Morphological and molecular characterisation of root-lesion nematodes (*Pratylenchus* spp.) (Rhabditida: Pratylenchidae) associated with apple in South Africa

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Summary. *Pratylenchus* spp. are widely known to cause damage to apple trees. A survey was conducted in all the major pome fruit production areas in South Africa, amounting to more than 100 sampling localities. Lesion nematodes detected in the samples were molecularly identified by PCR amplification and sequencing of the D2-D3 expansion segments of 28S, as well as ITS-rDNA and the cytochrome oxidase gene of mitochondrial DNA (*COI*). Viable lesion nematodes were handpicked from each sample and transferred to carrot discs for *in vitro* propagation. A sub-sample of each population was also preserved for morphological identification to species level and taxonomic studies. *Pratylenchus hippeastri* was detected in most of the sampled regions, except Villiersdorp. In some instances, mixed populations of *P. hippeastri*, *P. vulnus* and *P. penetrans* were found, but *P. hippeastri* was the most abundant. Morphological and molecular studies confirmed the identity of these species.

Key words: identification, morphometrics, phylogeny, survey, taxonomy.

Apple (*Malus pumila* Mill.) is an important deciduous fruit tree all over the world. In South Africa, apples are the largest component of the deciduous fruit crop with more than 20,000 ha harvested annually. South Africa is also the world's seventh-largest exporter of apples, with approximately 423, 394 MT being exported in 2019. The Western Cape province of South Africa, with its Mediterranean climate, is the largest production area (18,853 ha) for apples in the country (HORTGRO, 2019).

The root-lesion nematodes (*Pratylenchus* spp.) are the most common plant-parasitic nematodes present in apple orchards in South Africa. Apple trees, especially younger trees, infected with lesion nematodes have poor growth and yield declines gradually (Hugo & Storey, 2017). They are migratory endoparasites, causing severe damage by feeding and migrating through the cortical tissue. They live and reproduce in the roots, causing affected tissues to be more easily accessible to soil fungi (Loof, 1991). Worldwide, twelve species of the genus *Pratylenchus* Filipjev, 1936 have been reported as potential pathogens of apple (Castillo & Vovlas, 2007). In addition to the direct damage caused to the roots, *Pratylenchus* spp. have been implicated in apple replant disease (ARD), which presents as poor initial growth of young trees when old orchards are replanted. *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941 has been identified as being part of the ARD complex in Washington, USA (Mazzola, 1998); and *P. jordanensis* Hashim, 1983 has been involved in this disease in Queensland, Australia (Stirling *et al.*, 1995). In South Africa, the identity of the specific *Pratylenchus* species in the ARD complex is yet to be determined.

Twelve *Pratylenchus* species have been reported from South Africa (Marais, 2021). Results from an earlier survey, during which apple orchards were sampled in three production areas of the Western Cape, showed that six species of lesion nematodes were encountered frequently and occurred in 96% of the orchards (Hugo, 1994). *Pratylenchus flakkensis* Seinhorst, 1968 was the most abundant species, followed by *P. penetrans*, *P. pratensis* (De Man, 1880) Filipjev, 1936, *P. scribneri* Steiner, 1943, *P. vulnus* Allen & Jensen, 1951 and *P. zeae* Graham,

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1951. Samples taken from ARD soils in the Western Cape province of South Africa revealed the presence of *P. penetrans*, *P. scribneri* and *P. delattrei* Luc, 1958 (Tewoldemedhin *et al.*, 2011). According to the South African Plant-Parasitic Nematode Survey (SAPPNS), *P. crenatus* Loof, 1960, *P. delattrei*, *P. loosi* Loof, 1960, *P. neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941 and *P. thornei* Sher & Allen, 1953 have also been reported from apple orchards in South Africa (Marais, 2021). A survey of apple tree nurseries in 2009 suggested that lesion nematodes have been spread across apple production areas *via* rooted plant material, since it was found that 80% of nursery material had unacceptably high population levels of lesion nematodes (Storey, 2009). Additionally, more recent results from diagnostic samples (S. Storey, personal communication) suggest that a shift in the dynamics of populations encountered in apple orchards has taken place, creating the need for a new survey across the apple producing areas of South Africa.

Morphological diagnosis of the *Pratylenchus* spp. is problematic due to a lack of robust diagnostic characters, high morphological plasticity and incomplete taxonomic descriptions (Castillo & Vovlas, 2007; Subbotin *et al.*, 2008). Janssen *et al.* (2017), after studying the link between morphology and species-specific nuclear ribosomal and mitochondrial gene sequences of the *penetrans*-group, concluded that identification on morphology alone could be inconclusive for this group. *Pratylenchus hippeastri* Inserra, Troccoli, Gozel, Bernard, Dunn & Duncan, 2007 and undescribed species in the *hippeastri*-group are good examples of the cryptic nature of *Pratylenchus* species. Inserra *et al.* (2007) noted that the ranges of morphometric characters of *P. hippeastri* overlap with those of *P. scribneri* and *P. hexincisus* and that these species also share some morphological and biological features, such as two lip annuli, an empty spermatheca and similar lateral fields. Several specimens, which have previously been identified morphologically either as *P. scribneri*, *P. loosi* or *P. zeae*, have now been molecularly identified as undescribed species belonging to the *hippeastri*-group of species (De Luca *et al.*, 2010). Wang *et al.* (2016) also found that *P. hippeastri* is morphologically close to other *Pratylenchus* species, such as *P. scribneri* and *P. loosi*.

To improve the resolution and reliability of nematode phylogenetic and diagnostic studies, it should ideally be combined with molecular data (de Oliveira *et al.*, 2011). The use of DNA-based molecular techniques to aid in the identification of *Pratylenchus* species has become increasingly common in recent years. Sequencing and phylogenetic analyses of different fragments of the ribosomal gene cluster, including ITS (De Luca *et al.*, 2010; De Luca *et al.*, 2011), 18S (Subbotin *et al.*, 2008) and 28S rDNA (Al-Banna *et al.*, 2004; Subbotin *et al.*, 2008) have provided meaningful insight into the systematics of the group. More recently, Janssen *et al.* (2017) explored the cytochrome c oxidase subunit 1 (*COI*) gene of the mitochondrial genome as a barcode marker for *Pratylenchus*.

In this study, our aim was to survey the main apple production regions in South Africa and to identify and characterise the lesion nematode populations detected through morphological and molecular means.

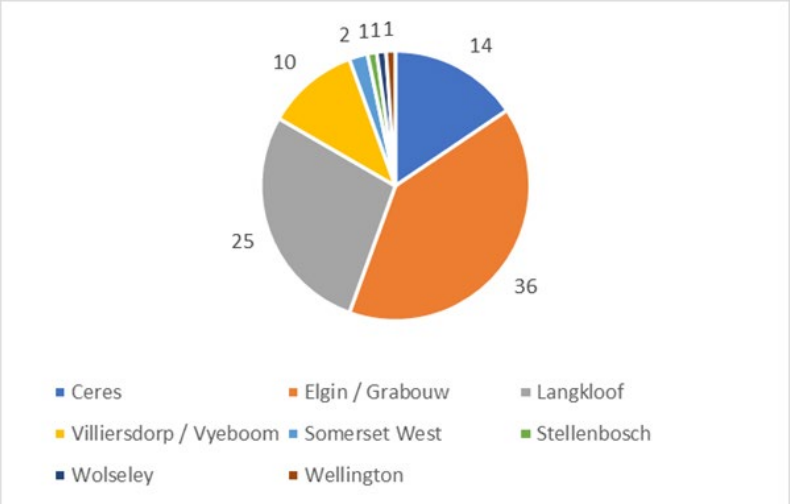

Fig. 1. Number of samples collected per apple production region, Western Cape province, South Africa.

Table 1. Populations of lesion nematodes (*Pratylenchus* spp.) detected in the present study from the Western and Eastern Cape provinces of South Africa.

Sample code	Production region	Molecular ID
164/18	Ceres	*P. hippeastri*
224/18	Vyeboom	*P. hippeastri*
368/18	Langkloof	*P. hippeastri*
2057/17	Ceres	*P. hippeastri*
2061/17	Ceres	*P. hippeastri*
2064/17	Ceres	*P. hippeastri*
2065/17	Ceres	*P. hippeastri*
2394/18	Langkloof	*P. hippeastri*
2771/17	Elgin	*P. hippeastri*
2777/17	Elgin	*P. hippeastri*
2778/17	Elgin	*P. hippeastri*
2779/17	Elgin	*P. hippeastri*
2783/17	Elgin	*P. hippeastri*
2960/17	Grabouw	*P. hippeastri*
2962/17	Grabouw	*P. hippeastri*
2963/17	Grabouw	*P. hippeastri*
2964/17	Grabouw	*P. hippeastri*
2968/17	Grabouw	*P. hippeastri*
2970/17	Grabouw	*P. hippeastri*
3195/18	Wellington	*P. hippeastri*
3210/18	Langkloof	*P. hippeastri*
4801/16	Ceres	*P. hippeastri*
4809/16	Ceres	*P. hippeastri*
2549/18	Villiersdorp	*P. vulnus*
3055/18	Langkloof	*P. penetrans*
3058/18	Langkloof	*P. hippeastri*
3481/18	Wolseley	*P. hippeastri*
Infruitec	Stellenbosch	*P. hippeastri*
AdK2	Ceres	*P. hippeastri*
B1	Ceres	*P. hippeastri*
B2	Ceres	*P. hippeastri*
C	Ceres	*P. hippeastri*
Ct	Ceres	*P. penetrans*
LT1	Somerset West	*P. hippeastri*
LT5	Somerset West	*P. vulnus*
4041/08	Villiersdorp	*P. penetrans*
4442/08	Vyeboom	*P. hippeastri*
B18	Grabouw	*P. hippeastri*
94f	Koue Bokkeveld/Ceres	*P. vulnus*
168/09	Grabouw	*P. hippeastri*
4822/08	Ceres	*P. hippeastri*

MATERIAL AND METHODS

Survey. Samples were collected from most of the major pome fruit production areas in the Western Cape province of South Africa, as defined in Hortgro's Key Deciduous Fruit Statistics (HORTGRO, 2019). These include Ceres, Langkloof, Elgin, Grabouw, Vyeboom, Villiersdorp, Somerset West, Wolseley, Stellenbosch and Wellington production areas (Fig. 1). Root samples were collected from 5-6 trees per orchard and pooled. Samples, including fine hair roots, were collected at the base of the tree up to 20 cm deep. Furthermore, samples collected from apple orchards that were submitted for analysis at a diagnostic laboratory (Nemlab) were also included in the survey.

Nematode populations. Only samples that contained a moderate to high number (> 50 g roots^{-1}) of lesion nematodes were used for further identification of the specimens through morphological and molecular means (Table 1). Only populations that were selected for morphological studies were cultured. Ten to twenty viable lesion nematodes were handpicked from selected samples and transferred to carrot discs for *in vitro* propagation (Coyne *et al.*, 2014).

Morphological and morphometric studies. Light microscopy. Female specimens were fixed in a heated 4% formaldehyde + 1% propionic acid (FPG) solution (Netscher & Seinhorst, 1969), dehydrated to anhydrous glycerin by using the short Seinhorst method (1959) and permanently mounted in anhydrous glycerin on glass slides. Measurements and drawings of the mounted specimens were done with an Olympus BX53F microscope, equipped with a drawing tube at 1000× magnification. Morphometrics according to those of De Man (1884) were used in descriptions. Facial patterns as described in Corbett & Clark (1983) and Castillo & Vovlas (2007) were followed. The specimens were deposited in the National Collection of Nematodes (NCN) at ARC-PHP, Biosystematics, Pretoria.

Scanning electron microscopy. For scanning electron microscope (SEM) studies, specimens were fixed, dehydrated in an ethanol series, critical point-dried, mounted on microscope stubs and coated with gold-palladium as described in Marais *et al.* (2017). The specimens were killed over a spirit flame, fixed in TAF and left for a week at room temperature. The specimens were then transferred to a range of ethanol solutions (70, 80, 90 and 96%) at 3 h intervals, repeating the 96% three times. The specimens were then critical point-dried using liquid carbon dioxide and transferred to copper foil conductive tape on a SEM viewing stub. The stub

was then coated with gold-palladium (66 and 33%, respectively). SEM were taken with a FEI Quanta FEG 250 electron microscope.

Molecular study. DNA amplification and sequencing. DNA was extracted from individual nematodes using a crude lysis method and the polymerase chain reaction (PCR) was performed as described in Knoetze *et al.* (2017). The forward primer D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and the reverse primer D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') (Subbotin *et al.*, 2006) were used to amplify the D2-D3 expansion segments of 28S rDNA. The forward primer rDNA1 (5'-TTG ATT ACG TCC CTG CCC TTT-3') and the reverse primer rDNA2 (5'-TTT CAC TCG CCG TTA CTA AGG-3') (Vrain *et al.*, 1992) were used for amplification of the ITS regions, including the 5.8S ribosomal gene. Partial amplification of *COI* was achieved using primers COIF (5'-GAT TTT TTG GKC ATC CWG ARG-3') and COIR (5'-CWA CAT AAT AAG TAT CAT G-3') (Lazarova *et al.*, 2006). PCR products were cleaned up and sequenced by Inqaba Biotechnical Industries (Pty) Ltd, using an ABI 3500xL Genetic Analyzer. Sequence assembly and editing were performed on the CLC DNA Workbench 8 (QIAGEN, Aarhus, Denmark).

Phylogenetic analysis. Sequences obtained in this study were compared to those of other *Pratylenchus* species deposited into the GenBank database. The selected sequences, as well as newly generated sequences, were aligned using the online version of MAFFT with default parameters (Katoh *et al.*, 2019). The GenBank accession numbers of the used sequences are indicated in the phylogenetic trees. The appropriate substitution model of DNA evolution that best fitted the data set was determined by the Bayesian information criterion (BIC) as well as the Akaike information criterion (AIC) with MEGA X (Kumar *et al.*, 2018).

The 28S dataset was analysed by using the maximum likelihood method and Kimura 2-parameter model (Kimura, 1980). A discrete gamma distribution was used to model evolutionary rate differences among sites. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The confidence intervals for the various branching patterns in the trees were measured with the bootstrap test (BS) with 1000 replicates (Felsenstein, 1985). Similarly, the ITS rDNA sequence dataset was analysed by using the maximum likelihood method and Tamura 3-parameter model (Tamura, 1992) and the *COI* dataset was analysed by using the maximum likelihood method and Hasegawa-Kishino-Yano model (Hasegawa *et al.*, 1985). Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

RESULTS

Survey. In this survey, only three species of root-lesion nematodes were detected in apple orchards of the Western Cape province of South Africa. *Pratylenchus hippeastri* was detected in all the sampled regions, except Villiersdorp (Fig. 2). In some instances, mixed populations of *P. hippeastri*, *P. vulnus* and *P. penetrans* were found, but *P. hippeastri* was by far the most dominant species (84.2% of samples), with *P. vulnus* and *P. penetrans* only being isolated from 9.17% of samples.

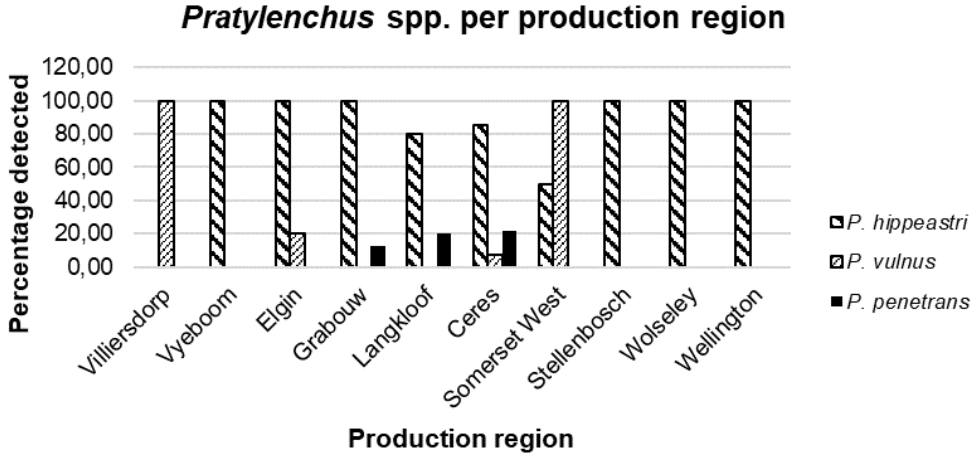

Fig. 2. *Pratylenchus* species detected per production region.

Table 2. Measurements of females, juveniles and one male of *Pratylenchus hippeastri* Inserra, Troccoli, Gozel, Bernard, Dunn & Duncan, 2007 from the Western and Eastern Cape provinces of South Africa. All measurements are in µm and in the form: mean ± s.d. (range).

Characters	Stellenbosch Apple Infruitec A		Stellenbosch Apple Infruitec 2A	Elgin Apple 2960	Ceres Apple 2057	Misgund Apple 3210		Stellenbosch Apple 3195	
	36 Females	2 Juveniles	16 Females	29 Females	4 Females	30 Females	2 Juveniles	18 Females	1 Male
L	510 ± 37.7 (408-598)	388, 395	517 ± 31.2 (470-589)	556 ± 50.9 (401-641)	582 ± 4.1 (541-623)	617 ± 23.2 (552-654)	360, 436	591 ± 39.7 (522-669)	498
a	27.1 ± 2.4 (21.4-32.4)	24.3, 26.9	23.9 ± 1.7 (21.3-26.3)	23.8 ± 2.9 (17.6-34.4)	27.5 ± 0.7 (26.9-28.6)	28.4 ± 1.9 (24.7-32.5)	23.3, 27.1	26.6 ± 3.1 (23.2-32.5)	32.2
b	4.1 ± 0.3 (3.6-4.7)	3.7, 3.6	4.2 ± 0.3 (3.7-4.6)	4.5 ± 0.4 (3.4-5.2)	4 ± 0.4 (3.6-4.5)	4.3 ± 0.2 (3.6-4.7)	3.3, 3.5	4.7 ± 0.3 (4.4-5.2)	3.9
b'	5.5 ± 0.4 (5-6.6)	4.5, 4.4	5.8 ± 0.3 (5.1-6.3)	6.3 ± 0.4 (5.6-7.3)	6 ± 0.4 (5.5-6.5)	6.2 ± 0.4 (5.6-7.1)	5.3, –	6.6 ± 0.4 (5.8-7)	5.6
c	17.7 ± 1.8 (14.4-22.5)	15.7, 15.8	18.1 ± 1.4 (15.7-21.7)	17.9 ± 2.1 (12.1-22.8)	18.9 ± 1.2 (17.5-20.4)	18.4 ± 1.5 (14.6-22.3)	13.6, 15.6	18.4 ± 1.6 (15.8-21.2)	18.8
c'	2.3 ± 0.3 (1.7-3.3)	2.1, 2.1	2 ± 0.2 (1.6-2.3)	2.1 ± 0.2 (1.7-2.6)	2.4 ± 0.3 (2.1-2.9)	2.4 ± 0.2 (1.9-2.8)	2.4, 2.5	2 ± 0.2 (1.8-2.5)	2.4
o	20 ± 3.4 (14.3-28.6)	21.6, 17.9	18.9 ± 2.7 (13.3-23)	23.7 ± 2.6 (19-28.8)	19.4 ± 0.7 (18.6-20)	19.8 ± 3.6 (13.6-25)	23.5, 20.5	16.8 ± 1.9 (14.3-19)	12.5
DGO	3 ± 0.6 (2-4)	3, 2.5	3 ± 0.5 (2-4)	3.5 ± 0.4 (3-4)	3 (3-3)	3 ± 0.5 (2-4)	2.9, 2.9	2.5 ± 0.3 (2-3)	2
V	77 ± 1.9 (71-81.2)	–	76 ± 1.6 (73-79)	76.5 ± 2.1 (69.8-80.2)	78 ± 1.1 (77-79)	78 ± 1.1 (74-80)	–	77 ± 0.8 (75-78)	–
OV %	46 ± 6.6 (32.5-57.7)	–	53 ± 3.1 (45-57.5)	55.3 ± 6.5 (37.2-63.7)	48 ± 9.2 (37-60)	48 ± 5.5 (35-58)	–	54.3 ± 9.7 (33.2-65.5)	–
OV length/primordium in juveniles	235 ± 39 (161-310)	109, 88	273 ± 20 (238-306)	307 ± 50.5 (149-371)	281 ± 56.4 (232-361)	293 ± 39.1 (192-356)	35, 82	321 ± 57 (188-377)	–
Stylet length	15 ± 0.6 (14-16)	13.5, 14	15.5 ± 0.8 (14-17.5)	15 ± 0.8 (14-17)	15 ± 0.6 (15-16)	15.5 ± 0.7 (15-17)	12.5, 14	15.5 ± 0.6 (15-17)	15
Conus	7 ± 0.4 (6-8)	6.5 (n = 1)	7.5 ± 0.6 (7-9)	7 ± 0.5 (6-8)	7 ± 0.4 (6.5-7.5)	7 ± 0.5 (6-8)	66.5	7 ± 0.5 (6.5-8.5)	–
Shaft	8 ± 0.3 (7-9)	7.5, 7	8 ± 0.4 (7.5-9)	8 ± 0.5 (7.5-9)	8 ± 0.5 (7.5-8.5)	8.5 ± 0.4 (8-9)	6.5, 7.5	8.5 ± 0.4 (7.5-9)	–
M	46.6 ± 2.1 43.4-51.4	–	47.4 ± 2.4 (43.7-51)	46.6 ± 2.4 (42.8-50)	47.3 ± 2.1 (46.5-50)	46 ± 2.3 (39-50)	47.2, 46.2	46 ± 2.4 (42.9-50)	–
Stylet knob height	2.5 ± 0.3 (2-3)	2.5, 3	2.5 ± 0.3 (2-3)	2.5 ± 0.3 (2-3)	2.5 ± 0.4 (2-3)	2.5 ± 0.4 (2-3)	2, 3	2.5 ± 0.4 (2-3)	–

Table 2 (continued). Measurements of females, juveniles and one male of *Pratylenchus hippeastri* Inserra, Troccoli, Gozel, Bernard, Dunn & Duncan, 2007 from the Western and Eastern Cape provinces of South Africa. All measurements are in µm and in the form: mean ± s.d. (range).

Stylet knob width	4 ± 0.4 (3-5)	4 (n = 1)	4 ± 0.5 (3-5)	4 ± 0.4 (3-5)	4 (4-4)	4 ± 0.4 (4-5)	3, 3.5	4 ± 0.4 (3.5-5)	–
Pharyngeal gland length	124 ± 6.7 (112-140)	107, 111	124 ± 8.4 (112-147)	124 ± 12.9 (103-159)	146 ± 5.7 (140-146)	142 ± 8.3 (126-153)	107, 126	128 ± 10.6 (110-150)	129
Excretory pore from anterior end	80 ± 6 (69-89)	67, 73	66 ± 10.4 (54-88)	77 ± 10.2 (56-96)	82 ± 4.4 (78-88)	96 ± 5.2 (79-103)	74, 83	78 ± 10.3 (63-96)	74
Pharyngeal overlap	31 ± 6 (21-48)	21, 22	33.5 ± 5.8 (25-50)	35.5 ± 7.5 (23-48.5)	48 ± 6.4 (43-57)	44 ± 8.5 (29-73)	39, –	36.5 ± 5.9 (28-48.5)	40.5
Width at mid-body	19 ± 1.5 (16-21)	16, 15	22 ± 1.1 (20-24)	24 ± 1.1 (22-26)	21 ± 1.4 (20-23)	22 ± 1.7 (18-26)	15.5, 16	23 ± 2.7 (18-29)	15.5
Width at anus	13 ± 1.1 (11-17)	12 (n = 1)	15 ± 1 (13-16)	15 ± 1 (14-18)	13 ± 1.8 (10-14)	14 ± 1.2 (12.5-17)	11, –	15.5 ± 1.3 (13-17)	11
Width at vulva	17 ± 1.4 (15-20.5)	–	21 ± 1.1 (19.1-22.8)	23 ± 1.6 (18-29)	18 ± 0.5 (17.5-18.5)	20 ± 1.4 (16-23)	–	21.5 ± 2.1 (17-23.5)	–
Width at excretory pore	–	–	18.5 ± 0.6 (17.5-20)	20 ± 0.9 (19-21)	17.5 ± 0.3 (17-18)	18 ± 1.2 (15.5-20.5)	–, 15.5	19 ± 0.9 (17-20)	14
Median bulb length	12.5 ± 1 (10-15)	11 (n = 1)	12 ± 0.9 (11-14)	12 ± 2.1 (11-15)	12 ± 1.7 (11-15)	13 ± 0.9 (11-15)	9.5, 9.5	12 ± 0.5 (11-14)	9.5
Valve length	3 ± 0.4 (2.5-4)	2, 2.5	3 ± 0.3 (3-3.5)	3 ± 0.4 (3-4)	3 (3-3)	3.25 ± 0.4 (3-4)	2, 3	3 ± 0.3 (3-4)	2
Median bulb width	10 ± 1.1 (8.5-13)	8, 9.5	11 ± 0.4 (10-12)	11 ± 0.8 (10-12.5)	10 ± 1.2 (9-11)	10 ± 0.9 (8-12)	8, 9	10.5 ± 1 (9-13)	8.5
Valve width	2 ± 0.6 (2-3)	2, 2.5	2.5 ± 0.3 (2-3)	2.5 ± 0.3 (2-3)	3 (3-3)	2.5 ± 0.3 (2-3)	2, 3	2.5 ± 0.3 (2-3)	2
Lip region width	8 ± 1.4 (7.5-9)	7.5 (n = 1)	8 ± 0.5 (7.5-9)	8 ± 0.5 (7.5-9)	8 (8-8)	8 ± 0.4 (7.5-9)	7.5, 7	8.5 ± 0.5 (7.5-9.5)	7
Lip region height	2.5 ± 0.2 (2-3)	2 (n = 1)	2 ± 0.3 (2-2.5)	2.5 ± 0.3 (2-3)	2.5 ± 0.2 (2-3)	2.5 ± 0.4 (2-3)	2.3, 5	2 ± 0.3 (2-3)	2
Annulus width	1 ± 0.2 (0.7-1.5)	0.7 (n = 1)	1.5 (n = 1)	1.5 ± 0.2 (1-2)	–	–	1.5	1.5 (n = 1)	–
Lateral field width	7 ± 1.4 (6-9)	6 (n = 1)	–	6, 6.5 (n = 2)	6 (6-6)	7 ± 0.6 (6-7.5)	4	7 ± 0.6 (6.5-7.5)	5
Tail length	29 ± 2.1 (24-36)	25 (n = 1)	29 ± 2.6 (22-34)	31 ± 3 (26.5-41)	31 ± 2.5 (27-33)	33.5 ± 2.4 (27-38)	26.5, 28	32 ± 2.6 (26.5-37.5)	26.5
Vulva to anus length	120 ± 12.2 (89-147)	–	93.5 ± 10.1 (78.5-105)	100 ± 15.9 (82-158)	95 ± 5.5 (90-103)	103.5 ± 8.6 (88-132)	–	101 ± 7 (92-112.5)	–
Vagina length	8.5 ± 0.6 (7.5-10)	–	8 ± 0.7 (7.5-9.5)	9 ± 1 (7.5-10)	8 ± 0.4 (7.5-8)	8 ± 0.5 (7-9)	–	9 ± 1.2 (7.5-11.5)	–

Table 2 (continued). Measurements of females, juveniles and one male of *Pratylenchus hippeastri* Inserra, Troccoli, Gozel, Bernard, Dunn & Duncan, 2007 from the Western and Eastern Cape provinces of South Africa. All measurements are in µm and in the form: mean ± s.d. (range).

Vagina length/vulval width %	49.4 ± 4.5 (40-57.1)	–	40.3 ± 3.3 (34.5-44.6)	39.5 ± 4.4 (32.5-48)	4 (n = 1)	40 ± 3.6 (32-46)	–	38.1 ± 4.1 (30.3-46.7)	–
Post-uterine sac length	29 ± 4.9 (20-39)	–	26 ± 6 (23.5-35)	40 ± 11 (24-71)	27 ± 6.2 (23-36)	29 ± 4.7 (19-40.5)	–	34 ± 8 (25-60)	–
Spermatheca length	13 ± 3.2 (8.5-20)	–	12 ± 2 (10-15)	12.5 ± 3 (7-17)	–	13.5 ± 3.9 (12.5-19)	–	12 ± 1.9 (10.5-15.5)	–
Spermatheca width	11 ± 1.7 (8-15)	–	10 ± 1.5 (8-13)	11.5 ± 2.9 (6-15.5)	–	12 ± 1.7 (9.5-15.5)	–	11 ± 1.8 (7.5-14)	–
1st Egg length	61 ± 3.6 (54-65)	–	64 ± 4 (58-70)	61 ± 5.3 (48.5-68.5)	67 (n = 1)	59 ± 4.2 (54-68)	–	61 ± 3.7 (54.5-65)	–
1st Egg width	23 ± 1 (21-24)	–	21 ± 1.2 (19.5-23)	25 ± 4.3 (21-35)	21 (n = 1)	22 ± 2.1 (18.5-25)	–	23 ± 1.3 (21-25)	–
2nd Egg length	–	–	–	52.5 ± 2.6 (51-54)	–	–	–	–	–
2nd Egg width	–	–	–	21.5 ± 0.8 (21-22)	–	–	–	–	–
3rd Egg length	–	–	–	59 (n = 1)	–	–	–	–	–
3rd Egg width	–	–	–	23.5 (n = 1)	–	–	–	–	–
No. tail annuli	23 ± 1 (18-34)	21 (n = 1)	22 ± 1.3 (21-25)	23 ± 2.2 (19-27)	22 ± 0.6 (22-23)	25 ± 1.9 (21-28)	21	23 ± 2.1 (20-28)	–
h	8 ± 1.1 (5-10)	0.7, 1.5	5 ± 0.9 (4-6.5)	5 ± 1.8 (4-8)	5 ± 0.5 (5-6)	5 ± 1.2 (3-6.5)	1	4.5 ± 0.7 (4-6)	–
Spicules length	–	–	–	–	–	–	–	–	17.5
Gubernaculum length	–	–	–	–	–	–	–	–	6

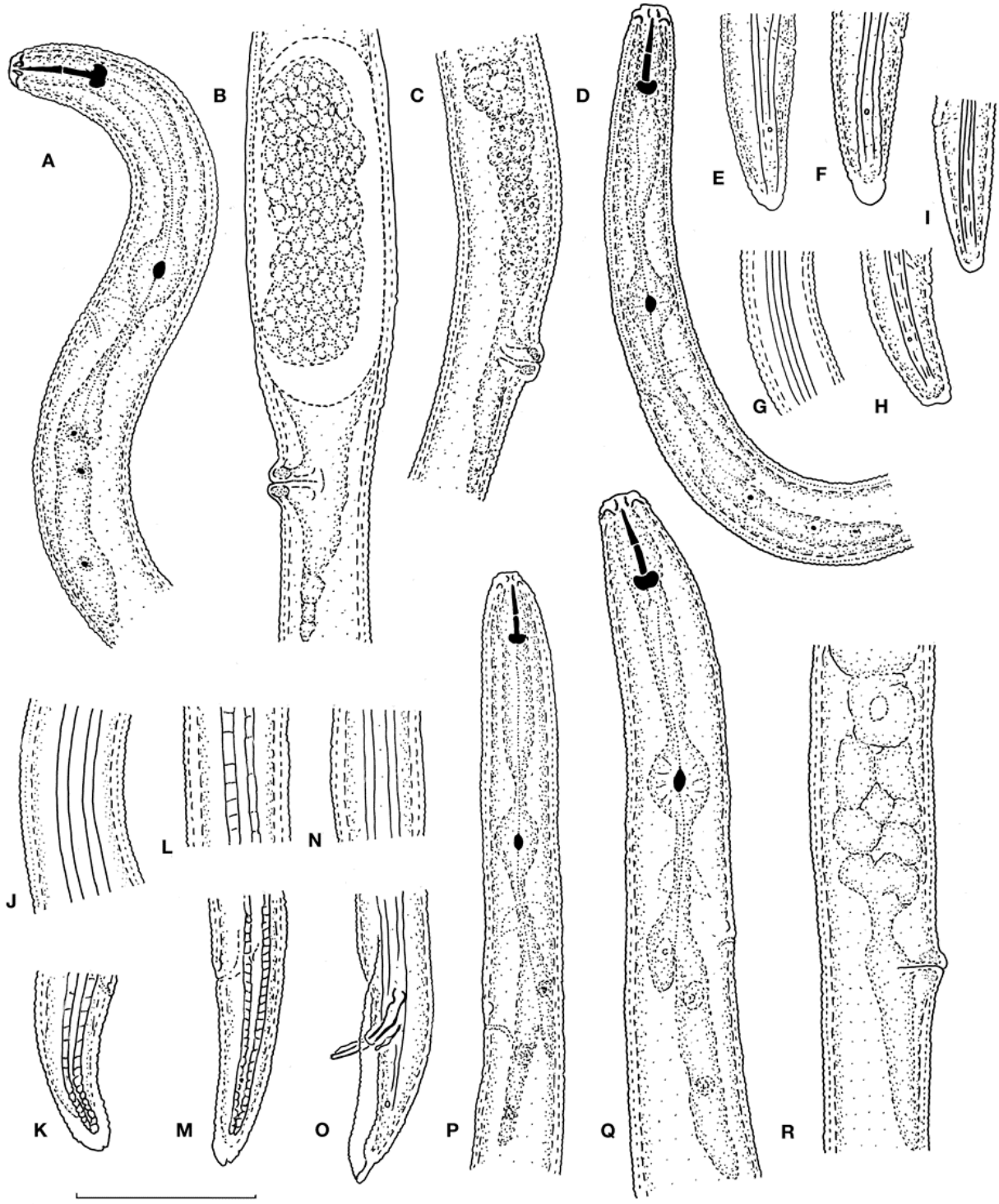

Fig. 3. *Pratylenchus hippeastri* Inserra, Troccoli, Gozel, Bernard, Dunn & Duncan, 2007 (isolate 2960). A & D: Pharynx of female; B: Vulval area with an egg; C: Vulval area with small, rounded, empty spermatheca; E & F, H & I: Female tail and the tip variations; G: Female lateral field at mid-body; J: Female lateral field at posterior body region; K: Female tail tip with areolated lateral field; L: Female lateral field near lip region; M: Female tail and lateral field; N: Male lateral field at mid-body; O: Male tail with spicules; P & Q: Pharynx of male; R: Female vulval area with empty spermatheca. Scale bars = 30 µm.

MORPHOLOGICAL CHARACTERISATION OF *PRATYLENCHUS HIPPEASTRI* FROM SOUTH AFRICA

Comparative measurements and morphological characteristics of females and males of *P. hippeastri* from apple in South Africa are reported in Tables 2, 3 and Figures 3, 4 and 7.

Female. Body slender. Form variable, ranging from almost straight to slightly ventrally or dorsally curved, S or circle shaped. Lip region low, flattened, slightly set off from body with two annuli. The first annulus slightly narrower and lower than the second annulus. Sometimes a faint third annulus could be seen on one side of the lip region. Facial pattern plain and smooth with all labial sectors fused with the oral disc. Stylet robust with well-developed knobs. The knobs rounded posteriorly, and flattened to variously indented anteriorly. Anterior and posterior cephalids not distinct, but where present, they are situated 2 to 3 and 7 to 10 annuli posterior to base of lip region. Hemizonid two to four annuli long at directly posterior to four annuli anterior to excretory pore. In specimens that are lying almost straight to slightly curved ventrad, the excretory pore is situated from middle of isthmus to opposite the basal part of pharyngeal gland, but in extremely curved specimens, the excretory pore is situated from middle of isthmus to opposite the middle of the median bulb. Pharyngeal gland overlapping the intestine ventrally with three distinct gland nuclei. The length of the overlap ranges from 21 to 73 µm. Lateral field with four lines, forming three bands. SEM photographs show outer bands to be faintly areolated over most part of the body, whilst the middle band has irregular faint areolations. Under the light microscope, the lateral fields were indistinct. The two outer bands continue past the phasmid right to tail tip and are well areolated, as seen on the SEM photographs, sometimes continuing around the tail tip. Length of ovary varies considerably. In some females, reaching anteriorly past the base of the pharyngeal gland. Spermatheca varying from small to larger rounded, rectangular to oblong in form, all with a small rounded cavity. All populations had empty spermatheca, except in the Infruitec (Stellenbosch) population where two specimens were found with sperm cells. Vulval lips distinctly protruding from the body outline, resulting in an elongated vagina. Several specimens had an egg in the ovary anterior to the vulva, while a few had two eggs anterior to the vulva and one female had two eggs anterior and one egg posterior to the vulva in the posterior uterine sac. This led to the thought that specimens with long post-uterine sacs might have an egg in them at some stage because the post-uterine sac of most females is much shorter and slender. Tail with 18 to 34 annuli with phasmid situated 10 to 19 annuli posterior to anus. In many specimens, the caudalid was distinct, one or two annuli long and situated mostly directly anterior to anus. Tail curved ventrad, tapering to a smooth, slightly rounded or flattened tip with a slight indent on the tip. Hyaline part of the tail fairly long.

Male. Rare. Only recovered in the 3195 (Wellington) population (none of the females had sperm in the spermatheca). Similar to females, except in sexual organs. Spicules 17.5 µm long and gubernaculum 6 µm long.

Diagnosis and relationships. The present specimens compare very well with those found in amaryllis described by Inserra *et al.* (2007). They found that *P. hippeastri* is morphologically very close to *P. scribneri*, *P. hexincisus*, *P.zeae* and *P. loosi*, while De Luca *et al.* (2010) described two species, *P. floridensis* and *P. parafloridensis*, and showed that they, as well as a few undescribed species (H1-H7), are also phylogenetically closely related to *P. hippeastri*. They stated that this close relationship indicates that they are representatives of a *P. hippeastri* species complex. Because the South African specimens were collected from apple, their measurements were compared separately to specimens described by Wang *et al.* (2016), also from apple, in Table 3. In the table, it can be seen that some of the characters differ in length, such as pharyngeal gland length, pharyngeal glands overlap, width of body at mid-body, vulva and anus, tail length, vulva to anus distance, post-uterine branch length, and number of tail annuli, but otherwise they compare very well.

Molecular characterisation of *Pratylenchus hippeastri* from South Africa. Phylogenetic relationships of the South African populations of *P. hippeastri* with other *Pratylenchus* species are shown in Figures 10, 11 and 12. In the tree derived from 28S rRNA sequences (Fig. 10), the five isolates of *P. hippeastri* form a clade with other *P. hippeastri* isolates obtained from the GenBank database, clearly separated from its closest relatives, *P. parafloridensis* and *P. floridensis*. The same trend can be observed in the trees derived from ITS rDNA sequences (Fig. 11) and *COX1* sequences (Fig. 12). The percentage of intraspecies variation between the sequences of the species occupying the same clades in the aforementioned trees was 0.0-0.7, 0.0-0.8 and 0.0-1.9%, respectively.

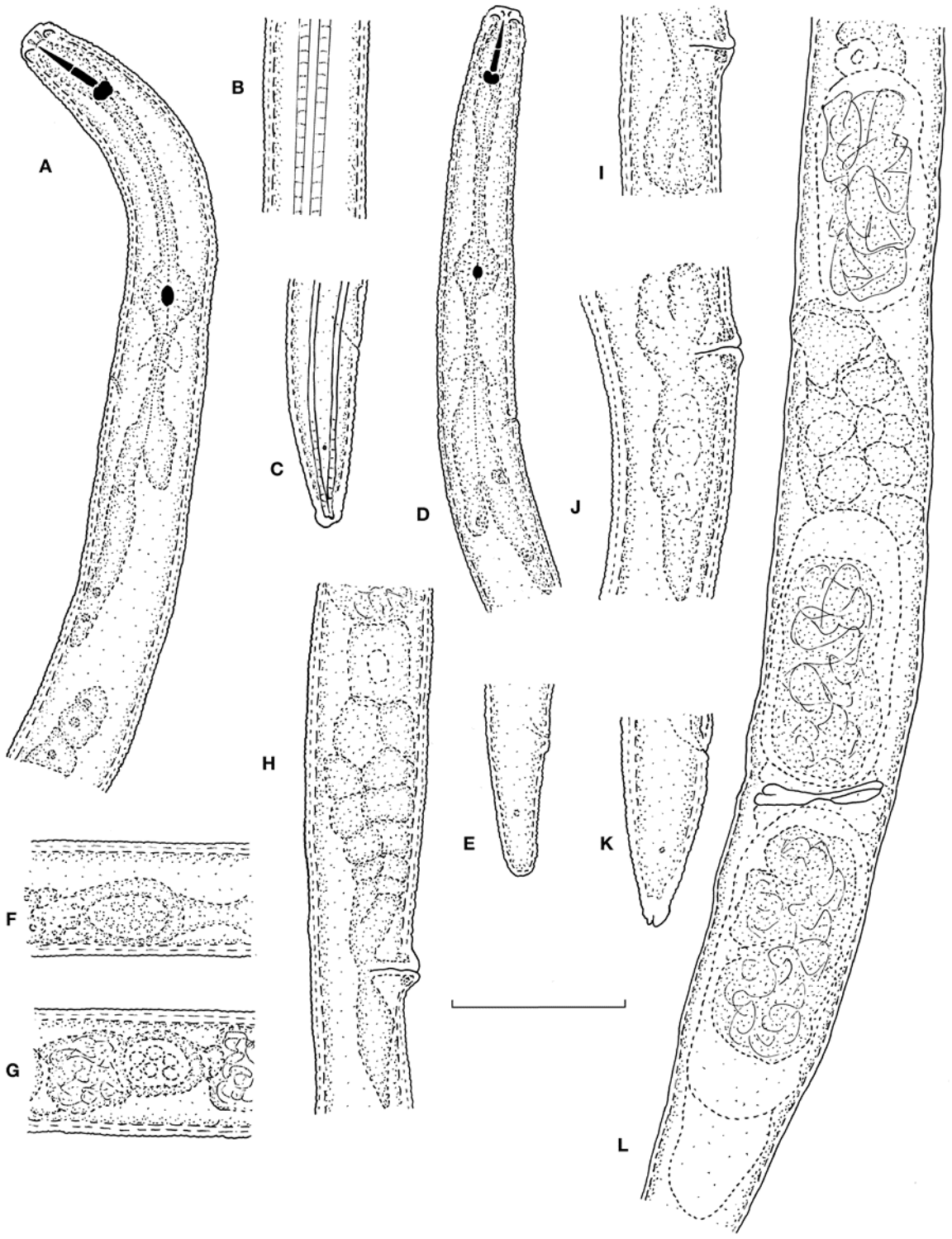

Fig. 4. *Pratylenchus hippeastri* Inserra, Troccoli, Gozel, Bernard, Dunn & Duncan, 2007 (isolate 2057). A: Pharynx of female; B: Lateral field at mid-body; C: Female tail; D: Pharynx of juvenile; E: Juvenile tail; F & G: Spermatheca of two females with sperm cells; H: Female with empty spermatheca; I & J: Two females with irregular post-uterine sacs; K: Female tail; L: Female with two eggs at anterior, and one egg at posterior to vulva. Scale bars = 30 µm.

Table 3. Measurements of females and males of *Pratylenchus hippeastri* Inserra, Troccoli, Gozel, Bernard, Dunn & Duncan, 2007 from the Western and Eastern Cape provinces of South Africa compared with that of the literature. All measurements are in µm and in the form; mean ± s.d. (range).

Characters	Stellenbosch, Ceres, Elgin Apple Infruitec, 2960, 2057, 3195, 3210			China Apple[a]	Florida, USA Bromeliads, Plantain, Hippeastrum[b]		Florida, USA Bromeliads[c]
	36 Females	2 Juveniles	1 Male	10 Females	48 Females	5 Males	22 Females
L	510 ± 37.7 (408-598)	388, 395	498	400.7-479.8	418-651	370-452	447-651
a	27.1 ± 2.4 (21.4-32.4)	24.3, 26.9	32.2	25-29.1	23.2-32.2	25.8-33.9	23.7-30.7
b	4.1 ± 0.3 (3.6-4.7)	3.7, 3.6	5.6	5-5.8	5.7-7.2	5.7-7.1	5.2-7.2
b'	5.5 ± 0.4 (5-6.6)	4.5, 4.4	3.9	2.8-3.5	3.4-5.3	3.2-3.8	3.3-5.3
c	17.7 ± 1.8 (14.4-22.5)	15.7, 15.8	18.8	15.6-20.5	13.6-23.3	16.1-19.3	15.7-23.3
c'	2.3 ± 0.3 (1.7-3.3)	2.1, 2.1	2.4	1.9-2.5	1.8-3.4	2.3-2.5	1.8-2.6
O	20 ± 3.4 (14.3-28.6)	21.6, 17.9	12.5	–	–	11.6-19	12.8-25.2
DGO	3 ± 0.6 (2-4)	3, 2.5	2	2.5-3.2	2.5-3	1.7-2.7	2-3.8
V	77 ± 1.9 (71-81.2)	–	–	76.4-80.2	75-79.6	–	75.7-79.6
OV %	46 ± 6.6 (32.5-57.7)	–	–	–	29-59	–	29-59
OV length/primordium in juveniles	235 ± 39 (161-310)	109, 88	–	–	–	–	108-387
Stylet length	15 ± 0.6 (14-16)	13.5, 14	15	14.4-15.6	14.5-17.5	14-14.7	15.1-16.7
Conus	7 ± 0.4 (6-8)	6, 5	–	7.1-7.6	6.5-8	–	–
Shaft	8 ± 0.4 (7-9)	7.5, 7	–	–	–	–	–
M			–			–	
Stylet knob height	2.5 ± 0.3 (2-3)	2.5, 3	–	1.6-1.9	1.5-3	1.7-2	–
Stylet knob width	4 ± 0.4 (3-5)	4	–	2.9-3.5	4-5	2.3-3	–
Pharyngeal gland length	124 ± 6.7 (112-140)	107, 111	129	126.3-148	116-147	117-132	–
Exp. from ant. end	80 ± 6 (69-89)	67, 73	74	74.9-83.1	70.9-99	66-76	77.4-99
Pharyngeal overlap	31 ± 6 (21-48)	21, 22	40.5	44.5-64	32-58	35-55	33-61.5
Width at mid-body	19 ± 1.5 (16-21)	16, 15	15.5	14.6-17.6	14.3-27	12.7-14.5	15.6-25.7
Width at anus	13 ± 1.1 (11-17)	12	11	9.5-12.1	10.2-16	9.3-10	10.7-16
Width at vulva	17 ± 1.4 (15-20.5)	–	–	13.3-16.2	12.2-24	–	14.2-24
Median bulb length	12.5 ± 1 (10-15)	11	9.5	–	–	10-11.3	–
Valve length	3 ± 0.4 (2.5-4)	2-2.5	2	–	–	–	–
Median bulb width	10 ± 1.1 (8.5-13)	8-9.5	8.5	–	–	7.3-8.7	–
Valve length	2 ± 0.6 (2-3)	2-2.5	2	–	–	–	–
Lip region width	8 ± 1.4 (7.5-9)	7.5	7	6.6-7.4	–	6-6.7	–
Lip region height	2.5 ± 0.2 (2-3)	2	2	1.5-2	–	–	–
Annulus width	1 ± 0.2 (0.7-1.5)	0.7	–	–	–	–	–
Lateral field width	7 ± 1.4 (6-9)	6	5	4.8-5.6	–	–	–
Tail length	29 ± 2.1 (24-36)	25	26.5	21.3-27	17-26	22-23.7	27.2-37.3

Table 3 (continued). Measurements of females and males of *Pratylenchus hippeastri* Inserra, Troccoli, Gozel, Bernard, Dunn & Duncan, 2007 from the Western and Eastern Cape provinces of South Africa compared with that of the literature. All measurements are in µm and in the form; mean ± s.d. (range).

Vulva to anus length	120 ± 12.2 (89-147)	–	–	61.5-85.1	65.3-112	–	70.5-109
Vagina length	8.5 ± 0.6 (7.5-10)	–	–	–	–	–	–
Vagina length/vulval width %	49.4 ± 4.5 (40-57.1)	–	–	–	–	–	–
PUB post-uterine sac length	29 ± 4.9 (20-39)	–	–	19.5-27.6	19-45	–	18.6-39.3
Spermatheca length	13 ± 3.2 (8.5-20)	–	–	–	–	–	–
Spermatheca width	11 ± 1.7 (8-15)	–	–	–	–	–	–
Egg length	61 ± 3.6 (54-65)	–	–	–	–	–	–
Egg width	23 ± 1 (21-24)	–	–	–	–	–	–
No. of tail annuli	23 ± 3.1 (18-34)	21	–	21-26	17-26	–	17-26
h	8 ± 1.1 (5-10)	0.7, 1.5	–	–	–	–	–
Spicules length	–	–	17.5	–	–	18-19	
Gubernaculum length	–	–	6	–	–	4.7-6	

a) Wang *et al.*, 2016; b) Inserra *et al.*, 2007; De Luca *et al.*, 2010; Gu *et al.*, 2014; c) Inserra *et al.*, 2007.

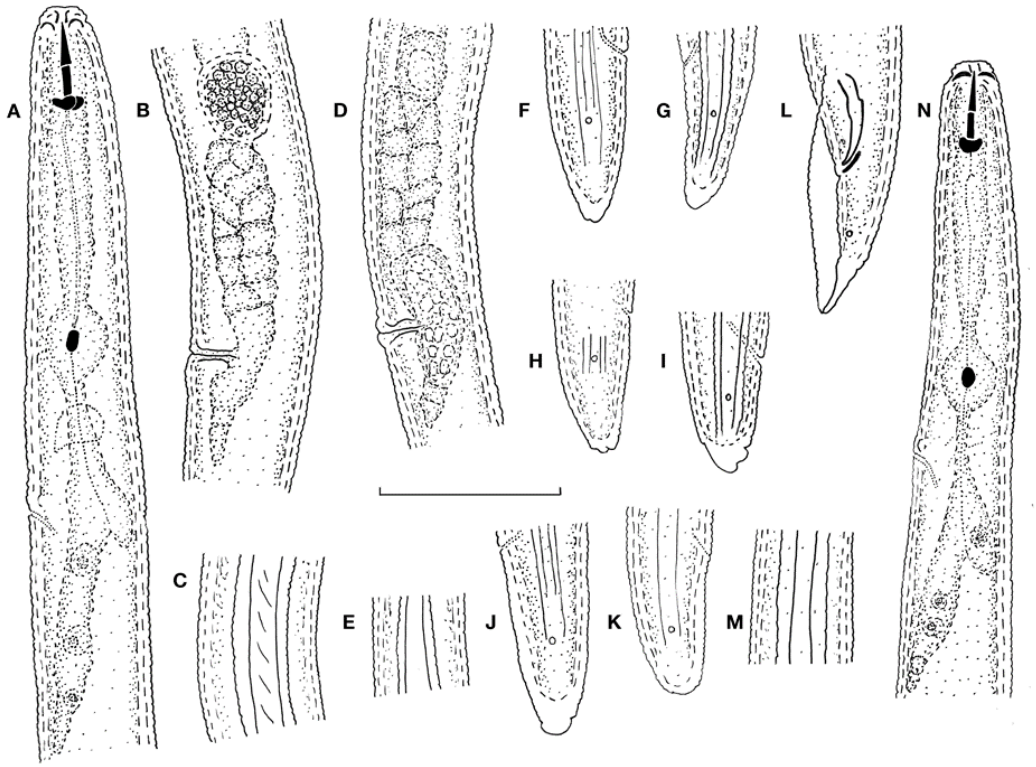

Fig. 5. *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941 (isolate Ct). A: Pharynx of female; B: Vulval area of female with filled spermatheca; C: Female lateral field at mid-body; D: Vulval area with empty spermatheca; E: Female lateral field on posterior part of body; F-K: Variations in female tail tip and ending of lateral field; L: Male tail; M: Male lateral field at mid-body; N: Pharynx of male. Scale bars = 30 µm.

MORPHOLOGICAL CHARACTERISATION OF *PRATYLENCHUS PENETRANS* FROM SOUTH AFRICA

Comparative measurements and morphological characteristics of females and males of *P. penetrans* from apple in South Africa are reported in Table 4 and Figures 5, 8 and 9.

Female. Body slightly ventrally curved after heat relaxation. Lip region flattened and slightly set off from body with three annuli. Facial pattern shows a distinct dumb-bell shaped pattern (Fig. 8A). The six labial pores adjacent to the mouth, the amphidial openings adjacent to the labial disc. Outer margins of labial framework heavily sclerotised and extended into the body about one annulus. Stylet with broadly rounded basal knobs, flattened to slightly hollow anteriorly. Hemizonid distinct, two to three annuli long and situated from directly anterior, to two annuli posterior to excretory pore. Anterior and posterior cephalids not distinct, but where present they are situated 2 to 3 and 7 to 9 annuli from base of lip region. Pharyngeal glands overlapping intestine ventrally with three distinct gland nuclei. Lateral field with four lines, not distinct, but appearing crenate over a large part of the body and occasionally with faint oblique striae along the central band. SEM photographs show the crenation and areolation of the outer and inner bands. There is a variation in the continuation of the lateral field lines past the phasmid. They are difficult to see and sometimes four continue past the phasmid and sometimes only two. The SEM photos show clearly two continuing and also some areolation on the tail posterior to the phasmid. Ovary not reflexed, comprising 32.0 to 57.4% of the body length and not reaching the pharyngeal overlap. Spermatheca rounded, mostly filled with rounded sperm cells. Vulval lips not markedly protruding from the body outline. Post-uterine sac 23 to 33 µm in length. Tail with 16 to 26 annuli with phasmid at near middle, or just posterior to middle of tail. Tail not markedly curved ventrad, gradually tapering to a slightly narrower rounded tip, not annulated, but frequently with irregularities. Hyaline part of tip fairly long, 4 to 6 µm.

Male. Male similar to female in most characters, except in sexual organs, but slightly shorter. Facial pattern similar to that of the female. Lateral field appears to be similar to that in female with faint and irregular areolations in the three bands. SEM of the tail shows that the dorsal line of the lateral field extends slightly past the anus.

Diagnosis and relationships. Following the keys of Loof (1978), Café-Filho & Huang (1989), Ryss (2002) and Geraert (2013), these specimens are very similar to *P. penetrans*. They are separated from *P. hippeastri* (also present in the same geographical area) by having a dumbbell-shaped facial pattern *vs* a smooth facial pattern; presence *vs* absence of males, rounded, filled spermatheca *vs* rectangular, empty spermatheca, less protruding vulval lips and a more posteriorly situated vulva (V = 75.7-83.0 *vs* 75.0-78.5%). *P. penetrans* has been cited as morphologically similar to *P. fallax* Seinhorst, 1968, *P. hexincisus* Taylor & Jenkins, 1957, *P. mediterraneus* Corbett, 1983, *P. pseudofallax* Café-Filho & Huang, 1989, *P. scribneri* Steiner in Sherbakoff & Stanley, 1943, *P. subpenetrans* Taylor & Jenkins, 1957 and *P. vulnus* Allen & Jensen, 1951, but when compared to all the descriptions of these species, there are distinct differences.

Molecular characterisation of *Pratylenchus penetrans* from South Africa. Phylogenetic relationships of the South African populations of *P. penetrans* with other *Pratylenchus* species are shown in Figures 10 and 12. In the tree derived from 28S rDNA sequences (Fig. 10), the isolates of *P. penetrans* form a clade that is a sister group to a clade, which includes *P. fallax* and *P. convallariae*. This grouping is even more defined in the tree derived from *COX1* sequences (Fig. 12).

MORPHOLOGICAL CHARACTERISATION OF *PRATYLENCHUS VULNUS* FROM SOUTH AFRICA

Comparative measurements and morphological characteristics of females and males of *P. vulnus* from apple in South Africa are reported in Table 5 and Figure 6.

Female. Body slender, variable in shape after heat relaxation, ranging from straight, slightly curved dorsally or ventrally into S and circle shaped. Lip region low, flattened or slightly rounded anteriorly, slightly set off from body. Facial distinctive dumb-bell shaped pattern of the sub-median segments with slightly lateral segments to complete the circle. Stylet knobs rounded posteriorly, and straight or slightly indented anteriorly. Cephalids situated 3 to 4 and 7 to 12 annuli from base of lip region. Hemizonid 2 to 3 annuli long and situated directly anterior to excretory pore. Pharyngeal glands overlapping intestine ventrally with three distinct gland nuclei. Length of overlap ranges from 18 to 44 µm. Lateral field with four lines and three bands, slightly areolate at mid-body or sometimes the outer bands are only crenate. Outer bands continue past the phasmid to tail tip. Spermatheca small and empty or large, oblong or rounded, thick-walled filled with a

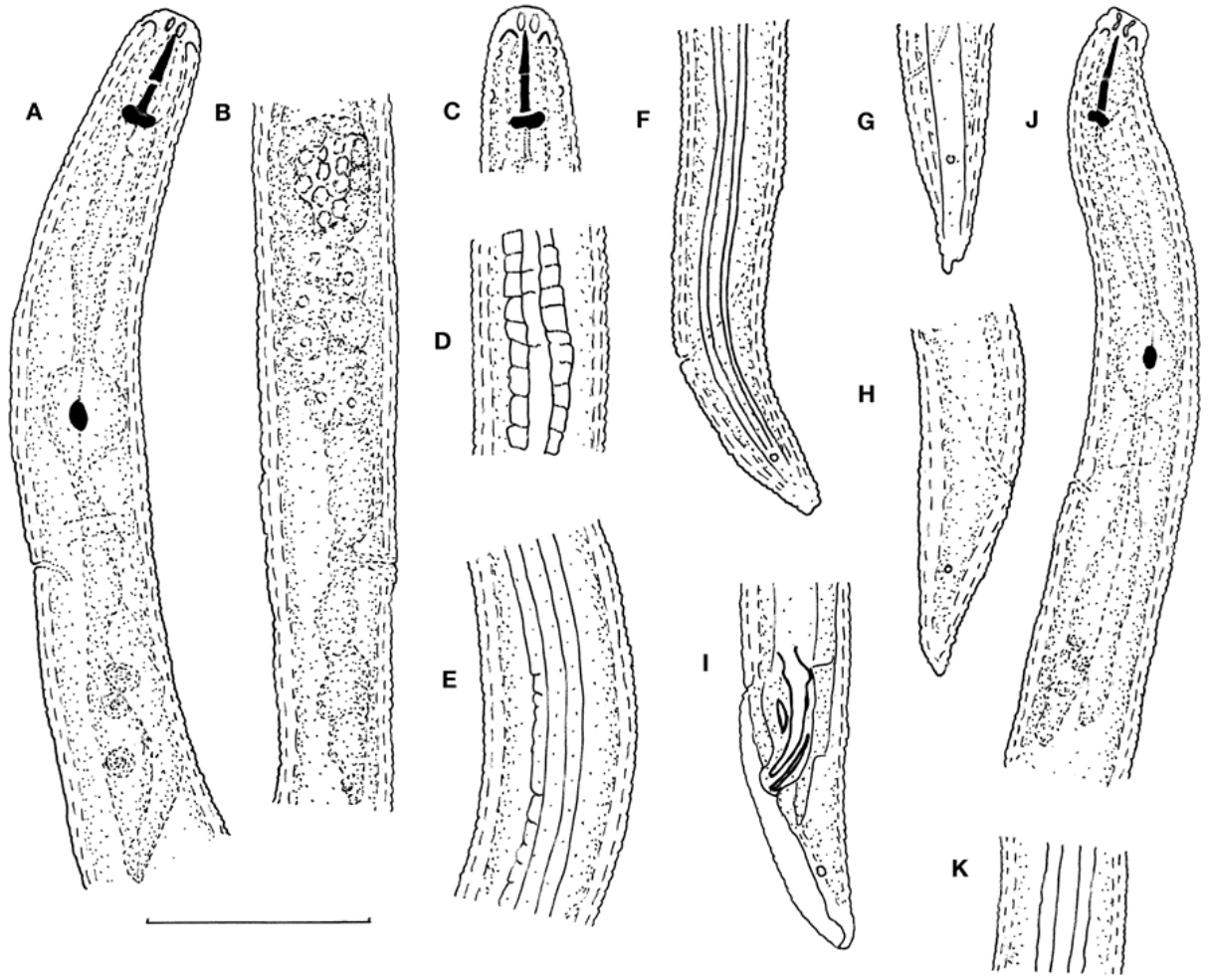

Fig. 6. *Pratylenchus vulnus* Allen & Jensen, 1951 (isolate 94f). A: Pharynx of female; B: Vulval area with filled spermatheca; C: Lip region of another female; D: Lateral field of female in anterior region; E: Lateral field of female at mid-body; F-H: Variations in female tail tip; I: Male tail; J: Pharynx of male; K: Male lateral field at mid-body. Scale bars = 30 µm.

few round sperm cells. Vulval lips not protruding much from the body outline. Post-uterine sac length ranging from 29 to 44 µm. Tail length varying from 22 to 32 µm, with 18 to 34 annuli with phasmid situated from 8 to 19 annuli posterior to anus. Caudalid rarely seen, situated directly anterior to anus. Tail mostly tapering to a finely rounded tip or sometimes a narrow, flattened tip, or irregular with a slight projection on the ventral side.

Male. Fairly common. Similar to female with regard to lip region, body form and pharyngeal region. Lateral field ends at the start of the bursa with outer lines more distinct and sometimes slightly areolate on body. Bursa enveloping the tail tip. Phasmid opposite to the middle of tail. Tail tapering to a finely rounded beak-like tip.

Diagnosis and relationships. Since the description of *P. vulnus* by Allen & Jensen (1951) from walnut in San Jose, California, USA, numerous scientists have done studies and descriptions of the species from numerous host plants in various countries in the world. Almost all of them remarked on the large variability in some of the characters such as structure of the post-uterine sac, shape of the spermatheca, width and make-up of the lateral field, variation in tail tip, body length *etc.* (Roman & Hirschman, 1969; Corbett & Clark, 1983; Doucet *et al.*, 1996, 2001). Doucet *et al.* (1996)

Table 4. Measurements of females and males of *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941 and one juvenile from apples in the Western Cape province of South Africa (Isolate Ct). All measurements are in µm and in the form; mean ± s.d. (range).

Characters	Females	Males	Juvenile
	9	15	1
L	531 ± 44 (479-609)	468 ± 36.8 (416-551)	430
a	22.7 ± 2.1 (19.7-27.3)	22.6 ± 2.3 (18.8-26.8)	22.9
b	4.4 ± 0.3 (4-5)	4.2 ± 0.3 (3.7-4.7)	3.7
b'	6.2 ± 0.5 (5.9-7)	5.7 ± 0.5 (4.9-6.7)	5.2
c	19.1 ± 3.5 (16.1-24.1)	19.8 ± 2.2 (17.1-25.9)	16.7
c'	2 ± 0.4 (1.5-3)	2 ± 0.4 (1.6-2.7)	1.9
O	22 ± 4.4 (14.3-27.8)	19.8 ± 4.7 (13.9-22.2)	22.2
DGO	3 ± 0.6 (2-4)	2.5 ± 0.4 (2-3)	3
V	80 ± 2.7 (75.5-83)	–	–
OV %	47.1 ± 9.9 (32-57.4)	–	–
OV length/primordium in juveniles	252 ± 61.7 (179-349)	–	–
Stylet length	14.5 ± 0.7 (13-15.5)	14 ± 0.9 (12-15)	13
Conus	7 ± 0.5 (6-8)	7 ± 0.6 (6-7.5)	6.5
Shaft	7.5 ± 0.3 (7-8)	7 ± 0.7 (6-8)	6.5
M	48.5 ± 2.2 (44.4-51.3)	48.1 ± 3.1 (44.4-52.9)	50
Stylet knob height	2 ± 0.4 (2-3)	2 ± 0.4 (2-2.5)	1.5
Stylet knob width	4 ± 0.6 (4-5.5)	4 ± 0.3 (3-4.5)	4
Pharyngeal gland length	125 ± 7.9 (115-139)	112 ± 8.3 (93-123.5)	114
Excretory pore from anterior end	79 ± 9 (63-90)	72 ± 5.7 (67-83)	72
Pharyngeal overlap	36 ± 3.4 (30-41)	30.5 ± 5 (23-41)	32
Width at mid-body	23.5 ± 2 (21-26.5)	21 ± 1.4 (19-23)	19
Width at anus	14.5 ± 1.3 (12.5-17)	12.5 ± 1.7 (11-17)	13
Width at vulva	21 ± 1.5 (19-23.5)	–	–
Median bulb length	12.5 ± 0.4 (12-13)	10 ± 0.9 (9.5-12.5)	10
Valve length	3.5 ± 0.3 (3-4)	2.5 ± 0.6 (1.5-3)	3
Median bulb width	11 ± 1.7 (9-14)	9 ± 0.7 (8-10)	9.5
Valve length	3 ± 0.5 (2-4)	2.5 ± 0.5 (1.5-3.5)	1.5
Lip region width	8 ± 0.3 (7.5-9)	7 ± 0.5 (6.5-8)	7.5
Lip region height	2.5 ± 0.4 (2-3)	2.5 ± 0.3 (2-3)	3
Annulus width	1.5 ± 0.2 (1-2)	1.5 ± 0.2 (1-1.5)	1
Lateral field width	8 ± 0.6 (7.5-9)	8 (n = 2)	–
Tail length	30 ± 5.6 (25-43)	24 ± 1.9 (20-26.5)	25.5
Vulva to anus length	81 ± 11 (67-97)	–	–
Vagina length	8.5 ± 1.2 (7-10)	–	–
Vagina length/vulval width %	39.5 ± 4.7 (31-45)	–	–
Post-uterine sac length	26 ± 3.4 (23-33)	–	–
Spermatheca length	13 ± 2.9 (8-17)	–	–
Spermatheca width	11 ± 2.2 (8-15)	–	–
Egg length	–	–	–
Egg width	–	–	–
No. of tail annuli	21 ± 3.5 (16-26)	–	25
h	4 ± 0.7 (4-6)	7.5 ± 1 (6-9.5)	1.5
Spicules length	–	17 ± 1.4 (13-18.5)	–
Gubernaculum length	–	5.5 ± 0.9 (4.5-7.5)	–

Table 5. Measurements of females and males of *Pratylenchus vulnus* Allen & Jensen, 1951 from apples in the Western Cape province of South Africa (isolate 94f). All measurements are in µm and in the form; mean ± s.d. (range).

Characters	Females	Males
	18	9
L	604 ± 46.8 (507-699)	508 ± 36.7 (424-554)
a	29.9 ± 1.8 (26.5-32.9)	29.3 ± 2.8 (25.8-34.2)
b	4.8 ± 0.3 (4.3-5.4)	7.2 ± 0.7 (6.4-8.7)
b'	6.4 ± 0.4 (5.8-7.3)	6 ± 0.5 (5.4-6.8)
c	22 ± 1.9 (17.7-25.5)	21 ± 2.1 (18-25.1)
c'	2.2 ± 0.2 (1.9-2.6)	2.2 ± 0.2 (1.9-2.5)
o	20.1 ± 2.3 (16.7-23.7)	15.2 ± 2.5 (10.5-18.4)
DGO	3 ± 0.2 (2.6-3.3)	2 ± 0.4 (1.5-2.6)
V	80 ± 1.5 (77.8-83.5)	–
OV %	36.3 ± 5.6 (27.5-45.4)	–
OV length	196 ± 80.5 (110-280)	–
Stylet length	14.5 ± 0.7 (13.2-15.5)	14 ± 0.5 (13.3-14.7)
Conus	7 ± 0.6 (5.9-8.1)	7 ± 0.5 (5.9-8.1)
Shaft	7.5 ± 0.5 (6.6-8.8)	7 ± 0.7 (5.9-8.1)
M	47.6 ± 2.8 (42.1-52.3)	50 ± 5 (43.4-55.6)
Stylet knob height	2.5 ± 0.3 (2.2-2.9)	2 ± 0.4 (1.5-2.6)
Stylet knob width	3 ± 0.4 (2.6-3.7)	3 ± 0.3 (2.6-3.7)
Pharyngeal gland length	126 ± 10.8 (111.7-144.1)	110 ± 8.5 (99.2-127.2)
Excretory pore from anterior end	81 ± 7.9 (66.2-91.9)	72 ± 6 (61.7-79.4)
Pharyngeal overlap	31 ± 5.5 (18.4-44.1)	25 ± 6.8 (19.8-39)
Width at mid-body	20 ± 1.1 (18.4-22.1)	17 ± 1.5 (15.4-19.8)
Width at anus	18.5 ± 1.4 (16.9-22.1)	11 ± 0.7 (9.6-11.8)
Width at vulva	12 ± 0.8 (10.7-13.2)	–
Median bulb length	12 ± 1.1 (10.3-14)	11 ± 1.4 (8.8-13.2)
Valve length	3.5 ± 0.5 (2.2-4.4)	3 ± 0.4 (2.2-3.7)
Median bulb width	10 ± 1 (8.5-11.8)	9 ± 0.9 (7.4-9.6)
Valve length	3 ± 0.3 (2.2-3.7)	2.5 ± 0.4 (2.2-2.9)
Lip region width	8 ± 0.7 (7.4-9.6)	7 ± 0.6 (6.2-8.1)
Lip region height	3 ± 0.5 (2.2-3.7)	2.5 ± 0.5 (1.8-3.3)
Annulus width	1.5 ± 0.2 (1.1-1.8)	1.4 ± 0.2 (1.1-1.5)
Lateral field width	7 ± 0.9 (5.8-8)	6 ± 0.9 (4.4-7)
Tail length	27.5 ± 2.6 (22.8-32.3)	24 ± 1.4 (22.1-26.5)
Vulva to anus length	90 ± 8.8 (74.2-100)	–
Vagina length	8 ± 0.6 (7-9.5)	–
Vagina length/vulval width %	41.3 ± 4.4 (33.3-49.1)	–
Post-uterine sac length	36.5 ± 4.3 (29.5-44)	–
Spermatheca length	19 ± 4.8 (11-26.5)	–
Spermatheca width	14 ± 2.5 (7.5-16)	–
Egg length	–	–
Egg width	–	–
No. of tail annuli	30 ± 4.4 (24-40)	–
h	3.5 ± 0.7 (3-4.5)	5.5 ± 1 (4.4-7.4)
Spicules length	–	18 ± 1.3 (16.2-19.8)
Gubernaculum length	–	8 ± 0.5 (7.4-8.8)

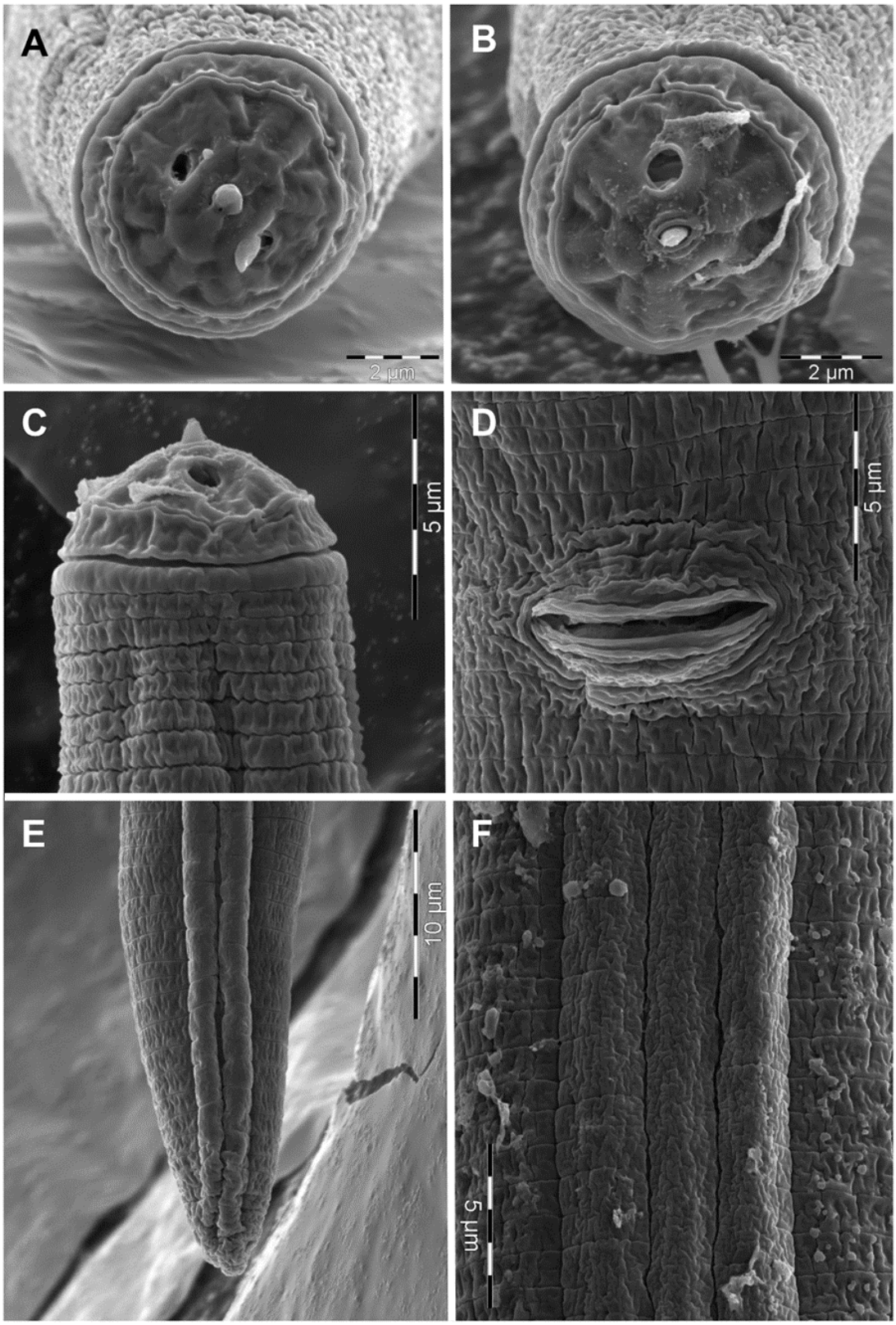

Fig. 7. *Pratylenchus hippeastri* Inserra, Troccoli, Gozel, Bernard, Dunn & Duncan, 2007 (isolate Infruitec). A & B: *En face* view of female lip region; C: Lateral view of female lip region; D: Vulva; E: Female tail; F: Lateral field at mid-body.

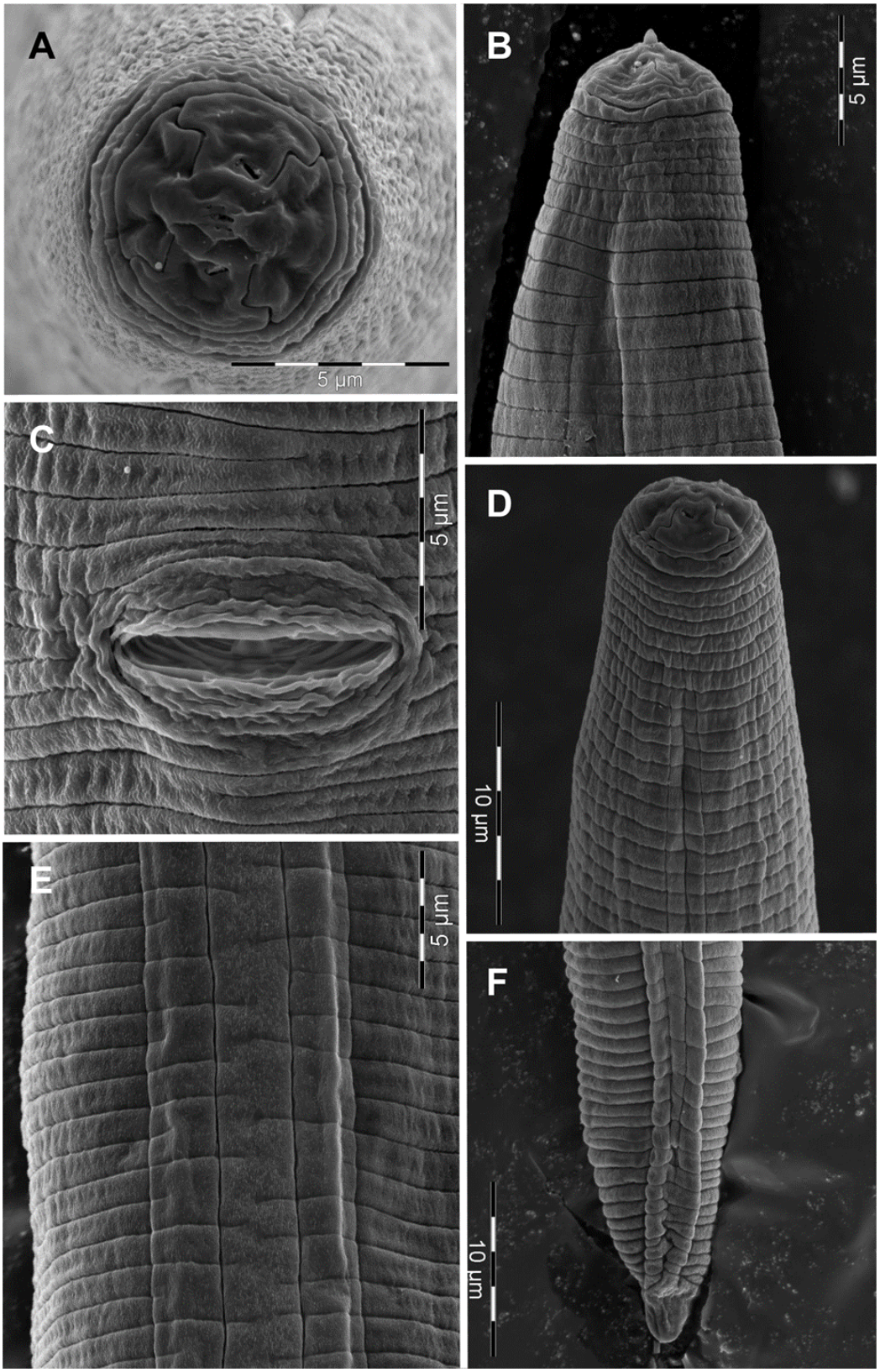

Fig. 8. *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941 female (isolate Ct). A: *En face* view of female lip region; B & D: Lateral view of female lip region; C: Vulva; E: Female lateral field at mid-body; F: Tail region of female.

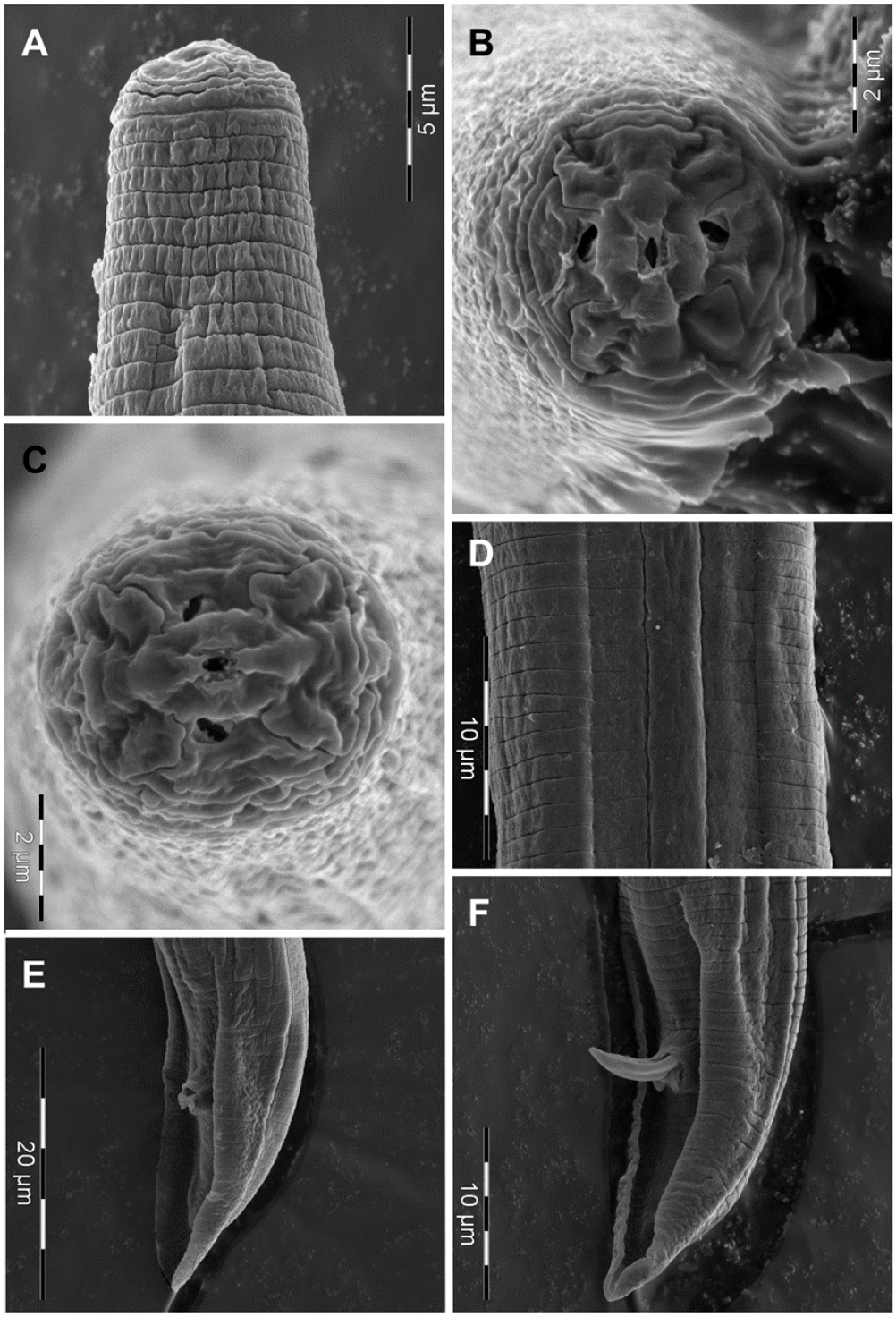

Fig. 9. *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941 male (isolate Ct). A: lateral view of male lip region; B & C: *En face* view of male lip region; D: Lateral field at mid-body; E & F: Male tail.

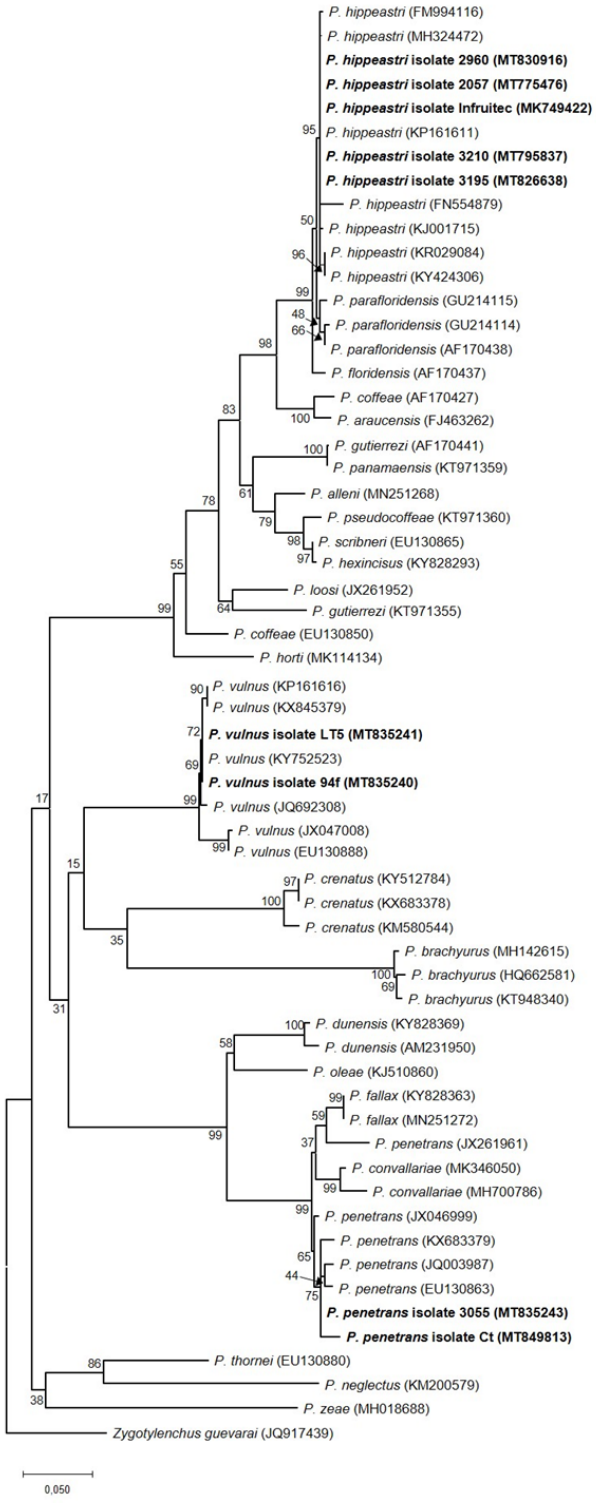

Fig. 10. Phylogenetic relationships of selected *Pratylenchus* species as inferred from the D2-D3 expansion segments of 28S rDNA by using the maximum likelihood method. The tree with the highest log likelihood (-7178.88) is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The percentage of trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

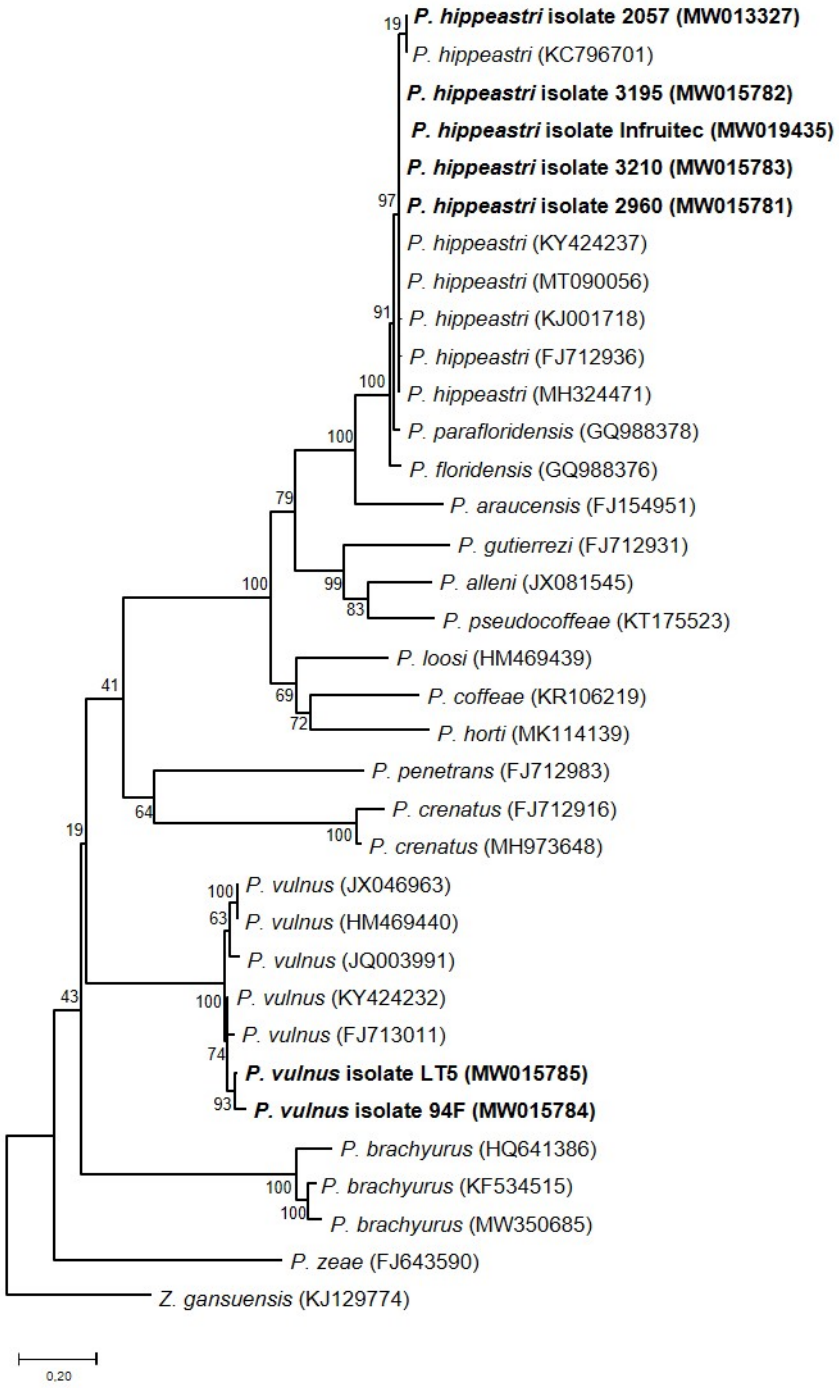

Fig. 11. Phylogenetic relationships of selected *Pratylenchus* species as inferred from ITS-rRNA sequences by using the maximum likelihood method. The tree with the highest log likelihood (-11267.08) is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The percentage of trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

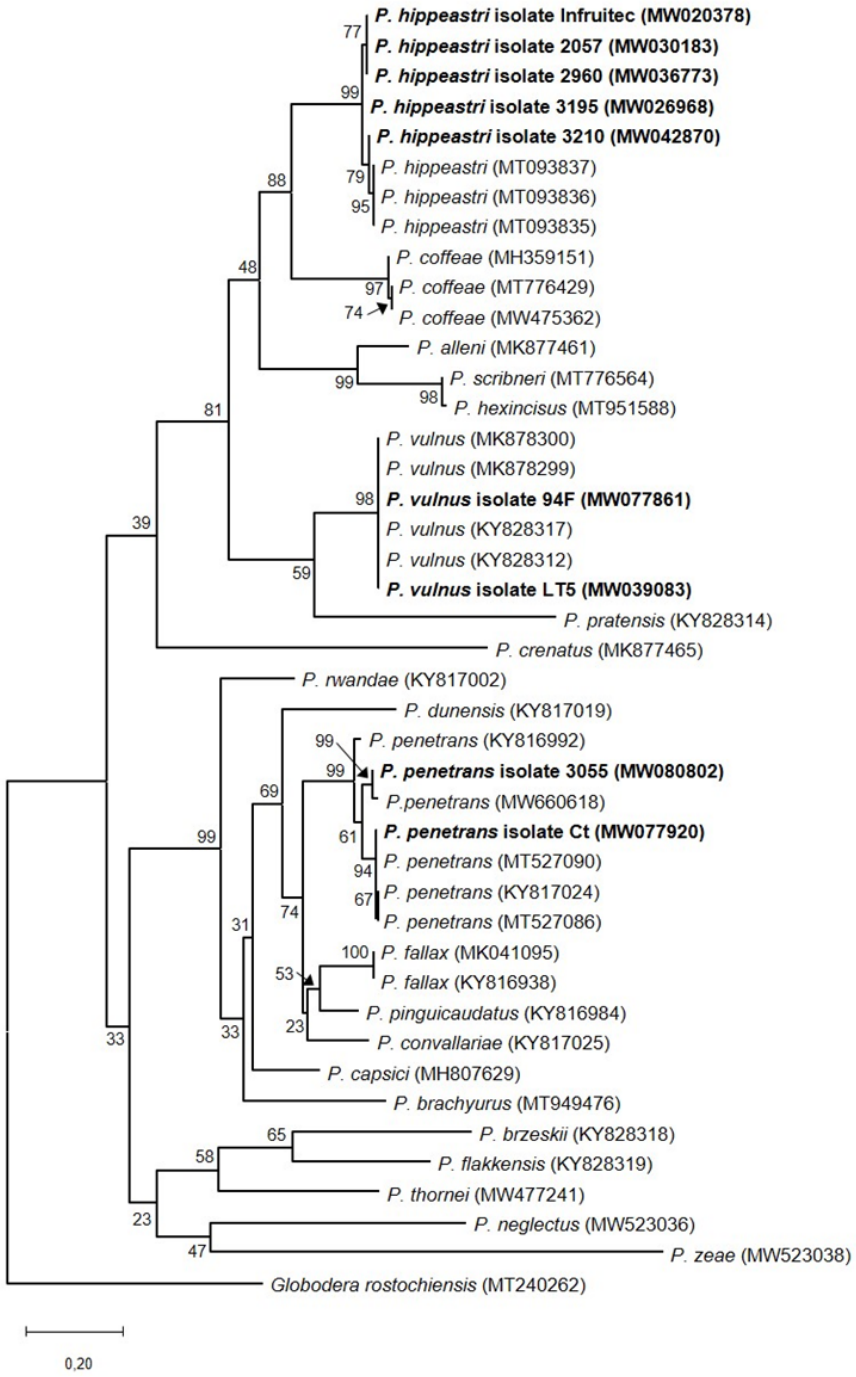

Fig. 12. Phylogenetic relationships of selected *Pratylenchus* species as inferred from the cytochrome oxidase gene of mitochondrial DNA by using the maximum likelihood method. The tree with the highest log likelihood (-28493.12) is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The percentage of trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

studied six isolates of *P. vulnus* from Europe and America. Differences were found in morphological characters such as structure of the post-uterine sac, shape of spermatheca and width of lateral fields, *etc*. They suggested that these differences could be influenced by geographic origins or hosts. However, in the absence of molecular data, the observed morphological differences should not be assigned to

the intraspecies variations (*e.g.*, Mortazavi & Pedram, 2020). Gao *et al.* (1997) found that females extracted from the roots of peaches were markedly longer and wider than ones from the soil around the same roots, while the males extracted from the roots were similar to those from the soil.

Molecular characterisation of *Pratylenchus vulnus* from South Africa. Phylogenetic relationships of the South African populations of *P. vulnus* with other *Pratylenchus* species are shown in Figures 10, 11 and 12. In the trees derived from 28S rRNA sequences, ITS rRNA sequences and *COX1* sequences, the populations of *P. vulnus* form a clearly defined clade with good bootstrap support with other *P. vulnus* isolates obtained from the GenBank sequence database.

DISCUSSION

Apple has been confirmed as a host for *P. hippeastri* (Knoetze *et al.*, 2019; Wang *et al.*, 2016). This root-lesion nematode was previously reported in association with various ornamentals such as amaryllis and bromeliads, in subtropical regions (De Luca *et al.*, 2010). These reports indicate that it could adapt to more temperate climates. In a recent study, *P. hippeastri* has been reported in association with grapevine, another deciduous plant (Handoo *et al.*, 2020).

The diversity of lesion nematodes on apples in South Africa is considerably less than expected. We hypothesise that the cryptic nature of these species could be responsible for earlier over-estimation of the species diversity of lesion nematodes in apple orchards. Regulatory and advisory diagnostic samples regularly contain very few specimens of lesion nematodes, which makes morphological identifications inaccurate. The ranges of morphometric characters of *P. hippeastri* overlap with several other species, like *P. scribneri*. Furthermore, some morphological and biological features such as having two lip annuli, empty spermatheca and similar lateral fields are shared between both species. Inserra *et al.* (2007) noted that the description of *P. scribneri* (from amaryllis) was incomplete, and consequent redescriptions of the species again in association with amaryllis have further confused the situation. De Luca *et al.* (2010) identified several populations that had already morphologically been identified as *P. scribneiri*, *P. loosi* and/or *P. zeae*, and found that they belong to undescribed species in the *hippeastri*-group. The species in this complex are morphologically very similar and molecular studies are necessary to delimit them.

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Резюме. Широко тзвестно, что нематоды рода *Pratylenchus* наносят вред яблоням. Обследование было проведено во всех основных районах выращивания семечковых фруктов в Южной Африке, охватив более 100 мест отбора проб. Нематоды, обнаруженные в образцах, были молекулярно идентифицированы с помощью ПЦР-амплификации и секвенирования сегментов D2-D3 28S, а также ITS-рДНК и гена цитохромоксидазы митохондриальной ДНК (COI). Живых нематод отбирали вручную из каждого образца и переносили на морковные диски для размножения *in vitro*. Подвыборка каждой популяции была также сохранена для морфологической идентификации до видового уровня и таксономических исследований. *Pratylenchus hippeastri* был обнаружен в большинстве обследованных районов, за исключением Вильерсдорпа. В некоторых случаях были обнаружены смешанные популяции *P. hippeastri*, *P. vulnus* и *P. penetrans*, но *P. hippeastri* был наиболее многочисленным. Морфологические и молекулярные исследования подтвердили принадлежность этих видов.