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The first morphological and molecular characterization of plant-parasitic nematodes in Rwanda, with description of new species of *Pratylenchus*

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The first morphological and molecular characterization of plant-parasitic nematodes in Rwanda, with description of new species of *Pratylenchus*

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Summary - Twenty one plant-parasitic nematodes genera representing eleven families were recovered from 41 soil and root samples collected from 15 crops in six different Rwandan agricultural zones. Morphologically and molecularly characterization was carried out on five populations of *Scutellonema paralabiatum*, three populations of *S. brachyurus*, 2 populations of *S. cavenessi*, one unidentified *Scutellonema* species, 2 populations of *Pratylenchus penetrans* and *P. goodeyi*. Special emphasis was given to the description and characterization of a new *Pratylenchus* species. *Pratylenchus* n. sp. can be distinguished from the other *Pratylenchus* species by combination of the following features of female: body slender with medium size (469-600µm); body cuticle in lateral field with four lines at pharynx level and posteriorly at level of phasmid in tail region, six to eight lines at mid body and six to ten at vulva region; the lateral lines at vulva region showing a slight oblique pattern; ridges in general smooth except in some specimens at phasmid level where they are annulated. Labial region with 3 lips annuli, the last lip annulus thicker than the first two and offset by a deep constriction from the rest of the body, e*n face view* showing no clear separation between subdorsal, subventral and lateral sectors. Stylet relatively short and slender (13-14.5 µm) with round to slightly concave anteriorly oriented knobs. Pharyngeal glands overlap 10.9-34.7 µm long. Female reproductive system with oval to slightly rounded spermatheca without sperm except for one specimen with sperm. Tail subcylindrical to conoid with high variability of tail tip from rounded, truncate to indented. Based on morphology and morphometrics the new species is most closely related to *P. oleae* but also closely resembles *P. delattrei, P. elamini, P. sudanensis, P. cruciferus, P. mediterraneus and P. microstylus.* Phylogenetic analysis based on the D2-D3 expansion region of 28S rDNA, 18S rDNA and COI of mitochondrial DNA confirmed its status as a new species within a well-supported clade including *P. oleae, P. convallariae, P. penetrans, P. brachyurus, P. arlington, P. dunensis, P. fallax* and *P. pinguicaudatus.*

Keywords - Morphology, morphometrics, description, SEM, molecular, D2-D3, Bayesian inference, COI of mtDNA, Phylogeny, taxonomy, Rwanda, *Scutellonema*, *Pratylenchus*.

Rwanda is a landlocked country usually called a country of thousands hills due to its dramatic undulating landscape. Its mountains range from volcanic at the northern fringe and rolling hills mostly in the central plateau, while the eastern part of the country is relatively flat with the altitude below 1500 m (Verdoodt & Van Ranst, 2003). It is a small East Central African country with 26,338 square kilometers, located between the 1°04' and 2°51' Southern latitudes and between the 28°53' and 30°53' Eastern longitudes. It has a tropical climate in most parts of the country and temperate climate at higher altitudes. It has altitude ranging from 900 to 4500 m above sea level. It has an average annual temperature ranging between 16^{o}C and 20^{o}C with no significant variations. The rainfall is well distributed in the whole year, even if it has some irregularities (Rurangwa, 2013).

Agriculture is a dominant economic activity in Rwanda and most of the population (88%) works in agriculture but production is only 36% to 40% of GDP (Rurangwa, 2013). Agriculture is based on rain fed and is largely practiced on small farms of relatively 0.5 hectare. The production is relatively low due to its subsistence statues. Its development is considered a key support for growth and considerably reduces poverty in the country. Rwandan government has introduced a series of agricultural reforms which require farmers to consolidate agricultural production by inducing monoculture of priority crops and planting of the same appropriate crop on neighboring field (Huggins, 2009).

Rwanda soils and climatic conditions differ in excess of short distances in response to relief, parent material and altitude. Soil parameters change from the top of hill slope to the lower slope and valley bottom (Habarurema & Steiner, 1997). Rwandan agriculture is founded on 12 different agricultural zones based on soil and climatic parameters (Delepierre, 1974; Gasana, 1990; Ndindabahizi & Gwabije, 1991; Verdoodt & Van Ranst, 2003). The agricultural soil quality varies from very poor in the eastern savannah region to excellent, for example, at the boarders of lake Kivu. Due to its wide range of climate different crops are grown and most of them are considered as priorities crops such as bananas (cooking, fruit and banana brew), cereals (maize, rice,…), roots and tubers (cassava, potatoes,..), legumes (beans, peas…), crops purely for export (tea, coffee, pyrethrum), vegetables (cabbages, carrots, tomatoes, onions,…).

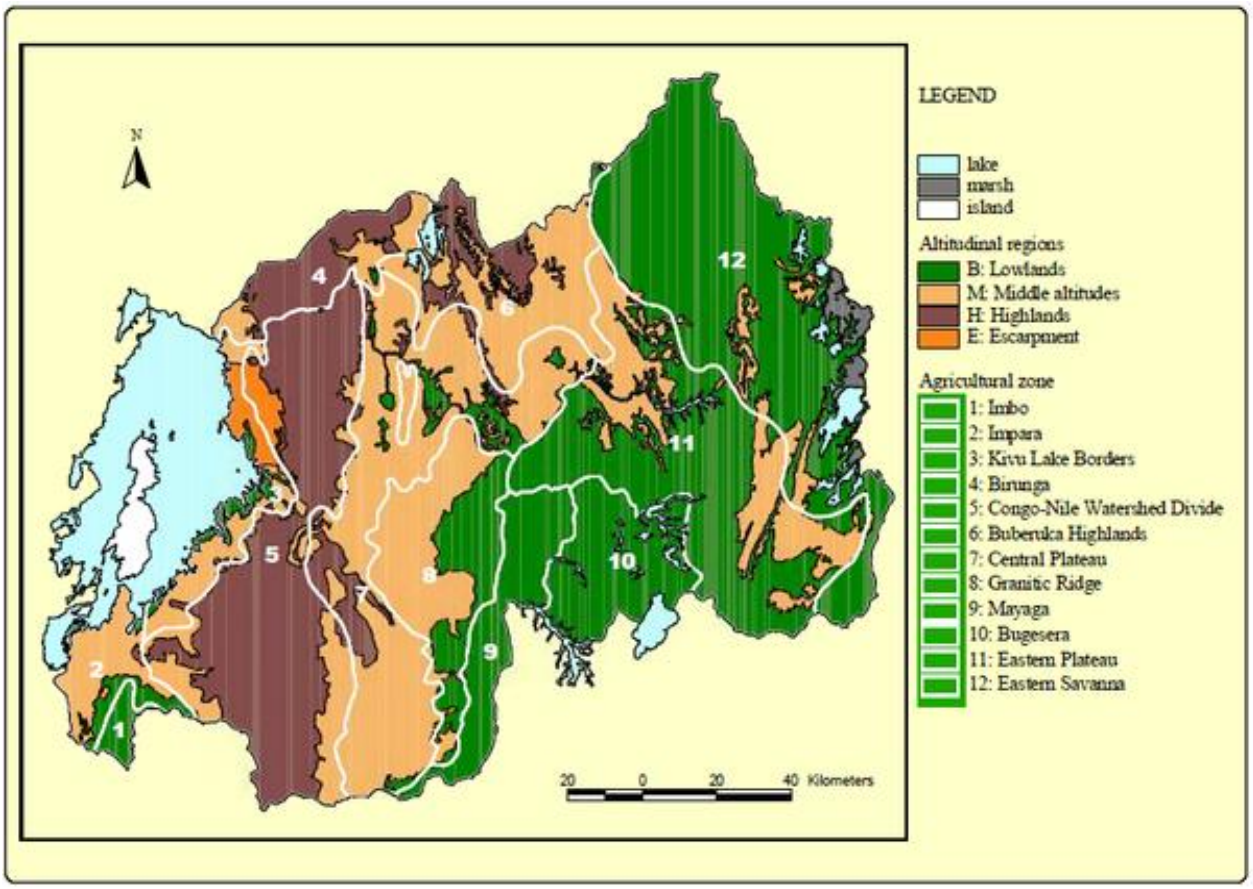


Fig. 1. Map of Rwanda with agricultural zones and altitudes of the regions. **Sources**: Verdoodt & Van Ranst, 2003

Among the major problems faced in agricultural production and productivity in Rwanda are the enormous crop losses caused by pests and other diseases. Moreover due to increase in Rwandan population and crop intensification program some of the techniques used in reducing crops loss are not in use. For example the fallow technique which is considered as one of the techniques in nematodes control is almost not in use. This can lead to high buildup of nematode population which may reach damaging level in crops. Plant-parasitic nematodes are known worldwide to reduce crop yield by feeding on and thus damaging different plant parts. Their damage is very difficult to determine since symptoms are often nonspecific. Most crops are attacked by more than one nematode species, additionally mixed populations of more than 2 species of one genus are found (Decraemer *et al.*, 2013). Their aboveground symptoms appear often similar to those caused by a poorly functioning root system (Bird & Koltai, 2000). Their economic impact on crops has been recently estimated at $80US billion per year. However this is likely to be an

under estimation of the true amount because many growers, particularly in developing countries, are unaware of nematodes as pests (Nicol *et al.*, 2011).

Despite nematode problems being encountered all over the world, in tropical and subtropical zones, very little research has been done, including Rwanda. Few crop related nematode investigations have been carried out; they are mostly related to banana and one research was on rice (Gaidashova *et al.*, 2004; Gaidashova *et al.*, 2008; Gaidashova *et al.*, 2009; Gaidashova *et al.*, 2010a; Gaidashova *et al.*, 2010b; Nsengimana *et al.*, 2012). Correct identification of plant-parasitic nematodes at species level is very important to carry out management of pest species and avoid spread of pathogens and non-adequate use of pesticides. Characteristics for identification of plant-parasitic nematodes are often limited because of their small size, their simple conserved morphology, inter- and intraspecific variability in morphological, morphometric, race and genetic features (Loubama *et al.*, 2007; Fonderie *et al.*, 2013).

The aims of this study was to integrate different approaches i.e. combinations of molecular and morphological data to identify plant-parasitic nematodes from Rwandan crops (potatoes, beans, maize, banana, tomatoes, carrot, cabbage, eggplant, onion, passion fruit, tamarillo and tea) and to search for new and unknown plant-parasitic nematodes species. More specific objectives for the first aim include (a) to analyze morphological, morphometric and molecular variation in and between different populations, (b) to establish phylogenetic relationship between the plant parasitic nematodes taxa in Rwanda with their relatives from other parts of the world. For the second aim, (a distinctive prominence effort during this study was attributed to the description of a new species of *Pratylenchus*, the main focus of this work. Under this major aim, the specific objectives. include (a) to describe the new species using a more holistic approach by combining morphological (based on light microscopy and scanning electron microscopy) and molecular observations, for (b) to differentiate the new species from other phenotypically similar species, (c*)* to analyze its phylogenetic relationship within the genus using different gene fragments of ribosomal and mitochondrial DNA (D2D3 of 28S, ITS, 18S of rDNA and COI of mtDNA)

Materials and Methods

NEMATODES SAMPLING

In total 41 soils and root samples were taken from fifteen different crops in six out of twelve agricultural zones of Rwanda. Sampling occurred randomly from fields of at least 25 m^2 in size of small scale farm holders. Sampled sites were selected based on approachability of the site and to cover priority crops from the different selected agricultural zones. The geographic coordinates of each of the sites were recorded using a Global Positioning System (GPS) so that we could map them precisely (see Fig. 3). Detailed information about location, sampled crops, and some ecological parameters (altitude, precipitation, agricultural zone) are presented in Table 1.

Table 1: Detailed information about sampling sites

Number of Samples	Agricultural zones	Altitude (m)	Average precipitation	Sector Sampled and coordinates	Crops sampled
9	Zone 3 Kivu lake border	1460-1900	1200	Kivumu (A) 1°47'40.5"S 29°18'46.5"E Nyabirasi (B) 1°46'41.8"S 29°19'20.1"E	Maize, banana, cassava, Passion fruit, tamariro, thea, cabbage
6	Zone 4 Birunga	1600-2500	1500	Nyakiriba (C) 1°39'34.1"S 29°22'08.5"E Kanzenze (D) 1°36'24.5"S 29°23'04.1"E	Bean, maize, potato, onion, carrot, cabbage
7	Zone 6 Buberuka highlands	1900-2300	1200	Byumba (E) 1°33'54.6"S 30°02'04.4"E Bushoki (F) 1°39'46.1"S 29°53'16.1"E	Thea, bean, carrot, cabbage, Tamariro, potato passparum
7	Zone 8 Granitic ridge	1500-1900	1200	Runda (G) 1°56'36.9"S 29°59'16.7"E Nyamabuye (H) 2°06'45.0"S 29°44'51.8"E Nyarugenge (I) 1°58'22.3"S 30°03'03.3"E	Potato, tomato, onion, cabbage, carrot, rice, passparum
4	Zone 10 Bugesera	1300-1500	900	Nyamata (J) 2°08'50.8"S 30°05'03.4"E	Cassava, banana eggplant, maize,
8	Zone 11 Eastern Plateau	1400-1800	950	Mukarange (K) 1°55'28.6"S 30°32'30.9"E Nyamirama (L) 1°57'16.6"S 30°31'16.9"E Kabuye (M) 1°53'30.9"S 30°03'50.5"E	Tomato, carrot, maize, cabbage, eggplant, rice

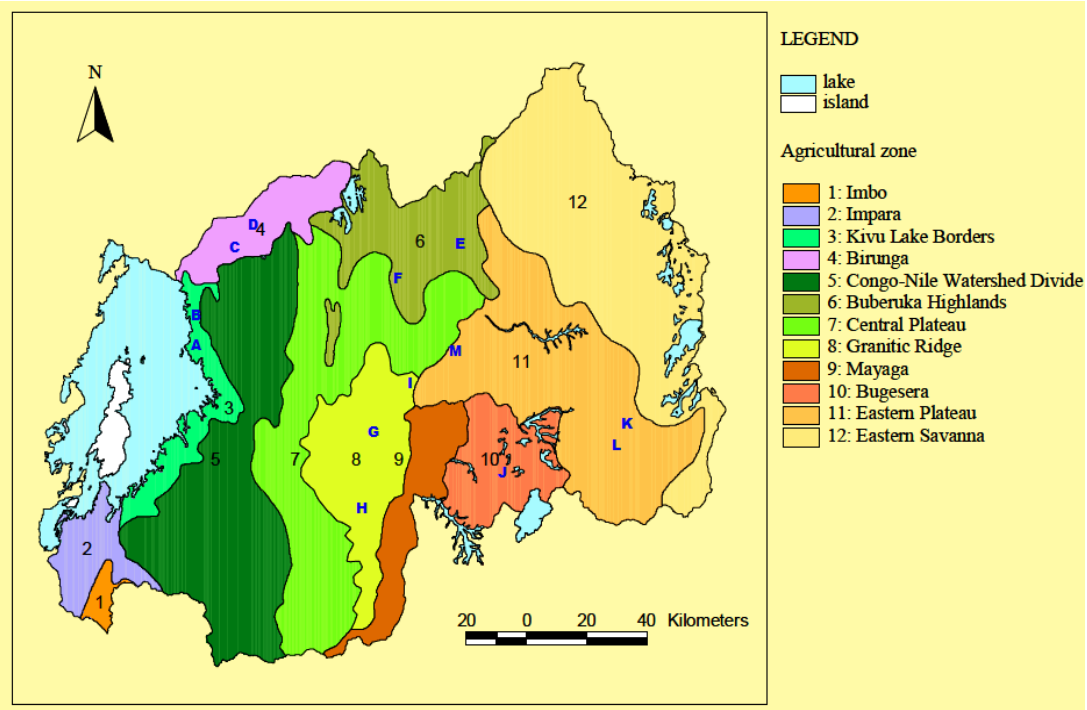


Fig. 2. Rwandan map showing locations from where samples were taken. Sampled places on map with agricultural zone. Sampled places represented by blue letters (A: Kivumu, B: Nyabirasi, C: Nyakiriba, D: Kanzenze, E: Byumba, F: Bushoki, G: Runda, H: Nyamabuye, I: Rwezamenyo, J: Nyamata, K: Mukarange, L: Nyamirama, M: Kabuye) **Sources**: Verdoodt & Van Ranst, 2003

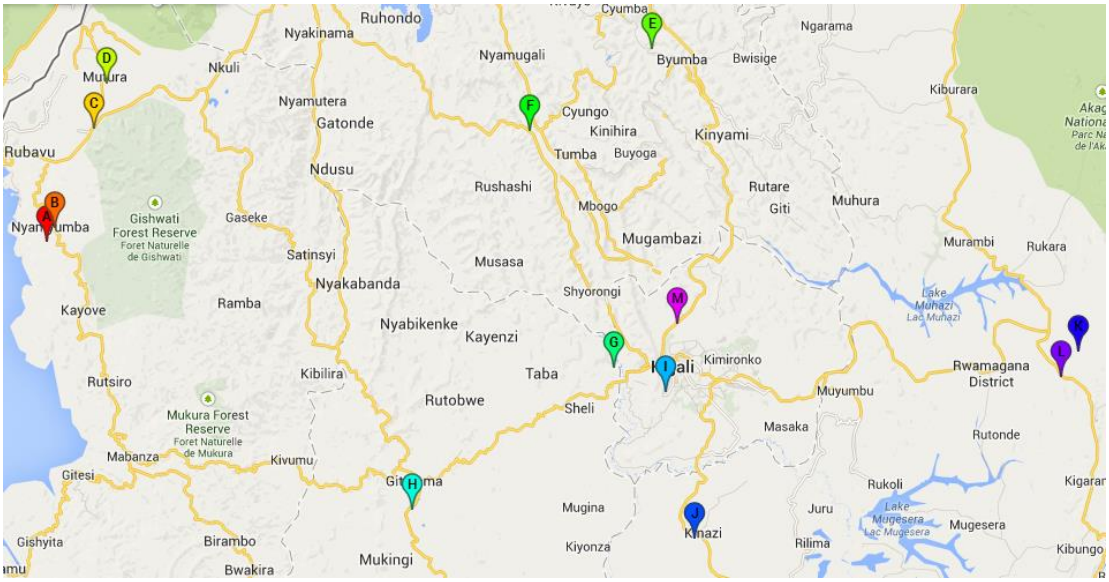


Fig. 3. Rwandan maps showing locations from where samples were taken. Map with GPS coordinates. The points with letters showing the exact sampled locations. (A: Kivumu, B:

Nyabirasi, C: Nyakiriba, D: Kanzenze, E: Byumba, F: Bushoki, G: Runda, H: Nyamabuye, I: Rwezamenyo, J: Nyamata, K: Mukarange, L: Nyamirama, M: Kabuye)

Sampling was done using a zigzag pattern. Samples were collected with a shovel from the upper 30 cm of soil and roots. During sampling process, ten soil and root subsamples of 200 grams from different spots within one are of a field crop were taken near plants showing moderate growing. From the well mixed collected bulk sample, a subsample of 500 grams was taken and placed in a plastic bag, labeled carefully with all necessary information and kept in a refrigerator at 4°C until extraction and further processing. Root knot galls infected with *Meloidogyne* were stored in eppendorf tube with an isotonic solution of 2% NaCl until their culture on tomato in green house.

NEMATODE EXTRACTION AND FIXATION

Nematodes extraction was done in Rwanda and Belgium. In Rwanda, nematode extraction from soil was done with Baermann tray method with slight modifications. Approximately 50 grams of each soil sample was used for extraction. After 3 days of extraction the nematode suspension was poured over a 25µm mesh sieve and fixed with DESS solution and collected in vials for later studies. For nematodes extraction in Belgium, Baermann tray method was used without modifications (Hooper, 2005) to extract soil samples and under mistyfier chamber as described in Viglierchio & Schmitt (1983) to extract endoparasitic nematodes from root samples.

For soil samples, nematodes were extracted from approximately 100 grams each time. After 4 to 5 days the extraction suspension was split into two equal parts. One part of the suspension was fixed with Trump's fixative to be used only for microscopic study while the other part of the suspension was fixed with DESS solution for molecular studies (Yoder *et al.*, 2006).

For root samples, roots were washed with water, chopped into small pieces and placed on tissue paper lined on a sieve. The sieves were placed on a glass funnel with water covering the bottom of the sieve and roots. After 3 days, nematodes were collected in a beakers from the tube attached to the funnels, for further processing. Labeling of extracts was done based on the information from plastic bag and including the date of extraction.

Plant-parasitic nematodes were handpicked from fresh extract suspension and collected in few drop of water in an embryo glass dish to be killed and fixed by adding 500 ml of Trump's fixative in an embryo glass dish containing a very small suspension of nematodes. The embryo

glass dish was placed in a microwave at 900 Watt for 5 seconds. The step of placing the embryo dish in microwave for 5 seconds was for the nematodes to die in a relaxed way. This step was replaced by what is done in the De Grisse (1969) method in the first step of killing and fixation where the solution one is heated up to 70^{o}C before use. The Trump's fixative was composed of 2% paraformaldehyde, 2.5% glutaldehyde in 0.1 M of Sorenson buffer (4.6 ml of 0.2 M $NaH_2PO_4.2H_2O$ + 15.4ml of NaH_2PO_4) (Dykstra, 1993). The Trump's fixative was used instead of the commonly used fixative of De Grisse (1969) in order to improve the quality of SEM pictures. The fixed nematodes were used for morphological studies with light and scanning electron microscopy.

Thereafter killing and fixation, nematodes were kept at 4^{o}C for at least 24 h for maximum penetration of the fixative before being processed to anhydrous glycerin according to Seinhorst (1959) and mounted on glass and Cobb slides for light microscopic study with Olympus BX51 DIC Microscope (Olympus Optical, Tokyo, Japan) equipped with an Olympus C5060Wz camera and Olympus optical CH30 RF 200 .

MORPHOLOGICAL CHARACTERIZATION

Tentative morphological and morphometric characterization and identifications was done on nematodes mounted on permanent and temporary Cobb slides and glass slides. Nematodes mounted on permanent slides were fixed with Trump's fixative while those on temporary slides were from fresh extracted materials and DESS fixed samples. Olympus BX51 DIC Microscope (Olympus Optical, Tokyo, Japan) equipped with an Olympus C5060Wz camera and Olympus optical CH30 RF 200 were used. Taxonomically important characters of the plant-parasitic nematodes were examined for identification at least up to genus level. In addition to the microphotographs taken, morphometric and allometric of all important taxonomical characters of nematodes were used for further identification up to species level. Of the new species found, drawings were made using a drawing tube of Olympus BX51 DIC Microscope. The holotype of the new species and some specimens of the other studied nematode species were video captured, by mimicking microfocal observation with light microscope based on video capturing editing procedures as developed by De Ley & Bert (2002).The resulted virtual specimens video are available on http://www.nematology.ugent.be/vce.html.

For SEM study nematodes specimens processed in anhydrous glycerin were used for nematode preparation scanning electron microscopy. Nematodes were picked from anhydrous glycerin and transferred into a small drop of glycerin in an embryo glass dish. One drop of water was added every 30 minutes until the dish was filled three to quarter and live it for the whole morning. In the afternoon the nematodes were transferred form the first embryo dish to another filled with four drops of distilled water and a 10 minutes ultrasonic treatment was done to remove as much as possible particles which were sticking to the body surface. After the nematodes were washed 3 times with Sorenson-buffer 0.1 M. Following was dehydration of nematodes with different concentrations of alcohols 30, 50, 75, 95 and 100% for the first four each for 20 minutes and the last one 10 min x 3 times in total 30 minutes (Dykstra, 1993). Nematodes were dried in critical point drying chamber (CPD020) with liquid CO2 and mounted on stubs with carbons tabs (double conductive tapes) and coated with gold of 25 nm before being observed under SEM microscope (JSM-840 EM; EOL, Tokyo, Japan) at 15 kV.

MOLECULAR CHARACTERIZATION

DNA extraction

Individual nematodes were picked from DESS and transferred to an embryo glass dishes containing 500 µl of double distilled water. One by one nematodes were picked from embryo glass dish and transferred to another dish containing 200 µl ddH2O 3 three times every 10 minutes to wash it and dehydrate. The washed nematodes were mounted on a temporary slide to gather all necessary morphological and morphometric information and video capture using an Olympus BX51 DIC Microscope before extracting DNA. DNA extraction was done in two ways the first was with NaOH and tween by mixing in a PCR tube 1 µl of sterile water, one nematode, 10 µl of NaOH 0.05 M and 1 µl of Tween. The mixture was incubated 15 minutes at 95^{o}C before adding 40 µl of PCR water to be kept at 4^{o}C for amplification. The second way was with worm lyse buffer mixture of (50 mM KCl, 10 mM Tris pH=8.3, 2.5 mM $MgCl_{2,}$ 0.45% NP 40 (Tergitol Sigma), 0.45% Tween 20) and protease K (1.2 mg/m) by transferring into eppendorf tube of 500 µl filed with 20 µl WLB nematodes cut into 2 to 3 pieces. Tubes were incubated at -20°C for 10 minutes after 1µl proteinase K (1.2 mg/ml) were added into the tube again incubated in PCR-machine for 1h at 65°C and 10 minutes at 95°C for enzyme deactivation. From PCR-machine

tubes were taken out and the suspension centrifuged for 1 min at a maximum speed (16,000 g) for debris to settle down.

DNA amplification, visualization and Sequencing

DNA amplification was done by preparing 30 µl PCR master mix comprising 23 µl water, 3 µl 10 x buffers, 2.4 µl MgCL2, 0.6 µl of dNTP (10 mM), 0.3 µl of reverse and forward primers, 0.06 µl of Toptaq and 1.2 to 5 µl of nematode template DNA. Primers used are summarized in a table below. PCR condition were 94°C for 4 minutes; a succession of 94°C for 30 secs; 56°C, 57°C and 58°C each for 30 secs; 72°C for 2 minutes up to 2 to 5 replications in order to be sure of having good DNA fragments, Then a succession of 94°C for 30 secs, 54°C for 30 secs and 72°C for 1 min up to 35 replications and lastly at 10°C for 10 min. PCR products were sized with 1 kb DNA ladder (Promega, Madison, WI, USA) and visualized on 1% agarose gel stained with 0.0003% ethidium bromide. Pictures were taken using UV light source. PCR products were sent for sequencing at Macrogen a commercial company. They were cleaned and sequenced with Big Dye 3.1 on ABI 3130XL genetic analyzer. Primers used were the same as the one used for PCR.

Table 2. Primers set used

Primer code	**Sequences(5→3)**	**Amplified gene**	**Genus sequence length (bp)**	**References**
D2Aadep	ACAAGTACTGTG AAGGAAAGTTG	D2D3 of 28S	*Pratylenchus*; 619, 688 *Scutellonema* (7 specimens) 461-700 *Tylenchorhynchus*; 481, 499 *Quinisulcius*, 678	Adapted from Subbotin *et al.* (2006)
D3Adep	TCAGAGGGAACC AGCTACTA			
JB3prat	TTTTTTGGGCATC CTGAAGTCTAT	COI of mitochondria l DNA	*Pratylenchus*; 291, 434 *Scutellonema* (10 specimens) 342-443 *Tylenchorhynchus*; 437, 438	Adapted from Derycke *et al.* (2010)
JB4prat	CCTATTCTTAAAA CATAATGAAAAT G			
G18	TGATCCWMCRGC AGGTTCAC	18S (part 1)	*Pratylenchus* 766	Adapted from Bert *et al.* (2008) (Blaxter *et al.*, 1998)
4R	GTATCTGATCGCK T CGAWC			
18P	TGATCCWMCRGC AGGTTCAC	18S (part 2)	*Pratylenchus* 644	(Bert *et al.*, 2008) (Blaxter *et al.*, 1998)
4F	CAAGGACGAWAG TWGAGG			

Phylogenetic Analysis

Phylogenetic analysis was done for the two genera *Pratylenchus* and *Scutellonema.* The sequences generated and used in the analysis are summarized in the table 3 below with more detailed information about them. The obtained sequences for each gene (D2-D3 of the expansion segment of 28S rDNA, 18S of rDNA and the COI of mtDNA) were assembled using Geneious Pro 7.1 created by Biomatters (http://www.geneious.com) and multiple sequence aligned together with sequences obtained from the gene bank of D2-D3 of 28S rDNA and 18S gene with MUSCLES programs (Edgar, 2004). For *Pratylenchus* species the D2D3 and 18S sequences used from the gene bank were mostly from Subbotin *et al.*(2008), Múnera *et al.*(2009) Palomares-Rius *et al.*(2014). For mitochondrial DNA sequences used to compare with the *Pratylenchus* n. sp. were provided by T. Janssen (unpublished data). For *Scutellonema* species both D2D3 and COI of mitochondrial DNA sequences used from the gene bank were mostly the from Van den Berg *et al.* (2013). Out-group taxa for *Pratylenchus* dataset used were selected based on the result of previously published data (Inserra *et al.*, 2007; Múnera *et al.*, 2009; Van den Berg *et al.*, 2013; Palomares-Rius *et al.*, 2014). For *Scutellonema* species out-groups used are the same as in Van den Berg *et al.* (2013) in addition to *Tylenchorhynchus spp., Quinisulcius spp.* and *Pratylenchus penetrans* sequences generated during this study.

For the aligned sequences G-BLOCK was used to eliminate poorly aligned positions (Castresana, 2000). The final multiple sequence aligned datasets were analyzed with Maximum likelihood using PHLML 3.0 (Guindon *et al.*, 2010) and Bayesian inference using MRBAYES 3.2 (Ronquist *et al.*, 2012) The programs MUSCLE, GBLOCKS, and PHYML3.0 used are found in SEAVIEW software package program (Gouy *et al.*, 2010). JMODELTEST 2.1.1 was used to determine the best fitting substitution model for our dataset. Maximum likelihood scores were evaluated using Akaike Information Criterion (AIC). BI analysis was done under general time-reversible model with rate variation across sites and a proportion of invariable sites (GTR+I+G). For each gene a random starting tree was run for chains of 10 millions generations. The Markov chains were sampled at intervals of 1000 generations. Two independent runs of 4 chains were done for each analysis. 25% of the results were removed as burn-in after convergence evaluation and the remaining were remained for further analysis. 50% majority rule consensus trees were generated using topology and are depicted in (Figs. 7, 8, 13, 14 and 15). Posterior probabilities are on the tree branches. Trees were visualized and rooted using FIGTREE v1.4 (2006–2012,

Andrew Rambaut, Institute of Evolutionary Biology; University of Edinburgh, UK) and converted into graphic files with Adobe Illustrator™ CS2. Phylogenetic trees of nematodes species presented were only limited to Bayesian inference. Nevertheless the trees topologies were the same for both Bayesian and Maximum likelihood approaches that we used. BI analysis was more emphasized on as this method has different important advantages compared to Maximum likelihood.

Table 3. Detailed information on sequences used in phylogenetic analysis

Molecular code of sequence	**Gene sequenced**	**Nematode species**	**Sample code**	**Agricultural zone**	**Crops sampled**
AL1	COI of mtDNA	*S. cavenessi*	ALN17	8	Onion
AL2	COI of mtDNA	*S. paralabiatum*	ALN17	8	Onion
AL4	COI of mtDNA	*S. paralabiatum*	ALN16	4	Onion
AL6	COI of mtDNA	*S. brachyurus*	ALN18	3	Banana
AL7	COI of mtDNA	*S. brachyurus*	ALN18	3	Banana
AL8	COI of mtDNA	*S. paralabiatum*	ALN18	3	Banana
AL9	D2D3 of 28S	*S. brachyurus*	ALN12	11	Tomato
AL10	D2D3 of 28S	*Scutellonema sp.*	ALN16	4	Onion
AL26	D2D3 of 28S	*S. cavenessi*	ALN7	8	Carrot
AL30	COI of mtDNA & D2D3 of 28S	*S. paralabiatum*	ALN17	8	Onion
AL31	COI of mtDNA & D2D3 of 28S	*P. cavenessi*	ALN17	8	Onion
AL34	COI of mtDNA & D2D3 of 28S	*S. cavenessi*	ALN18	3	Banana
AL35	COI of mtDNA & D2D3 of 28S	*Scutellonema sp.*	ALN16	4	Onion
AL37	COI of mtDNA & D2D3 of 28S	*Tylenchorhynchus sp.*	ALN10	8	Tomato
AL38	COI of mtDNA & D2D3 of 28S	*Tylenchorhynchus sp.*	ALN10	8	Tomato

143	COI of mtDNA	*P. penetrans*	ALN16	4	Onion
200	D2D3 of 28S	*P. penetrans*	ALN20	6	Potato
222	D2D3 of 28S	*P. goodeyi*	ALN23	6	Bean
277	D2D3 of 28S	*Quinisulcius*	ALN29	10	Maize
201	D2D3 of 28S & COI of mtDNA	*Pratylenchus* n. sp.	ALN29	10	Maize
202	D2D3 of 28S & COI of mtDNA	*Pratylenchus* n. sp.	ALN29	10	Maize
278	18S of rDNA	*Pratylenchus* n. sp.	ALN29	10	Maize

Results

MORPHOLOGICAL IDENTIFICATION

Nematode genera identification

Twenty one plant-parasitic nematodes genera (*Tylenchus, Lelenchus, Coslenchus* (and other unidentified Tylenchidae), *Tylenchorhynchus, Dolichodorus, Quinisulcius, Scutellonema, Rotylenchus, Helicotylenchus, Rotylenchulus, Hoplolaimus, Meloidogyne, Pratylenchus, Ditylenchus, Aphelenchus, Aphelenchoides, Criconema, Ogma, Hemicycliophora, Longidorus* and *Xiphinema*) representing eleven families (Tylenchidae, Anguinidae, Dolichodoridae, Hoplolaimidae, Meloidogynidae, Pratylenchidae, Aphelenchoididae, Hemicycliophoridae, Criconematidae, Aphelenchidae and Longidoridae) were recovered from 41 samples sampled from 13 different sites found in 6 different agricultural zones of Rwanda. Below is a list of detailed information about the nematodes identified according to sampled crops.

Table 4. Nematode taxa found in different crops

Nematodes genera found	**Crops**	**Sample code**	**Agricultural zone**	**Sampled area**
Tylenchorhynchus, Criconema Scutellonema, Tylenchidae *Helicotylenchus, Meloidogyne,*	Cabbage (*Brassica oleracea*)	ALN 1	11	Kayonza
Longidorus, Scutellonema		ALN 2	3	Nyabirasi

Quinisulcius, Pratylenchus, Aphelenchus		ALN 3	4	Nyakiriba
Scutellonema, Helicotylenchus, Meloidogyne, Quinisulcius, Ditylenchus		ALN 4	8	Runda
Scutellonema, Rotylenchus		ALN 5	6	Bushoki
Pratylenchus penetrans, S. paralabiatum, Longidorus	Carrot *(Daucus carrota)*	ALN6	4	Nyakiriba
S. cavenessi, P. penetrans, M. javanica, Tylenchidae		ALN7	8	Runda
Hemicycliophora, Scutellonema		ALN8	11	Mukarange
Longidorus, Scutellonema		ALN9	6	Bushoki
Scutellonema, Aphelenchus Tylenchorhynchus, Tylenchidae,	Tomato (*Solanum lycopersicum*)	ALN10	8	Runda
Helicotylenchus, Scutellonema, Ditylenchus, M. javanica		ALN11	11	Nyamirama
Tylenchorhynchus, Aphelenchus, S. brachyurus		ALN12	11	Kabuye
Aphelenchus, Meloidogyne, Longidorus, Ogma, Scutellonema, Helicotylenchus	Eggplant *(Solanum melongena)*	ALN13	11	Mukarange
Scutellonema 2sp., Meloidogyne		ALN14	10	Nyamata
Rotylenchus, Scutellonema, Aphelenchus, Aphelenchoides bicaudatus , Ditylenchus, Meloidogyne, Ogma, Longidorus		ALN15	11	Kabuye
S. paralabiatum ,Scutellonema sp., Aphelenchus, Helicotylenchus, P. penetrans	Onion (*Allium cepa*)	ALN16	4	Nyakiriba
S. paralabiatum, S. cavenessi, Aphelenchus, Tylenchorhynchus		ALN17	8	Runda
S. paralabiatum, S. brachyurus, Quinisulcius, Aphelenchus, Meloidogyne, Dolichodorus, Tylenchus, Ditylenchus,	Banana (*Musa spp.*)	ALN18	3	Kivumu

Tylenchorhynchus				
Helicotylenchus, Aphelenchus, Meloidogyne arenaria		ALN19	10	Nyamata
Aphelenchus, P. penetrans, Tylenchidae, *Rotylenchus, S. paralabiatum, S. brachyurus.*	Potato (*Solanum tuberosum)*	ALN20	6	Bushoki
Scutellonema, Xiphinema, Helicotylenchus, Dolichodorus, M. javanica, Tylenchidae		ALN21	8	Runda
Pratylenchus penetrans, Aphelenchus, Scutellonema, Rotylenchus, Tylenchidae		ALN22	4	Nyakiriba
Pratylenchus goodeyi, Aphelenchus, Scutellonema, Quinisulcius, Helicotylenchus	Beans (*Phaseolus vulgaris*)	ALN23	6	Bushoki
Meloidogyne, Ditylenchus dipsaci, Helicotylenchus, Tylenchidae		ALN24	4	Nyakiriba
Hemicycliophora, Rotylenchus Helicotylenchus, Scutellonema,	Maize *(Zea mays*)	ALN25	3	Nyabirasi
Hemicycliophora, Scutellonema, Helicotylenchus, Dolichodorus		ALN26	3	Kivumu
Aphelenchus, Scutellonema, Hoplolaimus		ALN27	4	Nyakiriba
Helicotylenchus, Quinisulcius, Scutellonema, Aphelenchus, Aphelenchoides,		ALN28	11	Nyamirama
Pratylenchus n. sp., Aphelenchus, Quinisulcius, Rotylenchus, Scutellonema		ALN29	11	Nyamata
Helicotylenchus, Tylenchidae	Pasparoom (*Paspalum conjugatum)*	ALN30	6	Gicumbi
Scutellonema, Aphelenchus		ALN31	10	Nyarugenge

Longidorus, Scutellonema, Tylenchidae, *Hemicycliophora*	Passion fruit *(Passiflora edulis)*	ALN32	3	Kivumu
Helicotylenchus, Scutellonema, Meloidogyne, Aphelenchus		ALN33	3	Nyabirasi
Tylenchidae, *Helicotylenchus, Xiphinema,*	Cassava *(Manihot esculenta)*	ALN34	3	Kivumu
Scutellonema, Longidorus, Hemicycliophora		ALN35	10	Nyamata
Hemicycliophora, Helicotylenchus, Ditylenchus	Thea *(Camellia senensis)*	ALN36	3	Nyabirasi
Scutellonema, Helicotylenchus, Aphelenchus, Coslenchus		ALN37	6	Byumba
Rotylenchus, Helicotylenchus, Meloidogyne, Tylenchidae, Ditylenchus, Rotylenchulus	Tamarillo *Cyphomandra crassifolia*	ALN38	6	Bushoki
Hemicycliophora, Tylenchidae, *Scutellonema, Xiphinema*		ALN39	3	Nyabirasi
No plant parasitic nematodes	Rice *(Oryza sativa)*	ALN40	11	Kabuye
No plant parasitic nematodes		ALN41	8	Muhanga

Nematodes species identification

Further identification both morphologically and molecularly up to species level was done for 2 genera *Scutellonema* and *Pratylenchus* from 9 different samples (ALN6, ALN7, ALN12, ALN16, ALN17, ALN18, ALN20, ALN23, and ALN29). The *Scutellonema* species were selected based on their high prevalence in the samples (78%). *Pratylenchus* species were chosen according to their economic importance. Two more genera *Quinisulcius* from ALN18 and *Tylenchorhynchus* from ALN10 were morphologically identified but no sequences were available in gene bank to support the identification; they represent new records for Rwanda and for Gene Bank. In total four species of *Scutellonema*, three species of *Pratylenchus* including one new species, one species of *Quinisulcius* and one species of *Tylenchorhynchus* were identified. For *Scutellonema* 5 populations of *S. paralabiatum* (Siddiqi & Sharma, 1994) from 4 different

agricultural zones (samples ALN16, ALN17, ALN20, ALN18 and ALN7), 3 populations of *S. brachyurus* (Steiner, 1938) Andrássy, 1958 from 3 different agro ecological zones (samples ALN12, ALN20 and ALN18) and 2 population of *S. cavenessi* (Sher, 1964) from 2 different crops of one agricultural zone (samples ALN 17 and ALN7) were identified. Two tentatively identified morphologically as *S. brachyurus* from two samples (ALN16 and ALN17) were not confirmed by molecular results. For the genus *Pratylenchus*, one new species from maize sample (sample ALN 29), *P. goodeyi* (Sher & Allen, 1953) from bean (sample ALN23) and 6 different populations of *P. penetrans* (Cobb, 1917) Filipjev, 1941 from samples ALN3, ALN6, ALN7, ALN16 and ALN20 were identified and both morphological and molecular data for the *Pratylenchus* n. sp., *P. goodeyi* and two *P. penetrans* confirmed the identification. Below are morphological, morphometrics and molecular data of the identified species.

MORPHOLOGICAL CHARACTERIZATION OF *SCUTELLONEMA* SPECIES

***Scutellonema paralabiatum* (Siddiqi & Sharma, 1994) populations**

Figure 4.

MEASUREMENTS

See Table 3

DESCRIPTION

Females

Population from onion Nyakiriba, agricultural zone 4, sample ALN 16, voucher slide UGnem-90

This nematode population largely agrees with the population from Van den Berg *et al.*(2003). They only differ in body appearance which becomes spiral after fixation (Fig.4B), long body length 763-843 µm, lip region high and flat, cephalic region continuous with body contour, with generally 6 to 7 annuli of 0.8 µm maximum width, cephalic framework strongly developed extending to 3 annuli anteriorly into the body (Fig. 4E), fine cuticle annulus with 1.3 µm of maximum width at vulva, stylet with slightly rounded or anterior concave knobs, vulva with short double epiptygma projecting above body surface in some specimens, tail broadly rounded to sub hemispherical with 11 to 12 annuli, scutellum above the anus.

Population from carrot Nyakiriba, agricultural zone 4, sample ALN7, voucher slide UGnem-92

In this nematode population, the morphological and morphometrics overlap with those of the specimens of sample ALN17. The main difference was observed in the rounded to pointed tails, the position of the scutellum at or above the anus level and a slightly shorter stylet (Fig. 4 H, K). They also largely agrees with the population from Van den Berg *et al.*(2003).

Population from onion Runda, agricultural zone 8, sample ALN17, voucher slide UGnem-91

This nematode population largely agrees with the population from Mekete *et al.* (2008) compared to those of Siddiqi & Sharma (1994) and Van den Berg *et al.* (2003). They differ in having body habitus ranging from irregular spiral to ventrally curved upon fixation (Fig.4 A), short body length ranging from 652 to736 µm, lip region slightly high and flat, cephalic region continuous with body contour, with 5 to 6 lip annuli of slight coarse annulus of 1.2 µm maximum wide, cephalic framework strongly developed extending 2 to 3 annuli anteriorly into the body (Fig. 4D), cuticles annuli at vulva fine of up to 1.5 µm maximum width, lateral field not areolated at mid-body and scutellum level but some specimens with the outer lateral line annulated (Fig. 4G), stylet long with rounded to anterior concave knobs, spermatheca not developed or developed but empty, vulva with pronounced double epiptygma not or in some specimens projecting above body surface, tail rounded with 10 to 12 annuli , scutellum at or posterior to the anus level (Fig. 4J).

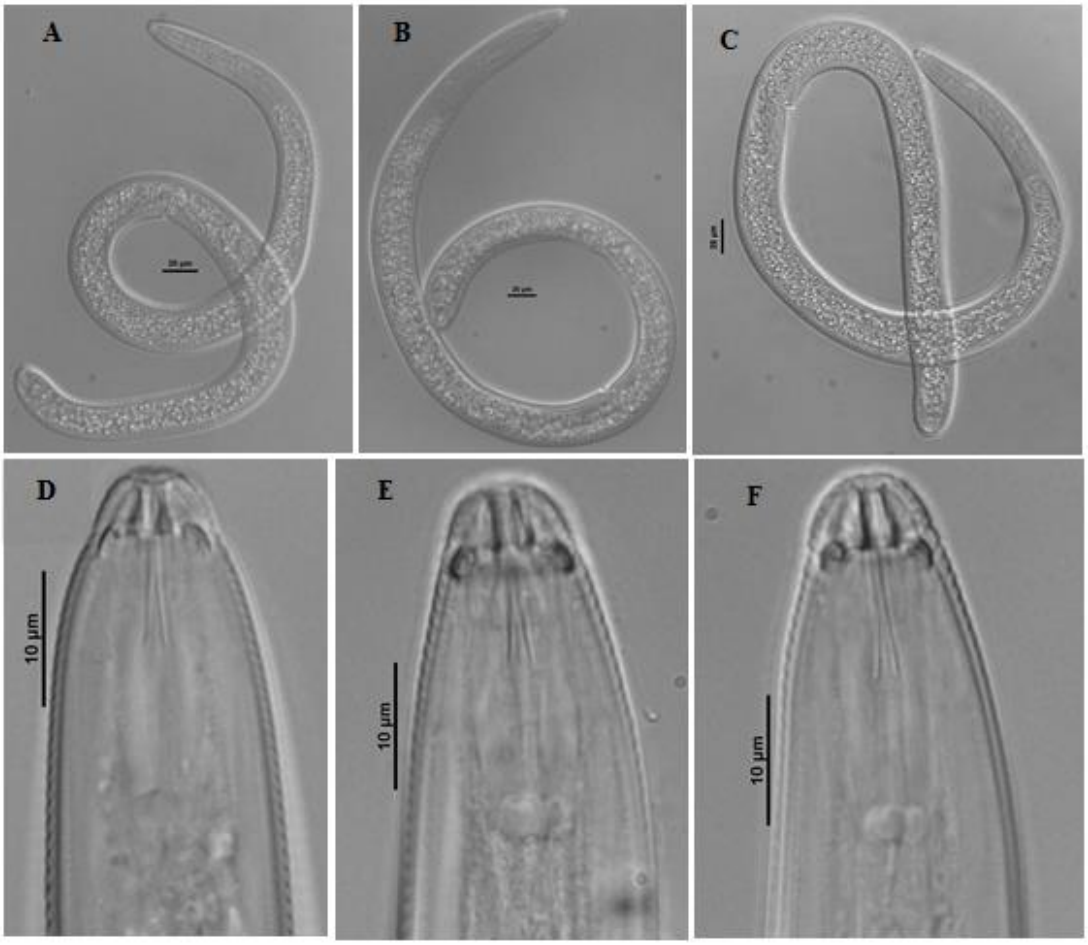
Population from potato Bushoki, agro-ecological zone 6, sample ALN20, voucher slide UGnem-94

This nematode population largely agrees with the population from Siddiqi & Sharma (1994) than those of Mekete *et al.*(2008), and Van den Berg *et al.* (2003) Body forming a spiral upon fixation, body length ranging from 701 to 822 µm, lip region high and flat, with 5 to 7 annuli of 1.4 µm maximum width, cephalic framework well developed extending 2 to 3 annuli anteriorly into the body (Fig. 4F), coarse cuticle annulus with 1,8 µm of maximum width at vulva, lateral field not areolated at mid-body and scutellum level but some specimens with the outer lateral line annulated (Fig. 4I), long stylet with rounded knobs, spermatheca not developed, vulva with

very long double epiptygma not or in some specimens projecting above body surface, tail narrowly rounded to hemispherical.(Fig. 4 L)

Population from banana Kivumu, agro-ecological zone 3, sample ALN 18, voucher slide UGnem-96

This nematode population largely agrees with the population from Van den Berg *et al.* (2003) but differs in body appearance, forming a spiral shape upon fixation (Fig. 4C), body 708-832 µm long, lip region high flat, generally with 6 to 7 annuli of 0.9 µm maximum width, cephalic framework highly developed extending over 3 annuli anteriorly, fine cuticle annulus with 1.4 µm of maximum width at vulva, lateral field not areolated at mid-body and scutellum level but some specimen with the outer lateral line annulated, long stylet with rounded to slightly concave knobs, spermatheca not developed, vulva with long double epiptygma in some specimens projecting above body surface, tail rounded to broadly rounded with 10 to 13 annuli, scutellum at or below anus level.



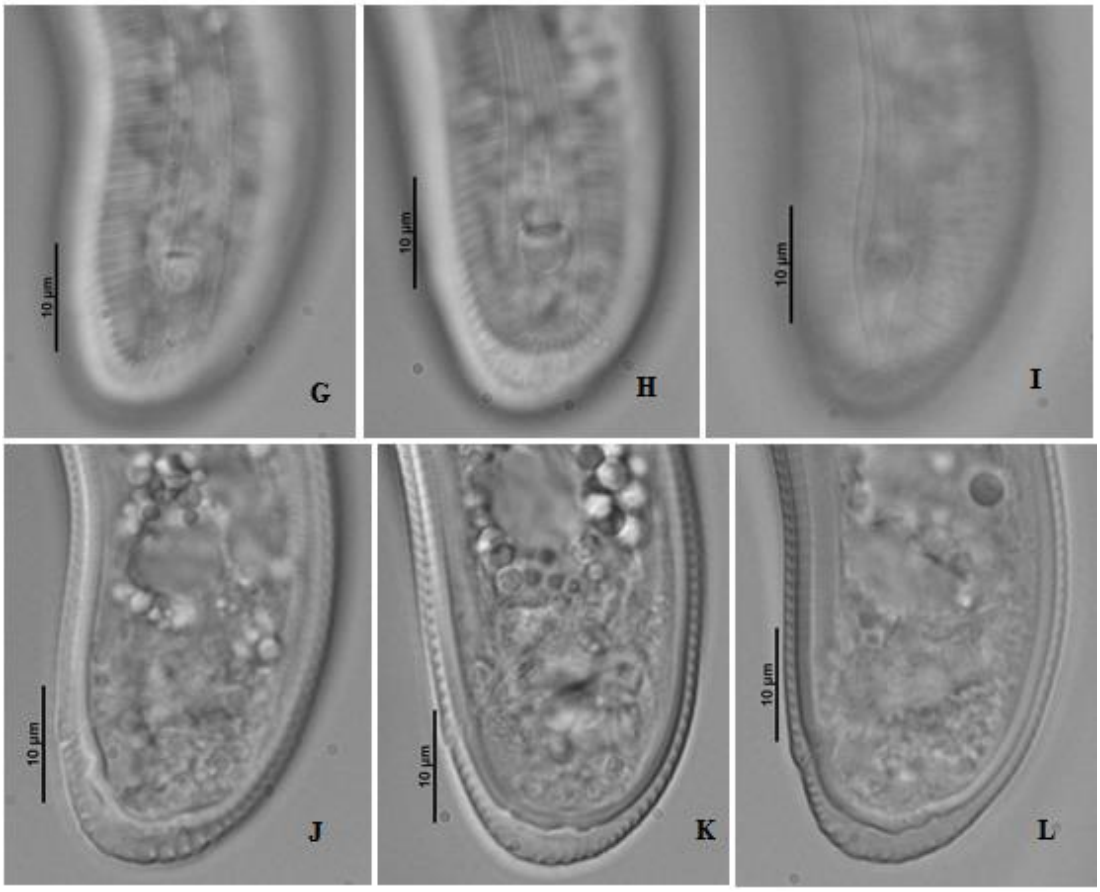


Fig. 4. Photomicrographs of *S. paralabiatum* population: A-C: body shape after fixation, D-E: Head region, F-G: Lateral lines at scutellum level, J-K: tail and tail annuli. A, D, G, J from samples ALN 17; B, E from AL16; C from ALN18; H, K from ALN7; F, I, L from ALN20 (Scale bars: A-C = 20 *µm*, D-L = 10 *µm*).

Table 5. Different *Scutellonema paralabiatum* populations measurements from 5 different localities. All measurements are in µm and in the format: mean ± s.d. (range)

	Sampled crop, code and locality					
Characters	Onion ALN16 Nyakiriba	Onion ALN17 Runda	Potato ALN20 Bushoki	Banana ALN 18 Kivumu	Carrot ALN7 Nyakiriba	*S. paralabiatum* from the literature*
n	4	10	12	3	8	
L	801 ± 36 (763-843)	707 ± 27.6 (652-736)	757 ± 32.9 (701-822)	773 ± 62 (708-832)	760 ± 31.6 (719-804)	578-870
a	24.9 ± 2.6 (21.2-27.2)	21.6 ± 1.5 (19.6-24)	34.2 ± 4.1 (25-40.7)	22.4 ± 1.5 (21-24.3)	30.5 ± 3.4 (23.3-35.4)	20.8-31.4

b	7.3 ± 0.4 (6.9-7.8)	7.7 ± 0.9 (6.6-9.1)	6.8 ± 0.4 (6.4-7.8)	7.3 ± 1.1 (6.6-8.6)	6.7 ± 0.4 (6.3-7.6)	4.9-20.98
b'	5.8 ± 0.4 (5.4-6.4)	6.3 ± 0.7 (5.3-7.6)	5.5 ± 0.2 (5.2-6.1)	5.7 ± 0.5 (5.3-6.3)	5.6 ± 0.3 (5.2-6.2)	5-7.2
c	78 ± 14.8 (66-97.8)	68.4 ± 3.4 (62.5-73)	59.6 ± 6.7 (49-71.4)	48.2 ± 5.2 (43.7-54)	54.4 ± 4.2 (501-61.7)	49-158
c'	0.5 ± 0.07 (0.4-0.6)	0.5 ± 0.02 (0.46-0.55)	0.6 ± 0.1 (0.4-0.7)	0.67 ± 0.1 (0.57-0.75)	0.5 ± 0.05 (0.5-0.64)	0.29-0.72
V	58.9 ± 1.8 (56.5-60.8)	57.5 ± 1.9 (53.6-60.7)	57 ± 1.7 (54-60.4)	56 ± 3.1 (52.9-59.1)	58.9 ± 2 (54.9-60.7)	51-61
m	48.6 ± 1.5 (47.1-50)	48.3 ± 4.5 (42.1-55.1)	46.5 ± 1.6 (44-48.5)	44.9 ± 1.4 (43.9-46.6)	46.4 ± 2.2 (42.6-48.5)	38-49.4
o	14 ± 1.2 (12.6-15.2)	14.9 ± 3.7 (10.3-229)	14.8 ± 2.6 (12-19.1)	14.7 ± 3.2 (11-16.7)	14.9 ± 1.5 (13.1-17.2)	14-31.9
S	1.2 ± 0.02 (0.9-1.4)	1.1 ± 0.1 (1-1.5)	1.3 ± 0.1 (1.2-1.5)	1.3 ± 0.1 (1.2-1.4)	1.3 ± 0.1 (1.2-1.5)	-
MB	64.8 ± 1.6 (63-66.2)	71.8 ± 5 (63.1-82)	70 ± 3.6 (63.5-75)	75.4 ± 4.2 (70.6-78)	67.3 ± 2.6 (62.9-71.4)	-
Distance from head to vulva	472 ±18.6 (455-497)	407 ± 19 (383-439)	431 ± 28 (402-497)	432 ± 11.4 (419-440)	440 ± 26 (411-488)	380-498
Tail length	10.4 ± 1.5 (8.4-11.6)	10.3 ± 0.4 (9.5-10.9)	12.8 ± 1.4 (10.6-15)	16.1 ± 1.5 (15.1-17.8)	14 ± 1.1 (12.9-15.5)	6-12
Anal body diameter	21.4 ± 15 (20-23.5)	20.5 ± 1.4 (17.7-22.5)	22±1,8 (19-24.4)	24.1 ± 2.8 (21.5-27)	24.6 ± 1.9 (21.5-27.9)	-
Maximum body diameter	32.5 ± 3.9 (30-38.4)	32.8 ± 2.5 (29.9-36.6)	30.6 ± 1.6 (28-34.4)	34.5 ± 3.1 (32.3-38)	33.9 ± 1.3 (31.1-35.5)	26-38
MB length from ant end	70.8 ± 1,1 (69.3-71.7)	66,7 ± 8,1 (55.9-86)	77.3 ± 3.1 (71-81.7)	80.8 ± 11.3 (68.2-90.3)	76.2 ± 3.5 (73.1-83.9)	61-84
NR length from ant. end	88.4 ±4.8 (84.3-95)	75.6 ± 8.6 (64.5-94.6)	93.9 ± 3.6 (88-99)	93.4 ± 9.8 (82.4-101)	93.1 ± 6.6 (86-105.4)	-
Pharynx lenght	109 ± 2.2 (108-113)	93.1 ± 10 (79.6-112)	111 ± 5 (105-120)	106.7 ± 9.8 (96.6-116)	113.3 ± 6.4 (105-125)	102-145
EP from anterior end	121 ± 4.8 (117-128)	101.4 ± 9.3 (84-118.3)	122 ± 4.5 (116-127)	121.1 ± 1.3 (120-123)	124.9 ± 4.8 (120-133)	100-135
Ant to pharynx gland end	138 ± 4.7 (131-142)	112.9 ± 12 (95-137.6)	136.7 ± 5.5 (131-148)	134.5 ± 2.7 (133-138)	135.8 ± 6.3 (129-146)	-
BD at MB	27 ± 7.4 (22-38)	21.6 ± 1.7 (19.4-24.7)	23.2 ± 1.7 (20.4-27)	25±1.5 (23.7-26.6)	25.5 ± 1.1 (24.7-27.9)	-
BD at pharynx end	28.2 ± 2.8 (24.6-36.9)	25.8 ± 3.6 (21.5-32.2)	26.7 ± 1.8 (23.7-29)	28.3 ± 1.6 (26.9-29.9)	27.4 ± 1.6 (25.8-30.1)	-
BD at overlap	29.7 ± 3.5 (27-34,9)	28.1 ±2.6 (23.4-32.2)	28.6 ± 1.5 (26-31.2)	29.8±1.4 (29-31)	29.5 ± 1.4 (27.9-31.2)	-
Medium bulb width	11.7 ± 0.3 (11.3-11.9)	11.4 ± 1.2 (10.3-13.7)	12.4 ± 0.9 (11-13.4)	12.6 ± 0.4 (12.2-13)	10.7 ± 0.9 (9.4-12.2)	10-11
Medium bulb height	13.4 ± 1.4 (12.1-15.1)	15.1 ± 2.1 (12.1-17.9)	14.5 ± 0.9 (13-16.3)	16.4 ±1.8 (14.6-18.3)	13.3 ± 0.9 (12.2-14.6)	14-15
Medium bulb valve height	3.2 ± 0.3 (2.9-3.7)	5.1 ± 1.1 (3.4-6.5)	3,6 ± 0.3 (3.3-4.1)	4.2 ± 0.6 (3.7-4.9)	3.2 ± 0.3 (2.8-3.7)	-
Medium bulb valve width	2.6 ± 0.1 (2.4-2.8)	2.8 ± 0.3 (2.3-3.4)	2.2 ± 0.3 (1.6-2.8)	3.3 ± 0.3 (3.1-3.7)	2.1 ± 0.2 (1.8-2.4)	-

DGO	3.7 ± 0.4 (3.2-4.2)	3.7 ± 0.7 (2.8-5.1)	3.8 ± 0.7 (3.3-5.3)	3.7 ± 0.9 (2.7-4.5)	3.7 ± 0.5 (3.3-4.5)	4 - 8
Stylet L	26.4 ± 1 (25.4-27.7)	25 ± 1.6 (22.2-27.4)	25.6 ± 1.2 (24.4-28)	25.3 ± 1.4 (24.3-26.8)	24.8 ± 1.5 (22.8-26.8)	21-28
Conus L	12.8 ± 0.7 (12.2-13.8)	11.6 ± 0.6 (11.1-12.8)	11.9 ± 0.6 (11-12.6)	11.4 ± 0.4 (11-11.8)	11.5 ± 1 (10.2-13)	-
shaft L	11.2 ± 0.5 (10.8-11.7)	10.6 ± 0.9 (8.6-11.9)	10.5 ± 0.5 (9.8-11.4)	10.7 ± 0.3 (10.4-11)	10.1 ± 0.4 (9.8-10.6)	-
Stylet knobs H	2.8 ± 0.3 (2.5-3.2)	3.1 ± 0.4 (2.4-3.4)	3.2 ± 0.5 (2.4-3.7)	3.3 ± 0.8 (2.4-4.1)	3.4 ± 0.5 (2.9-4.1)	2-4
Stylet knobs W	5.3 ± 0.6 (4.7-6)	5.4 ± 0.4 (4.9-5.9)	4.9 ± 0.4 (4.1-5.7)	5.6 ± 0.5 (5.1-6.1)	5.1 ± 0.5 (4.1-5.7)	4-6
BD at DGO	22.6 ± 3.7 (20.5-28.2)	17.8 ± 1.6 (14.6-2.3)	20.7 ± 1 (19.2-23)	21.1 ± 1.3 (20.3-22.6)	20.3 ± 0.9 (18.7-21.1)	-
BD at stylet	21.7 ± 3.9 (19.3-24.5)	17.1 ± 1.6 (14.6-19.5)	19.8 ± 1 (18-21.1)	19.3 ±0.6 (18.7-19.8)	18.6 ± 1.1 (17.3-20.3)	-
BD at conus	18.1 ± 2.9 (16.4-22.5)	14.3 ± 1.2 (12.2-15.9)	16.8 ± 0.9 (15-17.9)	16.7 ± 0.4 (16.3-17.1)	15.3 ± 0.5 (14.6-15.9)	-
Labial disc width	3.6 ± 0.6 (3.2-4.6)	3.9 ± 1 (2.8-5.7)	4.4 ± 0.6 (3.7-5.3)	3.5 ± 1 (2.3-4.1)	4.3 ± 0.4 (3.7-4.9)	-
Mid-lip width	9.2 ± 0.7 (8.4-10.2)	8.3 ± 1.1 (6.5-10.6)	9.8 ± 1.3 (7.7-13)	10.3 ± 1 (9.5-11.4)	9 ± 0.5 (8.5-9.8)	-
Basal lip width	11.5 ± 1 (10.6-12.9)	10.7 ± 1.4 (8.1-13)	12.5 ± 1.2 (10.6-15)	12.8 ± 0.4 (12.3-13)	11.6 ± 0.4 (11-12.2)	9-11
Lip region height	6.9 ± 0.1 (6.8-7)	7.1± 0.8 (5.9-8.6)	6.9 ± 0.8 (6.1-8.9)	6.3 ± 0.2 (6.1-6.5)	7.1 ± 0.6 (6.5-8.1)	4,5-6,5
Lateral field width	7.7 ± 07 (6.8-8.6)	7.2 ± 0.7 (6.4-8.6)	7 ± 0.4 (6.5-8.9)	6.9 ± 0.4 (6.5-7.3)	7.2 ± 0.5 (6.5-8.1)	5-7.5
Scutellum height	4.2 ± 05 (3.6-4.8)	3.9 ± 0.5 (2.8-4.7)	4.6 ± 0.5 (3.7-5.4)	5.1 ± 0.3 (4.8-5.3)	4.5 ± 0.4 (4.1-5.3)	3 – 5
Scutellum width	3.7 ± 0.4 (3.2-4.1)	3.7 ± 0.3 (3.4-4.3)	4.1 ± 0.5 (3.3-4.9)	4.4 ± 0.3 (4.1-4.6)	3.8 ± 0.6 (3.1-4.9)	-
Cut. annulus at lip region	0.8 ± 0.05 (0.7-0.8)	0.9±0.1 (0.8-1.2)	1.1 ± 0.15 (0.9-1.4)	0.8 ± 0.1 (0.7-0.9)	1.2 ± 0.1 (0.9-1.3)	-
Cut. annulus at vulva	1.2 ± 0.1 (1.1-1.3)	1.3 ± 0.1 (1.2-1.5)	1.5 ± 0.2 (1.2-1.8)	1.2 ± 0.1 (1.1-1.4)	1.4 ± 0.1 (1.2-1.5)	-
Cut. annulus at anus	0.8 ± 0.1 (0.7-1)	0.9 ± 0.2 (0.7-1.2)	1.5 ± 0.2 (0.8-1.2)	1.02 ± 0.02 (1.01-1.04)	1 ± 0.1 (0.81-1.22)	-
Epiptygma length	4.2 ± 0.5 (3.9-4.9)	3.6 ± 0.9 (1.1-4.4)	-	3.9 ± 0.3 (3.7-4.3)	-	-

(*****): Nematodes measurements compiled from Siddiqi & Sharma (1994), Van den Berg *et al.*(2003) and Mekete *et al.* (2008)

Scutellonema brachyurus (Steiner, 1938) Andrássy, 1958

Figure 5

MEASUREMENTS

See Table 4

DESCRIPTION

All populations found largely agree with the population from Sher (1964), which are syntypes, and with the population of Van den Berg (1973; 2013) from South Africa. The short descriptions provided below give the differences between the Rwandan populations.

Females

Population from Onion Nyakiriba, agro-ecological zone 5, sample ALN16, voucher slide UGnem-90

Body form ranging from spiral to an open C-shape after fixation (Fig. 5B), body length ranging from 619-816 µm, lip region generally high hemispherical, well to slightly set off with 3-5 annuli of 2.4 µm maximum width, labial framework moderate extending to end of first annulus anteriorly into the body (Fig. 5D), strong coarse cuticle with width of the annuli at vulva 2.3-2.7 µm, lateral field not areolated at midi-body and strongly areolated at scutellum level in different patterns (Fig. 5M), short stylet 24.3-27 µm long, metenchium slightly equal to telenchium with rounded basal knobs, spermatheca not developed, epiptygma folded into vagina not clear outside, tail rounded dorsally and pointed ventrally with 10-11 annuli (Fig. 5L), large scutellum situated below the anus.

Population from tomato Kabuye, agro-ecological zone 9, sample ALN12, voucher slide UGnem-95

Body form ranging from spiral, an open C-shape to curved ventrally after fixation (Fig. 4C), body length ranging from 788-857 µm, lip region high hemispherical separated from body by a slight constriction with 4-5 annuli of 0.8-1.3 µm width, labial framework weak some specimens not extending up to the end of the first annulus into the body (Fig. 5F), moderate coarse with width of the annuli at vulva 1.2-1.9 µm, lateral field with four lines, at mid-body for some specimens annulated, at scutellum level also some areolated other annulated, those areolation with different patterns (Fig. 4I), short to long stylet 23.6-30.5 µm, metenchium greater equal or small to telenchium stylet with oval knobs rounded posteriorly and some specimen slightly hollow anteriorly, oval spermatheca developed in some specimens filled with round sperms others empty, vulva without or with long to small epiptygma folded into the vagina, tail rounded

both side or only dorsally and the end pointed in some specimens with 11-13 annuli (Fig. 4H), scutellum oval of moderate size situated at or below anus.

Population from Potato Bushoki, agro-ecological zone 6, sample ALN20, voucher slide UGnem-94

Body form ranging from spiral to open C-shape upon heating, body length short ranging from 676-728 µm, lip region hemispherical slightly set off form the rest of the body with 3-4 annuli of 1.6-2.4 µm width, strong labial framework extending up to the second annulus into the body (Fig. 5G), moderate to strong coarse cuticle with annuli width ranging from 1.9-2.9 µm at vulva, lateral field with 4 lines, the field at mid body not areolated but areolated at the scutellum level with different patterns (Fig. 5O), stylet short of 22-25.7 µm long, metenchium greater or small to telenchium stylet knobs rounded to oval flattened on anterior surface and concave on the posterior side with dorsal esophageal gland opening fall from the posterior to base of stylet 4.9-6.1 µm, oval spermatheca developed in some specimens but empty, epiptygma folded into the vagina not clear outside, tail rounded to pointed at the tip with 7-9 annuli (Fig. 4N). Scutellum oval of small size situated below anus.

Population from banana Kivumu, agro-ecological zone 3, sample ALN18, voucher slide UGnem-96

Body form ranging from an open C shape, closed 6 or complete circle after fixation (Fig. 4A), body length long ranging 728-805 µm, lip region low to high, flat to hemispherical anterior (Fig. 5E) strongly to slightly set off from the rest of the body, with thin 5-7 lip annuli , moderate cephalic framework extending up to the second annulus into the body, moderate coarse cuticle with annuli width ranging from 1.4 to 2.4 µm at vulva , lateral field areolated at mid body for some specimens and areolated for all specimen at scutellum level with different patterns (Fig. 5K), stylet long 24.4-28.4 µm, metenchium longer than telenchium, stylet knobs flattened or forming a cup shaped anteriorly and rounded posteriorly, spermatheca not developed, vulva with epiptygma not projecting outside but folding into the vagina, tail rounded on both side or dorsally (Fig. 4J), scutellum slightly oval of moderate size situated at or below anus.

Male

Only one specimen found in this population and was similar to female apart from the reproductive system.

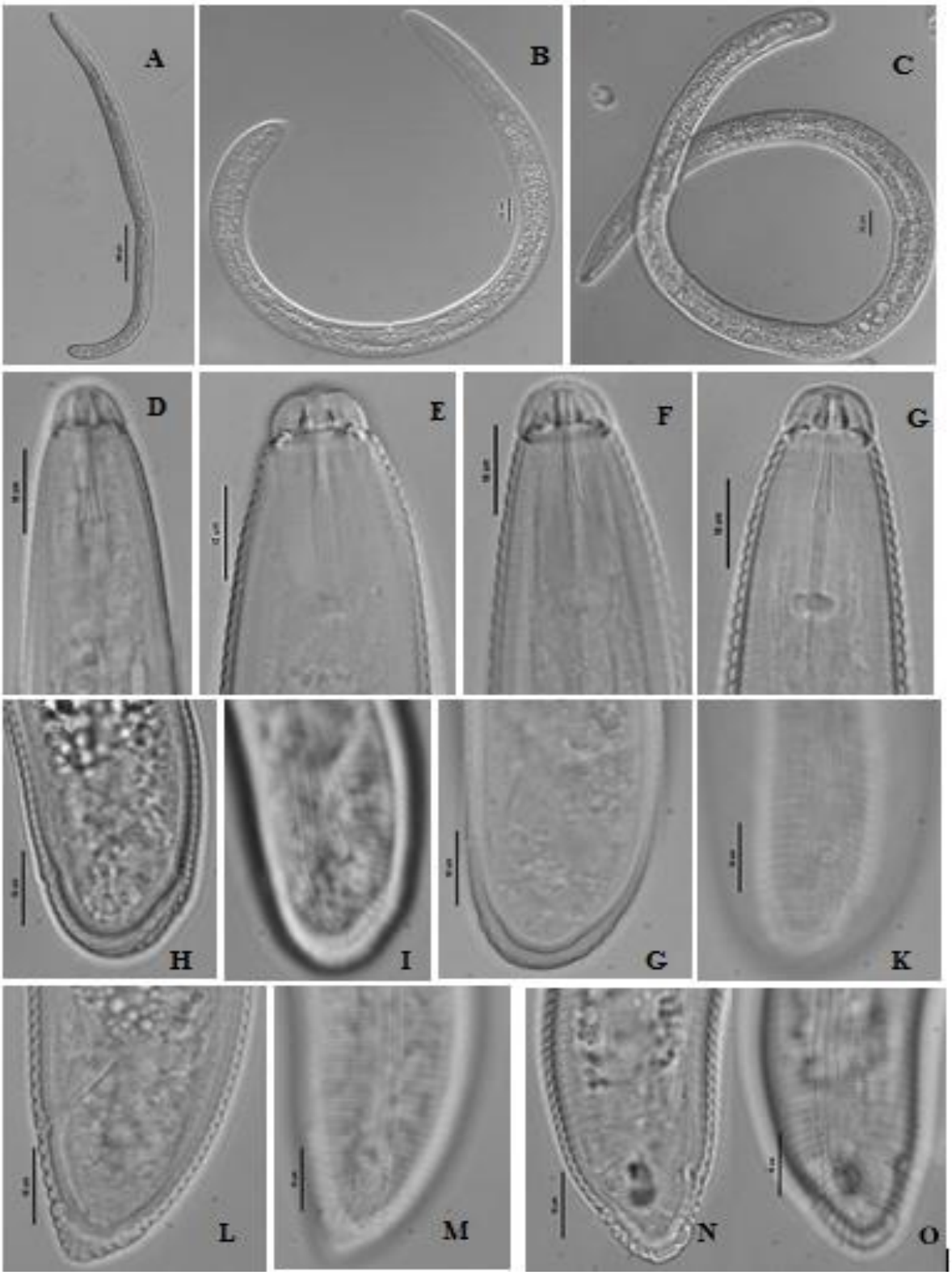


Fig. 5. Photomicrographs of *Scutellonema brachyurus*. A, B, C: Female entire body after fixation; D-G: Head region; I, K, M, and O: Lateral lines at scutellum level; H, G, L, N: Females

tails. A, E, G, K from ALN18 sample; N, O, G from ALN20; C, F, H, I from ALN12 sample; B, D, L, M from ALN 16 sample. (Scale bars: A = 100 *µm*, B-C = 20 *µm*, D-N = 10 *µm*)

Table 6. Different *Scutellonema brachyurus* populations measurements from 4 different localities. All measurements are in µm and in the format: mean ± s.d. (range).

	Sampled crop, code and locality				
Characters	Oignon ALN16 Nyakiriba	Tomato ALN12 Kabuye	Potato ALN20 Bushoki	Banana ALN18 Kivumu	*S. brachyurus* from the literature*
n	7	12	6	10	
L	686 ± 60.6 (639-816)	823 ± 22 (788-857)	709 ± 18.9 (676-728)	767 ± 26.5 (728-805)	500-1000
a	21.7 ± 1 (20.8-23.3)	24.1 ± 1.9 (21.5-26.5)	35.2 ± 3 (32.2-39.4)	31.3 ± 4.2 (23.1-35.6)	16.7-36
b	6.7 ± 0.5 (6.1-7.7)	7.1 ± 0.5 (6.4-8.1)	6.7 ± 0.3 (6.3-7.1)	7.2 ± 0.5 (6.5-7.8)	4.9-10.3
b'	5.3 ± 0.5 (4.8-6.2)	5.8 ± 0.4 (5.3-6.5)	5.6 ± 0.1 (5.5-5.9)	5.9 ± 0.3 (5.4-6.3)	4-9.1
c	42.7 ± 8.6 (35-60.9)	44 ± 3.8 (35.8-48)	45.1 ± 6.1 (35.7-53.7)	40.4 ± 6.1 (32.1-51.1)	37.2-127.7
c'	0.7 ± 0.1 (0.6-0.8)	0.7 ± 0.07 (0.6-0.8)	0.8 ± 0.08 (0.7-0.9)	0.8 ± 0.08 (0.7-0.9)	0.4-1
V	57.6 ± 0.8 (56.4-59)	56.6 ± 2.6 (52.3-59.6)	55.4 ± 1.4 (53-56.8)	57.5 ± 2.1 (54.6-61.4)	51-67
m	45.9 ± 1.1 (44.5-47.8)	45.5 ± 0.8 (44-47.2)	43.9 ± 1.1 (42.4-45.5)	45.7 ± 1.9 (42.9-48.6)	43-53
o	17.4 ± 1.8 (13.6-19.1)	17.1 ± 1.9 (13.4-20)	22.1 ± 1.6 (19.8-23.9)	15.9 ± 2.3 (12.8-20)	6.8-37.5
S	1.4 ± 0.1 (1.2-1.5)	1.6 ± 0.1 (1.3-1.7)	1.4 ± 0.1 (1.2-1.6)	1.4 ± 0.2 (1.2-1.6)	-
MB	64.5 ± 1.9 (62.3-68.6)	69.9 ± 3.2 (64.4-74)	68.5 ± 3.1 (65.3-73.9)	77.5 ± 4.5 (71.2-85)	-
Distance from head to vulva	395 ± 35.3 (360-469)	466 ± 32.4 (413-506)	391 ± 11.8 (377-402)	441 ± 23.9 (402-479)	-
Tail length	16,3 ± 1.8 (13.4-19.2)	18.8 ± 2.1 (17.2-23.7)	15.9 ± 2.1 (13.4-19.4)	19.3 ± 2.9 (15.1-24.7)	7-23
Anal body diameter	21.4 ± 1.1 (20.5-23.7)	25.6 ± 3.1 (21.5-32.3)	20.2 ± 1.4 (18.3-21.9)	24.1 ± 2.6 (21.5-29)	14-24.5
Maximumum body diameter	31.6 ± 3.3 (29.3-38.8)	34.3 ± 2.5 (30.1-38.7)	29.6 ± 1.1 (27.9-31.2)	32.1 ± 2.4 (29-36.6)	19.5-38
MB length from ant end	66.2 ± 3.5 (62.4-72.8)	80.7 ± 4.4 (73.1-86)	71.8 ± 2 (68.8-74.2)	82.1 ± 5.4 (73.1-91.4)	-
NR length from ant. end	83.2 ± 4.7 (77.4-91.8)	99.3 ± 8.2 (86-109.6)	86.3 ± 2.5 (83.8-90.3)	93.8 ± 5.5 (83.9-101.1)	-

Pharynx length	102.5 ± 2.8 (100.2-106.7	115.6 ± 7.5 (98.9-126.9)	105 ± 4.4 (99-109.7)	106 ± 4.6 (101.1-114)	104-165
Excretory Pore from ant. end	114.9 ± 8.8 (107-131)	128.7 ± 7.2 (111.8-137.6)	117.2 ± 3.8 (112-123)	118 ± 4.7 (112-124.7)	79-150
Ant.end to Phary.gland end	129 ± 10.3 (113.4-144)	141.2 ± 8.2 (122.6-152.7)	125.4 ± 2.6 (122.6-19)	129.5 ± 4.2 (122.6-138)	-
BD at MB	24.5 ± 2.7 (22.2-30)	21.8 ± 1.8 (19.4-25.8)	22.2 ± 1.3 (20.4-23.7)	23.3 ± 1.8 (21.5-26.7)	-
BD at pharynx end	26 ± 3.4 (22.9-33.3)	25.8 ± 3.6 (21.5-32.2)	25.4 ± 1.7 (23.7-28)	26.3 ± 1.9 (23.6-29)	-
BD at overlap	28.7 ± 4.1 (23.7-35.9)	28.1 ± 2.6 (23.4-32.2)	27.4 ± 1.6 (25.8-30.1)	28.5 ± 1.8 (25.8-31.2)	-
Medium bulb width	11 ± 0.7 (10.1-12.1)	10.8 ± 2 (7.7-15.4)	11.3 ± 0.8 (10.6-12.2)	12.1 ± 0.8 (10.6-13)	6.5-12.5
Medium bulb height	13.7 ± 0.8 (12.8-14.6)	14.2 ± 1.9 (12.2-18.6)	3.5 ± 0.3 (12.6-16.3)	14.9 ± 1.1 (13-16.7)	11-15.5
Medium bulb valve length	3.3 ± 0.5 (2.8-4.2)	3.6 ± 0.4 (3.3-4.5)	3.5 ± 0.3 (3.3-4.1)	4.6 ± 0.5 (3.7-5.3)	2-5.4
Medium bulb valve width	2.1 ± 0.2 (1.8-2.5)	2 ± 0.3 (1.6-2.4)	2.4 ± 0.4 (2-2.8)	2.9 ± 0.4 (2.4-3.7)	2-4
DGO	4,4 ± 0.4 (3.7-4.85)	4.8 ± 0.6 (4.1-5.7)	5.4 ± 0.5 (4.9-6.1)	4.2 ± 0.6 (3.3-4.9)	3-7
Stylet L	25,3 ± 1 (24.3-27)	28 ± 1.7 (23.6-30.5)	24.2 ± 1.6 (22-25.7)	26.7 ± 1.6 (24.4-28.4)	21-32
Conus L	11.6 ± 0.6 (11.1-13)	12.8 ±0.8 (10.4-13.7)	10.6 ± 0.9 (9.4-11.4)	12.2 ± 0.9 (10.6-13.8)	11.5-15.5
Shaft L	11.1 ± 0.7 (10.6-12.6)	12.4±0.8 (10.2-13.6)	10.2 ± 0.6 (9.2-10.9)	11.3 ± 0.9 (9.8-13)	13-16.5
Stylet knobs H	2.8 ± 0.3 (2.4-3.2)	2.9 ± 0.33 (2.4-3.3)	3.1 ± 0.4 (2.4-3.7)	3.4 ± 0.6 (2.9-4.9)	2-4
Stylet knobs W	4.4 ± 0.6 (3.6-5.2)	5.1 ± 0.6 (4.5-6.5)	5.2 ± 0.4 (4.9-5.7)	6.1 ± 0.7 (4.9-7.3)	5-7
BD at DGO	19.8 ± 1.7 (18.6-23.3)	17.7 ± 0.8 (16.3-18.7)	19 ± 1.5 (17.1-21.1)	20.3 ± 1.6 (17.9-22.8)	-
BD at stylet	18.6 ± 1.8 (17.2-22.3)	17 ± 0.9 (15.9-18.7)	17.2 ± 1.1 (15.9-18.3)	19 ± 1.5 (17.1-21.1)	-
BD at conus	14.7 ± 1.3 (13.1-16.9)	14.8 ± 1 (13.4-17.1)	14.2 ± 0.7 (13.4-15.5)	16.5 ± 1.4 (14.2-17.9)	-
Labial disc width	3.2 ± 0.5 (2.7-4)	3.3 ± 0.7 (2.4-4.6)	3.9 ± 0.6 (3.3-4.9)	4.5 ± 0.4 (3.7-4.9)	-
Mid-lip width	8.6 ± 0.4 (8-9.2)	7.6 ± 1 (5.6-9.3)	8.5 ± 0.6 (7.7-10)	10 ± 1.2 (8.5-1.8)	-
Basal lip width	10.7±0.3 (10.3-11.2)	10.2 ± 1.1 (8.5-12.6)	10.4 ± 1 (10-11.4)	12.2 ± 1.3 (10.6-13.8)	7.5-11
Lip region height	6.4 ± 0.3 (6-6.9)	5.4 ± 0.5 (4.9-6.1)	5.9 ± 0.6 (5.3-6.5)	6.3 ± 0.8 (4.9-7.3)	4-6.5

Latteral field width	7.4 ± 0.6 (6.3-8.1)	7.2 ± 0.5 (6.5-8.1)	7.2 ± 0.3 (6.9-7.7)	7.1 ± 0.4 (6.5-7.7)	4.5-7.5
Scutellum Height	4.8 ± 0.4 (4.2-5.5)	3.1 ± 0.3 (2.8-3.3)	3.7 ± 0.3 (3.3-4.1)	4.4 ± 0.4 (3.7-4.9)	3-6
Scutellum width	3.6 ± 0.3 (3.3-3.9)	3.6 ± 0.3 (3.3-4.1)	3.2 ± 0.3 (2.9-3.7)	3.7 ± 0.3 (3.2-4.1)	2.5-5
Cuticle annulus at lip region	1.9 ± 0.4 (1.4-2.4)	1.1 ± 0.2 (0.8-1.3)	2.1 ± 0.3 (1.6-2.4)	0.9 ± 0.3 (0.5-1.2)	-
Cuticle annulus at vulva	2.4 ± 0.2 (2.3-2.7)	1.6 ± 0.2 (1.2-1.9)	2.4 ± 0.4 (1.7-2.9)	1.75 ± 0.3 (1.4-2.2)	-
Cuticle annulus at anus	1.9 ± 0.1 (1.8-2.2)	1.3 ± 0.2 (0.9-1.6)	1.7 ± 0.2 (1.6-2.2)	1.3 ± 0.3 (0.9-1.8)	-

(***) :** Nematodes measurements compiled from Steiner (1938), Andrássy (1958), Sher (1964), Ali *et al.* (1973), Van den Berg (1973), Siddiqi (1974), Van den Berg (1998), Van den Berg *et al.* (2013)

Scutellonema cavenessi (Sher, 1964)

Figure 6

MEASUREMENTS

See Table 4

DESCRIPTION

Both populations described largely agree with the population from cotton of USA and Congo described by Elmiligy (1970) and population from coffee of South Africa population provided by Van den Berg (1973). The short descriptions mentioned below are the difference between the Rwandan populations.

Population from Onion Runda, agro-ecological zone 7, sample ALN 17, voucher slide UGnem-91

Female

Body form open C (Fig. 5B) to a closed spiral shape upon heating, body length ranging from 709-759 µm, coarse cuticle with annuli width ranging from 2.1-2.4 µm at vulva, lateral field areolated anterior, at midi-body and at scutellum level (Fig. 5J). Lip region high hemispherical flattened anteriorly deep set off from the rest of the body with fine 5-7 lip annuli, strong labial framework extending up to the third annulus into the body (Fig. 5E). Stylet well-developed with metenchium almost of the same length to the telenchium with rounded to flattened anteriorly

knobs, oval spermatheca with full rounded sperms, tail rounded with 8-9 annuli (Fig. 5K), big rounded scutellum of 4.1-5 µm situated above the anus (Fig. 5J)

Male

Similar to female apart from the reproductive system, presence of lobed caudal alae and a shorter stylet.

Population from carrot Runda, agro-ecological zone 7, sample ALN7, voucher slide UGnem-93

Female

Body open C (Fig. 5A), body length ranging from 674-855 µm, moderate to strong coarse cuticle with annulus width ranging from 1.2-2.6 µm at vulva, lateral field areolated the whole body from anterior, at mid body to scutellum level (Fig. 5K). Lip legion high dome-shaped deeply set off from the rest of the body with 5-7 lip annuli, strong labial framework extending up to the fourth annulus inside the body (Fig.5D). Stylet moderate to well developed with metenchium almost of the equal size with the telenchium stylet with knobs rounded, spermatheca rounded to oval with or without sperms, tail rounded on both side or dorsally with 9-11 annuli (Fig. 5G), big rounded scutellum of 3.2-5 µm situated at the anus (Fig. 5K).

Male

Similar to female apart from the reproductive system, lobed caudal alae and shorter stylet 20.3-23 µm compared to 26.5-28.9 µm in female (Fig.5 C, F, I, L) .

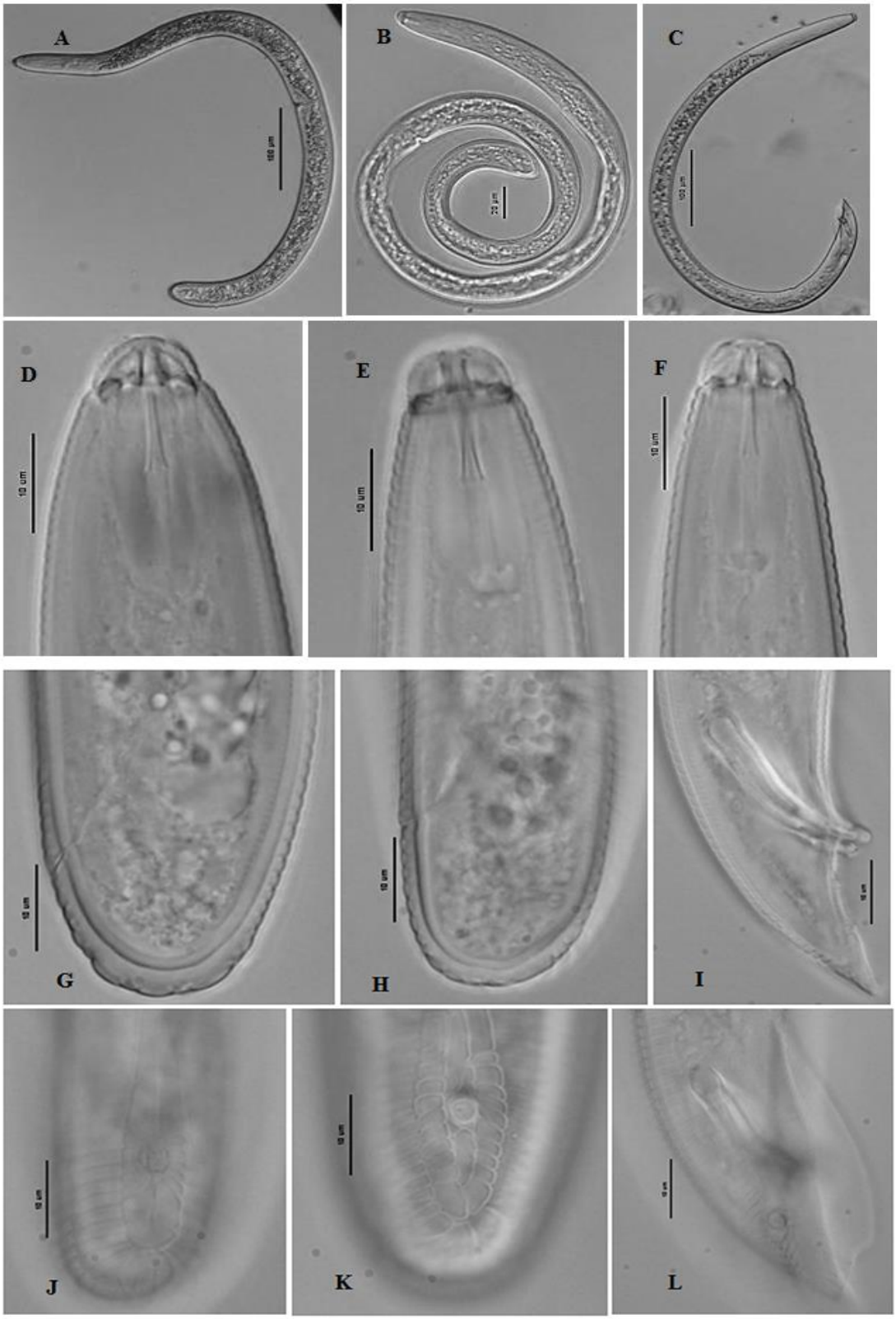


Fig. 6. Photomicrographs of *Scutellonema. cavenessi*. A, B: female entire body after fixation, C: male entire body after fixation; D, E: Female head; F: Male head; G, H: Female tail; I: Male tail;

J, K: female lateral lines at scutellum level; L: male scutellum. A, D, G, K from ALN17 females; B, E, H, J: From ALN7 females, C, F, I, L from ALN7 males. (Scale bars: A, C = 100 *µm*, B: 20 *µm*, D-I = 10 *µm*)

Table 7. Different *Scutellonema cavenessi* populations measurement. All measurements are in µm and in the format: mean ± s.d. (range).

	Sampled crop, code and locality					
	Onion ALN17 Runda		Carrot ALN7 Runda		*S. cavenessi* from the literature	
Characters	Female	Male	Female	Male	Female	Male
n	4	1	11	5	-	-
L	732 ± 21.2 (709-759)	769	775 ± 60.4 (674-855)	713 ± 34 (674-761)	600-900	500-800
a	22 ± 2.1 (19.9-24.8)	36	23.9 ± 3 (18.7-28.8)	33.3 ± 5,5 (28.7- 42)	17-30	17.9-29.4
b	9 ± 1.7 (6.8-10.8)	8.8	7.5 ± 0.9 (5.9-9.2)	6.8 ± 0.7 (6.3-8.1)	5.2-8.4	5.8-8.2
b'	6.7 ± 0.9 (5.5-7.5)	6.5	5.9 ± 0.7 (4.7-6.9)	5.4 ± 0.7 (4.7-6.4)	4.5-7.3	4.6-6.5
c	28.6 ± 1.9 (26.5 - 30.9)	41	51.9 ± 0.7 (35.4-74.8)	40.4 ± 1.6 (38.5-42.7)	27-57	22.1-55.2
c'	0.7 ± 0.16 (0.5-0.8)	0.9	0.7 ± 0.14 (0.5-0.9)	0.8 ± 0.2 (0.7-1.1)	0.57-1.02	1.2-1.9
V	55.6 ± 2.3 (52.6-58)	-	57.6 ± 2.2 (52.2-61.2)	-	52-62	-
m	50.1 ± 4 (45.5-55)	51	44.4 ± 3.5 (37.7-50)	47.6 ± 3 (43.5-51)	44.2-49	46.1-51
o	13.8 ± 1.1 (12.3-14.8)	16	18.5 ± 3.6 (12.7-23.7)	18 ± 5.6 (13.9-27.5)	28-34.5	20-33.3
S	1.3 ± 0.2 (1.1-1.6)	1.1	1.3 ± 0.2 (1.1-1.6)	1.2 ± 0.1 (1.1-1.3)	-	-
MB	71.6± 1.2 (70-72.7)	70	67.9 ± 4.3 (61.9-74)	66 ± 5.6 (61-75.3)	-	-
Distance head- vulva	406 ± 15 (393-428)	-	447 ± 38 (367-502)	-	-	-
Tail length	18 ± 4.5 (13.2-23.1)	18.8	15.4 ± 2.9 (10.5-19.8)	17.7 ± 1.3 (16-19)	23.5-31	-
Anal body diameter	25.7 ± 1.2 (24.7-27.3)	21.5	21.9 ± 1.8 (19-24.4)	21.8 ± 3.5 (17-26.5)	-	-
Max.body diameter	3.4 ± 2.3 (30.6-35.8)	34.9	32.6 ± 2.8 (29.1-37.6)	29.3 ± 3.1 (26.3-32.9)	-	-
Ant end to MB	59.7 ± 11 (50-75.3)	60.7	71 ± 10.9 (52.6-88.6)	69.5 ± 3.4 (65.4-73.2)	-	-

NR length from ant. end	68.9 ± 12 (59.3-86)	75.4	89 ± 11.8 (68.8-107)	88 ± 4.5 (80.5-92.6)	-	-
Pharynx lenght	83.5 ± 16.7 (70-107.5)	87.2	104.6 ±15.1 (76-127)	105.9 ± 10 (88.9-116)	-	-
EP from anterior end	93.3 ± 15.9 (81.7-116)	98	114.8 ± 13 (93.8-134)	115 ± 7 (105-124)	95-115	-
Ant end to Phary. gland L	110.2 ± 16 (98-133.3)	117.6	134 ± 19 (103-158.4)	133 ± 13.4 (111-143)	-	-
BD at MB	25.3 ± 3.3 (21.4-28.2)	27.8	27.9 ± 6.5 (18.6-35.7)	21.3 ± 2.3 (17.9-24.3)	-	-
BD at pharynx end	28 ± 3.6 (23.4-32.3)	31.6	24 ± 2.4 (20.1-28)	24.4 ± 2.9 (19.7-27.2)	-	-
BD at overlap	29.6 ± 3.8 (24.4-33.3)	33	25.9 ± 2.5 (22.2-29.4)	25.7 ± 2.9 (21.3-28.1)	-	-
Medium bulb width	11.3 ± 1.7 (9.6-13.8)	13,3	27.8 ± 3.2 (22.8-31.8)	10.5 ± 0.7 (9.5-11.5)	-	-
Medium bulb height	14 ± 2.6 (12-17.8)	15.3	11.2 ± 1.3 (8.7-13.1)	13.8 ± 0.7 (12.6-14.3)	-	-
Medium bulb valve Height	3.9 ± 2.6 (2.9-6.5)	3.16	13.7 ± 1.3 (11.3-15.5)	3.3 ± 0.8 (2.6-4.6)	-	-
Medium bulb valve weight	3.4 ± 0.6 (2-3.3)	2.32	3.1 ± 0.3 (2.6-3.6)	2.2 ± 0.6 (1.7-3.1)	-	-
DGO	3,5 ± 0.1 (3.3-3.6)	3.84	4.6 ± 1 (2.8-6)	3.6 ± 0.8 (2.9-4.8)	-	-
Stylet L	27.3 ± 1.1 (26.5-29)	23.6	25.2 ± 2 (22.6-29)	21.5 ± 1.2 (20.3-23)	20.6-28	20.6-25.5
Conus L	12.5 ± 0.6 (12-13.2)	11.93	11.2 ± 0.8 (10.1-12.9)	9.4 ± 0.6 (8.8-10.3)	-	-
Shaft L	12.1 ± 0.7 (11.4-13)	8.91	11.1 ± 0.8 (10.1-12.6)	9.5 ± 0.7 (8.8-10.3)	-	-
Stylet knobs H	2.7 ± 0.4 (2.6-3.3)	2.27	3 ± 0.7 (1.9-4)	2.3 ± 0.1 (2.2-2.5)	-	-
Stylet knobs W	4.3 ± 0.6 (3,5-4.9)	3.72	4.8 ± 0.9 (3.5-5.9)	3.6 ± 0.2 (3.2-3.8)	-	-
BD at DGO	22 ± 2.5 (19-24.4)	22	4.8 ± 0.9 (3.5-5.9)	18 ± 1.4 (15.8-19.3)	-	-
BD at stylet	21.3 ± 2.1 (19-23.5)	20.38	19.8 ± 2.1 (15.8-22.1)	17.2 ± 1.6 (14.4-18.6)	-	-
BD at conus	17.9 ± 1.4 (16.3-19)	15.78	18.8 ± 1.9 (15.2-21.6)	14.5 ± 1.4 (12.4-16)	-	-
Labial disc width	3.5 ± 1.5 (2.3-5.7)	2.84	15.5 ± 1.3 (13.2-17.4)	3.1 ± 0.3 (2.6-3.4)	-	-
Mid-lip width	9.8 ± 1.1 (8.8-11.4)	8.78	3.4 ± 0.4 (2.7-4.1)	8.9 ± 0.4 (8.5-9.3)	-	-
Basal lip width	11.3 ± 1.5 (10-13)	10.27	10.2 ± 1.3 (8-11.7)	9.7 ± 1.4 (7.4-10.9)	-	-

Lip region height	5.7 ± 1.1 (4.9-7.3)	5.48	5.9 ± 0.9 (4.8-7.7)	5.6 ± 0.6 (4.9-6.4)	-	-
Lateral field width	7.2 ± 0.6 (6.7-7.9)	7.08	6.8 ± 0.6 (6.2-7.8)	5.8 ± 0.8 (4.7-6.7)	-	-
Scutellum height	4.6 ± 0.4 (4.1-5)	4.65	4.3 ± 0.3 (3.8-5)	3.6 ± 0.1 (3.5-3.8)	-	-
Scutellum width	4.3 ± 0.4 (3.9-4.7)	4.44	3.7 ± 0.4 (3.2-4.3)	3.3 ± 0.2 (3.1-3.5)	-	-
Annulus at lip region	0.7 ± 0.2 (0.6-1)	0.85	1 ± 0.2 (0.7-1.5)	0.7 ± 0.1 (0.6-0.9)	-	-
Annulus at vulva	2.2 ± 0.2 (2.1-2.4)	-	1.8 ± 0.4 (1.2-2.6)	-	-	-
Annulus at anus	1.8 ± 0.2 (1.5-2)	1.89	1.3 ± 0.3 (0.9-1.9)	1.5 ± 0.3 (1.1-1.8)	-	-
Spermatheca height	18.6 ± 0.8 (18-19.3)	-	16.4 ± 3.5 (11.7-22.3)	-	-	-
Spermatheca width	17.4 ± 5.6 (14-23.9)	-	13.4 ± 2.4 (9.8-22.3)	-	-	-
Spicule lenght	-	27.9	-	30.8 ± 2.6 (26.6-33.2)	-	23.5-35
Spicule width	-	5.11	-	3.9 ± 0.5 (3.4-4.5)	-	-
Gubernaculum lenght	-	14.02	-	13.4 ± 1.7 (11.4-15.7)	-	7-17

(*): Nematodes measurements compiled from Elmiligy (1970), Sher (1964) and Van den Berg (1973)

MOLECULAR CHARACTERIZATION OF SCUTELLONEMA SPECIES

D2D3 expansion segment of ribosomal DNA

The polymerase chain reaction amplified a single DNA product. For seven different *Scutellonema* specimens the sequences generated for them were of approximately *ca* 580 bp (461-700) based on the good quality fragments sequenced. After sequences alignment and elimination of poorly aligned position 667 sites were selected for 32 species sequences aligned and 397 of the sites were selected without gaps of which 177(44.6%) were variable and 129 (32.5%) parsimony informative. The nucleotide differences between sequence of the *S. brachyurus* generated during this study (AL9) and the corresponding one in the gene bank used for the analysis were 8.1-9.5% for *Scutellonema brachyurus* type A, 5-6.6% for the *Scutellonema brachyurus* type B and 7.8% for *Scutellonema brachyurus* type D. The identity of the other *S. brachyurus* (AL10 and AL35) populations, as identified morphologically, could not confirmed

molecularly, since they were not member of the *S. brachyurus* clade. *S. paralabiatum* (AL30 and AL34) showed a considerable intraspecific variation .On 464 sites selected 25 nucleotides (5.5%) were divergent, no indels were found. *For S. cavenessi* (AL31 and AL26) intraspecific variation, out of 469 sites selected, 17 nucleotides (3.6%) were divergent, no indels.

Inter-specific variability between species done by pairwise comparison is pronounced. For the inter-specificity between *S. paralabiatum* (AL34) and *S. brachyurus* (AL9) on 527 sites selected 86 nucleotides (16.6%) were divergent. For *S. paralabiatum* (AL34) and *P. cavenessi* (AL26) out 532 sited selected 86 nucleotides (16.8%) were divergent. On 676 sites selected between *P. brachyurus* (AL9) and *P. cavenessi* (AL26) 89 nucleotides (13.9%) were divergent.

Based on the majority rule 50% consensus tree topology the phylogenetic relationships between different *Scutellonema* species are specified in Fig. 7. Three clades (I (A and B); II and III) are presented in roman number on the tree and letter for the subclades. The first with two subclade (A and B). The first subclade not very well supported with 75% of PP comprising *S. brachyurus* type A and *S. brachyurus* type B where AL9 (Rwandan population) is found, the second subclade with *Scutellonema* spp. type A, *Scutellonema* spp. type B and the contradicted morphological and molecular data for the two specimens identified as *S. brachyurus* morphologically, the second clade with the two identified *S. paralabiatum* from Rwanda. The third a moderately-supported clade (94% PP) with species *S. bradys*, *S. cavenessi* and other no identified *Scutellonema* sp. D from Burkina Faso.

COI of mitochondrial DNA

The polymerase chain reaction amplified a single DNA product. Ten sequences for four different species of *Scutellonema* generated were of approximately 342-443 bp based on the good quality fragments sequenced. They were used for were used for phylogenetic analysis together with 16 other sequences taken from the gene bank. After multiple sequences alignment and elimination of poorly aligned position, 394 sites were selected for the 32 species aligned of which 292 were complete without gap or N, 180 (61.6%) variable and 149 (51%) parsimony informative.

Intraspecific variability comparison between the two sequences of *S. brachyurus* generated (AL6 and AL7) was almost insignificant with only one nucleotide (0.2%) difference out of 431 sites selected. The nucleotide differences between sequences of the *S. brachyur*us generated during

this study and the corresponding one in the gene bank used for the analysis were 13-14.2% for *Scutellonema brachyurus* type A and 1.3-6.9% for the *Scutellonema brachyurus* type B. The intraspecific sequences variability comparison after aligning and elimination of poorly aligned position on 441 selected sites 38 nucleotides (10.7%) were diverging and 30 nucleotides (8.5%) were parsimony informative for *S. paralabiatum* species (AL2, AL4, AL8, AL30 and AL34). A pairwise comparison of the two *S. paralabiatum* sequences (AL2 and AL4) indicated a divergence of 29 nucleotides (6.7%) on 430 sites selected without indels. For *S. cavenessi* (AL1 and AL31) showed a divergence of 5 nucleotides (1.5%) on 343 nucleotides selected and one indel.

Pairwise comparison of interspecific sequences variability showed a prominent divergence. Comparison between *S. brachyurus* (AL6) and *S. paralabiatum* (AL8) on 430 sites selected after elimination of poorly aligned position 63 nucleotides (14.7%) and 2 indels was found. For *S. paralabiatum* (AL2) and *S. cavenessi* (AL1) on 430 sites selected 76 nucleotides (17.7%) were divergent and no indels found. Comparison between *S. brachyurus* (AL7) and *S. cavenessi* (AL31) on 348 sites selected 80 nucleotides (23.4%) were divergent and 6 indels.

Based on the majority rule 50% consensus tree topology generated for phylogenetic relationships analysis is presented in (Fig. 8). There are three clades presented in roman numbers on the tree (I; II and III). The first clade with two subclades the first with *S. brachyurus* type A, *Scutellonema* sp. A in a moderately supported clade of 92% PP; the second subclade with *S. brachyurus* type B including *S. brachyurus* specimens AL6 and AL7 from Rwanda, the contradicted morphological and molecular data for specimen identified as *S .brachyurus* morphologically and *Scutellonema* sp. B. the clade is poorly supported with 51% of PP. The second clade with 97% PP support contains *S. paralabiatum* from Rwanda and Ethiopia. The third clade is not well supported (67% of PP) and contains *Scutellonema* sp. D from Burkina Faso, *S. cavenessi* and *S. bradys.*

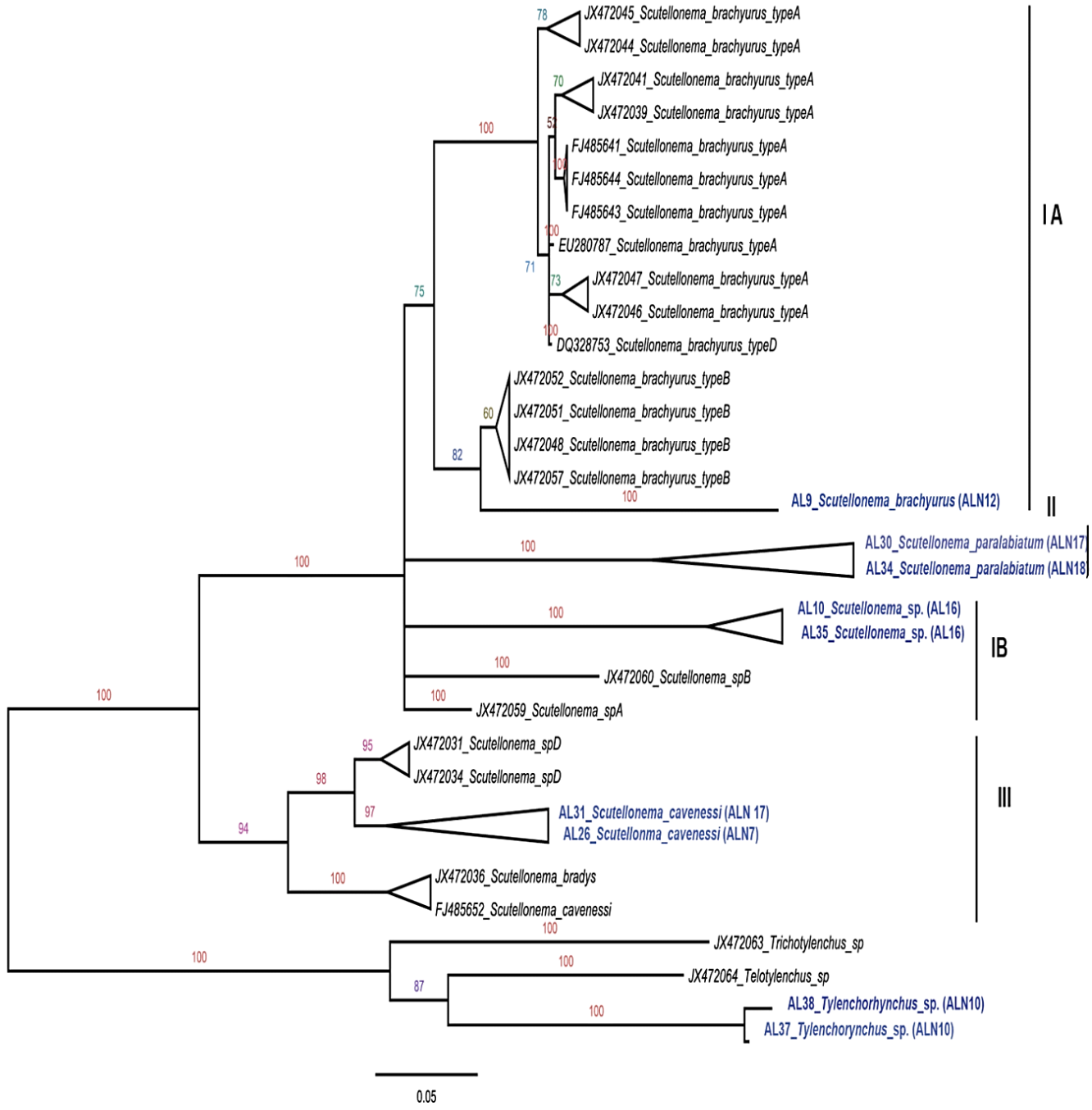


Fig. 7. Bayesian 50% majority rule consensus phylogenetic relationships tree of *Scutellonema* species based on the D2D3 expansion segment of rDNA sequences. Sequences alignments under GTR+I+G model. Posterior probabilities of more than 50% given for appropriate clade. Original generated sequences in this study are highlighted in different blue color. Roman numbers I, III, IV, V and VI indicate major clades as they were found by Subbotin *et al.* (2008). Letters A and B relate to nodes representing subclades individually resolved.

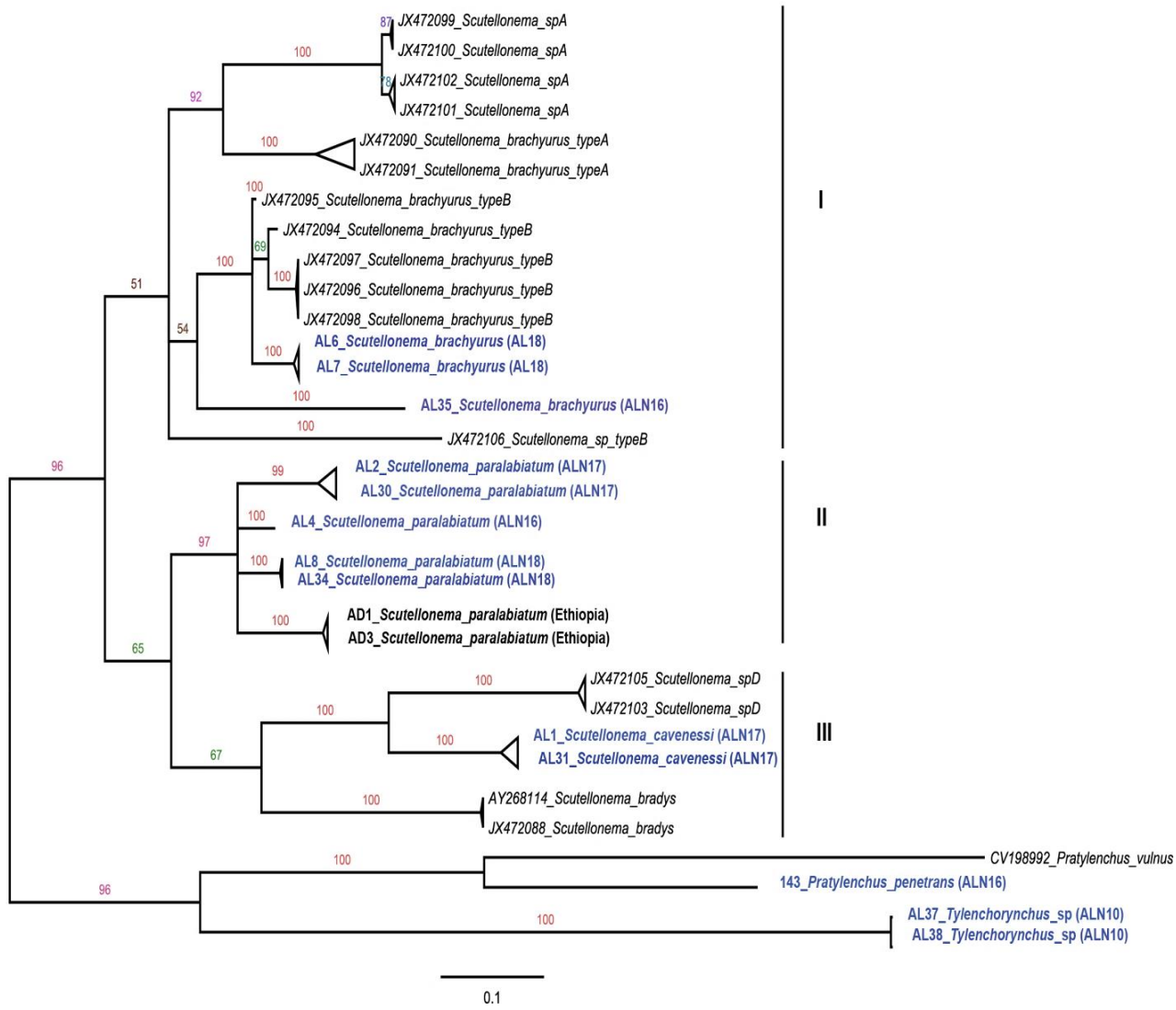


Fig. 8. Bayesian 50% majority rule consensus phylogenetic relationships tree of Scutellonema species based on COI of mitochondrial DNA sequences. Sequences alignment under GTR+I+G model. Posterior probabilities of more than 50% given for appropriate clade. Roman numbers I, III, IV, V and VI indicate major clades.

MORPHOLOGICAL CHARACTERIZATION OF *PRATYLENCHUS* FILIPJEV, 1936 SPECIES

***Pratylenchus* n. sp.**

(Figs 9-11)

MEASUREMENTS

See Table 2.

DESCRIPTION

Female

Body showing various postures when killed by heat, from straight, slightly curved ventrally, C-shaped to irregularly shape. Cuticle annuli at lip region, vulva and anus with 0.5-1.3 µm, 0.99-1.75 µm, and 0.95-1.93 µm wide respectively. Lateral field diameter occupying one to two quarters of the body diameter, marked by two to four incisures at the pharynx region and posterior at level of phasmid in tail region, six to eight at mid body and anus and six to ten vulva region where incisures arranged in a slightly oblique pattern in the central zone; all ridges smooth except for few specimens at phasmid level. The labial region flattened, offset by a deep constriction from the rest of the body, with three annuli of which the first being thicker that the others and fused with the oral disc in SEM observations. The *en face* view showing an oval oral aperture surrounded by 6 inner labial sensilla; subdorsal, subventral and lateral lip sectors fused with no division between submedian and lateral segments, amphidial aperture at the inner edge of the lateral segments. Lip region diameter about twice its height. Cephalic framework strongly sclerotized, extending toward the anterior border of the first annuli. Well-developed strong stylet, sclerotized conus and shaft of almost the same size with strongly developed rounded to slightly anteriorly concave basal knobs. Pharynx with dorso-pharyngeal gland opening at 2.7 to 3.6 µm posterior to stylet base. Round to slightly oval medium bulb occupying almost half of the corresponding body diameter with strong conspicuous valve of 1.7 to 2.6 µm wide. Hemizonid immediately anterior to the secretory-excretory pore or 1 annulus long, posterior to nerve ring; Secretory excretory pore and canal located to some extent anterior to pharyngo intestinal junction, pharyngeal glands with their nuclei in tandem, strong to slightly overlapping the intestine ventrally. Reproductive system with long, outstretched ovary, not reaching the pharyngeal gland overlap, oocytes arranged in a single row; oviduct narrow, spermatheca rounded to slightly oval, empty or with few sperm; uterus cells arranged in three rows of 4 cells (when seen laterally), vulva a transverse slit with cuticular folding at its edges; long post uterine sac with 20.3-26.5µm with differentiated cellular tissue at the distal end. Tail with 18-27 annuli, subcylindrical and conical towards the end, tail tip smooth and varying in shape from rounded to truncate or indented, phasmid in some specimens at mid tail level other below mid tail level.

Male

Not found, but probably rare as only one female out of 20 specimens found with sperm in

spermatheca and no male on 40 specimens extracted from approximately 300 gram of soil and root sample.

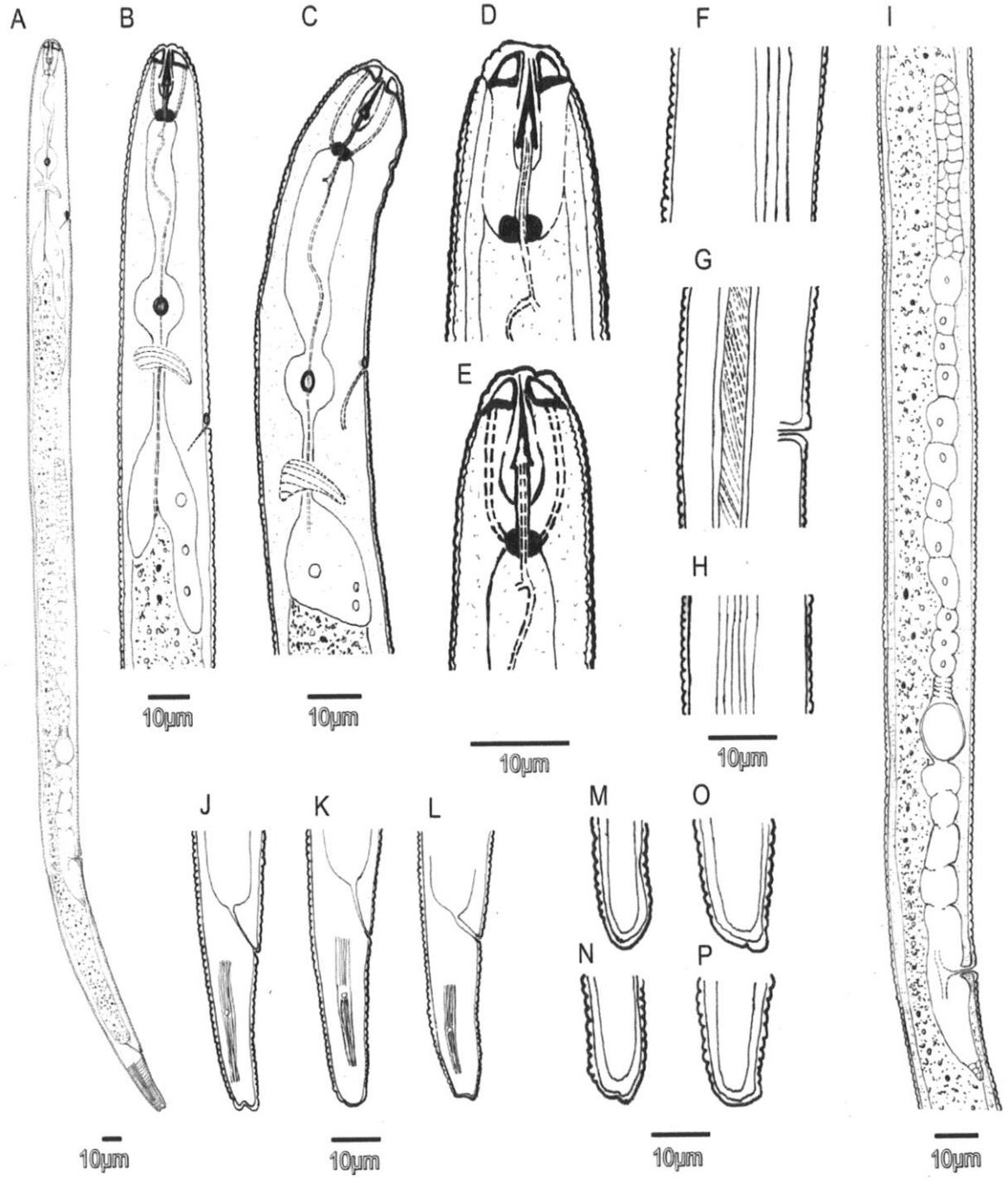


Fig. 9. Light microscopic drawings of *Pratylenchus* n. sp. A, B, E, F-J, O: Holotype. A: entire body; B-C: Neck region; D-E: Head region; F-H: Lateral lines variation (G: at pharynx level 4, H: at midi body, I: at vulva region in oblique patterns); I: Reproductive system; J-K: Variation in tail shape; M-P: Variation in tail tips (Scale bars = 10 μm)

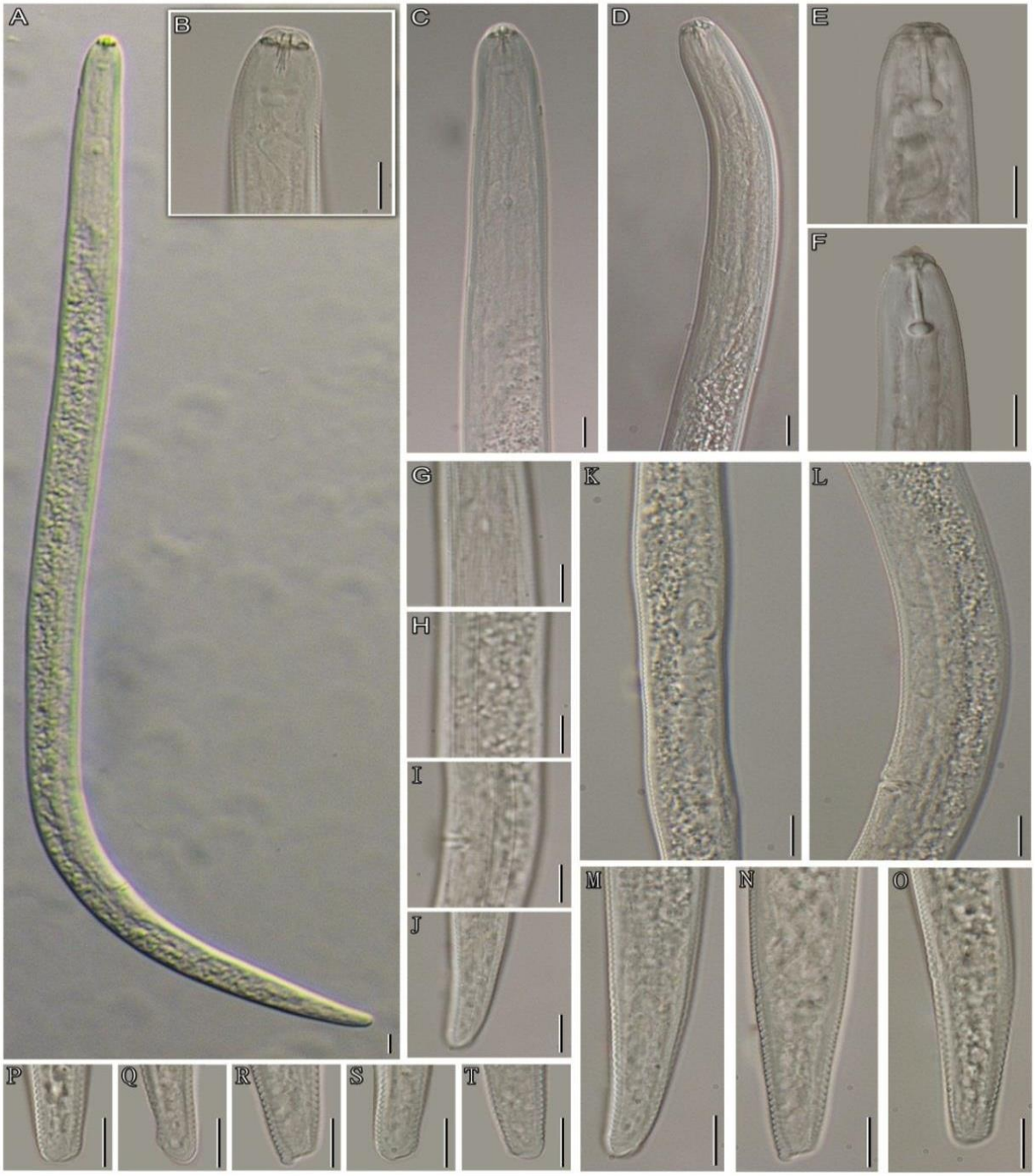


Fig. 10. Photomicrographs of *Pratylenchus* n. sp. A, B, C, G, H, I, J, L, M, P: Holotypes. D, E, F, K, N, O, Q, R, S, T: Paratypes. A: female entire body, B, E, F: Head region, C, D: Neck region, G-I: Lateral lines number and patterns variation (G: at pharynx level 4 lines, H: at midi body 6 lines, I: at vulva eight line in oblique patterns, J: at tail and phasmid level where outer line is annulated), M-O: Female tail shape, P-T: Female tail terminus variation. (Scale bars = 10 μm).

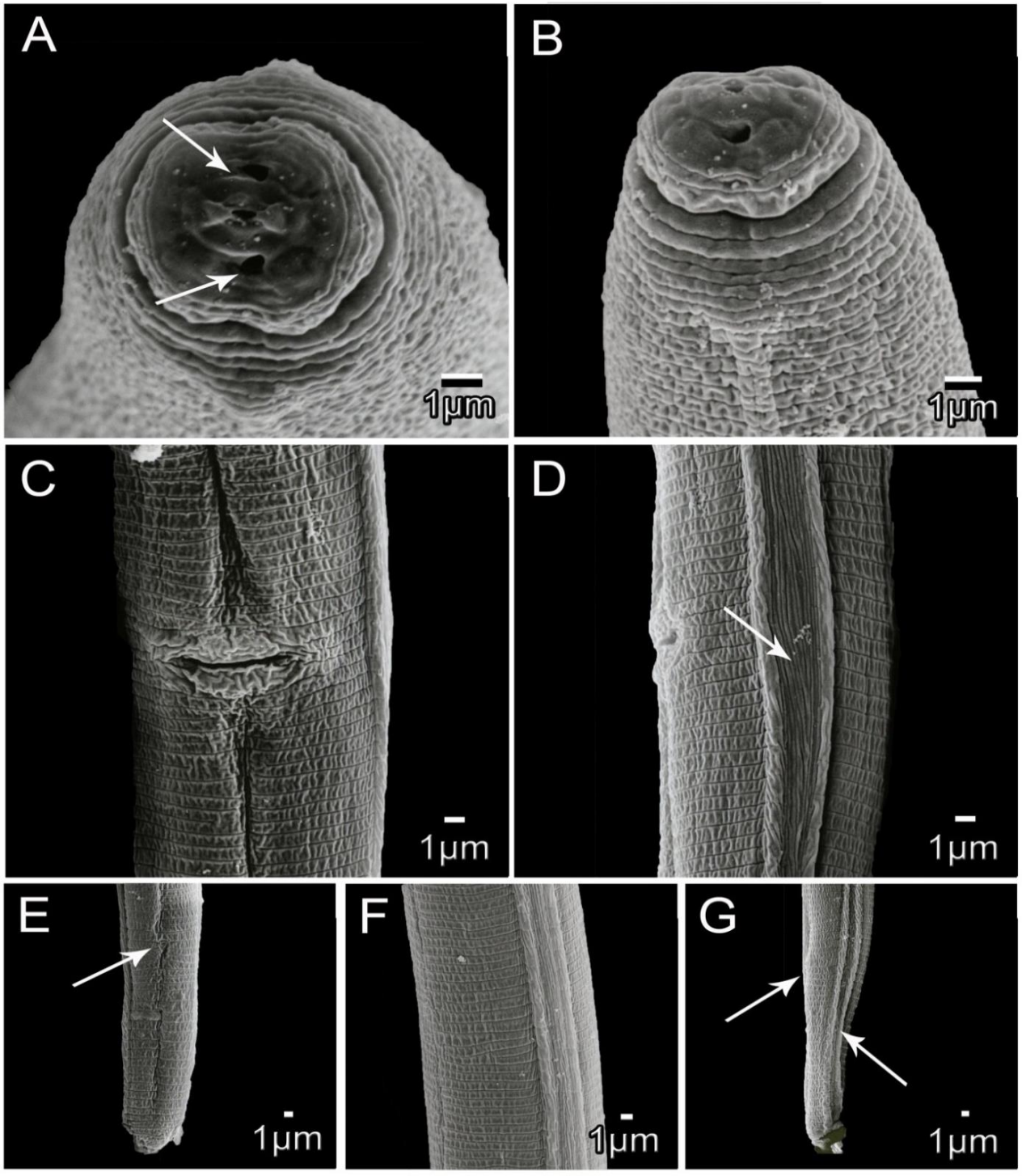


Fig. 11. SEM morphology of *Pratylenchus* n. sp. Female. A: en face view of lip region (arrows pointed to amphids); B: Labial region, oblique view; C: Vulva region; D: Lateral field at vulva region (Arrow showing oblique striations); E: female tail (arrow pointed to anus); F: Lateral field at pharynx; G: Lateral field at phasmid level (arrow pointed to anus and phasmid) (Scale bars = 1 *µm*).

Table 8. Morphometrics of *Pratylenchus* n. sp. females from maize Nyamata (ALN29). All measurements are in µm and in the format: mean ± s.d. (range).

Characters	Holotype	Paratypes
n		14
L	600	531 ± 44 (469-600)
a	22	24.5 ± 3.1 (19.8-29.6)
b	6.1	5.7 ± 0.4 (5.2-6.8)
b'	4.4	4.8 ± 0.6 (4-6.2)
c	17	16.3 ± 1.9 (12.8-20.1)
c'	2.5	2.4 ± 0.3 (1.8-2.7)
V	77.4	77.6 ± 1.4 (75.3-79.7)
m	40.3	47 ± 3.5 (41.2-52.2)
o	28	22.7 ± 2 (19.4-27.7)
S	0.84	0.85 ± 0.07 (0.75-1)
MB	60.4	61.2 ± 3.6 (54.4-68.5)
Distance from head to vulva	464.2	409 ± 40 (338-464)
Max. body diam.	26.9	21.6 ±1.8 (19-24.8)
Anterior end to center of metacorpus	59.9	54.7 ± 2.7 (50.9-59.6)
Anterior end to nerve ring	74.7	69.8 ± 4.6 (61.1-78.3)
Anterior end to secretory excretory pore	83.6	76 ± 12.2 (49.8-90.5)
Anterior to pharyngo intestinal junction	99.1	91.5 ± 6.6 (82.5-107.4)
Anterior end to pharyngeal gland lobe	135.1	111 ± 15.3 (80-132)
Pharyngeal overlap	26.2	22.5 ± 6.6 (10.9-34.7)
BD at metacorpus	22.1	19 ± 1.6 (17-21.9)
BD at pharyngo intestinal junction	23.2	19.8 ± 1.6 (17.2-22.9)
Diam. at vulva region	22.7	18.7 ± 1.8 (15.5-22)
Anterior genital tract length	205.9	142 ± 25.3 (95-184.5)
Post uterine sac	26.2	23.2 ± 1.8 (20.3-26.5)
Spermatheca-vagina	53.9	45.4 ± 9.2 (31-58.6)
Spermatheca height	15.3	13.3 ± 2.1 (9.7-17.8)
Spermatheca width	11.1	10.3 ± 1.9 (8-14.9)
Vulva to anus distance	98.9	79.7 ± 11.4 (64-101.4)
Anal body diam.	14.4	13.6 ± 1.7 (10.9-18)
Tail length	35.8	31.7 ± 2.9 (26.2-36.6)
Cuticle annuli at vulva	1.62	1.31 ± 0.19 (0.99-1.75)
Cuticle annuli at anus	1.18	1.34 ± 0.28 (0.95-1.93)
Lateral field width at vulva	5.6	6.1 ±1.3 (4.7- 9.4)
DGO from stylet base	3.7	3.1 ± 0.26 (2.74-3.6)
Stylet length	13.2	13.6 ± 0.5 (13-14.6)
Conus length	5.5	5.8 ± 0.3 (5.3-6.4)
Shaft length	5.3	5.5 ± 0.3 (5.1-6)

Stylet knob height	2.5	2.3 ± 0.25 (1.96-2.7)
Stylet knob width	4.7	4.7 ± 0.45 (3.49-5.1)
BD at stylet	15.8	16 ± 1.5 (13.8-18.6)
BD at conus	13.6	14.2 ± 1.02 (13-16.1)
Labial disc diameter	2.7	2.7 ± 0.3 (2.3-3.2)
Lip region diameter	9.1	9.4 ± 0.5 (8.5-10.4)
Lip region height	3.7	4 ± 0.6 (3.1-5)
Cuticle annulus at lip region	1.06	0.9 ± 0.2 (0.5-1.3)
Medium bulb width	12.6	10.2± 0,5 (9.5-11.1)
Medium bulb height	14.4	12.3 ± 1.1 (10.7-14.4)
Medium bulb valve Height	3.71	3.2 ± 0.4 (2.5-4.3)
Medium bulb valve width	3.1	2.3 ± 0.2 (1.7-2.6)

TYPE HOST AND LOCALITY

Pratylenchus n. sp., was collected from the rhizosphere and root of maize in a harvested field during the drought season, the period which covers the mid-June to the mid-October or vernacularly called "impeshyi", at Nyamata sector, Bugesera District, Eastern province of Rwanda. This location has a tropical climate with an average temperature of 20 to 30^{o}C, at an altitude of 1360 m and the average precipitation of 900 mm.

TYPE MATERIAL

One female holotype, five females and six juveniles paratypes were deposited in collection center. The Holotype female, 2 other females and 3 juveniles paratypes were deposited in the Museum voor Dierkunde University of Gent, K.L. Ledeganckstraat 35, 9000 Gent, Belgium under slide collection number……... Three females and 3 juveniles paratypes were deposited at National Plant Protection Organization, Wageningen Nematode Collection (WaNeCo), The Netherlands under the slide collection number…….

DIAGNOSIS AND RELATIONSHIPS

Pratylenchus n. sp. is characterized by: relatively long body, the labial region *en face view* flat undivided face Group 1 according to Corbett & Clark (1983), with no division between submedian and lateral segments, lip sectors amalgamated with the oval oral disc and the first annulus, followed by other two annuli, the third annulus thicker than the other two. Lip region offset by a deep constriction from the rest of the body. Short to long robust stylet with prominent

rounded or slightly anterior concave knobs. Short to long pharyngeal gland overlap. Number of lateral lines two at median bulb, four at the end of pharynx, six to eight at midi-body, six to ten at vulva region, and four to six posterior to the vulva region; all the lines smooth except in some specimens at phasmid level; the lateral line at vulva region in an oblique pattern. Oval to slightly rounded spermatheca generally empty. Tail subcylindrical to conoid with high variability of tail tip from rounded, truncate or indented; absence of male. The Code index, according to Castillo & Vovlas (2007) : A2, B1, C2, D3, E2, F3(4), G3(2), H1, I1(2), J3, K1 (variation in characters are given in brackets).

The dichotomous and tabulated identification key of Castillo & Vovlas (2007) were used to discover phenotypically similar species to the *Pratylenchus* n. sp. This species can be distinguished from the morphologically closely similar species with three lip annuli *P. cruciferus* Bajaj & Bhatti, 1984, *P. elamini* Zeidan & Geraert, 1991, *P. kumaoensis* Lal & Khan, 1990, *P. microstylus* Bajaj & Bhatti, 1984, *P. sudanensis* Loof & Yassin, 1971 *P. mediterraneus* Corbett, 1983, *P. delattrei*, Luc, 1958, *P. kralli* Ryss, 1982, *P. pratensisobrinus*, Bernard, 1984, *P. oleae* Juan, Ilhem, Najet, Carolina, Gracia & Castillo, 2014 and *P. pratensis* (de Man, 1880) Filipjev, 1936 by six to 10 lateral lines at vulva instead of four for the above species. Apart from *P. elamini* all the others have smooth bands of lateral lines at vulva region, Except *P. pratensisobrinus* all the others have a smooth tail tip. All have the same range of stylet length except *P. microstylus* with stylet less than 13 µm and *P. delattrei* with stylet up to 18 µm; the same range of vulva position ratio 75%-79.9% apart from *P. sudanensis* with vulva located at less than 75% of their body length from anterior end.

The table below presents the closely similar species to the *Pratylenchus* n. sp. and their differential character.

Table 3. Comparison of *Pratylenchus* n. sp. and its closely related *Pratylenchus* species. Characters in bold are different from the *Pratylenchus* n. sp.; based on the range used in tabulated identification key of Castillo & Vovlas (2007)

***Pratylenchus* species**	**Morphological and morphometrical differential traits**										
	Lip annuli	male	Stylet length in µm	Shape of spermatheca	Vulva position	PUS length in µm	Fem. tail shape	Female tail tip	Pharynx overlap L	Lateral field at vulva	Lateral field Structure at vulva
Pratylenchus n. sp.	3	Absent	13-14.6	Rounded or oval	75-80	20.3-26	Conoid, subcylindrical	Smooth	11-35	6-10	Smooth
P. cruciferus	3	Absent	15-16	**Indistinct**	76-81	18-30	Subcylindrical, cylindrical	Smooth	**31-51**	**4**	Smooth
P. elamini	3	Absent	13-14.5	oval	72-77	**30-35**	Conoid	Smooth	30-39.9	**4-5**	**Partially or completely areolated**
P. kumaoensis	3	Absent	14-15	**Indistinct**	**80-85**	20-24.9	Conoid	**Striated**	< 30	**4**	Smooth
P. delattrei	3	Absent	**16-18**	**Absent or reduced**	72-81	20-24.9	Subcylindrical	Smooth	< 30	**4**	Smooth
P. mediterraneus	3	**Present**	13-16	**Spherical**	77-80	18-25	Subcylindrical	Smooth	**25-55**	**4**	Smooth
P. pratensisobrinus	3	**Present**	15-17	Oval	75-80	20-24.9	Conoid	**Striated**	<30	**4**	Smooth
P. sudanensis	3	**Present**	13-14.9	Oval	<75	25-29.9	Subcylindrical	Smooth	30-39.9	4	Smooth
P. microstylus	3	Absent	**11-12**	**Indistinct**	75-77	16-19,9	Conoid	Smooth	28-46	**4**	Smooth
P. kralli	3	**Present**	13-15.9	**Rounded**	75-79.9	**< 16**	Conoid	Smooth	< 30	**4**	Smooth
P. oleae	3	Absent	14.5-17	**Rounded**	78-82	20-29.9	Conoid	**Striated**	22-36	**4**	**Partially or**

				or indistinct			to Subcylindrical				**completely areolated**
P. pratensis	3	**Present**	13-15.9	**Rectangular**	75-79.9	14-28	Conoid	**Striated**	<30	**4**	Smooth
P. vulnus	3	**Present**	13-18	**Rectangular**	78-80	25-29.9	Subcylindrical	Smooth	**40-50**	**4**	Smooth

In addition to the characters used in tabulated and dichotomous keys Castillo & Vovlas (2007) all other characters can be used to distinguish the above closely related species from *Pratylenchus* n. sp.

Morphologically *Pratylenchus* n. sp. is distinguished from *P. cruciferus* in having a short body (469-600 µm *vs* 650-790 µm); body with number of lateral lines changing in different parts of the body *vs* four the whole body. Stylet knobs round *vs* concave anteriorly in *P. cruciferus*; the position of hemizonid just anterior to excretory pore 1 annuli body *vs* 2 to 8 annuli.

Pratylenchus n. sp. differs from *P. delattrei* by slightly longer body 469-600 µm *vs* 340-570 µm; basal knobs rounded *vs* flattened; body with number of lateral lines changing in different parts of the body *vs* four the whole body; tail terminus variable from rounded, truncate or indented *vs* conical or rounded.

Pratylenchus n. sp. can be differentiated from *P. kralli* by number of lateral lines changing in different parts of the body *vs* four the whole body, spermatheca generally empty *vs* generally filled with sperms, post uterine sac with differentiated cellular tissue at the distal end *vs* presence of a rudiment branch of gonad occasionally observed with several small nuclei of somatic origin; tail tip variable from rounded, truncate to indented *vs* tail tip pointed showing a slight groove in direction of ventral surface of the body, sometimes serration seen in tip region.

Pratylenchus n. sp. can also be differentiated from *P. elamini* by slight longer body (469-600 µm *vs* 340-510 µm), coarse body cuticle (0.99-1.75 µm *vs* <1 µm) wide, number of lateral lines changing in different parts of the body *vs* four the whole body four or five lateral lines the whole body and at vulva partially or completely areolated. A round to oval medium bulb *vs*. an ellipsoid shape, short post-vulvar uterine sac (20.3-26.5 µm *vs*. 30-35 µm), tail tip variable in shape *vs*. a conical smooth terminus.

Pratylenchus n. sp. differs from *P. kumaoensis* by relatively longer body length (469-600 µm *vs* 390-480 µm) number of lateral lines changing in different parts of the body *vs* 4 lines the whole body, lip region offset by a deep constriction from the rest of the body *vs* lip region almost continuous with body contour.Tail terminus variable from rounded, truncate or indented *vs* conical or crenate.

Pratylenchus n. sp. differs from *P. mediterraneus* by body with different number of lateral lines, lateral lines along the whole body smooth, except at phasmid in some specimens *vs* four with the outer line crenate or occasionally areolated in outer bands, pharyngeal lobe overlapping intestine

only ventrally *vs* ventrally or laterally overlapping and occasionally showing to be in separate lobes rather being enclosed in a single lobe; the excretory pore anterior to pharyngo-intestinal junction *vs* being opposite to it; spermatheca empty *vs* being filled with sperm; tail not bent ventrally *vs* being strongly bent ventrally.

Pratylenchus n. sp. also differs from *P. microstylus* by relatively longer body of (469-600 µm *vs* 330-460 µm); body with lateral lines changing in different parts of the body *vs* four the whole body; long stylet with rounded knobs *vs* with anteriorly flattened knobs, intestine not overlapping rectum *vs* overlapping dorsally; tail terminus variable from rounded, truncate or indented *vs* only rounded.

Pratylenchus n. sp. differs from *P. sudanensis* by lateral line number changing along the whole body *vs* four to five in some specimens to the whole body, rounded knobs *vs* flattened or slightly concave; an oval to slight rounded spermatheca mostly empty *vs* large, elongated sometime spherical filled with sperms, tail terminus variable from rounded, truncate or indented *vs* broadly rounded to truncate.

Pratylenchus n. sp. also differs from *P. pratensisobrinus* by lateral line number changing the whole body parts *vs* four from anterior until the phasmid, below phasmid 3 lines, the lines with no regular markings indication between them, short stylet with rounded knobs *vs* capped anteriorly stylet knobs, tail terminus smooth variable from rounded, truncate *vs* coarse annulated or with large terminal annulus.

Pratylenchus n. sp. differ from *P. pratensis* by a slight coarse *vs* fine, inconspicuous cuticular annulation; variation of lateral lines the whole body *vs* four; tail tip annulated and variable in shape from rounded, truncate or indented *vs* tail tip not annulated usually oblique, symmetrically conoid or appearing slightly mucronate.

Morphologically *Pratylenchus* n. sp. differ from *P. oleae* by slightly long body of (469-600 µm *vs* 412-511 µm); long tail (26.2-37.5 µm *vs* 19-26.6 µm), variation of lateral lines the whole body *vs* lateral fields with four lines equidistant and completely areolated.

Among the closely related species to the *Pratylenchus* n. sp. comparing their lip region patterns based on SEM studies and the classification scheme according to Corbett & Clark (1983) showed that only *Pratylenchus* n. sp. and *P. elamini* have the same lip patterns but the lip region in *Pratylenchus* n. sp. is flattened *vs* dome-shaped for *P. elamini*. Both belong to the first group of Corbett & Clark (1983) while *P. mediterraneus* and *P. pratensis* are found in group three

characterized a distinctive dumbbell-shaped pattern of submedian segments with slightly small lateral segments to a complete cycle, and *P. oleae* is found in group two with lips fused with the oral disc in a dumb-bell pattern separated from lateral lip sectors by two almost straight incisures forming an obtuse angle (Subbotin *et al.*, 2008)

Pratylenchus goodeyi (Sher & Allen, 1953)

Figure 12.

Population from bean Bushoki, agro-ecological zone 6, sample ALN23, voucher slide UGnem-99

MEASUREMENTS

See Table 6

DESCRIPTION

Females

This population largely agrees with the population in Castillo & Vovlas (2007) and more specifically with those studied by Sakwe & Geraert (1994) from Cameroonian plantain. The slight differences are short body length. Lip region flattened slightly set of from the rest of the body with 3 lip annuli of 0.5-0.9 µm with instead of 4. Strong cephalic framework extending up to the second annulus inside the body (Fig. 9 A-D). Short stylet of 13.9-15.5 µm length with rounded to anterior flattened or cup shaped knobs. Large sub-rectangular to slightly oval spermatheca filled with sperms or empty, short post uterine sac of 13.3-17.1 µm length (Fig. 9 I-G). Tail conoid with 24-30 annuli (Fig. 9 I-K), tail tip smooth variable from pointed, truncate to irregular shape (Fig. 9 L-O)

Males

Similar to female apart from the reproductive system but with short stylet compared to female.

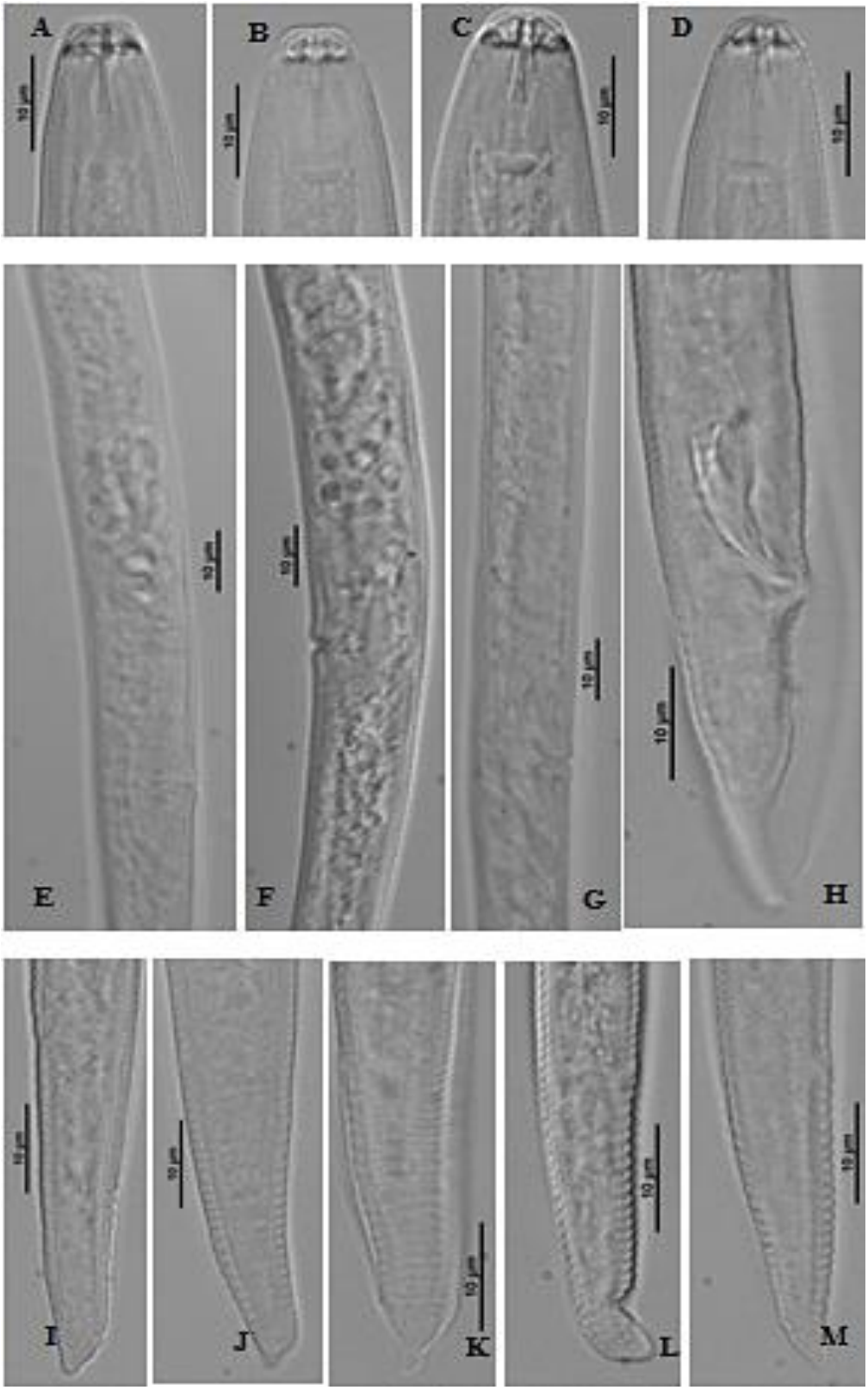


Fig. 12. Photomicrographs of *Pratylenchus goodeyi*. A-C: Female head region; D: male head region, E-G: spermatheca; H: male tail; I-K: Females tails; L-O: female tail (Scale bars = 10 μm)

Table 9. *Pratylenchus goodeyi* populations from bean Bushoki (agricultural zone 6) ALN23. All measurements are in µm and in the format: mean ± s.d. (range).

Characters	Females	Males	Females*	Males*
n	9	6	-	-
L	487 ± 48 (424-569)	440 ± 28 (393-464)	400-710	380-650
a	24.1 ± 2 (21.4-28.2)	24.6 ± 2 (22.1-26.5)	19.7-37	22.8-37
b	5.2 ± 0.6 (4.3-6.4)	5.3 ± 0.2 (4.9-5.5)	4.1-8.1	5.4-7.3
b'	4.3 ± 0.6 (3.8-5.6)	4.3 ± 0.2 (4.1-4.7)	2.9-5.3	3.8-5.1
c	15.2 ± 0.7 (14-16.2)	16.4 ± 1.4 (15.1-19)	13.8-20	12.5-25
c'	2.9 ± 0.2 (2.6-3.3)	2.3 ± 05 (1.4-2.7)	1.7-3.2	1.5-3.3
V	73 ± 1.5 (70.7-75.7)	-	72-79	-
m	44.4 ± 0.4 (43.7-45.1)	44.1 ± 1.2 (42-45.5)	-	-
o	17.5 ± 2.6 (13.4-21.3)	20.6 ± 2.2 (18-24.3)	-	-
S	1 ± 0.08 (0.9-1.2)	1.1 ± 0.1 (1.02-1.33)	-	-
MB	57.5 ± 4.1 (49.6-62.5)	60.4 ± 3.3 (55-64.5)	-	-
Distance of vulva-head	357 ± 38 (304-430)	-	-	-
Max. body diam.	20.3 ± 2.3 (17.4-24.9)	17.9 ± 1.5 (16.6-21)	-	-
Ant. end to MB	53.6 ± 2.4 (49.6-58.1)	50.1 ± 2.5 (46-53.3)	-	-
Ant. end to NR	67.8 ± 3.8 (61.8-73.3)	64 ± 3.2 (58.5-67.6)	61-74	60-68
Ant. end to EP	78.5 ± 4.6 (71.1-85.6)	73.7 ± 5 (68.9-82.5)	71-88	72-83
Ant. end to pharyngo-intestinal junction	93.7 ± 8.9 (86-108.4)	83.1 ± 5.8 (76-92.8)	-	-
Ant end to pharyngeal gland overlap	114 ± 12.4 (99-137.3)	103.1 ± 6.1 (97-110)	-	-
Pharyngeal gland L	31.6 ±3.6 (26.5-35.9)	30 ± 2.8 (26.2-33.7)	37-55	27-47
BD at medium bulb	17 ± 0.9 (15.1-19.4)	15.3 ± 0.8 (14-16.2)	-	-
BD at Pharynx end	17.5 ± 1.3 (15.6-19.4)	16.4 ± 1.1 (15-18.1)	-	-
Diam. at vulva region	16.8 ± 1.9 (14-19.7)	-	-	-
Anterior genital tract L	159 ± 207 (132-186)	-	-	-
Post uterine sac length	15.6 ± 1.2 (13.3-17.1)	-	-	-
Spermateca-vagina length	37.5 ± 7.8 (26.4-50.6)	-	-	-
Spermateca height	23.8 ± 7.9 (11.9-33.8)	-	-	-
Spermatheca width	10.6 ± 2.2 (7.8-13.4)	-	-	-
Vulva-anus length	96.7 ± 10.7 (81.6-111)	-	-	-
Anal body Diameter	11.1 ± 0.9 (9.4-12.4)	11.8 ±1.6 (10-14.6)	-	-
Tail L	32.1 ± 3.2 (28.2-38)	27.1 ± 3 (20.7-29.6)	25-28	27-34
Cuticle annulus at vulva	1.4 ± 0.2 (1.1-1.69)	-	-	-
Cuticle annulus at anus	1.2 ± 0.2 (1-1.6)	-	-	-
Lat. field width at vulva	5.2 ± 0.6 (4.1-6.2)	-	-	-
DGO	2.6 ± 0.3 (2-2.9)	2.8 ± 0.5 (2.1-3.4)	-	-
Stylet Length	14.7 ± 0.6 (13.9-15.5)	13.6 ± 0.6 (13-14.9)	14-18	13-16

Conus Length	6.5 ± 0.2 (6.1-6.9)	6 ± 0.3 (5.6-6.6)	-	-
shaft Length	6.2 ± 0.3 (5.8-6.7)	5.2 ± 0.6 (4.7-6.2)	-	-
Stylet knobs Height	1.9 ± 0.2 (1.62-2.25)	1.87 ± 0.1 (1.7-2.1)	-	-
Stylet knobs Width	3.8 ± 0.3 (3.4-4.3)	3 ± 0.4 (2.5-3.6)	-	-
BD at stylet	14.1 ± 0.9 (12.8-15.4)	12 ± 0.7 (11.2-12.9)	-	-
BD at conus	12 ± 0.7 (10.8-13)	10.3 ± 0.6 (9.5-10.9)	-	-
Labial disc diameter	2.2 ± 0.2 (2-2.5)	2.1 ± 0.2 (2-2.4)	-	-
Lip region diameter	8.5 ± 0.5 (7.5-9.1)	6.9 ± 1.5 (4-8.2)	-	-
Lip region height	4.2 ± 0.5 (3.4-4.8)	3 ± 1.2 (0.6-3.8)	-	-
Cuticle annulus at lip	0.7 ± 0.1 (0.5-0.9)	0.7 ± 0.1 (0.6-0.9)	-	-
Medium bulb width	9.3 ± 1 (7.9-10.7)	8.8 ± 0.4 (8.4-9.4)	-	-
Medium bulb height	12.1 ± 0.4 (11.5-12.8)	11.2 ± 0.8 (10-12.4)	-	-
Medium bulb valve H	3.4 ± 0.2 (3.1-3.7)	3 ± 0.3 (2.7-3.3)	-	-
Medium bulb valve W	2.2 ± 0.3 (1.7-2.6)	2 ± 0.2 (1.8-2.3)	-	-
Spicule lenght	-	15.8 ± 1 (14.6-17)	-	15-21
Spicule width at head	-	2.3 ± 0.3 (2-2.7)	-	-
Gubernaculum lenght	-	4,3 ± 0.3 (4-4.8)	-	5-6.5

***:** Nematodes measurements compiled from Sher & Allen (1953), Sakwe & Geraert (1994), Troccoli *et al.* (1996) and Ryss (1988)

MOLECULAR CHARACTERIZATION OF *PRATYLENCHUS* FILIPJEV, 1936 SPECIES

D2D3 expansion segment of ribosomal DNA

The polymerase chain reaction amplified a single DNA product of 620 and 690 bp based on the good quality fragments sequenced for the two specimens of *Pratylenchus* n. sp. studied. After multiple sequences alignment and elimination of poorly aligned position an average of 713 selected sites for 53 sequences were used for further analysis, in those sites 180 sites were complete without any gaps and of which 47% were variable and 33% were parsimony informative. Intraspecific comparison of the two *Pratylenchus* n. sp. sequences generated showed a divergence of 2.9% (15 nucleotides difference) and 14 indels. This difference was most likely due to the quality of one of the sequence which was not very good. Interspecific variation done by pairwise comparison between the long sequence of *Pratylenchus* n. sp. (201) and the molecular closely related species sequences of *P. oleae*, *P. penetrans*, *P. dunensis*, *P. arlington* and *P. fallax* varied form 9.1-14.3% (27-98 nucleotides differences based on sequences length). Pairwise comparison between *Pratylenchus* n. sp. and *P. penetrans* showed 13.9-14.3 % (95-98 nucleotides differences) and 8 indels to 691 selected sites. For *P. oleae*; 11.8% (78 nucleotides

difference) and 11 indels to 670 sites selected were found. For *P. dunensis*; 12.3-12.4% (84-85 nucleotides difference) and 8 indels for 693 selected sites were found. For *P. arlington* only D3 sequenced is the one used and there was 9.8% (29 nucleotides difference) and 2 indels for 298 selected sites were found. For *P. fallax*. Also D3 only was sequenced and used and there was 9.1% (27 nucleotides difference) and 1 indel for 297 sites selected were found

Based on the majority rule 50% consensus tree topology the phylogenetic relationships between *Pratylenchus* species are specified in (Fig. 13). Five major clades (I; III (A and B); IV; V and VI) are presented in roman number on the tree and letter for the subclades. Apart from the second clade which was previously presented by Subbotin *et al.* (2008) all the other clades are present. Excluding the third clade 4 others are supported with 100% of posterior probability. The first clade is composed with tropical species bearing two lip annuli and male presence such *P. loosi, P. coffeae, P. speijer* etc. The second clade comprises *P. pseudopratensis, P. vulnus, P. kumaoensis and P. crenatus*.which was individually resolved. The third clade comprises species that occur in both temperate and tropic climate, having or not male in their population, and bear mostly two lip annuli except *P. brachyurus* and *P. dunensis*. Those are *P. brachyurus, Pratylenchus* n. sp., *P. penetrans, P. fallax P. arlingtoni, P. oleae* and *P. dunensis* clade. This well supported clade (100% PP) includes the 2 specimens of the *Pratylenchus* n. sp., in a maximally supported sister positions to all other species of this clade except *P. brachyurus*, which is the most early diverging species of this clade. The fourth clade with also cosmopolitan species bearing two to three lip annuli and presence of male in their population. Those are *P. thornei, P. mediterraneus, P. brzeskii* and *P. neglectus.* The fifth clade comprise *P. delattrei, P. zeae, P. bhattii* and *P. goodeyi* also cosmopolitan species except *P. bhattii,* with 3 to 4 lip annuli and with male in their population excluding *P. delattrei* (Fig. 13).

18S of rDNA

The amplification yielded two DNA products which were concatenated and produced approximately 1590 bp based on the good quality fragment sequenced for one specimen of *Pratylenchus* n. sp. studied. The multiple alignment was done on 50 sequences. After elimination of poorly aligned position an average of 904 sites were selected. For these sites 452 were completes without gaps, 264 (58.4%) were variable and 99 (21.9%) were parsimony informative. Intraspecific comparison was not assessed as only one sequence was produced. Sequence

divergence at interspecific level detected by pairwise comparison for the species closely related to the *Pratylenchus* n. sp. varied from 7.9-22.3% (64-303 nucleotides difference based on sequence length). For *P. penetrans* divergence varied between 8.1-9.3% (64-74 nucleotides difference) and 6 indels for 774 to 798 sites selected. Sequence divergence for *P. oleae* was 22.3% (300 nucleotides difference) and 100 indels for 1445 sites selected. For *P. pinguicaudatus* divergence varied from 7.9-8.1% (61-62 nucleotides difference) and 6 indels for 770 sites selected. *P. brachyurus* diverged from *Pratylenchus* n. sp. 8.6% (66 nucleotides difference) and 16 indels for 785 selected sites.

According to the topology of the majority rule 50% consensus tree the phylogenetic relationships between *Pratylenchus* species are specified in (Fig. 14). Six clades (I (A and B); III (A and B) IV, V, VI, and VII) are presented in roman number on the tree and letter for the subclades with maximum and moderate support. The first clade is maximally supported and the taxa found in the clade are the same as what are found in the first clade of the D2-D3 of 28S rDNA tree with *P. japonicus* as the sister of the group and *P. pratensis* as a subclade which was individually resolved. The second clade with two individually resolved subclades maximally supported. The first subclade containing *P. kumaoensis* and *P. vulnus* and the second containing *P. crenatus*. The third clade is moderately supported. This clade comprises the *Pratylenchus* n. sp., which is sister to the group consisting of *P. convallariae, P. penetrans, P. pinguicaudatus* and *P. oleae*. As it was found in the D2-D3 of 28S tree also *P. brachyurus*, was found to be the earliest diverging species of this clade. The fourth clade containing *P. hispaniensis* and *P. neglectus* were estimated to be sister taxa but with moderate support of 84% of PP. The two species are sister of *P. thornei* with maximum support of 100% PP The fifth clade also is maximally supported with *P. goodeyi* as sister of *P. zeae*. The sixth clade poorly supported with only 64% of PP containing *P. bolivianus* and *Hirschmanniella loofi*.

COI of mitochondrial DNA

The PCR with JB4 prat and JB4 prat amplified one DNA product. For the *Pratylenchus* n. sp. one specimen yielded a short DNA fragment of 291 bp and the other specimen yielded a long fragment of 430 bp based on the good quality fragments sequenced. Multiple sequences alignment and elimination of poorly aligned position was done on 18 sequences and in average 445 sites were selected which among them 205 were complete without gaps, and in these

complete site 123 (60%) were variable, and 100 (49.3%) were parsimony informative. Pairwise comparison of the two *Pratylenchus* n. sp. sequences did not show any intraspecific variability. The interspecific pairwise comparison of sequences which are molecular closely related species to the *Pratylenchus* n. sp. *P. oleae, P. penetrans,* and *P. brachyurus* showed a divergence of 16.8-19.1% (64-83 nucleotides difference). For *P. penetrans* 17.7-18.4% (77-80 nucleotides difference) with no indels for 434-444 selected sites was noticed; *P. oleae* had 16.8% (64 nucleotides difference) with no indels for 381 sites selected and *P. brachyurus* had 19.1% (83 nucleotides difference) with 3 indels on 437 selected sites.

Based on the topology of the 50% majority rule consensus tree the phylogenetic relationships between the *Pratylenchus* species and the new species are given in (Fig 15). Three clades (III (A, B and C); IV and combination of member of clade V and VI in one clade) are presented in roman number on the tree and letter for the subclades. The first with three individually resolved and highly supported subclades. The first with *P. vulnus,* the second with *P. crenatus* the third with *P. neglectus*. The second clade also containing three subclades, the first not well supported (57% of PP) containing *P. brachyurus* and *P. oleae* clade, the second with the specimens of the *Pratylenchus* n. sp., maximally supported, the third with *P. penetrans* is 100% supported. The third major clade is not a well resolved and is poorly supported containing *P. thornei, P. goodeyi* and *P. brzeskii*. It is only supported with 68% of PP and *P. zeae* which is in a sister relationship with this clade also with 58% PP support.This clade contain members of clade IV and V as in Subbotin et al. (2008). (Fig 15).

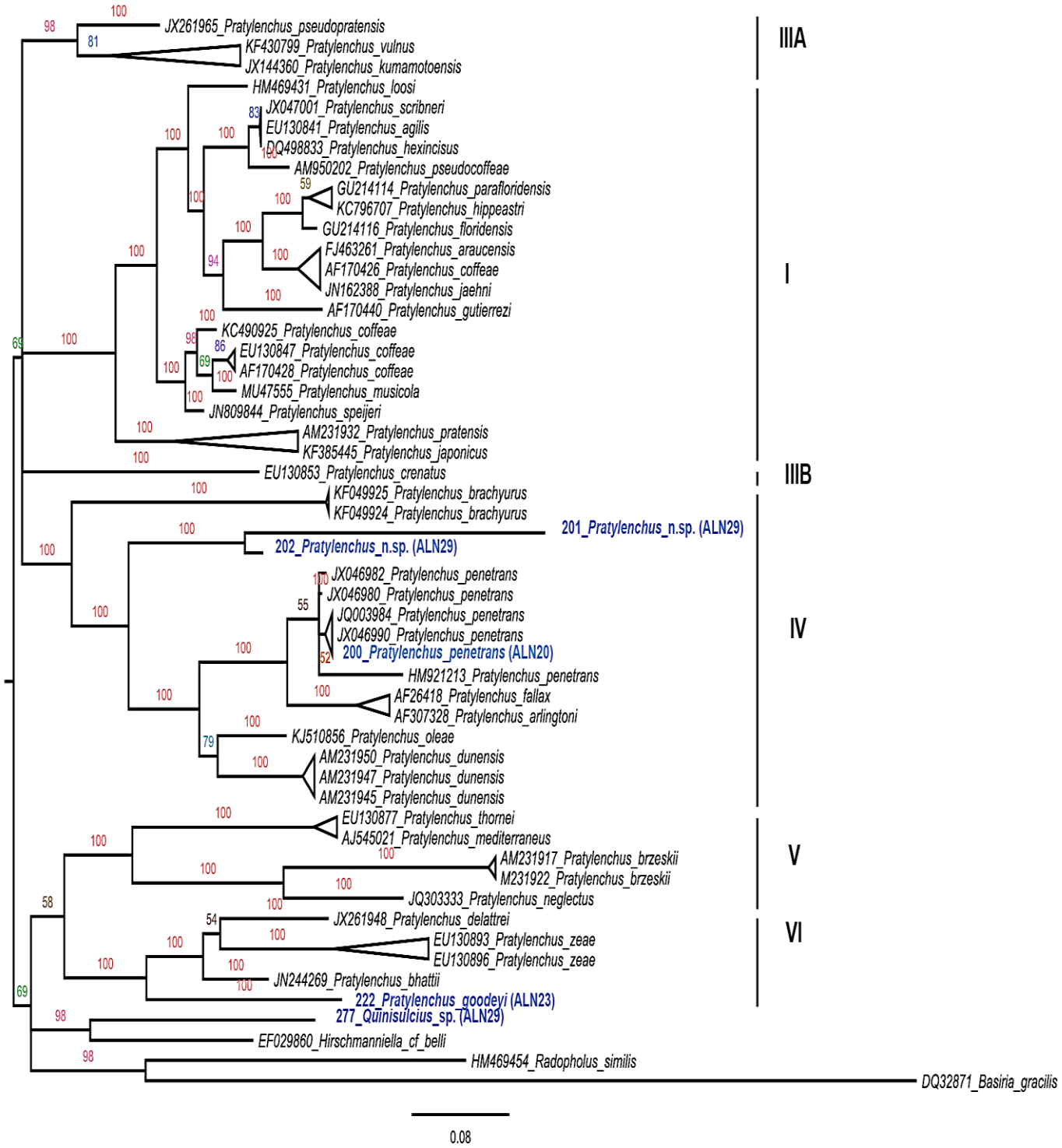


Fig. 13. Bayesian 50% majority rule consensus phylogenetic relationships tree of *Pratylenchus* n. sp., (two specimens) within other 47 *Pratylenchus* species based on D2-D3 of 28S rDNA gene sequences. Sequences aligned under GTR+I+G model. Posterior probabilities of more than 50% are given for appropriate clade. Original generated sequences in this study are highlighted in

different blue color. Roman numbers I, III, IV, V and VI indicate major clades as they were found by Subbotin *et al.* (2008). Letters A and B relate to nodes representing subclades individually resolved.

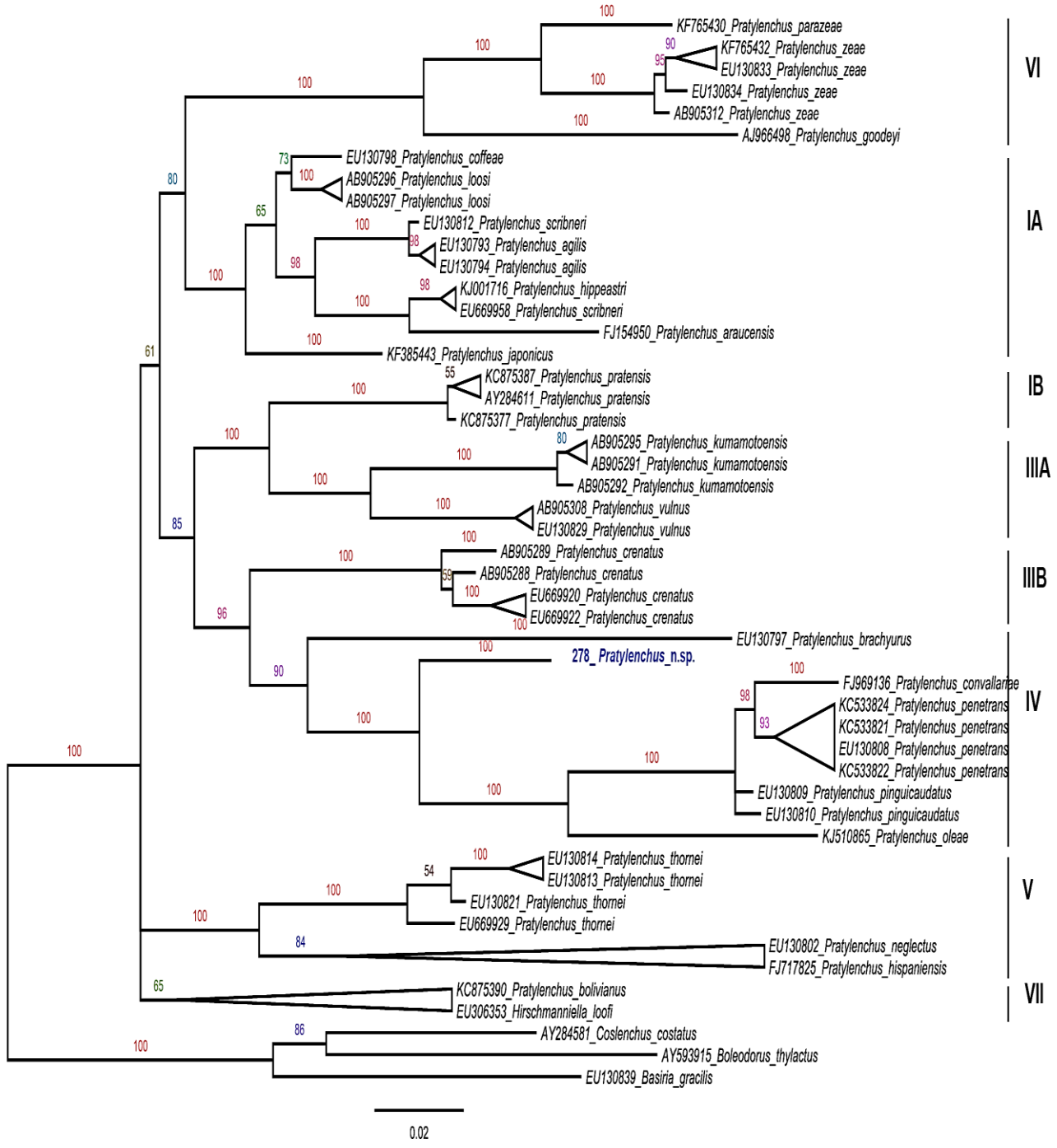


Fig. 14. Bayesian 50% majority rule consensus phylogenetic relationships tree of *Pratylenchus* n. sp., (1 specimen) within other 24 *Pratylenchus* species based partial 18S rDNA gene sequence

alignment. Sequences aligned under GTR+I+G model. Posterior probabilities of more than 50% are given for appropriate clade. New species highlighted in blue color. Roman numbers I, III, IV, V and VI indicate major clades as they were found by Subbotin *et al.* (2008). Letters A and B relate to nodes representing subclades individually resolved.

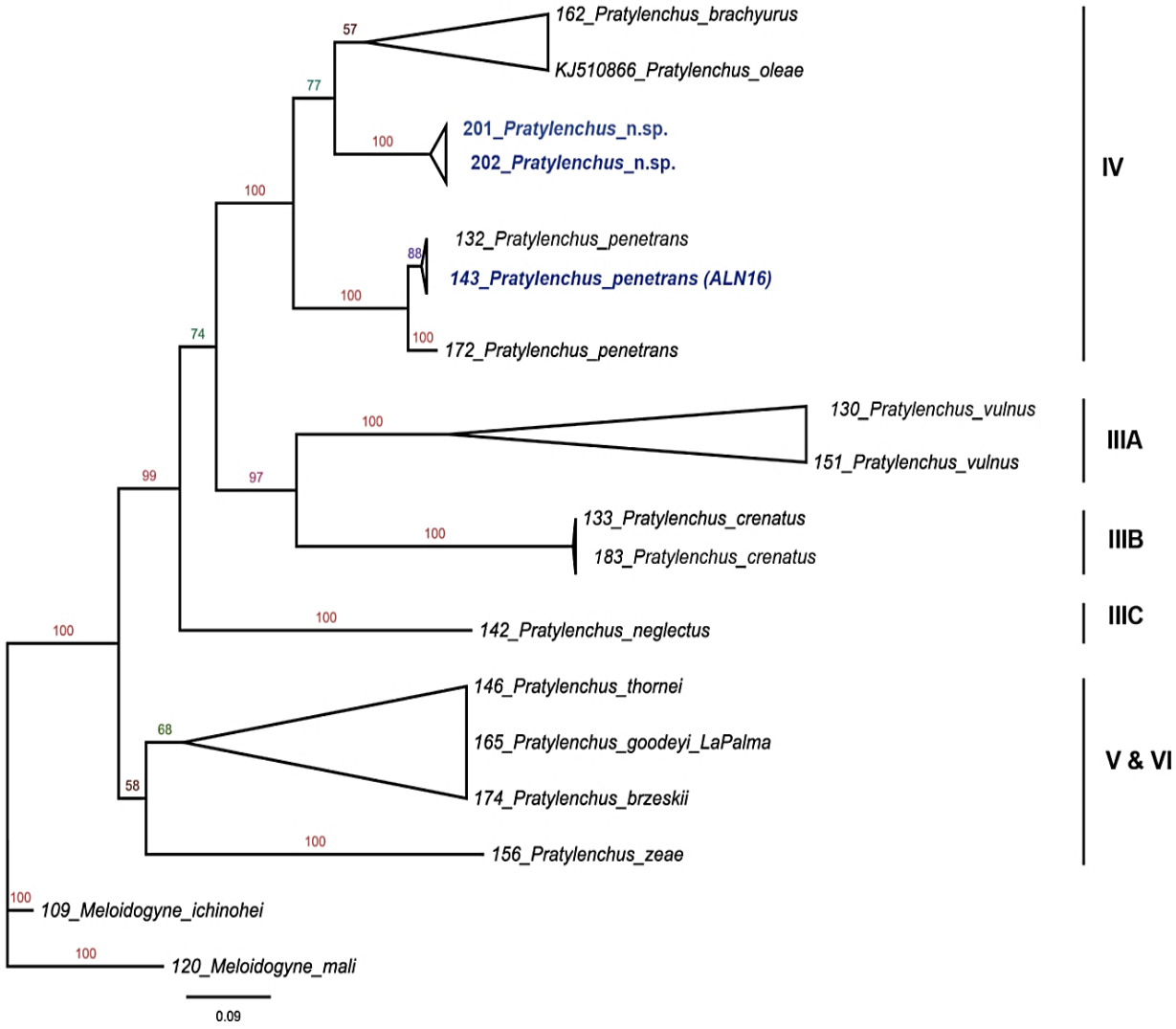


Fig. 15. Bayesian 50% majority rule consensus phylogenetic relationships tree of *Pratylenchus* n. sp. (2 specimens) within other 11 *Pratylenchus* species based on COI of mitochondrial DNA sequence alignment. Sequences aligned under GTR+I+G model. Posterior probabilities of more than 50% are given for appropriate clade. New species highlighted in blue color. Roman numbers I, III, IV, V and VI indicate major clades as they were found by Subbotin *et al.* (2008). Letters A to C relate to nodes representing subclades individually resolved.

Discussion

NEMATODE GENERA IDENTIFICATION

Crop loss and damage caused by nematodes is of potential importance and depends on wide range of factors such as population density, species or strain virulence and so on. Identification of plant-parasitic nematodes is very important before taking management decision. Identification of plant parasitic nematodes was done on fifteen crops sampled (5 vegetables crops (cabbage, carrot, onion, eggplant and tomato), two cereals (maize and rice), tuber crops (potato and cassava), bean as a food legume, tamarillo and passion fruits as fruit crops, banana, tea and *Paspalum.* Except *Meloidogyne, Pratylenchus, Scutellonema, Helicotylenchus, Hemicycliophora* and *Ogma* all the other genera are new records from Rwanda. All the previously identified genera were associated with banana crops. Of all 41 samples examined, the two rice samples didn't contain plant parasitic nematodes. The other samples have a minimum of two to a maximum of eight genera per sample.

The common absence of plant- parasitic nematodes and presence of few nematode specimens in general in Rwandan rice was also noticed by Nsengimana *et al.* (2012) and this was attributed to the high content of clay and the fluctuation of temperature which is 22^{o}C during the day and 15^{o}C during night in marshlands where rice is cultivated. Those may affect movement and reproduction of the nematodes. Except *Ogma* species which were found to be associated with vegetables (eggplants) for the first time, all other 13 genera found in the rhizosphere and root of Rwandan vegetables production were found to be associated with them in other part of the world (Knight *et al.*, 1997; Sikora & Fernandez, 2005; Mennan & Handoo, 2006; Waceke, 2007; Pedroche *et al.*, 2013; Haougui *et al.*, 2013). The ten genera found to be associated with Rwandan maize were also reported from other parts of the world (Addoh, 1971; Walters & Gevers, 1979; McDonald *et al.*, 2005; Guo & Shi, 2012; Singh *et al.*, 2013). Of the ten genera identified in banana samples, *Ditylenchus, Quinisulcius, Aphelenchus, Dolichodorus* and *Tylenchorhynchus* were not previously reported to be associated with banana in Rwanda Gaidashova *et al.* (2004). Nine different genera found in association with Rwandan potato were reported before in different parts of the world (Olthof *et al.*, 1982; Njuguna & Bridge, 1998; Scurrah *et al.*, 2005; Desgarennes *et al.*, 2009). Eight genera found on Rwandan bean crops were reported before to be associated with them somewhere else in the world (Bridge *et al.*, 1996;

Kimenju *et al.*, 1999; Sikora *et al.*, 2005). Five species were found to be associated with Rwandan cassava and all of them were also found to be associated with Ugandan cassava (Coyne *et al.*, 2003) and other parts of the world (Bridge *et al.*, 2005). Apart from the new association of *Hemicycliophora* and *Scutellonema* with tea in Rwanda, the other four genera found in Rwandan tea were reported before. (Kibet *et al.*, 2013; Gnanapragasam *et al.*, 2005). Of seven genera found to be associated with passion fruit, only *Meloidogyne* species were reported before. Only one study was found in which nematodes were identified to be associated with Tamalliro (Knight *et al.*, 1997). Apart from *Xiphinema* and *Meloidogyne* the other six genera found to be associated with it, is the first time to be reported. Out of 4 genera identified in Rwandan *Paspalum* one genus *Helicotylenchus* was known to be associated with this crop (Hixson & Crow, 2004).

In all samples studied *Scutellonema* species were the most frequently occurring with 78%, while *Meloidogyne*, the most economical important PPN in the world was only found in 27% of the samples. Cyst nematodes, although ranked second regarding economic importance, were not found in any of the samples. That is probably due to the fact that most of them have a narrow host range while in Rwanda crop rotation is mostly applicable. *Pratylenchus* species which are the third most important PPN were recovered in only 19.5% of all the samples. For most of the identified nematodes in the different crops their economic importance in crop production are not documented in Rwanda and most of them in the world.

NEMATODES SPECIES IDENTIFICATION

Species of Scutellonema Andrassy, 1958

Identification of *Scutellonema* species based only on morphological and morphometrics data is not always reliable due to many conserved characters, overlapping morphometrics and intraspecific variability as illustrated by our populations. The use of a holistic approach by combining morphological, morphometrics and molecular data is important for identification and validation the *Scutellonema* species. For example one of the *Scutellonema* species identified in ALN16 was misidentified morphologically and morphometrically as *S. brachyurus* but this was not supported by molecular analysis.

Based on the *Scutellonema* sequences available up to now for COI of mt DNA and D2D3 of 28S their phylogenetic analysis showed three separate groups based on Bayesian 50% majority rule consensus tree. The two tree topologies generated in this study largely agree with those found by

Van den Berg *et al.* (2013). They differ in the presence of one generated sequence during this study of different line of *Scutellonema* sp. which identity still has to be confirmed within the first clade, the *S. paralabiatum* which formed an individual clade and the combination of the *S. bradys* and *Scutellonema* sp. D in one clade together with *S. cavenessi*. In Van den Berg *et al.* (2013) *S. bradys* and *Scutellonema* sp. D formed two different clade individually. The results obtained in this analysis of ribosomal DNA and mitochondrial DNA still are showing the clear distinction based on geographical region in *S. brachyurus*. *S. brachyurus* from USA was grouped prior under type A and South African population where Rwandan population found was grouped under type B by Van den Berg *et al.* (2013). The previously identified *S. cavenessi* (FJ485652) by Bae *et al.* (2009) was found to be 100% similar to *S. bradys* (JX472036) identified by Van den Berg *et al.* (2013) based on D2D3 sequences. Relationship between them leaves an open question that possibly they were misidentified especially the *S cavenessi* due to the fact that it could cluster together with *S. cavenessi* from Rwanda which were confirmed by both Molecular and morphological data.

Comparing all the Rwandan population of *Scutellonema* species there was some morphological, morphometrical and molecular intraspecific variation. These variations sometimes were casting doubt in an only morphological identification as it was mentioned in the descriptions and molecular analysis of the current studied specimens.

The studied *S. paralabiatum* populations were similar to all the others in the literature in morphometrics characters as it was shown in table 5. The most important difference between the identified species in this study and those studied by Siddiqi & Sharma (1994), Mekete *et al.* (2008) and (Van den Berg *et al.*, 2003) are the presence of some specimens with annulation of lateral lines at scutellum level *vs* the absence of annulation for all the previously studied specimens. The common presence of specimens with 7 annuli at lip region *vs* rare presence of them for the previously studied and few differences in morphometrics characters such as the demanian ration (a) which was found large in two population

S. brachyurus specimens from Rwanda showed slight difference in morphological characters compared with those studied by Steiner (1938), Andrássy (1958), Sher (1964), Ali *et al.* (1973), Van den Berg (1973), Siddiqi (1974), Van den Berg (1998) and Van den Berg *et al.* (2013), such as the number of lip annuli which reach up to 7 in some specimens of the *S. brachyurus* ALN18 population instead of the 3 to 6 annuli observed before, the number of tail annuli which was

found to be low up to 7 annuli instead of 9 to 17 noticed by the authors mentioned above. For the morphometric characters the slight difference was found for the lateral line width, lip width and height, the short shaft and the pharynx length, the anal body diameter and the tail length. For the misidentified population (ALN16) all the morphological and morphometric data agrees with the previously *S. brachyurus* specimens studied.

S. cavenessi from Rwanda differ from those studied by Elmiligy (1970), Sher (1964) and Van den Berg (1973) for the female morphologically by few number of lip and tail annuli and a generally long stylet length, resulting in larger m and o ratio. The tail length was also short compared to the previously studied. For the demanian ratio b was bigger in both specimens population compared to the previously studied. For male the a ratio was big for the ALN7 population. The c' and o ratio were also small in both population compared to those in those studied in the literature.

Species of Pratylenchus Filipjev, 1936

Taxonomic discrimination of *Pratylenchus* species is not an easy task as they possess few morphological and morphometric inter-diagnostic characters and those available are highly variable at intraspecific level. That problem was again faced during discriminating *Pratylenchus* n. sp. and their closely related species where on only eleven important characters used in discriminating *Pratylenchus* species about 15 species, which have three lip annuli in common, were found to have only 2 to 3 characters difference with the *Pratylenchus* n. sp.,. Moreover to natural interspecific variability different other factors such as geographical origin, host, temperature, fixation procedure, different ages during analysis time, culture impact have effects of morphological and morphometrics data difference (Inserra *et al.*, 2001; Inserra *et al.*, 2007; Loubama *et al.*, 2007). This was also observed for the *P. goodeyi* from Rwandan bean and *P. goodeyi* from Cameroonian plantain where characters such as the stylet length, number of lip annuli and shape of spermatheca were found to be different.

Morphologically and molecularly *Pratylenchus* n. sp. is most similar to *P. oleae*. Also *P. penetrans* is closer to both of them molecularly. The high molecular resemblance of *P. oleae* and *P. penetrans* was also noticed by Palomares-Rius *et al.* (2014) in his phylogenetic analysis of the *P. oleae* with other *Pratylenchus* species. Even though molecular data separate the three species unambiguously, using morphological and morphometric characters to distinguish them have to

be done with care as almost only except for the stylet length all the other morphometrics overlap. Morphologically as well most of the characters are the same especially with *P. oleae.* The main morphological difference between *Pratylenchus* n. sp., *P. oleae* and *P. penetrans* are the number of lateral lines and the *enface view* of the undivided face which make the *Pratylenchus* n. sp. to fall in first group vs the divided face with dumb-bell shaped for *P. oleae* and *P. penetrans* which make them to fall in third group referring on how Corbett and Clark (1983) distinguished different *Pratylenchus* species based on face patterns. Nevertheless these morphological characters which help in discriminating between these species make identification more and more difficult as in most case the use of SEM during morphological studies is not done frequently. Morphological and morphometric identification of *P. goodeyi* showed a slight difference compared with those studied in monograph of Castillo &Vovlas (2007) such as the shape of spermatheca which was found to be slight oval without spermatheca and short stylet.

In all phylogenetic analysis the resulted trees have the same topology but the mtDNA tree have a clade containing the *Pratylenchus* species members which are found in both clades V and VI for the rDNA trees. All the analyzed species were found to be monophyletic, apart from *P. coffeae* in D2-D3 and *P. scribneri* which were found to be polyphyletic in 18S tree. However, the position of *P. scribneri* may be was due to misidentification but this still has to be approved. The high intraspecific molecular variability statue for *P. penetrans* and *P. coffeae* was clear and well pronounced in all tree topology. The *P. goodeyi* and *P. penetrans* identified from Rwanda were well resolved in the appropriate clades in all three topology. Based on 18S of rDNA tree the *Hirschmanniella loofi* which was one of taxa used as out-group again was found to be as an in-group together with *Pratylenchus* species (Fig. 5) which can be still a proof of suggesting that *Pratylenchus* genus is a paraphyletic group (Palomares-Rius *et al.*, 2014).

The D2-D3 expansion segment of 28S tree topology is in agreement with those of Subbotin *et al.* (2008) and Palomares-Rius *et al.*(2014) and all the clade are almost equally supported. The 18S tree as well are almost in agreement with to those of Múnera *et al.* (2009) and Subbotin *et al.* (2008). The clade which was not very well supported comprising *P. crenatus, P .penetrans, P. convallariae* and *P. vulnus* in Múnera *et al.* (2009) is found to be well supported and resolved in our analysis. The result is showing a clear separation of *P. crenatus* in one fully supported subclade, *P. vulnus* and *P. kumaoensis* in another well supported subclade and the same for *P. pratensis. P. penetrans* formed another clade together with *P. convallariae, P. brachyurus, P.*

pinguicaudatus, P. oleae and our *Pratylenchus* n. sp. The clades found in D2D3 and 18S tree are formed by the same species as well in Subbotin *et al.* (2008), Taheri *et al.* (2013) and Palomares-Rius *et al.* (2014).

Our results confirm Palomares-Rius *et al.* (2014), who already forwarded that the use of COI of mtDNA can result in good diagnosis and characterization of *Pratylenchus* species. Furthermore, we have shown that the use mitochondrial DNA resulted in well resolved trees. So as it was shown with the use of ribosomal DNA in the previously studies that *P. oleae and P. penetrans* have probably the same ancestor, and this was also supported by the use of mitochondrial gene where we can also add the *Pratylenchus* n. sp. and *P. brachyurus*

Referring to the above phylogenetic analysis the species which were found to be closely related to *Pratylenchus* n. sp. morphologically and which have already at least one sequence in the gene bank either of rDNA or mtDNA are P. *oleae*, *P. mediterraneus*, *P. kumaoensis*, and *P. pratensis.* In all of them only *P. oleae* clustered together in the same clade. However our results still shows that the combination of morphological data and molecular data especially the user of COI of mitochondrial DNA give a very good and reliable identification for *Pratylenchus* species. as it was demonstrated by Palomares-Rius *et al.* (2014)

Conclusion and recommendations

Diversity of plant-parasitic nematodes in Rwandan crops was with a minimum of two and maximum of eight PPN per small scale field crop. Most of the taxa were reported before to be associated with crops either in Rwanda or somewhere else in the world. The following taxa are the first records for Rwanda: *Meloidogyne* and *Pratylenchus,* the more economical important taxa, were found in in all sampled crops apart from cassava, tea, and rice. Their pathogenicity on crops still have to be approved in order to take control and management measure for them**.**

Different *Scutellonema* species were present in one sample and this was morphologically and molecularly confirmed. Morphological morphometric variations within and between *Scutellonema* species were noted. Those variability and overlaps changed according to geographical origin of the population. That lead to misidentification of one species morphologically but later molecular analysis provided the clear resolution about it. Integrated characterization is still supporting two clear groups of *S. brachyurus*. The Rwandan population is falling under South African population. Due to high variability in *Scutellonema* species for

identification up to species level combination of morphological, molecular and phylogenetic analysis are important steps and need to be applied as it validate the statue of the species.

Pratylenchus as an important economical important genus in both species number and wide distribution have to be properly identified in order to predict their risks on crop based on initial population. The status of one *Pratylenchus* n. sp. isolated from maize, *P. goodeyi* isolated from bean and *P. penetrans* isolated from different crops were approved by both morphological and molecular approaches. Pathogenicity of all identified species still has to be approved as apart from only *Pratylenchus goodeyi* from Rwandan banana with was associated with root necrosis (Gaidashova *et al.*, 2009) no other nematodes pathogenicity test was done. For phylogenetic study of *Pratylenchus* species more refined characters have to be explored and analyzed in order to get more insight to relationship between morphological and molecular data as most of the morphological characters evolved independently. It is only for the first clade where almost all important morphological characters and the molecular data agreed, all the other clades morphologically and molecular data do not correlate.

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