FACULTY OF SCIENCES

Thesis

Plant-parasitic nematodes of Arabica coffee (*Coffea arabica* L.) from southwest Ethiopia

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Preamble of this thesis

The original study plan of this thesis was:

1. To uncover plant-parasitic nematode diversity from six sampling sites (coffee fields) from which 30 soil samples were collected in total.

2. To identify the plant-parasitic nematode genera based on morphology from each soil sample and identify to species level wherever possible.

3. To link morphological data of the identified plant parasitic genera/species with molecular data such as partial ribosomal DNA and mitochondrial DNA sequences.

What has been done for this thesis:

1. Studied the plant-parasitic nematode diversity from all the sampling sites and soil samples based on morphology to genus level.

2. The most important plant-parasitic nematode genera/species from one sampling site (Ge16) (five-soil samples out of 30 soil samples) were identified based on morphological and molecular characterisation using D2-D3 of 28S rDNA, 18S rDNA and *COI* mtDNA:*Meloidodera* sp., *Paratylenchus leptos*, *Helicotylenchus multicinctus* and *Tylenchorhynchus* cfr. *zeae*.

What has not been done due to COVID-19 pandemic was:

1. Detailed morphological and molecular study of plant-parasitic nematode leading to species identification from the remaining soil samples from the five other sampling sites.

2. Linking of molecular information with plant-parasitic nematode genera/species identified from the remaining samples.

Plant-parasitic nematodes of Arabica coffee (*Coffea arabica* L) from southwest Ethiopia

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Summary – Coffee (*Coffea arabica* L.) is the most important cash crop grown in 51 countries of the world. The major regions of coffee production include South America, Africa, Asia and Oceania and Mexico and Central America. Ethiopia is the primary place of origin and centre of genetic diversity of *Coffea arabica* L. Plant-parasitic nematodes are known to cause serious damage to coffee production throughout the world. The diversity study of nematodes associated with coffee from southwest Ethiopia was studied based on 30 soil samples collected from six sampling sites from Gera and Gomma coffee growing districts in southwestern Ethiopia. In total, 11 plant-parasitic nematode taxa were recovered from the rhizosphere *viz*. *Paratylenchus leptos*, *Meloidodera* sp., *Helicotylenchus multicinctus*, *Tylenchorhynchus* cfr. *zeae*, *Belonolaimus* sp., *Xiphinema* sp., *Scutellonema* sp., *Longidorus* sp., *Rotylenchus* sp., *Criconemoides* and *Ogma* sp.. The taxa *Paratylenchus leptos*, *Helicotylenchus multicinctus*, and *Meloidodera* sp. were the most prevalent taxa found in respectively 93%, 93% and 87% of the soil samples with a mean intensity of 73 ± 62, 61 ± 44 and 193 ± 203 nematodes/100 ml. From one sampling site (Ge16), *Meloidodera* sp., *Paratylenchus leptos*, *Tylenchorhynchus* cfr. *zeae* and *Helicotylenchus multicinctus* were studied based on an integrated morphological and molecular (D2-D3 of 28S rDNA, 18S rDNA and *COI* mtDNA sequences) approach. In the present study, we provided the first sequences of the partial D2-D3 of 28S rDNA, 18S rDNA and *COI* mtDNA for *Meloidodera* sp. and D2-D3 of 28S rDNA and 18S rDNA for *Paratylenchus leptos* and *Tylenchorhynchus* cfr. *zeae* from nematodes associated with coffee and as well as from Ethiopia. Only juveniles were found of the *Meloidodera* population in this study which are molecularly different from all known species. *Meloidodera* was found for the first time from coffee and Africa, demonstrating its wide biogeographical distribution. *Paratylenchus leptos* and *Tylenchorhynchus* cfr. *zeae* were for the first time reported from coffee. The present populations of *Tylenchorhynchus* cfr. *zeae* were morphologically similar to the original description of *T. zeae* and the population from Spain and China but found molecularly different from these populations.

Keywords – 18S rDNA, *COI* mtDNA, D2-D3 of 28S rDNA, Diversity, *Helicotylenchus multicinctus*, *Meloidodera* sp., Molecular, Morphology, *Paratylenchus leptos*, Phylogeny, *Tylenchorhynchus* cfr. *zeae*.

Coffee (*Coffea arabica* L.) is the most important beverage and cash crop grown worldwide for its economic value contributing foreign exchange to many countries (Campos & Villain, 2005; Labouisse et al., 2008). Currently, it is grown in 51 countries in the tropical and sub-tropical regions with an estimated 125 million people depend directly or indirectly on it for their

livelihoods (Osorio, 2002; ICO, 2019). The production of *C. arabica* accounts for 75% of world trade while Robusta coffee contributes to about 30% with the highest production in South and Central America and East and Central Africa (Marsh, 2007). Brazil, however, controls more than 30% of the world's coffee production (Carlos et al., 2019). The major regions of coffee production in the world include South America (47%), Africa (25%), Asia and Oceania (24.%) and Mexico and Central America (16%) (ICO, 2014). The top coffee producing countries being Brazil, Vietnam, Colombia, Indonesia, Ethiopia, Honduras, India, Mexico and Peru (USDA, 2019a).

In the past two decades, the global coffee production increased significantly by 50% estimated to be 10.47 million metric tons with an export raised to7.056 million metric tons; mostly produced by smallholder farmers as demand for coffee consumption increased by 65% (ICO, 2019; USDA, 2019b). Most of the coffee-producing nation export their whole produce, receiving about US$ 20 billion exports annually and the annual income of the coffee sector as a whole is estimated to surpass US$ 220 billion, greater than 11 times the value of exports received by producing nations (ICO, 2019). In Africa, coffee generates substantial income for rural communities and is a primary source of income for an estimated 10 million households in twenty-five countries (ICO, 2015). However, coffee production in Africa is declining, by approximately 17% since the 1970's due to diseases and pests, and cost of management invested on them; while global coffee production and consumption have essentially doubled at a hastening rate over the last 50 years (ICO, 2014, 2015).

Ethiopia is the primary place of origin and centre of genetic diversity of *Coffea Arabica* L, with a huge potential to increase coffee production as it endowed with suitable agro-ecological conditions (Amamo, 2014; Deribe, 2019). Small landholder farmers contribute about 95% of Ethiopia's coffee production in four broad categories of production systems, namely: forest coffee accounts (5%), semi forest (35%), garden coffee (50%) and plantation coffees 10% (Labouisse et al., 2008; USDA, 2019b). Ethiopia attained the lion share of coffee production and export in Africa, with a total production about 449,229 metric tons per annum at an export capacity of about 240,000 metric tons on a total production area coverage estimated to 800,000 hectares, worth of $142.1 million in the US global coffee market (CSA, 2018; USDA, 2019b). In Ethiopia, *C. arabica* employs in rural areas to the livelihood over 15 million people contributing more than 30% of the country's foreign exchange earnings, over 5% of the GDP, 12% of the agricultural output and 10% of the government revenues (Amamo, 2014).

Several factors are challenging the coffee production and productivity most importantly is the losses due to pests and diseases (Le et al., 2019; Thiep et al., 2019). Arabica coffee is more sensitive to stresses whether biotic or abiotic than Robusta coffee (Kimani et al., 2002). The major fungal diseases of coffee are coffee leaf rust (*Hemileia vastatrix*), coffee berry disease (*Colletotrichum kahawae*) and coffee wilt disease (*Fusarium xylarioides*) (Waller et al., 2007). The two important bacterial coffee diseases are halo blight of coffee incited by *Pseudomonas syringae* pv. *Garcae* and coffee leaf scorch caused by the polyphagous bacterium *Xylella fastidiosa* gen. nov., sp. nov: which is Gram-Negative and Xylem Limited, Fastidious Plant Bacteria Related to *Xanthomonas* spp. (Wells et al., 1987). Stem and branch borers, berry feeders and flower feeding insects are the most important in coffee plantations (Waller et al., 2007). Of the various biotic factors, plant-parasitic nematodes, in particular, the genus *Pratylenchus* and *Meloidogyne* are

known to cause serious damage and yield loss throughout the world both on Arabica and Robusta coffee (Campos & Villain, 2005; Villain et al., 2013; Janssen et al., 2017; Le et al., 2019; Thiep et al., 2019).

In South and Central America, information on coffee nematode is adequately available better than any other coffee-producing part of the world and nematodes are known to be highly damaging pests of coffee plantations to the point of forcing to change coffee to other alternative cash crops, such as sugar cane and soybean (Campos & Villain, 2005; Stefanelo et al., 2019). Several nematodes of coffee are reported, *M. izalcoensis, M. coffeicola, M. javanica, M. paranensis, M. exigua, M. arenaria, M. hapla, M. arabicida* and *M. incognita* are the most widely distributed species reported from Brazil, Costa Rica, Nicaragua, Honduras, Guatemala and El Salvador (Hernandez et al., 2004; Campos & Villain, 2005; Villain et al., 2007; Garcia et al., 2009; Barbosa et al., 2010; Stefanelo et al., 2019). The yield losses caused by *M. exigua* alone estimated up to 10%-20% in Costa Rica and 45% in Brazil (Bertrand et al., 1997; Barbosa et al., 2004). Recently, *Radopholus reniformis,* and *Pratylenchus* spp. also reported from coffee nursery survey in Brazil (Willian et al., 2018).

In many African countries, plant-parasitic nematodes of the *Meloidogyne* spp. are reported as major constraints of coffee production including *M. incognita, M. javanica, M. izalcoensis, M. hapla, M. oteifae, M. ardenensis, M. mali*; in particular, *M. africana, M. decalineata, M. kikuyensis* and *M. megadora* in Tanzania and Kenya, resulting in yield loss up to 20% in Tanzania (Whitehead, 1959, 1968, 1969; Bridge, 1984; Almeida & Santos, 2002; Campos & Villain, 2005; Waller et al., 2007; Jorge et al., 2016; Janssen et al., 2017). Orisajo & Fademi (2012) and Maundu et al. (2014) also reported *Tylenchorhynchus* sp., *Pratylenchus* sp., *Tylenchulus* sp., *Tylenchus* sp., *Rotylenchus* sp., *Helicotylenchus* sp., *Xiphinema* sp., *Scutellonema* sp., *Hemicycliophora* sp., *Radopholus* sp and *Rotylenchulus* sp. nematodes associated with coffee in Nigeria and Kenya.

To alleviate the nematode challenges in coffee production, survey and resistance-screening test have been used as one of the most economical and practical nematode management strategies (Trinh et al., 2004; Oliveira et al., 2007; Trinh et al., 2009; Boisseau et al., 2009; Maundu et al., 2014; Le et al., 2019; Thiep et al., 2019). Muniz et al. (2009) reported *Coffea arabica* cv. Tupi Vermelho IAC 1669-33 showed resistance to the *M. exigua* in Brazil and Costa Rica evaluated under greenhouse conditions. Similarly, according to Trinh et al. (2012) resistance and tolerance to *P. coffeae* and *R. arabocoffeae* was found in *C. liberica* var. Dewevrei and Hong34. However, the Arabica coffee accessions from Ethiopia (KH1, KH13, KH20, KH21, KH29, and KH31) tested for resistance screening in Vietnam found a susceptible and good host for *P. coffeae* and *R. arabocoffeae* (Trinh et al., 2012). Trinh et al. (2011) also reported the planting of *Coffea arabica* cv. Catimor is possible one year after removal of the 5-year-old coffee plant in a field naturally infested by *Radopholus arabocoffeae* and *Pratylenchus coffeae* without further use of chemical control options. Screening for resistance and tolerance to *P. coffeae* and *R. arabocoffeae* in Vietnam are showing promising results for coffee-breeding (Trinh et al., 2012).

In Ethiopia, only a limited investigation on the identification of plant-parasitic nematodes started in the 1970s on some nematode species belonging to the taxa *Helicotylenchus, Heterodera, Meloidogyne* and *Pratylenchus* related with vegetable crops (O'Bannon, 1975; Bogale et al.,

2004). Afterwards, preliminary surveys and investigations were conducted by several researchers in the country on vegetable crops, cereals, pulses and oil crops, cut flowers, root crops and coffee (Marais et al., 2005; Mekete et al., 2008b; Van Den Berg & Mekete, 2010; Mekonnen et al., 2014; Meressa et al., 2014). Information about nematodes of vegetable crops is more available compared to other crops in Ethiopia (Abebe et al., 2015). According to a survey by Mandefro & Mekete (2002) root-knot nematodes were found to be the most dominant and widely distributed on tomato, pepper, onion, snap bean, cabbage, beetroot, carrot and potato. Several species of nematodes belonging to 15 taxa are reported from cereals, pulses and oil crops in Ethiopia (Abebe et al., 2015). For cut flowers, Meressa et al. (2014) identified 13 major plant-parasitic nematodes associated with rose, gypsophila, statice, freesia or carnation and *M. hapla* was found to be the most prevalent root-knot nematode. Although root and tuber crops play a very important role in food security in Ethiopia, the diseases of these crops are not yet investigated particularly for nematodes (Mandefro & Dagne, 2000). As initial reports indicated *M. incognita, M. javanica* and *M. ethiopica* are important species associated with Enset plant in Ethiopia (Mandefro & Dagne, 2000).

Although coffee is the backbone for the Ethiopian economy, taxonomic studies of coffee nematodes are almost non-existent (Abebe & Geraert, 1995; Mekete et al., 2008a). Only limited preliminary surveys based on morphological identifications of plant-parasitic nematodes associated with coffee Arabica had been conducted to genus and species level (Mekete et al., 2008a, b; Van Den Berg & Mekete, 2010). *H. steiner, H. dihystera, H. multicinctus, H californicus, H gerti, Q. capitatus, S. paralabiatum, R. unisexus, R. borealis, X. insigne, X. basilgoodeyi,* and *X. americanum* had been identified from coffee Arabica in Ethiopia based on morphology only (Mekete et al., 2008a, b; Van Den Berg & Mekete, 2010). To our knowledge, no work has been done that link traditional taxonomy and molecular barcodes to characterize coffee nematodes in Ethiopia. Knowing this, effective taxonomic skills to identify nematodes in Ethiopia is necessary. The combined traditional taxonomy and molecular barcode have a key role to identify plant-parasitic nematodes and determine the taxonomic position of the nematode. The integrative taxonomy facilitates identification and helps to develop an effective management strategy (Palomares-Rius et al., 2017). Therefore, the main objective of this research was to study the density and occurrence of plant-parasitic nematodes associated with Arabica coffee in southwest Ethiopia, morphological and molecular characterisation at the species level, link the most important species with informative molecular barcodes, and provide useful barcodes for the most important plant-parasitic nematodes.

Materials and methods

SOIL SAMPLING

Soil samples were collected from six sampling sites during July 2019 from coffee plants in Gera and Gomma districts, Jimma zone. The sites are located in the southwestern Ethiopia at GPS coordinates 7°50'37.9"N 36°37'30.7"E, 7°47'18.8"N36°39'03.3"E, 7°47'28.4"N36°24'49.6"E, 7°47'49.0"N36°24'42.8"E, 7°46'44.3"N36°19'55.4"E and 7°45'11.1"N 36°19'39.4"E (Fig. 1). Each

site of 30m^2 was divided into five grid cells of 10m^2 (Fig. 2). In each grid cell, four coffee shrubs were randomly selected for soil core sampling and five soil cores from the top 10 - 30 cm soil were taken using a 3 cm diameter auger from each of the four coffee shrubs and pooled into a single composite soil sample (Fig. 2). Hence, one composite soil sample consists of 4*5 = 20 cores. Five representative soil samples about 300 - 500 g were collected into a plastic bag from each sites resulting in 5 samples* 6 sites = 30 soil samples.

NEMATODE EXTRACTION

All samples were brought to Ghent University and stored at 4°C until extraction and further processing. Nematodes were extracted from 200 ml of soil from each sample using a Baermann tray method after 48 hours (Hooper et al., 2005; Gnamkoulamba et al., 2018; Shokoohi et al., 2019) and stored in water at 4°C until DNA extraction and morphological identification.

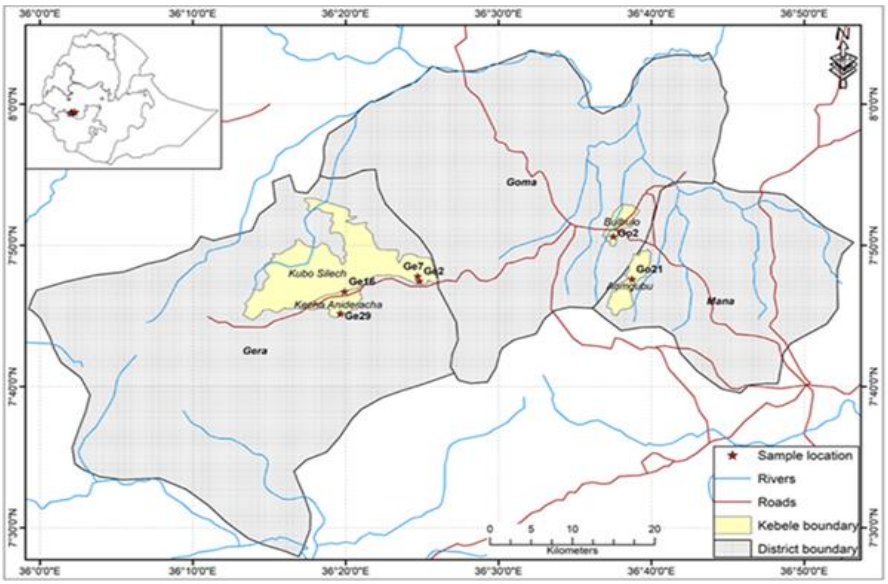

Fig. 1. Study area soil sampling sites (Go: Gomma; Ge: Gera sampling sites.).

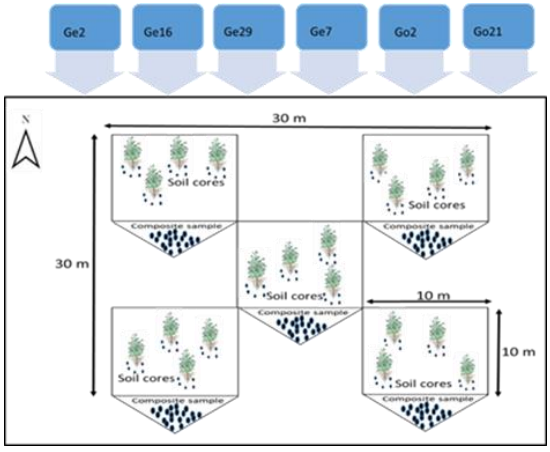

Fig. 2. Soil sampling sketch representing one soil-sampling site, the grids each represents one soil sample (5 grids = five samples.).

NEMATODE QUANTIFICATION

The nematode suspension was carefully stirred using a pipette blowing air for homogeneity before nematode counting. For individual nematode counting, 100 ml suspension pipetted from each sample and transferred to a counting dish of 100 squares. Then, nematodes within the two principal diagonals of the counting dish (20 out of 100 squares) were identified at the genus level using a stereomicroscope based on their morphological characters (Siddiqi, 2000; Hunt, et al., 2005; Hunt & Bert, 2012; Hunt & Palomares-Rius, 2012; Subbotin & Franco, 2012; Van Den Berg & Queneherve, 2012) and counted to estimate their intensity per sample. The prevalence and mean intensity per 100 ml of each genus was calculated using the formula below (Gnamkoulamba et al., 2018; Almohithef et al., 2020). The Tuky Test was used to tests the differences of the mean intensity of the nematode.

$$\text{Prevalence (\%)} = \frac{\text{positive samples containing nematode genus}}{\text{Total Number of samples collected}} * 100$$

$$\text{Mean Intensity } = \frac{\text{Number of individual nematode genus}}{\text{number of positive samples containing the genus}}$$

MORPHOLOGICAL AND MORPHOMETRIC CHARACTERISATION

Morphological and morphometric characterisation was done using fresh and fixed nematodes. A small suspension of nematodes was killed and fixed by adding a few drops of Trump's fixative (2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M Sorenson buffer (sodium phosphate buffer at pH 7.5) in an embryo glass block. The embryo glass block containing the nematodes suspension was heated in a microwave (700 W) for 3 s, left to rest for 1 h at 20°C and then kept for 24 hrs at 4°C for maximum penetration of the fixatives. Following the procedure described by Seinhorst (1959), the nematodes were gradually transferred to anhydrous glycerin for permanent slides and mounted in glass slides for light microscopy study as described by Singh et al. (2018). The nematodes were examined, measured and photographed using an Olympus BX51 DIC Microscope at the magnification 10X-100X (Olympus Optical), equipped with an Olympus C5060Wz camera and a drawing tube for morphological and morphometric characterization.

MOLECULAR CHARACTERISATION

Temporary slide (one fresh nematode per slide) was first made for digital light microscopy pictures as a morphological voucher to record all necessary morphological and morphometric data. Then, genomic DNA was extracted by cutting an individual specimen into 2-3 pieces and transferring it into a PCR tube with 20 µl of worm lysis buffer (50 mM KCl, 10 mM Tris at pH 8.3, 2.5 mM $MgCl_2$, 0.45% NP 40 (Tergitol Sigma), 0.45% Tween-20) and frozen for 10 min at −20°C. A 1 µl proteinase K (1.2 mg ml^{-1}) was added to each tube and then incubated in a PCR machine at 65°C for 1 h and 95°C (10 min) and finally centrifuged the lysate at 14000 rpm for 1 min. PCR assay was done by using 23 µl of Mastermix (17 µl Water; 2.5 µl 10x buffer; 2 µl $MgCl_2$; 2.5 µl Coralload; 0.5 µl dNTP (10 mM); 0.5 µl primer 1 and 2; 0.06 µl Top Taq) and 2 µl DNA template for each sample. The primers DP391/501 and D2A /D3B (forward/reverse) were used to

amplify the D2-D3 of 28S rDNA region. The PCR reaction was set for heating at 94℃ for 4 min, followed by 5 cycles of 94℃ for 30 s, 45°C for 30 s, and 72℃ for 2 min. This step was followed by 35 cycles of 94℃ for 30 s, 54°C for 30 s and 72℃ for 1 min and finished at 12℃ for 10 min. To amplify the 18S rDNA, the reaction conditions were: 94 ℃ for 5 min; 35 cycles of (94 ℃ for 1 minute; 52 ℃ for 1 minute 30 s; 68 ℃ for 2 min); 68 ℃ for 10 min. The cytochrome c oxidase subunit 1 (COI) gene fragment was amplified using the primers JB3/JB4; the reaction conditions were: denaturation at 94 ℃ for 5 min with 5 cycles of (94°C for 30 s; 54℃ for 30 s and temperature decreasing with 1°C for each cycle; 72℃ for 30 s) followed by 35 cycles of (94°C for 30 s; 50°C for 30 s; 72°C for 30 s), and a final extension of 10 min at 72°C. And the ITS1 rDNA was amplified using the primer TW81 and AB28 thermal profile: starting with 94℃ for 4 min; followed by 40 cycles at 94°C for 30 sec; annealing temperatures starting at 60°C for 30 s and 72°C for 1 min and finished at 10°C. All reactions were run in BIO-RAD, T100 Thermocycler, (BIO-RAD Laboratories, Inc., Singapore). Products (5 µL) were visualized on 1 % agarose gel electrophoresis stained with GelRed using a UV Transilluminator. After that, the successful PCR products were purified and sequenced in both directions at Macrogen Inc. (Amsterdam, The Netherlands). The details of the primers used and the respective references are mentioned in Table 1.

PHYLOGENETIC ANALYSIS

Sequences generated from both directions were assembled using assemble de novo in Geneious 10.0.9 (Biomatters Ltd company, New Zealand). The consensus sequence was subjected to BLAST search engine to check for similarities with other related sequences in the GenBank database (Altschul et al., 1997). Multiple DNA sequence alignment was made using MUSCLE in Geneious 10.0.9 with default parameters followed by manual trimming of the poorly aligned ends. Phylogenetic trees were constructed by using MrBayes 3.2.6 add-in of Geneious 10.0.9 with a nucleotide substitution under the GTR + G + I model. The Markov chain Monte Carlo (MCMC) were set with 1 x 10^6 generations, 4 runs and subsampling frequency of 500 generations. A 50% majority rule consensus trees were generated after discarding burn-in samples of 20% (Huelsenbeck & Ronquist, 2001).

Table 1. Details of the primers used and respective references.

Genes	Primers used, Forward/Reverse		References
	Forward	Reverse	
28S rDNA	**D2A** 5'-ACAAGTACCGTGAGGGAAAGTTG-3'	**D3B** 5'-TCGGAAGGAACCAGCTACTA-3'	(Subbotin et al., 2006) (De Ley et al., 1999)
	391 5'-AGCGGAGGAAAAGAAACTAA-3'	**591** 5'-TCGGAAGGAACCAGCTACTA-3'	(Nadler et al., 2006)
18S rDNA	**SSU18A** 5'-AAAGATTAAGCCATGCATG-3'	**SSU26R** 5'-CATTCTTGGCAAATGCTTTCG-3'	(Blaxter, 1998) (Floyd et al., 2002)
COI	**JB3** 5'-TTTTTTGGGCATCCTGAGGTTTAT-3'	**JB4** 5'-TAAAGAAAGAACATAATGAAAATG-3'	(Derycke et al., 2010) (Palomares-Rius et al., 2017)
ITS1-5.8 ITS2 rDNA	**TW81** 5'-GTTTCCGTAGGTGAACCTGC-3'	**AB28** 5'-ATATGCTTAAGTTCAGCGGGT-3'	(Subbotin et al., 2017; 2018; 2019)

Results

PREVALENCE AND MEAN INTENSITY OF NEMATODES ASSOCIATED WITH COFFEE

The survey resulted in all the 30 soil samples collected from the six sampling sites infested with plant-parasitic nematodes. Eleven nematode taxa including *Meloidodera* sp., *Paratylenchus leptos*, *Helicotylenchus multicinctus*, *Tylenchorhynchus* cfr. *zeae*, *Belonolaimus* sp., *Xiphinema* sp., *Scutellonema* sp., *Longidorus* sp., *Rotylenchus* sp., *Criconemoides* and *Ogma* sp. were recovered from coffee rhizosphere soil (Table 2).

Of the nematodes recovered, the taxa *Paratylenchus leptos*, *Helicotylenchus multicinctus*, *Meloidodera* sp. and *Xiphinema* sp. were the most important taxa found in respectively 28, 28, 26 and 25 soil samples and representing the highest prevalence, i.e. 93%, 93%, 87% and 83%, respectively. Of secondary importance are *Tylenchorhynchus* cfr. *zeae*, *Longidorus* sp., *Rotylenchus* sp. and *Criconemoides* recovered from 21, 20, 20 and 18 soil samples at the prevalence of 70%, 67%, 67% and 60%, respectively. Whereas, the nematode taxa *Scutellonema* sp., *Ogma* sp. and *Belonolaimus* sp. were less important and encountered at 27%, 20% and 13% recovered from 8, 6 and 4 soil samples, respectively (Table 2). Unlike the other taxa, these nematodes showed restricted distribution among the sampling sites (Table 3).

Based on the mean intensity of the nematode population, the nematode genus *Meloidodera* sp. was found to be the most abundant representing the highest mean intensity 193 ± 203 nematodes/100 ml; followed by *Paratylenchus leptos* and *Helicotylenchus multicinctus* at the intensity of 73 ± 62 and 61 ± 44 nematodes/ 100 ml, respectively. Other nematode taxa mainly represented at relatively low mean intensity were *Tylenchorhynchus* cfr. *zeae* and *Belonolaimus* sp. having 49 ± 40 and 33 ± 20 nematodes/100 ml, respectively. Whereas, the nematode taxa *Xiphinema* sp., *Scutellonema* sp. and *Longidorus* sp. were recovered at very low mean intensity 27 ± 16, 24 ± 18 and 20 ± 8 nematodes/100 ml, respectively (Table 2). However, the remaining three taxa, *Rotylenchus* sp., *Criconemoides* and *Ogma* sp. were detected at the lowest mean intensities of 17 ± 14, 13 ± 6 and 12 ± 4 nematodes/100 ml, respectively (Table 2 and Fig. 3).

Among the 11 taxa of nematodes identified, the site Ge7 and Go21 each hosted the most taxa 10, the sites Ge16, Ge2 and Ge29 each harboured nine taxa. The site Go2, however, contained seven of the taxa (Table 3). There was a significant difference between mean population intensity of *Paratylenchus leptos* at ($P = 0{,}003$), *Longidorus* sp. ($P = 0{,}036$) and *Tylenchorhynchus* cfr. *zeae* ($P = 0{,}035$); however, for the other taxa, there was no significant difference between mean population intensity at ($P \geq 0.05$) in the study sites.

Table 2. Mean intensity ± standard deviation/100 ml and prevalence of plant parasitic nematodes associated with *Coffeae arabica* L. in southwest Ethiopia.

Nematode taxa	Number of individuals in each site						Total population density	Mean intensity ± SD	Nematode taxa	Total samples	Positive samples	Prevalence (%)
	Ge16	Ge2	Ge29	Ge7	Go2	Go21						
Meloidodera sp.	440	630	1590	470	854	1030	5014	193 ± 203	*P. leptos*	30	28	93%
P. leptos	530	300	740	160	140	160	2030	73 ± 62	*H. multicinctus*	30	28	93%
H. multicinctus	500	320	200	230	140	320	1710	61 ± 44	*Meloidodera* sp.	30	26	87%
T. cfr. *zeae*	490	80	160	100	70	120	1020	49 ± 40	*Xiphinema* sp.	30	25	83%
Xiphinema sp.	170	80	120	140	110	50	670	27 ± 16	*T.* cfr. *zeae*	30	21	70%
Longidorus sp.	50	90	80	50	40	80	390	20 ± 8	*Longidorus* sp.	30	20	67%
Rotylenchus sp.	110	90	50	50	-	30	330	17 ± 14	*Rotylenchus* sp.	30	20	67%
Criconemoides	60	30	30	20	60	30	230	13 ± 6	*Criconemoides*	30	18	60%
Scutellonema sp.	-	40	70	-	-	80	190	24 ± 18	*Scutellonema* sp.	30	8	27%
Ogma sp.	30	-	-	20	-	20	70	12 ± 4	*Ogma* sp.	30	6	20%
Belonolaimus sp.	-	-	-	130	-	-	130	33 ± 20	*Belonolaimus* sp.	30	4	13%

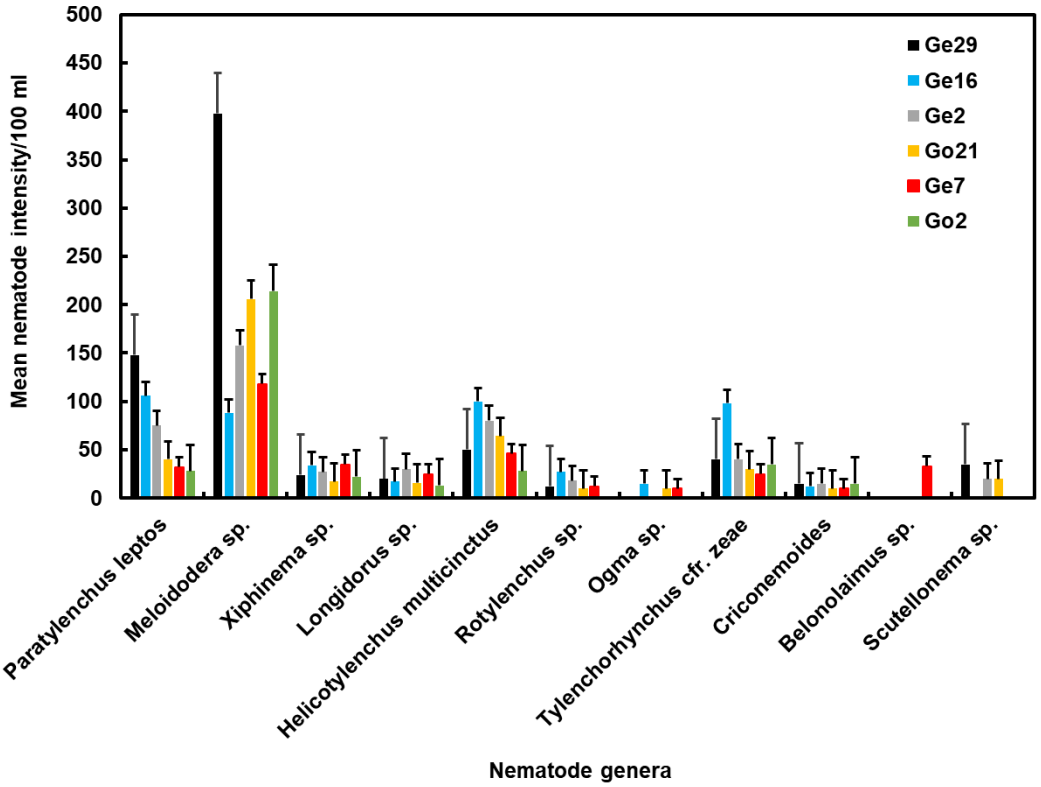

Fig. 3. Mean intensity of nematode taxa associated with *Coffea arabica* L. in each soil sampling sites (Ge: Gera; Go: Gomma) sampling sites, southwest Ethiopia.

Table 3. Occurrence of plant-parasitic nematode taxa in the six soil sampling sites associated with *Coffea arabica* L. in southwest Ethiopia.

Nematode taxa	Soil sampling sites					
	Ge7	Ge16	Ge2	Ge29	Go2	Go21
P. leptos	*****	*****	****	*****	*****	****
Meloidodera sp.	****	*****	****	****	****	*****
Xiphinema sp.	****	*****	***	*****	*****	***
Longidorus sp.	**	***	***	****	***	*****
H. multicinctus	*****	*****	****	****	*****	*****
Rotylenchus sp.	****	****	*****	****	-	***
Ogma sp.	**	**	-	-	-	**
T. cfr. *zeae*	****	*****	**	****	**	****
Criconemoides	**	*****	**	**	****	***
Belonolaimus sp.	****	-	-	-	-	-
Scutellonema sp.	-	-	**	**	-	****
Number of taxa	**10**	**9**	**9**	**9**	**7**	**10**

Note: Ge: Gera; Go: Gomma soil sampling sites, (*) represents number of composite samples containing nematodes (5 is max number), (-) specific genus not detected.

In this study, 11 taxa of nematodes were identified from the coffee rhizosphere. Morphological and molecular characterisations were performed for *Meloidodera* sp., *P. leptos*, *Tylenchorhynchus* cfr. *zeae* and *H. multicinctus* from one soil sampling site Ge16 (from five soil samples out of 30 soil samples).

MORPHOLOGICAL AND MOLECULAR CHARACTERISATION

***Paratylenchus leptos* Raski, 1975**

(Fig. 4)

MORPHOLOGICAL CHARACTERISATION

MEASUREMENTS

See Table 4.

DESCRIPTION

Female

Body small, ventrally curved, and open C-shape after heat relaxation. Lip region rounded with truncate anterior end and slightly offset. Cephalic framework moderately developed. Stylet moderately developed, knobs slightly sloping and slightly rounded ventrally. Pharynx with weakly to moderately developed and elongated median pharyngeal bulb containing a weak valve in most specimens. Isthmus not seen. Basal pharyngeal bulb elongated and pyriform in shape sometimes not visible in some specimens and not overlapping the intestine. Excretory pore same level with hemizonid, situated just anterior end of pharyngeal basal bulb about 57 µm from anterior end. Lateral field with three lines, outer two more distinct and a faint inner one. Vulva located about 80.8%, vulval anterior lip higher than posterior lip almost in all specimen. Cuticular flaps present except for some specimen. Ovary outstretched, spermatheca medium to large, oval shape, either empty or filled with round sperm cells. Post vulval uterine sac absent. Phasmid not seen. Tail conoid and rounded terminus curved ventrally, tapering gradually, and narrowing abruptly near the terminus forming a digitate appearance to the dorsal side. In most of the specimen anus obscure.

Male

Not found.

MOLECULAR CHARACTERISATION

D2-D3 of 28S rDNA

Three partial D2-D3 of 28S rDNA sequences of 543 bp, 578 bp and 527 bp long were produced with 0 - 2 bp variation (out of 567 bp). The multiple sequence alignment was 645 bp in length and included 48 other D2-D3 of 28S rDNA sequences of *Paratylenchus* from fifteen known and twenty-one unknown *Paratylenchus* species and four out-group taxa. The *P. leptos* formed a

maximally supported (PP = 1) monophyletic group, with a maximally supported sister relation to *P. leptos* and *P. rostrocaudatus* from China (Fig. 5). The present *P. leptos* has only 84.5 - 85.4% and 79.7 - 81.9% sequence similarity with *P. leptos* (KR270602) and *P. rostrocaudatus* (KR270601) differing by (77 - 85 bp and 105 - 112 bp), respectively.

18S rDNA

The amplification resulted in a single sequence of 812 bp long. The multiple sequence alignment was 1170 bp in length and contained 32 sequences from fifteen identified, eight unidentified *Paratylenchus* species and four outgroup taxa. The reconstructed phylogenetic tree showed a similar topology with the tree from D2-D3 of 28S rDNA (Fig. 6) where our *P. leptos* clustered with *P. leptos* and *P. rostrocaudatus* from China with maximum support (PP = 1). Sequence alignment revealed respectively the similarity of 97% and 96% differing by (31 bp and 36 bp) with *P. rostrocaudatus* (KR270598) and *P. leptos* (KR270599).

REMARKS

P. leptos was first described from *Piper* sp. in Brazil (Raski, 1975). In the same country, it was also detected from the rhizospheres of *Cocos nucifera*, *Musa acuminata* and *Brassica rapa* (Huang & Raski, 1987). Afterwards, it was reported from a humid forest in Guadeloupe (Van den Berg & Quénéhervé, 1999), from roses and grasses (Abebe & Geraert, 1995) in Ethiopia, grapevine in Iran (Esmaeili et al., 2016) and citrus in China (Zhuo et al., 2018).

Morphologically, the present population is very similar to the originally described specimen by Raski (1975) and aforementioned authorities descriptions of the species. However, some morphometric variations were observed between the present specimen and the studied populations of the species. The differences were having the slightly shorter body length (184 - 223 vs 200 - 260, 233 - 276, 204 - 260 and 250 -262 µm) compared to respectively the original description (Raski, 1975); Ethiopian specimens (Abebe & Geraert, 1995; Van den Berg et al., 2004) and Chinese populations (Zhuo et al., 2018). However, the body length is similar to the Brazilian specimens (184 - 223 vs 162 - 215 µm) (Huang & Raski, 1987). The shorter range of tail length (6.1 - 13.5 vs 11 - 17 and 17 - 21 µm) compared to the Iranian and the Chinese specimens, respectively.

The present specimens, based on the lateral field with three lines, presence of vulval flap and digitate tail terminus similar to *P. aquaticus* and *P. humilis*. Our specimens are also similar to *P hamatus* by the above-mentioned morphological characters except for the lateral field having four lines in *P hamatus*. Can be distinguished from *P. aquaticus* and *P. hamatus* by the post-vulva uterine sac (absent vs present in both *P. aquaticus* and *P. hamatus*) and by the shorter body size (184 - 223 vs 256 - 331 and 349 - 399 µm), respectively. Differed from *P. humilis* by shorter tail length (6.1 - 13.5 vs 12.0 - 17.0 µm) (Raski, 1975; Van Den Berg et al., 2014; Esmaeili & Heydari, 2017). This is the first report of *P. leptos* associated with coffee.

Our *P. leptos* is similar to the previously reported *P. leptos* from Ethiopia (Abebe & Geraert, 1995; Van den Berg et al., 2004). However, Abebe & Geraert (1995) did not give detail of the tail length. Also, in the original description, details of tail length and the anus was not mentioned that

makes it difficult to use it as a descriptive character and it is variable among the described species. In the recent identification of the *Paratylenchus* species, 117 valid species have been identified and divided into 11 groups based on the stylet length, lateral lines and presence or absence of advulval flaps (Ghaderi et al, 2014). According to Geraert (2011) and Ghaderi et al. (2014), our *P. leptos* well fitted into group two.

For the following reasons, the present *P. leptos* and its associated sequence are more likely to represent the genuine *P. leptos* than the Chines population. In the original description, the description by Abebe & Geraert (1995), Van den Berg & Quénéhervé (1999) and the present study the midline of the lateral line was indicated indistinct faint; however, in the Chinese population, the detail of the lateral line, except the number was not given. The round shape of the stylet knob of the present *P. leptos* is similar with the *P. leptos* described by Esmaeili et al. (2016) whereas in the Chinese population it was described as an oblate (Zhuo et al., 2018); geographically, our specimen is more closer to the original description of *P. leptos* and the climatic conditions of Ethiopia are more similar to Brazil than to China. The previous reports of *P. leptos* in Ethiopia could be an evidence for our *P. leptos*; but no previous report indicated the presence of this species in China other than Zhuo et al. (2018). However, molecular characterisation of this nematode from the type location (original specimen from Brazil) is needed to conclude if our sequences or the sequences from China represent the correct *P. leptos*.

In the Bayesian phylogenetic trees, the present *P. leptos* with the stylet shorter than 40 µm clustered together and formed a maximally supported clade with sequences having stylet less than 40 µm with *P. rostrocaudatus*, *P. leptos*, *P. aquaticus* and two *Paratylenchus* spp. (KF668516 and KF668517) identified as *P. shenzhenensis* in Zhuo et al. (2018) in the D2-D3 of 28S rDNA tree, and with *P. rostrocaudatus* (KR270598) and *P. leptos* (KR270599) in the 18S rDNA tree. The present sequence of *P. leptos* is well matched in both the D2-D3 of 28S rDNA and 18S rDNA phylogenetic tree with the morphological characterisation based on the stylet size, the three lateral lines and presence of advulval flaps.

Our finding is well supported with the report of Ghaderi et al. (2014), Zhuo et al. (2018) and Li et al. (2019) in that stylet length, advulval flaps and lateral lines were used as the most stable characteristics to identify *Paratylenchus* to species level fitted to *P. leptos*. As regard to morphological and morphometric congruence with the phylogenetic tree based on stylet length, we also accepted the synonymization of *Gracilacus* with *Paratylenchus*. The ITS rRNA sequence phylogenetic tree for *P. leptos* has been reported placing the sequence of *P. leptos* with fully supported clade with *P. rostrocaudatus* (KR270604), *Paratylenchus* sp. BC (KT258979) and *P. chongqingensis* (KM024367 - KM024369) (Zhuo et al., 2018; Li et al., 2019); whereas *COI* mtDNA sequences for *P. leptos* and other species of pin nematodes have not been sequenced at present time (Van Den Berg et al., 2014; Zhuo et al., 2018; Li et al., 2019).

Table 4. Morphometric data of *Paratylenchus leptos* associated with coffee from Ethiopia and comparison to other populations of *P. leptos*. All measurements are in µm and the form of mean ± SD (range). (data from soil sampling site Ge16).

Morphological characters	Ethiopia Present study	Brazil (Raski, 1975) (Type specimen)	Brazil (Huang & Raski, 1987)	Iran (Esmaeili et al., 2016)	China (Zhuo et al., 2018)	Ethiopia (Van den Berg et al., 2004)	Ethiopia (Abebe & Geraert,1995)
	Female	Female	Female	Female	Female	Female	Female
n	20	8	14	10	12	15	3
L	195 ± 10.1 (184 - 223)	220 (200 - 260)	188 ± 17 (162 - 215)	204 ± 15.8 (180 - 225)	256 ± 4.5 (250 - 262)	223 (204 - 260)	254 (233 - 276)
a	19.7 ± 1.5 (16.8-22.1)	17.0 (15 - 22)	21.9 ± 2.5 (18.8 - 26.8)	14.8 ± 1.4 (12.8 - 16.9)	24 ± 1.0 (23 - 26)	20.0 (15 - 24)	21.2
b	3.4 ± 0.2 (2.9 - 4)	3.2 (3.0 - 3.5)	3.2 ± 0.3 (2.8 - 3.8)	2.6 ± 0.3 (2.2 - 3.1)	4.3 ± 0.1 (4.2 - 4.5)	3.5 (3.3 - 4.3)	4.0
c	22.7 ± 4.8 (14.4 - 30.3)	-	17.8 ± 4.4 (10.3 - 22.3)	15.6 ± 2.2 (12.5 - 18.8)	13.5 ± 0.6 (12 - 15)	17.5 (15 - 19)	-
c'	1.9 ± 0.3 (1.6 - 2.5)	-	-	1.8 ± 0.3 (1.5 - 2.3)	3.0 ± 0.1 (2.5 - 3.1)	2.1 (1.8 - 2.6)	-
V%	80.8 ± 1.2 (78.5 - 83.3)	82 (77 - 84)	81.3 ± 0.9 (79.2 - 82.5)	77.7 ± 2.0 (76.0 - 82.5)	81 ± 1.3 (79 - 84)	82 (80 - 85)	79.3
MBW	10 ± 0.6 (8.9-11.1)	15 (14 -16)	-	13.9 ± 0.8 (13 - 15)	11 ± 0.4 (10 - 12)	12 (9 - 14)	12
Stylet length	20.1 ± 1 (18.3 - 22.7)	22 (20 - 23)	20 ±1.5 (18 - 24)	20.4 ± 1.8 (18 - 23)	23 ± 0.6 (22 - 24)	22.5 (20 - 26.5)	19.3
Cone length	14.2 ± 1.5 (13.2 - 16.3)	-	-	13.6 ± 1.6 (12 - 17)	-	-	-
Stylet knob	3.1 ± 0.4 (2.2 - 3.8)	-	-	-	-	-	-
Pharynx length	57.2 ± 3.1 (51.3 - 62.8)	-	59 ± 2 (51 - 63)	78.5 ± 2.3 (75 - 82)	59 ± 1.8 (55 - 62)	64 (60 - 68)	63.2
Anterior to SE pore	47.8 ± 3.7 (42.3 - 53.5)	55 (51 - 57)	50 ± 4.6 (38 - 58)	61.4 ± 2.6 (58 - 65)	54 ± 1.5 (52 - 57)	54 (51 - 61)	55.3
Anterior to vulva	158 ± 7.6 (149 - 175)	-	-	158 ± 11.8 (141 - 176)	-	-	-
Body width around anus	4.6 ± 0.6 (3.9 - 5.6)	-	-	7.4 ± 0.5 (7 - 8)	6.4 ± 0.2 (6.3 - 6.8)	-	-
Tail length	9.1 ± 2 (6.1 - 13.5)	-	-	13.3 ± 2.1 (11 - 17)	19 ± 0.8 (17 - 21)	13 (11 - 16)	-
m (cone length in% of stylet)	72 ± 8.7 (62.9 - 83.6)	-	-	66.6 ± 2.8 (65.0 - 73.9)	-	-	-
EP%L	24.5 ± 2 (21.7 - 29)	-	-	30.3 ± 3.0 (26.2 - 36.1)	-	-	-

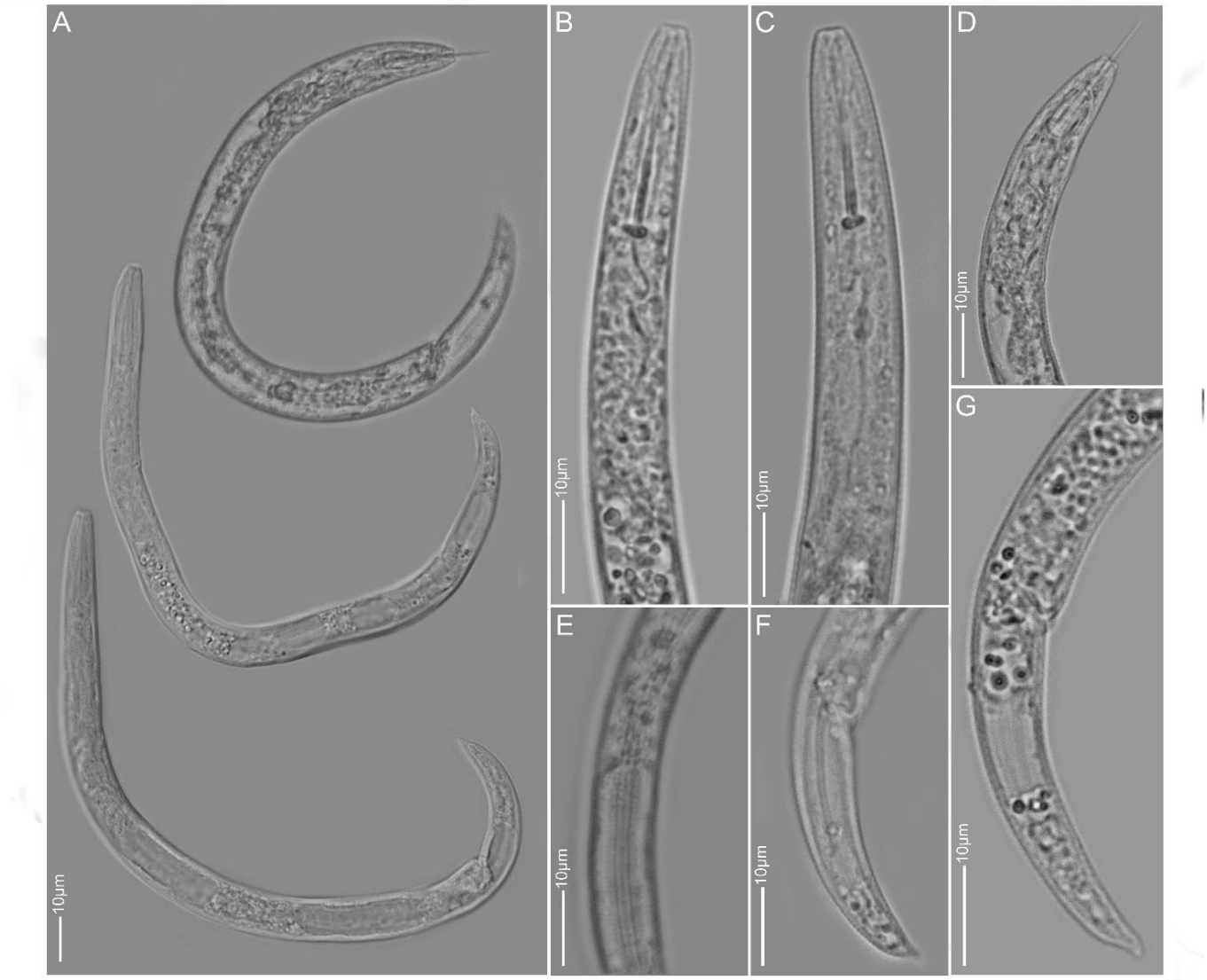

Fig. 4. Light microscopy of female *Paratylenchus leptos* from Ethiopia. A: Entire body; B-D: Anterior region (B-C: Head region and excretory pore; D: Stylet); E: Lateral line; F-G: Reproductive part showing vulva region, spermatheca and tail terminus.

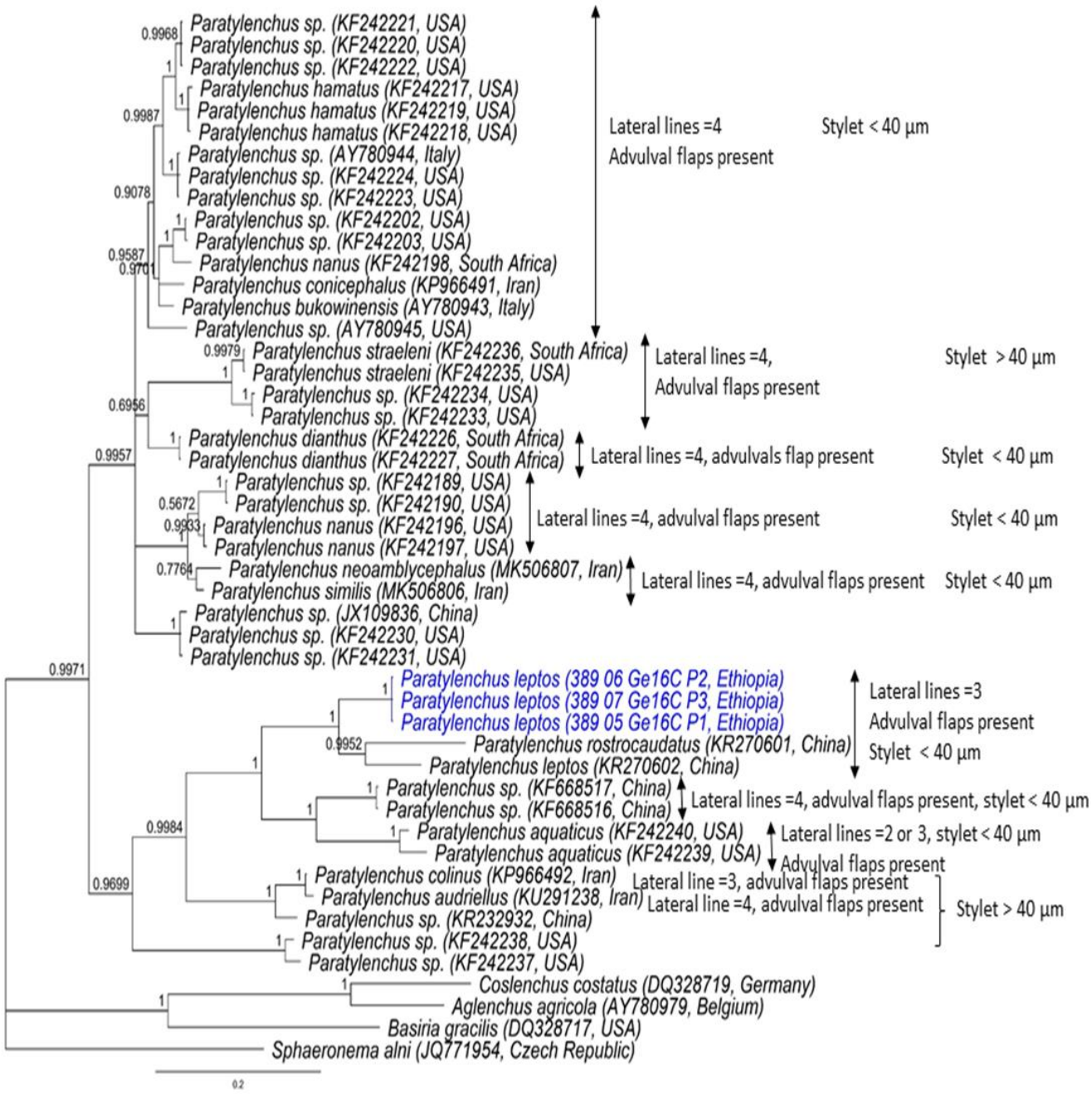

Fig. 5. Phylogenetic relationships of *Paratylenchus leptos* from Ethiopia and other *Paratylenchus* spp. Bayesian 50% majority rule consensus tree as inferred from the analysis of D2-D3 of 28S rDNA partial sequences under GTR+I+G model. Posterior probabilities of more than 50% are given for appropriate clades. The present sequences are shown in blue.

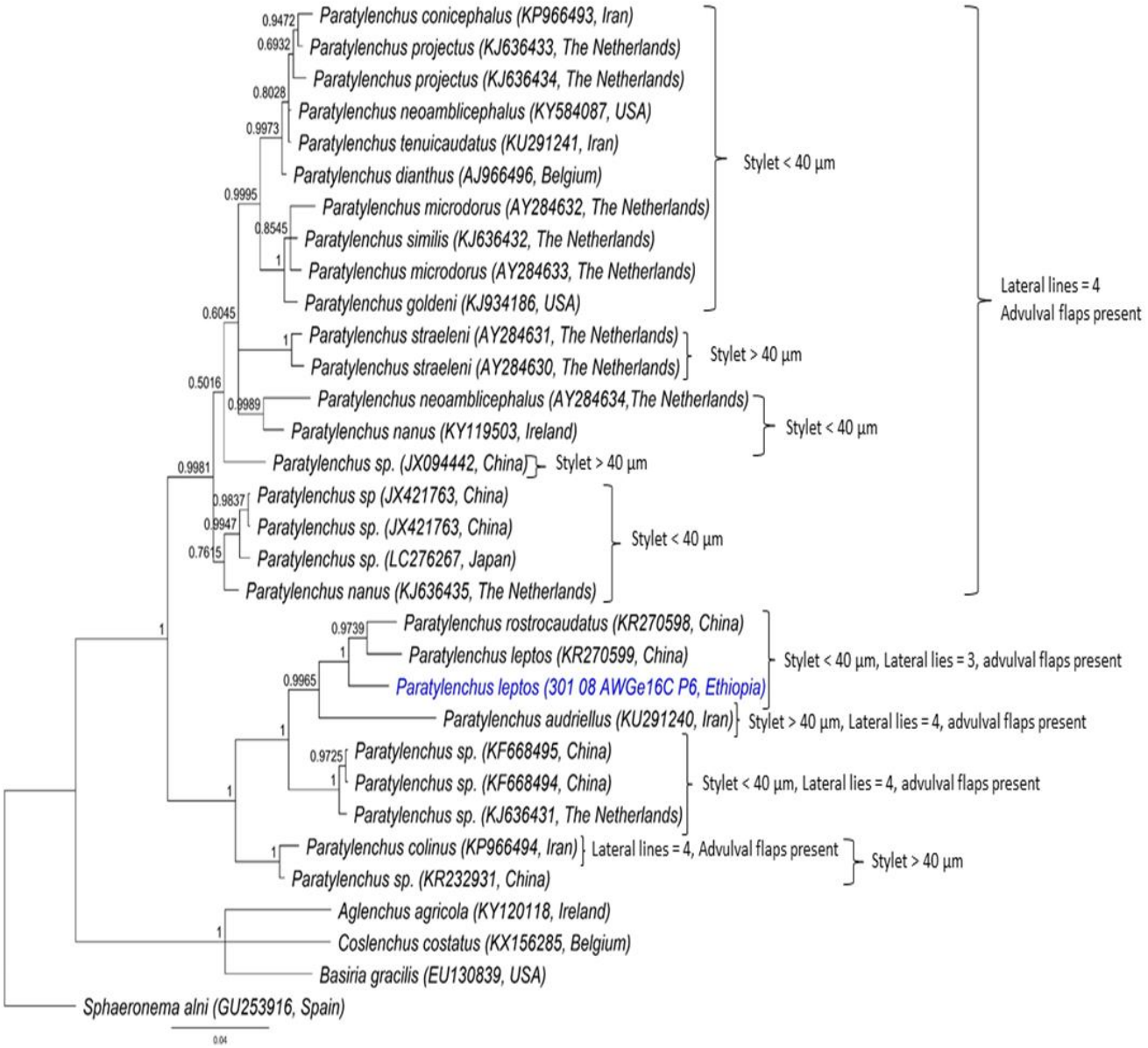

Fig. 6. Phylogenetic relationships of *Paratylenchus leptos* from Ethiopia and other *Paratylenchus* spp. Bayesian 50% majority rule consensus tree as inferred from the analysis of 18S rDNA partial sequences under GTR+I+G model. Posterior probabilities of more than 50% are given for appropriate clades. The present sequence is shown in blue.

***Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956)**

(Figs 7, 8)

MORPHOLOGICAL CHARACTERISATION

MEASUREMENTS

See Table 5.

DESCRIPTION

Female

The body usually in a spiral shape after heat relaxed. Lip region almost round not setoff, having 3.4 ± 0.4 µm (2.8 - 4.2 µm) height and 4.8 ± 0.5 µm (3.8 - 5.3 µm) width with four lip annuli; the labial framework was well sclerotized. Lateral fields with four incisures start areolation anteriorly around the median bulb and end around the posterior end of pharyngeal gland covering about one-fourth of midbody width. Stylet well developed, 21.0 - 24.7 µm long, having flattened knobs with slightly anteriorly directed. Median bulb oval in shape about 8.0 - 11.7 µm in length and 6.2 - 8.3 µm width. Excretory pore situated about 14.6 - 18.4 µm away from the head end. Hemizonid distinct, two to three annuli long located immediately anterior to excretory pore Vulva depressed transverse slit located at about 62.4% of the total body length. Reproductive system, didelphic with rounded spermatheca usually filled with round sperms. Tail slightly tapering with five to eight annuli long; having hemispherical annulated tail terminus without mucron. Phasmids conspicuous pore-like, two to six annuli anterior of the anus.

Male

Present and morphologically similar to the females except for the shorter body length and sexual characters. Testis not seen. Spicules paired, 19.0 - 20.4 µm long; gubernaculum simple and less than half of the spicule length measured about 7.0 - 8.3 µm long. Tail about 11.0 - 14.0 µm long ventrally curved with rounded projection.

MOLECULAR CHARACTERISATION

D2-D3 of 28S rDNA

Three sequences of D2-D3 of 28S rDNA were obtained with the length 441 bp, 596 bp and 625 bp varied from 0.2 - 0.9% (1 - 4 bp) among each other. Multiple alignments involved 65 sequences from twelve identified and one unidentified *Helicotylenchus* sp. with one out-group and was 645 bp in length. The present sequences of *H. multicinctus* formed a maximally supported clade that is sister to all other *H. multicinctus* sequences; all *H. multicinctus* sequences together form a well-supported clade (PP = 91) (Fig. 9). However, the present *H. multicinctus* showed only 96 - 98%, 92 - 93% and 91 - 93% similarity differing by (2 - 9 bp, 34 - 40 bp and 37- 43 bp) with the sequences of *H. multicinctus* from India (MG020141), South Africa (HM014290) and Brazil (MT239110), respectively. However, the sequences from our population are remarkably different from other *H. multicinctus* sequences forming an independent group found in the basal position of the clade.

REMARKS

H. multicinctus (Cobb, 1893) Golden, 1956 was described from Fiji from banana; since then, it has been reported from all biogeographical regions associated with different hosts including coffee in Ethiopia (Sher, 1966; Siddiqi, 1973; Sikora & Schlosser, 1973; Mcsorley & Parrado, 1986; Lamberti et al., 1987; Troccoli & Geraert, 1995; Philis, 1995; Bridge et al., 1996; Mani et al.,

1998; Marais et al, 2000; Marais et al., 2005; De Waele & Elsen, 2007; Mekete et al., 2008b; Tzortzakakis et al., 2017). Up to date 230 valid species of *Helicotylenchus* has been described from different countries in the world (Fotedar & Kaul, 1985; Uzma et al., 2015).

Morphologically, the present specimen corresponds well with the description of *H. multicinctus* by Sher (1966), Marais (2001), Tzortzakakis et al. (2017) and Marais et al. (2005) on the spiral body shape, the round lip region, the areolation of lateral lines, the oval median bulb, the rounded spermathecal, the position of the vulva, the hemispherical tail terminus without mucron, the pore-like phasmid and the presence of male population. However, some morphometric variations were observed between the present specimen and the previously studied populations of the *H. multicinctus*. The differences were the slightly longer body length (463 - 573 vs 470 - 530 µm) compared to the topotype specimen description of Sher (1966). The shorter body length (463 - 573 vs 476 - 654, 508 - 640 and 567 - 584 µm) compared to respectively the S. African (Marais, 2001); Greece (Tzortzakakis et al., 2017) and Ethiopian populations (Marais et al., 2005).

The present specimens showed some similarity to *H. dihystera* (the population from New Zealand), *H. pseudorobustus* (from Switzerland) and *H. microlobus* (from Iowa, USA) by the spiral body shape, the stylet length and the position of the vulva. However, it can be identified respectively by the shorter body length (463 - 573 vs 569 - 726, 675 - 81 and 752 - 797 µm), and the slightly shorter tail length (11 - 14 vs 12 - 17, 15 - 20 and 14 - 18 µm). Moreover, the present population differed from the above three specimens by having common characters of the shape of lip region (almost round vs hemispherical) and the male population (present vs absent) (Fortuner, 1984; Wouts & Yeates, 1994; Subbotin et al., 2015).

In the present study, there was a congruence between the morphological and molecular identification of *H. multicinctus*. Interestingly, the present *H. multicinctus* characterisation based on morphology and molecular well corresponds with the previous morphological and morphometric identification of *H. multicinctus* except for some difference in body length from similar agro-ecological area and host (Coffee) in southwest Ethiopia (Mekete et al., 2008a, b) and banana in south Ethiopia (Marais et al., 2005). Although the previous identifications of *H. multicinctus* from Ethiopia were only based on morphology and no molecular characterisation for better comparison, the present morphological and molecular identification can be used as evidence to strengthen the previously morphologically identified nematode as *H. multicinctus* could be correct identification. However, molecular characterisation from the type location and the Ethiopian population is required for molecular comparison. In the present study, remarkable molecular variation was observed within the sequences of *H. multicinctus*.

Fig. 7. Light microscopy of female *Helicotylenchus multicinctus* from Ethiopia. A-C: Anterior body region (A: Ventral overlap; B: Excretory pore; C: Head region and stylet); D, F: Lateral line; E: Vulva and spermatheca; G, K: Phasmids; H, L: Tail region showing anus and tail terminus; I-J: Entire body.

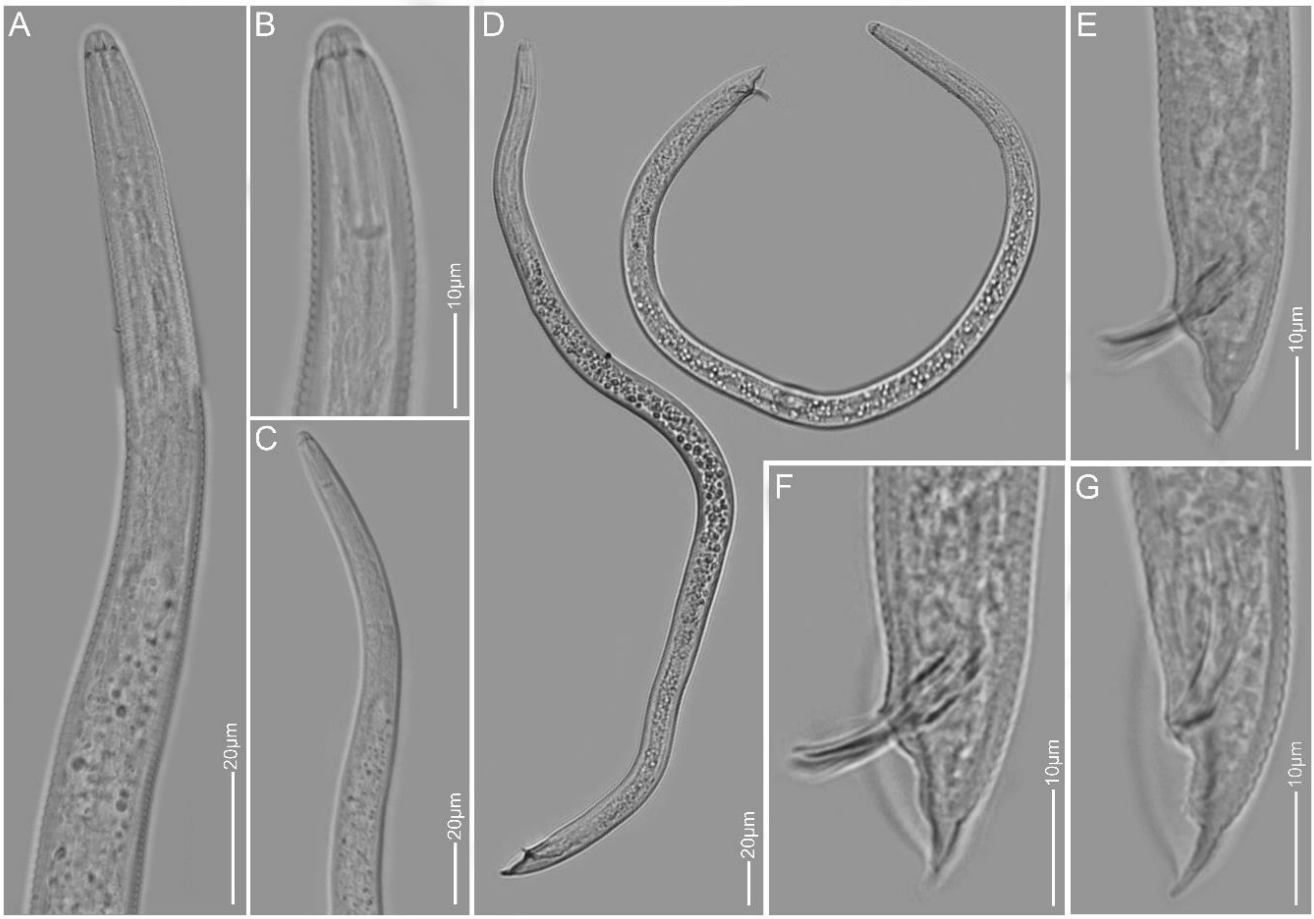

Fig. 8. Light microscopy of male *Helicotylenchus multicinctus* from Ethiopia. A-C: Anterior body region (A: Ventral overlap; B: Head region and stylet; C: Excretory pore); D: Entire body; E-G: Tail region showing spicule, bursa and tail terminus.

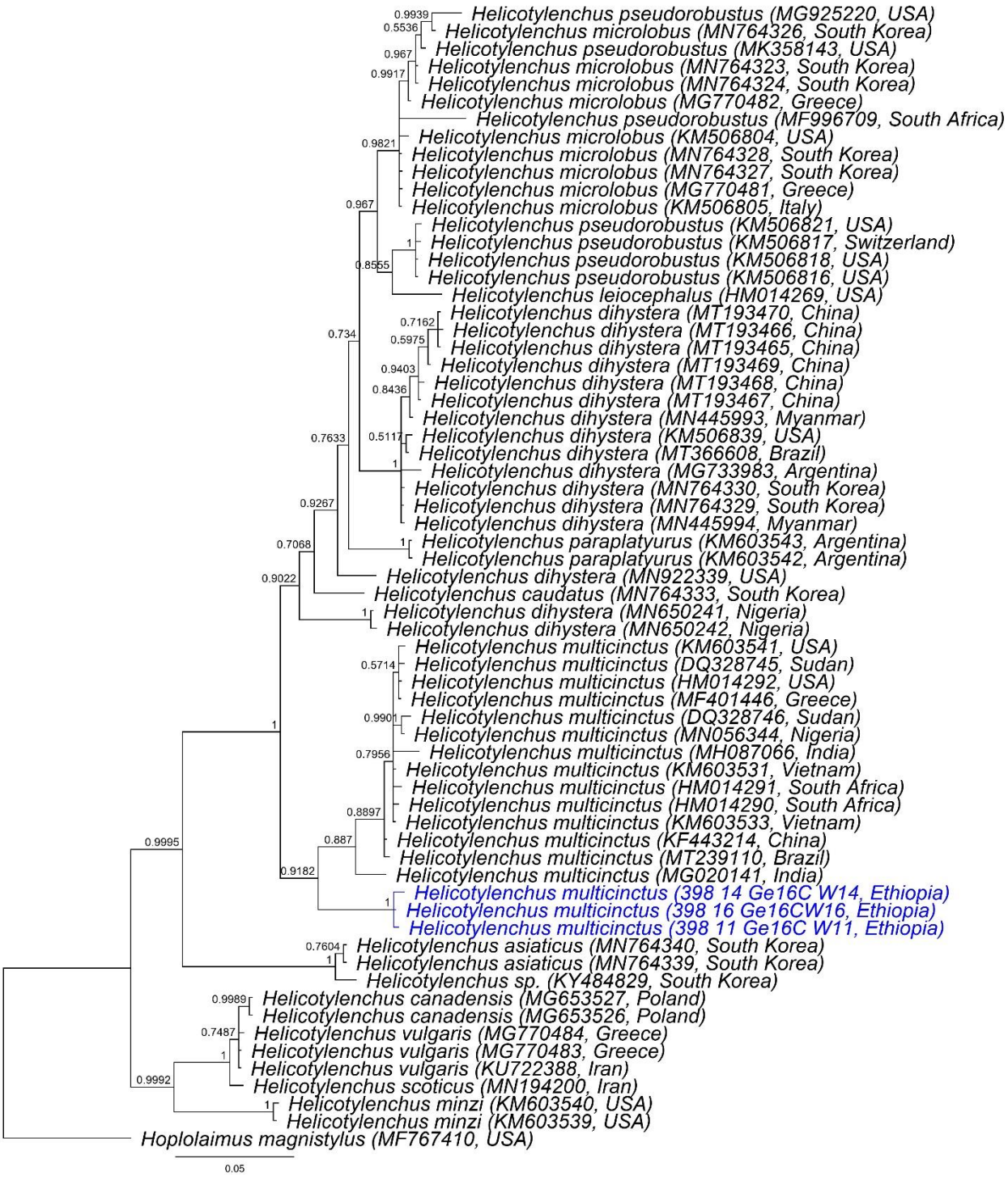

Fig. 9. Phylogenetic relationships of *H. multicinctus* from Ethiopia and other *Helicotylenchus* spp. Bayesian 50% majority rule consensus tree as inferred from the analysis of D2-D3 of 28S rDNA partial sequences under GTR+I+G model. Posterior probabilities of more than 50% are given for appropriate clades. The present sequences are shown in blue.

Table 5. Morphometric data of *Helicotylenchus multicinctus* associated with coffee from Ethiopia and comparison to other populations of *H. multicinctus*. All measurements are in µm and the form of mean ± SD (range). (data from soil sampling site Ge16).

Characters	Present study (Ethiopia)		(Cobb,1893) Golden, 1956	(Marais, 2001)	(Tzortzakakis et al., 2017)	(Marais et al., 2005)
	Female	Male	(Topo type after Sher, 1966)	South Africa	Greece	Ethiopia
n	10	6	3	46	4	2
L	506 ± 32.1 (463 - 573)	469 ± 36.2 (412 - 508)	470 - 530	547 ± 45.0 (476 - 654)	570 ± 65.4 (508 - 640)	567 - 584
a	26.6 ± 2.6 (22.8 - 31.0)	29.4 ± 3.2 (23.1 - 31.4)	24 - 30	25.5 ± 3.4 (17.7 - 32.5)	21.5 ± 1.8 (19.2 - 23.5)	27.4 - 29
b	4.1 ± 0.5 (3.3 - 4.7)	3.8 ± 0.3 (3.3 - 4.2)	4.7 - 5.4	5.0 ± 0.4 (4.3 - 5.3)	-	-
C	41.6 ± 4.1 (36.6 - 49.8)	33.3±4.1 (29.4 - 40.3)	35 - 46	45.9 ± 6.6 (33.8 - 58.5)	51.7 ± 4.0 (46.2 - 55.5)	46.9 - 57
c'	1.1 ± 0.1 (1.0 - 1.2)	1.3 ± 0.2 (1.1 - 1.5)	0.8 - 1.0	0.9 ± 0.2 (0.5 - 1.2)	1.0 ± 0.1 (0.9 - 1.1)	0.8 - 0.9
V%	62.4 ± 2.8 (59.2 - 67.8)	-	65 - 69	68 ± 3.1 (63 - 75)	65 ± 1.5 (64 - 67)	68 - 69
m%	50.8 ± 2.9 (45.5 - 56.3)	51.2 ± 3.8 (46.3 - 54.3)	-	49 ± 2.4 (44 - 54)		49
Lip region height	3.4 ± 0.4 (2.8 - 4.2)	3.3 ± 0.2 (3.0 - 3.5)	-	-	-	4
Lip region width	4.8 ± 0.5 (3.8 - 5.3)	4.7 ± 0.3 (4.2 - 5.0)	-	-	-	9
Number of lip annuli	4	4	-	-	-	
Stylet length	23.2 ± 1.1 (21.0 - 24.7)	20.6 ± 0.7 (20.0 - 21.6)	22 - 24	25 ± 1.9 (21 - 30)	22.3 ± 1.0 (21.0 - 23.0)	26 - 27
Stylet cone length	11.8 ± 1.0 (10.0 - 13.0)	10.6 ± 0.7 (10.0 - 11.4)	-	-	-	-
Stylet knob height	2.1 ± 0.2 (1.8 - 2.5)	2.0 ± 0.0 (2.0 - 2.0)	-	-	-	3
Stylet knob width	4.2 ± 0.4 (3.4 - 4.6)	3.5 ± 0.3 (3.0 - 3.8)	-	-	-	5
DGO	-	-	-	9 ± 1.5 (7 - 12)	8.8 ± 0.6 (8.0 - 9.5)	-
Median bulb length	10.1 ± 1.4 (8.0 - 11.7)	8.9 ± 1.0 (8.0 - 10)	-	-	-	11.0 -14.0
Median bulb width	7.4 ± 0.8	6.1 ± 0.1	-	-	-	9

	(6.2 - 8.3)	(6.0 - 6.2)				
Anterior to excretory pore	86.0 ± 4.1 (79.0 - 93.0)	82.7 ± 3.3 (79.6 - 86.0)	-	-	89.3 ± 4.8 (84.0 - 95.0)	-
Body width at excretory pore	16.1 ± 1.2 (14.6 - 18.4)	14.0 ± 1.0 (13.0 - 15.0)	-	-	-	19
Pharynx length	125 ± 16.8 (103 - 156)	124 ± 19.6 (99.0 - 151)	-	-	139 ± 10.0 (129 - 152)	-
Anterior to vulva	315 ± 26.7 (286 - 377)		-	-	-	-
Body width at vulva	19.8 ± 1.2 (18.0 - 21.9)		-	-	-	-
Body width at mid body	19.2 ± 1.7 (16.0 - 21.0)	16.1±1.2 (14.1 - 17.8)	-	-	-	20.0 - 21.0
Body width at anus	11.5 ± 0.3 (11.1 - 12.2)	10.7 ± 0.5 (10.0 - 11.1)	-	-	-	13.0 - 14.0
Number of lateral lines	4.0	4.0	-	-	-	-
Lateral field width	4.5 ± 0.6 (3.9 - 5.9)	-	-	-	-	5
Position of phasmid to anus	4 ± 1.5 (2 - 6)	-	-	-	-	-
Tail length	12.4 ± 1.1 (11.0 - 14.0)	14.2 ± 1.7 (12.0 - 16.9)	-	-	11.0 ± 0.8 (10.0 - 12.0)	(10 - 12)
Number of tail annuli	6.0 ± 1.3 (5.0 - 8.0)	-	-	-	8.0 ± 1.4 (7.0 - 10.0)	10
Tail hyaline region length	2.8 ± 0.2 (2.5 - 3.2)	-	-	-	-	-
Spicule length	-	19.6 ± 0.6 (19.0 - 20.4)	-	-	-	-
Gubernaculum	-	7.8 ± 0.7 (7.0 - 8.3)	-	-	-	-

***Meloidodera* sp.**

(Figs 10, 11)

MORPHOLOGICAL CHARACTERISATION

MEASUREMENTS

See Table 6.

DESCRIPTION

Second stage juvenile

Body tapering posteriorly and ventrally curved after heat-relaxed. Head region slightly setoff, having 7.7 ± 0.4 µm (6.6 - 8.8 µm) width and 4.9 ± 0.5 µm (4 - 5.8 µm) offset with three lip annuli. The labial framework was well developed and sclerotized. The lateral lips rounded, the labial disc oval in shape, the dorsal and ventral submedian lips elongated, the lateral and submedian lips separated. Stylet robust; having well developed and slightly anteriorly directed round knobs. The DGO behind the base of stylet knob about 5.9 ± 0.8 µm (4.9 - 7.1 µm). Median bulb ovoid, about 9.8 ± 1 µm (8-12.9 µm) in diameter and 11.4 ± 0.9 µm (9 - 13 µm) height containing bead-shaped valve plates. Hemizonid distinct and situated immediately anterior of excretory pore. Lateral field with four incisures indistinct areolation and reduced to three incisures to the most anterior body region and the posterior around the phasmid. Excretory pore located about 102 µm from anterior end. Tail conoid, gradually tapering with narrow round terminus. Hyaline part occupying about 52.8% of the tail length, Phasmid lens-like located three to four annuli anterior to the anus.

Female

Currently not found, research going on.

Male

Not found.

MOLECULAR CHARACTERISATION

D2-D3 of 28S rDNA

Five D2-D3 of 28S rDNA sequence were obtained with the length ranging 503 to 610 bp varied from 0.3 - 8.5% (2 - 45 bp) among each other. The resulting alignment of 692 bp involved 60 sequences including *H. martini* as an out-group. The present sequences of *Meloidodera* sp. formed a maximally supported clade (PP = 1) with *M. floridensis* and *M. mexicana* from USA and *M. astonei* from Mexico (Fig. 12). However, the position of some species of *Meloidodera* is not monophyletic in the phylogenetic tree; such as *M. alni* (DQ328706) from Belgium and *M. sikhotealiniensis* (MF425677) from Russia are clustered with *Cryphodera* spp. (all from Vietnam) (Fig. 12) in the maximally supported sister clade. Our *Meloidodera* sp. sequences found to be similar respectively about 77 - 85%, 78 - 86% and 78 - 86% differing by (82 - 125 bp, 77 - 118 bp

and 78 - 118 bp) with the sequences of *M. floridensis* (MF425684), *M. astonei* (MF425679) and *M. mexicana* (MF425680 - MF425683).

18S rDNA

Four sequences of 18S rDNA were obtained with the length ranging 818 to 834 bp varied from 0.06 - 0.21% (1 - 3 bp) among each other. The resulting alignment of 992 bp involved 30 sequences including one out-group. In the Bayesian inference phylogenetic tree (Fig. 13), the present sequences of *Meloidodera* sp. formed a maximally supported monophyletic clade (PP = 1) with *C. brinkmani* (Italy), *C. sinensis* (China) and two unidentified *Cryphodera* spp. (Thailand and Japan). The sequences of our *Meloidodera* sp. showed similarities respectively about 94%, 93 - 94%, and 93% differing by about (49 bp, 48 -54 bp and 52 bp) with the sequences of *C. brinkmani* (JQ965679), *Cryphodera* sp. (MK033149 and MK354241) and *C. sinensis* (JX566453).

COI mtDNA

Nine partial *COI* mtDNA sequences ranging from 322 - 437 bp were produced varied from 0 - 4.36% (0 - 27 bp) among each other. The multiple sequence alignment resulted in 453 bp involved 48 sequences including one out-group of *H. pseudorobustus*. In the Bayesian inference phylogenetic tree, the present *Meloidodera* sp. formed a well-supported clade (PP = 95) with *M. astonei* (Mexico), *M. Mexicana*, *M. floridensis* and *Meloidodera* sp. (USA) and *M. sikhotealiniensis* (Russia and Germany) (Fig. 14). However, without internal resolution. The multiple sequence alignment showed a similarity of 85 - 89%, 84 -89% and 80 - 87% differing by (20 - 46 bp, 20 - 48 bp and 26 - 73 bp) with the sequences of *M. astonei* (MF425727), *M. Mexicana* (MF425726) and *C. sinensis* (MF425737 - MF425741), respectively.

REMARKS

The genus *Meloidodera* was first described by Chitwood et al. (1956) in the USA as *Meloidodera floridensis* from *Pinus elliottii* Englm. Subsequently, species of this genus had been reported in Mexico (Cid Del Prado Vera, 1991; Cid Del Prado Vera & Rowe, 2000), Belgium (Subbotin et al., 2006), USA, Russia and Germany (Subbotin et al., 2017; Powers et al., 2019). The most closely related genus *Cryphodera* was first described by Colbran (1966) in Australia as *Cryphodera eucalypti* from *Eucalyptus major* (Maiden) Blakely. Followed, species of this genus had been described in New Zealand and Australia (Wouts, 1973), in the USA (Baldwin et al., 1983), India (Wouts, 1973; Bajaj et al., 1989), Japan (Wouts, 1973; Karssen & Van Aelst, 1999), S. Korea (Kang et al., 2019) and China (Zhuo et al., 2013; Gu et al., 2020). It has been also reported from Italy (Vovlas et al., 2013) and Vietnam (Nguyen et al., 2011). The finding of *Meloidodera* is the first report from coffee and the first record for Ethiopia.

The present second-stage juveniles are different from *Cryphodera* but they are similar to *Meloidodera* because they can be distinguished from all described *Cryphodera* species by having the number of lateral lines (4 vs 3) and the shorter range of tail length (41 - 51 vs 27 - 54, 52 - 68, 60 - 77, 52 - 65 and 61 - 77 µm) compared with *C. kalesari, C. japonicum, C. gayae, C. sinensis* and *C. brinkmani*, respectively (Table 6 and 7). The four lateral lines are reported to be useful in identifying juveniles of *Meloidodera* from the closer *Cryphodera* (Luc et al., 1988; Cid Del Prado

Vera, 1991; Cid Del Prado Vera & Rowe, 2000; Sturhan, 2010; Subbotin et al., 2017). In the re-examination of second-stage juveniles of *Cryphodera* spp. by Sturhan (2010), the six known *Cryphodera* spp. have been characterized by the presence of three lateral lines. The present *Meloidodera* differs from *C. eucalypti* by the body shape (ventrally curved vs straight) and the longer range of body length (364 - 470 vs 379 - 461 µm). It can be also differed from both *C. sinensis* and *C. brinkmani* by the shape of the tail terminus (narrow round vs pointed) and respectively by the shorter body length (364 - 470 vs 388 - 474 and 450 - 541 µm) (Karssen & Van Aelst, 1999; Zhuo et al., 2013).

Morphologically, the present *Meloidodera* correspond well with the descriptions of *Meloidodera* by Chitwood et al. (1956), Hooper (1960), Cid del Prodo Vera (1991), Cid del Prodo Vera & Rowe (2000) based on the slightly offset lip region, the presence of four lateral lines, the round stylet knobs, the oval median bulb, the conoid tail shape and the round tail terminus. However, some morphometric differences were observed between the present specimens and the previously studied *Meloidodera* species. It differs from both *M. floridensis* and *M. charis* respectively by the shorter range of body length (364 - 470 vs 500 - 559 and 406 - 508 µm). And, can differ from *M. mexicana* and *M. astonei* respectively by the longer body length (364 - 470 vs 202 - 404 and 292 - 344 µm) and the longer stylet length (26.1 - 30 vs 19 - 24.5 and 13 - 25 µm) (Cid Del Prado Vera, 1991; Cid Del Prado Vera & Rowe, 2000).

The genus *Meloidodera* can be differentiated by the shorter tail length respectively (< 36 µm vs 29 - 42, 76 - 110, 77 - 92, 50 - 86 and 44 - 61 µm) compared with all other taxa of *Atalodera, Ekphymatodera, Hylonema, Rhizonema* and *Sarisodera*; and the shorter body length compared with *Ekphymatodera, Hylonema* and *Rhizonema* respectively (500 - 559, 560 - 750, 600 - 730 and 410 - 770 µm) (Wouts & Sher, 1971; Luc et al., 1978; Cid del Prado Vera et al., 1983; Baldwin et al., 1989).

The present *Meloidodera* can be differentiated by having the shorter body length (364 - 470 vs 450 - 555, 560 - 750, 508 – 597, 600 - 730, 410 - 770 and 466 - 558 µm) compared with *Atalodera, Ekphymatodera, Bellodera, Hylonema, Rhizonema* and *Sarisodera,* respectively; but longer body length than *Camelodera* (364 - 470 vs 340 - 430 µm). Can be distinguished by the shorter tail length (41 - 51 vs 76 - 110, 77 - 92, 50 - 86 and 44 - 61 µm) compared with *Ekphymatodera, Hylonema, Rhizonema* and *Sarisodera* but longer tail length than *Atalodera* (41 - 51 vs 29 - 42 µm) (Table 8).

The *COI* mtDNA tree topology showed a change of sequence position of the present *Meloidodera* sp.; the position of *Meloidodera* in the *COI* mtDNA phylogenetic trees was not resolved. Unfortunately, there is no 18S rDNA partial sequence data available in the GenBank for *Meloidodera* sp. which is most likely the reason why our sequences are sister to *Cryphodera* spp. To the best of our knowledge, in the present study, we provided the first sequences of *Meloidodera* sp. for the partial D2-D3 of 28S rDNA, *COI* mtDNA and 18S rDNA.

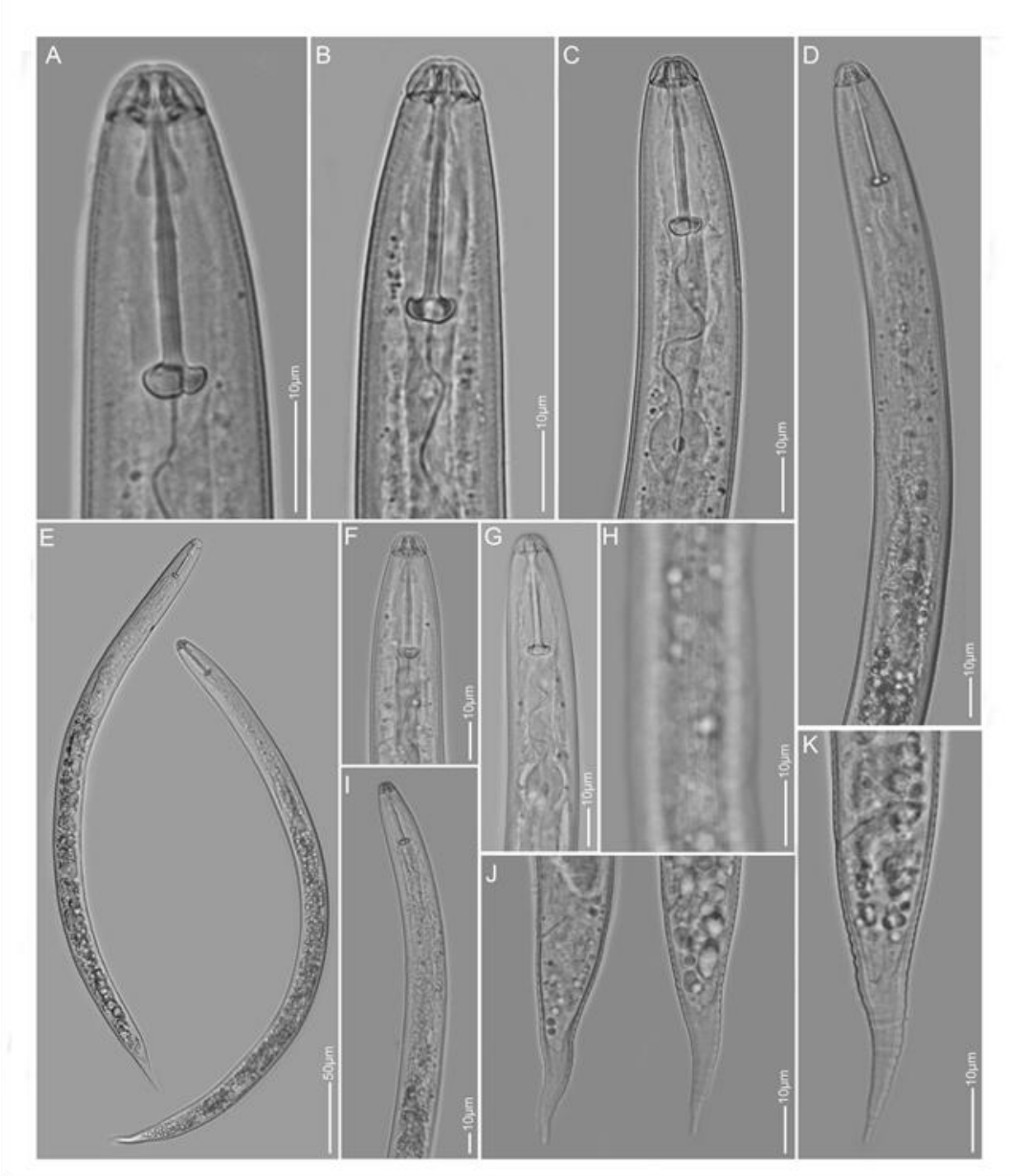

Fig. 10. Light microscopy of second-stage juvenile of *Meloidodera* sp. from Ethiopia. A, B, C, F, G: Anterior region (A, B, F: Head region, stylet and DGO; C, G: Median bulb); E: Entire body; H: Lateral line; D, I: Gland overlap and excretory pore; J-K: Tail region showing anus, hyaline region and tail terminus.

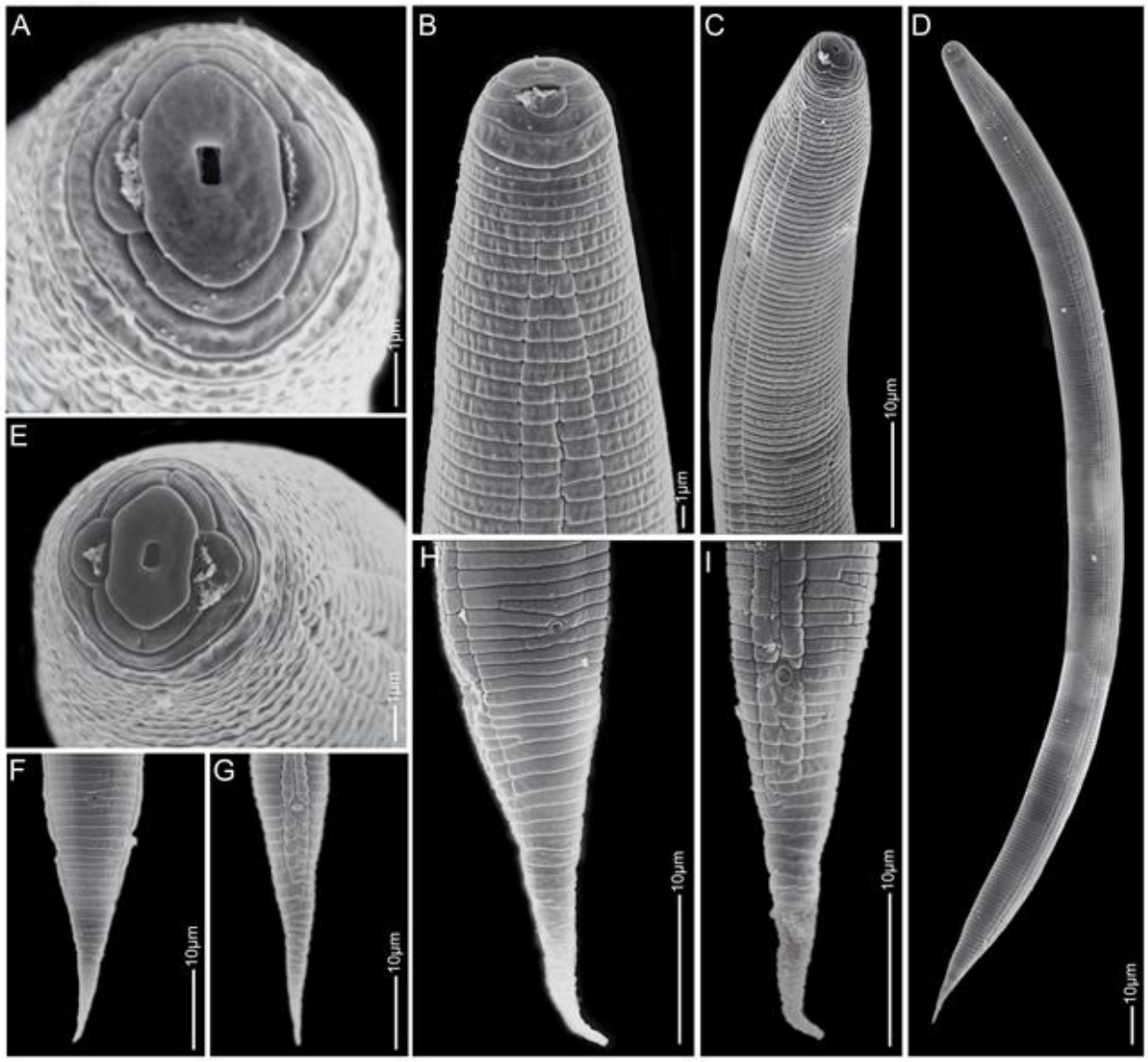

Fig. 11. Scanning electron microscopy of second-stage juvenile of *Meloidodera* sp. from Ethiopia. A, B, C, E: Anterior region (A, E: Basic pattern of cephalic region showing face view; B, C: Lateral view showing amphid openings); D: Entire body; F-G: Tail and anus; H-I: Phasmid and tail terminus.

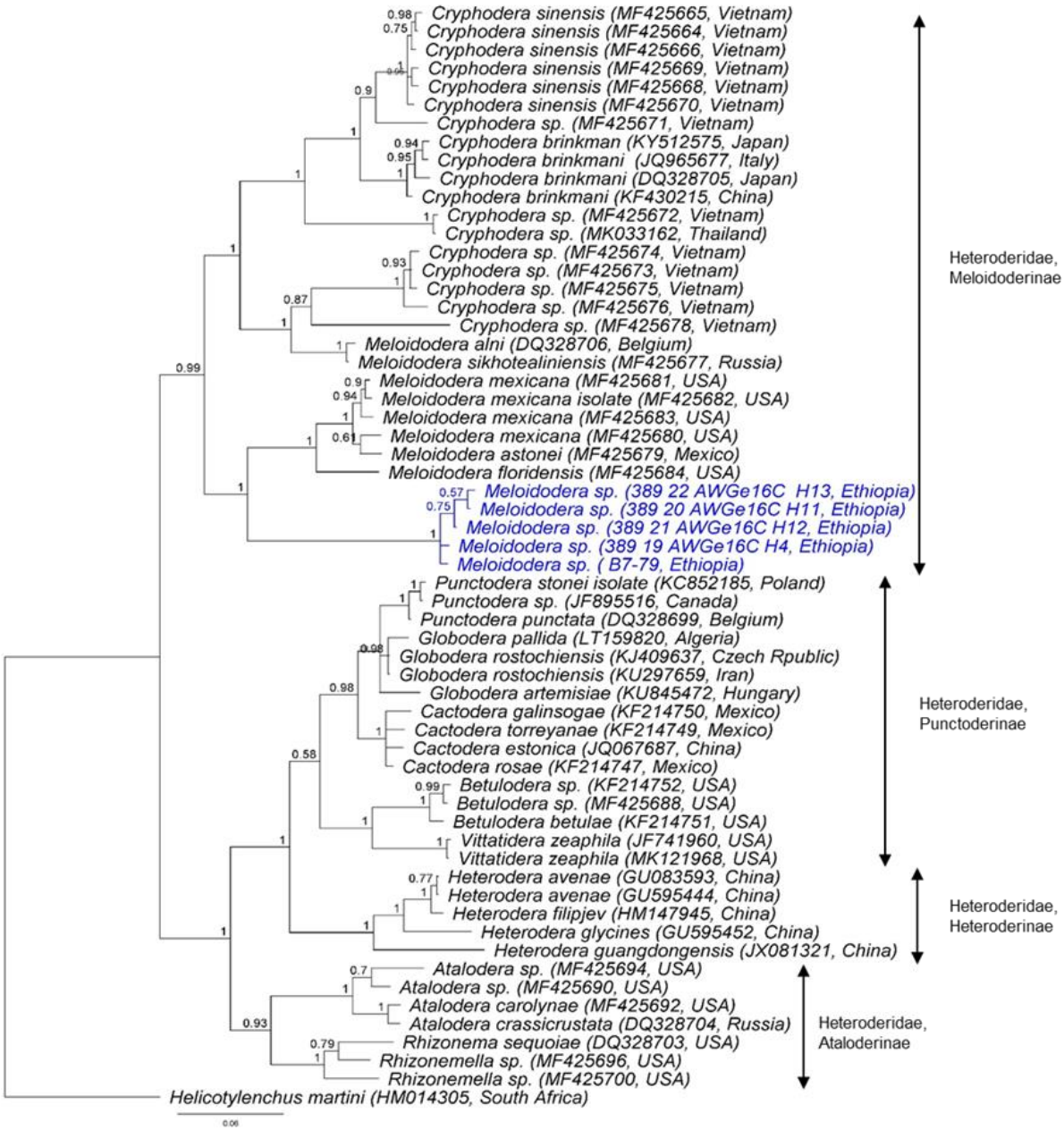

Fig. 12. Phylogenetic relationships of *Meloidodera* sp. from Ethiopia and other Heteroderidae spp. Bayesian 50% majority rule consensus tree as inferred from the analysis of D2-D3 of 28S rDNA gene under GTR+I+G model. Posterior probabilities of more than 50% are given for appropriate clades. The present sequences are shown in blue.

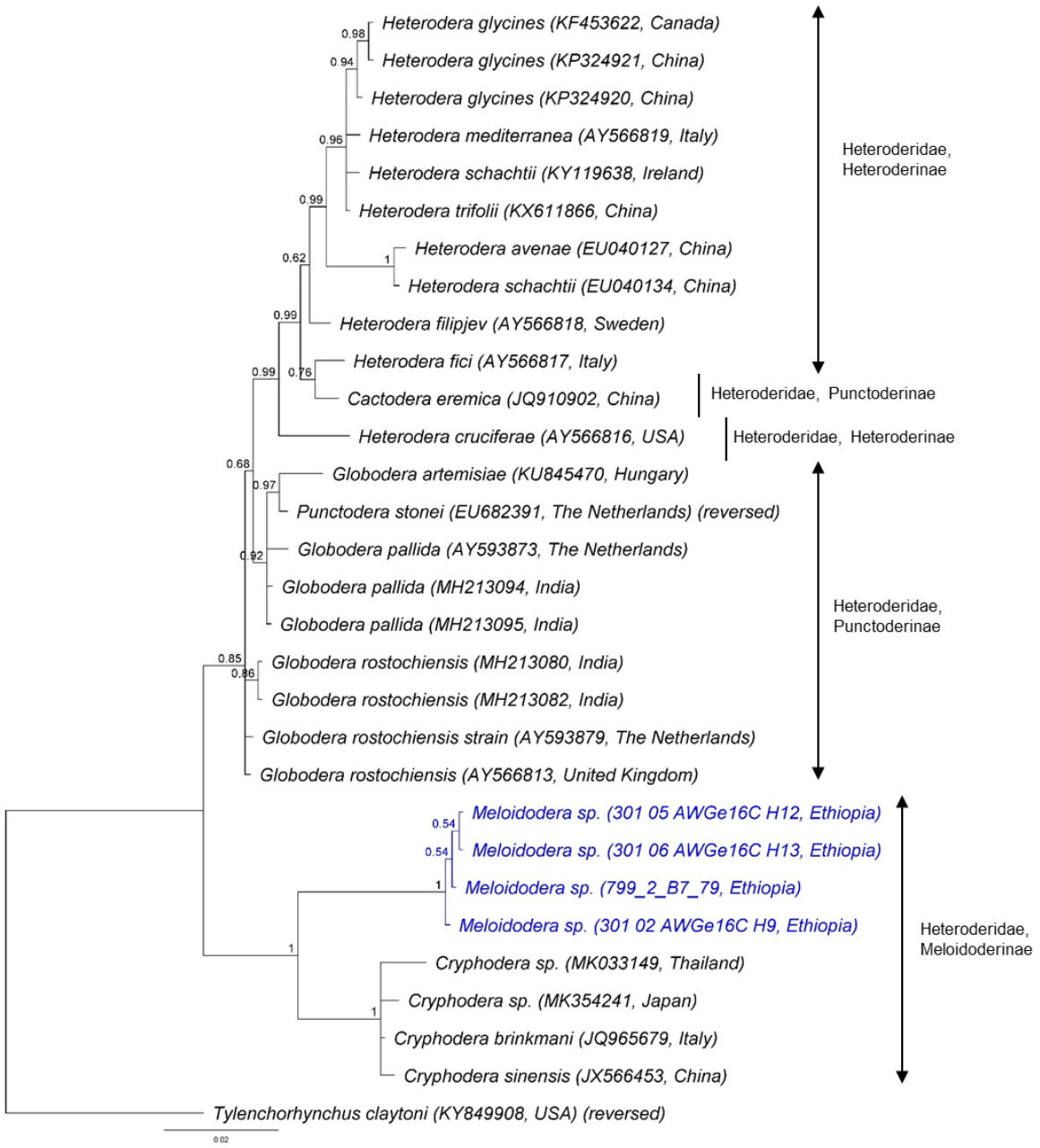

Fig. 13. Phylogenetic relationships of *Meloidodera* sp. from Ethiopia and other *Heteroderidae* spp. Bayesian 50% majority rule consensus tree as inferred from the analysis of partial 18S rDNA sequences under GTR+I+G model. Posterior probabilities of more than 50% are given for appropriate clades. The present sequences are shown in blue.

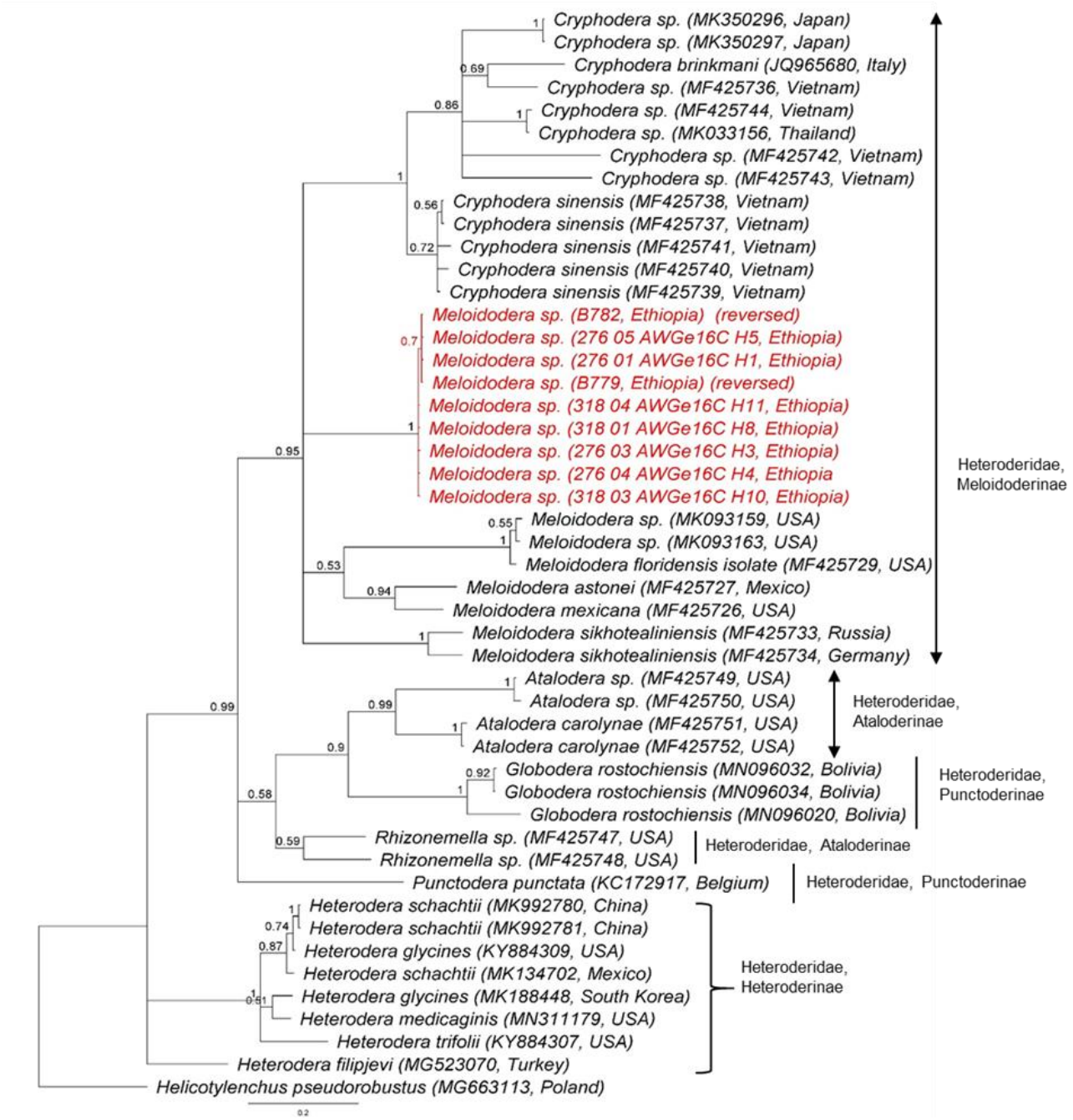

Fig. 14. Phylogenetic relationships of *Meloidodera* sp. from Ethiopia and other *Heteroderidae* spp. Bayesian 50% majority rule consensus tree as inferred from the analysis of partial *COI* mtDNA gene under GTR+I+G model. Posterior probabilities of more than 50% are given for appropriate clades. The present sequences are shown in red.

Table 6. Morphometric data of second stage juvenile of *Meloidodera* sp. associated with coffee from Ethiopia and comparison with other populations of *Meloidodera* spp. and *Cryphodera* spp. All measurements are in µm and the form of mean ± SD (range). (data from soil sampling site Ge16).

Characters	Present study	*M. floridensis* (Chitwood, Hannon & Esser, 1956) (Type specimen)	*M. charis*. Hooper (1960)	*M. mexicana* n.sp. (Cid del Prodo Vera, 1991)	*M. astonei*. (Cid del Prodo Vera & Rowe, 2000)	*C. eucalypti* (Colbran, 1966) (Type specimen)	*C. sinensis* (Zhuo et al., 2013)	*C. brinkmani* (Karssen & Van Aelst, 1999)
	Ethiopia	USA	USA	Mexico	Mexico	Australia	China	Japan
n	30	-	-	18	12	10	30	30
L	406 ± 27.8 (364 - 470)	500 - 559	460 (406 - 508)	330 ± 71.8 (202 - 404)	321 ± 13.9 (292 - 344)	379 - 461	435 ± 25.4 (388 - 474)	493 ± 23 (450 - 541)
a	21.4±1.7 (17.6-26.3)	26 - 29	23 (20.3 - 26.4)	19.6±1.3 (12.3 - 23.7)	20 ± 0.88 (17.4 - 21.6)	22.0 - 26.0-	22.6 ± 1.5 (19.4 - 25.9)	23.2 ± 1.3 (20.3 - 24.6)
b	3 ± 0.2 (2.6 - 3.4)	6.4 - 7.1	2.7 (2.5 - 3)	4 ± 0.24 (3.2 - 4.8)	3.4 ± 0.08 (3.2-3.6)	3.5 - 4.8-	3.9 ± 0.2 (3.7 - 4.4)	-
c	9.2 ± 0.6 (7.8-10.4)	8.4 - 10	9.9 (8.6 - 11.5)	10.2±0.45 (8.8 - 11.7)	8.6 ± 0.6 (6.6 -10.5)	7.9 - 9.8-	7.8 ± 0.5 (6.4 - 8.6)	7.1 ± 0.4 (6.4 - 7.8)
c'	3.3 ± 0.2 (2.9-3.6)	-	-	2.8 ± 0.30 (1.4 - 3.4)	3.4 ± 0.33 (2.9 - 5.0);	-	4.1 ± 0.3 (3.6 - 4.7)	4.7 ± 0.2 (4.2 - 5.3)
Mid body width	19.1 ± 1.6 (17-23.8)	19 - 21	19.7 (18 - 20)	-	-	-	19.3 ± 0.6 (18.0 - 20.0)	21.3 ± 0.6 (20.2 - 22.1)
Stylet length	27.9 ± 1.1 (26.1-30)	27 - 29	24 - 27	22 ± 0.79 (19 - 24.5)	21 ± 2.1 (13 - 25)	26 – 35.9	29.1 ± 0.8 (28.0 - 31.0)	33.3 ± 1.0 (31.6 - 35.4)
Stylet knob width	5.8 ± 0.3 (5.4 – 6.1)	6 - 7	4.9	-	-	4.0 – 5.0	5.9 ± 0.4 (5.0 - 6.5)	7.2± 0.3 (7.0 - 7.6)
Stylet knob length	2.8 ± 0.3 (2.4 - 3.3)	2 - 2.5	2.5	-	-	-	2.3 ± 0.2 (2.0–2.9)	3.6 ± 0.3 (3.2 - 3.8)
Stylet cone	14.6 ± 0.8 (13.1-15.8)	-	-	-	-	-	14.2 ± 0.7 (13.0 - 16.0)	-
Lip region height	4.9 ± 0.5 (4 - 5.8)	5	5	-	-	-	4.3 ± 0.3 (4.0 - 5.0)	5.5 ± 0.5 (5.1 - 6.3)
Lip region width	7.7 ± 0.4 (6.6 - 8.8)	10 - 11	10	-	-	-	9.3 ± 0.3 (9.0 - 10.0)	10.4 ± 0.3 (10.1 - 10.7)
Number of lip annuli	3	4	4	3	4	3	3	4
DGO	5.9 ± 0.8	4 - 6	3 - 4	4.3 ± 0.37	4.3 ± 0.45	4.3 - 5.0-	5.4 ± 0.7	5.8 ± 0.6

	(4.9 - 7.1)			(3.2 - 6.0)	(3.2 - 6.0)		(4.5 - 6.5)	(4.4 - 7.0)
Pharynx length	134 ± 8.3 (121-155)	-	-	113 ± 5.0 (100 - 136)	122 ± 5.2 (107 - 136)	-	164 ± 9.8 (147 - 181)	-
Anterion to median bulb valve	63.5 ± 4.4 (54.2-74)	-	-	-	-	-	75.3 ± 4.8 (66.0 - 88.6)	79 ± 4.3 (74 - 89)
Median bulb Valve length	2.7 ± 0.1 (2.6-2.9)	-	-	-	-	-	-	3.5 ± 0.3 (3.2 - 3.8)
Median bulb Valve width	2.2 ± 0.1 (2.1-2.4)	-	-	-	-	-	-	2.9 ± 0.4 (2.5 - 3.2)
Median bulb length	11.4 ± 0.9 (9-13)	-	10.7 - 12.3	-	-	-	13.4 ± 1.1 (11.0 - 15.0)	-
Median bulb width	9.8 ± 1 (8-12.9)	-	11.5 - 14	-	-	-	9.8 ± 1.0 (7.5 - 12.0)	-
Anterior to excretory pore	102 ± 4.7 (95.6 - 112)	108 - 110	94 - 109	76.5 ± 3.6 (68.5 - 92)	83 ± 3.3 (76 - 89)	-	104 ± 5.9 (91.7 - 113.2)	106 ± 5.5 (97 - 117)
Number of lateral lines	4	(4)	4	4	4	3	3	3
Tail length	44.5 ± 2.7 (40.3-50.5)	-	43 - 48	33 ± 1.8 (27 - 39)	37.6 ± 2.7 (32.4 - 49.6)	-	56.0 ± 3.2 (52.0 - 65.0)	69.7 ± 4.4 (61.3 - 76.5)
Hyaline length	23.5 ± 2.1 (19.3 - 27.9)	(< 36)	-	14.5 ± 0.97 (11 - 19)	16.7 ± 1.9 (14 - 26)	(> 25)	28.3 ± 2.3 (24.5 - 35.0)	37.2 ± 3.6 (30.3 - 42.3)
h%	52.8 ± 3.5 (46.4-60.9)	-	-	-	-	-	-	-
Body width at anus	13.5 ± 0.8 (12.1-14.9)	15 - 16	-	-	-	-	13.8 ± 0.7 (12.5 - 15.2)	-
Shape of phasmid	lens-like	circular, pore-like or disc shape	pore-like	lens-like	lens-like	lens-like	lens-like	lens-like
Shape of tail	conoid	elongate conoid	elongate conoid	conoid	conoid	elongate conoid	conoid	elongate conoid
Tail terminus	narrow round	blunt	bluntly round	round	acute	bluntly round	pointed	long pointed

Data referred to hyaline length (< 36) and (> 25); number of lateral lines (4) were taken from Siddiqi (2000).

Table 7. Comparative morphology and morphometric of the Ethiopian juvenile *Meloidodera* sp. and other juveniles of *Cryphodera* spp.

Characters	*Meloidodera* sp. Present study	*C. kalesari* (Bajaj et al.,1989)	*C. japonicum* (Gu et al., 2020)	*C. gayae* (Kang et al.,2019)	*C. cox* (Wouts,1973)	*C. nothophagi* Wouts (1973)	*C. podocarpi* Wouts (1973)
Body length	364 - 470 µm	353 - 424 µm	506 - 588 µm	424 - 525 µm	457 µm	450 µm	525 µm
Head region	slightly setoff	-	slightly offset	offset	-	-	-
Body shape	ventrally curved	almost straight	ventrally curved	straight	-	-	-
Stylet length	26 - 30 µm	24 - 29 µm	29 - 35 µm	27 - 31 µm	35 µm	34 µm	39 µm
Stylet knobs	round	concave	anterior flat	flat	pointing anteriorly	flat	pointing anteriorly
DGO	5 - 7 µm	3 - 4 µm	5 -7 µm	-	6 µm	6 µm	6 µm
Lateral lines	4	3	3	-	3	3	3
Tail length	41 - 51 µm	27-54 µm	52 - 68 µm	60 - 77 µm	54 µm	56 µm	69 µm
Tail shape	conoid	elongate-conoid	conoid	long conoid	-	-	-
Hyaline portion	20 - 28 µm	18 - 26 µm	half of tail length	32 - 48 µm	31 µm	30 µm	36 µm
Tail terminus	narrow round	-	rounded	sharply pointing	narrow rounded	narrow rounded	narrow rounded
Phasmid	lens-like	lens-like	lens-like	-	-	-	-
Phasmid location	anterior to anus	posterior to anus	posterior to anus	-	-	-	-

Data for *C. cox* Wouts (1973), *C. nothophagi* Wouts (1973) and *C. podocarpi* Wouts (1973) were taken from Kang et al. (2019)

Table 8. Comparative morphology and morphometric of the Ethiopian juvenile *Meloidodera* sp. and other juveniles of cystoid nematodes.

Characters	*Meloidodera* sp. Present study	*Atalodera* (Wouts & Sher., 1971)	*Camelodera* (Krall et al., 1988)	*Ekphymatodera* (Baldwin et al., 1989)	*Bellodera* (Wouts., 1985; Baldwin et al., 1983)	*Hylonema* (Luc et al., 1978)	*Rhizonema* (Cid del Prado Vera et al., 1983)	*Sarisodera* (Wouts & Sher, 1971)
Body length	364 - 470 µm	450 - 555 µm	340 - 430 µm	560 - 750 µm	508 - 597 µm	600 - 730 µm	410 - 770 µm	466 - 558 µm
Lip annuli	3 annuli	4 annuli	3 annuli	-	5	-	4 annuli	4 annuli
Stylet length	26 - 30 µm	23 - 25 µm	18 - 19 µm	28 - 32 µm	35 - 41 µm	33 - 40 µm	27 - 39 µm	39 - 43 µm
Stylet knobs	round	round	-	anchor shape	-	anchor shape	-	-
Lateral lines	4	3	4	4	4	3	4	4
Tail shape	conoid	-	conoid	-	conoid	elongate, conoid	conoid	conoid
Tail length	41 - 51 µm	29 - 42 µm	-	76 - 110 µm	40 - 51	77 - 92 µm	50 - 86 µm	44 - 61 µm
Phasmid	lens-like	lens like	pore-like	pore-like	lens-like	punctiform	lens-like	lens-like
Tail terminus	narrow round	-	large round	-	-	pointed	pointed	-
Phasmid location	anterior to anus	-	-	-	-	posterior to anus	posterior to anus	-
Hyaline portion	slightly above half tail	15 - 24 µm	< 1/3 of tail length	elongated 60 - 103 µm	about half of tail	very long 55 - 88 µm	30 - 52 µm	26 - 36 µm

Data for *Camelodera* (Krall et al., 1988) and *Bellodera* (Wouts, 1985) (tail details) were taken from Siddiqi (2000)

Tylenchorhynchus cfr. *zeae* Sethi & Swarup, 1968

(Figs 15, 16)

MORPHOLOGICAL CHARACTERISATION

MEASUREMENTS

See Table 9.

DESCRIPTION

Female

Body cylindrical, ventrally arcuate upon fixation. Head round and slightly offset, 5.8 ± 0.3 µm (5.2 - 6.4 µm) in diameter and 3.4 ± 0.3 µm (3.1 - 4 µm) height. Lip region contained three annules and the labial framework slightly sclerotized. Lateral line with four incisures occupying about ¼th body width having three delimiting bands and outer lines areolated. Stylet with rounded laterally directed knobs set-off from the shaft. DGO situated about 2.5 ± 0.2 µm posterior to the base of the spear knobs; excretory pore located anterior end of the basal bulb at about 89.2 µm from the head end, hemizonid located immediately anterior to the excretory pope. Median pharyngeal bulb relatively large and rounded, 8.6 - 10.7 µm in width and 10.5 - 13.5 µm length and well developed with distinct valve; basal bulb elongate pyriform shaped, offset from the intestine. Vulva with transverse slit, vulva protuberance absent and located about 56.7% of the total body length, vagina extending inward about 58 - 62% of corresponding body. Reproductive system didelphic, amphidelphic and ovaries outstretched; spermatheca relatively large and ovoid, either empty or filled with rounded sperm. Tail sub-cylindrical tapering regularly with 14 - 22 annules having relatively rounded smooth terminus. Post anal intestinal sac absent. Phasmid moderately circular located posterior to anus centred in between lateral lines in the middle of the anterior part of the tail.

Male

Found abundant and morphologically similar to the females except for sexual characters. Testis not seen. Spicules paired, 22.4 - 26.3 µm long and ventrally arcuate. Gubernaculum well-developed rod-shaped, top slightly curved and measures about half the length of the spicule, 12.1 - 14.8 µm long. Tail elongate conoid, about 32.1 - 48.1 µm long. Bursa enveloping complete tail. Tail terminus conoid and ventrally arcuate.

MOLECULAR CHARACTERISATION

D2-D3 of 28S rDNA

Two 100% identical D2-D3 of 28S rDNA partial sequences having 661 bp were obtained and the aligned sequence was 699 bp long contained 68 sequences from sixteen known and nine unknown *Tylenchorhynchus* spp. with one out-group. The Bayesian inference phylogenetic tree showed the sequences of the present *T.* cfr. *zeae* did not form a clade with *T. zeae* sequences (KM068058 and KJ461563 – KJ461566) of the Iranian and the Spanish population, but it formed

a highly supported clade with *T. annulatus, T. claytoni,* and three unidentified *Tylenchorhynchus* spp. with maximum support (PP = 99) (Fig 17). The sequence alignment result revealed respectively 93%, 92 - 93%, 90 - 91% and 90% sequence similarities differing by (46 bp, 46 - 56 bp, 54 - 59 and 62 bp) with *T. annulatus* (KJ475545 and KJ461532), *T. leviterminalis* (EU368591, KJ461550 and KJ475546 - KJ475548), *T. claytoni* (EU368589 and KJ461542 - KJ461542) and *T. zeae* (KM068058 and KJ461563 - KJ461566).

18S rDNA

Two 18S rDNA partial sequences of 590 bp and 780 bp were obtained varied from 0 - 0.8% (0 - 7 bp) among each other. Twenty-nine sequences were aligned from ten known *Tylenchorhynchus* spp and three known *Bitylenchus* spp. with one out-group taxon resulting in 757 bp. In the Bayesian phylogenetic tree (Fig.18), the present sequences of *T.* cfr. *zeae* were found in a well-supported clade (PP = 99) with the sequences of *T. claytoni, T. annulatus, T. agri, T. aduncus, T. leviterminalis, T. zeae* and *T. clarus.* However, our *T.* cfr. *zeae* sequences did not form a clade with the sequences of *T. zeae* from Spain (KJ461618 - KJ461619) and China (MG871204) (Fig.18). The sequence alignment showed similarities of 98.5%, 96 - 98%, and 97% differing by (11 bp, 11 -14 bp and 9 bp) with the sequences of *T. zeae* (KJ461618 - KJ461619 and MG871204), *T. claytoni* from the USA (EU368587 and KY849908) and *T. annulatus* from Japan (LC540651 and LC540653), respectively.

REMARKS

T. zeae was originally described from *Zea mays* in India (Sethi & Swarup, 1968). Subsequently, it was reported from olive and grapevine in Spain (Arias & Romero, 1979; Handoo et al., 2014), from *Z. mays,* cauliflower and cabbage in Taiwan and Gansu provinces of China (Chen et al., 2007; Xu et al., 2020) and from *Ziziphus zizyphus* in Iran (Alvani et al., 2017).

Morphological and morphometric data of the present specimen are coincident with the original description of *T.* zeae (Sethi & Swarup, 1968) and is also similar to the populations described by Chen et al. (2007), Handoo et al. (2014), Alvani et al. (2017) and Xu et al. (2020). However, some differences were noticed compared to the original description such as longer range of body length (487 - 731 vs 530 - 640 µm), fewer lip annuli (3 vs 4) and shorter mean tail length (34.1 vs 46 µm). In comparison to the specimen studied from Iran, the present specimen was characterised by the shorter range of body length (487 - 731 vs 541 - 752 µm) and slightly the shorter range of tail length (28.5 - 41.1 vs 30 - 47 µm). However, remarkably the longer range of body length (487 - 731 vs 458 - 556 µm) and slightly the longer range of tail length (28.5 - 41.1 vs 28.48 - 34.54 µm) compared to the population studied from China. To the best of our knowledge, the present specimen is the first report of *T.* cfr. *zeae* associated with coffee and from Ethiopia.

The present *T.* cfr. *zeae* is similar to *T. leviterminalis* and *T. annulatus* in the ventrally curved body shape, in the round median bulb and the smooth tail terminus. Also similar to *T. claytoni* in the above-mentioned characters except for the spheroid shape of the median bulb and the annulated tail terminus of *T. claytoni*. It can be differentiated from *T. leviterminalis* by its lip region (round and slightly offset vs hemispherical and continuous) and shape of the basal bulb (elongate pyriform

vs saccate) (Vovlas & Cheng, 1988; Takayuki & Yukio, 1991; Handoo et al., 2014; Pham et al., 2014; Mwamula et al., 2020). The present population can be differed from *T. claytoni* by the body shape (ventrally curved vs straight or only slightly ventrally curved) and the shorter mean body length (594 µm vs 697 µm) (Handoo, 2000; Khan et al., 2008; Geraert, 2011). Can be distinguished from *T. annulatus* by its lip region (round and slightly offset vs truncate and continuous) and male population (present vs absent) (Takayuki & Yukio, 1991; Handoo et al., 2014; Mwamula et al., 2020).

In D2-D3 of 28S rDNA and 18S rDNA, the present sequences of *T.* cfr. *zeae* showed close sequence similarity to *T. annulatus, T. claytoni* and *T. leviterminalis* and they are different from the sequences of *T. zeae* and did not form a monophyletic group with other *T zeae* sequences in the GeneBank disagreeing with the morphological description. However, it showed morphological resemblance most close to *T.* zeae than to *T. annulatus, T. leviterminalis* and *T. claytoni.* Concerning this disagreement, the presence of cryptic species has been suggested as the cause of the difference, and therefore the topotype molecular data to be used as an integrative reference for species delineation (Subbotin et al., 2015; Janssen et al., 2017; Palomares-Rius et al., 2018). Therefore, *T. zeae* could be possibly a cryptic species.

In the present study, we noticed the position of *T. zeae* in different places in the 18S rDNA tree and considerable sequence variations in D2-D3 of 28S rDNA between our *T.* cfr. *zeae*, and sequences of *T. zeae* deposited in the GenBank. However, it cannot be concluded that the sequences in the GenBank or the present sequences to be the exact representation of *T. zeae*. Therefore, sequence comparison is needed with the sequence of the type specimen of *T. zeae* from the type location. We also suggest there is a need to compare the slides of the present *T.* cfr. *zeae* and the type specimen slides of *T. zeae* and *T. annulatus* to make a comparison of important morphological features. Given the close morphological resemblance of the present *T.* cfr. *zeae* with the original description of *T. zeae* by Sethi & Swarup (1968) and report of Handoo et al. (2014), Alvani et al. (2017) and Mwamula et al. (2020), it is however highly likely the present specimen to be tentatively identified as *T.* cfr. *zeae*.

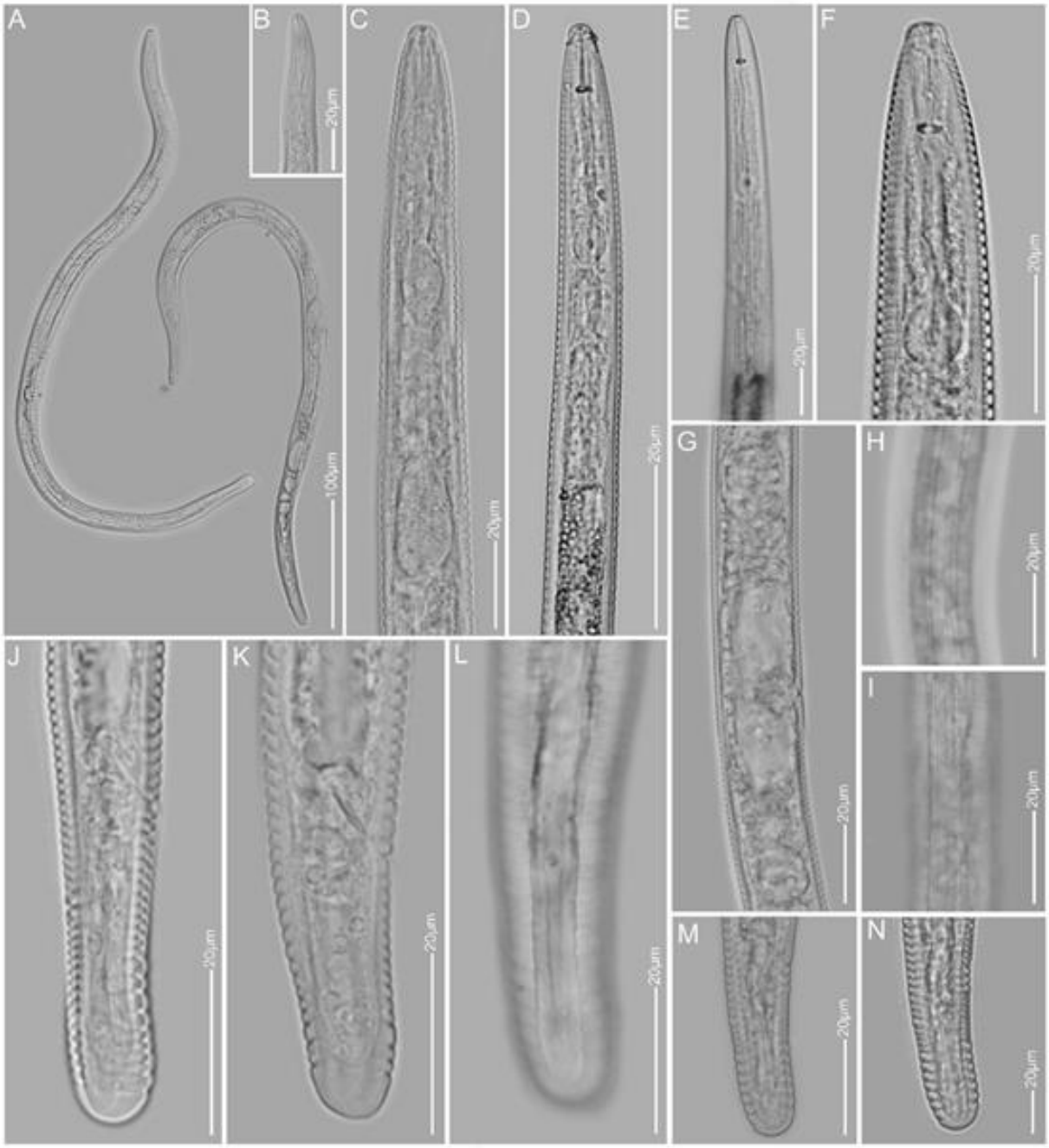

Fig. 15. Light microscopy of female *Tylenchorhynchus* cfr. *zeae* from Ethiopia. A: Entire body; B-F: Anterior region (B, F: Head region; C-E: Basal bulb, median bulb and stylet); G: Reproductive part showing vulva region and spermatheca; H-I: Lateral line; J-N: Posterior region (J-K: Anus and tail annules; M-N: Tail terminus; L: Phasmid.).

Fig. 16. Light microscopy of male *Tylenchorhynchus* cfr. *zeae* from Ethiopia A: Entire body; B-D: Anterior region (B: Median bulb and stylet; C-D: Head region); E: Lateral line; F-G: Tail region showing spicule, bursa and tail terminus.

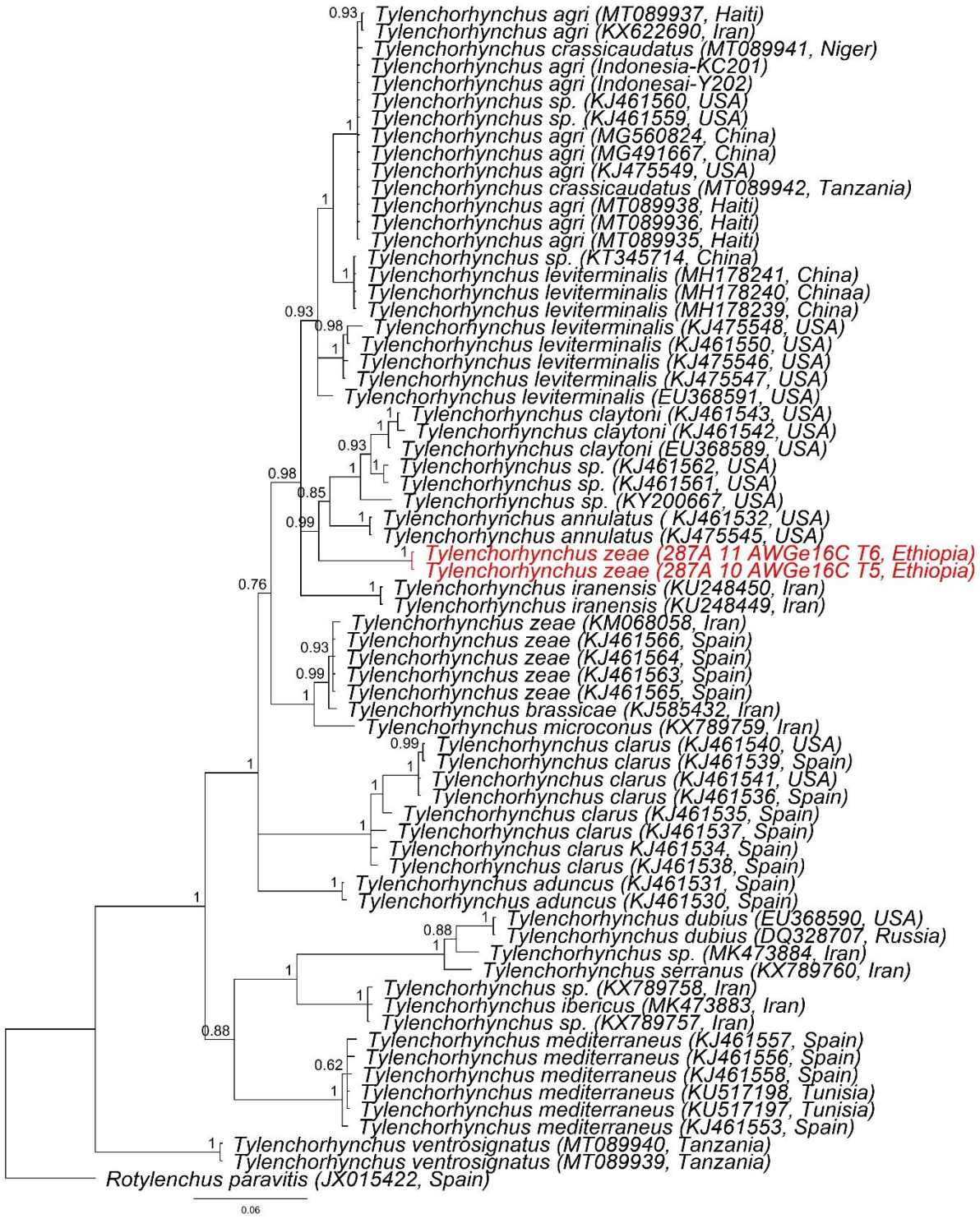

Fig. 17. Phylogenetic relationships of *Tylenchorhynchus* cfr. *zeae* from Ethiopia and other *Tylenchorhynchus* spp. Bayesian 50% majority rule consensus tree as inferred from the analysis of D2-D3 of 28S rDNA partial sequences under GTR+I+G model. Posterior probabilities of more than 60% are given for appropriate clades. The present sequences are shown in red.

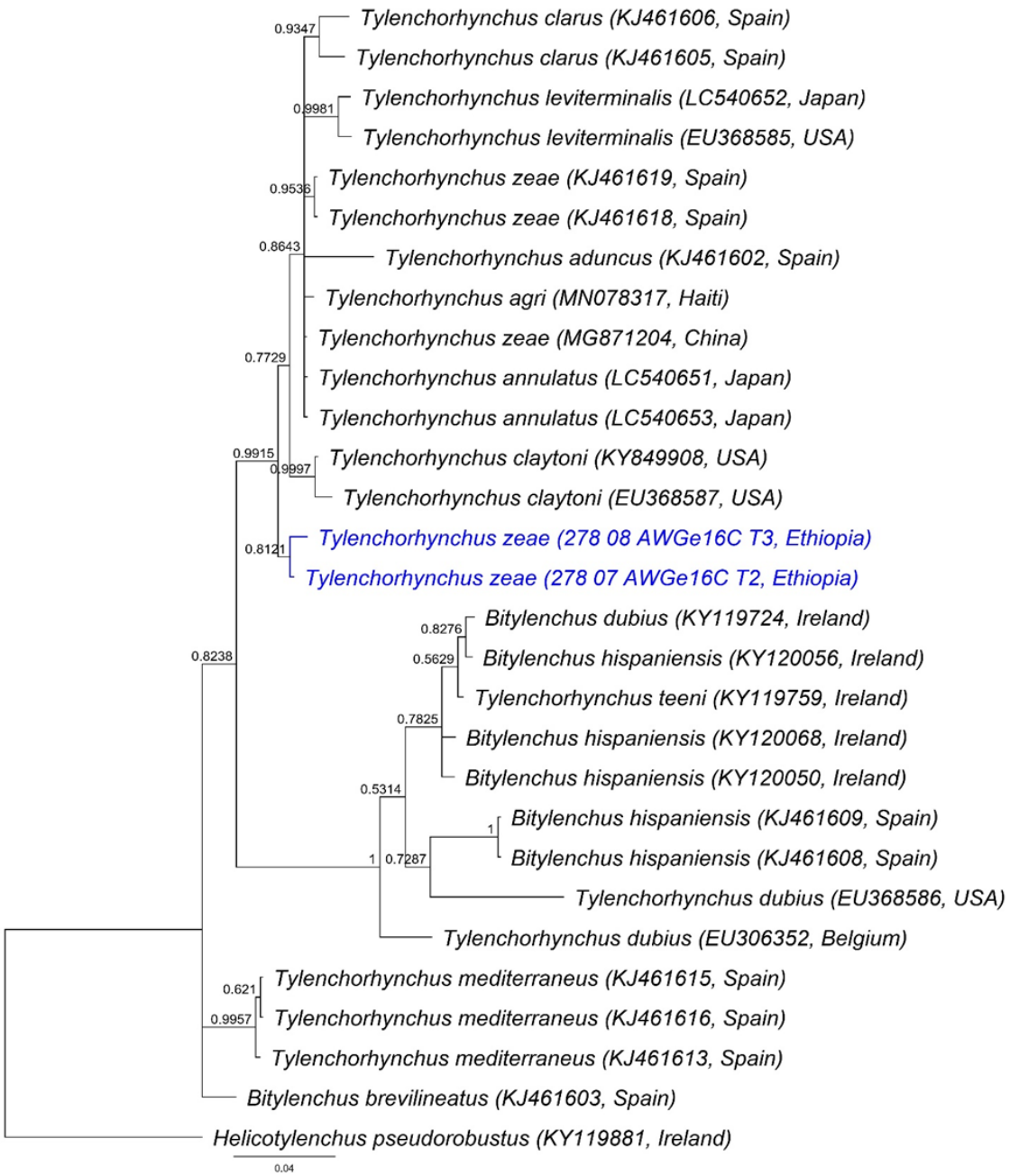

Fig. 18. Phylogenetic relationships of *Tylenchorhynchus* cfr. *zeae* from Ethiopia and other *Tylenchorhynchus* spp. Bayesian 50% majority rule consensus tree as inferred from the analysis of 18S rDNA partial sequences under GTR+I+G model. Posterior probabilities of more than 50% are given for appropriate clades. The present sequence is shown in blue.

Table 9. Morphometric data of *Tylenchorhynchus* cfr. *zeae* associated with coffee from Ethiopia and comparison to other populations of *T. zeae*. All measurements are in µm and the form of mean ± SD (range). (data from soil sampling site Ge16).

Morphological characters	Ethiopia Present study		India (Sethi & Swarup,1968)	Iran (Alvani et al., 2017)	China (Xu et al., 2020)
	Female	Male	Female	Female	Female
n	15	10		8	20
L	594 ± 73.6 (487-731)	610 ± 73.2 (511-742)	530-640	644 ± 72.8 (541-752)	493 ± 34.36 (458-556)
a	29.8 ± 2.9 (25.5-35.2)	30.7 ± 2 (27.5-33.5)	26 - 34	29.5 ± 1.8 (25.6-31.3)	25.98 ±2.1 (22.24-28.72)
b	4.9 ± 0.4 (4.2- 5.4)	5.1 ± 0.5 (4.3-4.8)	-	5.1 ± 0.5 (4.2-5.9)	4.81 ± 0.35 (4.23-5.5)
c	17.7 ± 2.1 (15.2-23.4)	15.6 ± 1.8 (12.9-19.6)	14-20	17.1 ± 2 (13.3-19.2)	15.76 ± 1.19 (13.46-17.59)
c'	2.8 ± 0.3 (2.2-3.1)	2.7 ± 0.2 (2.3-3.1)	2.6	2.7 ± 0.3 (2.3-3.2)	2.69 ± 0.29 (2.31-3.23)
V%	56.7 ± 2.1 (54.9-63.7)	-	57-61	58.6 ± 1.1 (57.1-60.1)	57.59 ± 4.66 (50.00-66.11)
EP%L	14.9 ± 0.9 (13.4-16.8)	13.9 ± 1.7 (11.7-16.7)	-	-	-
MBW	20 ± 1.4 (16.6 -21)	19.9 ± 1.9 (16.4-23.7)	19	21.8 ± 2.4 (18-24.5)	-
Stylet length	17.9 ± 1.1 (15.1-19.1)	18 ± 0.6 (17-19)	17-20	18.6 ± 0.3 (18.5-19)	16.02 ± 0.55 (15.36-17.29)
Stylet cone length	9.5 ± 0.3 (9.1-10)	9.3 ± 0.5 (8.1-9.8)	-	-	-
Stylet knob width	4 ± 0.5 (3.2-4.8)	3.8 ± 0.6 (3.1-4.8)	-	-	-
Stylet knob length	1.9 ± 0.2 (1.5-2.3)	2 ± 0.3 (1.4-2.5)	-	-	-
Lip region width	5.8 ± 0.3 (5.2-6.4)	5.8 ± 0.3 (5.4-6.3)	-	-	-
Lip region length	3.4 ± 0.3 (3.1-4)	3.4 ± 0.2 (3.1-3.9)	-	-	-
Lip annuli number	3	3	4	-	-
DGO	2.5 ± 0.2 (2.3-2.7)	-	2-3	-	-
Pharynx length	122 ± 14 (107-158)	118 ± 6.3 (105-127)	121	125 ± 12.1 (105-143)	-
Anterior to valve	58.1 ± 4 (52.3-68.3)	56.9 ± 3.7 (49.2-62.3)	-		-
Anterior to SE pore	89.2 ± 11.4 (76.7-115)	83.7 ± 6 (75.6-98.7)	-	90.2 ± 13.0 (69-110)	-
Median bulb width	9.7 ± 0.7 (8.6-10.7)	9 ± 0.6 (8-9.7)	-	-	-
Median bulb length	11.9 ± 0.8 (10.5-13.5)	11.3 ± 0.8 (9.9-12.7)	-	-	-
Anterior to vulva	337 ± 41.1 (273-418)	-	-	377 ± 39.4 (324-431)	-
Body width at vulva	19.8 ± 1.9 (16.9-22.5)	-	-	21.2 ± 2.1 (18-24)	-
Body width at anus	12.2 ± 1 (10.6-14.1)	14.3 ± 0.9 (13.2-16.4)	-	13.6 ± 1.2 (12-16)	-
Tail length	34.1 ± 3.9 (28.5-41.1)	39.3 ± 4.8 (32.1-48.1)	46	38 ± 6.1 (30-47)	31.13 ± 2.16 (28.48-34.54)
Tail annuli number	18 ± 2.9 (14-22)	-	16	-	-
Number of lateral line	4	4	4	-	-
m (cone length in% of stylet)	52.9 ± 1.7 (50.3-55.8)	51.4 ± 1.8 (47.4-53.6)	-	-	-
Spicule length	-	24.3 ± 1.1 (22.4-26.3)	-	21 ± 1.4 (20-22)	-
Gubernaculum length	-	13.2 ± 0.7 (12.1-14.8)	-	7.5 ± 0.7 (7-8	-

Discussion

The genus *Meloidodera* recovered in this study is the first report from coffee and Ethiopia. However, related taxa of this nematode (*Heterodera* sp.) had been reported from coffee (Nguyen & Nguyen 2001; Mekete et al., 2008a; Trinh et al., 2009). Currently, the genus *Meloidodera* contains eight valid species (Baldwin, 1992), of which only three nematode species, *M. floridensis*, *M. charis* and *M. mexicana* have been reported to be agriculturally important pests of *Capsicum annuum* cv. Pasilla, *Solanum lycopersicum*, *Zea mays*, *Phaseolus vulgaris*, *Abelmoschus esculentus* and *Cucurbita pepo* (Inserra & Vovlas, 1986; Cid Del Prado Vera, 1991; Fuentes et al., 1997). Subbotin et al. (2017) indicated a lesser economic significance of cystoid nematodes species in agriculture compared to the cyst nematodes. However, our study indicates the potential importance of this genus for coffee, while no evidence has been found about the host status of *Meloidodera* species to coffee. The four lateral lines of juvenile of *Meloidodera sp.*is the main constant character for identification. However, variability in phasmids with or without lens-like structure among second-stage juvenile of *Meloidodera sp.* had been observed (Baldwin, 1986; Luc et al., 1988; Sturhan, 2010). Hence, *M. astonei, M. charis* and *M. belli* had been identified to have a pore-like phasmid structure (Wouts, 1973; Cid Del Prado Vera & Rowe, 2000). Unfortunately, female of this nematode were not detected in the three soil samples checked, with the hope of getting more information from the ongoing research at Ghent and Jimma University on the rest of the soil samples. However, coffee roots were not checked due to lack of permission from farmers' coffee trees.

P. leptos has been already reported from Ethiopia associated with grass and roses (Abebe & Geraert 1995). Other species of this genus such *as P. aculentus*, *P. mutabilis* and *P. peperpotti* in India (Kumar, 1988; Souza, 2008) and *P. holdemani* in El Salvador, *P. besoekianus* in Indonesia and *P. minutus* in Hawaii had been also reported from coffee (Schenck & Schmitt, 1992; Souza, 2008). In our study, *P. leptos* was found in the highest prevalence of 93 % and mean intensity of 73 nematodes/100 ml; whereas, in Vietnam, a nematode of this genus the *P. nawadus* was recovered from coffee with very low intensity of 16 nematodes/250 ml and prevalence of 1% (Trinh et al., 2009). *Paratylenchus* species could be identified with known stable characters including the position of the vulva, head shape and conus length (Esser,1992l; Brzeski & Hanel, 2000; Ghaderi et al., 2014). Whereas post-vulval uterine sac, tail shape, tail terminus, tail length and the ratios related to tail are not reliable in species identification (Ghaderi et al., 2014; Van Den Berg et al., 2014). To the best of our knowledge, *P. leptos* is herewith reported for the first time from coffee.

The *H. multicinctus* was recovered at the high prevalence of 93% and the mean intensity of 61 nematodes/100 ml. It has been previously reported from coffee with a 73% prevalence and intensity of 373 nematodes/100 ml and from banana in Ethiopia (Mekete et al., 2008a, b; Marais et al., 2005). Similarly, *Helicotylenchus* was found from coffee and other hosts in Brazil, Hawaii, India and Vietnam (Fortuner et al., 1981; Mcsorley & Parrado, 1986; Schenck & Schmitt, 1992; Volvas et al., 1995; Marais, 2001; Campos & Villain, 2005; Decraemer & Hunt, 2006; De Waele & Elsen, 2007; Subbotin et al., 2011, 2015; Tzortzakakis et al., 2017; Mutala'Liah et al., 2018). Few characters such as body length, vulva position, stylet length, the shape of stylet knobs, shape

of the head, shape of the tail, position of phasmid to anus, presence/absence of male are considered to be reliable to identify *Helicotylenchus* species (Fotedar & Kaul, 1985; Uzma et al., 2015). According to Fortuner et al. (1981), although the morphological identification of spiral nematodes studied so far reported to be less reliable and contradicts with the molecular results, Subbotin et al. (2011) and Tzortzakakis et al. (2017) indicated the identification of *H. multicinctus* based on morphology and molecular data exhibited congruence, which is confirmed in the current study.

The present study revealed a prevalence of 70% and a mean intensity of 49 nematodes/100 ml of *T.* cfr. *zeae*; whereas, Al-Hazmi et al. (2009) reported *Tylenchorhynchus* sp. at the very low prevalence of 8% and intensity of 30 nematodes/200 ml from Arabica coffee. However, it was indicated that no further studies have been conducted on the parasitic status of *Tylenchorhynchus* to coffee (Campos & Villain, 2005; Mekete et al., 2008a). As far as the morphological identification of *Tylenchorhynchus* is concerned, the stylet length, vulva position, tail length, tail shape, tail annuli, head annuli, presence/absence of post anal intestinal sac and presence/absence of male reported being an important character used in the species identification (Handoo, 2000; Geraert, 2011). In our study, the specimen has been identified as *Tylenchorhynchus* cfr. *zeae* based on the above mentioned morphological characters (Handoo, 2000; Geraert, 2011; Handoo et al., 2014; Alvani et al., 2017).

In our study, *Xiphinema* sp. was detected at a prevalence of 83% and a mean intensity of 27 nematodes/100 ml. Mekete et al. (2008a, b) reported *X. insigne, X. basilgoodeyi* and *X. americanum 'a'* and *'b'* from coffee in Ethiopia at the low prevalence of 42% and high intensity of 174 nematodes/100 ml. Similarly, Trinh et al. (2009) from Vietnam reported *X. diffusum, X. elongatum* and *X. brasiliense* at a very low prevalence respectively 31%, 1% and 1% and an intensity of 15, 18 and 5 nematodes/250 ml from coffee. Other taxa identified in the present study included *Longidorus* sp., *Rotylenchus* sp., *Criconemoides*, *Scutellonema* sp., *Ogma* sp. and *Belonolaimus* sp. These taxa had been reported to parasitize coffee plants (Campos & Villain, 2005; Souza, 2008; Mekete et al., 2008a, b; Trinh et al., 2009; Maundu et al., 2014). Mekete et al. (2008a, b) reported *Rotylenchus unisexus* and *Scutellonema paralabiatum* associated with coffee from Ethiopia. However, information regarding coffee-parasite relationship is lacking (Souza, 2008). To the best of our knowledge, *Belonolaimus* sp. is the first record from coffee and as well as from Ethiopia.

Conclusions

The present diversity and taxonomic study of plant-parasitic nematodes have confirmed the occurrence and wide infestation of plant-parasitic nematodes associated with coffee in Ethiopia and provided new insights into the presence of plant-parasitic nematodes in coffee. In the present study, we identified eight nematodes to genus level and three nematodes to species level. In this study, we provided the first sequences of the partial D2-D3 of 28S rDNA, 18S rDNA and *COI* mtDNA for *Meloidodera* sp. and D2-D3 of 28S rDNA and 18S rDNA for *Paratylenchus leptos* and *Tylenchorhynchus* cfr. *zeae* from nematodes associated with coffee and as well as from Ethiopia. The result of this study not only provided valuable information on the integrated taxonomy of coffee nematodes as the first step to plan appropriate nematode management

strategies in Ethiopia but also provide additional evidence of the wide biogeographical distribution of *Meloidodera* sp., which is for the first time reported from Africa and coffee and *P. leptos* and *T.* cfr. *zeae* which are for the first time reported from coffee. Finally, further research should be conducted on the other important plant-parasitic nematodes based on integrated nematode identification and their effects on coffee.

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