequence databases. Based on the BLAST results, sequences were aligned by eye into files containing DNA sequences representing the phylogenetic breadth of the GFC (11). Maximum parsimony, Neighbor-Joining and Maximum Likelihood analyses were performed using the PAUP\* phylogenetics package (beta versions 4.0b9-11: (13)). For maximum parsimony analyses, alignment gaps were considered missing sites, and heuristic searches were performed using random sequence addition (10 replicates) and TBR branch-swapping. Neighbor-joining analyses were performed using the Kimura 2-parameter model. Bootstrapping was performed using simple sequence addition, with MAXTREES set at 100,000 trees.

# Results

## Growth and morphology on PDA and SNA.

Colonies on PDA and SNA were pale orange 4-14 days after plating depending on the isolate. The orange color was more intense in the center of the colony and faded to pale orange to white at the growing margins. Colony appearance on the reverse was the same. Orange spore-forming pinnodes were produced in concentric rings by day 4. Hyaline mycelium was sparse and oppressed. Aerial mycelium bore sporodochia on which macroconidia were formed. Microconidia were also formed on aerial mycelium but not in sporodochia. Mean growth rates were significantly different among isolates on both PDA and SNA (Table 2).

**Table 2.** Mean growth rates (mm/day) of CWD isolates grown on PDA and SNA

Isolate	Growth Rate (mm/day)	
	PDA	SNA
A1	3.45 bcd*	3.58 a-d
A2	4.20 a	4.00 ab
B1	3.83 abc	4.8 a
B2	370 a-d	3.20 cd
C1	3.83 abc	3.83 a-d
C2	4.12 a	3.79 a-d
D1	4.16 a	3.70 a-d
D2	3.91 ab	3.70 a-d
E1	3.50 bcd	3.16 d
E2	3.87 abc	3.16 d
E3	3.45 bcd	3.25 bcd
F	4.16a	4.33 a
G1	3.25 d	3.62 a-d
G2	3.95 ab	3.95 abc
H	4.04 a	4.25 a
K	3.37 ad	3.62 a-d

\* values in a column followed by the same letter are not significantly different at P=0.05.

## Spore shape and size.

Macroconidia and microconidia were produced on both PDA and SNA (Fig. 2). Macroconidia had 1-4 septa and were strongly curved with hooked apices and marked foot cells. They ranged in size (length x width) from 12.5 – 45.5 x 2.8 – 3.8 μm on PDA and 12.5 – 47.5 x 2.5 – 4.5 μm on SNA. Microconidia were unicellular, allantoid and formed 0-1 septa. They ranged in size from 5.0 – 10.0 x 2.0 – 2.7 μm on PDA and 5.0 – 12.5 x 2.0 – 2.7 μm. Size differences between isolates were not statistically significant.

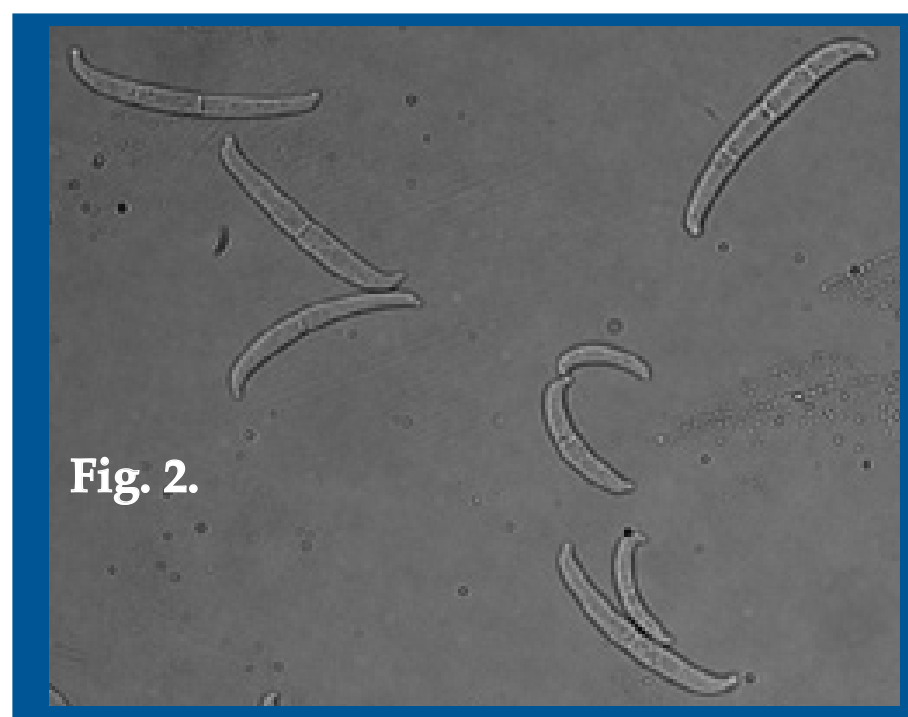


Fig. 2.

## Pathogenicity tests.

All the isolates were pathogenic on clones 1s/2, 1s/3, and 223/32 and 257/53 with disease incidence varying among the clones (Table 3). Isolates A1, B1, C1, E1, E3, F, G1 and G2 were non-pathogenic on clone 258/24(0). Symptoms on inoculated plants included wilting of leaves followed by defoliation, dieback from the apices and eventually death of plants. Latent period varied among isolates and clones as indicated in Table 3.

**Table 3.** Percent incidence and latent period of CWD isolates from Uganda on cultivars of *Coffea canephora* grown under screen house conditions. Ten plants of each cultivar were used with three replications.

	cv. 1st/s		cv. 1s/3		cv. 223/32		cv. 257/53		cv. 258/24(0)	
	L.P.	% Inc.	L.P.	% Inc.	L.P.	% Inc.	L.P.	% Inc.	L.P.	% Inc.
A1	45	100	54	83.3	46	91.7	55	100	N.S.	0
A2	50	100	44	100	49	100	54	82.2	65	100
B1	58	100	63	88.9	50	91.7	54	94.4	N.S.	0
B2	51	100	44	100	49	100	53	100	63	91.7
C1	58	88.2	44	100	47	100	49	100	N.S.	0
C2	45	100	56	94.4	57	100	50	100	50	33.3
D1	68	88.8	54	94.4	51	100	40	100	56	8.3
D2	61	94.4	46	100	55	100	47	100	56	8.3
E1	61	77.7	54	94.4	49	83.6	58	91.7	N.S.	0
E2	59	100	57	100	46	100	63	66.6	57	100
E3	51	100	49	94.4	47	100	43	100	N.S.	0
F	47	88.9	57	94.4	53	100	50	100	N.S.	0
G1	60	100	52	94.4	46	91.7	53	100	N.S.	0
G2	65	100	56	100	57	100	58	100	N.S.	0
H	65	88.9	58	94.4	50	91.7	41	100	51	25.0
K	55	100	66	88.3	51	100	52	100	56	100

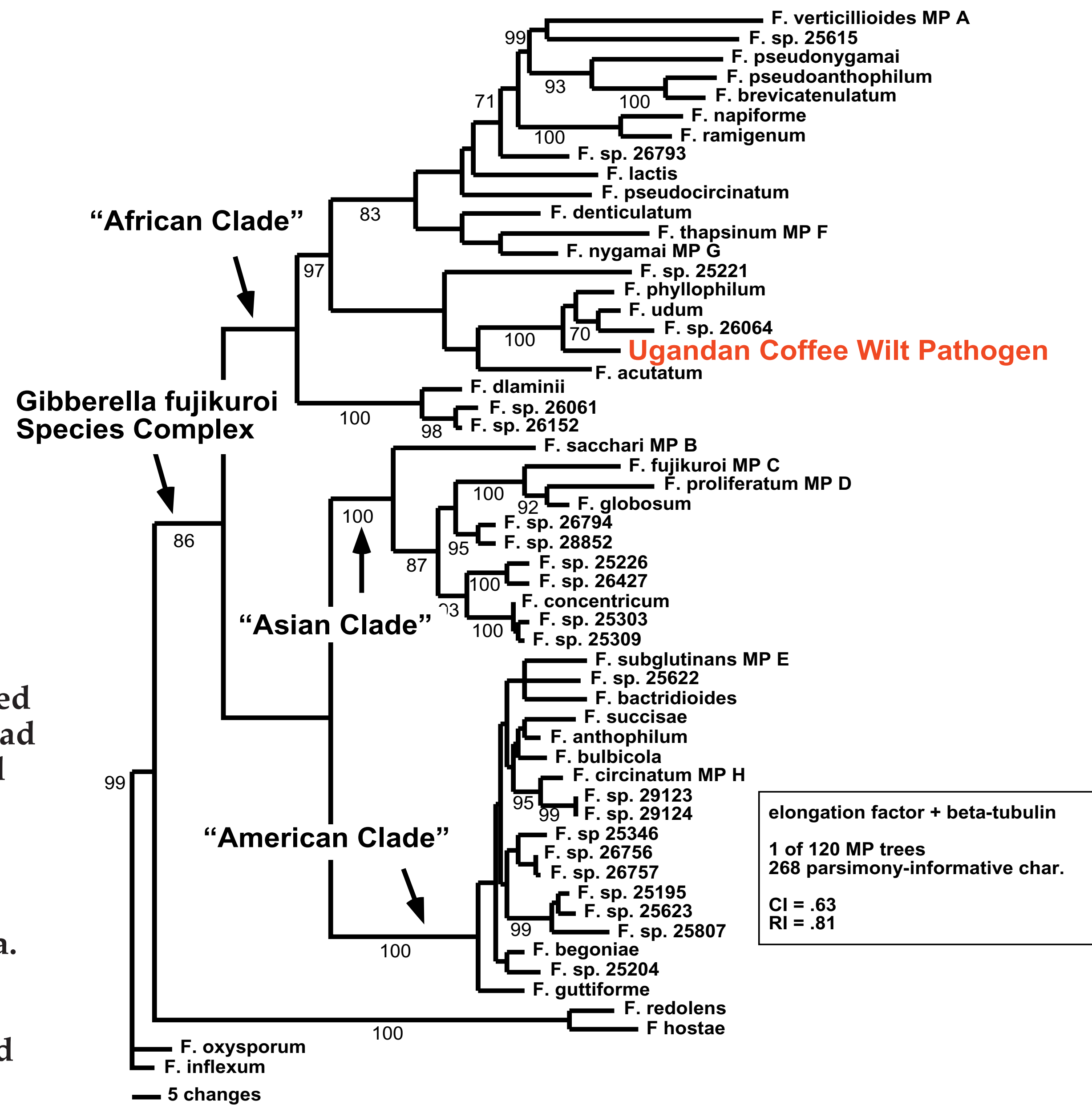
L.P. = Latent period, % Inc. = Percent wilt incidence, N.S. = No wilt symptoms.

## Phylogenetic analysis.

PCR with primers specific for the *tef* and *benA* genes generated a single amplified fragment for each gene of approximately 690 bp and 1000 bp respectively. All CWD isolates from Uganda had identical *tef* and *benA* DNA sequences. *tef* sequences compared with sequences in the GenBank database using BLAST matched most closely with members of the African clade of the GFC, particularly *F. udum* and *F. phyllophilum*. Based on this result, representative *tef* and *benA* sequences were

added manually to an alignment of sequences representing the known phylogenetic breadth of the GFC. Maximum parsimony analyses of the *benA* and *tef* data sets yielded 51,608 and 280 most parsimonious trees, respectively (not shown). The strict consensi of both sets of trees showed a clade including the CWD isolates, *F. udum*, *F. phyllophilum* and an unnamed species, designated as *Fusarium* sp. 26064 (11). A combined analysis of the *benA* and *tef* datasets yielded 120 most parsimonious trees (Fig. 3), which showed a strongly supported clade that includes the Ugandan CWD sequences and those from these three species

**Figure 3.** Phylogenetic relationship of the CWD isolates and the *Gibberella fujikuroi* species complex as inferred from a maximum parsimony analysis of the *tef* and *benA* gene regions. "American", "African" and "Asian" clades are marked. Species associated with known mating populations (biological species) are labeled with their MP designations. Undescribed phylogenetic species are labeled as *Fusarium* sp., with their corresponding accession numbers in the USDA NRRL culture collection, Peoria, IL. Numbers below branches represent bootstrap values based on 1000 replicates.



# Conclusions

- Morphological and cultural analyses were useful for initial characterization of the isolates and served to differentiate the CWD pathogen from other *Fusarium* species commonly associated with robusta coffee in Uganda.
- Colony growth and spore shape and size varied little among isolates making it difficult to separate isolates into distinct groups.
- The Ugandan CWD isolates proved to be members of the African Clade of the GFC complex of which *F. xylarioides* is not a member.
- The Ugandan CWD isolates showed enough of a morphological connection to descriptions of *F. xylarioides* that they could easily be mistaken for this species. A comprehensive morphological and molecular analysis involving a diverse set of isolates is needed to straighten out this difficult problem.

## References

- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acid Res.* 25:3389-3402.
- Ewing, B., and Green, P. 1998. Base-calling of automated sequencer traces using Phred. II. Error probabilities. *Genome Res.* 8(3):186-94.
- Ewing, B., Hillier, L., Wendt, M.C., and Green, P. 1998. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. *Genome Res.* 8(3):175-85.
- Hakiza, G.J. and Mwebesa, L. (1997). Proposed Strategies for the Control of Tracheomyces in Uganda. Proceedings of the First Regional Workshop on Coffee Wilt Disease (Tracheomyces). International Conference Centre, Kampala Uganda. 28 - 30 July, 1997. Hakiza, G.J., Birikunzira, J.B. and Musoli, P. (Eds.). Pp. 83 - 87.
- Lee, S.B and J.W. Taylor. 1990. Isolation of DNA from fungal mycelia and single spores. In M.A. Innis, D.H. Gelfand, J.T. Sninsky, and T.J. White (ed.), PCR Protocols: a guide to method and applications. Academic press, San Diego, Ca.
- National Environment Management Authority (NEMA) (2001). The State of the Environment Report for Uganda. Published by NEMA, P.O. Box 22255, Kampala, Uganda. Pp 5 - 7.
- Nelson, P.E., Toussoun, T.A., and Marasas, W.F.O. 1983. *Fusarium* species. An illustrated manual for identification. Pennsylvania State University Press, University Park, Pa.
- Ngategize, P.K., Nabbumba, R. and Kayobyo, G. (2001). Economic Background. In: Agriculture in Uganda, Volume 1. Mukibi, J.K. (ed.). Published by Fountain Publishers Ltd., P.O. Box 488, Kampala, Uganda. Pp. 200 - 215.
- O'Donnell, K., and Cigelnik, E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Mol. Phyl. Evol.* 7: 103-116.
- O'Donnell, K., Kistler, H.C., Cigelnik, E., and Plötz, R.C. 1998b. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proc Natl Acad Sci USA* 95: 2044-2049.
- O'Donnell, K., Nirenberg, H.L., Aoki, T., and Cigelnik, E. 2000. A multigene phylogeny of the *Gibberella fujikuroi* species complex: detection of additional phylogenetically distinct species. *Mycoscience* 41: 61-78.
- Ojacor, F.A. (2001). Introduction and Organisation. In: Agriculture in Uganda. Volume 1. Mukibi, J.K. (Ed.). Published by Fountain Publishers Ltd. P.O. Box 488, Kampala, Uganda. Pp. 1 - 4.
- Swofford, D.L. 2002. PAUP\*: Phylogenetic Analysis Using Parsimony (and Other Methods) 4.0 Beta. Sinauer and Associates, Sunderland, MA.
- Uganda Coffee Development Authority (UCDA) (2000). Annual Report Volume 9. P.O. Box 7267, Kampala, Uganda.