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Morphological and Molecular Identification of *Pratylenchus goodeyi* Associated with Banana in Tanzania

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Abstract

Banana (*Musa* sp. [L]) is an important staple food and cash crop for about 30 percent of the total population in Tanzania. Root lesion nematodes, particularly *Pratylenchus goodeyi*, are among the major pests affecting banana production in Tanzania. Morphological, morphometric and molecular approaches are required for accurate identification of nematodes. In this study, 80 *P. goodeyi* isolates from major banana growing areas in Tanzania were identified. Sequence analysis of part of the small subunit (SSU), large subunit (LSU) and internal transcribed spacers (ITS) regions of ribosomal DNA (rDNA) confirmed the identity of *P. goodeyi*. DNA sequences were analysed using Geneious version 11.0 software and phylogenetic relationship was constructed using Bayesian inference (BI) method. *P. goodeyi* identified in this study resembles *P. goodeyi* identified from other parts of the world and their sequences clustered closely (98.6 – 99.9 % similarity) with other *P. goodeyi* sequences in the GenBank. The results obtained from this study will provide an insight on the presence of harmful root lesion nematodes in Tanzanian bananas, contributing to further studies aimed at developing efficient nematode management strategies in Tanzania.

Keywords: Genetic variation, Morphology, *Musa* sp., Root lesion nematode.

Introduction

In Tanzania, banana is cultivated by smallholder farmers in approximately 0.5 ha per household (Mbwana and Rukazambuga 1998). The total area under banana cultivation in Tanzania constitutes about 350,000 ha (FAO 2015), distributed through several major agro-ecological zones: The Lake Zone (Kagera and Mara regions), Northern Zone (Arusha and Kilimanjaro regions), Southern Highlands (Mbeya and Ruvuma regions), Eastern Zone (Morogoro and Tanga regions), Western Zone (Kigoma region) and Zanzibar islands (Unguja and Pemba) (FAOSTAT 2013). The largest proportions of the varieties grown are of AAA genome types commonly referred to as East African Highland Bananas (EAHB-AAA). But there are also AAB genome types such as French horn (mzuzu); ABB types such as bluggoe (bokoboko) and kisukari; AB types such as Ney poovan (kanana) and AA genotypes such as mchare (mshare) (Perrier et al. 2009).

Banana cultivation is constrained by many factors, particularly pests and diseases. The common pests of banana in Tanzania are banana weevils and plant parasitic nematodes (PPNs), altogether are greatly favored by the environmental conditions characterized by warm and/or humid weather. Plant parasitic

nematodes, particularly root lesion nematodes (*Pratylenchus goodeyi*), are among the major damaging pests in banana and other crops (Gowen and Quénéhervé 1990, Jones et al. 2013, Sikora et al. 2018). They cause primary infections to plants, which then favor secondary infections by fungi and bacteria (Bridge and Starr 2007, Smiley 2015). Yield losses by nematodes on banana are estimated to be up to 50% annually in East Africa (Coyne 2009). Nematodes are widely distributed in banana worldwide (Talwana et al. 2003, Bridge and Starr 2007, Tuyet et al. 2012). These pathogens are obligate plant parasites, known to be confined in cooler higher altitudes of about 1500 m.a.s.l. and above (Kamira et al. 2013). However, the findings from Luambano et al. (2017) and Rajab et al. (1999) identified *Pratylenchus* spp. in low-humidity areas of East Africa including the Zanzibar islands and coastal areas of Tanzania. A study conducted by Luambano et al. (2019) identified *P. coffeae* in mainland Tanzania, where it was not found before.

Thus, this necessitated further studies on the identification of root lesion nematodes affecting bananas in Tanzania. Thorough identification of root lesion nematodes is important so as to design appropriate management strategies such as diagnostic tools.

Other several nematode species have been identified in banana growing areas such as *Pratylenchus coffeae* and *Pratylenchus goodeyi* (root lesion nematodes), *Radopholus similis*, *Helicotylenchus multicinctus* (spiral nematodes), and *Meloidogyne* sp. *(M. incognita* and *M. javanica* [root knot nematodes]) (Brooks 2004, Coyne 2009).

Although a morphological method is required for initial identification of nematodes, confirmation with a molecular method is important (Van Den Berg et al. 2014, Wang et al. 2016, Handoo et al. 2016), especially when there is insufficient capacity to identify nematodes morphologically (such as lack of efficient microscope and skilled taxonomist). Molecular techniques are more reliable than morphological. Ribosomal DNA region have been successfully used for molecular identification of plant parasitic nematodes (PPN) and has been confirmed as a reliable tool for this purpose (Kolombia et al. 2017). The current study was carried out to identify *P. goodeyi* affecting banana in Tanzania, through morphological, morphometric and molecular methods. For better management of *P. goodeyi* and other PPNs, clear identification of PPN species is important for proper planning of management such as development of quick diagnostic kits.

Materials and Methods

Sample collection

A survey was conducted between January and May, 2015 in major banana growing zones of Tanzania: Southern Highlands Zone (Ruvuma and Mbeya region), Northern Zone (Kilimanjaro and Arusha region), Lake Zone (Kagera region) and Zanzibar Islands (Unguja and Pemba). A total of 104 fields were surveyed (10 to 24 fields per region). Variation in number of fields surveyed was due to availability of banana fields. Some districts/villages within the same region surveyed did not have banana fields as they relied on other agricultural crops. Root samples (314) were collected using random sampling procedure, whereby three samples were collected from each banana field. Each sample was collected by digging a hole of 20 cm wide with 20 cm depth. Ten roots sized 10-15 cm were collected following the procedure described by De Waele and Speijer (2001). The samples were packed in labelled plastic bags, stored in a cool box and then shipped to the Tanzania Agricultural Research Institute (TARI), Kibaha laboratory. The samples were kept at 10 °C and nematodes extraction was done at least not more than two days later.

Sample preparation and nematode extraction from banana roots

Banana root samples with necrosis and lesions in some parts of the root (5 g each) were washed thoroughly with tap water, then rinsed with sterilized double distilled water (sddH_2O) to remove any adhering soils. Nematodes were extracted from the infected banana roots using a modified Bearman's method (Coyne et al. 2007). The modification included chopping the roots into 1–1.5 cm pieces, blending and pouring into plastic sieves lined with two layers of paper napkins. The mixture was incubated in a covered plastic plate at 28 °C for 24 h. Other procedures were followed as described in Coyne et al. (2007).

Morphological and morphometric examination

A total of 80 *P. goodeyi* isolates were identified morphologically and morphometrically as described by Roy et al. (2018) and Tuyet et al. (2012). Morphological identification of nematodes was initially done on a dissecting microscope (Leica MZ 9.5, Heerbrugg, Switzerland), to obtain the required samples for further identification. *P. goodeyi* observations were done through their main distinguishing characteristics which include: presence of long stylet length with rounded stylet knobs (anteriorly flattened knobs); flattened lip region and ventral overlap of esophageal gland over intestine; median esophageal bulb oval shaped or rounded in shape; excretory opening situated at 80 to 90 µm from the anterior end; anteriorly flattened, conoid knobs and ventrally concave tail; vulva positioned near posterior end at 71% to 83%; presence of smooth rounded tail terminus, large spermatheca filled with sperm and annulated tail tip in females. *Pratylenchus goodeyi* males have slender bodies compared to female bodies, with large stylet and round stylet knobs. The *P. goodeyi* males have single testes and slender spicules. The spicules are also curved with the bursa that envelop the tail tips.

A single nematode having characteristics associated with *P. goodeyi* was picked and placed on a glass slide containing 10-20 µl of sddH_2O. Further observations of each specimen were done on a compound microscope (Leica 2500, Leica Microsystems CMS GmbH, Wetzler, Germany) at 20x magnification for conformation of the species. Therefore, from each zone, 20 nematodes (10 females and 10 males) were examined, making 4 populations of *P. goodeyi*.

For the morphometric identification, a coverslip was placed on a glass slide containing nematodes. The nematodes were briefly heated by placing the glass slide containing the nematodes on the hot plate (1 – 2 s) to keep them at relaxed and straight position for easy measurements. Each nematode was measured under the compound microscope connected to a camera and a computer, under 20× magnification. Twenty (20) nematodes from each population (10 females and 10 males) were measured based on parameters described by Handoo et al. (2016). Immersion oil was later added on the covered glass slide to enable identification at 100× magnifications. Nematodes were then photographed under 100 × magnification.

Molecular identification

DNA extraction

Genomic DNA extraction from nematodes was done according to the protocol by Ye et al. (2015). A single root lesion nematode previously used for morphological analysis was removed from the glass microscope slide and placed in 10 µl of extraction buffer: 1M TE buffer (Tris-hydrochloride pH 8.0, 2.5 mM Ethylenediaminetetraacetic acid). Each individual nematode was macerated and added with 40 µl of TE buffer. The mixture was then pipetted into a 1.5-ml sterile Eppendorf tube and stored at −20 °C for further analysis.

PCR amplification and sequencing

Polymerase chain reaction (PCR) with universal primers 18S965/18S1573R and rDNA2/rDNA1.58S (Table 1) that amplify the

small sub unit (18S) and ITS 1 regions of the rDNA of plant parasitic nematodes, respectively, were initially used for preliminary identification of *P. goodeyi*. Genomic DNAs extracted from nematodes were used as templates for PCR. The PCR products obtained were sent for nucleotide sequencing at the North Carolina State University (NCSU) for confirmation purposes.

Specific primers for *Pratylenchus* sp. were designed using Primer3 (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) software. The designed primers (Table 1) amplify the ITS1, ITS2 and LSU rDNA regions of *P. goodeyi*. These primers targeted the conserved regions of the rDNA (Figure 1) and were obtained by aligning numerous representative species of *P. goodeyi*. The primers were analyzed on the integrated DNA technologies (IDT) software at https://eu.idtdna.com/pages prior to synthesis by the Bioneer (Bioneer Corporation, Daejeon, Republic of South Korea). PCR with newly designed primers was done according to Luambano et al. (2019). The PCR was done in a reaction mixture of 25 µl containing 9.5 µl of molecular grade water, 12.5 µl 2X Apex *Taq* red master mix DNA polymerase (Genesee Scientific Corporation, San Diego, CA, USA), 0.5 µl of each 10 µM forward and reverse primer and 2 µl of DNA template. The PCR conditions were as follows: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, distinct annealing temperatures described in Table 1 for 45 s, extension at 72 °C for 1 min and then a final extension at 72 °C for 10 min. The amplified PCR products were analyzed on 1% agarose gel electrophoresis then were sent to NCSU for nucleotide sequencing.

Data management and analysis

Morphometric data obtained from *P. goodeyi* measurements were analyzed using the GENSTAT software (14th edition, VSN International Ltd, Hemel Hempstead, UK) by analysis of variance to compare the means through least significance at $P < 0.005$.

Sequence analysis and phylogeny

Obtained *P. goodeyi* sequences were used for comparison with other related sequences available in GenBank through National Center for Biotechnology Information (NCBI) BLASTn homology search. Multiple sequence alignment was done by multiple sequence comparison by log-expectation (MUSCLE) in Geneious version 11.0 software according to Kearse et al. (2012). Phylogenetic trees were constructed using Bayesian inference method. The base frequencies, proportions of invariable sites, gamma distribution shape parameters and substitution rates were used in phylogenetic analyses. The model selected as the best fit for SSU, LSU and ITS dataset was the Hasegawa–Kishino–Yano (HKY) + I, which accept occurrence of nucleotides at different frequencies, hence different rates of transitions and transversions. The *Helicotylenchus* sp. were selected as outgroups for all datasets.

Table 1: List of primers used for PCR and DNA sequencing of *Pratylenchus* sp

Forward primer		Reverse primers		Gene	Annealing °C	Size (bp)	Reference
Name	Sequence	Name	Sequence				
rDNA2	TTGATTACGTTCC CTGCCCTTT	rDNA1.58S	ACGAGCCGAGT GATCCACCG	ITS	55	496	(Cherry et al. 1997)
18S965	GGCGATCAGATA CCGCCCTAGTT	18S1573R	TACAAAGGGCA GGGACGTAAT	18S	55	950	(Harris et al. 2005)
PCITSFor	GTGGATCACTCG GCTCGTAG	PCITSRev	CAGCGGGTATT CACGTCTGA	ITS	49	493	This study
PC1LSUF	ACAAGTACCGTG AGGGAAAGTTG	PC1LSUR	TCGGARGGAAC CAGCTACTA	28S	61	781	This study
P1BF	GATTACGTCCCTG CCCTTTGT	P1BR	CTGCTCAACAA CGCACAGAC	ITS	61	510	This study
Prg1F	CCTGCTGTTGTTG GAAACCA	Prg2R	AGGCGTCAACT CACAACCAT	ITS	49	631	This study
Prg2F	TGTCTTCGTCCGT GACTGTG	Prg5R	ATGCTTAAGTTC AGCGGGTG	ITS	61	545	This study

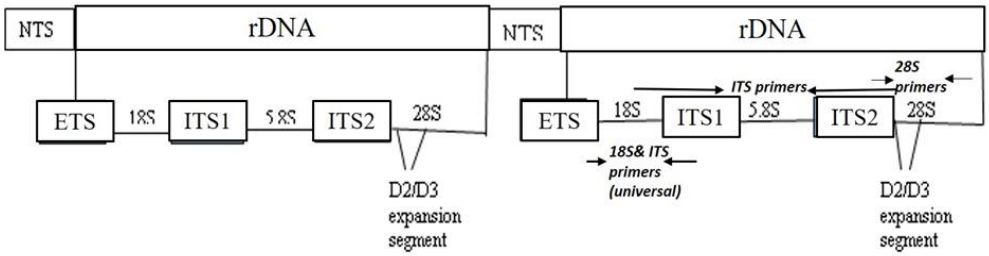

Figure 1: Genome arrangement of ribosomal DNA sequences in nematodes showing the primers location (indicated by arrows) within the coding regions of the 18S small subunit (SSU), 5.8S and 28S large subunit (LSU); non-coding regions of the internal transcribed spacer 1 & 2 (ITS 1 and 2), external transcribed spacer (ETS) regions and external non-transcribed spacer (NTS) region.

Results

Morphology and morphometrics

Pratylenchus goodeyi (Figure 2) was present in all sampled regions. The mean stylet lengths observed in this study were between 11.3 ± 0.8 and 11.9 ± 0.8 µm for *P. goodeyi* females and 10.5 ± 0.8 and 11.1 ± 0.8 µm for *P. goodeyi* males compared to the standard measurements of 17–20 µm (females) and 12.7–16.0 µm (males) (Tables 2 and 3). The mean (± standard deviation) body lengths of *P. goodeyi* females and males from the Northern Zone (522.5 ± 15.9 and 473.8 ± 50.5 µm. respectively) were higher than the mean body lengths from other zones. The measurements of the esophageal lengths in both *P. goodeyi* females and males from the Northern Zone were 146.6 ± 11.2 and 110.1 ± 12.2 µm, respectively. The *P. goodeyi* females from Zanzibar had the shortest esophageal length compared to *P. goodeyi* females from other zones (Table 2). In addition, the average body width of females collected from the Lake zone was higher (22.5 ± 2.4 µm.) than other *P.*

goodeyi females from other zones. In contrast, the highest distance from cloaca to anterior most part of testis (T), tail length (t), maximum body width (W) and distance from basal knobs of stylet to dorsal pharyngeal gland orifice (DGO) were observed in *P. goodeyi* males collected from the Northern Zone, whereas the lowest average varied within different *P. goodeyi* male population studied (Table 3). Measurements of *P. goodeyi* body length/maximum body width (a), body length/distance from anterior end to pharyngo-intestinal junction (b), body length/distance from anterior end to posterior end of pharyngeal gland (c') and DGO ratios in both females and males showed very little variations between the populations collected both from Zanzibar Islands and other agro-ecological zones in mainland Tanzania. The highest distance of vulva from anterior end expressed as percentage of body length (%) was obtained from *P. goodeyi* females collected from the Southern Highlands, followed by the Northern Zone.

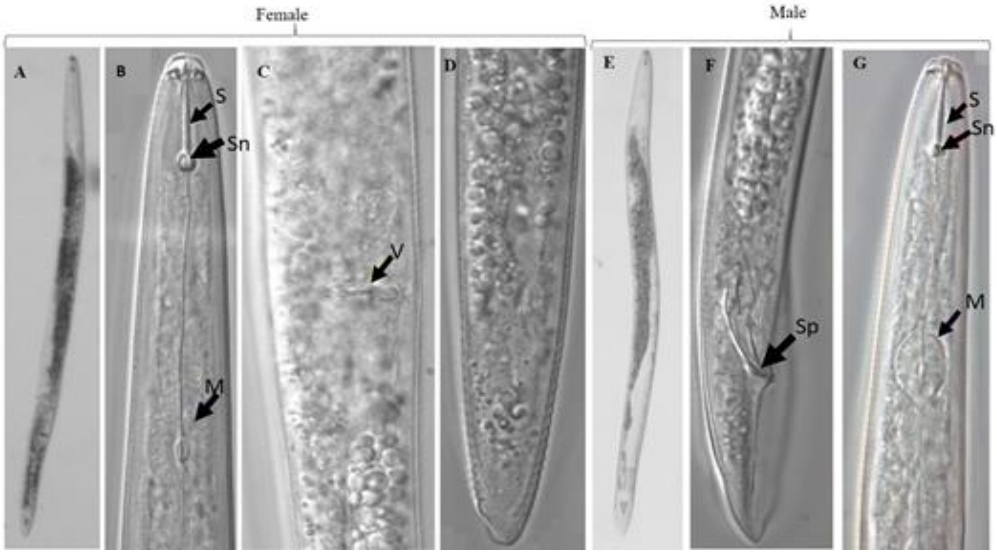

Figure 2: Micrographs of representative specimens of *Pratylenchus goodeyi* female and male collected from Tanzanian banana fields: **A** and **E** = female and male entire body structure; **B** and **G** = pharyngeal region with stylet & stylet knobs (shown by black arrows labelled; S and Sn, respectively) and median bulb (black arrow labelled M); **C, D** and **F** = posterior end showing tail region, with position of vulva in female and a spicule in male (shown by black arrows labelled V and Sp, respectively). Scale bars: **A** and **E** = 50 µm; **B, C, D, F** and **G** = 25 µm.

Molecular identification

Nucleotide sequencing successfully confirmed the identity of *P. goodeyi*. Phylogenetic analysis of SSU, ITS 1 & 2 and LSU rDNA sequences of *P. goodeyi* isolated from Tanzania showed close similarity (98.6 to 99.9%) to other related species in the NCBI GenBank (Figures 3, 4 and 5). *P. goodeyi* isolated in this study consistently clustered into similar clade with other *P. goodeyi* isolated from elsewhere in the world and away from other *Pratylenchus* species.

Table 2: Morphometrics of *Pratylenchus goodeyi* female populations collected from Tanzania; All measurements are expressed in µm and presented in the form mean ± standard deviation (range), *n* = 10

Parameters	Lake Zone	Northern Zone	Southern Highlands Zone	Zanzibar
L	515.1 ± 15.9	522.5 ± 15.9	511.4 ± 15.9	505.3 ± 15.9
	(487 – 710)	(487 – 710)	(487 – 710)	(487 – 710)
a	23.0 ± 3.2	24.3 ± 3.3	23.8 ± 4.0	23.8 ± 4.1
	(19.7 – 29.5)	(19.7 – 29.5)	(19.7 – 29.5)	(19.7 – 29.5)
b	6.2 ± 1.2	5.8 ± 0.9	7.3 ± 0.7	6.6 ± 0.9
	(4.1 – 8.1)	(4.1 – 8.1)	(4.1 – 8.1)	(4.1 – 8.1)
b'	5.2 ± 0.9	4.9 ± 0.8	5.8 ± 1.1	5.4 ± 0.7
	(2.9 – 5.3)	(2.9 – 5.3)	(2.9 – 5.3)	(2.9 – 5.3)
c	15.7 ± 0.2	13.8 ± 0.3	14.6 ± 0.4	17.5 ± 0.4
	(13.8 – 20.0)	(13.8 – 20.0)	(13.8 – 20.0)	(13.8 – 20.0)
c'	2.4 ± 0.3	3.0 ± 0.2	2.7 ± 0.4	2.3 ± 0.4
	(1.9 – 3.2)	(1.9 – 3.2)	(1.9 – 3.2)	(1.9 – 3.2)
Sl	11.3 ± 0.8	11.5 ± 0.8	11.9 ± 0.8	11.6 ± 0.8
	(17 – 20)	(17 – 20)	(17 – 20)	(17 – 20)
V	73.5 ± 0.4	72.6 ± 0.8	78.5 ± 1.4	78.3 ± 1.4
	(73 – 79)	(73 – 75)	(73 – 75)	(73 – 75)
W	22.5 ± 2.4	20.9 ± 2.4	21.0 ± 2.4	21.2 ± 2.4
	(17.8 – 28.7)	(17.8 – 28.7)	(17.8 – 28.7)	(17.8 – 28.7)
t	33.1 ± 5.0	36.0 ± 5.0	34.5 ± 6.0	28.4 ± 6.0
	(27.7 – 41)	(27.7 – 41)	(27.7 – 41)	(27.7 – 41)
OL	142.3 ± 11.2	146.6 ± 11.2	131.5 ± 11.2	128.7 ± 11.2
	116 – 166	116 – 166	116 – 166	116 – 166
DGO	2.7 ± 0.5	2.1 ± 0.6	2.0 ± 1.3	2.2 ± 1.5
	(2.3 – 4.3)	(2.3 – 4.3)	(2.3 – 4.3)	(2.3 – 4.3)

L = body length as measured along the axis; a = body length/maximum body width; b = body length/distance from anterior end to pharyngo-intestinal junction; b´ = body length/distance from anterior end to posterior end of pharyngeal gland; c = body length/tail length; c´ = tail length/body width at anus (female) or cloaca (male); Sl = stylet length; V = distance of vulva from anterior end expressed as percentage of body length (%); W = maximum body width; t = tail length; T = distance from cloaca to anterior most part of testis; OL = oesophageal length; DGO = distance from basal knobs of stylet to dorsal pharyngeal gland orifice.

Table 3: Morphometrics of *Pratylenchus goodeyi* male populations collected from Tanzania; All measurements are expressed in µm and presented in the form mean ± standard deviation (range), *n* = 10

Parameters[a]	Lake Zone	Northern Zone	Southern Highlands Zone	Zanzibar
L	446.0 ± 50.5	473.8 ± 50.5	430.8 ± 49.1	437.3 ± 50.1
	(380 – 526)	(380 – 526)	(380 – 526)	(380 – 526)
a	27 ± 2.4	26.8 ± 2.2	29.3 ± 2.2	26.1 ± 2.0
	(24 – 31.7)	(24 – 31.7)	(24 – 31.7)	(24 – 31.7)
b	6.4 ± 0.4	5.9 ± 0.4	6.0 ± 0.4	6.4 ± 0.2
	(5.8 – 7.3)	(5.8 – 7.3)	(5.8 – 7.3)	(5.8 – 7.3)
b'	5.3 ± 0.5	4.4 ± 0.6	5.0 ± 0.4	5.2 ± 0.5
	(2.9 – 5.3)	(2.9 – 5.3)	(2.9 – 5.3)	(2.9 – 5.3)
c	14.2 ± 1.8	13.9 ± 1.4	10.6 ± 0.8	9.3 ± 0.8
	(12.5 – 17.2)	(12.5 – 17.2)	(12.5 – 17.2)	(12.5 – 17.2)
c'	2.8 ± 0.4	3.4 ± 0.2	3.4 ± 0.2	3.6 ± 0.5
	(3.12 – 4.13)	(3.12 – 4.13)	(3.12 – 4.13)	(3.12 – 4.13)
Sl	10.5 ± 0.8	10.6 ± 0.8	11.1 ± 0.8	10.7 ± 0.8
	(12.7 – 16.0)	(12.7 – 16.0)	(12.7 – 16.0)	(12.7 – 16.0)
T	187.7 ± 12.2	197.0 ± 13.0	106.1 ± 12.5	141.8 ± 12.1
	(174 – 256)	(174 – 256)	(174 – 256)	(174 – 256)
W	18.8 ± 2.3	19.7 ± 2.3	13.6 ± 2.3	14.7 ± 2.3
	(15.2 – 19.5)	(15.2 – 19.5)	(15.2 – 19.5)	(15.2 – 19.5)
t	31.9 ± 2.0	32.2 ± 2.4	31.1 ± 2.0	30.8 ± 1.0
	(28.4 – 35)	(28.4 – 35)	(28.4 – 35)	(28.4 – 35)
OL	93.8 ± 12.1	110.1 ± 12.2	106.4 ± 12.2	102.5 ± 12.0
	(93 – 128)	(93 – 128)	(93 – 128)	(93 – 128)
DGO	2.9 ± 0.8	3.1 ± 0.5	2.9 ± 0.9	3.0 ± 0.8
	(2.4 – 3.6)	(2.4 – 3.6)	(2.4 – 3.6)	(2.4 – 3.6)

[a]Definitions of the abbreviations are provided under Table 2.

Discussion

Identification of the root lesion nematodes (*P. goodeyi*) from various major banana growing areas was achieved in this study. *P. goodeyi* were closely related, with few morphological differences. The results from morphological and morphometric identification of *P. goodeyi* isolated from Tanzania were consistent with reports from elsewhere (Uehara et al. 1999), except for the differences in female and male mean stylet lengths in all the four agro-ecological zones, that were less than the commonly reported ones.

Root lesion nematodes were not commonly reported in the low and warm altitudes until the observation by Luambano et al. (2017). Earlier reports of *Pratylenchus* sp. in East Africa including Tanzania dates back to 1980's (Sikora et al. 1989, Speijer and Bosch 1996). However, *P. goodeyi* were previously observed in the highland and cooler climates (Bridge et al. 1995, Prasad and Reddy 2000). This current observation suggests that, *P. goodeyi* in banana are spreading to areas in which they were not previously found (Luambano et al. 2017), and thus increasing

threat to banana and other crop production. These findings also suggest continuous spread of *P. goodeyi* in banana, possibly owing to increased adaptation of the nematodes to hostile environments. Thus, there is a need for developing simple and rapid diagnostic tools for detection of *Pratylenchus* sp. for proper nematode management strategies. Identification of *P. goodeyi* in this study required both morphological and molecular techniques because of interspecies overlap and intraspecies variations in the PPNs morphological and molecular characteristics. The PCR-based method and sequencing employed in this study confirmed the presence of these damaging banana nematodes in Tanzania.

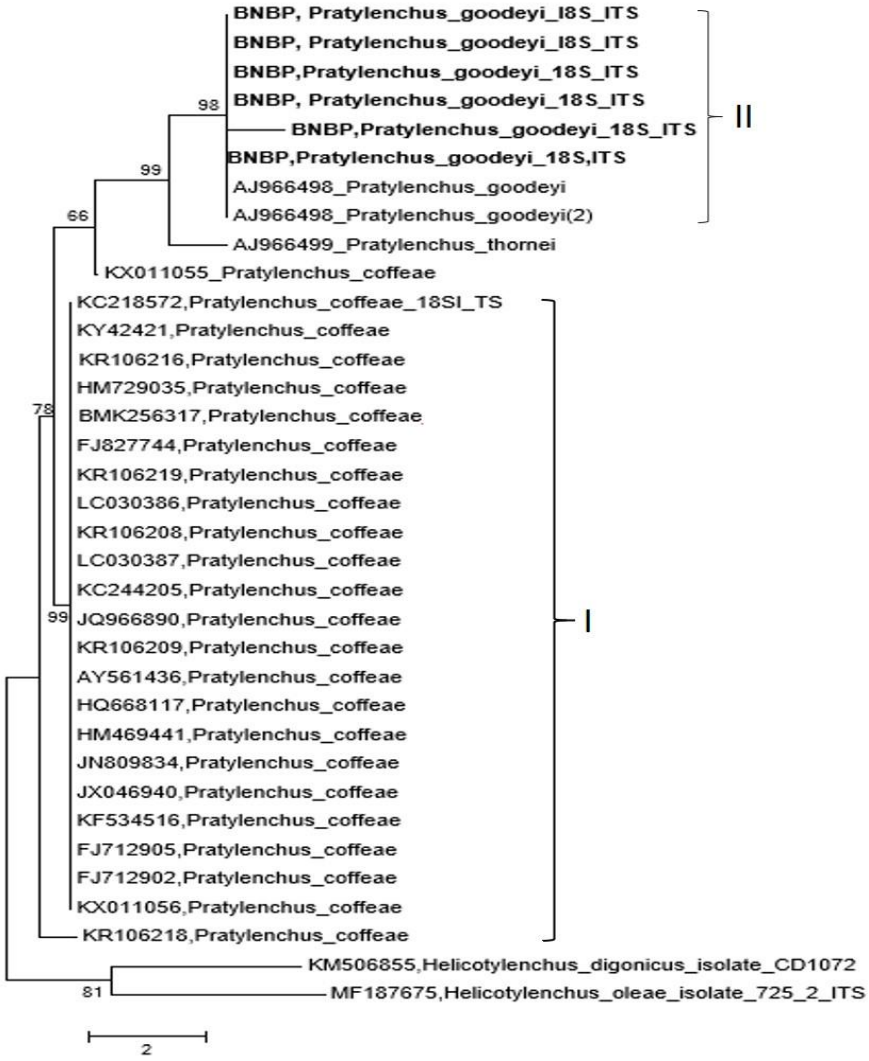

Figure 3: Bayesian consensus tree inferred from SSU-ITS 1 (18S-ITS 1) of *P. goodeyi* sequences and related species sequences under HKY + I model test. Posterior probability values exceeding 50% are given an appropriate clade. Sequences obtained from this study are bolded.

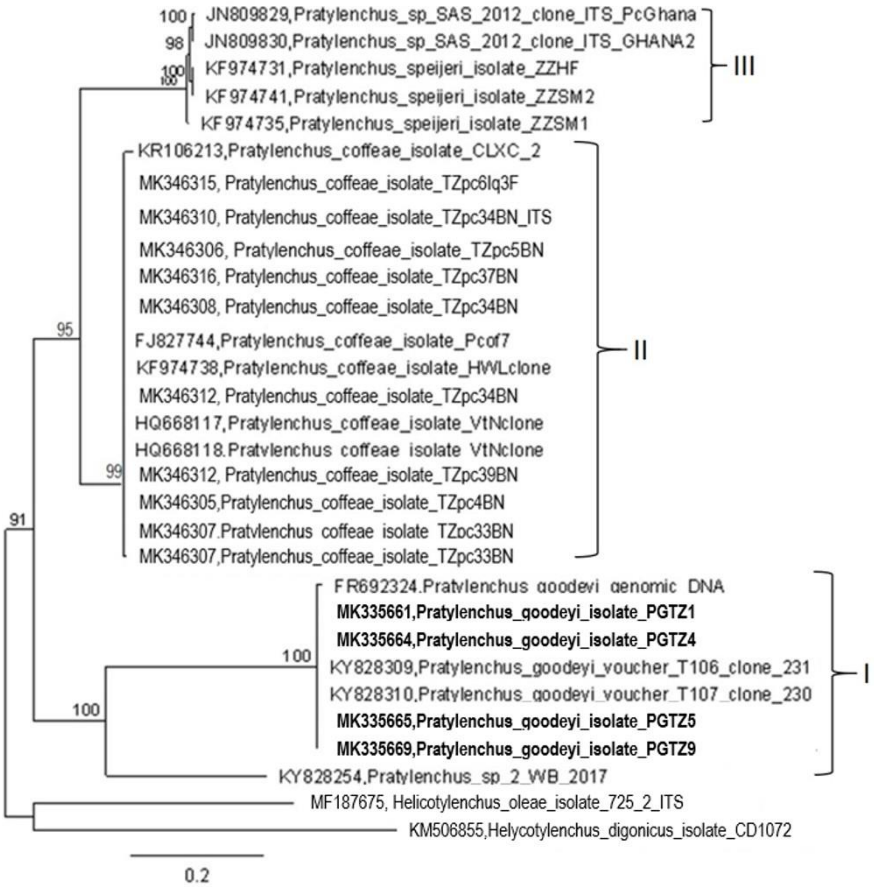

Figure 4: Bayesian consensus tree inferred from ITS 1 & 2 rDNA region of *P. goodeyi* populations and related species under HKY + I model test. Posterior probability values exceeding 50% are given an appropriate clade. Sequences obtained from this study are bolded.

Molecular identification of *P. goodeyi* through PCR and sequencing of SSU, ITS 1 & 2 and LSU rDNA regions demonstrated the importance of utilizing the PCR-based technique for rapid and accurate detection of nematodes affecting crops. Phylogenetic analysis with other related sequences from GenBank clustered the *P. goodeyi* together, while dissimilar species of the same genus were clustered differently, indicating that *Pratylenchus* species are distantly related despite of their common evolutionary origin.

The isolates collected from major banana growing zones in Tanzania clustered together (in all SSU, ITS and LSU sequences) with those from Europe. For instance, through ITS 1 & 2 sequences, *P. goodey* isolates from Zanzibar (MK335669) and Northern Zone (MK335665) were closely related to *P. goodeyi* sequences (FR692324) isolated from Europe (Portugal); *P. goodeyi* sequences (MK335661 and MK355664) isolated from Southern Highlands Zone and Lake Zone, respectively were closely related to sequences KY828309 and KY828310 isolated from

Europe (Netherlands). Similarly, all *P. goodeyi* LSU rDNA sequences from this study were also related to other *P. goodeyi* LSU sequences from Europe. These results imply that the *P. goodeyi* isolated from this study are similar to isolates from Europe, thus indicate great distribution and/or continuous spreading of these nematode species globally Al-Banna et al. 2004, De Luca et al. 2011, Wang et al. 2016, Handoo et al. 2016).

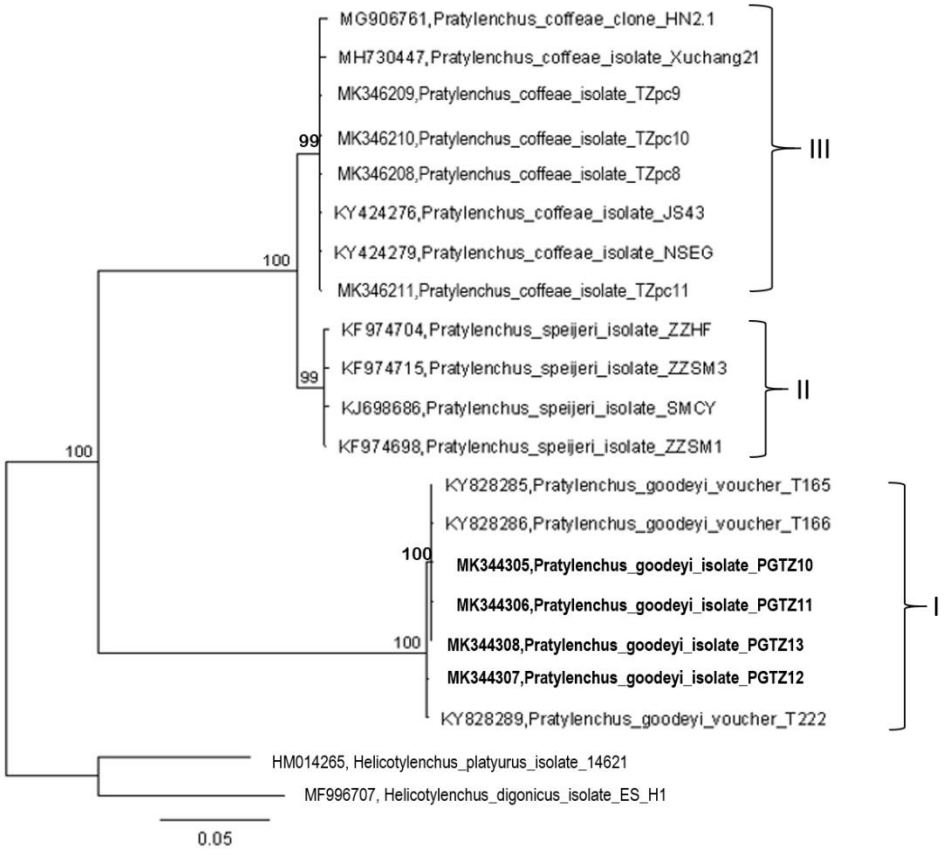

Figure 5: Bayesian consensus tree inferred from LSU rDNA region of *P. goodeyi* populations and related species under HKY + I model test. Posterior probability values exceeding 50% are given an appropriate clade. Sequences obtained from this study are bolded.

The *P. goodeyi* species specific primers designed and used in this study can be used for rapid nematodes identification to generate information for creating awareness of these pests to farmers and to develop sustainable PPN management strategies essential for the improvement of banana production. In this study, very little sequence variations were observed within *P. goodey*i from different populations, suggesting the inter-specific variations. These results are consistent with other studies by De Luca et al. (2004) and Waeyenberge et al. (2000) who reported the presence of inter-specific variations in *Pratylenchus spp*. from different geographical regions.

Sequences from the rDNA region are closely related among root lesion nematodes and are important for species discrimination (Al-Banna et al. 2004). Therefore,

morphological features alone are not enough for the identification of nematodes to genus and species level. Studies by Wang et al. (2016) and Troccoli et al. (2008) characterized *Pratylenchus* species using morphological and molecular approaches, and both studies were able to accurately discriminate nematode species. Sequence and phylogenetic analysis demonstrated a strong relationship between *P. goodeyi* from this study and *P. goodeyi* from GenBank.

Conclusion

The *P. goodeyi* were successfully identified in Tanzania and they are among the damaging pests in bananas. The *P. goodeyi* populations identified using morphological and molecular approaches in our study were closely related. The study also confirmed recent findings by Luambano et al. (2017) of this species presence in low warm altitudes where they have not been found before. Simple molecular diagnostic kit for PPNs such as *P. goodeyi* is important for rapid and accurate detection of *P. goodeyi* in bananas and in other crops. Strictly regulations for transferring banana planting materials should be enforced to avoid introduction of new strains/biotypes of *P. goodeyi*. These efforts may help to avoid or minimize unintentional nematode spread and subsequent crop losses. Further studies to assess virulence and economic losses caused by different populations of *P. goodeyi* from different agro-ecologies are required. The findings of this research will contribute in developing efficient nematode management strategies or in studies aimed at preventing, reducing or eradicating nematodes, especially *P. goodeyi* and other PPNs infestations to improve banana production and other crops in Tanzania.

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