IDENTIFICATION AND MOLECULAR PHYLOGENETICS OF *LASIODIPLODIA PARVA* ASSOCIATTED WITH WHITE, YAM (*DIOSCOREA ROTUNDATA* L.) IN GHANA

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Abstract

Ten isolates of the fungus obtained from diseased yam tubers were identified using morphological characterisation, complemented with phylogenetic study, involving sequences of the rDNA-ITS region and part of the beta-tubulin gene of isolates. The pathogenicity of the isolates was tested on healthy yam tubers. The conidial morphology and size indicated that the isolates were *Lasiodiplodia parva*. In the phylogram, the isolates clustered with the type strain of *L. parva* and other *L. parva* strains of confirmed identities, in clade supported by a high bootstrap value. The fungus was able to cause the disease symptoms on artificially inoculated tubers, showing that it was responsible for the disease symptoms. The results showed that *L. parva* was responsible for the hard rot disease of yam in storage in Ghana, rather than the *L*. theobromae cited in the literature.

Introduction

Yam (*Dioscorea* spp.) is one of the most important staple foods in West Africa serving as an important food security crop. The crop is cultivated also in some regions across Asia, south and central America, the Caribbean and the Pacific (Degras. 1993). It serves as an important source of carbohydrates in the diets of most people in the tropics. In the producer countries, the crop serves as a major source of income to farmers. Yam is also an important foreign exchange earner in these producer countries. Together with other root and tuber crops, yam contributes to 50 per cent of Ghana's agricultural GDP (MoFA, 2010).

The production of yams in Ghana is constrained by the incidence of pests and diseases. One of the major diseases affecting the crop is tuber rot during storage. Rot is a major factor limiting the post-harvest life of yams and losses can be very high. It was estimated that rots could cause about 10-30 per cent losses to retailers and 10-40 per cent losses to exporters (Cornelius, 1998). Recent observations suggest that the recorded losses may be higher. Post-harvest yam rot losses significantly affect farmers' and traders' income, food security and seed yams stored for planting. The quality of yam tubers is affected by rots, which makes them unappealing to consumers (Atta, 2013).

Several plant pathogens have been implicated in the rots of yams in Ghana. Nine fungal spoilage microorganisms, including *Aspergillus flavus*, *Aspergillus niger*, *Lasiodiplodia theobromae* (syn. *Botryodiplodia theobromae*) *Fusarium culmorum, Fusarium oxysporum*,

Penicillium brevicompactum, *Penicillium sp*. and *Rhizopus stolonifer* have been reported on yams in Ghana (Aboagye-Nuamah *et al*., 2005). In addition, the bacterium, *Erwinia carotova,* and the nematode, *Scutellonema brady*s, have also been reported as causing tuber rots in yam (Aboagye-Nuamah *et al*., 2005). Along with *F. oxysporum* and *R. stolonifer, L. theobromae* has been identified as one of the most frequently occurring fungal species on yams in Ghana (Aboagye-Nuamah *et al*., 2005). *L. theobromae* was the only first recorded fungus associated with yam rots in Ghana (Dade & Wright, 1930).

Lasiodiplodia theobromae is reported to be cosmopolitan in nature and is commonly found in tropical and sub-tropical regions of the world (Marques *et al*., 2013;). The fungus is associated with several plant hosts either as a parasite, saprophyte or endophyte (Alves *et* al., 2008; Slipper & Wingfield, 2007). In Ghana, it has been reported on mango (Honger *et al*., 2015), coconut and citrus (Offei, Cornelius & Sakyi-Dawson, 2008; Oduro, 2000). It has also been reported on cash crops such as cocoa and yams (Twumasi *et al*., 2014).

Morphologically, *L. theobromae* can be distin-guished from other related species by the presence of paraphysis within the pycnidia, the production of hyaline and aseptate immature conidia, which turn brown and become septated with longitudinal striations on the conidial walls (Hyde, Nilson & Alias, 2014; Phillips *et al*., 2013; Sutton, 1980). In recent times it has been observed that there are several cryptic species among *L. theobromae*. Among these is *L. parva*, whose ex-type was originally isolated from cassava field soils. *L. parva* has been distinguished from *L. theobromae* using both morphological and molecular characteristics (Alves *et al*., 2008). Though the *L. parva* fungus has not been identified on any crop in Ghana, some isolates of the fungus, identified as *L. theobromae* on yam, using morphological features, were presumed to be *L. parva,* based on their spore sizes.

Although morphological features can be used to delineate unknown species to some extent, the general plasticity of these features means morphology cannot be relied upon entirely for the accurate delineation of species and often needs molecular characterisation for certainty. For satisfactory control of a postharvest disease to be achieved, accurate identification of the causal agent is essential. In this study, therefore, morphological characteristics were complemented with sequence analysis of phylogenetically informative genes for the identification of the presumed *L. parva* species causing the black rot disease of yams in Ghana.

Isolation of *Lasiodiplodia* species

Yam tubers of the pona variety showing the characteristic black, dry rots were collected from the Kokomba, Kaneshie and Madina yam markets in the Greater Accra Region and the Yendi market in Tamale in the Northern Region of Ghana. The rots observed on the infected tubers appeared in different shades of grey to black. Some tubers showing the rots were pulvurent and were breaking into pieces. The rots were all dry in nature and in most cases, the rotten portions of the tubers were only observed after the whole tuber had been sliced with a knife. These were sent to the Plant Pathology Laboratory of the Department of Crop Science, University of Ghana, Legon, Accra (5.6508° N, 0.1869° W) for the isolation of the causal agents.

Causal agents were first isolated on water agar (20 g l^{-1}; Oxoid, Basingstoke, UK) and then on potato dextrose agar (PDA, 39 g l^{-1}; Oxoid). Each medium was autoclaved at 121 ºC for 15 min, cooled and poured into clean sterilized plates to set. Samples of the diseased yam tubers were washed under running water and air-dried. A sterile scalpel was used to incise about 5 mm x 3 mm portions of the tubers from the advancing edge of the disease lesion. These were surface sterilised with 1 per cent sodium hypochlorite, washed in sterile distilled water to flush off the chemical and blotted dry using a tissue paper. Surface sterilized tissues pieces were plated singly on water agar plates and incubated for 5 days in the laboratory at 25-27 ºC and relative humidity of 60-65 per cent under alternating light/dark regimes of 12 h each. The developing

mycelium of the fungus was then sub-cultured on PDA and incubated for 7 days under the same ambient conditions in the laboratory after which the mycelium and conidia were mounted on slides using plain lactophenol and viewed under compound microscope (Optical Technologies, Asia). The nature of the growth on PDA and shape of the conidia of the isolated fungi were used for identification to genus level. Monoconidial cultures of selected *Lasiodiplodia* species were then established and used for further studies.

Cultural and morphological studies of *Lasiodiplodia* species

Mycelial discs (4 mm diameter), taken from actively sporulating areas near the growing edge of 7-day-old cultures of each isolate, were transferred unto PDA and incubated at 25-27 °C and RH of 60-65 per cent. Colony diameter was measured daily for 4 days when the mycelia had covered the entire plate and growth rate was calculated as the 4-day average of mean daily growth (millimetres per day). Mycelium and conidia were mounted on slides and the number of septum, length and width of 50 conidia harvested from the cultures were recorded and means calculated according to the methods of Alves *et al*. (2008). Ten isolates selected at random and three, plates, representing three replications of each isolate, were used in the study.

Molecular characterisation

Polymerase chain reaction. Isolates of the *Lasiodiplodia* species obtained from yams in Ghana were selected at random and DNA was extracted using the Sigma's GenFlute Plant Genomic DNA Miniprep Kit (St. Louis, MO, USA), following the manufacturer's instructions. DNA extracted was used as templates in PCR. The PCR were carried out using two primers pairs, ITS1/ITS4, to amplify the entire internal transcribed spacer region (White *et al*., 1990), and Bt2a/Bt2b (Glass & Donaldson, 1995) to amplify part of the beta tubulin gene. The PCR reaction mixture was made up of 2 µl target DNA, 5 µl of 10X PCR buffer (Invitrogen, Carlsbad, CA), 2.5 µl of deoxynucleoside-triphosphate mix (2.5 mm each), 0.25 µl bovine serum albumin (20 mg/ml), 2 µl each of the forward and reverse primer, 1.8 µl of magnesium chloride (50 mm) and 0.2 µl of taq polymerase (Invitrogen, Carlsbad, CA) added to 34.25 µl of double distilled water.

Each PCR was performed in a total reaction volume of 50µl. The reaction was carried out in a Thermo Hybaid PXE Thermal Cycler (Thermo Electron Corporation, USA). The reaction cycles were denaturing for 2 min at 94°C followed by 35 cycles of 1min at 94°C, 1 min at 55°C, 2 min at 72°C and a final extension of 10 min at 72°C. Amplification products were separated by 1.5% w/v agarose gel (Invitrogen, Carlsbad, CA), stained with Ethidium bromide or gel red alongside 1.0 kb marker at 100 V for about 1.5 hours. Bands were observed under UV light and Polaroid photographs taken using the Gene Flash Documentation System (Snygene Bio Imaging).

Sequencing of amplified products

The PCR amplified product of the ITS region and part of the beta tubulin gene of isolates were sent to ETON Bioscience Laboratory at Raleigh in North Carolina for purification and sequencing. Ten picomole of each primer was used to sequence the product from both directions. Sequences were entered into the BIOEDIT software and edited.

Sequence analysis and phylogenetic studies of the ITS region and the Beta tubulin gene

The assembled sequences of ITS region of the isolates were used in a Basic Local Alignment Search (BLAST) (http://blast.ncbi.nlm.nih.gov/BLAST.cgi). After this, the sequences, together with those obtained for the beta tubulin gene, were used in a phylogenetic analysis as below.

Phylogenetic analysis. The sequences of the internal transcribed spacer region (ITS) and partial beta tubulin gene of 27 isolates were used in the phylogenetic studies. These were made up of 10 isolates from the rotten yams collected in this study sequences of which were deposited in

the GenBank (Table 1), and 17 sequences of ex-types and isolates of confirmed identities (downloaded from EMBL database) (Table 2). The downloaded sequences included that of *Diplodia seriata* which was used as the out-group. The sequences of the different gene regions of the isolates were aligned using Clustal W. Three data sets were prepared and analysed: data set based on rDNA-ITS region (data set 1), data set based on partial beta tubulin gene (data set 2) and combination of the rDNA-ITS region and the beta tubulin gene. The multiple sequence alignments obtained were used in a phylogenetic analysis using MEGA5 (Tamura *et al*., 2011).

The Maximum Parsimony (MP) analysis was performed according to the method of Nei & Kumar, (2000). The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei & Kumar, 2000) with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). The tree was drawn to scale with branch length calculated using the average pathway method (Nei & Kumar, 2000) and were in units of number of changes over the whole sequence. All positions containing gaps and missing data were eliminated from the data set (complete deletion option). Clade stability of the tree resulting from maximum parsimony analysis was assessed by bootstrap analysis with 1000 replicates (Felsenstein, 1985).

Pathogenicity tests

Pathogenicity of the isolated fungi was tested on healthy yam tubers of the pona variety**.** Selected yam tubers were surface sterilized with 1 per cent sodium hypochlorite and air-dried. One 7 mm deep hole was aseptically punched with cork-borer (4 mm diameter) on the tuber and a plug of the isolate, taken from the actively sporulating areas of a 7-day old culture was placed into each of the holes. Tubers inoculated with sterile PDA as a control check. Tuber plugs removed from punched holes were replaced and covered with parafilm. Each isolate was used to inoculate three different tubers to serve as three replicates. The inoculated tubers were incubated at the prevailing relative humidity and temperature conditions in the laboratory (60-65% RH and 23-25 °C). Inoculated tubers were observed daily till symptoms appeared. The pathogen which was able to induce similar hard rot symptoms on the artificially inoculated tubers was considered the causal agent of the disease. Subsequently, it was re-isolated from the disease lesions to confirm Koch's postulates and authenticate the pathogen as the causal agent of the disease.

Results

Cultural and morphological characteristics of isolated Lasiodiplodia species

Each of the ten isolates selected at random for morphological characterisation, produced mycelium which was initially white and turned dark as the culture aged. Mycelia grew and filled the entire 9 mm plate in 4 days (Fig. 1A). The hyphae were initially hyaline and turned dark, and were septated. Two types of conidia; mature and immature were observed. The immature conidia were hyaline, aseptate, granular, ovoid and thick- walled (Fig. 1B). The matured ones were uniseptate, brown walled and had longitudinal striations (Fig. 1C). These conidia were produced in dark coloured pycnidia. The conidial dimensions ranged from 15.5 -18.4 µm x 8.9-11.6 µm (Table 3).

Basic Local Alignment Search (BLAST)

Blast search using the assembled nucleotide sequences of the rDNA-ITS region of the isolates obtained in this study showed they were similar to either *L. theobromae* or *L. parva*. The percentage similarity was 100 per cent between isolates obtained from this study and the *L. theobromae* isolates L3 (accession number KR 260793.1), and RSGV/PD02 (accession number HM 466960.2) and *L. parva* type strain CBS 456.78 (accession number NR111265.1).

Sequences and phylogenetic analysis of the ITS region and β-tubulin gene

An approximately 600 bp product of the ITS region and 312 bp of part of the β-tubulin gene were amplified from the isolates using the primer pairs ITS1/ITS4 and Bt2a/Bt2b,

respectively. The assembled sequences were 535 bp and 300 bp long for the ITS region and the partial β-tubulin gene, respectively. The individual gene trees obtained with the rDNA-ITS region and partial β-tubulin gene data sets were broadly congruent. The most parsimonious tree obtained with the concatenated sequences of the two genes is shown as Fig. 2.

The tree length was 86, the consistency index was 0.823529, the retention index was 0.910000, and the composite index was 0.814767 (0.749412) for all sites and parsimony-informative sites (in parentheses). The bootstrap values in percentages are shown next to the branches. There were 877 sites in the final data set. All the 10 isolates collected from yams in Ghana, clustered with the ex-type strain of *L. parva* and other *L. parva* isolates with confirmed identities. The clade was supported with a high bootstrap value of 68% (Fig. 2). This indicated that all the isolates from yam were *L. parva*. The type strain of *L. theobromae* and other *L. theobromae* isolates of confirmed identities also clustered together in a clade supported by a high bootstrap value of 98% (Fig. 2). None of the isolates from yam clustered in the *L. theobromae* clade.

Pathogenicity test

All isolates of the pathogen were able to induce the dry black rot symptoms on the inoculated yam tubers**.** Initial necrotic areas were observed on the fruits at the point of inoculation, a day after inoculation. These were seen on all inoculated tubers. When the inoculated tubers were cut opened, the characteristic rot symptoms induced by the fungus in the flesh of the yam tubers were observed**.** The rot symptoms were not seen on the control tubers (Fig. 4). The fungus isolated from the artificially induced symptoms was similar to what was used for the artificial inoculations.

Discussion

The nature of rot symptoms observed in this study has been reported to be characteristic of two plant pathogenic fungi, namely *Rosellina bunodes* and *Lasiodiplodia theobromae* (syn. *Botrodiplodia theobromae* (IITA, 1993). The cultural and morphological features of the isolated fungi, particularly the nature of the mature spores that were uniseptate, brown walled and had longitudinal striations was an indication that it belonged to the genus *Lasiodiplodia* (Phillips *et al*., 2008). The isolates produced conidia of sizes 15.5 - 18.4 µm in length and 8.9 - 11.6 µm in width. These conidia dimensions are within the range prescribed for *L. parva*, whose spore dimensions have been reported to range from 16 - 23.5 µm in length and 10.5-13 µm in width (Alves *et al*., 2008). The conidia of the isolates obtained were much smaller than what has been reported for *L. theobromae* or other distinct species such as *L. psuedotheobromae* and *L. venezuelensis*. Therefore, based on conidial sizes, the isolates in this study could be deemed to be *L. parva*.

Though morphological features such as conidial dimensions have been found to be reliable in differentiating among species previously identified as *Lasiodiplodia*, the method is most of the time complemented with molecular methods (Rosado *et al*., 2015; Phillips *et al*., 2008; Alves *et al.*, 2008). In this study, analysis of the nucleotide sequences of some phylogenetically informative genes was carried out, to complement the morphological identification of *L. parva* isolates obtained from yam. The nucleotide sequences of the rDNA-ITS region, that has been used severally as barcode for species identification in fungi (Weir, Johnston & Damm 2012), was obtained from the isolates in this study and compared to those of species reported worldwide, using a BLAST search. It was observed that the isolates obtained from this study could be either species of *L. theobromae* or *L. parva* as their nucleotide sequences matched those of these distinct species. In line with normal practices of identifying unknowns based on BLAST search, the isolates in this study could be either *L. parva* (consistent with the results of the morphological characterisation) or could be *L. theobromae*.

The BLAST search results identified the isolates as either *L. parva* or *L. theobromae* and

there was the need to clear this ambiguity. Therefore, multiple gene analysis involving the nucleotide sequences of the rDNA-ITS region and partial beta tubulin genes was carried out. These two gene region have been found to be phylogentically informative and have been combined with other genes such as the translation elongation factor-1 and the nuclear ribosomal SSU gene in species delineation within the genera *Botryosphaeriaceae* (Rosado *et al*., 2015; Phillips *et al*., 2008; Alves *et al.*, 2008). Therefore, a combination of these two gene was found to be robust enough to distinguish between *L. theobromae* and *L. parva*. Type strains of *L. theobromae* and other *L. theobromae* isolates of confirmed identities were included for comparison purposes. In the phylogram obtained with the multiple sequence alignment, the *L. theobromae* isolates clustered together in a well-supported clade that did not include any of the isolates from the yams in Ghana (Fig. 2). This effectively confirmed that the fungi isolated in Ghana were not *L. theobromae*. On the other hand, all isolates of the fungi obtained in this study clustered with the ex-type strain and other strains of *L. parva*, of confirmed identities (Fig. 2). This underscores the view point that the isolates in this study were *L. parva.*

The *L. parva* clade was formed close to the *L. theobromae* clade, an indication that the two species, though distinct are closely related. The type strain of *L. parva* was isolated from cassava field soils, and, therefore, the pathogen is more likely to also affect yams which are often cultivated in the same soils as cassava in Ghana. Incidence of *L. parva*, as a pathogen of yam, has not been previously reported in Ghana. However, the nature of the symptoms caused by *L. parva* on both the natural and artificially inoculated yam tubers, were indicative that it was the same pathogen that had previously been identified as *L. theobromae*.

The identification of *L. parva* strains as *L. theobromae* may not be confined to Ghana alone. According to Alves *et al* (2008), it had long been conjuctured that *L. theobromae* was composed of a number of cryptic species after Punithalingam (1976) reported that the pathogen was widespread in occurrence and had a large number of hosts with mycelial and conidial morphological variability. This eventually led to the identification of several distinct species originally identified as *L. theobromae* including *L. parva* and *L. psuedotheobromae* (Alves *et al*. 2008; Pavlic *et al*. 2004; Burgess *et al*. 2006). Prior to the identification of these cryptic species of *L. theobromae*, both *L. parva* and *L. psuedotheobromae* were identified as *L. theobromae*. This could be the reason why *L. parva* infecting yam in Ghana and probably elsewhere had been wrongly named as *L. theobromae*. The finding in the present study necessitates the updating of the plant pathogen checklist in Ghana.

Conclusion

In this study, *L. parva* was identified as a causal agent of yam tuber rot in in storage in Ghana. The use of conidial morphology, complemented with the sequence analysis of the rDNA-ITS region and the partial beta tubulin gene, confirmed the identity of the fungus. The ability of *L. parva* to cause the disease on artificially inoculated yam tubers confirmed that the fungus was responsible for the disease. BLAST search using the nucleotide sequences of the rDNA-ITS region of the *L. parva* isolates showed their nucleotide sequences were similar to that of *L. theobromae*. This and the fact they produced conidia of similar morphology might have led to the identification of the strains as *L. theobromae* in Ghana and probably elsewhere. The findings in this study corroborates earlier reports elsewhere that the *L. theobromae* is made up of distinct discrete species including *L. parva*. The update of the current checklist of plant pathogens in Ghana, to reflect the findings in this paper is, therefore, recommended to reflect this finding.

TABLE 1

Isolates of Lasiodiplodia *species used in the study and their GenBank accession numbers*

Strain identification	*Host*	*Locality*	*Accession numbers*	
			ITS	**β-tubulin**
YBT-GH1	*Dioscorea rotundata*	Ghana	KX227550	KX227560
YBT-GH2	*Dioscorea rotundata*	Ghana	KX227551	KX227561
YBT-GH3	*Dioscorea rotunduta*	Ghana	KX227552	KX227562
YBT-GH4	*Dioscorea rotundata*	Ghana	KX227553	KX227563
YBT-GH5	*Dioscorea rotundata*	Ghana	KX227554	KX227564
YBT-GH6	*Dioscorea rotundata*	Ghana	KX227555	KX227565
YBT-GH7	*Dioscorea rotundata*	Ghana	KX227556	KX227566
YBT-GH8	*Dioscorea rotundata*	Ghana	KX227557	KX227567
YBT-GH9	*Dioscorea rotundata*	Ghana	KX227558	KX227568
YBT-GH10	*Dioscorea rotundata*	Ghana	KX227559	KX227569

TABLE 2

A list of isolates downloaded from EMBL Database and used in the study and their GenBank accession numbers

Species	*Strain identification*	*Host*	*Country*	*GenBank accession numbers*	
				ITS	**β-tubulin**
D. seriata	CBS 112555	*Vitis vinifera*	Portugal	AY259094	DQ458867
L. gonubiensis	CBS 115812	*Syzygium cordatum*	S. Africa	DQ458892	DQ458860
L. gonubiensis	CBS 116355,	*Syzygium cordatum*	S. Africa	AY639594	EU673126
L. missouriana	UCD2199MO	*Vitis vinifera*	USA	JX010251	JX009572
L. missouriana	UCD2193MO	*Vitis vinifera*	USA	JX010205	JX009564
L. parva	CBS 356.59*	Cassava field soil	Colombia	FJ972612	JX009584
L. parva	CBS 494.78	Cassava field soil	Colombia	EF622084	EU673114
L. parva	CBS 494.78	Cassava field soil	Colombia	EF622083	KP872419
L. psuedotheobromae	CBS 447.62	*Citrus sp.*	Suriname	EF622081	EU673112
L. psuedotheobromae	CBS116459*	*Gmelina arborea*	Costa Rica	EF622077	EU673111
L. subglobosa	CMM3872	*Jathropha curcas*	Unknown	KF234558	KF254942
L. subglobosa	CMM4046	*Jathropha curcas*	Unknown	KF234560	KF254944
L. theobromae	CAA 006	*Vitis vinifera*	USA	DQ458891	DQ458859
L. theobromae	CBS124.13	Fruit along coral reef coast	USA	DQ458890	DQ458858
L. theobromae	CBS 164.96*	Fruit along coral reef coast	New Guinea	AY640255	EU673110
L. viticola	UCD2604MO	*Vitis vinifera*	USA	HQ288228	HQ288307
L. viticola	UCD2553AR	*Vitis vinifera*	USA	HQ288227	HQ288306
L. rubropurpurea	CBS118740	*Eucalyptus grandis*	Queensland	DQ103553	EU673136

*=Type strain

TABLE 3

Conidial types and dimensions and pathogenicity tests of Lasiodiplodia *isolates from yam.*

Isolate designation	*Conidium septation*		*Mean mature conidium dimensions(μm)*	*Pathogenicity on yam*
	Immature	Mature		
YBT-GH1	No septation	1-septate	17.2 x 10.3	+
YBT-GH2	No septation	1-septate	16.3 x 8.9	+
YBT-GH3	No septation	1-septate	15.8 x 10.1	+
YBT-GH4	No septation	1-septate	16.7 x 9.9	+
YBT-GH5	No septation	1-septate	18.4 x 9.1	+
YBT-GH6	No septation	1-septate	17.6 x 10.2	+
YBT-GH7	No septation	1-septate	17.2 x 11.6	+
YBT-GH8	No septation	1-septate	16.7 x 9.8	+
YBT-GH9	No septation	1-septate	15.5 x 11.1	+
YBT-GH10	No sepatation	1-septate	16.4 x 9.6	+

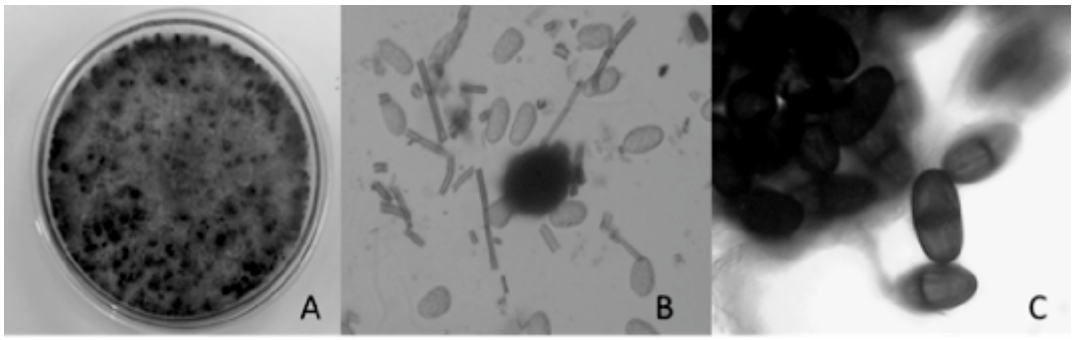

Fig. 1.C*ultural and morphological characteristics of* Lasiodiplodia *isolates obtained from yam.*

A = Mycelia growth on PDA, B = Hyaline, non-septate spores, C = dark coloured, septate spores

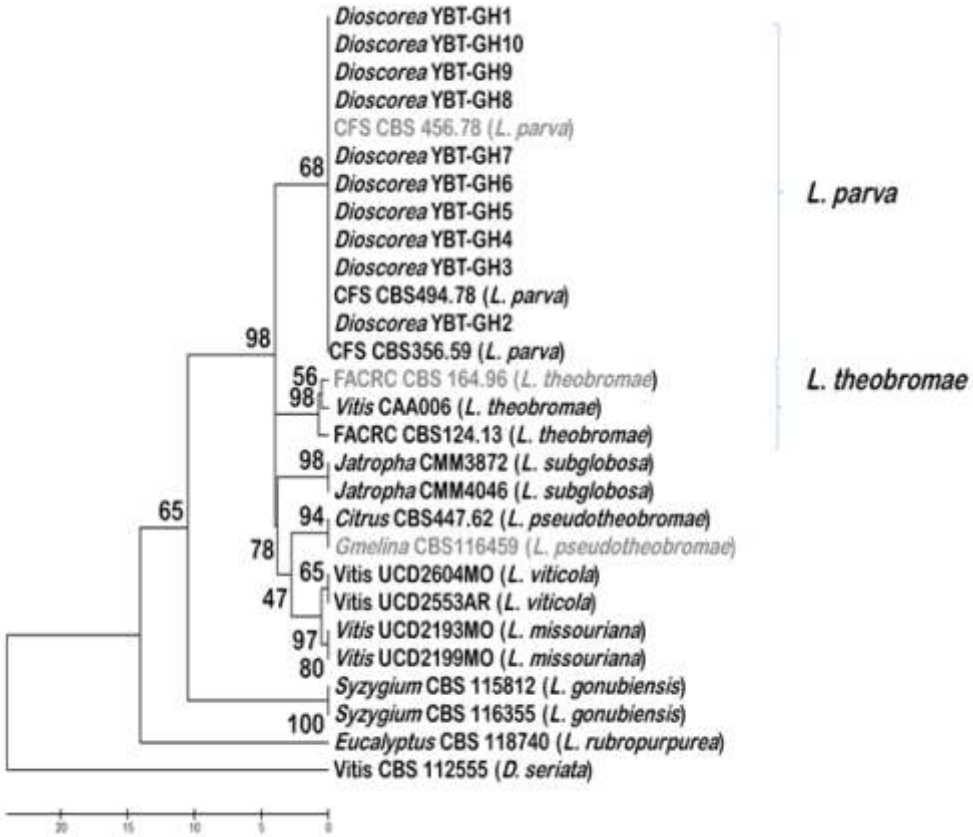

Fig. 2. Phylogram constructed from the multiple sequence alignment

Maximum parsimony phylogram constructed from the multiple sequence alignment of the combined nucleotide sequences of the ITS region and β-tubulin gene gene. *D. seriata* was used as the out group. With the exception of strains whose names were preceded by YBT-GH, all strains are either isolates of confirmed identities or are ex-type strains, with names of ex-type strains in grey. Sequences of ex-type strains or strains with confirmed identities were downloaded from the EMBL database. The genus name of host, strain identification and species name of isolates have been provided (CFC=Cassava field soil; FACRC=Fruits along coral reef coast)

Fig. 4.
Characteristic dark brown rot symptoms induced in the flesh of yam by L. parva *isolate inoculated through wounds.* Note the absence of expanded lesion on control tubers (Left) and the extended lesions on the inoculated tuber (Right).

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