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Diversity of plant-parasitic nematodes associated with coffee and soybean in Kenya with description of known and putative new species

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Diversity of plant-parasitic nematodes associated with coffee and soybean in Kenya with description of known and putative new species

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Pre-amble

Due to the covid-19 pandemic and the restricted access to the lab, it was not possible to analyse root samples from coffee and soybean and therefore only soil samples were analysed. Moreover, only few specimens of the respective nematode populations were described. However, David Kihoro and myself conducted a detailed overview of Kenyan terrestrial nematofauna (***see addendum 2.)*** during this study to replace the time lost for the laboratory work.

General summary - Coffee and soybeans are exceptionally important crops to millions of peoples' livelihoods, especially in the developing countries and they play a huge role in providing food security globally. However, despite the benefits of the two crops, their yield is immensely reduced by pests and diseases, including plant-parasitic nematodes (PPN). Furthermore, comprehensive data on the diversity and distribution of the plant parasitic nematodes associated with coffee and soybean in Kenya are missing. Therefore, the main objective of this research was to study the prevalence and density of PPN associated with coffee and soybeans in Kenya, and characterise the most important species using morphologically and molecularly methods. A total of 14 genera were identified from coffee (8) and soybean (6). *Meloidogyne* spp. dominated 90 % of the coffee farms investigated with high densities (146 nematodes^{-1} 100 cm^3) in all the farms. Out of the six genera found to be associated with soybean, *Rotylenchus*, was the most frequently encountered PPN. Molecular characterisation of the most important species based on the D2-D3 of the LSU rRNA, partial SSU rRNA and COI genes combined with morphometric and morphological characterisation were made for some nematode populations. They include *Rotylenchus* cfr *robustus, Scutellonema brachyurus* and *Helicotylenchus dihystera* from soybean and *Rotylenchulus macrosoma* from coffee. Moreover, a new species of *Pratylenchus* associated with coffee has been described based on morphological and molecular approaches. Finally, an overview of terrestrial nematofauna of Kenya is provided.

Key words – LSU, molecular, morphology, morphometrics, nematofauna, PPN, rRNA, SSU, terrestrial

Coffee and soybean are among the most valuable cash and food crops in Kenya, as earners of foreign currency and source of food. A continued decline in the productivity of these crops has been usually attributed to abiotic, biotic and socio-economic factors (Nzesya, 2012). However, yields of both crops are

severely constrained by the presence of a myriad of pests and diseases, including plant-parasitic nematodes (PPN) causing increased food insecurity (Sikora et al., 2018).

Coffee is an important cash crop, especially in developing countries (Kufa et al., 2011) and its production forms the economic backbone of many countries worldwide. It is exported by over fifty countries making it the second world most traded export commodity after petroleum (Aerts et al., 2011) . The two main coffee varieties which make up the largest coffee trade are *Coffea arabica* and *C. canephora* and they as well contribute approximately 90% of the worldwide coffee production (Davis et al., 2012). However, of all coffee produced commercially 70% is mainly Arabica coffee whose origin is Ethiopia. Arabica coffee's wild variety is currently only grown in Ethiopia, Uganda, and Kenya (Koebler, 2013).

In Kenya coffee supports about 700,000 households representing approximately 4.2 million people representing 10% of Kenyan population. It is fourth in foreign exchange earnings after tourism, tea and horticulture industries which translates approximately into 30 % of the total foreign exchange (Karanja, 2002) . It earned the country $ 154 million in the year 2009/2010 and has earned an average of $ 98 million per year for the last five years (CBK 2011). However, in spite of the fact that coffee plays an invaluable role in the economic development of Kenya, a continuous decline in its quality and quantity has been registered due to increased myriad of pests and diseases including PPNs amongst other factors (Hammond & Onsongo, 2010).

PPNs are a major limiting factor in coffee producing areas worldwide (Campos & Villain, 2005). *Meloidogyne* spp. (root-knot nematodes) and *Pratylenchus* spp. (root lesion nematodes) are the predominant genera and are widely distributed in coffee plantations, causing great economic losses to both farmers and industry (Campos & Villain, 2005). However, many other genera have also been found associated with coffee trees worldwide (Campos & Villain, 2005; Sikora et al., 2018).

The damage caused by PPN on coffee is not well documented in Africa mostly because of lack of attention to the crop and its associated PPN (Sikora et al., 2018). *Meloidogyne* spp. are amongst the most dominant plant-parasitic nematodes attacking coffee production in Kenya (Nzesya et al., 2014) besides *Pratylenchus* spp. and *Tylenchulus* spp. Nevertheless, comprehensive information regarding species associated with

coffee in Kenya is lacking because most of PPN are identified to genera level only (Nzesya et al., 2014). Lack of understanding of what PPNs species associated with coffee limits the management strategies of the particular PPNs and they attribute unknown yield loss.

Soybean *(Glycine max (L.) Merril))* is ranked as the number two oil crop after sunflower in Kenya and is one of the most important oilseed crops in the world. It is a multipurpose crop, ideal for human and livestock feeding and for sustainable cereal production. It has the highest protein content (40-42 %) and second only to groundnut in oil content (18-22 %) among food legumes (Wynstra, 1986). However, the average yield of soybean in Kenya is 0.2-0.5 tha^{-1} compared to other soybean-growing countries like Brazil that have as high as 4 tha^{-1} (Lesueur et al., 2011). This has been attributed to low soil fertility, particularly N and P deficiencies due to soil acidity, intensive cropping, lack of farm inputs used by farmers and effects of pest and diseases including PPN (Mahasi et al., 2011).

About 100 PPN species, representing several genera, are reported to be associated with soybean (Sikora et al., 2018). The major PPN species that cause substantial damage to soybean include, *Meloidogyne* spp. (Koenning, 2015), *Heterodera glycines* (Kim et al., 2011), *Pratylenchus* spp. and *Rotylenchulus reniformis* (Fabia, et al., 2016). Of these, *Meloidogyne* spp. are regarded among the most damaging to soybean (Fourie et al., 2001; Sikora et al., 2018). However, there is no published information regarding PPN associated with soybean in Kenya.

The economic consequences of crops due to direct damage caused by PPNs is very huge and therefore correct PPN identification is a prerequisite to effective applications of management options (Tadigiri et al., 2005). Most of the quantitative and qualitative yield losses caused by PPN which approximates to $172 million worldwide (Abad et al., 2008) are due to lack of expertise in nematology (Tadigiri et al., 2005) amongst other factors such as lack of PPNs awareness and the economic damage they cause.The problem with PPN is that they are so morphological minimalistic (De Ley et al., 2005) which therefore requires well-trained taxonomists and high-resolution light and electron microscopy for their identification. Also, the traditional identification of plant-parasitic nematode species by morphology and morphometric studies is very difficult because of their high morphological intraspecific variability that can lead to considerable overlap of many characteristics and their ambiguous interpretation. For this reason, it is essential to

implement also approaches to ensure accurate species identification, such as DNA barcoding, which facilitate identification.

DNA-based approaches have been successfully developed and used for molecular diagnostics and diversity of PPN species. The genes involved include, SSU rRNA, LSU rRNA, and ITS1 and ITS2 rDNA regions (Waeyenberge et al., 2000; Handoo, Carta & Skantar, 2008). Mitochondrial genes are also promising reliable barcodes especially for identification of PPN (Subbotin et al., 2013; Pagan et al., 2015). The Nad5 gene fragment is a promising barcode for clade I or tropical *Meloidogyne* spp.(Janssen et al., 2016).

Therefore, the objective of the present work was to: 1) Morphologically and molecularly identify and characterise known PPN associated with coffee and soybean in Kenya; 2) Determine the density and prevalence of nematodes to assess their potential impact in both soybean and coffee; 3) describe new species (see separate paper (**Addendum 1**): *Morphological and molecular characterisation of Pratylenchus sp. n. (Pratylenchidae), a root-lesion nematode associated with coffee in Kenya)*); 4) Provide an overview of Kenyan nematofauna (see separate paper (**Addendum 2**): *An overview of terrestrial nematodes in Kenya*)).

Materials and Methods

Sampling

Coffee

To obtain information on potentially harmful PPNs associated with coffee species, we conducted an extensive survey in two main coffee producing counties (Kiambu and Kirinyaga) on January and February 2020 (Fig. 1). In each county, five coffee farms were randomly selected for sampling. In each farm, five coffee plants were selected and then 2 samples (one from soil and one from roots) were taken using a shovel per plant from the top 20 cm of weed-free soil from three different places under the tree canopy at an approximate distance of 50-70 cm from the stem. That makes 10 samples per farm which translates to a total of 25 soil and 25 root samples per county (Kirinyaga and Kiambu).

Soybean

A survey of 5 soybean-producing counties in western part of Kenya was conducted (Fig.1) in November 2019 to obtain information on potentially harmful PPNs associated with soybean. With the aid of extension officer personnel, fields being representative of the various cultural systems (i.e. rotation, cropping sequence, and tillage practices) were selected in each county. Sampling area was predetermined by the size of the soybean field, from which approximately 1000 g of soil was collected. Soil samples were taken using a sampling shovel to an approximate depth of 15-20 cm in a zig-zag pattern across the area sampled. One bulk soil sample was collected per field, regardless of its size. Approximately 8-10 root systems and rhizosphere soil depending on the size of the farm composed each sample. As a result, a total of 50 samples (25 root and 25 soil), each weighing at least 1000 g, were collected.

Nematode extraction

For every soil sample collected, nematode extraction was conducted in 300 ml of soil using a modified Baermann funnel technique (Coyne et al., 2018) and nematodes were collected after 24-72 hours. The roots from each sample were carefully washed under running tap water and gently blotted dry with a kitchen towel; clean roots were then chopped into small pieces followed by weighing 15 g of the chopped roots. The living vermiform nematodes were extracted using the modified Baermann funnel technique as employed for the soil samples. These nematodes were collected by washing the samples over 38 µm aperture sieve.

Density, prevalence and prominence analysis

Nematodes were identified to the genus level and counted under a stereoscopic microscope at ×40 magnification. When necessary, observations for species identifications were made with a phase contrast Olympus BX50 DIC Microscope (Olympus Optical, Tokyo, Japan). The PPNs incidence was assessed by determining the **prevalence**, calculated as the number of samples having a particular nematode species divided by the number total samples examined and expressed as a percentage. Density was presented by determining the **mean density**, calculated as the number of individuals of a particular nematode species in the positive samples divided by the number of positive samples), (Boag, 1993). **Prominence** was calculated according to formula provided by Al-Hazmi et al (2009)

Morphological study

Specimens were identified by light microscopy using morphology and morphometric characters. Individual juveniles, females and/or males of each population were fixed using 4% formalin with 1% glycerin at 70°C (Seinhorst, 1966). The fixed nematodes were gradually transferred to anhydrous glycerin for permanent slides, following the protocol of Seinhorst (1959a) and mounted on glass slide for light microscopy study. Measurements and light micrographs were taken with an Olympus BX50 DIC Microscope (Olympus Optical, Tokyo, Japan) connected to an Olympus C5060Wz camera; ImageJ software version 1.51. was used to take measurements.

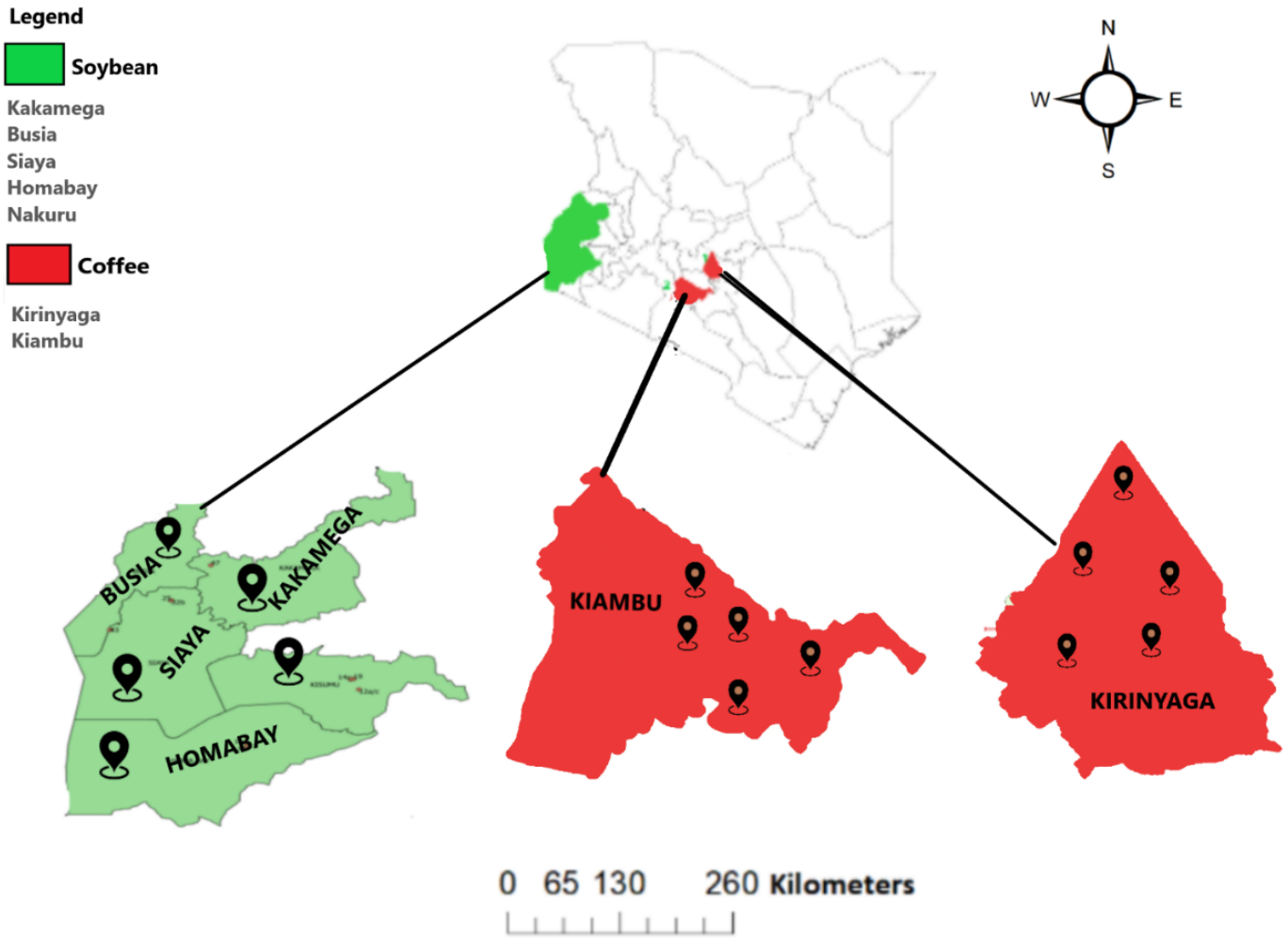

Figure 1. Map showing nematode sampling locations.

DNA extraction, PCR and sequencing

DNA extraction

Morphological vouchers

Individual live specimens were handpicked into a drop of distilled water and used for preparation of morphological vouchers of temporary mounts using light microscopy.

DNA extraction with Worm lysis buffer (WLB)

After morphological vouchers, individual nematodes were cut in 2 pieces with a sterile picking needle. The pieces were put into a 200µl eppendorf tube with 20 µl of WLB (50mM KCl; 10mM Tris pH=8.3; 2.5mM MgCl2; 0.45% NP 40 (Tergitol Sigma); 0.45% Tween 20) and were frozen for at least 10 min at −20°C. Then 1µl proteinase K (1.2mg/ml) was added to the sample to aid in digesting any proteins present that would contaminate the DNA and to protect the nucleic acids from nuclease attack. Then the sample were incubated in the thermocycler for 1 hour at 65°C and 10 minutes at 95°C and finally centrifuged for one minute at 15,000 rpm (Singh et al., 2018).

Polymerase Chain Reaction (PCR)

PCR were carried out in 25 µl volumes PCR reaction with different primers depending on the target gene (Table 2). PCR mix shown in table (Table 1) was added to each tube. The PCR reactions were run in a PTC-100 Thermocycler (Bio-Rad). PCR cycling conditions for each gene were as below.

18S rDNA region

The 5′-end of the 18S rDNA region was amplified using the primers 18A/26R Initial denaturation at 94°C for 4 min, followed by 5 cycles of denaturation at 94°C for 1 min, annealing temperatures starting at 52°C for 1 min and 30 s (decreasing by 1°C per cycle), and 68°C for 2 min for extension. This step was followed by 35 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 1 min and finished at 10°C for 10 min.

28S rDNA region

The 5′-end of the 28S rDNA region was amplified using the primers DP391/501 (Nadler et al., 2006) with the PCR reaction started at 94°C for 5 min, followed by 5 cycles of 94°C for 30 s, 45°C for 30 s, and 72°C for 2 min. This step was followed by 35 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min and finished at 12°C for 10 min.

cytochrome c oxidase subunit 1 (COI mtDNA) gene

The cytochrome c oxidase subunit 1 (COI mtDNA) gene was amplified using the primers JB3/JB4 (Derycke et al., 2010); initial denaturation of 5 min at 94 °C, 5 cycles of (94°C for 30 s; 54°C for 30 s and temperature decreasing with 1°C for each cycle; 72 °C for 30 s) followed by 35 cycles of (94°C for 30 s; 50°C for 30 s; 72°C for30 s), and a final extension of 10 min at 72°C.

Mitochondrial DNA, NAD 5

The Nad5 gene was amplified using the primers F2/R1 (Janssen et al., 2016). Initial denaturation of 2 min at 94 °C, followed by 94°C for 1 min, 45 °C for 30 s and 72 °C for 1 min, followed by 40 cycles of 72 °C for 10 s and 12 °C ∞.

PCR Visualization

PCR products were visualized on UV light source after gel electrophoresis conducted with 1% agarose gel stained with GelRed. Only successful PCR products were submitted for sequencing by commercial Macrogen company (https://dna.macrogen.com).

Table 1. The PCR cocktail used in this study

Reagent	**Final concentration (µl)**
Nuclease free water	17.00
dNTP (10 mM)	0.50
MgCl2	2.00
Foward Primer	0,50
Reverse Primer	0,50
10x Buffer	2.50
CoralLoad	2.50
TopTaq	0.05
DNA	2.00

Table 2. Primers used in this study

Genes	**Forward primers**	**Reverse Primers**	**References**
18S rRNA	**SSU18A** 5'-AAAGATTAAGCCATGCATG-3'	**SSU26R** 5'-CATTCTTGGCAAATGCTTTCG -3'	(Mayer et al., 2007)

28S rRNA	**D2A** 5'-ACAAGTACCGTGAGGGAAAGTTG-3'	**D3B** 5'-TCGGAAGGAACCAGCTACTA-3'	(De Ley et al., 1999)
	391f 5'-AGCGGAGGAAAAGAAACTAA-3'	**501** 5'-TCGGAAGGAACCAGCTACTA-3'	(Nadler et al., 2006)
COI	**JB3** 5'-TTTTTTGGGCATCCTGAGGTTTAT-3'	**JB4** 5'-TAAAGAAAGAACATAATGAAAATG-3'	(Palomares-Rius et al., 2017)
NAD 5	**F2** 5'-TATTTTTTGTTTGAGATATATTAG-3')	**R1** 5'-CGTGAATCTTGATTTTCCATTTTT-3')	(Janssen 2016)

Sequence and phylogenetic analyses

Forward and reverse sequences for each sample generated in this study were assembled using Geneious 7.0.6 (https://www.geneious.com). Consensus sequences obtained were used to search for similar sequences in GenBank (http://www.ncbi.nlm.nih.gov) through BLAST. The alignment was done by muscle (Edgar, 2004) (Built-in Geneious) programme. The poorly aligned regions of the alignments were manually removed. The BI was performed with MrBayes 3.2.6 Add-in in Geneious R11 (Huelsenbeck, 2001) under general time-reversible model with rate variation across sites and a proportion of invariable sites (GTR + I + G) (Abadi et al., 2019). The Markov chains were set with 1 × 10^6 generations, four runs, 20% burn-in, and subsampling frequency of 500 generations (Huelsenbeck, 2001). Trees were visualised and rooted using FIGTREE v1.4.

RESULTS

Density and prevalence analysis

Coffee

Eight PPN genera and representatives of the family Criconematidae were found associated with coffee growing regions of Kiambu and Kirinyaga in Kenya (Table 1). *Meloidogyne* was the most encountered genus with a prevalence of 60% and average density of 146 nematodes/100 cm^3 soil followed by *Tylenchulus* (24%) with an average density of 117 individuals $^{-1}$ 100 cm^3.The least encountered taxa were *Trichodorus* and *Criconematidae,* 4% each (Table 3, Figure 2,3). The mean density of the analysed taxa associated with coffee in this study ranged from 10 to 200 indivinduals^{-1}100 cm^3 soil (Table 3).

Table 3. Abundance and prevalence of PPNs associated with coffee in Kirinyaga and Kiambu counties

Taxon	Prevalence (%)	Nematode density (100 cm^3) soil			Prominence
		Mean	Min	max	
Meloidogyne	60	146	109	346	113
Tylenchulus	24	117	153	306	57
Pratylenchus	16	62	67	102	18
Xiphinema	16	10	15	15	4
Paratylenchus	8	200	132	268	57
Rotylenchulus	8	174	142	205	49
Helicotylenchus	6	93	25	68	13
Trichodorus	4	56	56	56	11
Criconematidae	4	17	17	17	3

* Prevalence = Number of positive samples containing a given taxon divided by total samples × 100.

** Mean density = Mean number of vermiform nematodes / 100 cm^3 soil in the positive samples.

***Prominence = Density× SQRT(Prevalence), based on absolute density and absolute frequency in the positive samples

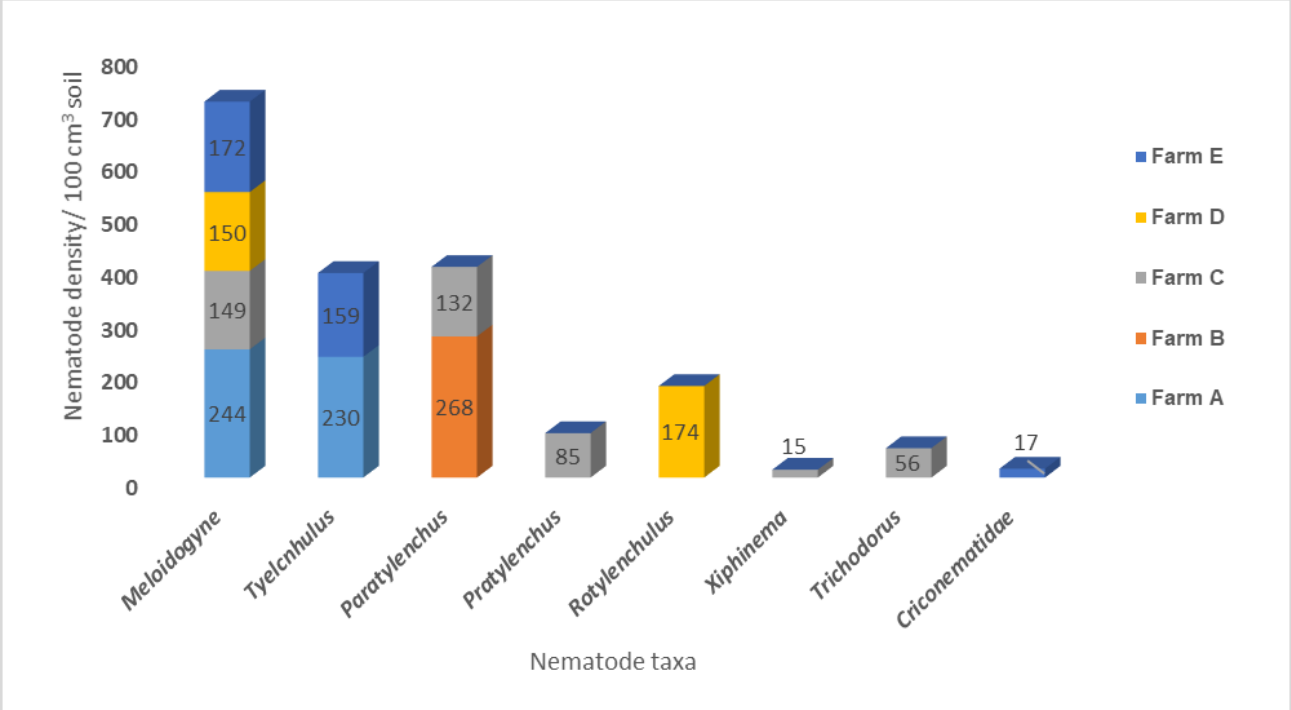

Figure 2.Mean density of PPNs associated with coffee in Kirinyaga county.

In Kirinyaga county, eight PPN taxa were recorded of which *Meloidogyne* was dominant in almost all the farms sampled with the highest density (244) in farm A (Figure 2). Farm C had the highest nematode diversity (5 taxa), namely; *Meloidogyne, Paratylenchus, Pratylenchus, Xiphinema, and Trichodorus,* followed by farm E (3 taxa): *Meloidogyne*, *Tylenchulus* and *Criconematidae.*

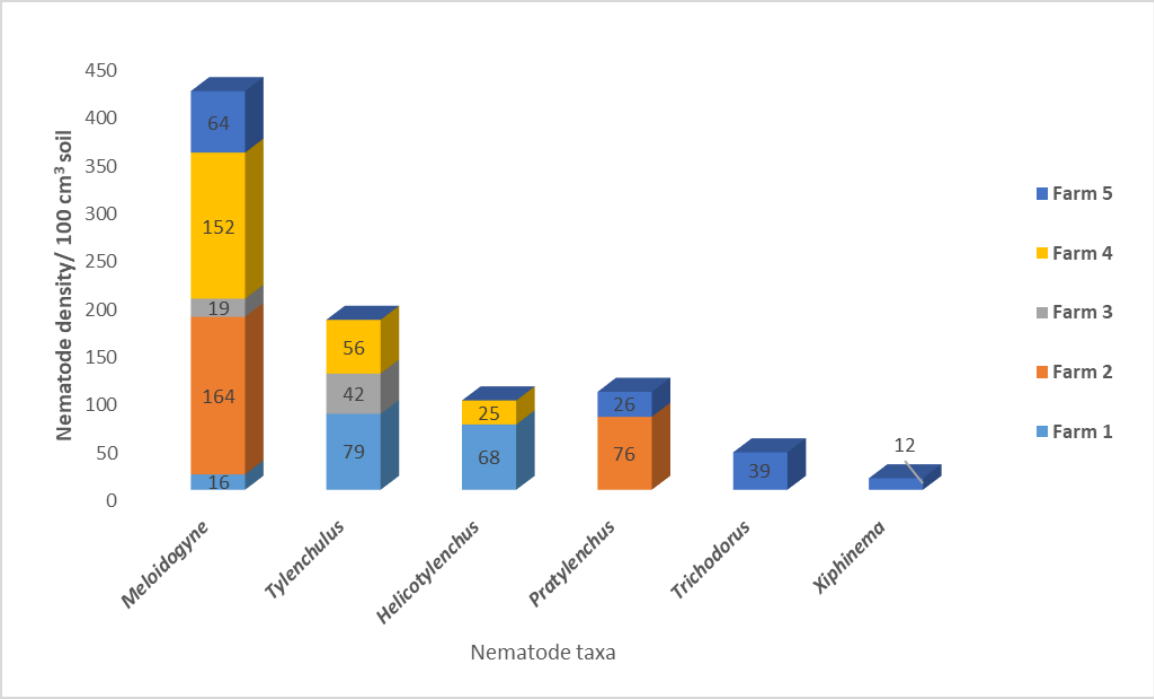

Figure 3. Mean density of PPNs associated with coffee in Kiambu county.

In Kiambu county (Figure 3), six PPN were recorded and *Meloidogyne* dominated all the farms sampled with the highest average density (152 nematodes /100cm^3) in farm 4. The highest nematode diversity on genus level (4 out 6) was recorded in Farm 5, which includes; *Meloidogyne, Tylenchulus, Pratylenchus* and *Xiphinema* while farm 2 had the least nematode diversity with only two taxa recorded, namely; *Meloidogyne* and *Pratylenchus.*

Soybean

Six PPN genera were identified across the 25 soil samples collected from soybean agricultural field sites in five counties. *Rotylenchus* was the most prevalent (69%), most prominent (124) and had the highest density while *Pratylenchus* had the lowest prevalence (6 %) (Table 4).

Table 4. Density, frequency and prominence values of plant-parasitic nematodes identified from soil samples of Soybean.

Taxon	Prevalence (%)	Nematode density (100 cm^3) soil			Prominence
		Mean	Min	Max	
Rotylenchus	69	149	41	261	124
Helicotylenchus	31	102	23	299	57
Meloidogyne	25	119	53	246	60
Scutellonema	19	122	78	152	53
Criconematidae	13	14	13	15	5
Pratylenchus	6	78	78	78	19

* Prevalence = Number of positive samples containing a genus ÷ number of collected samples × 100.

** Mean density = Mean number of vermiform nematodes / 100 cm^3 soil in the positive samples.

*** Prominence = Density× SQRT(*frequency*), based on absolute density and absolute frequency in the positive samples.

Busia and Bungoma had the most taxa with 6 and 5 taxa, respectively (Table 5) while Nakuru had the least with only 2 taxa. *Rotylenchus* was present in all the counties and with relatively high prevalence values followed by *Meloidogyne* (absent in Kakamega and Nakuru), *Scutellonema* (absent in Bungoma and Nakuru) and *Helicotylenchus* (absent in Siaya and Nakuru).

Table 5. Density, frequency and prominence values of plant-parasitic nematodes of Soybean in different counties

Counties	Taxon	Prevalence (%)	Nematode density (100 cm^3) soil			Prominence
			Mean	Min	Max	
Busia	*Helicotylenchus*	83	88	23	103	80
	Rotylenchus	50	149	67	249	105
	Pratylenchus	50	145	98	205	101
	Scutellonema	33	157	98	215	90
	Meloidogyne	26	115	81	148	70
	Criconematidae	17	78	78	78	32
Bungoma	*Xiphinema*	67	20	13	27	16
	Rotylenchus	46	70	23	116	47
	Meloidogyne	33	42	24	37	24
	Helicotylenchus	29	31	31	31	17
	Criconematidae	13	15	15	15	5
Siaya	*Rotylenchus*	67	96	48	243	78
	Meloidogyne	67	57	31	82	46
	Scutellonema	33	214	78	136	122
Kakamega	*Rotylenchus*	75	164	41	261	142
	scutellonema	25	152	152	152	76
	Helicotylenchus	25	299	299	299	150
	Pratylenchus	25	78	78	78	39
Nakuru	*Rotylenchus*	75	176	140	201	152
	Criconematidae	25	13	13	13	7

Morphological and molecular characterisations of five PPN species associated with coffee and soybean

From the identified nematodes, molecular characterisation based on the D2-D3 of the LSU rRNA, partial SSU rRNA, *COI* and *Nad5* genes combined with morphometric and morphological characterisation were made for five species, namely: *Rotylenchus* sp. n*, Scutellonema brachyurus* and *Helicotylenchus dihystera* from soybean soil samples and*, Pratylenchus* n. sp (see separate paper) and *Rotylenchulus macrosoma* from coffee soil samples. Other identified nematodes were not described to species here due to Covid -19 pandemic which limited ability to access the laboratory.

Rotylenchus sp. n

Rotylenchus sp. n were collected from soil and root rhizosphere of soybean in Busia county, Kenya. The population was retrieved from two soil samples (DGS15 and DGS26) from the following GPS coordinates, 0° 34' 26.5224" N, 34° 11' 34.8828" E and 0° 34' 38.2224" N, 34° 11' 32.316" E respectively.

Morphological characterisation

Female

Lip region hemispherical, offset, with 6 distinct annuli. Cephalic framework strongly sclerotized. Stylet robust, 30-32 µm long. Lateral field with four lines areolated at pharyngeal region only. Spermatheca rounded, without sperms. Vulva with distinct epiptygma. Phasmids located on the 7-9 annuli anterior to anal level. Tail rounded, more curved on dorsal side, with annulated tip.

Measurements

See table 6.

Table 6. Morphometric data of *Rotylenchus* sp. n. from soybean soil rhizosphere. All measurements are in µm (except for ratio) and in the form: mean±s.d. (range).

Character	*Rotylenchus* sp. n
n	6
L	929 ± 50.8 (868 – 991)
a	27.0 ± 1.65 (25.3 – 30.3)
b’	6.6 ± 0.34 (6.1 – 7.0)
c	50.2 ± 4.1 (44.9 – 55.3)
c’	0.81 ± 0.06 (0.7– 0.9)
V	59 ± 0.04 (55 – 65)
Lip height	5.7 ± 0.1 (5.6 – 5.9)
Lip diam.	10.9 ± 1.2 (8.5 – 12.3)
Stylet length	31.1 ± 0.8 (29.7 – 32.1)
Conus length	16.5 ± 0.8 (15.1 – 17.2)
Shaft length	11.2 ± 1.4 (9.0 – 13.0)
Knob height	3.2 ± 0.18 (3.2 – 3.8)
Knob diam.	5.1 ± 0.25 (4.8 – 5.5)
Dorsal gland opening from stylet base	3.7± 0.24 (3.4 – 4.1)
Anterior end to secretory-excretory pore	132 ± 3.1 (129 – 138)
Anterior end to the end of pharyngeal gland	140 ± 7.1 (126 – 147)
Max body diam.	34.6 ± 2.8 (31.8 – 39.2)
Vulval body diam.	26.9 ± 3.1 (21.5 – 31.6)
Anal body diam.	23.2 ± 1.6 (20.6 – 25.3)
Tail length	18.6 ± 0.83 (17.7–19.7)

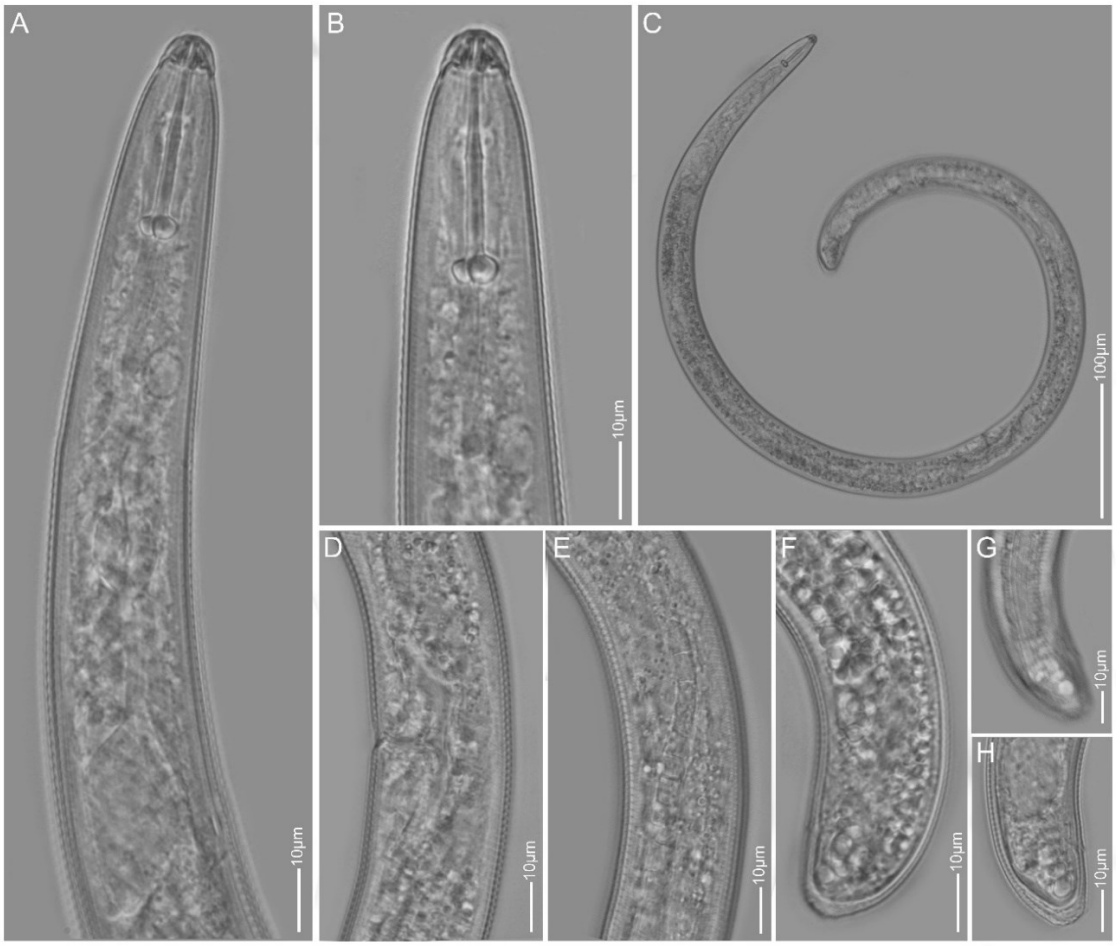

Figure 4. The LM pictures of *Rotylenchus robustus* A: Pharyngeal region; B: Lip region; C: Entire body; D, E: Vulva region; F, G, H: Tail region.

Molecular characterisation

Three sequences of the partial SSU rDNA products were obtained with intraspecific variations of 0.44 - 0.95% (4 - 9 bp). The partial SSU rDNA alignment was 952 bp in length. Our population was found together in a maximally supported clade with *R. robustus* from Ethiopia (MK348059) (Fig. 5). However, not in clade together with *R. robustus* from Belgium and the Netherlands (GenBank KJ636398, KJ636397, AJ966503, KJ636429,). Our population is 99.43 - 99.89% % (1 –5 bp different) and only 92.57 – 93.52% (59-69 bp differences) similar to the *R. robustus* from Belgium and the Netherlands.

18S

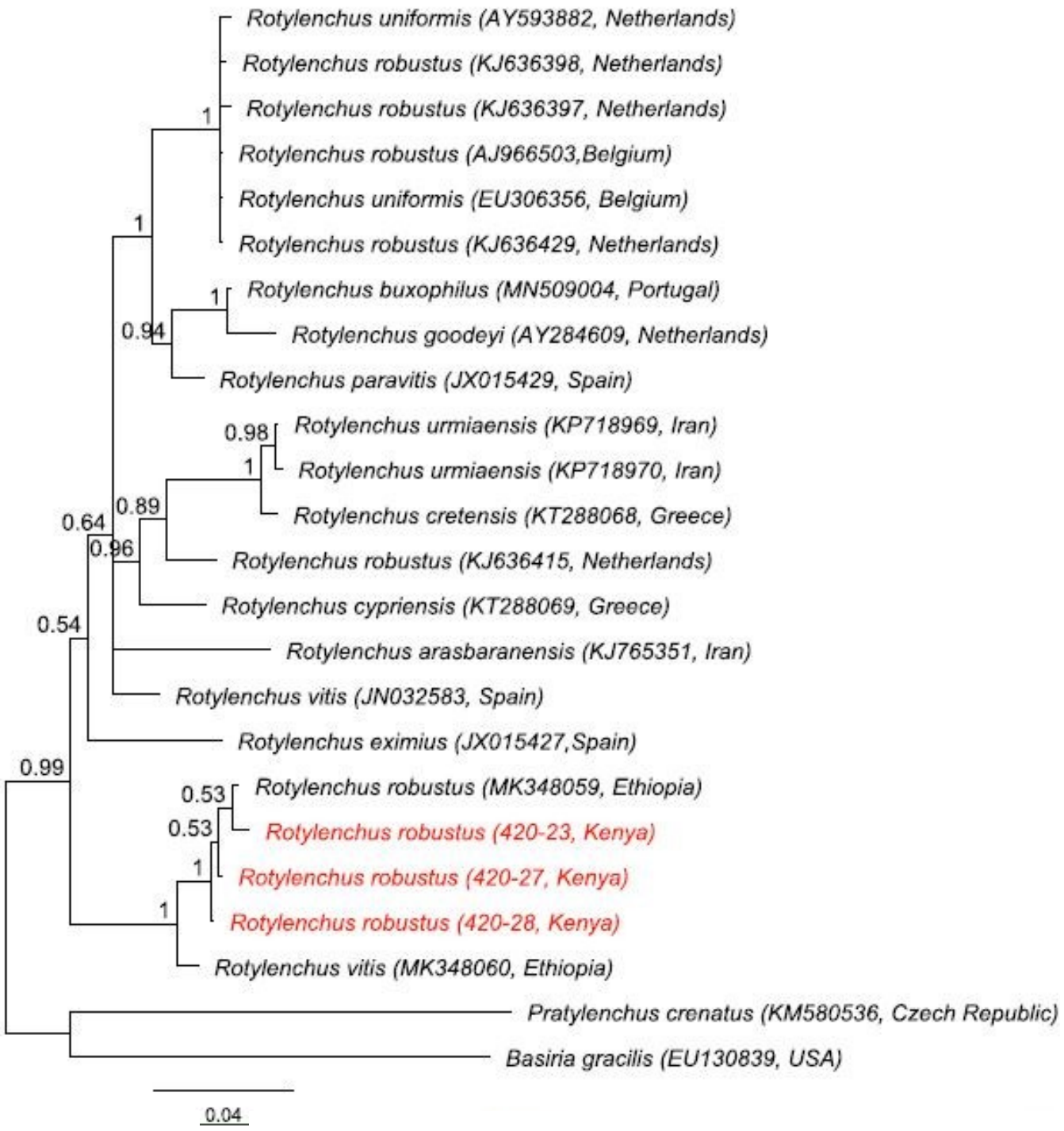

Figure 5. The LM pictures of *Rotylenchus* sp. n A: Pharyngeal region; B: Lip region; C: Entire body; D, E: Vulva region; F, G, H: Tail region.

Remarks

The morphology and the morphometrics of *Rotylenchus* sp. n from Kenya were in agreement with the description of the type population of *R. robustus* by de Man (1876) and the re-description of Filip'ev (1936). However, based on molecular analyses, the *Rotylenchus* sp. n and Ethiopian *R. robustus* population formed a maximally supported clade but it was clearly different from the Belgium and Netherland *R. robustus* populations (Fig 5). It was interesting to note that the SSU sequences of *R. robustus* originating from Netherlands, Belgium and Ethiopia are not linked to morphological data and are most likely mislabeled or misidentified which validates the importance of linking morphological data with DNA sequences of the same specimen in order to prevent a sequence misidentifications and mislabeling.

This study hypothesizes three options based on the molecular analysis; first, the Belgian and Netherland sequences were mislabeled, which therefore means that the Ethiopian and *Rotylenchus* sp. n are the valid *R. robustus.* Second, the Netherland and Belgium sequences are the genuine *R. robustus* based on the fact that they were collected near to the type location which means that the Ethiopian and our population are new species and thirdly, *R. robustus* is a cryptic species complex. Further clarifications on *Rotylenchus* sp. n should be done based on other molecular genes such as LSU rDNA, *COI* mtDNA and ITS since this study was not able to provide that information due to the inability to access the laboratory facilities during COVID-19 pandemic. This will be the first report of *Rotylenchus* spp. associated with soybean in Kenya given that it has been reported in soybean from other countries (Elhady et al., 2018; Sikora et al., 2018).

***Scutellonema brachyurus* (Steiner, 1938) Andrássy, 1958**

S. brachyurus were collected from soil and root rhizosphere of soybean in Busia county, Kenya. The population was retrieved from one soil sample (DGS23) from the following GPS coordinates, “0° 34' 53.1012" N, 34° 11' 17.4588" E”.

Morphological traits and measurements of the female Kenyan population of *S. brachyurus* is in agreement with the type population of Steiner (1938). Van Den Berg et al (2013) described two types of *S. brachyurus*; type A and type B representing American population and African population respectively. The comparison of the Kenyan population with the American and African population revealed that the Kenyan population

matched those of South Africa and differed with the American population based on the following characters; lip region with 4 to 6 annuli vs mainly three, rarely 4 to 5 annuli; 4 to 12 blocks on basal annulus vs 8 to 20 blocks; secretory–excretory pore located opposite anterior part to mid-region of overlapping pharyngeal lobe vs from rarely opposite mid-isthmus to mostly opposite the posterior part of pharyngeal gland lobe up to its posterior border (Van Den Berg et al., 2013) (Table 7)

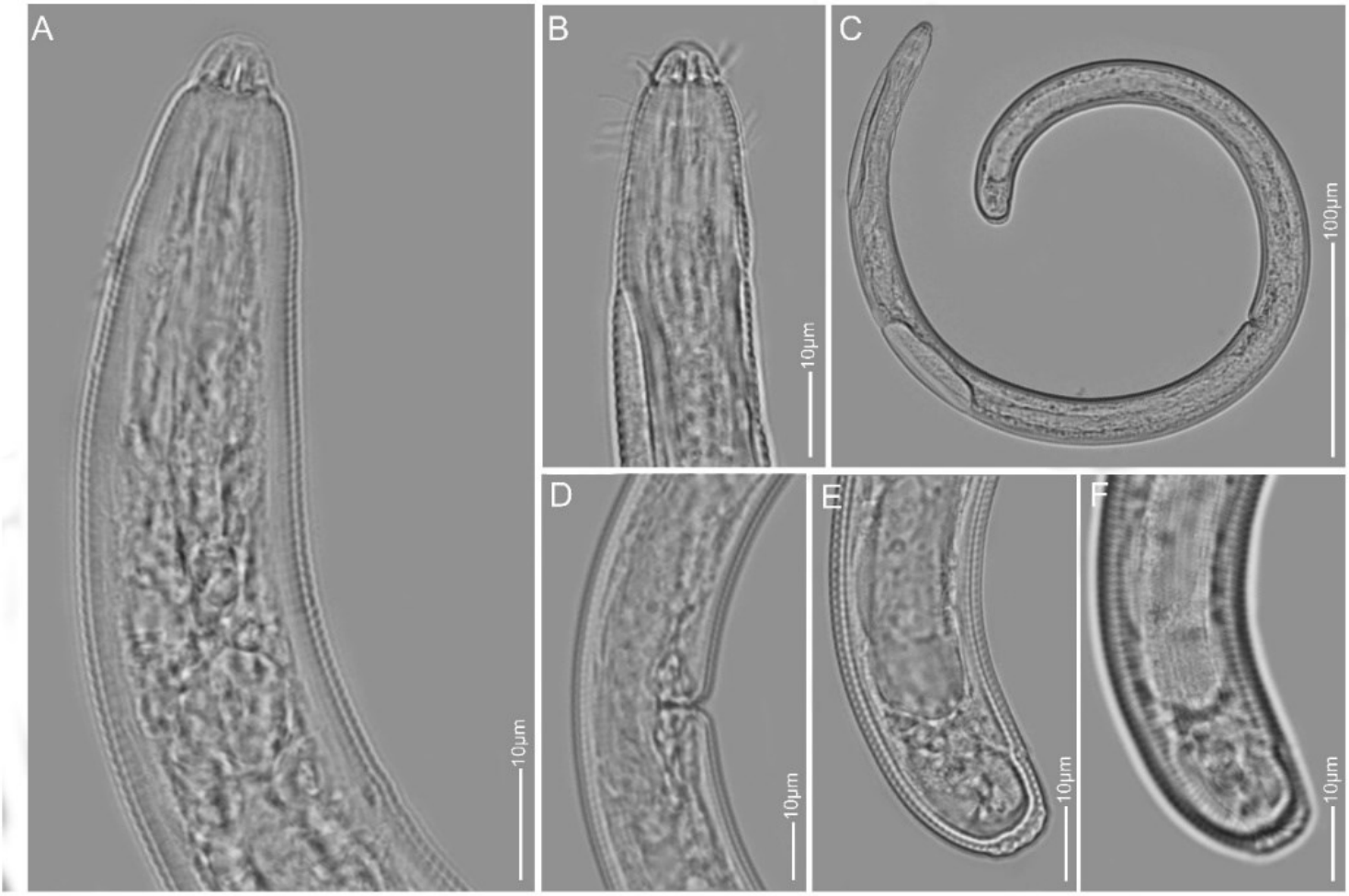

Figure 6. The LM pictures of *Scutellonema brachyurus* A: Pharyngeal region; B: Lip region; C: Entire body; D, E: Vulva region; F, G, H: Tail region.

Table 7.Measurements of *Scutellonema brachyurus* from soybean soil rhizosphere , American and South African populations. All measurements are in µm and in the form: mean ± s.d. (range) for Kenyan population and (median) range for both American and South African populations.

Character	Kenyan population of *S. brachyurus* (This study)	American populations of *S. brachyurus* (Type A)[1]	South African populations of *S. brachyurus* (Type B)[a]
N	6	-	-
L	768 ± 30.3 (715 - 803)	(715-756) 611-805	(777-820) 692-920
A	23.2 ± 2.9 (20 -27.2)	(21-21.7) 19.5-23.1	(26.3-28.5) 23-31.8
b'	5.6 ± 0.52 (5.0 – 6.3)	(6.1-6.3) 5.5-7.4	(7-7.5) 6.2-8.8
C	56.8 ± 4.8 (48.3-64.0)	(62.6-69) 56.5-95.3	(49.7-67.5) 42.9-95.5
c'	0.65 ± 0.07 (0.6 – 0.77)	(0.5) 0.4-0.6	(0.7-0.9) 0.5-1.0
V	60 ± 0.02 (57 - 64)	(59-60) 57-62	(57-58) 53-60
Lip height	5.1 ± 0.5 (4.4 – 5.9)	(5.5) 5-6.5	(5) 4.5-6.5
Lip diam.	8.3 ± 0.7 (7.4 – 9.6)	(9.5-10) 9-11	(8-8.5) 7.5-10.5
Stylet length	29.9 ± 0.7 (28.9 -31.2)	(28.5-29) 27-30.5	(28-29.5) 25.5-32
Conus length	14.1 ± 0.7 (12.8 – 14.7)	(13.5-14.5) 12-15.5	(13-14) 11.5-15.5
Shaft length	12.7 ± 0.6 (12 – 13.6)	(14.5-15) 13.5-16	(15) 13-16.5
Knob height	3.1 ± 0.5 (2.5 - 4)	(3.5) 3.0-3.5	(3) 2-4
Dorsal gland opening from stylet base	4.9 ± 0.0.9 (3.9 – 6.3)	(5-6) 3-7	(5-6) 3.5-6.5
Anterior end to secretory-excretory pore	130 ± 9.4 (122 -140)	(109-127) 103-136	(115-127) 101-150
Anterior end to the end of pharyngeal gland	136 ± 9.4 (125 - 153)	(147) 133-156	(128-140) 114-165
Pharyngeal gland overlap	25.2 ± 2.8 (19.5 – 28.3)	(26-27.5) 10.5-37	(19.5-23.5) 10.5-34.5
Max body diam.	33.5 ± 3.3 (29.3 -37.9)	(33-35) 29.5-38	(28-31) 19.5-38
Anal body diam.	21.1 ± 3.2 (16.1 -25.5)	(18.5-22.5) 17-24.5	(18-21) 14-23.5
Tail length	13.6 ± 0.9 (12.5 – 14.8)	(11-12) 7.5-14	(12.5-16.5) 7.5-23
Scutellum length	4.4 ± 0.5 (3.7 - 5.1)	(4) 3.5-5	(3.5-4.5) 3-5

[1] (Van Den Berg et al., 2013)

Molecular characterisation

Two LSU rDNA sequences were obtained with the length of 1060 and 1063 bp. The length of Muscle alignment was 1612 positions, and 1063 positions were retained in the final dataset. The intraspecific variation of our population sequences of was 0.2 % (3 bp difference). The Kenyan population was found together in a maximally supported clade with the South African populations of *S. brachyurus* (JX472049, JX472048, JX472056, JX472057). The Kenyan population was 95.3-99.85 % (4-37 bp difference) and only 91.5-94.7 % (34-44 bp difference) similar to the African and American populations respectively.

Four COI mtDNA sequences were obtained (442 bp in length) whose analysis involved 39 nucleotide sequences was found together in a maximally supported paraphyletic clade with Rwanda and South African populations (KY639327, JX472095, JX472096, JX472097) and Kenyan population from ornamentals (unpublished sequences). It was 93-100 % (0-28 bp difference) and 85.4-86.5 % (43-48 bp difference) similar to the African and American populations respectively.

Remarks

The Kenyan population clearly belongs to *S. brachyurus* type B based on both morphological and molecular analyses in which it formed a clade with South African population and Kenyan population (unpublished sequences) from ornamentals (Type B) in both COI and LSU rDNA phylogenetic trees (Fig. 7 & 8).

***Rotylenchulus macrosoma* Dasgupta, Raski & Sher, 1968**

R. macrosoma population was collected from soil and root rhizosphere of coffee in Kirinyaga county, Kenya. The population was retrieved from one soil sample (DGC19) with the following GPS coordinates, “0° 30' 35.1576" N, 37° 18' 25.4988" E”.

Morphological characterisation

Male

Body shape usually in closed C-shape when heat-relaxed. Lip region conoid-rounded not offset, finely annulated. Labial framework well developed, extending two or three annuli posterior from basal annulus. Stylet long and well developed with cone usually slightly shorter than shaft. Stylet knobs rounded, sloping

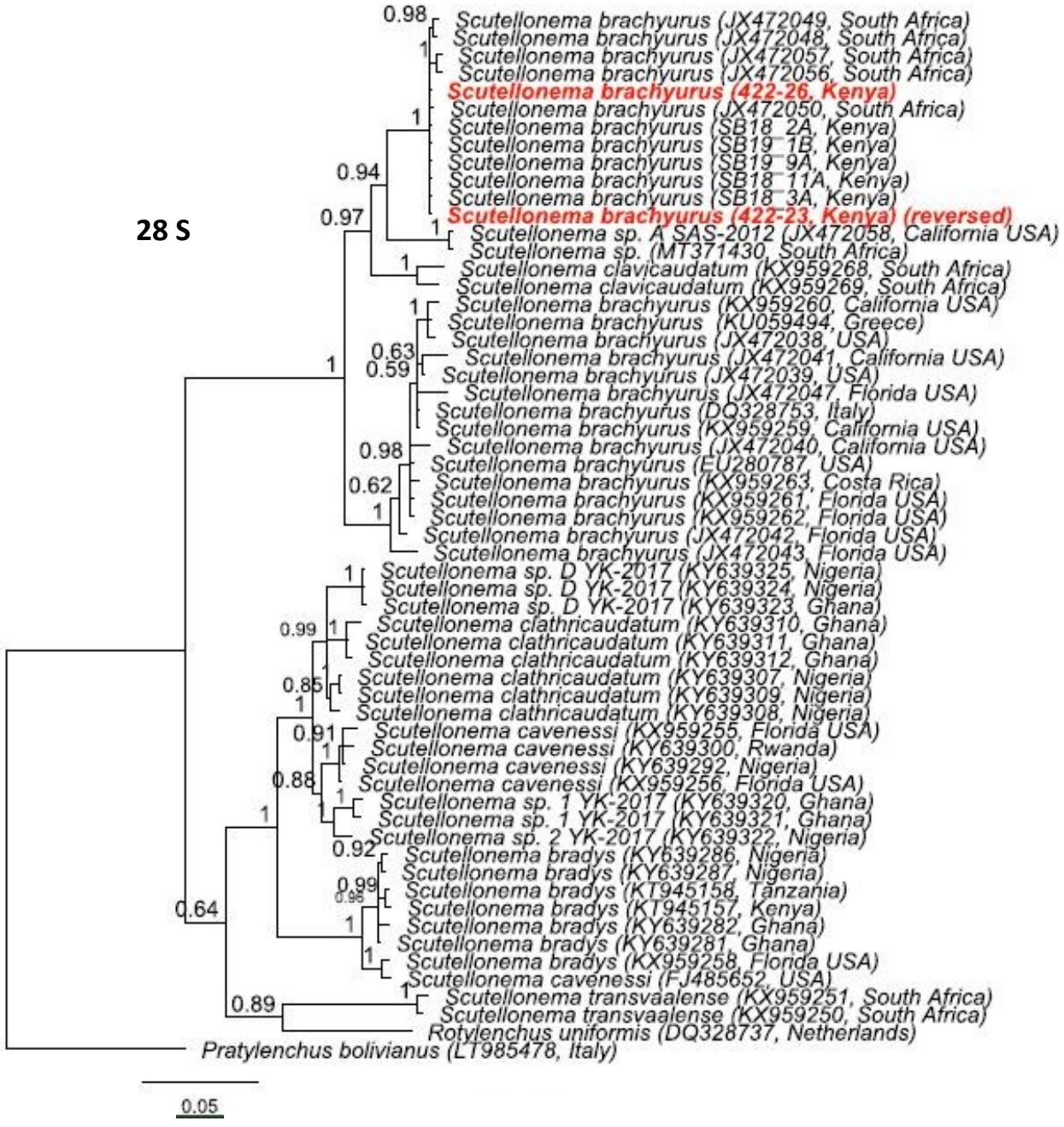

Figure 7. Phylogenetic relationships of *Scutellonema brachyurus* with 59 Scutellonema spp. Bayesian 50% majority consensus tree as inferred from D2-D3 expansion segments of 28S rDNA sequences analysed with GTR + I + G model. The branch support is indicated by posterior probabilities. The *Scutellonema brachyurus* in this study species is highlighted in red.

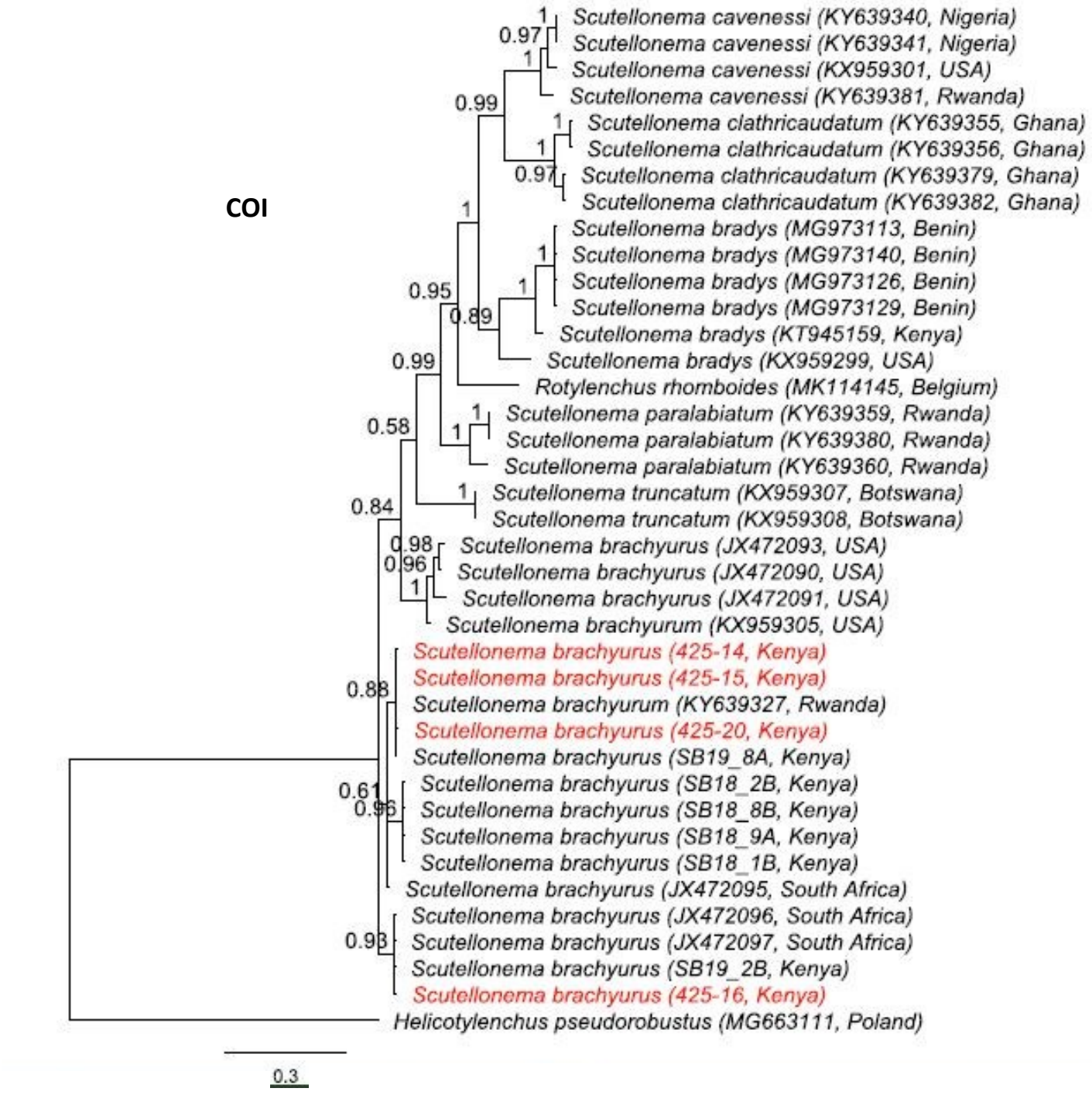

Figure 8. Phylogenetic relationships of *Scutellonema brachyurus* with 39 *Scutellonema spp*. Bayesian 50% majority consensus tree as inferred from COI mtDNA sequences analysed with GTR + I + G model. The branch support is indicated by posterior probabilities. The *Scutellonema brachyurus* in this study species is highlighted in red.

posteriorly. Dorsal pharyngeal gland opening situated ca 1.5 stylet lengths posterior to base of stylet. Median bulb rounded-oval, large, with prominent valves. Secretory-excretory pore situated from opposite middle of

isthmus to opposite anterior part of pharyngeal lobe. Hemizonid indistinct, two or three annuli long, situated from opposite to two annuli anterior to excretory pore. Pharyngeal glands overlapping intestine laterally and mostly ventrally. field distinct with four lines and three equal bands. Tail broadly rounded with rounded tip. Gubernaculum and spicules well developed, ventrally arcuate.

Mature/immature female not found.

Table 8. Morphometric data of *Rotylenchulus macrosoma*. from soil rhizosphere of coffee. All measurements are in µm (except for ratio) and in the form: mean ± s.d. (range).

Character	Kenyan population
n	4
L	465 ± 23.3 (433 – 485)
a	30.4 ± 2.1 (27.3 – 32.0)
b’	3.6 ± 0.23 (3.3 – 3.9)
c	17.3 ± 1.3 (15.9 – 18.6)
c’	2.5 ± 0.18 (2.3 – 2.7)
Lip diam.	6.1 ± 0.24 (5.8 – 6.3)
Stylet length	15.3 ± 1.1 (14.2 – 16.7)
Dorsal gland opening from stylet base	19.9 ± 1.2 (18.7 – 21.5)
Anterior end to secretory-excretory pore	75.7 ± 4.4 (69.4 – 79.7)
Anterior end to nerve ring	60.0 ± 8.2 (50.8 – 70.7)
Anterior end to the end of pharyngeal gland	129 ± 7.5 (118 – 136)
Max body diam.	15.3 ± 0.34 (15.1 – 15.9)
Anal body diam.	10.7 ± 0.53 (10.1 – 11.3)
Tail length	26.9 ± 1.6 (26.0 – 29.3)
Hyaline	9.0± 0.59 (8.6 – 9.9)
Spicule	20.6 ± 0.72 (19.8 – 21.5)
Gubernaculum	8.3 ± 0.63 (7.8 – 9.2)

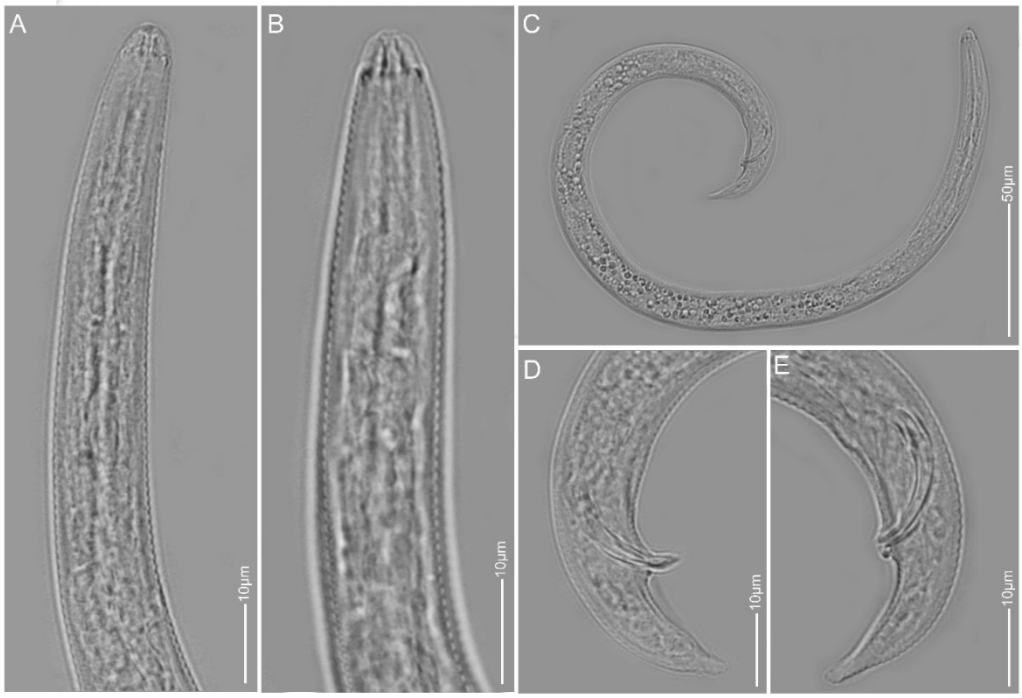

Figure 4. The LM pictures of *Rotylenchulus macrosoma* A: Pharyngeal region; B: Lip region; C: Entire body; D, E: Tail region.

Molecular characterisation

One LSU rDNA sequence was obtained with the length of 994 bp. The length of Muscle alignment was 1234 positions, and 994 positions were retained in the final dataset. Our population was found together in a maximally supported clade with 5 Spain and 2 Greece *R. macrosoma* populations (KT003749, KY 992807, KT003750, KT003751, KT003748 from Spain and KY992796, KY992793 from Greece) and not with maximally supported clade of two other *R. macrosoma* from Greece and one from Spain (KY992795, KY992797 from Greece and KY992805 from Spain). Our population was 94.11-95.32 % (33-41 bp difference) similar with the 7 *R. macrosoma* populations from Spain and Greece it formed a clade with and only 83.52-83.81 % (113-115 bp difference) similar with the other three *R. macrosoma* from Greece and Spain.

Three COI mtDNA sequences were obtained with of 447 bp. Our population was found together in a maximally supported clade with 7 other *R. macrosoma* populations form Greece and Spain (KT003724, KT003725, KY992849 from Spain and KY992845, KY992847, KY992848, KY992846 from Greece). Our

population had an intraspecific variation of 97.6-99.1% (6-12 bp difference) and was 85.7-89.2 % (42-56 bp difference) similar with the 7 *R. macrosoma* populations from Greece and Spain.

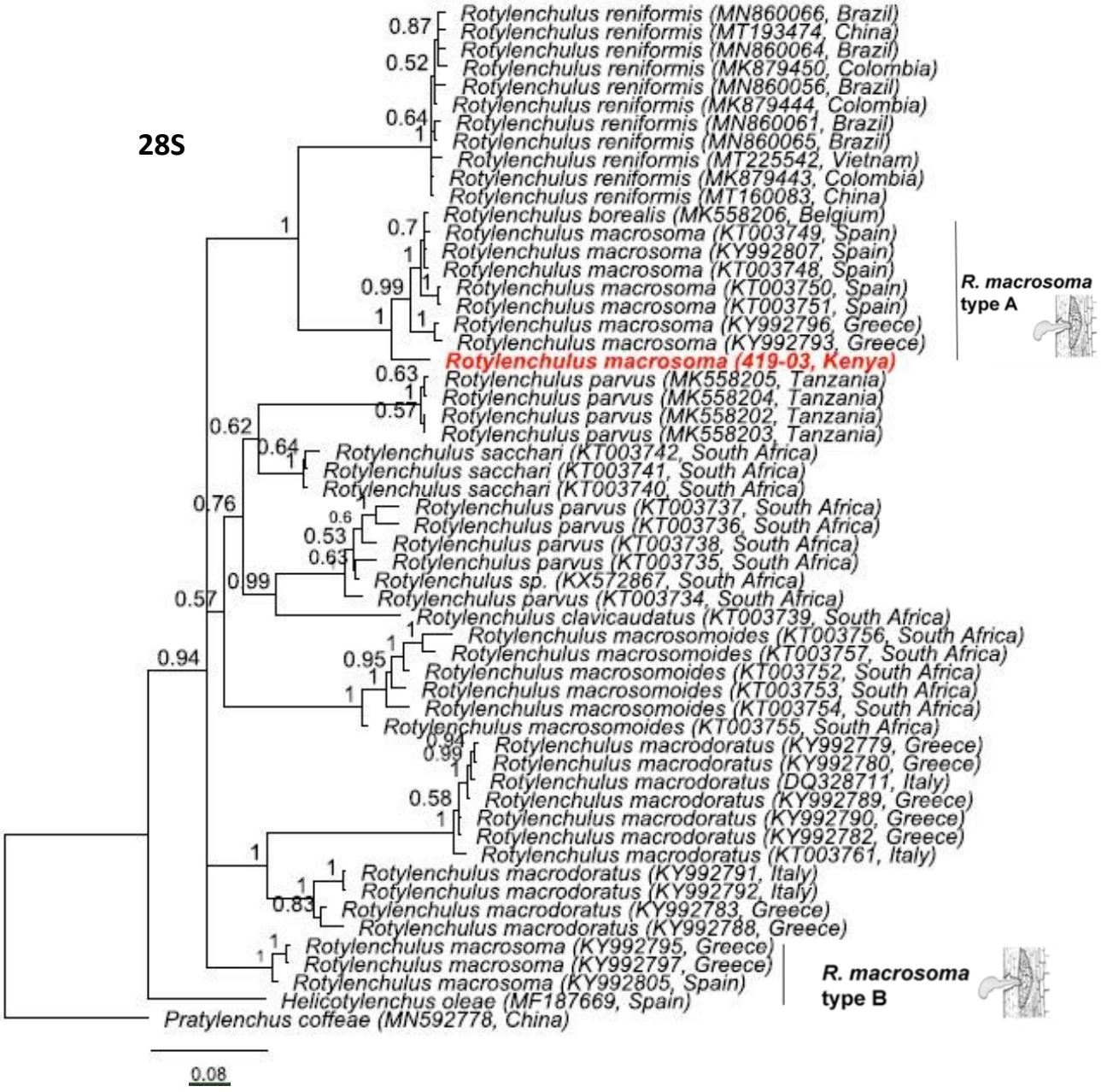

Figure 5. Phylogenetic relationships of *Rotylenchulus macrosoma* with 56 *Rotylenchulus* spp. Bayesian 50% majority consensus tree as inferred from D2-D3 expansion segments of 28S rDNA sequences analysed with GTR + I + G model. The branch support is indicated by posterior probabilities. The *Rotylenchulus macrosoma* in this study species is highlighted in red.

COI

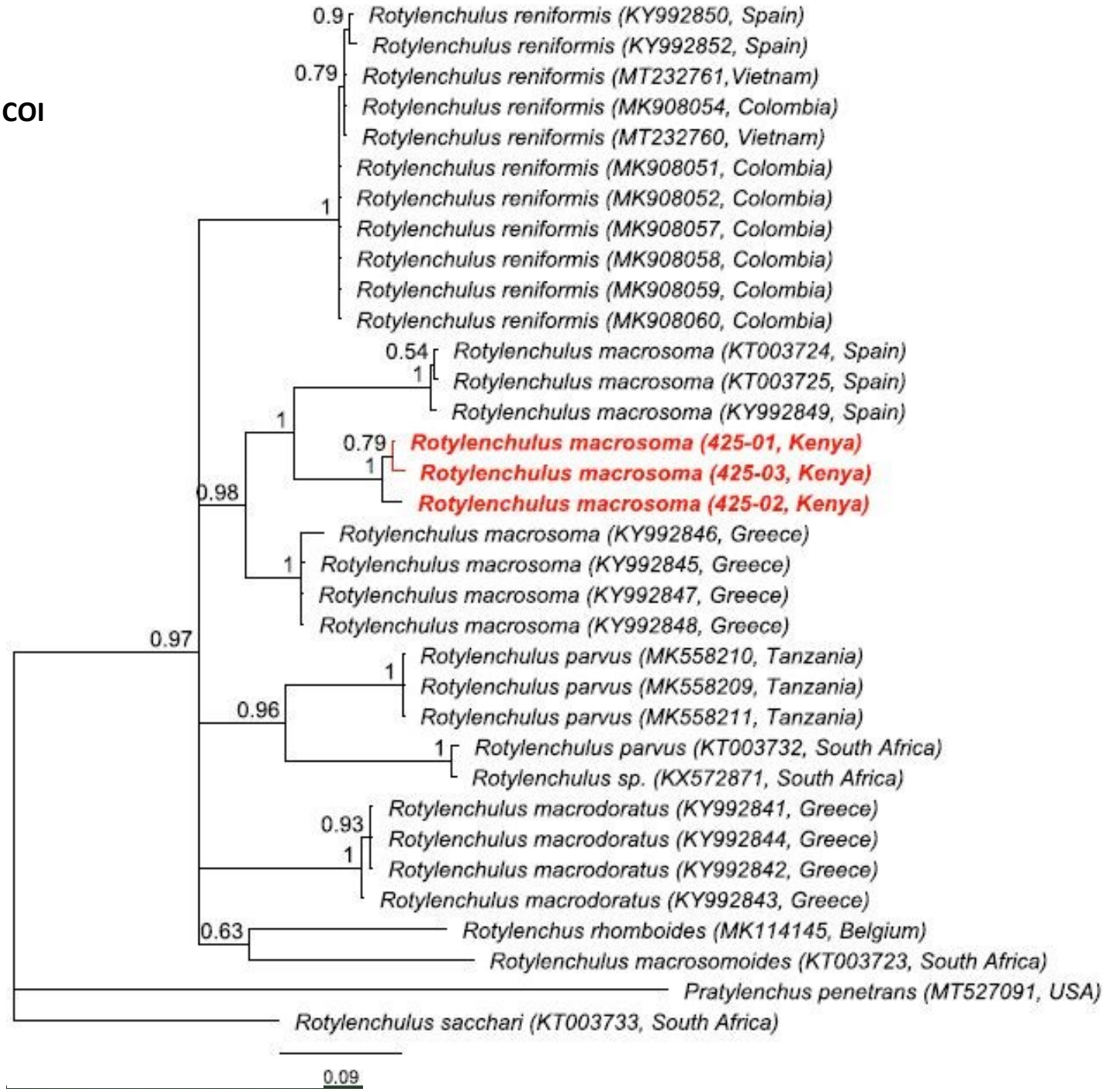

Figure 6. Phylogenetic relationships of *Rotylenchulus macrosoma* with 59 *Rotylenchulus spp*. Bayesian 50% majority consensus tree as inferred from *COI* mtDNA sequences analysed with GTR + I + G model. The branch support is indicated by posterior probabilities. The *Rotylenchulus macrosoma* in this study species is highlighted in red.

REMARKS

Morphological traits, morphometrics and molecular analysis of *R. macrosoma* from Kenyan population was in full agreement with the type population of Dasgupta et al (1968). Two types, Type A and Type B, of *R. macrosoma* has been revealed by several studies (Van Den Berg et al., 2016; Palomares-Rius et al., 2018) (Fig 10). Our population is clearly type A based on LSU phylogenetic tree because it formed a maximally supported clade with type A population from Greece and Spain. However, two distinct types of rRNA operons have been reported in *Rotylenchulus* spp. (Van Den Berg et al., 2016). This phenomenon could have occurred in our study which means that using the universal D2A and D3B primer set of the D2-D3 of LSU rRNA, only type A was amplified but not type B. Therefore, our study agrees with Van Den Berg et al., (2016) that further PCR study using various universal rRNA gene primers and sequencing of the whole *Rotylenchulus* genome could help to discover existence of rRNA gene types in *Rotylenchulus* spp. This study provides the first report of *R. macrosoma* associated with coffee in Kenya.

***Helicotylenchus dihystera* (Cobb, 1883) Sher, 1961**

H. dihystera population was collected from soil and root rhizosphere of soybean in Kirinyaga county, Kenya. The population was retrieved from one soil sample (DGS24) with the following GPS coordinates, "0° 34' 47.1324'' N, 34° 10' 55.7616'' E"

Female description

Habitus C-shaped to spiral. Labial region rounded to slightly flattened anteriorly with 4-7 annuli. Labial framework and stylet moderate. Stylet knobs rounded, flattened, indented or sloping anteriorly. Pharyngeal glands overlapping ventrally. Vulva situated medially to post medially with two outstretched genital tracts. Spermatheca empty. Lateral field with four lines, phasmids punctiform, situated from opposite to 19 annuli anterior to anus. Tail with 8-22 ventral annuli, asymmetrically rounded, dorsally curved with or without projection.

Molecular characterisation

One LSU rDNA sequence was obtained with the length of 987 bp. The alignment containing 51 sequences was initially 1143 positions but 987 positions were retained in the final dataset. The Kenyan population was

found together in a maximally supported clade with other sequences of *H. dihystera* deposited into GenBank with an interspecific variation of 3.8- 4.7% (28-32 bp difference).

One 18S rDNA sequence was obtained with the length of 879 bp. The Kenyan population was found together in a maximally supported clade with other sequences of *H. dihystera* deposited into GenBank with an interspecific variation of 0.8 –2.4% (7-25 bp difference).

Figure 7.. The LM pictures of *Helicotylenchus dihystera* A: Entire body; B: Lip and Pharyngeal region; C: Vulval region; D: Tail region

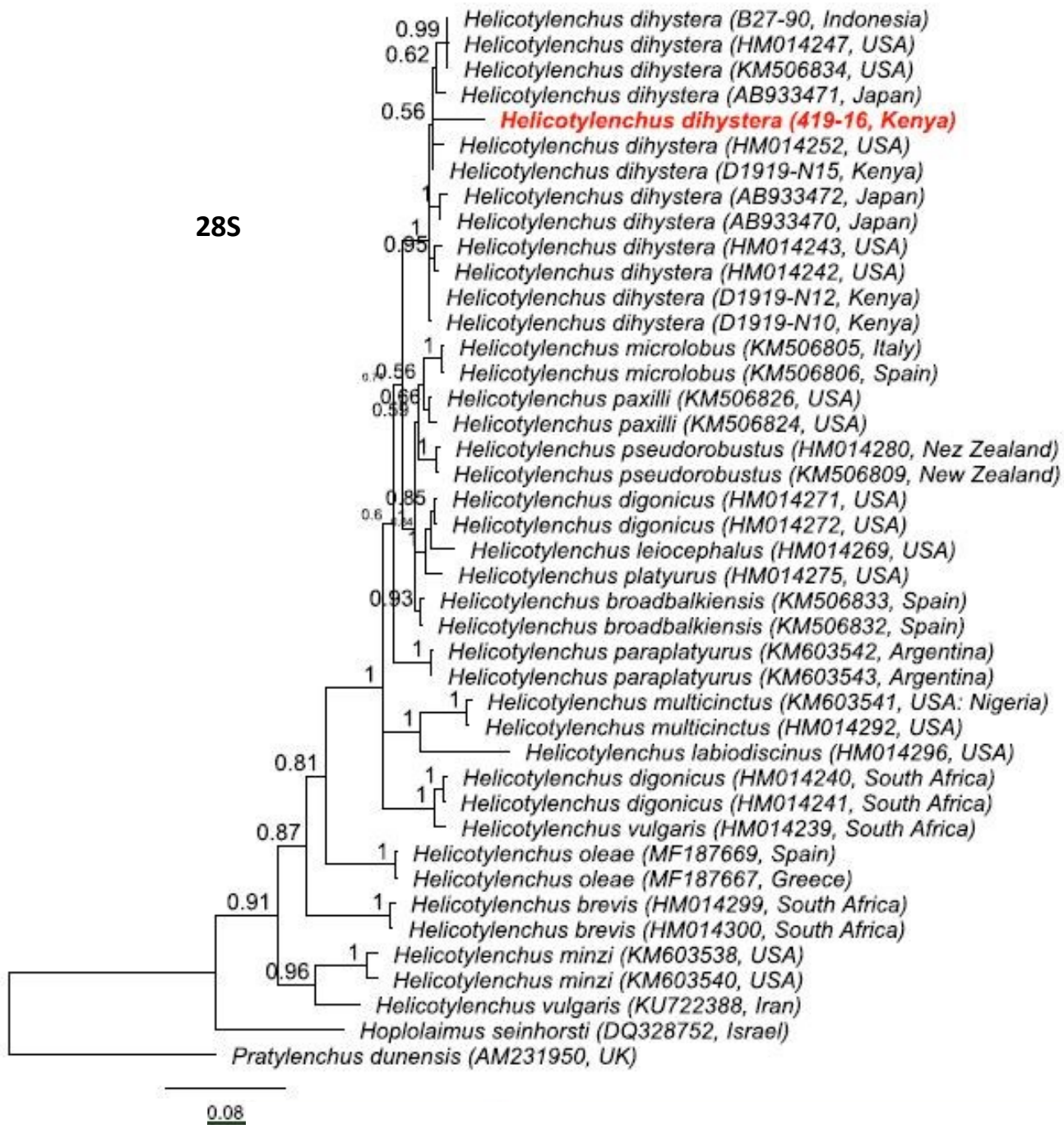

Figure 8. Phylogenetic relationships of *Helicotylenchus dihystera* with 51 *Helicotylenchus spp*. Bayesian 50% majority consensus tree as inferred from D2-D3 expansion segments of 28S rDNA sequences analysed with GTR + I + G model. The branch support is indicated by posterior probabilities. The *Helicotylenchus dihystera* in this study species is highlighted in red.

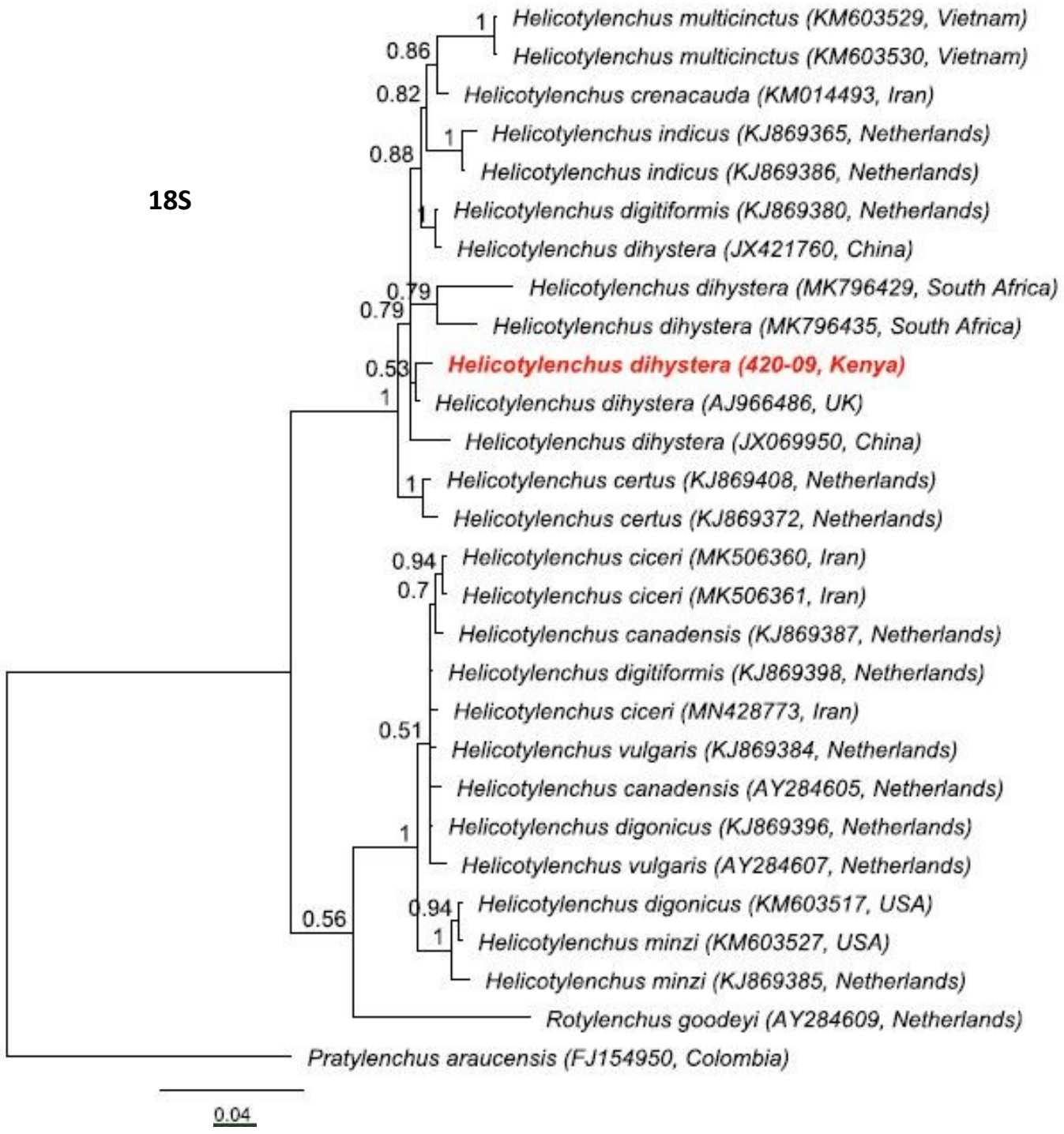

Figure 9. Phylogenetic relationships of *Helicotylenchus dihystera* with 27 *Helicotylenchus spp*. Bayesian 50% majority consensus tree as inferred from 18S rDNA sequences analysed with GTR + I + G model. The branch support is indicated by posterior probabilities. The *Helicotylenchus dihystera* in this study species is highlighted in red.

Remarks

Our specimens were identified as *H. dihystera* based on morphological traits and molecular analysis which is in agreement with the original description of Sher (1961) and Fortuner et al. (1981).

***Pratylenchus* n. sp. *(see addendum 1)* retrieved from soil rhizosphere of coffee in Kirinyaga county, Kenya**

Overview of Kenya nematofauna (see addendum 2)

Discussion

PPN associated with Coffee

Numerous genera and species of nematodes have been associated with coffee worldwide. *Meloidogyne* and *Pratylenchus* are the major genera whose damage to the crop causes great losses to the growers worldwide (Campos & Villain, 2005). Other PPN associated with coffee but whose yield loss, pathogenicity and pathogenicity are yet to be recorded include; *Gracilacus, Caloosia, Criconemoides, Discocriconemella, Helicotylenchus, Hemicriconemoides, Hoplolaimus, Longidorus, Ogma, Paratrichodorus, Aorolaimus, Rotylenchus, Scutellonema, Trichodorus*, *Tylenchorhynchus, Paratylenchus* and *Xiphinema* (Sikora et al., 2018).

Amongst the PPN recovered from soil samples of coffee in this study, genera belonging to *Meloidogyne* spp., *Tylenchulus* and *Pratylenchus* spp. were the most prevalent and prominent with high densities across the two regions surveyed. The three PPN have been reported previously to be associated with coffee in Kenya (Nzesya et al., 2014) and are known to cause great losses to growers worldwide (Campos & Villain, 2005; Trinh et al., 2009; Nzesya et al., 2014; Sikora et al.,2018). Other PPN genera found in association with coffee plants were *Xiphinema, Paratylenchus, Trichodorus* and representatives of the family Criconematidae but their damage on coffee has not yet been recorded (Campos & Villain, 2005; Sikora et al., 2018). This study provides the first report of *Rotylenchulus macrosoma* associated with coffee in Kenya.

Out of the six genera found to be associated with soybean, *Rotylenchus*, *Helicotylenchus*, *Meloidogyne* and *Scutellonema* were frequently present in numbers enough to reduce the soybean yield. These genera have been reported to affect the growth of soybeans hence reduced crop yield (Lima et al., 2016). All of the

nematode species observed from Kenya soybean field samples have previously been reported associated with soybean in other countries (Fourie et al., 2001; Lima et al., 2016; Sikora et al., 2018).

Meloidogyne spp. are considered responsible for most damage on soybean world-wide followed by *Heterodera glycines* (not detected in this study) (Sikora et al., 2018). In our study, *Meloidogyne* spp. had an average density of 119 nematodes/100 cm^3 which is a potential threat to soybean production according to Fourie et al., (2001) that reported average density of 103 individuals (*Meloidogyne)* per 200 cm^3 of soil and estimated to cause a significant yield loss. *M. incognita* has a wider distribution and is considered as the most important RKN species associated with soybean worldwide followed by *M. javanica* (Lima et al., 2016).

Pratylenchus spp. are also considered to be an economically important genus associated with soybean (Schmitt et al., 1981; Sikora et al., 2018). It may be more important than the relatively low mean soil densities (78 nematodes/100 cm^3) would designate. Root samples would probably have given a more definite indication of their damage potentials. It has been reported that *Pratylenchus* populations in soybean tend to increase slowly in roots until the stage when pods fill where their densities increase rapidly and can cause *ca* 30-50 % yield loss (Franchini et al., 2014; Fabia et al., 2016; Lima et al., 2016).

Two member members of Hoplolaimidae (*Scutellonema* and *Helicotylenchus*) that were identified to species level in this study, *Scutellonema brachyurus* and *Helicotylenchus dihystera* have recently been considered as emerging nematodes potentially threatening soybean production worldwide (Machado et al., 2019). Both *S. brachyurus* and *H. dihystera* had high average densities (122 and 102 nematodes/100cm^3) respectively. Machado et al, (2019) projected that they could reduce soybean production because of their high densities in both roots and soil. This is the first report of *S. brachyurus* and *H. dihystera* in association with soybean in Kenya.

This study has revealed that there are a significant number of PPN threatening coffee and soybean yield in Kenya. Moreover, unveils the existence of important PPN species in coffee and soybean which are not yet discovered e.g. *Pratylenchus* n. sp in coffee. It is therefore necessary for growers and researchers to carry out an intensive survey on both crops in order to describe important PPNs to be able to come up with sustainable management approaches to reduce the negative impact pf PPN on coffee and soybean.

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Addendum 1

Morphological and molecular characterisation of *Pratylenchus* sp. n. (Pratylenchidae), a root-lesion nematode associated with coffee in Kenya

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Summary - During a survey of plant-parasitic nematodes associated with coffee in Kenya, a new species of the root-lesion nematode was recovered. The new species was identified based on morphology and morphometrics and further characterised based on sequences of the D2D3 expansion domains of LSU rRNA and SSU rRNA genes. The combination of these analyses confirmed that this nematode is different from other previously described root-lesion nematodes. The females of *Pratylenchus* n. sp. are characterised by the following traits: body stout; lateral field areolated with four incisures at mid-body and decreasing to three or two lines toward tail end; *En face* view characterised by plain, undivided face with no division between the submedian and lateral segments when observed under SEM; stylet stout 13.4-15.7 *μ*m, conus *ca* 0.5 stylet length, strong shaft, stylet knobs well pronounced and anteriorly flattened to rounded; spermatheca rectangular with round sperms; tail subcylindrical and conoid towards the tip with 26-28 annuli. Males present and similar to females but with a weaker stylet. The matrix code of *Pratylenchus* new sp. according to Castillo & Vovlas (2007) is A2, B2, C2, D4, E1, F2, G2, H3, I2, J1, K2.

Keywords – LSU, morphology, morphometrics, phylogeny, rDNA, root-lesion nematode, SSU, taxonomy.

Root-lesion nematodes (RLNs) *Pratylenchus spp*. are considered the most common, damaging, and major parasites of coffee besides *Meloidogyne* spp. worldwide (Villain, Hernández & Anzueto, 2008; Rivillas, Villain & Bertrand, 2015). The *Pratylenchus* spp. that have been reported to attack coffee include; *P. brachyurus*, *P. coffeae*, *P. delattrei*, *P. goodeyi*, *P. gutierrezi*, *P. loosi*, *P neglectus*, *P. panamaensis*, *P. penetrans*, *P. pratensis*, *P. vulnus*, and *P. zeae*. However, *P. coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941, is the most prevalent and destructive RLN on coffee worldwide (Campos & Villain, 2005; Handoo, Carta & Skantar, 2008; Inomoto & Oliveira, 2008)

To date, 104 valid species have been described (Nguyen et al., 2019). The recent species described after Geraert (2013) include*; P. oleae* Palomares-Rius, Guesmi, Horrigue- Raouani, Cantalapiedra-Navarrete, Liébanas & Castillo, 2014, *P. quasitereoides* Hodda, Collins, Vanstone, Hartley, Wanjura & Kehoe, 2014, *P. Parazeae* Wang, Zhuo, Ye & Liao, 2015, *and P. haiduongensis* Nguyen, Le, Nguyen, Nguyen, Liébanas & Trinh, 2017 (see Hodda et al., 2014; Palomares-Rius et al., 2014; Wang et al., 2015; Nguyen et al., 2017*), P. Rwandae* Singh, Nyiragatare, Janssen, Couvreur & Bert, 2018, *P. horti* Nguyen, Trinh, Couvreur, Singh, Decraemer & Bert, 2019.

In Kenya, the diversity of RLNs is relatively well examined with 9 reported species, namely: *Pratylenchus scribneri* Steiner, Sherbakoff & Stanley, 1943, *Pratylenchus neglectus* (Rensch, 1924)) Filipjev & Schuurmans Stekhoven, 1941, *Pratylenchus loosi* Loof, 1960, *Pratylenchus brachyurus,* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941, *Pratylenchus coffeae*, *Pratylenchus goodeyi* Sher & Allen, 1953, *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941, *Pratylenchus sudanensis* Loof & Yassin, 1971, and *Pratylenchus zeae* Graham, 1951.

Although Castillo & Vovlas (2007), developed a very useful tabular identification key for *Pratylenchus species* based on 11 main morphological characteristics, facilitated by a Cluster analysis (Nguyen et al., 2019), in the morphological identification of *Pratylenchus spp.* is still a challenge and has resulted in multiple of the populations to remain unidentified (Campos & Villain, 2005). Several morphological characters are important for identification but are subject of intraspecific variation, including the shape of lip region, the shape of the spermatheca, structure of PUS, shape of female tail and terminus, shape of stylet knobs and structure of lateral field (Loof, 1991; Inserra et al., 1998) which are caused by environmental factors, type of host, or geographical origin (Doucet et al., 2001; Loubana et al., 2007).

To overcome such problems, the addition of molecular and phylogenetic approaches to the morphological analyses has aided in identification to species level and to allow the detection of cryptic species throughout plant-parasitic nematode groups (Palomares-Rius, 2014). DNA-based techniques such as the use of SSU rDNA, ITS rDNA, D2-D3 of LSU rDNA, and *COI* mtDNA regions as molecular markers have been able to distinguish among species of *Pratylenchus* (Blaxter et al., 1998; Subbotin et al., 2003, 2007; Subbotin, Moens

& Perry, 2006; Holterman et al., 2009; Janssen, Karssen, Orlando, et al., 2017). However, molecular approaches have their pitfalls as discussed by Janssen et al. (2017) such as the presence of unlabeled, misassembled, mislabeled, and misidentified sequences from GenBank. Therefore, it is essential to include both molecular and morphological approaches in the characterisation and identification of *Pratylenchus spp.* (Palomares-Rius, 2014).

The purpose of this study was to characterise molecularly and morphologically a new species of *Pratylenchus* from Kenya associated with coffee.

Materials and methods

SAMPLING AND NEMATODE EXTRACTION

Pratylenchus n. sp. was collected from the rhizosphere of coffee at Mukengeria coffee factory farm, Kerugoya district in Kirinyaga county during the hot-dry season (December-February). The location is characterised by a warm and temperate climate, an altitude of 1562 above sea level, average annual rainfall of 1412 mm, and an average annual temperature of 18.7 °C. The GPS coordinates of the location are 0° 30' 35.2" N 37° 18' 25.5" E.

The samples were collected using a shovel from the upper 30 cm of soil and roots and stored at 4°C until extraction and further processing. However, only specimens from soil were included in the analyses. Nematodes were extracted from the soil by using a modified Baermann funnel technique (Coyne *et al.*, 2018). For molecular work, some nematodes were stored in DESS as a backup (0.25 M disodium EDTA at pH 8.0, 20% dimethyl sulfoxide, and saturated NaCl) (Yoder et al., 2006).

MORPHOLOGICAL CHARACTERISATION

For morphological characterisations, a small suspension of nematodes in an embryo glass block were killed and fixed using 4% formalin with 1% glycerin at 70 °C (Seinhorst, 1966). The fixed nematodes were gradually transferred to anhydrous glycerin following the protocol of Seinhorst (1959) and mounted on a glass slide for light microscopy study. Measurements and light micrographs were taken with an Olympus BX50 DIC Microscope (Olympus Optical, Tokyo, Japan) connected to an Olympus C5060Wz camera; the ImageJ software version 1.51. was used to take measurements.

For scanning electron microscopy (SEM), specimens fixed in Trump's fixative were washed in 0.1 M phosphate buffer (pH 7.5) and dehydrated in a graded series of ethanol solutions, critical point-dried with liquid CO2, mounted on stubs with carbon tabs (double conductive tapes), coated with gold of 25 nm, and photographed with a JSM-840 EM (JEOL) at 12 kV (Nguyen et al., 2019).

MOLECULAR CHARACTERISATION

The living nematodes were used to make temporary slides (one specimen per slide) for taking digital light microscope pictures as morphological vouchers. In the next step, the single nematode was taken out of the temporary slide, washed with distilled water for 10 min, cut into 2-3 pieces and put together into an Eppendorf tube with 20 µl of WLB (50 mM KCl;10 mM Tris pH 8.3; 2.5 mM MgCl2; 0.45% NP-40 (Tergitol Sigma); 0.45% Tween-20). Subsequently, the samples were incubated at −20°C for at least 10 min, followed by adding 1 µl proteinase K (1.2 mg ml−1) before incubation in a PCR machine for 1 h at 65°C and 10 min at 95°C and centrifugation for 1 min at 20 800 g. Finally, the samples were stored at −20°C before running PCR (Singh et al., 2018).

The primers DP391/501 were used to amplify the 5'-end of the D2-D3 of 28S rDNA region, 5'-AGCGGAGGAAAAGAAACTAA-3' / 5'-TCGGAAGGAACCAGCTACTA-3' (Nadler et al., 2006) with the PCR reaction started at 94°C for 4 min, followed by 5 cycles of 94°C for 30 s, 45°C for 30 s, and 72°C for 2 min. This step was followed by 35 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 1 min and finished at 12°C for 10 min. For the 18S rDNA region, the 5′-end was amplified using the primers 18A/26R, 5'-AAAGATTAAGCCATGCATG-3' / 5'-CATTCTTGGCAAATGCTTTCG -3' (Mayer et al., 2007) with initial denaturation at 94°C for 4 min, followed by 5 cycles of denaturation at 94°C for 1 min, annealing temperatures starting at 52°C for 1 min and 30 s (decreasing by 1°C per cycle), and 68°C for 2 min for extension. This step was followed by 35 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 1 min and finished at 10°C for 10 min. The PCR products were sequenced by Macrogen (https://dna.macrogen.com).

The consensus sequences were obtained by assembling forward and backward sequences using Geneious R11 (www.geneious.com). The BLAST search was used to check for closely related sequences of other species on GenBank (Altschul et al., 1997). Multiple alignments were made from selected sequences by

using MUSCLE in MEGA 7 (Hall, 2011). The poorly aligned regions of the alignments were manually cut. The BI was performed with MrBayes 3.2.6 Add-in in Geneious R11 (Huelsenbeck, 2001) under the general time-reversible model with rate variation across sites and a proportion of invariable sites (GTR + I + G). The Markov chains were set with 1 × 106 generations, four runs, 20% burn-in, and a subsampling frequency of 500 generations (Huelsenbeck, 2001). Trees were visualized and rooted using FIGTREE v1.4.

Results

***Pratylenchus* n. sp**.

(Fig 1)

MEASUREMENTS (See Table 1.)

DESCRIPTION

Female

Body stout, straight to slightly curved ventrally after fixation Lateral field marked with four incisures at mid-body, inner band wider than 2 narrowed outer bands, areolation present distinctly at pharyngeal and tail regions, but indistinctly at vulva regions. Labial region continuous with body contour and highly sclerotized, flattened in front, with two to three annuli, the lip is 2.3-4.1 (3.6 ± 0.8) *µ*m high, 6.8-7.9 *µ*m wide. *En face* view characterised by plain, undivided face with no division between the submedian and lateral segments classified into group one (Castillo & Vovlas, 2007). Stylet stout 13.4-15.7 *µ*m, conus *ca* 0.5 stylet length, a strong shaft, stylet knobs well pronounced, and anteriorly flattened to rounded. Dorsal pharyngeal gland orifice at 2.0-2.8 µm posterior to stylet base. Round to slightly oval medium bulb occupying almost half of corresponding body diam. with a strong conspicuous valve. Hemizonid *ca* two annuli wide and situated one or two annuli anterior to excretory pore. Reproductive system well developed. Ovary with a single row of oocytes. Spermatheca rectangular with round sperms. Postvulval uterine sac *ca* one-fourth to one-third of the vulva-anus distance. Tail subcylindrical and conoid towards the tip with 26-28 annuli. Phasmid at *ca* 14-15 annuli from the tail tip.

Male

Similar to females, with small differences for all non-sexual characters. Relatively short stylet length of 12.4-13.2 µm. Spicules 15.6 (15-16.2) µm long. Gubernaculum ventrally curved, 3-6 µm long. Single testis

outstretched anteriorly. Phasmids near mid-tail, extending into the bursa. Bursa enclosing the tail about the level of the spicule head.

Table 1. Morphometric data of *Pratylenchus* n. sp. from glycerin-fixed specimens. All measurements are in µm (except for ratio) and in the form: mean±s.d. (range).

Character	Female		Male
	Holotype	Paratypes	Paratypes
N	-	5	3
L	520	503 ± 69 (414 -601)	437 ± 10.2 (425 - 450)
A	26.8	26 ± 2.9 (22 -29.5)	27 ± 0.82 (26-28)
b'	5.7	4.5 ± 0.96 (3.2 - 5.7)	3.7 ± 0.40 (3.2 – 4.2)
c	15.0	16.0 ± 1.1 (15.0-17.3)	18.8 ± 1.5 (17.2 – 20.9)
c'	2.8	2.8 ± 0.5 (2.3 – 3.4)	2.4 ± 0.12 (2.3 – 2.6)
V	71	74 ± 0.02 (71 - 77)	-
Lip height	4.1	3.6 ± 0.8 (2.3 - 4.1)	3.5 ± 0.22 (3.3 – 3.8)
Lip diam.	7.4	7.4 ± 0.4 (6.8 - 7.9)	6.9 ± 0.34 (6.4 – 7.2)
Stylet length	13.4	14.5 ± 1.0 (13.4 -15.7)	12.8 ± 0.33 (12.4 – 13.2)
Conus length	4.5	5.6 ± 0.9 (4.5 - 6.8)	4.3 ± 0.37 (3.9 – 4.8)
Shaft length	7.4	6.5 ± 0.7 (6.5 - 7.4)	6.5 ± 0.51 (6 – 7.2)
Knob height	2.1	2.4 ± 0.4 (2.1 - 3.0)	2.23 ± 0.12 (2.1 – 2.4)
Dorsal gland opening from stylet base	2.8	2.4 ± 0.3 (2.0 – 2.8)	2.73 ± 0.33 (2.3 – 3.1)
Anterior end to secretory-excretory pore	85.7	86 ± 10.7 (70 -100)	76.1 ± 2.78 (73 – 79)
Anterior end to nerve ring	65.4	68 ± 6.3 (60.8 -74.6)	65.5 ± 2.58 (62.5 – 68.8)
Anterior end to the end of pharyngeal gland	90.8	114 ± 16.8 (91 - 131)	98.7 ± 3.07 (95 – 103)
Pharyngeal gland overlap	39.3	39.7 ± 3.3 (36.7 – 45.3)	-
PUS (posterior uterine sac)	19.4	18.1 ± 0.5 (17.6 – 18.8)	-
Max body diam.	19.4	19.3 ± 0.7 (18.9 -20.4)	15.7 ±1.60 (13.5 – 17.3)
Vulva body diam.	19.8	18.9 ± 1.36 (17.2 -20.6)	-
Anal body diam.	12.2	11.3 ± 0.9 (10.8 -12.2)	12.3 ± 0.70 (11.5 – 13.1)
Tail length	34.6	31.5 ± 5.2 (24.8 - 36.7)	-
Hyaline length	4.9	5.1 ± 0.3 (4.9 - 5.3)	-
Tail annuli number	26	25.3± 0.94 (24.0 -26.0)	-
Spicule length (arc)	-	-	15.6 ± 0.50 (15 – 16.2)

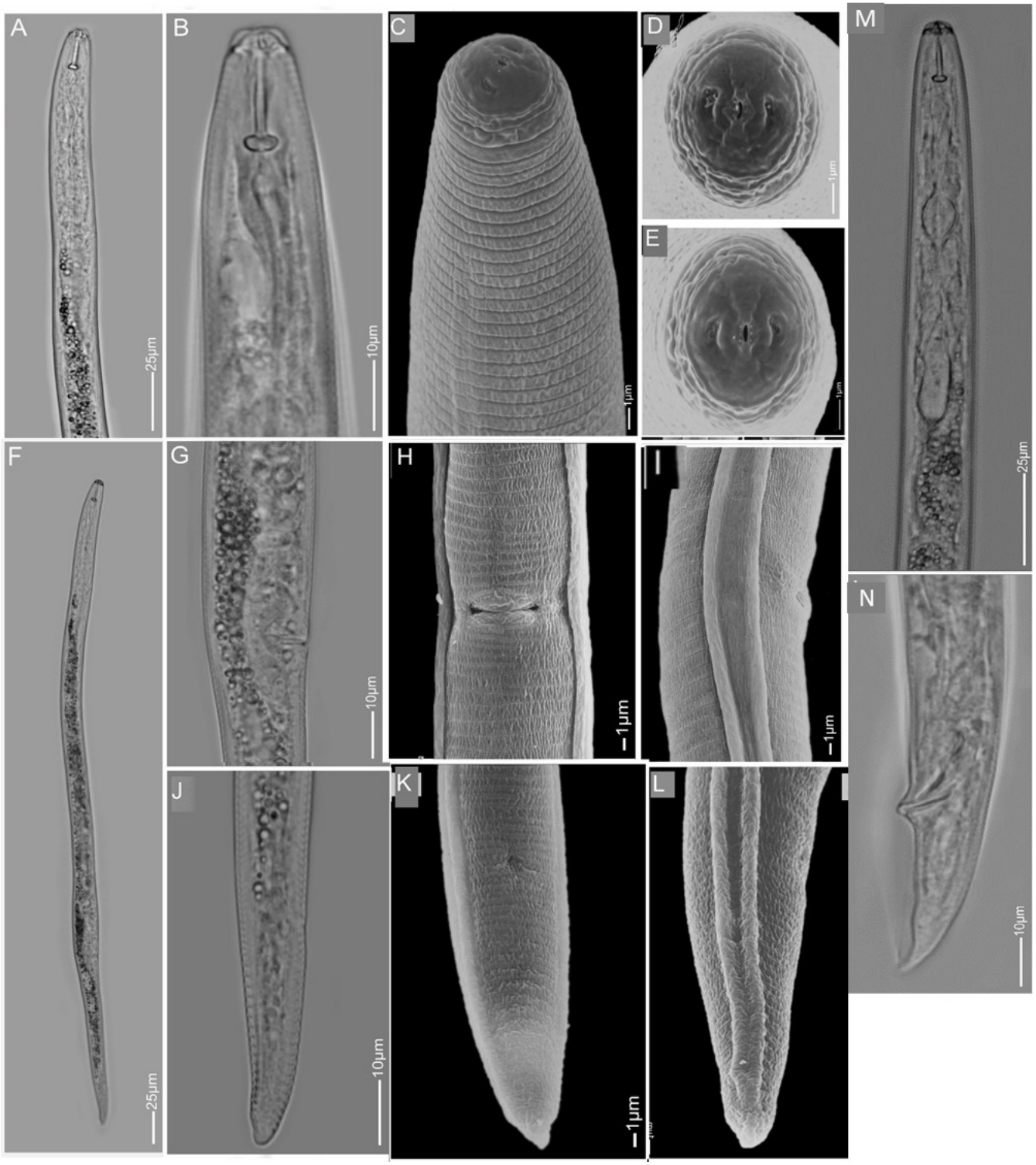

Figure 10.The LM and SEM pictures of *Pratylenchus* n. sp. A: Pharyngeal region; B, C: Lip region; D, E: En face view; G, H: Vulva region; F: Entire body; I: Lateral field at vulva region; J-L: Tail region. M-N: Male. M: Head and Pharyngeal region; N: Tail region.

TYPE HOST AND LOCALITY

Pratylenchus n. sp. was collected from the rhizosphere and root of coffee at Mukengeria coffee factory farm, Kerugoya district in Kirinyaga county during the hot-dry season (December-February). The location is characterised by a warm and temperate climate, an altitude of 1562 above sea level, average annual rainfall of 1412 mm, and an average annual temperature of 18.7 °C. The GPS coordinates of the location are 0° 30' 35.2" N 37° 18' 25.5" E.

TYPE MATERIAL

Holotype female, two females paratypes are included a slide number UGMD XX and deposited at Ghent University Museum, Zoology Collections. Additional paratypes (2 females in one slide) are available in the UGent Nematode Collection (slide number UGnem-XXX) of the Nematology Research Unit, Department of Biology, Ghent University, Ghent, Belgium.

DIAGNOSIS AND RELATIONSHIPS

Pratylenchus n. sp. is characterised by a combination of the following morphological features: stout body; plain and smooth face, lip region with three lip annuli; stylet 13.4 -15.7 µm long with anteriorly flattened to rounded knobs; lateral field marked by four incisures, outer lateral ridges areolated at vulval level; V = 74, ovary with a single row of oocytes, rectangular spermatheca filled with round sperm sometimes empty, relatively short PUS; subcylindrical tail with varied pointed shape of the terminus, phasmids at mid-tail. Males are common. Code index, according to Castillo and Vovlas (2007): A2, B2, C2, D4, E1, F2, G2, H3, I2, J1, K2.

Pratylenchus n. sp. is different from all other species according to Geraert (2013) as well as the comparison with the more recently described species (Singh et al., 2018) and (Nguyen et al., 2019).

Comparison of *Pratylenchus* n. sp. with 103 other species using the web-based key of Nguyen et al, (2019) showed that *Pratylenchus* n. sp. is most similar to *P. sudanensis* Loof & Yassin, 1971; *P. loofi* Singh & Jain, 1984; *P. bhattii,* Siddiqi, Dabur & Bajaj, 1991; *P. araucensis,* Múnera, Bert & Decraemer, 2009 and *P. goodeyi,* Sher & Allen, 1953 (Bray-Curtis similarity > 88 %).

Table 9. Comparison of matrix codes of the tabular key for *Pratylenchus spp*. identification, proposed by Castillo & Vovlas (2007), between putative *Pratylenchus* n. sp (asterisk) and its morphologically and molecularly related species.

species	A	B	C	D	E	F	G	H	I	J	K
Pratylenchus* n. sp.	**2**	**2**	**2**	**4**	**1**	**2**	**2**	**3**	**2**	**1**	**2**
P. sudanensis	2	2	2	**3**	1	**4**	2	**1**	2	1	**1**
P. loofi	2	**1**	2	**3**	**2**	**1**	2	**1**	2	1	**1**
P. bhattii	2	2	2	**2**	1	2	2	**1**	2	1	**1**
P. araucensis	**1**	2	2	**3**	**2**	2	**3**	**1**	**4**	1	2
P. zeae	2	2	**3**	**1**	1	**5**	**3**	3	2	1	**1**
P. parazeae	2	**1**	**3**	**3**	1	**6**	2	**1**	**3**	1	2
P. delattrei	2	**1**	**3**	**1**	**2**	**3**	**3**	**1**	**1**	1	**1**
P. haiduongensis	2	**1**	**4**	**1,3**	1	**5,6**	**1,**2	**1,4**	**1,2,3,4**	1	**1,**2
P. goodeyi	**3**	2	**3**	4	1	**1**	**3**	3	**3**	1	2

Pratylenchus n. sp. differs from *P. sudanensis* by rectangular spermatheca *vs* oval to rounded, shorter PUS (17.6-18.8 vs 25-29.9 µm), pointed vs smooth female tail tip, partially areolated lateral field structure at vulval region vs smooth bands.

It differs from *P. loofi* by the presence of male vs male absent, rectangular spermatheca *vs* oval to rounded, more anterior vulva position (71-77 vs 75-79 %), longer PUS (17.6-18.8 vs less than 16 µm), pointed vs smooth female tail tip, partially areolated lateral field structure at vulval region vs smooth bands.

It differs from *P. bhattii* by rectangular *vs* rounded to spherical spermatheca, pointed vs smooth female tail tip, partially areolated lateral field structure at vulval region vs smooth bands.

It differs from *P. araucensis* by three vs two lip annuli, rectangular *vs* oval to rounded spermatheca, more anterior vulva position (71-77 vs 75-79 %), subcylindrical vs conoid female tail shape, pointed vs smooth female tail tip, shorter pharyngeal overlapping length (36.7- 45.3 vs greater than 50 µm).

It differs from *P. goodeyi* by three vs four lip annuli, shorter stylet length (13.4 -15.7 vs 17 µm), longer PUS (17.6-18.8 vs less than 16 µm), subcylindrical vs conoid female tail shape, relatively shorter pharyngeal overlapping length (36.7-45.3 vs 40-50 µm).

Pratylenchus n. sp. is also clearly morphological different from the molecularly closely related species. Our new species differs from *P. zeae* by shorter stylet length (13.4-15.7 vs 16-17.9 µm), rectangular

spermatheca vs absent to reduced spermatheca, shorter PUS (17.6-18.8 vs 30-35 µm), subcylindrical vs conoid female tail, partially areolated vs smooth lateral field structure at the vulval region.

The new species differs from *P. parazeae* by presence vs absence of males, shorter stylet length (13.4 - 15.7 vs 16-17.9 µm), rectangular vs oval spermatheca, shorter PUS (17.6-18.8 vs more than 35 µm), pointed vs smooth tail tip, relatively shorter pharyngeal overlapping length (36.7- 45.3 vs 40-50 µm.

It differs from *P. delattrei* by presence vs absence of males, shorter stylet length (13.4 -15.7 vs 16-17.9 µm), rectangular spermatheca vs absent to reduced spermatheca, more anterior vulval position (71-77 vs 75-79%), shorter PUS (17.6-18.8 vs 20-24.9 µm), subcylindrical vs conoid female tail, pointed vs smooth tail tip, longer pharyngeal overlapping length (36.7-45.3 vs less than 30 µm) and partially areolated vs smooth lateral field structure at the vulval region.

MOLECULAR CHARACTERISATION

D2D3 expansion domains of LSU rDNA

Two LSU D2D3 sequences were obtained with intraspecific variations of 2.2 % (21 bp). Fig. 2 represents a phylogenetic relationship of *Pratylenchus* n. sp. with an alignment (1074 bp) of 65 *Pratylenchus* sequences from 30 species and three outgroups (*Belonolaimus longicaudatus* DQ328710, USA; *Coslenchus costatus* DQ328719, Germany*; Basiria gracilis* DQ328717, USA). Our new species formed a maximally supported clade with *P. bhattii* Siddiqi, Dabur & Bajaj, 1991 (JN244269, JN244270) with a similarity of 94.3-94.7 % (38-40 bp difference). This clade was sister to a maximally supported clade of *P. parazeae* Wang, Zhuo, Ye & Liao, 2015*,* P. *zeae* Graham, 1951, *P. delattrei* Luc, 1958 and *P. haiduongensis* Nguyen, Le, Nguyen, Nguyen, Liébanas & Trinh, 2017. Our new species differed respectively 7.3-9.2 % (51-63 bp difference), 10.6-13.8% (76-95 bp), 7.4-9.2% (53-65 bp) and 16.6-21.2% (113-148 bp) with these species.

Partial sequence of SSU rDNA

The SSU rDNA alignment included 56 sequences of 25 *Pratylenchus* species and three outgroups (*Basiria gracilis*, EU130839; *Coslenchus costatus*, AY284581; *Beleodorus thylactus*, AY593915). A maximally supported clade (1 pp) of *Pratylenchus* n. sp. was formed with an intraspecific variation of 0.3 % (3 bp

difference). This clade was sister to a maximally supported clade of 5 sequences of *P. parazeae* Wang, Zhuo, Ye & Liao, 2015 and 4 sequences of *P. zeae* Graham, 1951 with interspecific difference of 3.0-4.2 % (24-36 bp difference) and 5.2-5.7% (45-48 bp difference) respectively (Fig.3).

Figure 2. Phylogenetic relationships of *Pratylenchus* n. sp. with 30 *Pratylenchus* spp. Bayesian 50% majority consensus tree as inferred from D2-D3 expansion segments of 28S rDNA sequences analyzed with GTR + I + G model. The branch support is indicated by posterior probabilities. The newly described species is highlighted in red.

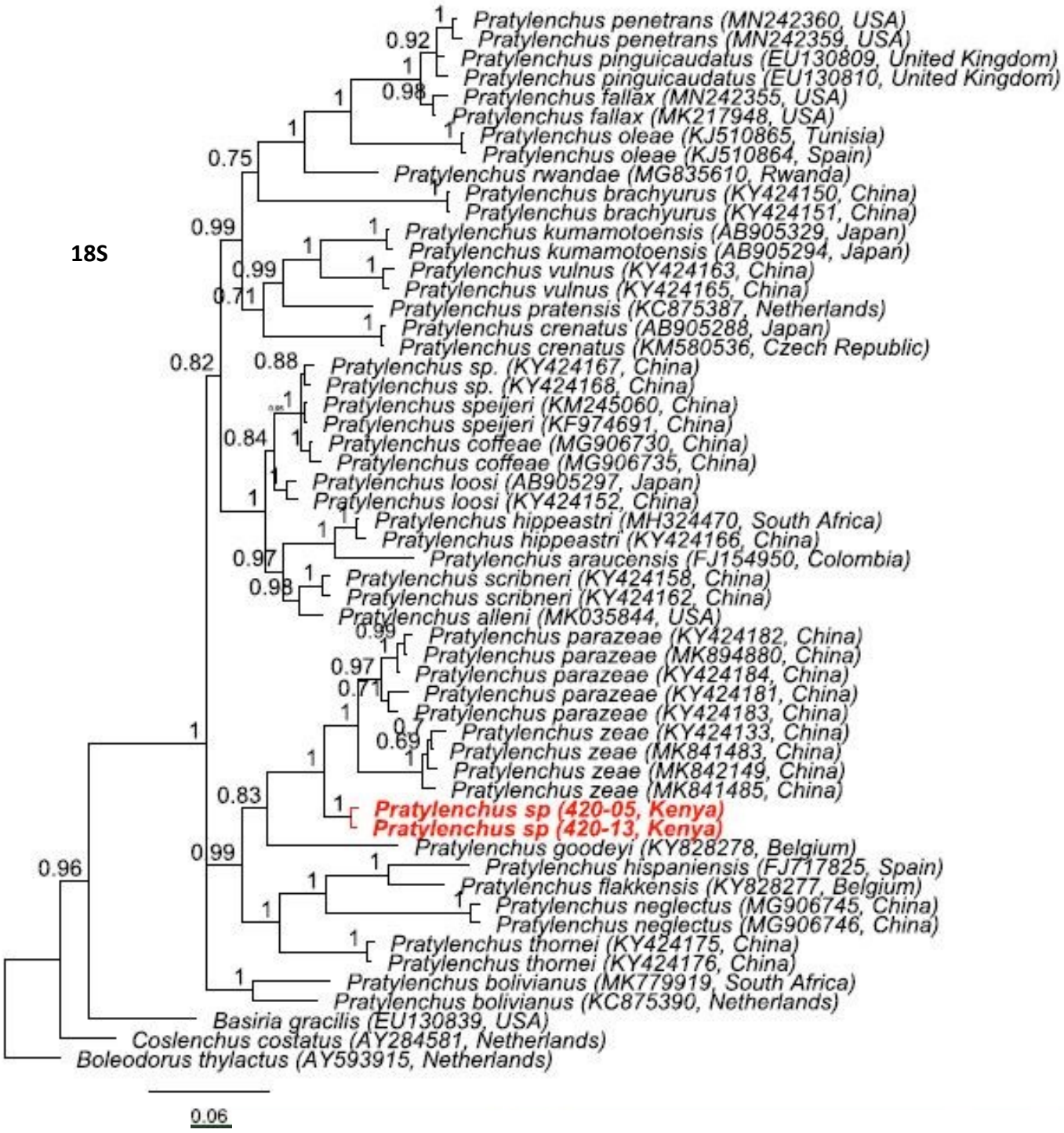

Figure 3. Phylogenetic relationships of *Pratylenchus* n. sp. with 22 *Pratylenchus* spp. Bayesian 50% majority consensus tree as inferred from 18S of rDNA sequences analyzed with the GTR +I +G model. The branch support is indicated by posterior probabilities. The newly described species is highlighted in red.

Discussion

Plant-parasitic nematodes are a major limiting factor in coffee-producing areas worldwide (Campos & Villain, 2005) due to the significant damage they cause to the crops hence resulting in important economic losses for coffee farmers and local economies. Numerous genera and species of nematodes have been associated with coffee worldwide with *Meloidogyne* and *Pratylenchus* being the major genera whose damage to the crop causes great losses to the growers worldwide (Campos & Villain, 2005).*Pratylenchus spp*. are reported as the major parasites of Robusta and Arabica coffee in all major coffee-producing countries (Souza, 2008; Villain et al, 2008; Rivillas, Villain & Bertrand, 2015). The species considered to be associated with coffee include; *Pratylenchus coffeae, P. brachyurus*, *P. delattrei*, *P. goodeyi*, *P. gutierrezi*, *P. loosi*, *P. neglectus*, *P. panamaensis*, *P. penetrans*, *P. pratensis*, *P. vulnus* and *P. zeae* (Sikora et al., 2018b). Among these species, *P. brachyurus* and *P. coffeae* are the most widespread in the tropics (Inomoto & Oliveira, 2008).

In this study, *Pratylenchus* n. sp. associated with coffee in Kenya was differentiated from all other described species through molecular analyses based on ribosomal DNA small subunit (SSU), D2D3 expansion domains of large subunit (LSU D2D3) combined with morphometrics and morphological approaches. The impact of the new species in coffee in terms of the damages it causes remains to be investigated. However, based on the *Pratylenchus* spp. prevalence and average density, 16% and 54 nematodes^{-1} 100cm^3 soil respectively, it can be predicted that the *Pratylenchus* n. sp could be a potential threat to coffee. A study conducted by Villain (2000) proved that densities of 125 nematodes/g of the root, yielded *ca* 0.5 ton/ha compared to 15 nematodes/g of the root which yielded *ca* 6 ton/ha.

It has been shown that *Pratylenchus* spp. may cause extensive damage to coffee (Campos et al., 2005; Sikora et al., 2018a). For instance, *P. coffeae* is considered a serious parasite of coffee in Indonesia causing an average yield loss of 29-78 % (Souza, 2008). The coffee plant exhibits the following symptoms when it has been attacked by *Pratylenchus*; stunted growth, leaf fall, chlorosis, continuous plant decay which depends on the intensity of the nematode species, the agro-ecological conditions the coffee cultivar and age. *Pratylenchus* density plays a great role in the prediction of potential damage to coffee.

Given the economic significance of coffee to the countries that produce it, decisions on sustainable management of root-lesion nematode will be required. Therefore, it is necessary to have subsequent supplementary studies to determine both the density and diversity of *Pratylenchus* spp. associated with coffee by the use of an integrated taxonomic approach by making use of molecular tools, morphological and pathogenicity assessments. This will aid in reevaluating the worldwide distribution of *Pratylenchus* spp. and develop coffee germplasm resistant to these damaging nematodes for the ultimate benefit of coffee producers globally.

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Addendum 2

An overview of terrestrial nematodes in Kenya

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summary - A study of terrestrial nematodes from Kiambu (coffee, roses, and pineapple), Kirinyaga (coffee), Western Kenya (Soybean) (10 taxa) and a literature review of the nematofauna in Kenya (116 taxa) resulted in a checklist of plant-parasitic, entomopathogenic, and free-living nematodes from different geographical habitats of Kenya. Of the 126 taxa, 67 to the genus and 59 to species level (plant-parasitic nematodes: 29 genera, 37 species; free-living nematodes: 38 genera,16 species; entomopathogenic nematodes:6 species). This study revealed four new records for Kenya: *Scutellonema brachyurus, Rotylenchus* n. sp and *Rotylenchulus macrosoma.*

Key words: Checklist, Kenya, nematodes, nematofauna, terrestrial, overview, PPN

Agriculture is the backbone of Kenya's economy contributing about 25% of the GDP[1]. Nevertheless, agriculture is faced by a myriad of pests and diseases including plant-parasitic nematodes (PPN) responsible for billions of dollars' losses annually [2]. Distribution and diversity of terrestrial nematodes is of great concern in tropical and subtropical environments considering the low level of awareness on their biogeography and systematics[3]. Though Kenya shows significant heterogeneity of milieu and biogeographical sectors, there is minimal data on current information on the distribution of terrestrial nematodes, and mainly represented by ecological surveys in which only the genus level of identification has been adopted. Here is a presentation of the first checklist of terrestrial (free-living, plant-parasitic and entomopathogenic) nematodes from Kenya including records from literature and new records from the current study on different crops or cropping systems.

Materials and methods

Soil and root samples were collected from the rhizosphere of coffee, pineapple, roses and soybean fields using a zigzag pattern.[4] Individual samples collected were geolocated with Garmin GPS device, kept in

ziplock bags, and labeled for transit to the laboratory. The samples (each 100cc soil and 5g roots) were extracted following Whitehead method.[5] Specimens were identified under the light microscope using morphological characters and morphometric data.

Specimens were identified under the light microscope using morphological characters and morphometric data. Identification was based on adults and juveniles (RKN) of individual populations which were fixed in 4% formalin with 1% glycerin at 70°C[9] and processed to anhydrous glycerin following the glycerin-ethanol method.[10] Measurements and photomicrographs were taken with the aid of an Olympus BX50 DIC Microscope (Olympus Optical, Tokyo, Japan) connected to an Olympus C5060Wz camera. For molecular identification, prior to DNA extraction, individual live specimens were handpicked into a drop of distilled water and used for preparation of morphological vouchers of temporary mounts using light microscopy. Following the Singh protocol[6], individual specimens whose morphological information has been recorded were cut and transferred into PCR tubes with 20 µl of worm lysis buffer for DNA extraction. The primer sets SSU18A/SSU26R[7] was used for amplification of the 18S rDNA; D2A/D3B amplified the 28S rDNA and JB3/JB4.5[8] amplified the COI mtDNA. The primers F2/R1[9] were used to amplify the *NAD5* gene for root-knot nematodes. Successful PCR amplicons were sequenced by Macrogen Inc. Additionally, available data of terrestrial nematodes from literature in Kenya were extracted and included in this study.

Results and discussion

Current study data

The study resulted in identification of 10 plant-parasitic nematodes (PPN) (5 genera and 5 species) associated with coffee, pineapple, roses or soybean (Table 1).

Helicotylenchus dihystera was found to be similar to the original description and D2D3 sequences (Accession XX) were 100% similar to *H. dihystera* sequence (MN445997) from China. *Meloidogyne javanica* NAD5 sequences (Accession XX) were all identical to each other and to the reference polymorphic nucleotide position sequence (KU372392) from Janssen *et al*. (2016). *Rotylenchulus macrosoma* was found to be similar to the original description and D2D3 (Accession XX) and COI sequences (Accession XX) were 95.32 % (33 bp difference) and 89.23% similar (42 bp difference) with the most similar *R.*

macrosoma sequences (KT003749 and KT003724) respectively. Our *R. macrosoma* population was maximally supported within the clade of type A. *Rotylenchus* n. sp was in agreement with the original description of *R. robustus* but molecularly it was 99.43 - 99.89% % (1 –5 bp different) similar to *R. robustus* sequence (MK348059) from Ethiopia, but only 92.57 – 93.52% (59-69 bp differences) similar to the *R. robustus* from Belgium and the Netherlands. *Scutellonema brachyurus* D2D3 (Accession XX) and *COI* (Accession XX) sequences was found to be morphologically similar to the description of the South African *S. brachyurus* populations and the D2D3 sequences were 100% similar to *S. brachyurus* (JX472052) and 100% similar to *S. brachyurus* (KY639327) for the *COI* sequence. *Pratylenchus* n. sp. was found morphologically and molecularly different from all known species. The closest relatives to this species using LSU rDNA were; *P. bhattii*, *P. parazeae,* P. *zeae*, *P. delattrei* and *P. haiduongensis* with an interspecific variation of 5.7-5.3.7 (38-40 bp difference), 7.3-9.2% (51-63 bp difference), 10.6-13.8% (76-95 bp), 7.4-9.2% (53-65 bp) and 16.6-21.2% (113-148 bp) respectively.

The taxa identified to genera level included; *Xiphinema, Tylenchulus, Paratylenchus* sp.*, Trichodorus, Criconema.* Four out of the six identified species are new reports in Kenya: *Scutellonema brachyurus, Rotylenchus* n. sp *and Rotylenchulus macrosoma.*

Literature review records

A total of 116 taxa (54 species and 62 genera) were retrieved from literature which includes; PPN, free-living and entomopathogenic nematodes (Table1).

Table 1. List of the nematode taxa from Kenya. The superscript numbers refer to the references where the taxa are reported. Crops, locations and nematode species in bold are records from the present study

NO.	Plant-parasitic nematodes	Host crops, Location
	A. Migratory endo-parasitic nematodes*	

* Some of the taxa here can either be migratory endo-parasitic or ecto-parasitic nematodes

1.	*Helicotylenchus* sp. Steiner, 1945	Cabbage, Kiambu[10]; Coffee, Kiambu[11]; Maize, Kirinyaga[12]; Rice, Kirinyaga[13]; Maize & Bean, Chuka[14]; Sweet potato, Kisii[15]
2.	*Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961	Irish potato, Central[16], Sweet Potato, Coast[16], Banana, Central Kenya[17], **Soybean, Western Kenya**, **Pineapple, Kiambu**
3.	*Helicotylenchus microcephalus* Steiner, 1945	Sweet potato, Central[16]
4.	*Helicotylenchus multicinctus* (Cobb,1893) Golden, 1956	Banana, Central[18]
5.	*Hoplolaimus* sp. Von Daday, 1905	Cabbage, Kiambu[10], Coffee, Kiambu[11], Rice, Kirinyaga[13], Maize & Bean, Chuka[14], Sweet potato, Teso[15]
6.	*Paratylenchus* sp. Micoletzky, 1922	Sweet potato, Busia & Teso[15], cabbage, Kiambu[10], Sugarcane, western Kenya[19], **coffee, Kirinyaga, Kiambu**
7.	*Pratylenchus* sp. Filipjev, 1936	Coffee, Kiambu[11], Maize, Kirinyaga[12], Rice, Kirinyaga[13], Maize & Bean, Chuka[14], Sweet potato, Embu & Kisii[15], **Coffee, Kirinyaga**
8.	*Pratylenchus scribneri* Steiner, Sherbakoff &Stanley, 1943	Cabbage, Kiambu[10]
9.	*Pratylenchus neglectus* (Rensch, 1924)) Filipjev & Schuurmans Stekhoven, 1941	Cabbage, Kiambu[10]
10.	*Pratylenchus loosi* Loof, 1960	Cabbage, Kiambu[10]
11.	*Pratylenchus brachyurus,* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941	Cabbage, Kiambu[10], Maize, western Kenya[20]
12.	*Pratylenchus coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941	Pyrethrum, Kisii[21]
13.	*Pratylenchus goodeyi* Sher & Allen, 1953	Banana, Central Kenya[17]

14.	*Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941	Pyrethrum, Kisii[21]
15.	*Pratylenchus sudanensis* Loof & Yassin, 1971	Sweet potato, Central
16.	*Pratylenchus zeae* Graham, 1951	Maize, Western Kenya[20]; Cabbage, Kiambu[10]
17.	*Peltamigratus* sp. Sher, 1964	Cabbage, Nyandarua[22]
18.	*Radopholus* sp. Thorne, 1949	Coffee, Kiambu[11]
19.	*Radopholus similis* (Cobb, 1893) Thorne, 1949	Banana, Western Kenya[17,18]
20.	*Rotylenchus* sp. Filipjev, 1936	Coffee, Kiambu[11], Rice, Kirinyaga[13], Maize & Bean, Chuka[14], Banana, Central Kenya[17], Sweet potato, Embu[15]
21.	*Rotylenchus reniformis* (Thorne, 1949) Loof & Oostenbrink, 1958	Irish potato, Central [16], Sweet potato, Coast Kenya[16]
22.	*Rotylenchus unisexus* Sher, 1965	Sweet potato, central[16]
23.	***Rotylenchus* n. sp.**	**Coffee, Kiambu**
24.	*Scutellonema* sp. (Steiner, 1937) Andrassy, 1958	Cabbage, Kiambu[10], Coffee, Kiambu[11], Maize, Kirinyaga[12], Rice, Kirinyaga[13], Maize & Bean, Chuka[14], Sweet potato, Makueni[15]
25.	***Scutellonema brachyurus***, (Steiner, 1938) Andrassy, 1958	**Pineapple, Kiambu , Soybean, Western Kenya**
26.	*Scutellonema bradys* (Steiner & LeHew, 1937) Andrassy, 1958	Yam, Mukuyu[23]
27.	*Scutellonema anum* Andrassy, 1958	Irish potato, Central[16]
28.	*Scutellonema labiatum* Andrassy, 1958	Sweet potato, Central[16]
	B. Ectoparasitic nematodes	
29.	*Aphelenchoides* sp. Fischer, 1894	Coffee, Kiambu[11], Maize, Kirinyaga[12], Rice, Kirinyaga[13] & Coast[24], Carnation, Kericho[25]
30.	*Aphelenchoides besseyi* Christie, 1942	Rice, Coast [26]
31.	*Aphelenchus* sp. Bastian, 1865	Coffee, Kiambu[11], Maize, Kirinyaga[12], Carnation, Kericho[25]

32.	*Belonolaimus* sp. Steiner, 1949	coffee, Sugarcane, Cabbage, Kiambu[10]
33.	*Ditylenchus* sp. Filipjev, 1936	Maize, Kirinyaga[12], Rice, Kwale[13], Banana, Kakamega[17]
34.	*Ditylenchus dipsaci (*Kuhn, 1857) Filipjev, 1936	Irish potato, Central Kenya??[16]
35.	*Ditylenchus destructor* Thorne, 1945	Irish potato, Central[16]
36.	*Paratrichodorus* sp. Siddiqi, 1974	Coffee, Kiambu[11], Maize, Kirinyaga[12]
37.	*Paratrichodorus allius* (Jensen, 1963) Siddiqi 1974	Irish potato, Central[15,16]
38.	*Paralongidorus* sp. Siddiqi, Hooper and Khan, 1963	Rice, Kwale[13]
39.	*Trichodorus* sp. Cobb, 1913	Sugarcane, Cabbage, Kiambu[10], Coffee, Kiambu[11], Maize, Kirinyaga[12], Rice, Kirinyaga[13], Maize & Bean, Chuka[14], Sweet potato, Makueni[15]
40.	*Tylenchorhynchus* sp. Cobb, 1913	Coffee, Kiambu[11], Cabbage, Kiambu[10], Maize, Kirinyaga[12], Rice, Kirinyaga[13], Maize & Bean, Chuka[14], Banana, Kakamega[17], Sweet potato, Kisii[15]
41.	*Quinisulcius* sp. Siddiqi, 1971	Cabbage, Kiambu[10]
42.	*Hemicriconemoides* sp. Chitwood & Birchfield, 1957	Cabbage, Kiambu[10], Coffee, Kiambu[11], Rice, Kirinyaga[13]
43.	*Criconema* sp. Hofmanner & Menzel, 1914	Coffee, Kiambu[11], Maize, Kirinyaga[12]
44.	*Criconemella* sp. De Grisse & Loof, 1965	Sweet potato, Makueni[15], Rice, Kisumu[13]
45.	*Mesocriconema* sp. Andrassy, 1965	Maize, Kirinyaga[12], Rice, Kirinyaga[13]
46.	*Hemicycliophora* sp. De Man, 1921	Cabbage, Kiambu[10], Coffee, Kiambu[11], Maize, Kirinyaga[12], Rice, Kirinyaga[13], Banana, Central Kenya[17]

47.	*Longidorus* sp. Micoletzky, 1922	Carnation –Coffee, Kiambu[11], Maize, Kirinyaga[12], Rice, Kirinyaga[13], Maize & Bean, Chuka[14]
48.	*Xiphinema* sp. Cobb, 1913	Cabbage, Kiambu[10], Coffee, Kiambu[11], Maize, Kirinyaga[12], Rice, Kirinyaga[13], Maize & Bean, Chuka[14], **coffee, Kirinyaga, ornamentals, Kiambu**
	C. Sedentary endoparasitic nematodes	
49.	*Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959	Irish potato, Nyandarua [27,28]
50.	*Globodera pallida* Stone, 1973	Irish potato, Nyandarua[28,29]
51.	*Heterodera* sp. Schmidt, 1871	Rice, Kirinyaga[13]
52.	*Meloidogyne* sp. Goldi, 1887	Sweet potato, Busia[15,16] , Common bean, Kiambu & Kakamega[30], Cassava, Mtwapa[31],, Cabbage, , Embu & Nyandarua [10,22], Banana[18], Sugarcane[19], Coffee, Kiambu[11], **Kirinyaga**, Maize, Kirinyaga[12], Rice, Kirinyaga[13], Maize & Bean, Chuka[14], Banana, Central Kenya[17]
53.	*Meloidogyne enterolobii* Young & Eisenback, 1983	African nightshade, Kiambu[32], Sweet potato, Central Kenya[33]
54.	*Meloidogyne hapla* Chitwood, 1949	whitetop weed, Tigoni[34], Sweet potato, Central Kenya[33], Pyrethrum, Kisii[21]
55.	*Meloidogyne incognita* (Kofoid &White, 1919) Chitwood, 1949	Irish potato, Central[16] ,Sweet potato, Central Kenya[16,33], Tea, Nyanza[35] Cassava[36]
56.	*Meloidogyne javanica* (Treub, 1885) Chitwood, 1949	Irish potatoes, Sweet potatoes, Nyanza & Coast[16], whitetop weed, Tigoni[34], Cassava[36], **Coffee, Kiambu**
57.	*Meloidogyne arenaria* Chitwood (1949)	Tomatoes, Central Kenya[37]
58.	*Meloidogyne kikuyensis* De Grisse,1960	Kikuyu grass, Muguga[38]
59.	*Rotylenchulus* sp. Linford & Oliveira, 1940	Maize, Kirinyaga[12], Rice, Kirinyaga[13], Sweet potato, Busia[15],
60.	*Rotylenchulus clavicaudatus* Dasgupta, Raski & Sher, 1968	Sweet potato, Coast, Nyanza[16]
61.	***Rotylenchulus macrosoma*** Dasgupta, Raski & Sher, 1968	**Coffee, Kiambu**

62.	*Rotylenchulus parvus* (Williams, 1960) Sher, 1961	Sweet potato, Nyanza[16]
63.	*Rotylenchulus reniformis* Linford & Oliveira, 1940	Sweet potato, Central Kenya[16]
64.	*Rotylenchulus variabilis* Dasgupta, Raski & Sher, 1968	Sweet potato, Central Kenya[16]
65.	*Tylenchulus* sp. Cobb, 1913	Maize, Kirinyaga[12], **Coffee, Kiambu**
66.	*Tylenchulus semipenetrans* Cobb, 1913	Coffee, Kiambu[11,39]
	Entomopathogenic	**Host crops, Location**
	A. Entomopathogenic	
67.	*Heterorhabditis bacteriophora* Poinar, 1976	Uknown[40]
68.	*Heterorhabditis indica* Poinar, Karunakar & David, 1992	Mombasa[40,41]
69.	*Heterorhabditis taysearae* Shamseldean, El-Sooud, Abd-Elgawad & Saleh, 1996	Central, Rift Valley[41]
70.	*Steinernema karii* Waturu, Hunt & Reid, 1997	Rift Valley[40,42], Kirinyaga[43]
71.	*Steinernema weiseri* Mracek, Sturhan & Reid, 2003	Rift Valley[42]
72.	*Steinernema yirgalemense* Nguyen, Tesfamariam, Gozel, Gaugler & Adams, 2005	Rift Valley[42]
	B. non-parasitic	
73.	*Filenchus* sp. Andrassy, 1954	Sweet potato ,Central[15], Maize, Kirinyaga[12], Rice, Kirinyaga[13], Maize & Bean, Chuka[14]
74.	*Tylenchus* sp. Bastian, 1865	Coffee, Kiambu[11], Maize, Kirinyaga[12], Rice, Kirinyaga[13], Maize & Bean, Chuka[14], Sweet potato, Makueni[15]
75.	*Polenchus* sp. Andrassy, 1980	Cabbage, Kiambu[10]
76.	*Mesorhabditis* sp. Osche, 1952	Maize, Kirinyaga[12]
77.	*Protorhabditis* sp. Osche, 1952	Maize, Kirinyaga[12]
78.	*Alaimus* sp. De Man, 1880	Coffee, Kiambu[11], Maize, Kirinyaga[12]
79.	*Alaimus asifkhalili* Mulk & Coomans, 1979	Mount Kenya[44]
80.	*Alaimus postamphidius* Coomans & Khan,1981	Mount Kenya[44]
81.	*Alaimus thompsoni* Mulk & Coomans, 1979	Mount Kenya[44]

82.	*Acrobeles* sp. Linstow, 1877	Carnation ,Kericho[25], Coffee, Kiambu[11],Maize, Kirinyaga[12]
83.	*Bunonema* sp. Jagerskiold, 1905	Carnation, Kericho[25], Coffee, Kiambu[11]
84.	*Cephalobus* sp. Bastian, 1865	Carnation, Kericho[25], Coffee, Kiambu[11]
85.	*Panagrolaimus* sp. Fuchs, 1930	Maize, Kirinyaga[12]
86.	*Cervidellus* sp. Thorne, 1937	Maize, Kirinyaga[12]
87.	*Aphanolaimus* sp. De Man, 1880	Maize, Kirinyaga[12]
88.	*Chiloplacus* sp. Thorne, 1937	Maize, Kirinyaga[12]
89.	*Chromadora* sp. Bastian, 1865	Carnation, Kericho[25], Coffee, Kiambu[11]
90.	*Coslenchus* sp. Siddiqi, 1978	Cabbage, Kiambu[10], Rice, Kwale[13]
91.	*Diplogasterid* sp. Micoletzky 1922	Maize, Kirinyaga[12]
92.	*Dorylaimus* sp. De Man, 1876	Maize, Kirinyaga[12]
93.	*Mesodorylaimus* sp. Andrassy 1959	Maize, Kirinyaga[12]
94.	*Discolaimus* sp. Cobb, 1913	Maize, Kirinyaga[12]
95.	*Eucephalobus* sp. Steiner, 1936	Carnation, Kericho[25], Coffee, Kiambu[11], Maize, Kirinyaga[12]
96.	*Eudorylaimus* sp. Andrassy, 1959	Maize, Kirinyaga[12]
97.	*Clarkus papillatus* (Bastian, 1965) Jairajpuri, 1970	Mount Kenya[45]
98.	*Coomansus parvus* (De Man, 1880) Jairajpuri & Kahn, 1977	Mount Kenya[45]
99.	*Iotonchus indicus* (Cobb 1916) Altherr, 1950	Coffee, Kiambu[11], Mount Kenya[45]
100	*Seinura* sp. Fuchs, 1931	Maize, Kirinyaga[12]
101	*Mononchus* sp. Bastian, 1865	Coffee, Kiambu[11], Maize, Kirinyaga[12], Carnation, Kericho[25]
102	*Prismatolaimus* sp. De Man, 1880	Maize, Kirinyaga[12]
103	*Mononchus truncatus* Bastian, 1865	Mount Kenya[45]
104	*Mylonchulus nainitalensis* Jairajpuri, 1970	Mount Kenya[45]
105	*Plectus* sp. Bastian, 1865	Coffee, Kiambu[11], Maize, Kirinyaga[12], Carnation, Kericho[25]
106	*Plectus acuminatus* Bastian, 1865	Mount Kenya[46]

107	*Plectus armatus* Butschli, 1873	Mount Kenya[46]
108	*Plectus globocephalus* Bastian, 1865	Mount Kenya[46]
109	*Plectus longicaudatus* Bastian, 1865	Mount Kenya[46]
110	*Plectus palustris* De Man 1880	Mount Kenya[46]
111	*Plectus parietinus* Bastian, 1865	Mount Kenya[46]
112	*Plectus parvus* Bastian, 1865	Mount Kenya[46]
113	*Tripyla* sp. Bastian, 1865	Maize, Kirinyaga[12]
114	*Prionchulus muscorum* (Dujardin, 1845) Wu and Hoeppli, 1929	Mount Kenya[45]
115	*Drilocephalobus* sp. Coomans and Goodey, 1965	Maize, Kirinyaga[12]
116	*Heterocephalobus* sp. (Brzseki 1960) Brzseki, 1961	Carnation, Kericho[25], Maize, Kirinyaga[12]
117	*Aporcelaimellus* sp. Heyns, 1965	Maize, Kirinyaga[12]
118	*Psilenchus* sp. De Man, 1921	Maize, Kirinyaga[12]
119	*Labronema* sp. Thorne, 1939	Maize, Kirinyaga[12], Carnation, Kericho[25]
120	*Geomonhystera* sp. Andrássy, 1981	Maize, Kirinyaga[12]
121	*Monhystera* sp. Bastian, 1865	Maize & bean, Chuka[14]
122	*Nygolaimus* sp. Cobb, 1913	Carnation, Kericho[25], Maize, Kirinyaga[12]
123	*Oscheius* sp. Andrassy, 1976	Maize & bean, Chuka[14]
124	*Prodorylaimus* sp. Andrassy, 1959	Maize, Kirinyaga[12], Coffee, Kiambu[11], Carnation, Kericho[25]
125	*Rhabditis* sp. Dujardin, 1845	Maize, Kirinyaga[12], bean, Coffee, Kiambu[11], Carnation, Kericho[25]
126	*Wilsonema* sp. Cobb, 1913	Maize, Kirinyaga[12]

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