BRILL

Nematology 00 (2016) 1-21

Nematology
brill.com/nemy

Diversity of root-lesion nematodes (*Pratylenchus* spp.) associated with wheat (*Triticum aestivum* and *T. durum*) in Morocco

Fouad MOKRINI [1,2,3], Lieven WAEYENBERGE [2], Nicole VIAENE [2,4], Fouad ABBAD ANDALOUSSI [1] and Maurice MOENS [2,3,*]

[1] *National Institute of Agronomic Research (INRA), Regional Centre of Agadir, Avenue des FAR, 24 Inezgane, Agadir, Morocco*
[2] *Institute for Agricultural and Fisheries Research, Plant, Crop Protection, Burg. Van Gansberghelaan 96, B-9820 Merelbeke, Belgium*
[3] *Laboratory for Agrozoology, Ghent University, Coupure links 653, B-9000 Ghent, Belgium*
[4] *Department of Biology, Ghent University, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium*

Received: 3 February 2016; revised: 4 May 2016
Accepted for publication: 4 May 2016; available online: **???**

Summary – Root-lesion nematodes (*Pratylenchus*) have a worldwide distribution and cause severe production constraints on numerous important crops. During a survey of the wheat-growing area of Morocco, 17 populations of root-lesion nematode were collected. They were identified on the basis of their morphological and morphometric characters, and by molecular methods. Microscopic observations of females and males demonstrated the occurrence of *P. penetrans* in 13 of the 17 samples; *P. thornei* and *P. pseudocoffeae* were detected in four samples from Zaers and a single sample from Settat, respectively. A duplex PCR primer set was used to confirm the presence of *P. penetrans* while the species-specific forward primer PTHO and the common reverse primer D3B were used for *P. thornei*. For the remaining populations, the D2-D3 expansion segments of the 28S rRNA gene were amplified and the obtained sequences were compared with those of *Pratylenchus* species in the GenBank database. This comparison confirmed the morphological identifications and revealed a population of *P. pinguicaudatus*. The study of the phylogenetic relationship of the Moroccan *Pratylenchus* populations showed a high similarity (99-100%) between all *P. penetrans* populations. The population dynamics of six *Pratylenchus* populations from Morocco were evaluated on carrot disk cultures at 4, 8 and 12 weeks after inoculation, and at 10, 15, 20 and 25°C. The optimum temperature for reproduction of all populations was 20°C. After 8 weeks at this temperature, nematode numbers increased up to 458-fold, 310-fold and 252-fold for the four populations of *P. penetrans*, the *P. thornei* and the *P. pseudocoffeae* population, respectively.

Keywords – D2-D3, molecular, morphology, morphometrics, phylogeny, Pratylenchidae, rDNA, reproductive fitness.

Cereals constitute the world's most important source of food. Amongst them, wheat (*Triticum aestivum* L. and *T. durum* Desf.) occupies the top position in terms of production, acreage and source of nutrition, particularly in developing countries (Nicol *et al*., 2011). By 2030, the world production of cereals is expected to have increased to 8 billion tons; the world production of *T. aestivum* is expected to increase from 584 million tons (average 1995-1999) to 860 million tons (Hossain & Teixeira Da Silva, 2012). In Morocco, wheat production (*T. aestivum* and *T. durum*) in 2012-2013 was estimated at 3.8 million tons, ranking the country 15th among the wheat-producing countries (Anonymous, 2013).

Cereals are exposed to biotic and abiotic stresses. Among the biotic stresses, plant-parasitic nematodes play an important role in decreasing crop yield (Nicol, 2002; Nicol & Rivoal, 2008). The damage they cause to cereals has been estimated at $US80 billion per year (Nicol *et al*., 2011). Root-lesion nematodes (RLN; *Pratylenchus* spp.) are widespread and one of the most important groups of plant-parasitic nematodes (Jones *et al*., 2013). Significant economic losses due to RLN have been reported from Europe (Lasserre *et al*., 1994), Australia (Thompson *et al*., 1993, 2008) and the USA (Smiley *et al*., 2004).

Eight *Pratylenchus* species affect roots of cereals (Rivoal & Cook, 1993). Among them, *P. thornei* Sher

* Corresponding author, e-mail: maurice.moens@ilvo.vlaanderen.be

DOI 10.1163/15685411-00002993

& Allen, *P. neglectus* (Rensch) Filipjev & Schuurmans Stekhoven, *P. penetrans* (Cobb) Filipjev & Schuurmans Stekhoven and *P. crenatus* Loof have a worldwide distribution, and sometimes co-exist (Nicol *et al*., 2003). In cereal-growing areas of Morocco, *Pratylenchus* spp. are the most important plant-parasitic nematodes (Meskine & Abbad Andaloussi, 1992; Mokrini *et al*., 2012). They cause extensive necrosis of the roots with consequent crop losses. *Pratylenchus penetrans* was recovered from 70% of the soil samples taken during a nematode survey of wheat-growing areas, with population densities of 32-123 nematodes (100 ml soil)$^{-1}$ and 67-102 (nematodes g root)$^{-1}$ (Mokrini *et al*., 2012).

Traditionally, identification of *Pratylenchus* species relies on morphological characters of the adult stages (Loof, 1991; Handoo *et al*., 2001). However, diversity in morphology and morphometrics among and within the species has been reported frequently as the result of different environmental conditions, including host plant (*e.g.*, Román & Hirschmann, 1969; Doucet *et al*., 2001). This morphological diversity makes diagnosing of *Pratylenchus* species time consuming and technically difficult.

Diversity also exists at the genetic level between and within *Pratylenchus* species (Orui, 1996; Uehara *et al*., 1998; Waeyenberge *et al*., 2000). It allows separation of species on the basis of different DNA fragments and makes molecular characterisation a useful complement to morphological identification (Orui, 1996; Waeyenberge *et al*., 2000; Mizukubo *et al*., 2003). Genetic variation further allows the study of the phylogeny of the pratylenchids (Subbotin *et al*., 2008) by comparing sequences of the ITS rDNA (*e.g.*, Waeyenberge *et al*., 2000; Mizukubo *et al*., 2003; de la Peña *et al*., 2006) or the D2-D3 segment of 28S rDNA (*e.g.*, Al-Banna *et al*., 2004; De Luca *et al*., 2004; Subbotin *et al*., 2008). Intraspecific variation in the ITS rDNA has been observed in several *Pratylenchus* species (Uehara *et al*., 1998; Waeyenberge *et al*., 2000; Mizukubo *et al*., 2003).

Earlier research also revealed differences in pathogenicity between *Pratylenchus* populations; *e.g.*, *P. vulnus* Allen & Jensen on peach almond hybrids and apple rootstocks (Pinochet *et al*., 1993), *P. coffeae* Goodey on sweet potato (Mizukubo & Sano, 1997) and *P. neglectus* on potato (Hafez *et al*., 1999). Trinh *et al*. (2011) demonstrated the high pathogenicity of Vietnamese isolates of *P. coffeae* on Arabica coffee. The isolates differed in reproductive fitness, which was linked to their pathogenicity. Reproductive fitness is one of the major components of pathogenicity (Shaner *et al*., 1992); it is an important feature for the assessment and understanding of disease reactions of plants to pathogens. To compare the reproductive fitness of *Pratylenchus* populations, the use of *in vitro* monoxenic cultures, such as carrot disk cultures (Moody *et al*., 1973), offers a suitable approach since this technique provides homogenous environmental conditions including a constant temperature.

To our knowledge, Moroccan RLN have only been studied with respect to their morphology (Meskine & Abbad Andaloussi, 1992; Mokrini *et al*., 2009); hence, there is no information available on the diversity of their morphometrics and genetics, nor of their potential pathogenicity. For this reason, we conducted a survey of the major wheat-growing areas in Morocco, with the following main objectives: *i*) to collect, identify and compare *Pratylenchus* species and populations using morphological, morphometric and molecular approaches including species-specific PCR and sequencing of the D2-D3 28S rDNA expansion segments; and *ii*) to assess the diversity in population dynamics and optimum temperature for reproduction between Moroccan *Pratylenchus* species and populations by examining their *in vitro* reproduction.

Materials and methods

Pratylenchus populations

A nematode survey was conducted in 2011 in four cereal-growing areas of Morocco, *i.e.*, Zaers, Chaouia, Gharb and Sais (Table 1). For each region, 15-20 fields were sampled, yielding a total of 75 soil and root samples. Samples were taken where plants showed chlorotic leaves and poor growth. Each sample was composed of 15 subsamples randomly collected in the rhizosphere of several plants at a depth of 5-20 cm. The sample was thoroughly mixed before *ca* 2000 g was taken to the laboratory. Nematodes were extracted from 100 g of soil and 10 g of roots (when present) using an automated zonal centrifuge (Hendrickx, 1995). The extracts were examined for the presence of the genus *Pratylenchus* using a compound microscope; a total of 17 *Pratylenchus* populations were obtained.

Morphological identification

From each isolate, adults were recovered from carrot disks, fixed (Netscher & Seinhorst, 1969) and transferred to anhydrous glycerin (Seinhorst, 1959). Ten females or males were transferred to a drop of glycerin and covered

Table 1. Species and populations of *Pratylenchus* collected during a survey in wheat-producing areas of Morocco (2011).

Code	Location	Area	Morphological identification*	Identification using PCR with specific primer		Result of sequencing*
				P. penetrans	*P. thornei*	
PZ1 (6)	Ain El Aouda	Rabat	Pp, Pth	+	+*	Pp, Pth
PZ2 (6)	Ain El Aouda	Rabat	Pth	−	+	Pth
PZ3 (6)	Merchouch	Rabat	Pp, Pth	+	+	Pp, Pth
PZ4 (5)	Merchouch	Rabat	Pp, Pth	+	+	Pp, Pth
PZ7 (5)	INRA Settat	Settat	/	−	−	Pping
PZ8 (4)	Sid Laaydi	Settat	/	−	−	Pping
PZ12 (1)	Oulad Said	Rabat	Pp	+	/	Pp
PZ18 (1)	Oulad Said	Rabat	Pp	+	/	Pp
PZ19 (2)	Oulad Said	Rabat	Pp	+	−	Pp
PC1 (4)	INRA Settat	Settat	Ppseu	−	−	Ppseu
PC2 (2)	INRA Settat	Settat	Pp	+	−	Pp
PC3 (2)	Berrechid	Berrechid	Pp	+	−	Pp
PC20 (2)	Mediouna	Casablanca	Pp	+	−	Pp
PG18 (2)	Sidi Slimane	Sidi Slimane	Pp	+	−	Pp
PS12 (1)	Ain Jmaa	Meknes	Pp	+	/	Pp
PS14 (2)	Mhaya	Fes	Pp	+	−	Pp
PS20 (1)	Ain Taoujdate	Fes	Pp	+	/	Pp

Values in parentheses are the number of nematode specimen used for DNA extraction. +: positive for the mentioned species; −: negative for the mentioned species; /: not checked

* Pp: *Pratylenchus penetrans*; Pth: *P. thornei*; Pping: *P. pinguicaudatus*; Ppseu: *P. pseudocoffeae*.

with a cover slip. The nematodes were identified using the morphometrics and the morphological features of ten females and ten males as suggested by Ryss (1988) and Castillo & Vovlas (2007). Measurements were taken with an Olympus BX51 compound microscope equipped with an Olympus image-capture system and software (Cell^D).

Molecular observations

DNA extraction

For each of the populations, DNA was extracted from 1-6 individuals separately, depending on the number of nematodes available. Nematodes were transferred to an Eppendorf tube containing 25 μl double distilled water and 25 μl nematode lysis buffer (final concentration: 200 mM NaCl, 200 mM Tris-HCl (pH 8), 1% mercaptoethanol and 800 μg proteinase K). The tubes were incubated at 65°C for 1.5 h and 99°C for 5 min, consecutively (Holterman *et al*., 2006). A total of 52 DNA extracts were obtained from 17 populations (Table 1). They were stored at −20°C or used immediately for DNA amplification.

PCR with species-specific primers

The species-specific primers PpenA (5′-TGA CTA TAT GAC ACA TTT RAA CTT G-3′) and AB28 (5′-ATA TGC TTA AGT TCA GCG GGA-3′) (Waeyenberge *et al*., 2009) together with the universal primers (De Ley *et al*., 1999) D3A (5′-GAC CCG TCT TGA AAC ACG GA-3′) and D3B (5′-TCG GAA GGA ACC AGC TAC TA-3′) were used to detect *P. penetrans* in all 52 DNA extracts. The DNA extracts not identified as *P. penetrans* were used in a PCR with the species-specific forward primer PTHO (5′-TAG GGC AGT AGG TTG TCG GC-3′) along with the universal reverse primer D3B (Al-Banna *et al*., 2004) to detect *P. thornei*.

To detect *P. penetrans*, 2 μl of DNA extract (see above) were added to the PCR reaction mixture containing 21 μl ddH_2O, 25 μl 2× DreamTaq PCR Master Mix (Fermentas Life Sciences) and 1 μM of each of the primers Ppen, AB28, D3A and D3B. The thermal cycler program consisted of 5 min at 95°C, 35 cycles of 30 s at 94°C, 30 s at 56°C and 60 s at 72°C, followed by a final elongation step of 8 min at 72°C. For the detection of *P. thornei*, 2 μl of the DNA extract was added to the PCR reaction mixture containing 22 μl ddH_2O,

25 μl 2× DreamTaq PCR Master Mix (Fermentas Life Sciences), and 1 μM of both primers PTHO and D3B. The program of the thermal cycler consisted of 8 min at 95°C; 35 cycles of 30 s at 94°C, 45 s at 60°C and 1 min at 72°C, followed by a final elongation step of 8 min at 72°C. Five μl of each PCR product was mixed then with 1 μl of 6× loading buffer (Fermentas Life Sciences) and loaded on a 1.5% standard TAE buffered agarose gel. After electrophoresis (100 V, 40 min), the gel was stained with ethidium bromide (0.1 μg ml^{-1}) for 20 min, visualised and photographed under UV-light. The remaining PCR product was stored at −20°C.

Sequencing of D2-D3 expansion segments

For DNA extracts where the species-specific PCR for both *P. penetrans* and *P. thornei* were negative, the D2-D3 region was amplified with the forward D2A (5′-ACA AGTACC GTG AGG GAA AGT TG-3′) and reverse D3B (5′-TCG GAA GGA ACCAGC TAC TA-3′) primers according to De Ley *et al*. (1999), and subsequently sequenced. Additionally, to confirm the positive results obtained by the species-specific PCR, we sequenced the PCR-product from one DNA extract of each population identified as *P. penetrans* or *P. thornei*, except for two populations of *P. thornei* of which extracts were depleted.

In each case, 2 μl DNA extract (see above) was added to the PCR reaction mixture containing 22 μl ddH_2O, 25 μl 2× DreamTaq PCR Master Mix (Fermentas Life Sciences) and 1 μM of both primers (D2A and D3B). The thermal cycler program consisted of 5 min at 95°C; 40 cycles of 30 s at 94°C, 45 s at 55°C and 1 min at 72°C; this was followed by a final elongation step of 7 min at 72°C. After PCR amplification, 5 μl of each PCR product was mixed with 1 μl of 6× loading buffer (Fermentas Life Sciences) and loaded on a 1.5% standard TAE buffered agarose gel. After electrophoresis (100 V, 40 min) the gel was stained with ethidium bromide (0.1 μg ml^{-1}) for 20 min, visualised and photographed under UV-light. The remaining PCR product was stored at −20°C. The amplification and electrophoresis process were repeated and both PCR products were pooled for purification. The purification process was done as described by the manufacturer's instructions (Wizard® SV Gel and PCR Clean-Up System Kit, Promega). The purified PCR products were sequenced (Macrogen) in both directions to obtain overlapping sequences of the forward and reverse DNA strands. The sequences were edited and analysed using the software packages Chromas 2.00 (Technelysium) and BioEdit 7.0.4.1 (Hall, 1999). Finally, all sequences were blasted in GenBank (Sequin v. 9.00, http://www.ncbi.nlm.nih.gov/) to reveal their identity.

Phylogenetic analysis

The obtained sequences were compiled with sequences of *Pratylenchus* species available in GenBank. The sequences were aligned with Clustal W (Thompson *et al*., 1994) and visually checked. Differences between sequences were estimated using the DNA distance option provided by BioEdit sequence alignment editor (Hall, 1999). The alignment was imported into the software package Mega 5.0 (Tamura *et al*., 2011); after checking 24 different nucleotide substitution models, the model with the lowest BIC score was retained for constructing a 60% consensus Maximum Likelihood tree. *Radopholus similis* (Cobb) Thorne (KF234235) was added as outgroup. Bootstrap analysis with 100 replicates was performed to assess the degree of support for each clade on the tree.

Reproductive fitness

Nematode cultures

Starting from single females, four populations of *P. penetrans* (PZ3, PC2, PG18 and PS20), one population of *P. thornei* (PZ2) and one of *P. pseudocoffeae* Mizukubo (PC1) were multiplied *in vitro*. Each population was established on carrot disks (Moody *et al*., 1973), which were inoculated with one gravid female per disk and kept in an incubator at 21-22°C. When needed, the nematodes were extracted from the carrot disks on a Baermann funnel in a mistifier chamber. Vigorous nematode inoculum was available 2 months later.

To assess population dynamics at different times and temperatures, batches of 120 carrot disks of each of the six selected populations were prepared. Twenty gravid females were inoculated per carrot disk and kept in a Petri dish sealed with Parafilm. Disks were incubated in the dark at 10, 15, 20 or 25°C for 4, 8 or 12 weeks; they were completely randomised in the incubators. After every incubation time, ten carrot disks were used for evaluation of the nematode reproduction. The experiment was repeated to confirm the results.

Assessment of nematode reproduction

Nematodes were extracted from the carrot disks at the end of the incubation periods. Living mobile stages were collected after 48 h on a Baermann funnel in a mistifier chamber (Hallman & Viaene, 2013). Then, the same carrot disks were macerated in a blender for 1 min before nematode eggs were extracted using an automated

centrifuge (Hendrickx, 1995) to collect the remaining immobile nematodes. The sum of the number of eggs, juveniles, females and males was the final population density (P_f) that was used to compute the reproduction factor (R_f) = P_f/P_i (P_i: initial nematode population density = inoculum density = 20 females).

Statistical analysis

The morphometric data of males and females were analysed in a canonical discriminant analysis (CDA) to investigate the distinctness of the populations. Data from both experiments on reproductive fitness were not significantly different and were therefore pooled for analysis, resulting in 20 replicates per combination (population × time × temperature). Data were analysed using SPSS 21 software. Numbers of female, male, eggs, juveniles and final total population density were subjected to a three-way ANOVA. Subsequently, the means were separated using Tukey's Honestly Significant Difference test ($P < 0.05$).

Results

The survey yielded 17 *Pratylenchus* populations (Table 1). Ten populations were monospecific for *P. penetrans*, one for *P. thornei*, one for *P. pseudocoffeae*, and three populations contained a mixture of two species (*P. penetrans* and *P. thornei*). Two populations, PZ7 and PZ8, from the Zaers region yielded only eight and five females, respectively. These limited numbers failed to multiply on carrot-disks; hence, morphometrics are not available for these populations. They were molecularly characterised as *P. pinguicaudatus* Corbett.

Morphology and morphometrics

Pratylenchus penetrans (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941

Morphometrics

See Tables 2, 3.

Description

Female

Body slender and vermiform, straight to slightly curved ventrally when killed. Lip region set off, flat anteriorly with rounded outer margins, bearing three annules. Lateral field marked by four incisures. Stylet robust with rounded knobs, sometimes flattened anteriorly. Excretory pore at 79.6 (74.0-81.5) μm from anterior extremity (averages of 13 populations), located 2-3 annules posterior to hemizonid. Spermatheca rounded, filled with sperm. Postuterine sac longer than body diam. Vulva located at 78.1 (75.0-80.4)% of body length (average values of 13 populations). Tail generally cylindrical, 28.3 (26.0-30.5) μm long. Tail smooth, with hemispherical end, comprising 20-28 annules on ventral side.

Male

Morphologically similar to female, but smaller for all non-sexual characters. Stylet knobs variable in shape. Lateral field with four lines ending on bursa, spicules slender, gubernaculum ventrally curved. Bursa enclosing tail.

Remarks

This species (13 populations) was found in the four cereal-growing areas of the country. The morphological features and the morphometrics of these populations were similar to other populations described by Loof (1960), Román & Hirschmann (1969) and Townshend (1991), except for variation of the a-ratio of populations PZ12 and PZ18. Females of these two populations showed the greatest a-ratio of all the females examined. The populations showed different degrees of variability in morphology within the population. The most variable character was the tail terminus of females of *P. penetrans*; within population PC2, this feature ranged from smooth to distinctly crenate. In three out of 17 populations *P. penetrans* was found mixed with *P. thornei*.

Pratylenchus thornei Sher & Allen, 1953

Morphometrics

See Table 4.

Description

Female

Lip region relatively high, composed of three annules, not offset from body. Body contracted ventrally posterior to vulva. Lateral field marked by four incisures. Stylet moderately stout, with rounded to anteriorly flattened basal knobs. Tail terminus bluntly rounded to truncate. Vulva at 76.7 (74-78)% of body length (average of four populations). Spermatheca difficult to observe, without spermatozoa when visible. Excretory pore 83 (80-85) μm

Table 2. Morphometric characters of females of *Pratylenchus penetrans* populations from different localities in Morocco. All measurements (n = 10) are in μm and in the form: mean ± s.d. (range).

Character	Population													Loof (1960)
	PZ1*	PZ4	PZ18	PZ19	PZ3	PZ12	PC2	PC3	PC20	PG18	PS12	PS14	PS20	
L	605 ± 33.7 (567-678)	592 ± 22.5 (550-622)	612 ± 53 (530-693)	585 ± 50 (512-646)	605 ± 26.4 (570-655)	601 ± 66 (517-710)	556 ± 43 (490-612)	630 ± 37 (586-710)	556 ± 38 (512-625)	601 ± 49 (512-690)	578 ± 52 (461-643)	569 ± 41 (490-621)	586 ± 30 (522.3-621)	343-811
a	32.2 ± 2.1 (29.3-36)	31.1 ± 2.7 (26.7-35.1)	33.1 ± 2.1 (30.1-36.8)	31.8 ± 2.3 (27.8-33.9)	33.1 ± 1.5 (30.0-35.0)	32.8 ± 3.2 (28.4-37.9)	29.2 ± 1.6 (26.7-31.8)	30.6 ± 2.1 (27.4-34)	30.9 ± 2.2 (27.3-34.9)	31.3 ± 2.8 (27.1-35.2)	29.7 ± 4.1 (24-34.8)	29.9 ± 2.3 (27-33.9)	30.8 ± 3.1 (26.3-35.8)	19-32
b	6.6 ± 0.4 (6-7.6)	6.6 ± 0.2 (6.3-6.8)	7.2 ± 0.8 (6-8.7)	7.0 ± 0.6 (5.9-7.8)	6.9 ± 0.4 (6.4-7.9)	7.1 ± 0.7 (5.9.-8.5)	6.6 ± 0.7 (5.4-7.5)	7.7 ± 0.6 (7.1-8.7)	7.7 ± 0.6 (6.8-8.9)	7.2 ± 0.6 (6.2-8.5)	6.8 ± 0.6 (5.4-7.6)	6.9 ± 0.6 (6.1-7.6)	7.3 ± 0.5 (6.6-8)	5.3-7.9
b′	4.9 ± 0.2 (4.5-5.3)	4.4 ± 0.1 (4.3-4.6)	4.7 ± 0.4 (4.1-5.4)	4.6 ± 0.4 (3.9-5.1)	4.9 ± 0.3 (4.5-5.3)	4.9 ± 0.3 (4-5.8)	4.4 ± 0.6 (4-5)	5.0 ± 0.3 (4-5.9)	4.4 ± 0.3 (4-5)	4.7 ± 0.4 (4-5.8)	4.5 ± 0.4 (4-5)	4.5 ± 0.5 (4-5)	4.8 ± 0.4 (4-5.6)	
c	21.5 ± 1.5 (18.7-23.6)	21.7 ± 1.8 (19.1-25.6)	22.9 ± 3.3 (17.5-27.4)	20.7 ± 3.1 (17.3-28.3)	21.9 ± 1.7 (19.7-24.3)	21.8 ± 3 (18.6-26.9)	20 ± 1.5 (17.5-23)	20.6 ± 1.4 (17.3-22.1)	24.9 ± 5.8 (20-36.2)	19.7 ± 1.2 (17.9-21.4)	19 ± 1.1 (16.9-20.7)	19.6 ± 1.7 (15.7-22)	21.2 ± 1.6 (18.5-23.4)	15-24
c′	2.2 ± 0.2 (1.8-2.6)	2.1 ± 0.2 (1.7-2.5)	2.2 ± 0.2 (1.9-2.6)	2.3 ± 0.2 (2.1-2.9)	2.3 ± 0.2 (1.8-2.5)	2.2 ± 0.2 (1.8-2.4)	2 ± 0.2 (1.8-2.3)	2.3 ± 0.3 (1.7-2.8)	2.2 ± 0.3 (1.8-2.9)	2.1 ± 0.3 (1.5-2.7)	2.2 ± 0.2 (1.8-2.7)	1.9 ± 0.1 (1.7-2.1)	2 ± 0.2 (1.7-2.3)	
V	80.4 ± 1.1 (78.0-82.0)	79.2 ± 0.7 (77.0-80.0)	80.1 ± 0.7 (78.0-82.0)	78.7 ± 1.3 (76.0-80.0)	79.9 ± 0.5 (77.0-81.0)	78.7 ± 1.1 (76.0-82.0)	75.0 ± 1.3 (74.0-79.0)	77.8 ± 0.9 (76.0-80.0)	78.5 ± 1.7 (77.0-8.01)	78 ± 1.2 (76.0-80.0)	76.2 ± 0.3 (75.0-78.0)	76.2 ± 1.6 (75.0-78.9)	77 ± 2.1 (75.0-80.0)	75-84
Stylet length	15.6 ± 0.7 (14.6-17.2)	15.7 ± 0.5 (15.0-16.2)	16.3 ± 0.6 (15.8-17.4)	15.9 ± 0.5 (14.9-16.8)	16.3 ± 0.5 (15.6-17.4)	16.0 ± 0.4 (15.2-16.7)	15.6 ± 0.5 (14.8-16.3)	16.1 ± 0.9 (14.8-17.5)	16.0 ± 0.8 (14.8-17.2)	16.2 ± 0.7 (15.1-17.4)	16.1 ± 0.5 (15.2-17.2)	15.6 ± 0.5 (15.0-16.8)	16.3 ± 0.5 (15.6-17.2)	15-17
DGO	2.5 ± 0.3 (2.2-3.1)	2.9 ± 0.3 (2.4-3.3)	2.6 ± 0.2 (2.1-2.9)	2.7 ± 0.3 (2.1-3.2)	2.7 ± 0.2 (2.3-3.1)	2.6 ± 0.2 (2.1-2.9)	2.7 ± 0.4 (2.1-3.4)	2.5 ± 0.4 (2.1-3.3)	2.3 ± 0.1 (2.1-2.6)	2.8 ± 0.3 (2.3-3.4)	2.6 ± 0.3 (2.1-3.1)	2.8 ± 0.3 (2.5-3.2)	2.7 ± 0.3 (2.2-3.1)	
Max. body diam.	19 ± 0.8 (17.6-20.4)	19.1 ± 1.7 (17.6-21)	18.4 ± 0.8 (17.5-19.9)	18.3 ± 0.5 (17.3-19.2)	18.2 ± 0.6 (17.5-19.2)	18.3 ± 0.6 (17.5-19.7)	19 ± 0.7 (17.8-20.3)	20.6 ± 1.6 (17.3-22.7)	20.2 ± 1 (18.9-22.5)	19.2 ± 1.5 (16.7-21.5)	19.6 ± 2.2 (17-22.7)	19.1 ± 1.8 (16.7-22)	19.1 ± 1.5 (17.4-21.9)	
Excretory pore	81.2 ± 1.7 (78.8-84.2)	80.9 ± 1.4 (79.3-84.2)	81.2 ± 0.8 (79.4-82.3)	81.1 ± 1.1 (79.5-83.1)	81.5 ± 0.5 (80.6-82.4)	81.3 ± 1.2 (79.2-83)	75.7 ± 2.1 (72.0-78.0)	74.0 ± 2.5 (70.0-79.0)	75.3 ± 1.4 (73.0-78.0)	81.4 ± 1.2 (79.7-83.0)	81.2 ± 1.5 (78.2-83.0)	80.7 ± 1.6 (78.3-83.0)	80.3 ± 2.7 (76.3-85.0)	
L/excretory pore	7.4 ± 0.4 (7.0-8.3)	7.3 ± 0.2 (6.8-7.6)	7.5 ± 0.7 (6.5-8.7)	7.2 ± 0.6 (6.2-8.0)	7.4 ± 0.3 (6.9-7.9)	7.4 ± 0.8 (6.3-8.8)	7.3 ± 0.5 (6.3-7.9)	8.5 ± 0.6 (7.7-9.7)	8.3 ± 0.4 (7.6-8.9)	7.4 ± 0.6 (6.1-8.5)	7.1 ± 0.7 (5.6-8.1)	7.1 ± 0.5 (6.0-7.8)	7.3 ± 0.3 (6.6-7.8)	
Pharynx length	82.7 ± 5.7 (75.4-92.4)	88.9 ± 3.5 (84.2-94.3)	85.1 ± 4.2 (77.2-90.1)	83.0 ± 3.4 (79.0-89.3)	86.6 ± 3.8 (78.4-93.1)	84.2 ± 4.1 (78.4-88.4)	83.9 ± 3.8 (76.3-90.3)	81.0 ± 4.6 (74.3-87.0)	81.2 ± 4.4 (74.1-87.0)	82.8 ± 3.5 (76.0-87.1)	84.3 ± 3.1 (79.3-89.1)	82.5 ± 3.2 (78.2-87.0)	80.1 ± 3 (76.1-86.7)	
Pharyngeal overlap	39.3 ± 5.7 (28.5-46.2)	42.7 ± 2.9 (36.4-47)	43.2 ± 2.4 (39-47.2)	44.4 ± 2.2 (39.5-47.3)	34.7 ± 4.7 (28.4-44)	38.3 ± 3.8 (32.5-43.3)	42.6 ± 3.2 (38.2-47.1)	45 ± 2.1 (41.2-48.2)	44.6 ± 1.9 (42.2-47.6)	45.4 ± 1.7 (42-47.2)	42.5 ± 2.1 (39-46.3)	44.3 ± 1.7 (41.8-47.0)	42.7 ± 2.6 (39.3-47.2)	
Ovary	210 ± 16.6 (190-240)	200 ± 6.6 (192-211)	216 ± 7.6 (204-226)	232 ± 4.1 (227-241)	233 ± 5.6 (220-240.3)	229 ± 5.8 (223-239)	197 ± 3.5 (192-203)	193 ± 1.9 (190-197)	194 ± 2.5 (192-199)	204 ± 6.8 (192-213)	234 ± 10.2 (225-253)	234 ± 5.2 (223-241)	226 ± 9.6 (213-241)	
Post-uterine sac	28.0 ± 2.4 (25.1-32.3)	28.1 ± 1.9 (25.5-31.4)	26.5 ± 0.9 (24.7-27.5)	29.8 ± 0.7 (28.9-31.4)	30.9 ± 1.6 (28.8-34.4)	30.5 ± 1.3 (28.6-32.6)	29.2 ± 2.2 (25.5-32.3)	26.4 ± 1.8 (23.5-28.5)	26.7 ± 2.5 (22.1-29.8)	26.9 ± 2.9 (23.4-31.4)	27.3 ± 1.8 (23.4-29.4)	26.9 ± 1.7 (23.5-29)	26.2 ± 1.7 (22.1-28.4)	
Tail	28.3 ± 2.7 (24.5-31.5)	27.5 ± 2.8 (23.3-32.4)	27.1 ± 3.1 (23.4-33.2)	28.8 ± 4.6 (18.2-33.8)	27.7 ± 2.7 (23.8-32.8)	27.7 ± 1.8 (24.3-29.8)	28 ± 2.3 (23.9-31.5)	29.6 ± 2.9 (25.9-34.9)	26 ± 5.2 (18.4-32.5)	30.5 ± 2.9 (27.4-35.2)	30.4 ± 2.4 (27.3-35.4)	29.1 ± 1.9 (26.3-32)	27.7 ± 1.1 (25.8-29.8)	
Vulva to anus distance	90 ± 5.1 (83-101)	98 ± 6.1 (89-105)	93 ± 7.2 (83-103)	95 ± 6 (87-104)	93 ± 4.6 (85-98)	99 ± 6.3 (87-109)	111 ± 6.5 (98-119)	110 ± 6.1 (102-119)	109 ± 4.6 (101-116)	107 ± 8.3 (93-117)	106 ± 8.9 (92-117)	105 ± 7.2 (93-113)	102 ± 9.8 (91-117)	
Lip diam.	7.6 ± 0.4 (7.0-8.2)	7.2 ± 0.4 (6.5-8.1)	6.6 ± 0.5 (6.1-7.7)	7.7 ± 0.4 (6.8-8.1)	7.1 ± 0.6 (6.1-8.1)	6.9 ± 0.4 (6.5-8.1)	7.2 ± 0.2 (6.8-7.7)	7 ± 0.6 (6.2-8.1)	7.2 ± 0.5 (6.5-8.4)	6.9 ± 0.5 (6.2-8.1)	7.6 ± 0.3 (7.2-8.3)	7.5 ± 0.6 (6.6-8.3)	7.3 ± 0.2 (6.8-7.5)	
Lip height	2.4 ± 0.3 (2.1-3.1)	2.6 ± 0.2 (2.1-3.1)	2.4 ± 0.3 (2.1-3.1)	2.1 ± 0.2 (1.8-2.4)	2.4 ± 0.3 (2.1-2.9)	2.3 ± 0.3 (1.9-2.9)	2.4 ± 0.2 (2.1-2.8)	2.3 ± 0.2 (1.9-2.9)	2.6 ± 0.4 (2.1-3.2)	2.9 ± 0.2 (2.5-3.2)	2.4 ± 0.3 (1.9-2.8)	2.2 ± 0.2 (1.9-2.6)	2.4 ± 0.3 (1.9-2.8)	

For codes for populations, see Table 1.

Table 3. Morphometric characters of males of *Pratylenchus penetrans* populations from different localities in Morocco. All measurements (n = 10) are in μm and in the form: mean ± s.d. (range).

Character	Population													Loof (1960)
	PZ1	PZ4	PZ18	PZ19	PZ3	PZ12	PC2	PC3	PC20	PG18	PS12	PS14	PS20	
L	495 ± 22.6 (455-522)	502 ± 11.7 (481-521)	501 ± 7.4 (490-511)	516 ± 18.3 (494-560)	501 ± 5.2 (493-513)	501 ± 4.5 (495-508)	509 ± 8.2 (498-523)	499 ± 13.8 (481-531)	502 ± 8.5 (490-512)	499 ± 7.6 (485-509)	498 ± 15.5 (467-509)	509 ± 6.1 (498-519)	502 ± 6 (490-511)	305-574
a	27.5 ± 1.3 (25.0-29.0)	27.4 ± 1.1 (25.8-29)	27.7 ± 0.8 (26.5-29.0)	28.7 ± 1.6 (26-32)	27.9 ± 1.2 (26-29)	27.9 ± 0.6 (26.8-29.2)	28.4 ± 1.2 (27-30.2)	28.7 ± 1.3 (26.5-30.1)	28.2 ± 1.2 (26-29.4)	27.4 ± 0.7 (26-28.2)	27.3 ± 1.2 (25.4-29.4)	28.4 ± 0.8 (26.9-29.9)	27.8 ± 0.6 (26-29)	23-34
b	7.3 ± 0.3 (6.8-7.8)	6.8 ± 0.3 (6.4-7.5)	6.8 ± 0.3 (6.3-7.2)	7.7 ± 0.4 (7-8.6)	7.4 ± 0.2 (7-7.7)	7.6 ± 0.2 (7.3-8)	7.6 ± 0.2 (7.3-8)	7.3 ± 0.3 (6.9-8.1)	7.4 ± 0.3 (7-7.9)	7.3 ± 0.2 (7-7.6)	7.4 ± 0.3 (6.9-7.7)	7.5 ± 0.3 (7.1-8)	7.3 ± 0.2 (7-7.6)	5-8
b′	4.4 ± 0.2 (4.0-4.7)	4.4 ± 0.1 (4.2-4.7)	4.3 ± 0.1 (4.2-4.5)	4.6 ± 0.2 (4.4-4.9)	4.4 ± 0.1 (4-4.7)	4.5 ± 0.1 (4.4-4.7)	4.6 ± 0.1 (4.4-4.8)	4.5 ± 0.2 (4-4.9)	4.5 ± 0.1 (4.4-4.7)	4.5 ± 0.1 (4.3-4.7)	4.5 ± 0.2 (4.2-4.7)	4.5 ± 0.1 (4.2-4.7)	4.4 ± 0.1 (4-4.7)	
C	19.6 ± 1.3 (17-21.5)	20.3 ± 1.2 (18.3-22.0)	19.7 ± 1.1 (18.2-21.6)	20.2 ± 1.3 (17.8-22.9)	19.7 ± 0.6 (18.0-20.4)	20 ± 0.8 (18.8-21.5)	20.3 ± 0.7 (19.0-22.0)	19.9 ± 0.9 (18.0-22.0)	19.9 ± 0.8 (18.5-21.0)	20.1 ± 0.4 (19.0-21.0)	19.5 ± 0.9 (18.0-21.1)	20.1 ± 0.4 (19.6-21.0)	19.3 ± 0.8 (18.0-21.0)	16-22
c′	1.9 ± 0.1 (1.8-2.2)	2.1 ± 0.2 (1.9-2.4)	2.2 ± 0.2 (1.8-2.5)	2.0 ± 0.1 (1.8-2.4)	2.0 ± 0.1 (1-2.2)	2.0 ± 0.1 (1.8-2.2)	2.0 ± 0.1 (1.8-2.3)	2.1 ± 0.1 (1.9-2.2)	2.1 ± 0.1 (1.9-2.3)	2.0 ± 0.2 (1.8-2.4)	2.1 ± 0.2 (1.9-2.4)	1.9 ± 0.1 (1.8-2.2)	2.0 ± 0.1 (1.0-2.2)	
Stylet length	15.6 ± 0.3 (14.9-16)	15.5 ± 0.6 (14.9-16.3)	15.8 ± 0.5 (14.8-16.5)	16.0 ± 0.5 (14.9-17)	16.0 ± 0.4 (15.0-16.8)	16.0 ± 0.6 (15.3-17.1)	15.5 ± 0.5 (14.8-16.2)	15.2 ± 0.6 (14.3-16.1)	15.9 ± 0.5 (15.0-16.9)	15.4 ± 0.4 (14.9-16)	15.5 ± 0.5 (14.9-16.2)	15.1 ± 0.5 (14.3-16.1)	15.6 ± 0.4 (14.2-16.4)	
DGO	1.9 ± 0.2 (1.7-2.2)	1.8 ± 0.1 (1.6-2.1)	1.9 ± 0.1 (1.8-2.2)	2.0 ± 0.1 (1.8-2.2)	1.9 ± 0.1 (1.0-2.0)	1.9 ± 0.1 (1.7-2.1)	1.9 ± 0.1 (1.7-2.1)	1.9 ± 0.1 (1.7-2.1)	2.0 ± 0.1 (1.8-2.1)	1.9 ± 0.1 (1.7-2.1)	1.8 ± 0.1 (1.6-2.1)	1.9 ± 0.2 (1.7-2.2)	1.9 ± 0.1 (1.0-2.0)	
Max. body diam.	18.2 ± 0.5 (17.4-19)	18.3 ± 0.5 (17.8-19.5)	18.0 ± 0.5 (17.2-19.3)	18.0 ± 0.4 (17.2-18.8)	17.9 ± 0.7 (16.0-19.0)	17.9 ± 0.4 (17.4-18.7)	17.9 ± 0.6 (17.0-18.6)	17.4 ± 0.6 (16.7-18.4)	17.8 ± 0.7 (16.8-19)	18.2 ± 0.3 (17.0-18.9)	18.2 ± 0.4 (17.0-18.8)	17.9 ± 0.5 (17.4-19.0)	18 ± 0.4 (17.0-18.5)	
Excretory pore	77.3 ± 2.5 (74.0-81.0)	75.5 ± 3.6 (69.4-80.4)	75.2 ± 2.7 (71.0-78.4)	76.6 ± 3.4 (70.0-81.0)	76.1 ± 3 (71.0-80.0)	73.7 ± 2.5 (69.4-77.4)	72.8 ± 1.8 (70.4-75.3)	73.4 ± 1.9 (70.0-76.0)	76.4 ± 4.9 (70.6-87)	73.2 ± 2.5 (69.5-77)	72.8 ± 2.7 (69.0-77.0)	72.2 ± 1.9 (69.7-74.6)	72.9 ± 2.8 (69.0-77.0)	
L/excretory pore	6.4 ± 0.4 (5.7-6.9)	6.6 ± 0.4 (6.1-7.5)	6.7 ± 0.2 (6.3-7.1)	6.7 ± 0.4 (6.3-7.4)	6.6 ± 0.3 (6.2-7.2)	6.8 ± 0.2 (6.5-7.2)	7 ± 0.2 (6.7-7.3)	6.8 ± 0.2 (6.5-7.4)	6.6 ± 0.4 (5.6-7.1)	6.8 ± 0.3 (6.4-7.2)	6.8 ± 0.4 (6.0-7.0)	7.0 ± 0.2 (6.6-7.3)	6.9 ± 0.2 (6.5-7.4)	
Pharynx length	67.6 ± 3.3 (62.0-72.6)	73.8 ± 2.8 (69.8-78.2)	73.7 ± 4.1 (68.3-80.2)	67.3 ± 2.8 (60.0-70.0)	67.2 ± 1.2 (63.0-70.0)	66.3 ± 1.5 (62.8-67.8)	67.3 ± 2.2 (63.8-70.1)	68.1 ± 2.3 (63.6-71.6)	68.2 ± 2.3 (63.0-70.8)	68.2 ± 2.2 (65.0-71.0)	67.7 ± 2.8 (63.0-71.0)	67.7 ± 3 (62.7-71)	68.4 ± 1.2 (66.0-70.0)	
Pharyngeal overlap	43.7 ± 2.2 (41.0-45.6)	40.2 ± 2.6 (34.4-44.2)	41.4 ± 2.7 (37.1-45.6)	44.8 ± 2.4 (39.0-47.0)	45.4 ± 2.1 (42.0-49.0)	44.1 ± 1.2 (41.8-45.8)	43.5 ± 3.2 (38.7-47.0)	42.9 ± 1.7 (39.8-45.8)	43.3 ± 2.3 (38.0-45.7)	42.7 ± 2.1 (39.0-45.6)	43.3 ± 2.1 (39.0-45.0)	43.9 ± 1.1 (42.7-45.9)	44.4 ± 2.4 (39.0-47.0)	
T	41.9 ± 2.8 (38.0-46.0)	41.5 ± 1.3 (40.2-44.1)	40.7 ± 0.5 (40.0-41.5)	40 ± 1.5 (37.0-42.0)	41.8 ± 1.3 (40.0-44.0)	41.6 ± 1.2 (39.9-43.5)	41.4 ± 0.9 (39.0-42.5)	41.6 ± 1.4 (39.0-43.0)	40.9 ± 1 (39.0-42.0)	41.2 ± 1.1 (39.0-42.6)	41.9 ± 1.2 (40.1-44.4)	41.2 ± 0.8 (40-42.9)	41.6 ± 1.2 (39.1-42.8)	
Testis	207 ± 5.7 (199-215)	208 ± 4 (201-216)	203 ± 3.6 (199-211)	206 ± 4.8 (199-212)	209.5 ± 6.1 (201-220)	208 ± 5.6 (201-216)	211 ± 2.7 (208-217)	207 ± 5.3 (201-218)	205.7 ± 3.3 (201-210)	205 ± 5.1 (196-211)	208 ± 4.7 (199-213)	209 ± 4.3 (203-219)	208 ± 4.7 (199-213)	
Tail length	25.3 ± 1 (23.9-27.0)	24.7 ± 1.5 (22.2-27.1)	25.2 ± 1.5 (23.0-27.0)	25.6 ± 1.2 (23.9-27.8)	25.5 ± 0.9 (24.0-27.0)	25 ± 1 (23.7-26.7)	25.1 ± 0.7 (23.8-26.4)	25.1 ± 0.8 (23.9-26.0)	25.2 ± 1 (23.0-27.6)	24.8 ± 0.5 (23.0-25.8)	25.5 ± 1.4 (23.5-27.5)	25.3 ± 0.5 (24.6-26.0)	25.9 ± 1.1 (23.0-27.0)	
Lip diam.	7.1 ± 0.1 (6.9-7.4)	7.1 ± 0.2 (6.8-7.4)	7.1 ± 0.3 (6.5-7.6)	7.1 ± 0.1 (6.8-7.3)	7 ± 0.2 (6.7-7.2)	7 ± 0.1 (6.8-7.2)	6.9 ± 0.2 (6.6-7.3)	7.1 ± 0.2 (6.8-7.5)	7.1 ± 0.2 (6.8-7.5)	7.1 ± 0.2 (6.9-7.4)	7 ± 0.3 (6.0-7.5)	7.1 ± 0.1 (6.9-7.4)	7.0 ± 0.2 (6.0-7.6)	
Lip height	1.9 ± 0.3 (1.6-2.6)	2.4 ± 0.4 (1.8-2.9)	2.1 ± 0.2 (1.8-2.5)	2.1 ± 0.2 (1.8-2.4)	2.3 ± 0.2 (1.9-2.5)	2.1 ± 0.2 (1.8-2.4)	2.2 ± 0.2 (1.9-2.5)	2.3 ± 0.2 (1.8-2.5)	2.2 ± 0.2 (1.8-2.6)	2.2 ± 0.2 (1.9-2.5)	2.2 ± 0.2 (2.0-2.5)	2.2 ± 0.2 (1.9-2.5)	2.2 ± 0.2 (1.7-2.5)	
Spicule	14.7 ± 1.1 (13.0-16.3)	14.7 ± 1.1 (12.9-16.4)	14.9 ± 0.7 (14.0-16.6)	14.9 ± 0.6 (13.6-15.9)	14.8 ± 0.6 (13.4-15.0)	14.3 ± 0.7 (13.0-16.4)	14.5 ± 0.6 (13.8-15.3)	14.3 ± 0.6 (13.4-15.1)	14.6 ± 0.4 (13.0-15.3)	14.9 ± 0.6 (13.0-15.8)	15.2 ± 0.6 (14.5-16.3)	15.1 ± 0.6 (13.5-15.7)	15.5 ± 0.7 (14.2-16.4)	14-17
Gubernaculum	3.9 ± 0.2 (3.6-4.3)	4.8 ± 0.1 (4.6-5.0)	4.3 ± 0.4 (3.8-5.1)	4.3 ± 0.3 (3.9-4.7)	4.3 ± 0.3 (4.0-5.0)	4.1 ± 0.3 (3.8-4.6)	4.3 ± 0.2 (3.8-4.6)	4.1 ± 0.2 (3.8-4.6)	4.2 ± 0.3 (3.7-4.6)	4 ± 0.2 (3.8-4.4)	4.1 ± 0.1 (3.9-4.3)	4.2 ± 0.2 (3.9-4.7)	4.5 ± 0.2 (3.8-4.9)	3.9-4.2

For codes for populations, see Table 1.

Table 4. Morphometrics of females of four *Pratylenchus thornei* populations collected in Morocco. All measurements (n = 10) are in μm and in the form: mean ± s.d. (range).

Character	Population				
	PZ1	PZ2	PZ3	PZ4	Sher & Allen (1953)
L	522 ± 55 (429-595)	603 ± 17.9 (571-626)	554 ± 38.2 (468-601)	550 ± 30.3 (489-608)	450-770
a	30 ± 5.7 (21-39)	32.8 ± 2.2 (30.1-34.6)	31.5 ± 2.6 (28.6-36.7)	31.4 ± 2.8 (27-36)	26-36
b	7.4 ± 0.6 (6.5-8.1)	8.8 ± 0.3 (8.3-9.2)	7.8 ± 0.7 (6.4-8.7)	7.5 ± 0.5 (6.8-8.5)	5.5-8
b′	4.8 ± 0.7 (3.7-5.8)	4.8 ± 0.3 (4.3-5.2)	4.7 ± 0.5 (3.9-5.5)	5.1 ± 0.4 (4.7-5.8)	
c	16.7 ± 1.8 (14.5-20.2)	20.1 ± 1.6 (17.8-22.9)	17.8 ± 1.5 (15.9-20.5)	17.7 ± 1.6 (15.9-20.4)	18-22
c′	2.0 ± 0.2 (1.7-2.4)	1.9 ± 0.2 (1.7-2.3)	1.9 ± 0.2 (1.6-2.3)	2.2 ± 0.2 (1.9-2.5)	
V	74.4 ± 2.6 (70.4-79.1)	78.5 ± 1.5 (75.5-80.4)	77.4 ± 1.7 (73.6-79.8)	76.8 ± 1.9 (73.4-79.7)	73-80
Stylet length	17.2 ± 1.1 (15.5-18.5)	16.1 ± 1.1 (15-18.3)	16.2 ± 0.4 (15.7-16.8)	17.5 ± 0.9 (15.5-18.5)	17-19
DGO	2.0 ± 0.1 (1.9-2.2)	2.1 ± 0.1 (1.9-2.4)	2.1 ± 0.1 (1.9-2.3)	2.1 ± 0.1 (1.8-2.3)	
Max. body diam.	17.7 ± 2.2 (13-19.8)	18.4 ± 0.9 (16.9-19.8)	17.6 ± 1.3 (15.7-19.7)	17.6 ± 0.9 (16.3-19.3)	
Excretory pore	80.8 ± 3.1 (75.4-86.3)	84.2 ± 2.6 (79.3-87.2)	84.9 ± 3 (79.3-88.2)	82.0 ± 2.4 (78.4-86.7)	
L/excretory pore	6.5 ± 0.7 (5.2-7.5)	7.1 ± 0.3 (6.6-7.7)	6.5 ± 0.5 (5.4-7.6)	6.7 ± 0.5 (5.6-7.6)	
Pharynx length	70.6 ± 3.3 (63.7-74.4)	68.4 ± 2.3 (64.3-71.5)	70.7 ± 2.4 (66.8-74.6)	73.5 ± 2.8 (69.8-78.3)	
Pharyngeal overlap	40.7 ± 3 (34.0-44.1)	40.1 ± 2.2 (36.4-43.5)	41.2 ± 1.8 (37.8-44.2)	43.4 ± 2.6 (39.8-46.7)	
Post-uterine sac	26.2 ± 0.5 (25.3-27)	26.3 ± 0.5 (25.4-27.4)	26.4 ± 0.5 (25.7-27.1)	26.3 ± 0.5 (25.7-27.3)	
Tail	31.1 ± 2.2 (28.5-35)	30.2 ± 2.5 (26.3-34)	31.1 ± 2.2 (27.9-34.2)	31.2 ± 2.4 (28.7-34.5)	
Vulva to anus distance	101 ± 8.1 (88-111)	99 ± 6.6 (91-110)	93 ± 4.9 (87-103)	95 ± 6.6 (86-107)	
Lip diam.	7.7 ± 0.2 (7.4-8.1)	7.7 ± 0.2 (7.4-8.0)	7.6 ± 0.2 (7.4-7.9)	7.7 ± 0.1 (7.5-7.9)	
Lip height	2.7 ± 0.1 (2.6-3.0)	2.8 ± 0.1 (2.5-3.0)	2.7 ± 0.1 (2.6-2.9)	2.7 ± 0.2 (2.5-3.0)	

For codes for populations, see Table 1.

(average of four populations) posterior to head, immediately posterior to hemizonid. Tail 18-25 annules, bluntly rounded, without striations around terminus.

Male

Absent in each of the four populations.

Remarks

This species was detected in four populations (PZ1, PZ2, PZ3 and PZ4). The populations were morphologically and morphometrically similar to populations described previously (Sher & Allen, 1953; Handoo & Gol-

den, 1989; Pourjam *et al*., 1997). The morphometrics of females of these populations matched the original description with the exception of PZ1, PZ2 and PZ4, for which we observed a c-ratio different from the original description. A similar observation was made for the b-ratio of females of PZ2.

Pratylenchus pseudocoffeae Mizukubo, 1992

Morphometrics

See Table 5.

Description

Female

Body straight with posterior half slightly curved ventrally when heat-relaxed. Lip region with three annuli, relatively low, flattened, 2-3 μm high, 6-9 μm wide. Stylet knobs massive, shape variable but mostly broadly rounded or flattened anteriorly. Excretory pore at 87 (86-90) μm, located slightly anterior to level of cardia. Hemizonid flat, 2-3 annuli long, at level of excretory pore. Vulva located at 81.8 (78-83.4)% of body length. Spermatheca oblong, packed with sperm. Post-vulval uterine sac less than two vulval body diam. long. Tail sub-hemispherical or bluntly pointed with smooth terminus.

Male

Body generally straight when heat-relaxed, similar to female except for sexual dimorphism. Stylet length slightly shorter than in female (Table 5). Stylet knob shape mostly broadly rounded. Labial region with three annuli. Spicules arcuate, slightly longer than stylet. Gubernaculum simple.

Remarks

Only one population of *P. pseudocoffeae* was detected (Settat, Chaouia region). The morphometric and morphological characters corresponded to those reported by Mizukubo (1992).

Canonical discriminant analysis (CDA)

Using a combination of 14 morphometric characters for the females and 13 morphometric characters for the males (Table 6), the CDA clearly separated the three species from one another (Fig. 1). Seven morphometric traits of females (L, a, b, c, body diam., excretory pore and tail length) provided the most useful taxonomic characters for identification and discrimination (Table 6). The CDA for male morphometric characters equally allowed the separation of the two species of *Pratylenchus*; in this case four characters (a, b, body diam. and excretory pore) were the most valuable for species separation.

Molecular characterisation

Species-specific PCR and sequences

The duplex PCR with the PpenA/AB28 and D3A/D3B primer pairs amplified a single band of 340 bp for all 52 DNA extracts. A second specific band of 660 bp was present only for PZ1, PZ3, PZ4, PZ12, PZ18, PZ19, PC2, PC20, PG18, PS12, PS14 and PS20 (Table 1; Fig. 2). This means that out of 52 DNA extracts (17 populations), 23 extracts (12 populations) were identified as *P. penetrans*. Two DNA extracts of PC3 were not detected by the species-specific PCR for *P. penetrans*, although they were earlier identified morphologically as *P. penetrans*. For the remaining 29 extracts (nine populations) not identified as *P. penetrans*, the *P. thornei*-specific primer pair PHTO/D3B amplified a specific band of 288 bp for four DNA extracts originating from four populations (PZ1, PZ2, PZ3 and PZ4) (Fig. 3). Populations PZ1, PZ3 and PZ4 were composed of two species (*P. penetrans* and *P. thornei*) (Figs 2-4; Table 1).

The comparison of the sequences of the D2-D3 28S rDNA expansion segments of the *P. penetrans* and *P. thornei* populations and three unidentified populations of *Pratylenchus* spp. (PZ7, PZ8 and PC1) from Morocco among themselves and with sequences of other *Pratylenchus* species available in GenBank is presented in Figure 4. This comparison confirmed the identification of *P. penetrans* and *P. thornei* using both morphological features and species-specific PCR. In addition, the sequences revealed the two DNA extracts of PC3 to be *P. penetrans*; earlier they were morphologically identified as *P. penetrans* but not detected by the species-specific PCR.

Thirteen sequences of the D2-D3 28S rDNA expansion segments were similar (99-100%) to the sequences of *P. penetrans* published in GenBank, two sequences (PZ1 and PZ3) were similar (99%) to *P. thornei*, four sequences (three individuals of PZ8 and PZ7) were similar (96-99%) to *P. pinguicaudatus*, and one sequence (PC1) was similar (98%) to *P. pseudocoffeae*. The comparison of the D2-D3 28S rDNA expansion segments at intra-population level showed no divergence between PC3, PS12 and PS14; very low sequence divergence (0-0.3%) was observed for the

Table 5. Morphometric characters of female and male *Pratylenchus pseudocoffeae* population PC1 collected in Morocco. All measurements are in μm and in the form: mean ± s.d. (range).

Character	Male		Female	
	PC1	Mizukubo (1992)	PC1	Mizukubo (1992)
n	10	11	10	50
L	480 ± 16 (451-502)	490 (390-540)	508 ± 14.9 (480-523)	510 (410-620)
a	30.8 ± 1.3 (28.0-32.0)	30.6 (25.6-37.0)	27.8 ± 2.3 (25-30.7)	27.5 (22.6-32.1)
b	5.9 ± 0.2 (5.6-6.2)	6.1 (4.6-6.9)	5.9 ± 0.2 (5.3-6.2)	5.8 (4.7-7.0)
b′	3.3 ± 0.1 (3.2-3.4)	3.3 (2.9-3.8)	3.7 ± 0.1 (3.5-3.9)	3.1 (2.6-3.4)
c	19.9 ± 2 (17.4-24.5)	20.3 (18.1-23.9)	20.1 ± 0.8 (18.2-20.8)	19.3 (18-20)
c′	1.9 ± 0.1 (1.7-2.2)	2.4 (1.8-2.9)	2.4 ± 0.1 (2.2-2.7)	2.4 (1.8-2.5)
V	–	–	81.8 ± 0.4 (78.0-83.4)	81 (79-82)
Stylet length	14.8 ± 0.4 (14-15.5)	15.0 (14.0-15.5)	16 ± 0.4 (15.4-16.6)	16 (15-17)
DGO	2.7 ± 0.3 (2.2-3.2)	–	2.5 ± 0.2 (2.1-2.8)	2.5 (2.1-2.8)
Max. body diam.	15.5 ± 0.4 (15.0-16.1)	–	17.6 ± 1.6 (15.9-20.8)	–
Excretory pore	83.4 ± 0.2 (82.4-86.1)	–	87.8 ± 1.2 (86.2-90.1)	–
L/excretory pore	5.6 ± 0.2 (5.2-5.9)	–	5.7 ± 0.1 (5.4-6.1)	–
Pharynx length	81.6 ± 2.9 (76.4-85)	–	85.7 ± 1.2 (81.4-90.1)	–
Vulva to anus distance	–	–	66.5 ± 3.6 (61.3-72.3)	–
Tail	24.3 ± 2 (20.0-27.4)	24.0	25.2 ± 0.8 (24.0-26.4)	–
Spicule	17.1 ± 0.4 (16.2-17.8)	16.5	–	–
Gubernaculum	4.4 ± 0.2 (4.1-4.8)	5.0	–	–

other *P. penetrans* populations (Table 7). The D2-D3 sequence of the Moroccan population of *P. pinguicaudatus* had over 96% similarity with a sequence of this species deposited in GenBank (AJ545014) originating from a population from Tunisia. The sequence comparison of the two *P. thornei* populations from Morocco with the sequence of four *P. thornei* populations available in GenBank revealed the Moroccan sequences to be identical to the *P. thornei* sequence from Spain (EU130873). The relationships with other *Pratylenchus* species were measured through Bayesian Inference (BI) analysis. The obtained alignment presented 604 characters of which 181 were parsimony informative. On the basis of the topology of the calculated majority rule, 60% consensus Maximum Likelihood tree for all populations studied with addition of 13 *Pratylenchus* spp. from GenBank, two major groups of pratylenchids were revealed (Fig. 4). In Group I (bootstrap value = 75%), three subgroups were found. The first group (Ia) comprised all *P. penetrans* populations from Morocco together with four other *P.*

Table 6. Standardised coefficients for canonical variants of *Pratylenchus* spp. for females and males.

Selected character	Females		Males	
	Root 1	Root 2	Root 1	Root 2
L	0.5	−1.89	−0.47	−0.44
a	0.18	1.53	0.33	−0.19
b	−1.2	−0.1	0.2	0.6
b′	−0.005	−0.023	−0.28	1.02
c	0.34	−0.22	0.23	−0.16
c′	0.35	0.008	−0.12	−0.13
V	0.4	0.94	–	–
Stylet length	−0.16	0.02	−0.21	−0.15
DGO	0.29	0.045	–	–
Max. body diam.	0.23	1.108	−0.007	−0.45
Excretory pore	−0.31	0.72	−0.008	−1.11
Pharynx overlap	0.13	0.04	0.15	0.1
Post-uterine sac	0.13	0.0004	–	–
Tail	−0.36	0.16	0.61	0.85
Spicule	–	–	0.24	0.18
Gubernaculum	–	–	0.21	0.1

penetrans populations available in GenBank (bootstrap value = 99%). The second subgroup (Ib) comprised only *P. fallax* (AF264181). The third subgroup (Ic) comprised four populations of *P. pinguicaudatus*. Group II (bootstrap value = 90%) held three subgroups. Subgroup IIa (bootstrap value = 99%) contained the *P. pseudocoffeae* population from Morocco together with a Chinese population of the same species (AF170444). Subgroup IIb contained a *P. coffeae* population from China (JX046966). Finally, subgroup IIc (bootstrap value = 99%) contained all *P. thornei* (Moroccan and other) populations. The *P. thornei* populations from Morocco (PZ1, PZ3) clustered with a Spanish population of the same species (EU130873).

Reproductive fitness

Significant differences in reproduction were observed between populations ($F = 19.1$; df = 5; $P < 0.0001$), temperature ($F = 5022$; df = 3; $P < 0.0001$) and time ($F = 4888.8$; df = 2; $P < 0.0001$). Also the interactions populations × temperature ($F = 76.4$; df = 15; $P < 0.0001$), population × time ($F = 22.9$; df = 10; $P < 0.0001$) and temperature × time ($F = 4888.8$; df = 6; $P < 0.0001$) were significant. Additionally, there was a significant three-way interaction between populations, temperature and time ($F = 50.7$; df = 30; $P < 0.0001$).

At 10°C, all populations had increased 8 and 12 weeks after inoculation (WAI), but not at 4 WAI (Table 8). The highest reproduction factor (R_f) at this temperature was found 12 WAI for *P. penetrans* PZ3 but was merely 3.7. The fewest nematodes ($R_f = 0.4$) were retrieved 4 WAI for *P. thornei* PZ2. At this temperature, all final populations consisted of females, juveniles, males and eggs, except for *P. thornei* PZ2 in which no males were found. In all populations the fraction of eggs was the highest at each of the three observation times and varied between 61 and 92% (Fig. 5).

At 15°C, more nematodes were extracted than inoculated for all populations, even after only 4 WAI (Table 8). The greatest reproduction was found 12 WAI for *P. penetrans* PC2 ($R_f = 51.3$) followed by *P. penetrans* PZ3 ($R_f = 45.5$); *P. pseudocoffeae* PC1 and *P. thornei* PZ2 had the lowest final R_f (21.7 and 26.9, respectively). At this temperature, all populations consisted of eggs, juveniles, females and males at all time points, except for *P. thornei* PZ2 for which no males were found. At 4 WAI, the proportion of eggs in the total population varied between 29 (*P. penetrans* PC2) and 39% (*P. penetrans* PS20). This percentage had increased by 8 WAI and ranged between 70 (*P. pseudocoffeae* PC1) and 82% (*P. penetrans* PC2 and PZ3). At 12 WAI, the percentage of eggs had decreased again to about the same numbers as at 4 WAI (Fig. 6). The remainder of the nematodes consisted of a mixture of juveniles, males and females in variable proportions, depending on the population.

At 20 and 25°C, the R_f of all populations differed significantly between incubation times ($P < 0.05$) (Table 8). At 8 WAI, the R_f was greatest for all populations at both temperatures. The highest R_f was found 8 WAI at 20°C for *P. penetrans* PG18 ($R_f = 458.6$). At 25°C, all populations had multiplied already by a factor between 5.8 (PZ2) and 10.9 (PG18) 4 WAI (Table 8). At this temperature, at 8 WAI, *P. pseudocoffeae* PC1 had increased most ($R_f = 217$) while the R_f of the other populations was significantly less and varied between 65 (PS20) and 81 (PC2). The final R_f at 25°C (12 WAI) ranged from 22 (*P. penetrans* PS20) to 111 (*P. pseudocoffeae* PC1). The final populations at 20 and 25°C consisted of females, juveniles, males (except *P. thornei*) and eggs; there was a greater percentage of females at 20 and 25°C than at the lower temperatures (Fig. 6).

Discussion

Our survey of *Pratylenchus* in wheat-growing areas of Morocco yielded four species, *viz.*, *P. penetrans*, *P. thornei*, *P. pinguicaudatus* and *P. pseudocoffeae*. The

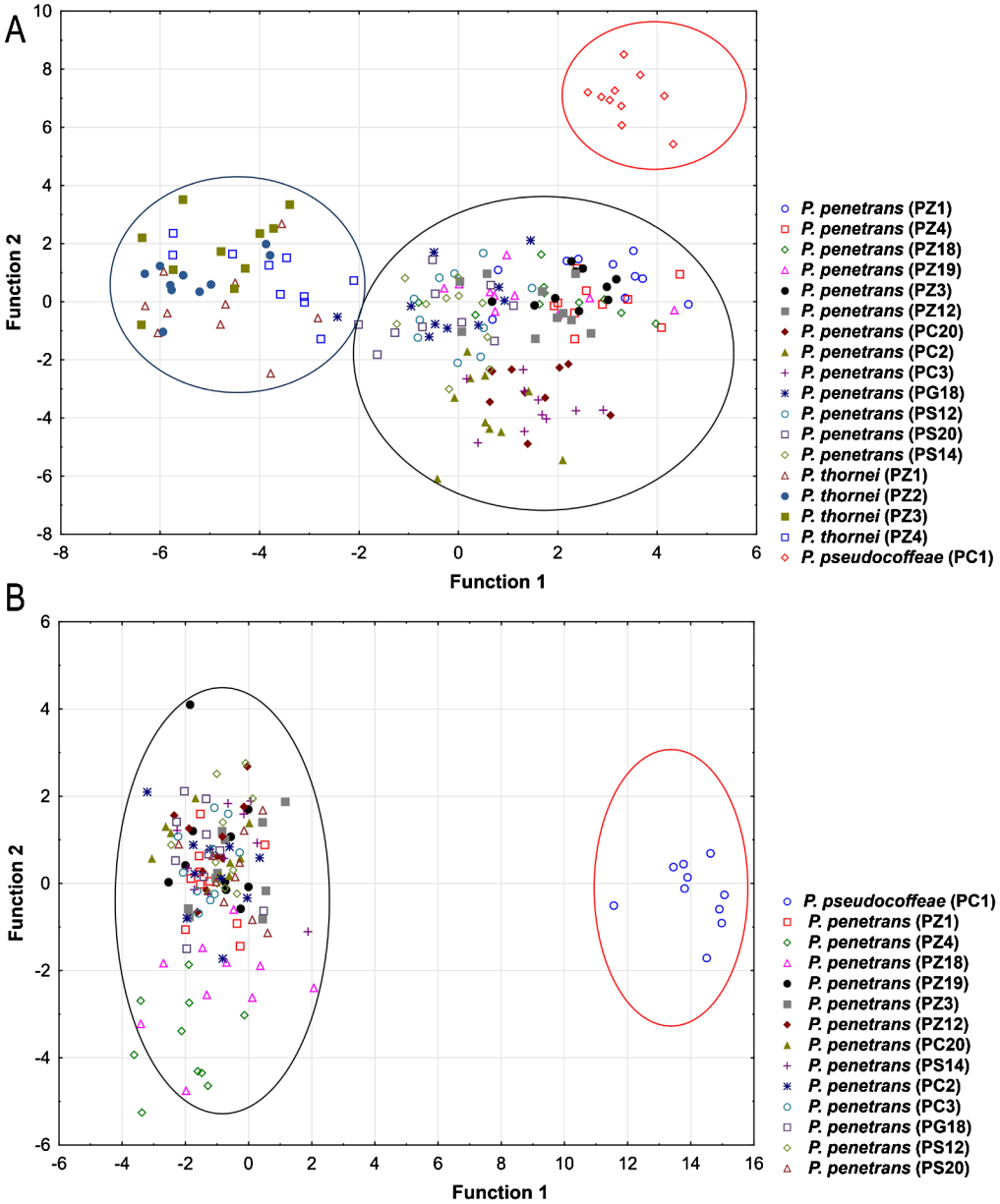

Fig. 1. The two main canonical discriminant functions between 18 *Pratylenchus* populations from Morocco for females (A) and males (B), based on 14 morphological characters (see Table 6).

presence of these nematodes indicates potential damage to wheat and the need for appropriate management strategies. Previous surveys in the area had only revealed the presence of *P. penetrans* and *P. thornei* (Ammati, 1987; Meskine & Abbad Andaloussi, 1992; Mokrini *et al*., 2009). That means that the presence of *P. pinguicaudatus* and *P. pseudocoffeae* in Morocco is reported herein for the first time. Both, *P. penetrans* and *P. thornei* are economically important and considered serious pests of many hosts, including cereals (Castillo & Vovlas, 2007). However, nothing is known about the importance of *P. pinguicaudatus* and *P. pseudocoffeae* in cereals.

Traditionally, identification of *Pratylenchus* species relies on the morphology and morphometrics of females

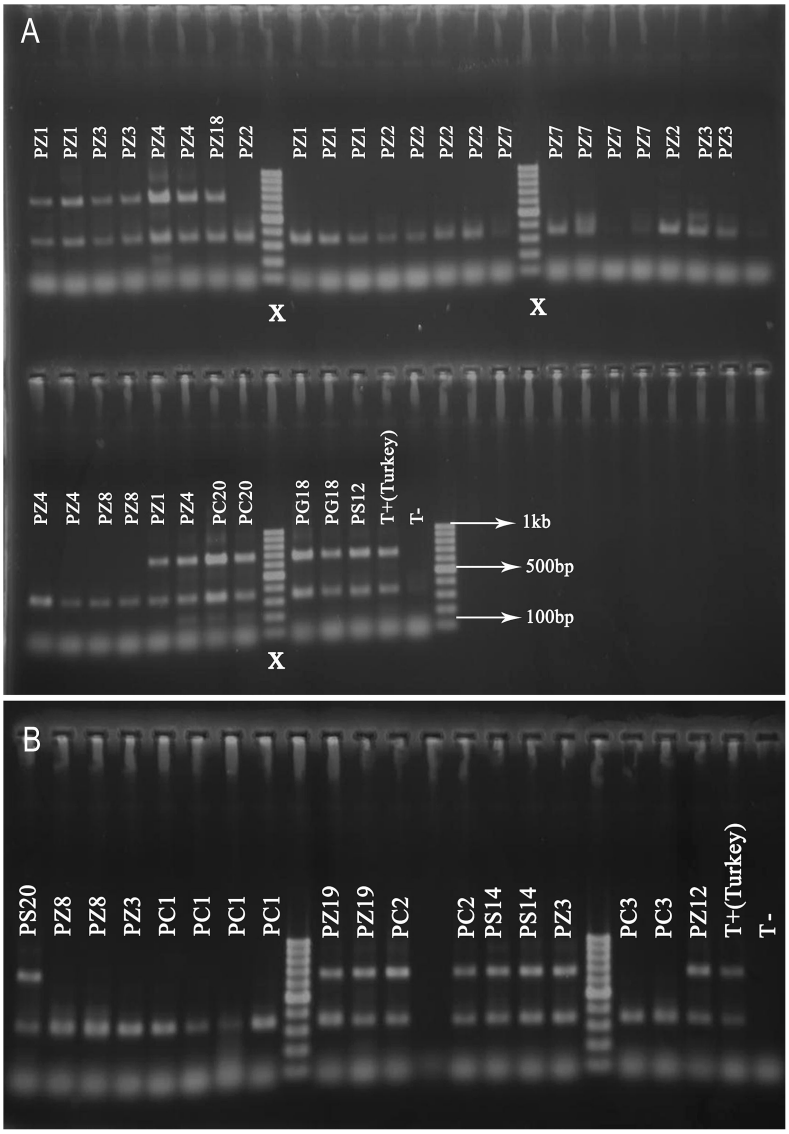

Fig. 2. A, B: Result of *Pratylenchus penetrans* species-specific duplex PCR (Waeyenberge *et al*., 2009) using all DNA extracts mentioned in Table 1. X = 100 bp DNA ladder (Promega Benelux); T−: negative control; T+: positive control.

and males (when present). Variability in morphology is frequently observed among species of the genus *Pratylenchus* (*e.g.*, Doucet *et al*., 2001; de la Peña *et al*., 2007; Troccoli *et al*., 2008). However, individual species subjected to varying environmental conditions and different host plants also differ in many morphometric relationships (Román & Hirschmann, 1969).

The CDA of morphometric data revealed the most important characteristics to discriminate *P. penetrans*, *P. thornei* and *P. pseudocoffeae*. Seven female characters allowed discrimination of these three species, whereas four male characters separated *P. penetrans* from *P. pseudocoffeae* (no males for *P. thornei*). Body diam. and distance from anterior end to excretory pore were the most important discriminating characters for the females and males. CDA has been used successfully to analyse morphometric data of *Pratylenchus* spp. Tuyet *et al*. (2013) reported that CDA enabled separation of ten populations of *P. coffeae* from Vietnam into three groups. The body length, b-ratio and distance from

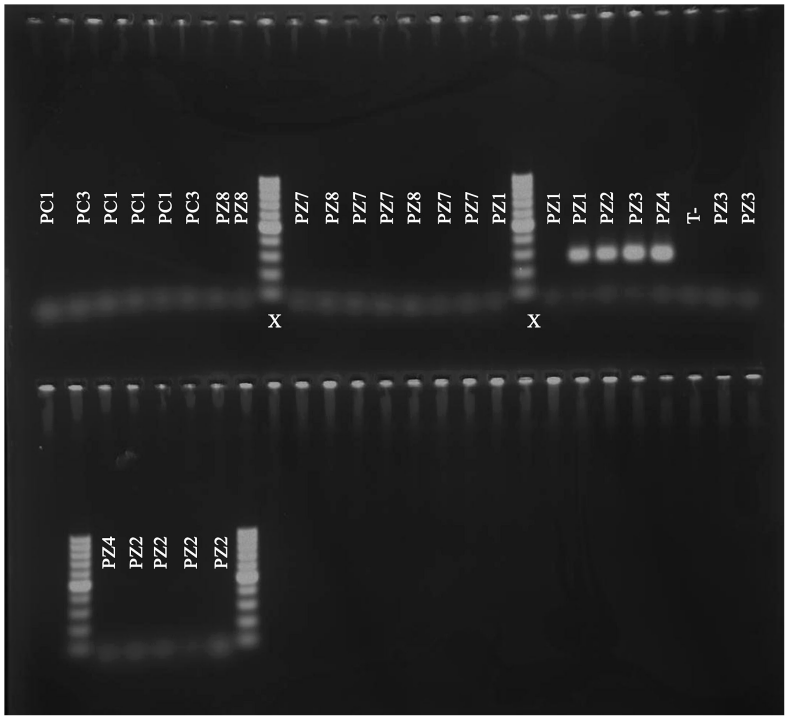

Fig. 3. *Pratylenchus thornei* species-specific primers PTHO/D3B (Al-Banna *et al*., 2004) using DNA from 29 DNA extracts. X = 100 bp DNA ladder; T−: negative control.

anterior end to the excretory pore were the most important characters of males to distinguish the populations in these groups. Unlike the findings of Tuyet *et al*. (2013), the Moroccan populations of *P. penetrans* did not segregate into groups.

Species-specific primers for PCR have been developed to complement the traditional species identification of *P. penetrans* (Uehara *et al*., 1998; Waeyenberge *et al*., 2009), *P. thornei* (Al-Banna *et al*., 2004; Troccoli *et al*., 2008) and *P. neglectus* (Yan *et al*., 2008). Waeyenberge *et al*. (2009) demonstrated that the primers were able to detect a very large number of isolates of *P. penetrans* originating from all over the world. With the exception of two DNA extracts from the Chaouia region of Morocco (PC3), the PCR confirmed the morphological identification of 12 of our populations as *P. penetrans*. The non-identification of the two individuals of PC3 might have been caused by a polymorphism at the binding site of the primers. Waeyenberge *et al*. (2009) commented on problems associated with species-specific primers, such as variation between sequences of isolates from different geographical origins. Sogut & Devran (2011) showed that the duplex PCR (PpenA, AB28/D3A, D3B) developed by Waeyenberge *et al*. (2009) did not always yield the two expected amplification products yet they demonstrated that the single primer pair (PpenA/AB28) successfully identified *P. penetrans*. When using the species-specific primers developed for *P. thornei* (Al-Banna *et al*., 2004), we obtained the characteristic band of 288 bp for four populations, confirming their morphological identification. The same primer set was successfully used in other research (Sogut & Devran, 2011; Fayazi *et al*., 2012) and seems to be universal.

During the last two decades, sequences of the D2-D3 region have been commonly used to separate nematodes at species level, including pratylenchids (*e.g.*, Duncan *et al*., 1999; Carta *et al*., 2001; De Luca *et al*., 2004; de la Peña *et al*., 2007; Inserra *et al*., 2007; Subbotin *et al*., 2008). Previous studies (Duncan *et al*., 1999; Al-Banna *et al*., 2004; Waeyenberge *et al*., 2009) demonstrated that this region is stable in length (303 bp) within *Pratylenchus* and is therefore a suitable region for studying the phylogeny of the genus. The number of clades and their composition vary with both the gene(s) and the number of *Pratylenchus* spp. studied (Carta *et al*., 2001; De Luca *et al*., 2004; de la Peña *et al*., 2007; Subbotin *et al*., 2008). The D2-D3 sequences

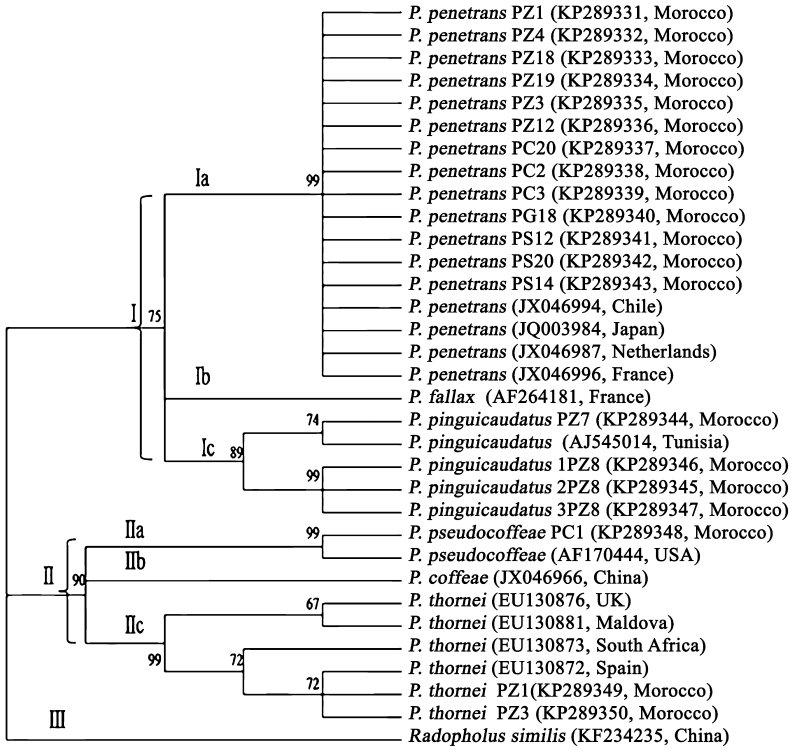

Fig. 4. Maximum likelihood tree (60% majority rule) obtained from the analysis of the alignment of 20 sequences of *Pratylenchus penetrans*, *P. thornei*, *P. pseudocoffeae* and *P. pinguicaudatus* with addition of *Pratylenchus* sequences obtained from GenBank.

Table 7. Pairwise distances between 13 *Pratylenchus penetrans* populations from Morocco, based on D2-D3 28S rDNA expansion segments sequences.

Population		% similarity												
		1	2	3	4	5	6	7	8	9	10	11	12	13
PZ1	1		100	99.8	100	100	99.8	100	100	99.8	100	99.8	100	99.8
PZ4	2			99.8	100	100	99.8	100	100	99.8	100	99.8	100	99.8
PZ18	3				99.8	99.8	99.7	99.8	99.8	99.8	99.8	99.8	99.8	99.8
PZ19	4					100	99.8	100	100	99.8	100	99.8	100	99.8
PZ3	5						99.8	100	100	99.8	100	99.8	100	99.8
PZ12	6							99.8	99.8	99.7	99.8	99.7	99.8	99.7
PC20	7								100	99.8	100	99.8	100	99.8
PC2	8									99.8	100	99.8	100	99.8
PC3	9										99.8	100	99.8	100
PG8	10											99.8	100	99.8
PS12	11												99.8	100
PS20	12													99.8
PS14	13													

For codes, see Table 1.

Table 8. Effect of time and temperature on the *in vitro* reproduction (R_f) on carrot disks of six *Pratylenchus* populations belonging to different species from Morocco (n = 20).

T (°C)	R_f					
	PZ3	PC2	PG18	PS20	PZ2	PC1
4 weeks						
10	0.5a*	0.8a	0.5b	0.5b	0.4b	0.6b
15	3.7a	3.4a	3.9a	4.1a	3.6a	3.8a
20	6.5ab	5.7c	6.8a	6.1bc	3.6e	4.7d
25	10.4a	8.7b	10.9a	9b	5.8c	8b
8 weeks						
10	1.3b	1.4b	1.6b	1b	1.2b	3.2a
15	41.8a	41.1a	44.7a	39.9a	39.8a	27.1b
20	350cd	413.5ab	458.6a	394.4bc	310.2d	252.3e
25	68.6bc	80.8b	77.9b	64.9c	70.7bc	217.3a
12 weeks						
10	3.7a	3.1ab	2.8b	2.7b	3b	2.9b
15	45.5b	51.3a	39.2c	35c	26.9d	21.7d
20	121b	112.1bc	115.2b	118.5b	103.7c	146.5a
25	25.8c	38.3c	23.8c	21.8c	68b	111a

Carrot disks inoculated with 20 females and incubated at 10, 15, 20 or 25°C. Observations made 4, 8 or 12 weeks after inoculation. For codes, see Table 1.

* Means in rows at 4, 8 and 12 weeks after inoculation followed by the same letter do not differ significantly according to Tukey's test ($P < 0.05$).

of the Moroccan pratylenchids showed a high interspecific difference. *Pratylenchus penetrans* and *P. pinguicaudatus* were the closest species with 9.1% nucleotide divergence for the compared sequences. However, *P. penetrans* and *P. pseudocoffeae* showed a divergence of 25.4%. In the phylogenetic tree inferred from the D2-D3 region, the Moroccan populations of *P. penetrans* are localised in a large clade with *P. fallax* and *P. pinguicaudatus*, while *P. pseudocoffeae* is grouped with *P. thornei*. The topology of the consensus tree obtained in this study is relatively similar to the one obtained by Subbotin *et al*. (2008). Previous research using the D2-D3 region or the 18S rDNA gene as molecular marker (Subbotin *et al*., 2008; De Luca *et al*., 2011), always grouped *P. penetrans* with *P. pinguicaudatus* and *P. fallax*. However, Carta *et al*. (2001), when using the ITS region as marker, showed *P. fallax* to be close to *P. penetrans*, thereby confirming that *P. fallax* and *P. penetrans* are two different species, as suggested by previous studies (Perry *et al*., 1980; Ibrahim *et al*., 1994, Waeyenberge *et al*., 2000). A similar grouping was observed by De Luca *et al*. (2004) using D2-D3 sequences. The clustering of *P. penetrans* with *P. pinguicaudatus* indicates a close phylogenetic relationship. The D2-D3 sequences obtained for the two populations of *P. thornei* (PZ3 and PZ4) were identical to each other and also to a sequence in GenBank from a *P. thornei* population from Spain (EU130873). Similar results were obtained when Subbotin *et al*. (2008) compared five *P. thornei* populations from different countries in the Mediterranean area (Italy, Morocco, Tunisia and Spain).

At intraspecific level the D2-D3 sequences showed very low diversity (*P. penetrans*: 0-0.3%, *P. thornei*: 0-0.9%, *P. pinguicaudatus*: 0-4.3%, *P. pseudocoffeae*: 0.3%). Earlier, de la Peña *et al*. (2007) reported similar low divergences of D2-D3 sequences of *P. pratensis* (0-1.4%) and *P. dunensis* de la Peña, Moens, van Aelst & Karssen (1.7%). Clearly, subspecific divergence is common in pratylenchids. The relatively greater differences obtained for *P. pinguicaudatus* are difficult to explain. However, the absence of different geographical isolates of this species, and of additional sequences in the database, does not allow any conclusion concerning these two sequences.

The multiplication of a selected number of six populations (*P. penetrans*: PZ3, PC2, PG18 and PS20, *P. thornei*: PZ2, and *P. pseudocoffeae*: PC1) was clearly influenced by the population, the temperature and the incubation time. The significant interaction between these three pa-

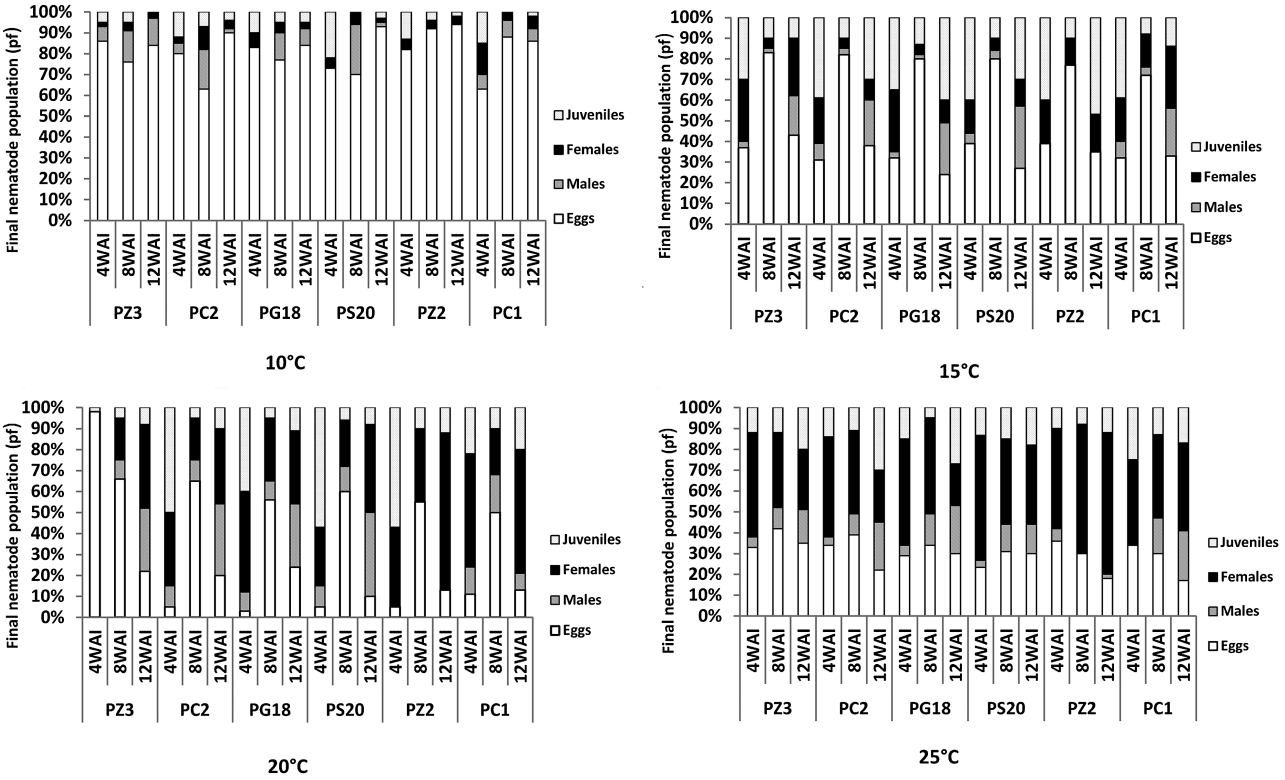

Fig. 5. Effect of time and temperature on the *in vitro* population composition on carrot disks of six *Pratylenchus* populations belonging to different species (n = 20). Carrot disks inoculated with 20 females and incubated at 10, 15, 20 or 25°C. Observations were made 4, 8 or 12 weeks after inoculation. For codes see Table 1.

rameters indicated that the populations did not increase in a similar way with temperature and time. When kept at 10°C for 4 weeks, none of the populations was able to increase in number. Obviously, 10°C is close to the lower limit of the temperature range allowing multiplication of Moroccan *Pratylenchus* spp. Kodira & Ferris (1992) showed that *P. neglectus* reproduced at 10°C on excised barley roots in Petri dishes, but data on multiplication of *Pratylenchus* populations in carrot disks at this temperature are not available. Next to the availability of suitable host plants, the geographic distribution of pratylenchids is related to their temperature requirements (Castillo & Vovlas, 2007). In Morocco, the minimum soil temperature throughout the cereal production cycle (November to June) varies in the four regions surveyed. In Chaouia, the minimum temperatures range from 5 (January) to 20°C (at the end of winter) (Balaghi *et al*., 2013). This range is favourable for the development of RLN as we observed an increase in *Pratylenchus* populations on carrot disks 8 WAI at 10°C and even earlier at 15 and 20°C. Penetration of nematodes into the root system, which can seriously affect plant growth, is expected at tillering, which occurs from February onwards when temperatures are above 10°C. (Meskine & Abbad Andaloussi, 1992). In the Zaers and Gharb regions, minimum temperatures are above 10°C during the elongation stage which allows nematode penetration during this period. However, in the Saiss region, minimum temperatures are close to 0°C, but temperatures exceed 10°C by the end of March, corresponding to the ear emergence stage, thereby promoting nematode penetration of plant roots. Consequently, wheat production is more prone to RLN damage in Chaouia.

Amongst the temperatures studied, 20°C is clearly the optimum. In general, this value is in agreement with studies on the effects of temperature on the reproduction of several *Pratylenchus* species reported by several authors (Ascota & Malek, 1979; Castillo *et al*., 1995, 1996a, b; Thompson *et al*., 2015). As several studies have showed a relationship between reproductive fitness and pathogenic-

ity (Sarah *et al*., 1993; Fallas *et al*., 1995; Trinh *et al*., 2011), we can assume that at temperatures where high reproduction on carrot disks was noted damage to cereal crops can be expected. In Morocco, temperatures around 20°C coincide with the elongation and heading stages of the wheat crop (Balaghi *et al*., 2013). Lesion nematode attacks during this period affect both the vegetative growth and the grain filling and can result in a significant reduction in grain production quantity and quality.

In our study, all three species of *Pratylenchus* multiplied very well on carrot disks at 20°C. However, the total numbers recovered per carrot disk varied with the species and population. Intraspecific differences in reproductive fitness of the four populations of *P. penetrans* were noticed at different temperatures. This difference among *P. penetrans* populations may be due to adaptation of the population to its environment (*e.g.*, climate, temperature, soil type) (Dao, 1970; Moens & Perry, 2009).

Fewer nematodes were retrieved 12 WAI than 8 WAI at 20 and 25°C in all six populations. This decrease in nematodes is probably caused by exhaustion of the food source. Such a decline has been observed in other carrot disk experiments and is influenced by the initial nematode population density, temperature and duration of the experiment (*e.g.*, Stoffelen *et al*., 1999; Tuyet *et al*., 2013). At 20 and 25°C we noticed some deterioration of the carrot tissues 12 WAI, as well as nematodes migrating away from the carrot disks. This suggests that the high nematode densities resulted in the breakdown of carrot tissue as many plant cells were punctured by nematodes. The effect of temperature on the proportion of the different developmental stages was remarkable. At 10°C, the nematode community on the carrot disks was mainly composed of eggs, even at 12 WAI. As the temperature increased, the prominent presence of eggs was much reduced in favour of juveniles, females and males (but not for PZ2 as the taxon has no males). Based on the high number of females in all populations at higher temperatures, the life cycle of most individuals was probably not completed at 10°C. However, at 20 and 25°C, the life cycle was already completed within 4 WAI, whilst egg laying had resumed. A similar life cycle duration at 20-25°C has been reported for *P. thornei* from Spain by Castillo *et al*. (1995). No males were observed for *P. thornei* (PZ2). Absence of males in populations of *P. thornei* has been reported previously (Pinochet *et al*., 1992; Castillo *et al*., 1995, 1998).

The results presented here are the first providing integrated morphometric, morphological and molecular characterisation of RLN populations from Morocco. Observations of nematode reproduction in carrot disks further showed clear differences between species and populations. These results provide basic information to develop a research program that aims at establishing a control strategy against lesion nematodes. Additional experiments on nematode multiplication on wheat are necessary to confirm the observations in the laboratory on carrot disks and the pathogenicity of *P. penetrans* and *P. thornei* on wheat needs to be examined.

Acknowledgements

The first author thanks the awarding of a Ph.D. scholarship from the Islamic Development Bank (IDB). The authors wish to thank warmly Ms Nancy de Sutter for maintaining the *Pratylenchus* populations and helping with laboratory work. The reviewers are thanked for their constructive suggestions to improve the quality of the manuscript.

References

Al-Banna, L., Ploeg, A.T., Williamson, V.M. & Kaloshian, I. (2004). Discrimination of six *Pratylenchus* species using PCR and species-specific primers. *Journal of Nematology* 36, 142-146.

Ammati, M. (1987). Nematode status on food legumes in Morocco. In: Saxena, M.C., Sikora, R.A. & Srivastava, J.P. (Eds). *Nematodes parasitic to cereals and legumes in temperate semi-arid regions*. Aleppo, Syria, International Centre for Agricultural Research in the Dry Areas (ICARDA), pp. 169-172.

Anonymous (2013). Moroccan Ministry of Agriculture, report no. MO1303.7.

Ascota, N. & Malek, R.B. (1979). Influence of temperature on population development of eight species of *Pratylenchus* on soybean. *Journal of Nematology* 11, 229-232.

Balaghi, R., Jliben, M., Tychon, B. & Eerens, H. (2013). *Agro-meteorological cereal yield forecasting in Morocco*. Rabat, Morocco, INRA.

Carta, L.K., Skantar, A.M. & Handoo, Z.A. (2001). Molecular, morphological and thermal characters of 19 *Pratylenchus* spp. and relatives using the D3 segment of the nuclear LSU rRNA gene. *Nematropica* 31, 193-207.

Castillo, P. & Vovlas, N. (2007). Pratylenchus *(Nematoda: Pratylenchidae): diagnosis, biology, pathogenicity and management*. *Nematology Monographs and Perspectives 6* (Series Editors: Hunt, D.J. & Perry, R.N.). Leiden, The Netherlands, Brill.

Castillo, P., Jimenez Diaz, R.M., Gomez-Barcina, A. & Vovlas, N. (1995). Parasitism of the root-lesion nematode *Pratylenchus thornei* on chickpea. *Plant Pathology* 44, 728-733.

Castillo, P., Gomez-Barcina, A. & Jimenez-Diaz, R.M. (1996a). Plant parasitic nematodes associated with chickpea in southern Spain and effect of soil temperature on reproduction of *Pratylenchus thornei*. *Nematologica* 42, 211-219.

Castillo, P., Gomez-Barcina, A. & Jimenez-Diaz, R.M. (1996b). The effect of temperature on hatching and penetration of chickpea roots by *Pratylenchus thornei*. *Plant Pathology* 45, 310-315.

Castillo, P., Vovlas, N. & Jimenez-Diaz, R.M. (1998). Pathogenicity and histopathology of *Pratylenchus thornei* populations on selected chickpea genotypes. *Plant Pathology* 47, 370-376.

Dao, D.F. (1970). Climatic influence of temperature on distribution pattern of plant parasitic and soil inhabiting nematodes. *Mededelingen van de Landbouwhogeschool, Wageningen* 70, 187.

de la Peña, E., Moens, M., Van Aelst, A. & Karssen, G. (2006). Description of *Pratylenchus dunensis* sp. n. (Nematoda: Pratylenchidae) a root-lesion nematode associated with the dune grass *Ammophila arenaria*. *Nematology* 8, 79-88.

de la Peña, E., Karssen, G. & Moens, M. (2007). Distribution and diversity of root-lesion nematodes (*Pratylenchus* spp.) associated with *Ammophila arenaria* in coastal dunes of western Europe. *Nematology* 9, 881-901.

De Ley, P., Felix, M.A., Frisse, L.M., Nadler, S.A., Sternberg, P.W. & Thomas, W.K. (1999). Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology* 2, 591-612.

De Luca, F., Fanelli, E., Di Vito, M., Reyes, A. & De Giorgi, C. (2004). Comparison of the sequences of the D3 expansion of the 26S ribosomal genes reveals different degrees of heterogeneity in different populations and species of *Pratylenchus* from the Mediterranean region. *European Journal of Plant Pathology* 110, 949-957.

De Luca, F., Reyes, A., Troccoli, A. & Castillo, P. (2011). Molecular variability and phylogenetic relationships among different species and populations of *Pratylenchus* (Nematoda: Pratylenchidae) as inferred from the analysis of the ITS-rDNA. *European Journal of Plant Pathology* 130, 415-426.

Doucet, M.E., Lax, P., Di Rienzo, J.A., Pinochet, J. & Baujard, P. (2001). Temperature-induced morphological variability in an isolate of *Pratylenchus vulnus* Allen & Jensen, 1951 (Nematoda: Tylenchida). *Nematology* 3, 1-8.

Duncan, L.W., Inserra, R.N., Thomas, W.K., Dunn, D., Mustika, L., Frisse Mendes, M.L., Morris, K. & Kaplan, D.T. (1999). Molecular and morphological analysis of isolates of *Pratylenchus coffeae* and closely related species. *Nematropica* 29, 61-80.

Fallas, G.A., Sarah, J.L. & Fargette, M. (1995). Reproductive fitness and pathogenicity of eight *Radopholus similis* populations on banana plants (Musa AAA cv. Poyo). *Nematropica* 25, 135-141.

Fayazi, F., Farokh-Nejd, R., Ahmad, A.R. & Bahmani, Z. (2012). Molecular and morphometric identification of *P. thornei* and *P. neglectus* in southwest of Iran. *Plant Pathology and Microbiology* 3, 123.

Hafez, S.I., Al-Rehiayani, S., Thornton, M. & Sundararaj, P. (1999). Differentiation of two geographically isolated populations of *Pratylenchus neglectus* based on their parasitism of potato and interaction with *Verticillium dahlia*. *Nematropica* 29, 25-36.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis. *Nucleic Acids Symposium Series* 41, 95-98.

Hallman, J. & Viaene, N. (2013). PM 7/119(1) nematode extraction. *EPPO Bulletin* 43, 471-495.

Handoo, Z.A. & Golden, A.M. (1989). A key and diagnostic compendium to the species of the genus *Pratylenchus* Filipjev, 1936 (lesion nematodes). *Journal of Nematology* 21, 202-218.

Handoo, Z.A., Carta, L.K. & Skantar, A.M. (2001). Morphological and molecular characterisation of *Pratylenchus arlingtoni* n. sp., *P. convallariae* and *P. fallax* (Nematoda: Pratylenchidae). *Nematology* 3, 607-618.

Hendrickx, G. (1995). An automated apparatus for extracting free-living nematode stages from soil. *Nematologica* 41, 308.

Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T. & Holovachov, O. (2006). Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Biology and Evolution* 23, 1792-1800.

Hossain, A. & Teixeira Da Silva, J.A. (2012). Phenology, growth and yield of three wheat (*Triticum aestivum* L.) varieties as affected by high temperature stress. *Notulae Scientia Biologicae* 4, 97-109.

Ibrahim, S.K., Perry, R.N., Burrows, P.R. & Hooper, D.J. (1994). Differentiation of species and populations of *Aphelenchoides* and of *Ditylenchus angustus* using a fragment of ribosomal DNA. *Journal of Nematology* 26, 412-421.

Inserra, R.N., Troccoli, A., Gozel, U., Bernard, E.C., Dunn, D. & Duncan, L.W. (2007). *Pratylenchus hippeastri* n. sp. (Nematoda: Pratylenchidae) from amaryllis in Florida with notes on *P. scribneri* and *P. hexincisus*. *Nematology* 9, 25-42.

Jones, J.T., Haegeman, A., Danchin, E.G.J., Gaur, H.S., Helder, J., Jones, M.G.K., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J., Wesemael, W.M.L. *et al.* (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology* 14, 946-961.

Kodira, C.U. & Ferris, H. (1992). Effect of temperature on *Pratylenchus neglectus* and on its pathogenicity to barley. *Journal of Nematology* 24, 504-511.

Lasserre, F., Rivoal, R. & Cook, R. (1994). Interactions between *Heterodera avenae* and *Pratylenchus neglectus* on wheat. *Journal of Nematology* 26, 336-344.

Loof, P.A.A. (1960). Taxonomic studies on the genus *Pratylenchus* (Nematoda: Pratylenchidae). *Tijdschrift voor Plantenziekten* 66, 29-90.

Loof, P.A.A. (1991). The family Pratylenchidae Thorne, 1949. In: Nickle, W.R. (Ed.). *Manual of agricultural nematology*. New York, NY, USA, Marcel Dekker, pp. 126-134.

Meskine, M. & Abbad Andaloussi, F. (1992). Importance et répartition des nématodes phytoparasites associés aux cultures de blé et orge au Maroc. *Al Awamia* 80, 123-134.

Mizukubo, T. (1992). *Pratylenchus pseudocoffeae* n. sp. (Nematoda: Pratylenchidae) from compositae plants in Japan. *Applied Entomology and Zoology* 27, 437-444.

Mizukubo, T. & Sano, Z. (1997). *Pratylenchus coffeae* virulent races on sweet potato. *Research Front* 4, 2.

Mizukubo, T., Oriu, Y., Hanada, K. & Sano, Z. (2003). Micro evolutionary trend in *Pratylenchus coffeae* sensu stricto (Nematoda: Pratylenchidae): the diversity in PCR-RFLP phenotype, compatibility on host plants and reproductive segregation. *Japanese Journal of Nematology* 33, 57-76.

Moens, M. & Perry, R.N. (2009). Migratory plant endoparasitic nematodes: a group rich in contrasts and divergence. *Annual Review of Phytopathology* 47, 313-332.

Mokrini, F., Abbad Andaloussi, F., Alaoui, Y. & Troccoli, A. (2009). Importance and distribution of the main cereal nematodes in Morocco. In: *Proceedings of the first workshop of the International Cereal Cyst Nematode Initiative, 21-23 October 2009, Antalya, Turkey*. Ankara, Turkey, CIMMYT, pp. 45-50.

Mokrini, F., Waeyenberge, L., Viaene, N. & Moens, M. (2012). Occurrence of nematodes of the *Heterodera avenae* group and *Pratylenchus* spp. on wheat and barley in Morocco. In: *31st international symposium of the European Society of Nematologists, 23-27 September 2012, Adana, Turkey*, p. 107. [Abstr.]

Moody, E.H., Lownsbery, B.F. & Ahmed, J.M. (1973). Culture of the root-lesion nematode *Pratylenchus vulnus* on carrot disks. *Journal of Nematology* 19, 125-126.

Netscher, C. & Seinhorst, J.W. (1969). Propionic acid better than acetic acid for killing nematodes. *Nematologica* 15, 286.

Nicol, J.M. (2002). Important nematode pests. In: Curtis, B.C., Rajaram, S. & Macpherson, H.G. (Eds). *Bread wheat improvement and production*. *Plant Production and Protection Series 30*. Rome, Italy, FAO, pp. 345-366.

Nicol, J.M. & Rivoal, R. (2008). Global knowledge and its application for the integrated control and management of nematodes on wheat. In: Ciancio, A. & Mukerji, K.G. (Eds). *Integrated management and biocontrol of vegetable and grain crops nematodes*, *Vol. 2*. Dordrecht, The Netherlands, Springer, pp. 243-287.

Nicol, J., Rivoal, R., Taylor, S. & Zaharieva, M. (2003). Global importance of cyst (*Heterodera* spp.) and lesion nematodes (*Pratylenchus* spp.) on cereals: distribution, yield loss, use of host resistance and integration of molecular tools. In: Cook, R. & Hunt, D.J. (Eds). *Proceedings of the fourth international congress of nematology, 8-13 June 2002, Tenerife, Spain*. *Nematology Monographs and Perspectives 2* (Series Editors: Cook, R. & Hunt, D.J.). Leiden, The Netherlands, Brill, pp. 1-19.

Nicol, J.M., Turner, S.J., Coyne, D.L., Nijs, L.D., Hockland, S. & Maafi, Z.T. (2011). Current nematode threats to world agriculture. In: Jones, J., Gheysen, G. & Fennol, C. (Eds). *Genomics and molecular genetics of plant-nematode interactions*. Dordrecht, The Netherlands, Springer, pp. 21-43.

Orui, Y. (1996). Discrimination of the main *Pratylenchus* species (Nematoda: Pratylenchidae) in Japan by PCR-RFLP analysis. *Applied Entomology and Zoology* 31, 505-514.

Perry, R.N., Plowright, R.A. & Webb, R.M. (1980). Mating between *Pratylenchus penetrans* and *P. fallax* in sterile culture. *Nematologica* 26, 125-129.

Pinochet, J., Verdejo, A., Soler, S. & Canals, J. (1992). Host range of a population of *Pratylenchus vulnus* in commercial fruit, nut, citrus, and grape rootstocks in Spain. *Journal of Nematology* 24, 693-698.

Pinochet, J., Fernandez, C., Esmenjaud, P. & Doucet, M.E. (1993). Effects of six *Pratylenchus vulnus* isolates on the growth of peach-almond and apple rootstocks. *Journal of Nematology* 25, 843-861.

Pourjam, E., Kheiri, A. & Geraert, E. (1997). The genus *Pratylenchus* Filip'jev, 1936 (Tylenchida: Pratylenchidae) from north of Iran. *Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent* 62, 741-756.

Rivoal, R. & Cook, R. (1993). Nematode pests of cereals. In: Evans, K., Trudgill, D.L. & Webster, J.M. (Eds). *Plant parasitic nematodes in temperate agriculture*. Wallingford, UK, CABI Publishing, pp. 259-303.

Román, J. & Hirschmann, H. (1969). Morphology and morphometrics of six species of *Pratylenchus*. *Journal of Nematology* 1, 363-386.

Ryss, A.Y. (1988). *World fauna of the root parasitic nematodes of the family Pratylenchidae (Tylenchida)*. Leningrad, USSR, Nauka.

Sarah, J.-L., Sabatini, C. & Boisseau, M. (1993). Differences in pathogenicity to banana (*Musa* sp., cv. Poyo) among populations of *Radopholus similis* from different production areas of the world. *Nematropica* 23, 75-79.

Seinhorst, J.W. (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* 4, 67-69.

Shaner, G., Stromberg, E.L. & Lacy, G.H. (1992). Nomenclature and concepts of pathogenicity and virulence. *Annual Review of Phytopathology* 30, 47-66.

Sher, S.A. & Allen, M.W. (1953). Revision of the genus *Pratylenchus* (Nematoda: Tylenchidae). *University of California Publications in Zoology* 57, 441-447.

Smiley, R.W., Merrifield, K., Patterson, L.M., Whittaker, R.G., Gourlie, J.A. & Easley, S.A. (2004). Nematodes in dryland field crops in the semiarid Pacific Northwest United States. *Journal of Nematology* 36, 54-68.

Sogut, M.A. & Devran, Z. (2011). Distribution and molecular identification of root-lesion nematodes in temperature fruit orchards of Turkey. *Nematropica* 41, 91-98.

Stoffelen, R., Jimenez, M.I., Dierckxsens, C., Tam, V.T.T., Swennen, R. & De Waele, D. (1999). Effect of time and inoculum density on the reproductive fitness of 138 *Pratylenchus coffeae* and *Radopholus similis* populations on carrot disks. *Nematology* 1, 243-250.

Subbotin, S.A., Ragsdale, E.J., Mullens, T., Roberts, P.A., Mundo-Ocampo, M. & Baldwin, J.G. (2008). A phylogenetic framework for root-lesion nematodes of the genus *Pratylenchus* (Nematoda): evidence from 18S and D2-D3 expansion segments of 28S ribosomal RNA genes and morphological characters. *Molecular Phylogenetics and Evolution* 48, 491-505.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolution genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28, 2731-2739.

Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 11, 4673-4680.

Thompson, J.P., Clewett, T.G. & O'Reilly, M.M. (1993). Tolerance and resistance in wheat to *Pratylenchus thornei*. In: Pratylenchus *workshop, 9th biennial conference of the Australasian Plant Pathology Society, July 1993, Hobart, Tasmania*, pp. 43-48.

Thompson, J.P., Owen, K.J., Stirling, G.R. & Bell, M.J. (2008). Root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*): a review of recent progress in managing a significant pest of grain crops in northern Australia. *Australasian Plant Pathology* 37, 235-242.

Thompson, J.P., Clewett, T.G. & O'Reilly, M.M. (2015). Temperature response of root-lesion nematode (*Pratylenchus thornei*) reproduction on wheat cultivars has implications for resistance screening and wheat production. *Annals of Applied Biology* 167, 1-10.

Townshend, J.L. (1991). Morphological observations of *Pratylenchus penetrans* from celery and strawberry in southern Ontario. *Journal of Nematology* 23, 205-209.

Trinh, P.Q., Wesemael, W.M.L., Nguyen, S.T.T., Nguyen, C.N. & Moens, M. (2011). Pathogenicity and reproductive fitness of *Pratylenchus coffeae* and *Radopholus arabocoffeae* on arabica coffee seedlings (*Coffea arabica* vs. Catimor) in Vietnam. *European Journal of Plant Pathology* 130, 45-57.

Troccoli, A., De Luca, F., Handoo, Z.A. & Di Vito, M. (2008). Morphological and molecular characterization of *Pratylenchus lentis* n. sp. (Nematoda: Pratylenchidae) from Sicily. *Journal of Nematology* 40, 190-196.

Tuyet, N.T., Elsen, A., Nhi, H.H. & De Waele, D. (2013). Effect of temperature on the in vitro reproductive fitness of *Pratylenchus coffeae* populations from Vietnam. *Archives of Phytopathology and Plant Protection* 46, 1-13.

Uehara, T.M., Mizukubo, T., Kushida, A. & Momota, Y. (1998). Identification of *P. coffeae* and *P. loosi* using specific primers for PCR amplification of ribosomal DNA. *Nematologica* 44, 357-368.

Waeyenberge, L., Ryss, A., Moens, M., Pinochet, J. & Vrain, T.C. (2000). Molecular characterisation of 18 *Pratylenchus* species using ribosomal DNA restriction fragment length polymorphism. *Nematology* 2, 135-142.

Waeyenberge, L., Viaene, N. & Moens, M. (2009). Species-specific duplex PCR for the detection of *Pratylenchus penetrans*. *Nematology* 11, 847-857.

Yan, G., Smiley, R.W., Okubara, P.A., Skantar, A., Easley, S.A., Sheedy, J.G. & Thompson, A.L. (2008). Detection and discrimination of *Pratylenchus neglectus* and *P. thornei* in DNA extracts from soil. *Plant Disease* 92, 1480-1487.