



## Morphometric and Molecular Characterization of Isolates of the Root Lesion Nematode, *Pratylenchus loosi* Infecting Tea in Sri Lanka

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### ABSTRACT

The Root-lesion nematode, *Pratylenchus loosi* Loof inhabits all tea growing regions of Sri Lanka depicting a variety of symptoms and damage severities to affected tea. Though much has been researched on biology and control measures, isolate characterization has not been attempted. Hence, male and female morphometric variations and molecular characterization of *P. loosi* populations isolated from different agro-ecological regions in Sri Lanka viz. PL1 (Cecilton, Balangoda), PL2 (Delmar, Halgranoya), PL3 (Hapugastenna, Ratnapura) PL4 (Mahadowa, Passara), PL5 (Nawalapitiya) and PL6 (Richiland, Deniyaya) were studied. Female morphometrics of *P. loosi* showed intraspecific variation and clustered in four groups in Principal Component Analysis where PL1 and PL5 were closely-related while PL3 and PL6 clustered separately with the exception of PL2 and PL4. Sequence analysis of the D2/D3 expansion segments of the 28S rDNA gene of the *P. loosi* populations revealed that PL3 and PL6 were closely-related while PL1, PL4 and PL5 were relatively distant. Sequences of the ITS region of rDNA placed PL3 and PL6 in a single clade. The isolates PL1, PL2, PL4 and PL5 were relatively distantly-related and PL2 and PL4 were relatively distant from the other populations. Molecular characterization further validated the relatedness of PL1 and PL5, PL3 and PL6 and, PL2 and PL4 obtained from the morphometric data. The divergence in *P. loosi* populations shown in this study supports evidence of intra specific isolates resulting different symptomological expressions and thus implies need of specific management strategies in managing nematode infestations in tea plantations in Sri Lanka.

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## INTRODUCTION

Tea, [*Camellia sinensis* (L) O. Kunze (Theaceae)], is the major plantation crop grown in Sri Lanka. Plant parasitic nematodes are considered one of the key pests limiting establishment, growth and productivity of tea. *Pratylenchus loosi* is the most predominant nematode species causing economic damage to tea cultivation in different agro climatic regions in Sri Lanka (Gnanapragasam and Mohotti, 2005). Mohotti (1998 and 2002) indicated that there may be several *P. loosi* isolates after studying populations of the nematodes from Sri Lanka, Japan, Iran and Florida. Mizukubo (1998) introduced the *P. loosi* species complex which appears to include phylogenetically distinct isolates based on four Japanese isolates and a Sri Lankan isolate. However, possible intraspecific variations of different populations present in different localities in Sri Lanka have not been studied.

In the past decades, several molecular tools have been deployed to identify and compare nematodes, at the species level and between populations of the same species. For species of the genus *Pratylenchus*, a remarkable difference exists in the sizes of the ITS region of rRNA of the nematodes; so far, the difference between the smallest and the largest amplified ITS regions is about 350 bases (Castillo and Vovlas, 2007). In addition, intraspecific variation in the ITS region has

been observed in this genus, particularly within populations of *Pratylenchus coffeae* and *Pratylenchus vulnus* (Orui, 1996; Uehara et al., 1998; Waeyenberge et al., 2000; Mizukubo et al., 2003, Begum et al., 2019). Sequences of the D2/D3 expansion segment of the 28S rDNA gene has also been used to distinguish between *Pratylenchus* spp and, has been shown to be an important and strong molecular tool for nematode diagnostics (Handoo et al., 2001; Subbotin et al., 2006; de la Pena et al., 2007).

This study was, therefore, undertaken with the objective of using morphometrics and molecular data to characterize *P. loosi* populations collected from six geographic locations in Sri Lanka causing varying symptomolgical expressions and damage to tea.

## MATERIALS AND METHODS

### Selection of sampling sites

Sampling sites to collect *P. loosi* populations to study the morphometrics and molecular data were selected by reviewing history of records maintained by the Nematology Laboratory of the Tea Research Institute, Sri Lanka. Accordingly, six locations representing different elevations with higher *P. loosi* populations were recognized and sampled in this study (Table 1).

**Table 1: Details of the sampling sites**

Code No.	Location	Latitude	Altitude	AER	Elevation (m amsl)
PL1	Cecilton Estate, Balangoda	6°43'60"N	80°39'0"E	IM2b	780
PL2	Delmar Estate, Ragala	7°0'0"N	80°52'0"E	IU2	1400
PL3	Hapugastenna Estate, Ratnapuara	6°69'3"N	80°38'6"E	WM1a	810
PL4	Mahadowa Estate, Passara	7°0'0"N	81°10'0"E	IU3c	1280
PL5	Nawalapitiya (Small holder land)	7°3'0"N	80°32'0"E	WM2a	550
PL6	Richiland Estate, Deniyaya	6°19'28"N	80°33'43"E	WM1a	470

### Morphometric characterization of *P. Loosi* populations

The *P. loosi* specimens were collected from the sampling sites described in Table 1 and the slides were prepared as per the standard protocols in Seinhorst (1959). For the study, 20 female and 12 male specimens of *P. loosi* were used for each site. Specimens were observed under OPTIKA B 500 microscope, and all morphometric measurements were done with Optika Vision Pro plus (Version 2.7) software. The measurements taken were as follows;

#### Female

Body length (L), Maximum body width (w), Body width at anus, Tail length, Head end – vulva, Head end – anus, Anus – phasmid, Phasmid - tail end, Stylet length, Stylet base – DOGO, Body length / maximum body width, Body length / maximum body width, Body length / tail length, Tail length / body width at anus, Tail length / body width at anus, Distance to vulva to anterior end/ Body length \* 100% (V (%)), Head end – vulva/ head end – anus\* 100% V' (%).

#### Male

Body length (L), Maximum body width (w), Tail length, Stylet length, Spicule, Body length / maximum body width, Body length / maximum body width, Body length / tail length, Volume ( $\mu\text{m}^3$ ) and weight ( $\mu\text{g}$ ).

De Man's formula for male and female morphometrics were calculated based on Siddiqi (1986).

#### Data analysis

The data were statistically analysed using SAS 9.1. Principle Component Analysis was used to make an objective assessment of the relative similarity among the populations using fourteen (14) morphometric characters for the females.

### Molecular characterization of *P. loosi* populations

The *P. loosi* populations used for molecular study were collected from the locations described in Table 1. The molecular work (e.g. PCRs, cloning, sequencing, sequence analyses and DNA homology work) of this study was carried out at the State Agricultural Biotechnology Centre (SABC), Murdoch University, Perth, Australia.

DNA extraction from single nematodes was done using phenol chloroform method and methods described by Marek *et al.* (2014) and Wayenberg *et al.* (2000).

#### PCR of the D2/D3 rDNA

Genomic DNA of single adult *P. loosi* nematodes were used for PCRs. Primers D2-1 (5'-GACCCGTCTTGAAACACGGA-3') and D3-2 (5'-CGGAAGGAACCAGCTACTA-3') were used for amplification of the D2/D3 expansion region of the 28S RNA gene. All PCRs were made up to 20  $\mu\text{l}$  with nuclease-free water and contained 10.0  $\mu\text{l}$  Go Taq Green master mix (Promega Corp, Australia), 1.0  $\mu\text{l}$  each of the primers D2-1 and D3-2 and 2.0  $\mu\text{l}$  of DNA template. Thermal cycling was done using an initial denaturation at 95 °C for 5 min, followed by 50 amplification cycles (denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min) and a final step at 72 °C for 10 min. Amplified products were observed on 1% TAE-buffered agarose gels, stained with SyBr Safe DNA gel stain (Invitrogen, USA) and visualized with a Trans illuminator. PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, USA) according to the manufacturer's protocol.

#### PCR of the ITS region

The partial 18S-ITS1-5.8S-ITS2-partial 28S of the rDNA was amplified from genomic DNA of single adult *P. loosi* nematodes using the

primer pair 26S (5'-TCCTCCGCTAAATGATATGC-3' and 18S (5'-GTAACAAGGTAGCTGTAGG-3'). The PCR conditions and the thermal cycling profiles were the same as those used for amplification of the D2/D3 rDNA PCR above.

Ligation of the purified PCR products to the pGEM-T cloning vector and transformation of *E. coli* JM109 with the ligated products were as per the manufacturer's protocol (Promega Corp). This was followed by confirmation of successful ligation by PCR of the colonies, isolation of plasmid DNA, restriction digestion of recombinant plasmids, and sequencing of plasmid DNA using standard molecular biology protocols (Sambrook et al., 1989).

### Sequence alignments and analyses

The software, Finch TV 1.4.0 (Geospiza) was used to visualize and edit erroneous sequences. Clustal omega and MEGA 7.0.26 (Kumar et al., 2016) were used to align the edited sequences and to develop phylogenetic trees respectively.

### Phylogenetic analyses

Phylogenetic trees were constructed using sequences of the D2/D3 28S rDNA expansion segment and ITS region separately using the Neighbour-Joining and Maximum Parsimony (MP) algorithms. Bootstrap analyses with 1000 replicates were performed to assess the degree of support for each clade on the trees. Sequences of similar regions of the respective rDNA for *P. coffeae* (GenBank Accession numbers HQ688681.1 and KF974721.1) were used as out-group taxon for the phylogenetic analyses.

## RESULTS AND DISCUSSION

### Morphometric characterization of *P. loosi* populations

The specimens of males and females conformed closely to the earlier descriptions

of *P. loosi*, but there were noteworthy variations (Tables 2 and 3).

### Female populations of *P. loosi*

Table 2 presents the data compared with morphometrics reported earlier for *P. loosi*. The data showed that the measurements of the populations studied were in the ranges of previously reported *P. loosi* and confirmed their taxonomic identity. Although some may be significant, the deviations in the morphometric characters among the populations were acceptable for *Pratylenchus* species.

The least variable characters were considered in the comparison of different *P. loosi* populations. The results of this study showed evidence of existence of a significant intraspecific variation in the morphometric characters of *P. loosi* found in Sri Lanka.

### Body length (L)

The results showed that 'L' of the study population PL 4 is significantly higher (552µm) than that of the other populations. However, the mean 'L' of each of the populations and for all the populations were in the range of body lengths reported by Loof (1960), Duncan et al. (1999), Inserra et al. (2001) and Wu et al. (2002). Also, the average body length of the populations was similar to that reported for *P. loosi* identified from pasture grasses in central Florida (Inserra et al. 1996).

### Distance from vulva to anterior end/ body Length \* 100% - (V%)

The distance from the vulva to the anterior end/Body Length \* 100% (V%) is the one of the most important morphometric parameters used for characterizing *Pratylenchus* species. Several studies have demonstrated that V ratio is less modified by biotic or abiotic factors and the ratio is a reliable diagnostic character for the genus.

This was confirmed in our study which indicated a very small coefficient of variation (2.96%) as presented in Table 2. The PL 4 population had a lower V ratio (77.05%) while the PL 3 population had a significantly higher V ratio (84.01%) than the other populations.

#### **Maximum body width**

The populations from the PL 2, PL 3, PL 5 and PL 6 locations had a higher maximum body widths compared to *P. loosi* previously identified by Mohotti (1998) from several locations in Sri Lanka. In contrast, PL 4 population were on average 3  $\mu\text{m}$  thinner.

#### **Stylet length**

Roman and Hirschmann (1969) and Loof (1991) explained that the appearance of the stylet knobs may change during storage or after mounting in glycerine implying the diagnostic value of this character may not always be reliable. For this reason, detailed work on stylet morphology was not carried out.

However, in our study, the average stylet length of all the populations showed low coefficient of variation (6.53%), ranging from 13.47 to 16.48  $\mu\text{m}$ . The data showed that stylet length of all the study populations were in the range of that reported by Loof (1960) and Seinhorst (1977) of *P. loosi* identified. The PL 3 and PL 4 populations had comparatively longer stylet lengths than those of PL 2 and PL 5.

Our study showed that some morphometric features, such as the stylet length and V ratio, were less modified by biotic or abiotic factors and are probably more reliable as diagnostic characters for the *Pratylenchus* genus.

#### **Male populations of *P. loosi***

Males were abundant in all the populations studied. Coefficients of variation of all the male characters showed similar trends as in the female characters. Variation in volume and weight were however less in the males of

the same population. The data from this study and comparison with existing references are presented in Table 3.

#### **Tail length**

The tail length of the males of the populations studied was shorter than those to described by Mohotti (1998) except for the populations from PL 4 and PL 5. Similarly, the tail lengths of males from all the locations studied were shorter than in isolates from Taiwan (Wu *et al.* 2002).

#### **Body length (L)**

The 'L' of the PL 4 populations showed similar trends as in the females with highest 'L' of 436.50  $\mu\text{m}$ . Also, the male 'L' was statistically not significantly different among the populations ( $P \leq 0.05$ ). However, the mean values fall within the range in Sri Lankan populations of *P. loosi* previously described by Mohotti (1998) and generally did not conform to the findings of Handoo and Golden (1989) and Wu *et al.* (2002).

#### **Maximum body width**

Our data revealed that the maximum body width was highest in the PL 4 nematodes (12.83  $\mu\text{m}$ ) and lowest in PL 6 nematodes (12.16  $\mu\text{m}$ ). The average maximum body width of the nematodes of the six populations were in the range of those described by Mohotti (1998) but not for those described by Wu *et al.* (2002). For descriptive determination of the *P. loosi* isolates, morphometry of the female specimens was used (Figure 1).

#### **Principal component analysis**

Fourteen (14) morphometric characters of the female nematodes were used for Principal Component Analysis. The six populations were clearly separated into four groups. The PL 4, Mahadowa population and the PL 2, Delmar populations were in separate groups compared to the other populations. The PL 1 and PL 5 were clustered into one group while PL 3 and PL 6 clustered into another.

Table 2: Female morphometrics of *Pratylenchus loosi* populations

Character	Mean values of the study populations											Existing References on <i>P. loosi</i>				
	PL1	PL2	PL3	PL4	PL5	PL6	CV%	Loof, 1960	Seinhorst, 1977	Inserra et al. 1996	Mohotti, 1998	Duncan et al., 1999	Inserra et al., 2001	Wu et al., 2002		
L	491.39 ±53.1	513.16 ±25.2	519.75 ±50.0	552.00 ±48.09	530.09 ±35.62	531.76 ±35.8	3.85	430- 640	575	570- 680	391-540	430- 615	430- 620	490- 710		
Max. body width	17.77 ±2.8	21.42 ±3.0	19.47 ±4.1	15.29 ±0.38	18.99 ±3.11	18.59 ±4.5	6.23	-	-	-	13.3- 18.3	16-31	-	-		
body width at anus	12.82 ±1.3	13.08 ±1.1	11.91 ±1.9	11.81 ±1.0	12.31 ±1.46	12.33 ±0.9	10.63	-	-	-	8.6-12.5	-	9.8-15	-		
tail length	26.05 ±4.4	27.88 ±4.5	22.92 ±1.2	31.82 ±0.85	31.83 ±4.88	23.87 ±5.3	6.97	-	-	-	25.0- 30.2	24.5- 31.0	24.5-36	28.1- 40.6		
head end - vulva	405.84 ±55.3	435.04 ±8.5	430.75 ±5.7	425.59 ±49.2	437.17 ±57.39	439.22 ±40.2	6.87	-	-	-	294-434	-	-	-		
head end - anus	444.75 ±68.3	488.61 ±26.6	489.62 ±38.1	540.59 ±46.2	504.01 ±80.49	512.84 ±25.3	6.80	-	-	-	393-503	-	-	-		
anus - phasmid	11.74 ±0.8	11.94 ±0.3	10.83 ±0.3	10.74 ±0.54	11.10 ±0.56	10.92 ±3.5	15.55	-	-	-	16.5	--	-	-		
phasmid - tail end	12.64 ±0.9	12.39 ±0.7	10.72 ±0.4	10.66 ±0.46	15.27 ±1.12	11.80 ±1.2	10.47	-	-	-	14.3	-	-	-		
stylet length	13.47 ±1.5	13.94 ±1.0	15.01 ±1.1	16.48 ±1.03	15.27 ±1.63	15.62 ±1.6	6.53	14-18	14-18	15.0- 15.6	14.7- 16.3	15-16	15-17.5	16.3- 19		
stylet base - DOGO	22.32 ±0.7	21.85 ±1.9	23.09 ±1.0	32.28 ±0.01	24.28 ±0.56	25.15 ±1.9	8.01	-	-	-	28.9	-	-	-		

Table 2: Female morphometrics of *Pratylenchus loosi* populations (continued)

Character	Mean values of the study populations										Existing References on <i>P. loosi</i>				
	PL1	PL2	PL3	PL4	PL5	PL6	CV%	Loof, 1960	Seinhorst, 1977	Insera et al, 1996	Mohotti, 1998	Duncan et al., 1999	Inserra et al., 2001	Wu et al., 2002	
a ratio	28.13 ±4.7	24.60 ±3.3	27.22 ±5.4	36.13 ±3.38	30.14 ±3.11	31.74 ±5.4	13.57	19.5- 36	31.9	26.7- 34.8	26.2-29.9	-	-	-	
c ratio	19.26 ±3.5	19.07 ±3.4	22.45 ±2.3	23.74 ±3.06	24.44 ±3.03	26.21 ±5.7	11.45	16-25	19.9	17.2- 22.0	18.0	16.2- 20.5	15.1- 22.5		
c' ratio	2.04 ±0.3	2.15 ±0.5	1.97 ±0.3	1.40 ±0.14	1.85 ±0.20	1.78 ±0.4	14.21	-	-	2.4-2.9		-			
V (%)	82.42 ±4.5	83.96 ±4.0	84.01 ±3.9	77.05 ±4.57	82.43 ±8.62	82.46 ±5.2	2.96	78.5- 85	82.5	77.5- 82.1	77.8-81.6	78.5- 82.5	78.2- 84.7		
V' (%)	91.55 ±3.8	89.23 ±4.2	88.25 ±7.3	78.71 ±5.43	85.07 ±4.52	83.44 ±7.9	6.67	-	-	86.5		-			
Volume (µm <sup>3</sup> )	94.30 5 ±141 74	143,40 9 ±1439 2	120,25 4 ±19403	75,923 ±7346	123,759 ±41050	121,3 39 ±175 26	21.03	-	-	45,665- 107,320		-			
Weight (µg)	0.10 ±0.04	0.15 ±0.05	0.13 ±0.06	0.08 ±0.01	0.13 ±0.04	0.13 ±0.08	25.56	-	-	0.05-0.11		-			

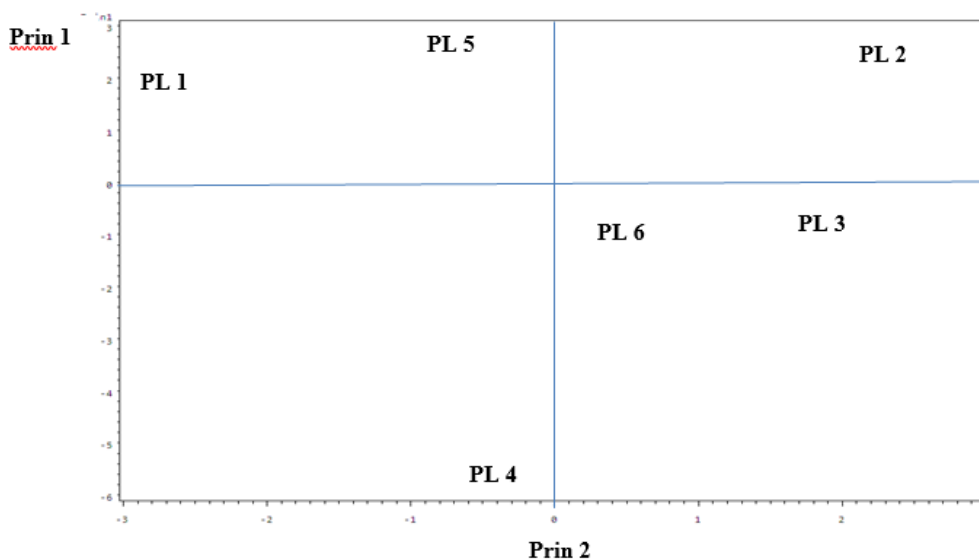
All measurements are given in µm. CV%- coefficient of variation. The figures represent the mean from 20 nematodes and are presented as mean ± standard deviation

Table 3: Male morphometrics of *Pratylenchus loosi* populations

Character	Mean values of the study populations						Existing References on <i>P. loosi</i>			
	PL1	PL2	PL3	PL4	PL5	PL6	CV%	Handoo and Golden, 1989	Mohotti, 1998	Wu et al., 2002
L	427.80 ±23.6	424.50 ±25.6	422.50 ±23.1	436.50 ±23.6	426.50 ±30.9	423.00 ±5.12	6.68	480-640	410-470	490-710
Max. body width	12.58 ±2.05	12.68 ±3.15	12.78 ±2.65	12.83 ±2.89	12.57 ±2.87	12.16 ±2.58	8.43		11.4-15.6	16.3-26.3
tail length	22.54 ±2.05	21.91 ±4.12	21.38 ±1.89	22.69 ±2.45	22.53 ±5.41	21.20 ±2.35	6.45		22.5-30.9	28.13-40.63
Stylet length	15.75 ±3.69	13.84 ±2.63	14.35 ±3.41	14.44 ±3.15	15.47 ±2.89	13.66 ±1.89	5.38	14-18	15.0-16.4	16.3-19.0
Spicule	13.67 ±1.52	14.11 ±1.85	16.52 ±4.12	17.02 ±4.65	13.65 ±3.12	18.12 ±2.56	14.69		13.9-20.0	
a ratio	34.19 ±3.65	33.55 ±2.69	33.24 ±4.12	34.23 ±4.32	34.03 ±3.18	32.28 ±3.45	16.58	28-36	26.3-38.6	24.4-35.7
c ratio	19.01 ±4.12	19.46 ±3.12	19.77 ±3.58	19.27 ±1.85	18.96 ±4.56	19.97 ±3.33	14.89		14.3-20.4	
Volume ( $\mu\text{m}^3$ )	39971.45 ±1569	40216.88 ±2030	40876.00 ±2089	42573.05 ±1385	39745.06 ±2365	43405.97 ±3251	28.69		33637-63846	
Weight ( $\mu\text{g}$ )	0.04 ±0.001	0.04 ±0.001	0.04 ±0.001	0.05 ±0.002	0.04 ±0.001	0.05 ±0.001	27.92		0.04-0.07	

All measurements are given in  $\mu\text{m}$ . CV%- coefficient of variation. The figures represent the mean from minimum of 12 nematodes and are presented as mean  $\pm$  standard deviation





**Figure 1: Principal Component Analysis of the fourteen female morphometric characters of *P. loosi* populations of PL 1-PL6**

In summary, the female morphometrics of the six populations of *P. loosi* measured in this study indicates there is intraspecific variation in the nematodes (Table 2 and Figure 1). However, the PCA results did not clearly separate the populations into clusters based on their geographic locations. Generally, the specimens could be confirmed as *P. loosi* species as their morphometric features were well within the ranges of typical measurements reported for this species. The slight variations in the morphometric parameters allowed the six populations to be clustered into four distinct groups. The results revealed that PL 1 and PL 5 populations are closely-related, as were the PL 3 and PL 6 populations. However, the separation of PL 2 and PL 4 populations into individual separate groups indicate they may be morphometrically different from the other four populations.

### **Molecular characterization of *P. loosi* populations**

#### **PCR of D2/D3 rDNA**

The amplification of the D2/D3 Large Sub Unit of rDNA expansion segments yielded 345

bp fragments for all the isolates except for nematodes from PL 2 where there was no amplification.

Results of the homology search with available DNA databases of *P. loosi* species are given in Table 5. Representative sequences from all the locations best matched with *P. loosi* with sequence identities ranging from 96 % - 100%. The individual sequences from each location showed some intra-species variation depicting potential isolates of *P. loosi*.

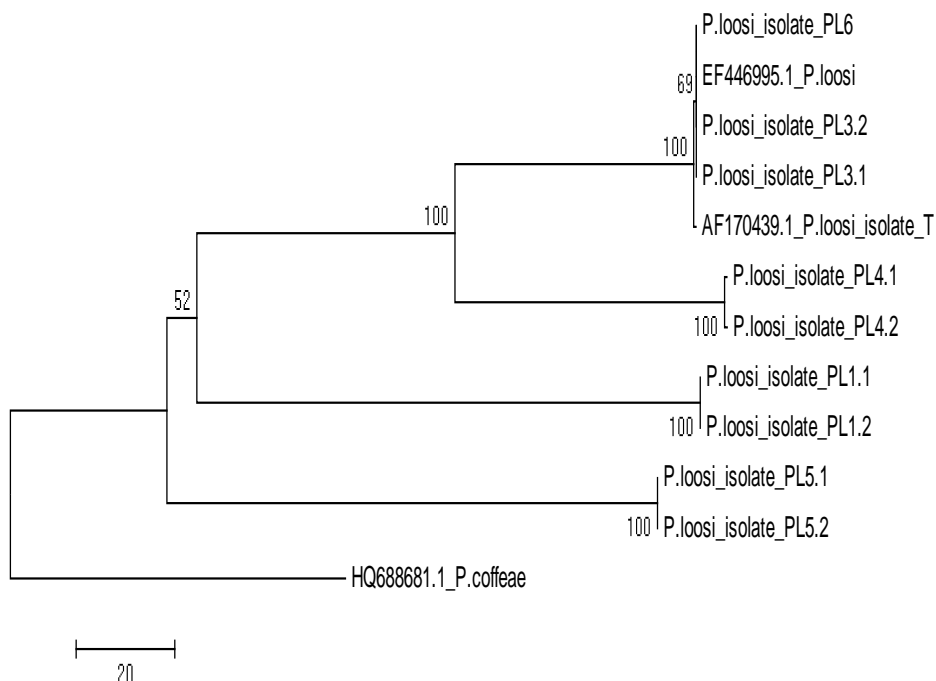
#### **Phylogenetic analysis of D2/D3 sequences**

The Neighbor-Joining trees constructed using the sequences of D2/D3 28S rDNA expansion segment are shown in Figure 2.

The five populations were clustered into different phylogenetic clades with nematodes from populations PL 6 and PL 3 closely-related to each other than the others. Sequences of the nematodes of the PL 3 and PL 6 populations were grouped with EF446995.1, a sequence of *P. loosi* isolated from Iran but were more distantly-related to AF170439.1 *P. loosi* isolate T which was described as a Sri Lankan isolate. The rest of the populations existed as well separated

clusters, indicating the distant relationship they may have with PL3 and PL6 populations. The sequence divergence of the nematodes of the different populations were low based on the pairwise mean distances between sequences of the D2/D3 28S rDNA expansion segments. The best matching sequences on

Genbank are presented in Table 5. The sequences from the same location were in the same clade confirming there were no sequence differences among *P. loosi* isolates from the same location; sequences with the same number PL designation (e.g. PL5.1 and PL5.2) were from the same location.



**Figure 2: Neighbor joining tree of *P. loosi* populations studied, other *Pratylenchus* populations obtained from GenBank based on the sequence alignment of the D2/D3 28S rDNA expansion segments**

### PCR of ITS Region

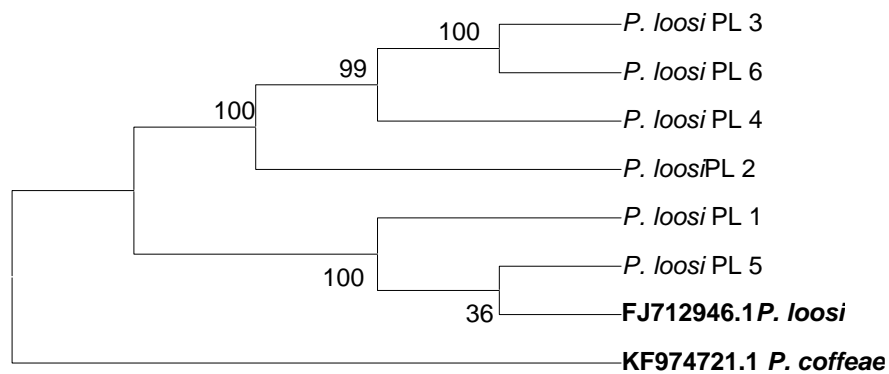
ITS sequences of nematodes analysed showed a 100% homology and 100% query coverage with *P. loosi* 18S rRNA sequences deposited in the GenBank Database and confirmed the identity of the six populations studied (Table 4).

Based on the phylogenetic trees (Figures 3 and 4) constructed using the Maximum Parsimony and Neighbor-Joining methods, the PL 3 and PL 6 populations clustered together and formed a single clade. Nematodes of the PL4 population clustered separately but showed a genetic

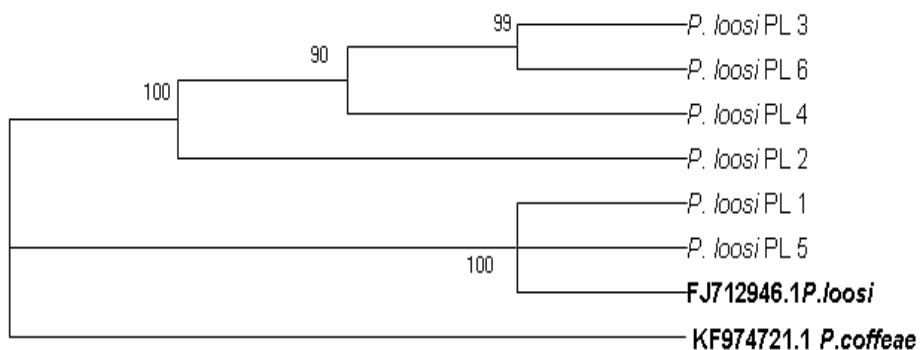
relationship closer to the PL 3 and PL 6 populations. The PL 2 nematodes clustered separately but showed a closer relationship to PL 4 and PL 3 and PL 6 populations than those of the PL1 and PL 5 populations. Both analyses methods showed that PL 1 and PL 5 clustered together and formed a clade with FJ712946.1 *P. loosi* isolate. Therefore, the results clearly indicated there is some level of intra-species variation in the *P. loosi* isolates obtained from the different locations in Sri Lanka.

**Table 4: Details of the homology search of the rDNA sequences**

Sample Name	Description of the highest homologue	Query cover	E value	Identity	Accession
PL1	<i>Pratylenchus loosi</i> isolate Jan-1 28S ribosomal RNA gene, partial sequence	100%	3e-171	99%	KF430797.1
PL3	<i>Pratylenchus loosi</i> isolate EX11 28S ribosomal RNA gene, partial sequence	100%	0.0	100%	KY424292.1
PL4	<i>Pratylenchus loosi</i> isolate P11 28S ribosomal RNA gene, partial sequence	100%	5e-143	100%	EF446996.1
PL5	<i>Pratylenchus loosi</i> isolate Jan-1 28S ribosomal RNA gene, partial sequence	99%	2e-153	96%	KF430797.1
PL6	<i>Pratylenchus loosi</i> isolate EX11 28S ribosomal RNA gene, partial sequence	100%	0.0	100%	KY424292.1



**Figure 3: Maximum Parsimony tree of *P. loosi* populations (PL 1 - PL 6), other *Pratylenchus* populations obtained from GenBank based on the sequence alignment of the ITS region**



**Figure 4: Neighbor Joining tree of *P. loosi* populations studied, other *Pratylenchus* populations obtained from GenBank based on the sequence alignment of the ITS region.**

Table 5: Details of the homology search of the DNA sequences of the PCR products with ITS sequences deposited at GenBank

Sample Name	Description of the highest homologue	Query cover	E value	Identity	Accession
PL 1	<i>Pratylenchus loosi</i> clone 46-2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	100%	0.0	100%	KT184921.1
PL 2	<i>Pratylenchus loosi</i> 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	100%	0.0	100%	FJ799118.1
PL 3	<i>Pratylenchus loosi</i> isolate GuJP 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	100%	0.0	100%	KY424223.1
PL 4	<i>Pratylenchus loosi</i> genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: Shizu1, clone: No.2	100%	0.0	100%	LC030321.1
PL 5	<i>Pratylenchus loosi</i> clone 46-2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	100%	0.0	100%	KT184921.1
PL 6	<i>Pratylenchus loosi</i> isolate GuJP 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	100%	0.0	100%	KY424223.1

## CONCLUSIONS

Overall results showed evidence of existence of morphotypes in the six *P. loosi* populations infesting tea in Sri Lanka. It also showed some divergence in the molecular data of the nematodes. Whether these divergence amount to differences in infestation patterns and severity and persistence of the nematodes in different regions of Sri Lanka with varied soil and environmental factors needs further investigation. Results of such investigations are important as they will indicate whether specific management strategies are required to control damage caused by the nematodes in each of the tea-growing areas in Sri Lanka.

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