Morphological and molecular characterisation of Pratylenchus arlingtoni n. sp., P. convallariae and P. fallax (Nematoda: Pratylenchidae)

Article *in* Nematology · December 2001

DOI: 10.1163/156854101753389220 · Source: OAI

3 authors:

Zafar Handoo
United States Department of Agriculture
243 PUBLICATIONS **2,791** CITATIONS
SEE PROFILE

Lynn Carta
180 PUBLICATIONS **2,561** CITATIONS
SEE PROFILE

Andrea Skantar
United States Department of Agriculture
171 PUBLICATIONS **1,634** CITATIONS
SEE PROFILE

Nematology, 2001, Vol. 3(6), 607-618

Morphological and molecular characterisation of *Pratylenchus arlingtoni* n. sp., *P. convallariae* and *P. fallax* (Nematoda: Pratylenchidae)

Zafar A. HANDOO, Lynn K. CARTA * and Andrea M. SKANTAR

United States Department of Agriculture, ARS, Nematology Laboratory, Beltsville, MD 20705, USA

Received: 17 October 2000; revised: 19 June 2001
Accepted for publication: 23 July 2001

Summary – *Pratylenchus arlingtoni* n. sp. from the rhizosphere of grasses *Poa pratensis* and *Festuca arundinacea* at Arlington National Cemetery, VA, USA is characterised by six to eight lines in the lateral field, and pyriform to slightly overlapping pharyngeal glands. Morphological comparisons are made with lesion nematodes having similar morphometrics, six lateral lines, or crenate tail tips. Molecular sequences of the LS 28S rDNA were generated for the new species as well as *P. fallax* and *P. convallariae*. The new species differs by only 1% from identical sequences found in *P. fallax* and *P. convallariae*.

Keywords – *Festuca arundinacea*, lesion nematode, pathogenicity, *Poa pratensis*, *Pratylenchus crenatus*, quarantine.

During a search for nematodes in Arlington National Cemetery, Arlington, VA, USA, we discovered a new lesion nematode from soil around grass roots. This new species described hereunder as *Pratylenchus arlingtoni* n. sp. had some morphological similarities to crenate-tailed *Pratylenchus crenatus* Loof, 1960 and *Pratylenchus fallax* Seinhorst, 1968, two other lesion nematode species found in turf in North America and Europe (Yu *et al.*, 1998).

The purpose of this study was to morphologically and molecularly describe the new nematode and compare it with nematodes having similar characters, such as *P. fallax* and *P. convallariae* Seinhorst, 1959. One of these characters was the D3 region of the 28S rDNA, which encodes part of the Large Subunit (LS) rRNA (Baldwin *et al*., 1997) recently used to characterise other species of *Pratylenchus* and their phylogenetic relationships (Al-Banna *et al.,* 1997; Duncan *et al.*, 1999).

Materials and methods

MORPHOLOGICAL CHARACTERISATION

P. arlingtoni n. sp. was found in turf (*Poa pratensis* and *Festuca arundinacea*) soil from Arlington National Cemetery, Arlington, VA, USA, in November, 1999. *P. convallariae* and *P. fallax* were isolated from intercepted lily of the valley shipments (March, 1999 and January, 2000, respectively, from a company in Europe) after collection at JFK International Airport, Jamaica, New York by Bernice Medina and dispatched to us, for identification, by Alan Towson, USDA, Animal and Plant Health Inspection Service (APHIS).

Nematodes were extracted from soil with sieves or from chopped, infested roots over filter paper in Baermann funnels. Specimens were then handpicked and fixed in 3% formalin. Some specimens were studied in fixative on temporary slide mounts, others in permanent glycerine mounts (Golden, 1990), or viewed live, with or without 5 mM sodium azide for narcotisation on an agar pad (Stiernagle, 1999). Examinations were made with a compound light microscope and morphometric data obtained with an ocular micrometer. All measurements are in micrometers (μm) unless otherwise specified. Light microscopic images of live and fixed nematodes were taken with the Bioquant ver. 3.2 imaging system (Biometrics, Inc., Nashville, TN, USA) on a Leitz Ortholux microscope. Differential interference contrast (DIC) images of live nematodes were taken with an imaging system employing Image-Pro Plus ver 3.0 (I-Cube Image Analysis/Image Processing, Crofton, MD, USA) on a Zeiss Ultraphot II microscope equipped with DIC optics. Nema-

* Corresponding author, e-mail: cartal@ba.ars.usda.gov

todes were processed for scanning electron microscopy (SEM) in room temperature 0.05 phosphate-buffered (pH 6.8) 3% glutaraldehyde (12 h) and 2% osmium tetroxide (2 h) and viewed after ethanol dehydration, critical-point drying and gold-palladium coating on a Jeol JSM-T300 microscope at 20 kV.

Template preparation

Nematode extracts were prepared by the procedure of Williams *et al.* (1992). A single nematode was placed in 10 μl of digestion buffer (10 mM Tris pH 8.2; 2.5 mM $MgCl_2$; 50 mM KCl; 0.45% Tween 20; 0.05% gelatin; 60 μg/ml proteinase K) and frozen at -70°C for 15 min to several days. The extracts were thawed, overlaid with a drop of mineral oil, and warmed to 60°C for 1 h. Proteinase K was denatured by heating to 95°C for 15 min.

Amplification and sequencing

The D3 expansion region of 28S rDNA (345 nucleotide base pairs (bp) raw sequence) was amplified separately from two adult nematodes of each species, using hot-start reactions as described by Chou *et al.* (1992) with the following modifications. Manufacturer-supplied Display-TAQ buffer (PGC Scientific, Gaithersburg, MD, USA), 250 μM dNTPs, 4 mM $MgCl_2$, and 600 μM of each ribosomal DNA primer, originally designed by W. Kelley Thomas, University of Missouri, Kansas City, MO, USA. Primers D3A (5′-GACCCGTCTTGAAACACGGA-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (Baldwin *et al.*, 1997) were added to the bottom of 0.5 ml thin-wall microcentrifuge tubes. A drop (*ca* 25 μl) of paraffin wax was overlaid and allowed to cool, forming an even barrier. The remaining TAQ buffer, template and Display TAQ were then layered on top of the wax. Cycling conditions were: 94°C, 3 min (to allow hot start); 94°C, 1 min; 52°C, 1 min; 72°C, 1 min, ×35 cycles; 72°C, 10 min. Reactions were analysed by gel electrophoresis.

DNA sequences were obtained by sequencing PCR products directly or by sequencing cloned PCR products. For direct sequencing, whole nematode extracts (10 μl) were included in the PCR reactions to generate a sufficient amount of PCR product. Prior to sequencing, the DNA was purified using the Qiaquick PCR purification kit (Qiagen, Valencia, CA, USA). Sequencing reactions included 100 ng PCR product and 3.2 pmol D3A or D3B primer. To generate cloned PCR products for sequencing, 2 μl nematode extract was included in each PCR reaction. The resultant PCR products were cloned into the vector pCR2.1 using the Topo-TA Cloning kit (Invitrogen, Carlsbad, CA, USA). Plasmid DNA was purified from bacterial cultures using Wizard Preps (Promega, Madison, WI, USA). The sequencing reactions contained 200 ng plasmid template and the M13 forward or M13 reverse primers. All BigDye Terminator cycle sequencing was performed using an ABI 377 Sequencer (PE-Applied Biosystems, Foster City, CA, USA).

Negative controls included reactions with water or a mock extract (no nematode) instead of DNA. A reaction containing 5 ng *Meloidogyne javanica* genomic DNA was included as a positive control. To confirm the authenticity of the sequences obtained, PCR amplification and DNA sequencing were performed on two individuals from the same nematode population. To account for the possibility of PCR-generated errors in the cloned PCR products, we compared the sequences from two or more clones obtained from the same nematode extract. If ambiguities were detected between clones, sequences derived directly from PCR products were used to resolve the conflict.

The sequences for *P. arlingtoni* n. sp., *P. convallariae* and *P. fallax* have been deposited in the GenBank database (National Center for Biotechnology Information, National Library of Medicine, National Institute of Health, Bethesda, MD, USA, http://www.ncbi.nlm.nih.gov) as AF307328, AF196351, and AF264181, respectively.

Alignment

From the 345 bp of raw sequence, 305 bp were used in the final alignment. The new sequences were aligned with the Clustal W (ver 1.4) program (Clustal W, WWW Service at the European Bioinformatics Institute, Rodrigo Lopez, Services Programme, http://www2.ebi.ac.uk/clustalw; Thompson *et al.*, 1994) with all *Pratylenchus* sequences in the GenBank database. The closest sequence to the new species from the GenBank database is also shown in the alignment provided here (Fig. 5). Positions are numbered where 1 corresponds to number 3324 of the *Caenorhabditis elegans* 28S rRNA gene (Ellis *et al.*, 1986; Al-Banna *et al.*, 1997). The number and position of nucleotide differences among the four taxa were noted.

Table 1. *Morphometrics of* Pratylenchus arlingtoni *n. sp. females. (All measurements in μm.) Values are means ± standard deviation. Values in parentheses indicate ranges.*

$n = 20$	Holotype	Paratypes	
L	423	455 ± 37	(405 - 535)
a	24.8	27 ± 3	(21 - 33)
b	4.0	4.4 ± 0.4	(4.1 - 5.3)
c	20.6	22 ± 2	(18 - 28)
V	81.5	82.3 ± 1.2	(81 - 86)
Stylet length	17	16.8 ± 0.5	(16 - 17.5)
c′	2.0	1.3 ± 0.1	(1.1 - 1.5)
Tail annule number	21	20.9 ± 1.8	(19 - 25)
Lateral line number	–	–	(6 - 8) aerolate extremes
Post-uterine sac	25	19.4 ± 2.8	(15 - 25)
Vulval width	15	15.9 ± 1.1	(14 - 17.5)
Vulva-Anus distance	53	55.9 ± 7.1	(43 - 70)
Post-uterine sac/Vulval width	1.7	–	(1.5 - 2.5)
Post-uterine sac/Vulva-anus × 100	47	–	(28 - 42)
Pharyngeal overlap	–	20 ± 7.8	(6 - 31) pyriform to overlap
Males/spermatheca	–	Not found/oval	
Lip number, shape	–	3, slightly offset	
Excretory pore position	–	Pharyngeal-intestinal junction	
Excretory pore-lips	–	70 ± 10	(48 - 85)
Body diam.	15	17 ± 1.4	(15 - 20)
Pharynx length	–	101 ± 3.5	(93 - 107)
Tail length	–	21 ± 1.8	(15 - 23)
Stylet knob height	–	2.7 ± 0.3	(2.5 - 3.0)
Stylet knob width	–	4.6 ± 0.2	(4.5 - 5.0)
Stylet knob width/height	–	1.7 ± 0.1	(1.6 - 1.8)
Head diam.	–	8.1 + 0.4	(7.5 - 9.0)
Head height	–	2.8 ± 0.3	(2.5 - 3.0)
Head diam./height	–	2.8 ± 0.3	(2.6 - 3.2)

Pratylenchus arlingtoni* n. sp.

(Figs 1 - 3, 5)

Measurements

Females

See Table 1.

Description

Female

Body vermiform with some tapering at extremities. Lip region slightly offset, three annules. Head framework extending inward for two or three annules. Anterior body annules measuring 1.2 μm, tail annules measuring 0.9 μm. Stylet knobs broadly rounded, with anterior outer edges directed slightly forward. Dorsal pharyngeal gland opening at 2-3 μm behind the stylet knobs. Lateral field beginning behind the level of the stylet as four narrow crenate lines, widening to five by the level of the median bulb, and six to eight by the anterior intestine level through to the vulva. Four lines extending to the phasmid just posterior to the vulva, after which three lines extend a few annules short of the tail tip. Lateral field sometimes aerolated at the extremes. Pharyngeal glands pyriform in approximately 25% of the specimens or slightly overlapping in others. Excretory pore and canal located within an area slightly above or below the pharyngeal-intestinal junction. Vulva elevated, the slit extending inward about 70% of the body diam. Anterior gonad with a single row of oocytes and extending forward for nearly three times the distance between the vulva and anus. An egg (36-cell) within the body measuring 75 × 21 μm; another (4-cell) 64 × 14 μm.

* From Arlington, VA, USA, type locality.

Oval spermatheca, 17 μm in length with apparent sperm cells of 1.4 - 2.2 μm diam., 74 μm anterior to the vulva (one specimen out of at least 200). Distance from vulva to spermatheca, 120% of the distance between vulva and anus. Post-uterine sac generally undifferentiated, with a discernible tip cell or four columnar cells at the dorsal arch sometimes present. Phasmid located 10 to 15 annules above the tail tip. Tail terminus coarsely annulated, with variable shape from conoid, clavate to truncate, or sometimes bifid.

Male

Not found.

Type host and locality

Roots of turf (*Poa pratensis*, blue grass and *Festuca arundinacea*, tall fescue) under *Quercus* spp. (oaks) at Old Section 27, Arlington Cemetery, Arlington, VA, USA. This section of the cemetery was from the old Arlington Farm, uncultivated since the 1800s.

Type specimens

Holotype

Slide T-543p deposited in the United States Department of Agriculture Nematode Collection (USDANC), Beltsville, MD, USA.

Paratypes (females)

Same data and repository as holotype. Slides T-4918p-4921p with 15 mixed stage females and slides T-4922p-4924p with single adult females. Others deposited in the nematode collections at The University of California Davis, Davis, CA, USA; The Canadian Food Inspection Agency, Centre for Plant Quarantine Pests, Ontario, Canada; Rothamsted Experimental Station, Rothamsted, UK; Muséum National d'Histoire Naturelle, Laboratoire des Vers, Paris, France; Wageningen University and Research Centre, Landbouwhogeschool, Wageningen, The Netherlands, and the Zoological Institute, Russian Academy of Sciences, St Petersburg, Russia.

Diagnosis and relationships

P. arlingtoni n. sp. is unique among lesion nematode species in having six to eight lateral lines on specimens from the pharyngeal to the vulval region, and pharyngeal glands with a pyriform basal bulb to a shallow overlap. It also has a crenate tail tip, an elevated vulva, no observed males, and a very rare, oval spermatheca.

P. arlingtoni n. sp. shares a variably crenate tail with *P. cerealis* Haque, 1965, *P. convallariae* Seinhorst, 1959,

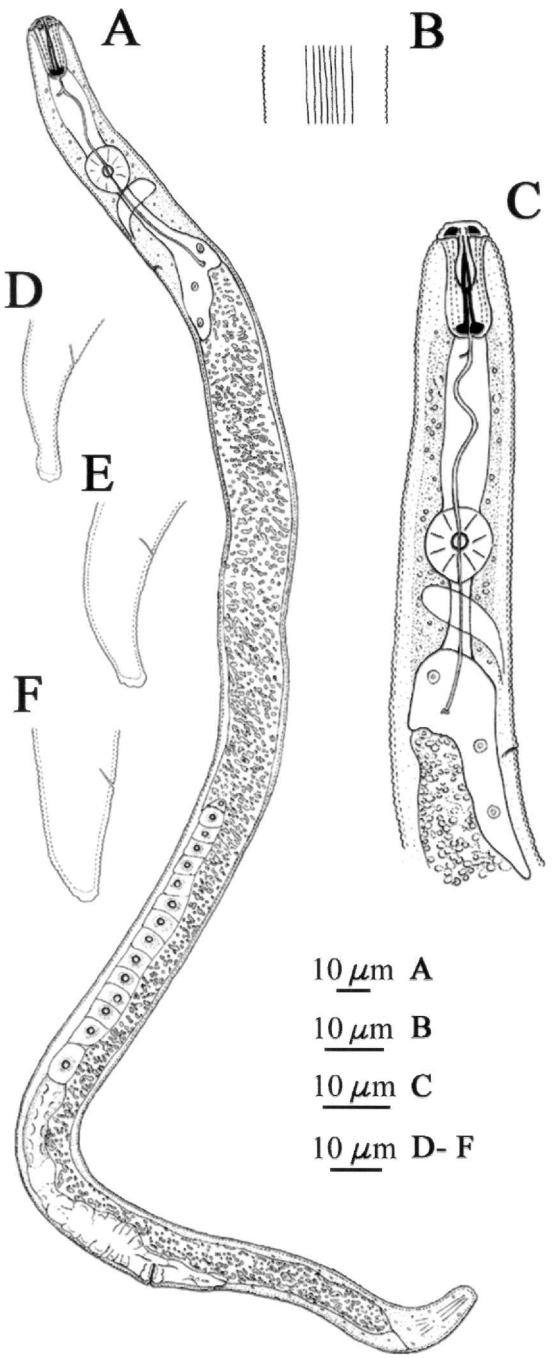

Fig. 1. Pratylenchus arlingtoni *n. sp. (lateral views) A: Body; B: Lateral field, midbody; C: Head and pharynx; D - F: Tail.*

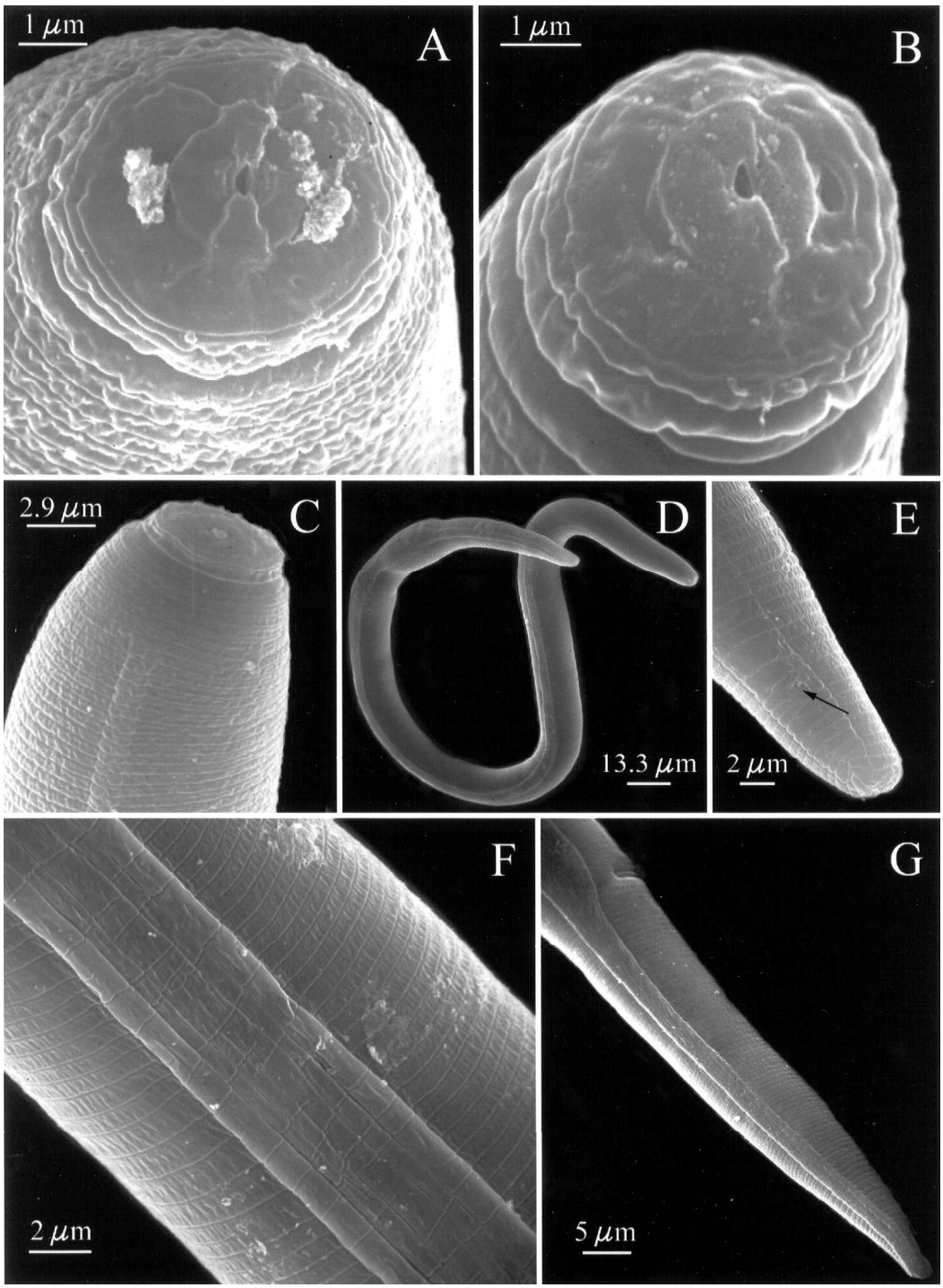

Fig. 2. Pratylenchus arlingtoni *n. sp. Scanning electron micrographs. A, B: Lip region (face view) with and without amphid secretion; C: Lip region (lateral view); D: Body, E: Tail and phasmid opening (arrow); F: Lateral field; G: Tail region (lateral view).*

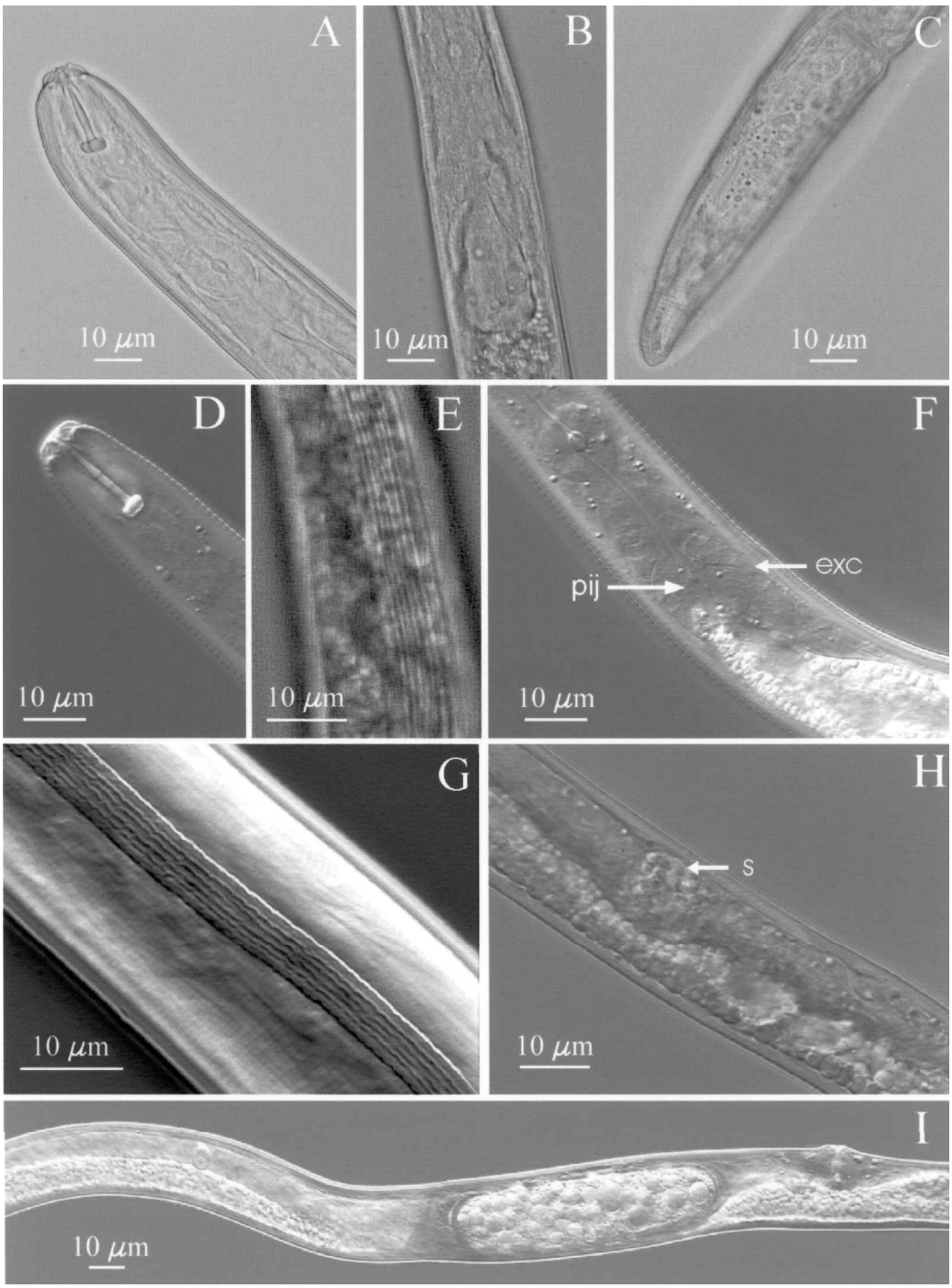

Fig. 3. Pratylenchus arlingtoni *n. sp. A: Head end (lateral) with median bulb, light microscopy (LM); B: Pharynx, pyriform basal bulb (ventrolateral view), LM; C: Female tail (lateral view), LM; D: Head (lateral view), Differential interference contrast (DIC); E: Lateral field, midbody LM; F: Pharyngeal-intestinal junction (pij) and excretory canal (exc) (lateral view), DIC; G: Lateral field, mid-body, DIC; H: Spermatheca with sperm (lateral view), DIC; I: Female gonad with vulva, egg, post-uterine sac (lateral view), DIC.*

P. crenatus Loof, 1960, *P. fallax* Seinhorst, 1968, *P. gibbicaudatus* Minagawa, 1982, *P. gutierrezi* Golden, López & Vílchez, 1992, *P. pratensis* (de Man, 1880) Filipjev, 1936, *P. pseudofallax* Café-Filho & Huang, 1989, *P. roseus* Zarina & Maqbool, 1998, *P. teres* Khan & Singh, 1974, and *P. yassini* Zeidan & Geraert, 1991.

P. crenatus, P. roseus and *P. teres* may also have up to six lateral lines. *P. arlingtoni* n. sp. is morphologically most similar to *P. crenatus,* but differs by longer pharynx (93 - 107 *vs* 57 - 78 μm), pyriform to slightly overlapping basal pharyngeal glands (6 - 31 *vs* 17 - 38 μm), somewhat lower b value (4.1 - 5.3 *vs* 4.9 - 7.0 μm in *P. crenatus*; Loof, 1960; Torres & Chaves, 1999; Urek, 1999), smaller post-uterine sac length relative to vulval-anal distance (PUS/VA $\times$ 100: 28 - 42 *vs* 40 - 50), lower c′ value (1.1 - 1.5 *vs* 1.6 - 2.9), more elevated vulva, and six to eight lateral lines compared to four to six lines. The excretory pore in *P. arlingtoni* n. sp. is often below the pharyngeal-intestinal junction (p-ij), and is above the p-ij in *P. crenatus. P. arlingtoni* n. sp. lacks a vulval membrane, has a longer stylet (16 - 17.5 *vs* 15 - 16 μm) and a shorter pharyngeal overlap (6 - 31 *vs* 118 - 130 μm) compared to *P. roseus. P. arlingtoni* n. sp. differs from *P. teres* by more posterior vulva (V = 81 - 86 *vs* 69 - 78), fewer tail annules (19 - 25 *vs* 24 - 30), shorter pharyngeal overlap (6 - 31 *vs* 58 μm), longer PUS/VA $\times$ 100 (28 - 42 *vs* 18) and six to eight lateral lines rather than only six.

When *P. arlingtoni* n. sp. is compared to both *P. convallariae* and *P. fallax,* it has a lower b value (4.1 - 5.3 *vs* 6.0 - 9.0 and 5.2 - 6.7, respectively), and more posterior vulva (V = 81 - 86 *vs* 78 - 81 and 77 - 81, respectively), lacks males and has two to four more lateral lines. *P. arlingtoni* n. sp. also has more tail annules (19 - 25 *vs* 16 - 19) than *P. convallariae.* The new species has a longer stylet (16.0 - 17.5 *vs* 15.0 - 15.5 μm) more offset lip region, and coarser tail annulation than *P. fallax.* Differences from *P. fallax* also apply to *P. pseudofallax* (Café-Filho & Huang, 1989). *P. arlingtoni* n. sp. differs from *P. penetrans* by having a crenate tail in all specimens, lack of observed males, lower b value (4.1 - 5.3 *vs* 5.3 - 7.9), shorter pharyngeal overlap (6 - 31 *vs* 32 - 65 μm), and longer PUS/VW (1.5 - 2.5 *vs* 1.0 - 1.5).

Observations on intercepted *Pratylenchus fallax* and *P. convallariae*

P. fallax is common in sandy or sandy-peat soils (Seinhorst, 1977) around grass and ornamentals of many European countries (Webb, 1990) as well as the Canadian provinces of Quebec and Ontario (Yu *et al.*, 1998). However, *P. fallax* was found in the United States only in 1974 on strawberries in Iowa (Norton, 1984). Based on its geographic distribution and pathogenicity to barley and maize in Europe (Corbett, 1970, 1972), it is under US quarantine restriction (Joseph Cavey, pers. comm.).

A morphologically related species, *P. convallariae* Seinhorst, 1959 is a non-endemic quality pest, common in light, sandy soils (Seinhorst, 1959) from *Convallaria majalis* (lily of the valley) shipments from northern European countries to the United States. It was detected at least six times at the USDA Nematology Laboratory from late 1998 to late 2000. Because of its limited geographic distribution but long-term presence in the United States, it is not subject to regulatory action (Joseph Cavey, pers. comm.).

P. fallax was originally distinguished from *P. convallariae* by its shorter body length, narrower, more numerous tail annules, and lower male to female ratio (Seinhorst, 1968). *P. fallax* was synonymised by Frederick and Tarjan (1989) with *P. cerealis* Haque, 1965 because of the belief, shared by Loof (1978), that the flattened condition of the type specimens accounted for an artificially much lower a ratio. However, the original description of *P. fallax* also distinguished it from *P. cerealis* by a longer body length (0.42 - 0.56 *vs* 0.39 - 0.43 mm), more posterior excretory pore (between nerve ring and pharyngeal-intestinal valve *vs* at level of median bulb in *P. cerealis*), a longer tail, and more obscure, flatter lip annules (Seinhorst, 1968).

Recently, both *P. fallax* and *P. convallariae* were detected in a shipment of *C. majalis* from France, through the Netherlands and destined for the state of Ohio, USA. Mounted specimens of original paratypes of *P. fallax* were compared with *P. convallariae* from a previous interception. Intermediate morphological forms were also noticed between the typical *P. convallariae* and *P. fallax.* Photographs and supplemental measurements of representative members of each species are provided and compared with previous descriptions.

The *P. fallax* population from France had typical species-diagnostic characters of crenate tail terminus in all members of the population (compared to *P. penetrans*), oblique, central lateral field striations, body length (0.48 - 50.5 *vs* 0.50 - 0.56 mm in original description *vs* 0.58 - 0.61 mm for *P. convallariae*), rounded to anchor-shaped stylet knobs compared to more tulip-shaped knobs in *P. convallariae*, tail annule numbers (19 - 26 *vs* 16 - 19 in *P. convallariae*), and rare males to distinguish it from *P. convallariae* (Table 2, Fig. 4) or *P. penetrans* where males are more numerous. Stylet lengths in this popula-

Table 2. *Morphometrics of* Pratylenchus fallax *and* P. convallariae *(after Seinhorst, 1959*[1]*, 1968*[2]*, 1977*[3]*; Loof, 1991*[4]*. * = measure derived from original description). Values are in μm, and those followed by (n = 10) were derived by the authors, and are presented as range followed by mean ± standard deviation.*

Measure	*P. fallax* Seinhorst, 1968			*P. convallariae* Seinhorst, 1959		
L	420 - 560			580 - 610		
a	24 - 33			23 - 27		
b	5.2 - 6.7			6.0 - 9.0		
c	18 - 24			17 - 28		
V	77 - 81			78 - 81		
Stylet length	15 - 15.5			16 - 17		
Tail annule number	16 - 26			16 - 19		
Lateral line number	4, centre often oblique			4		
Post-uterine sac/Vulval width	1.0 - 1.6			1.4 - 2.0		
Post-uterine sac/Vulva-anus × 100	25 - 33			21 - 25		
Pharyngeal overlap	20 - 44 (31)[4]			31 - 55 (41)[4]		
Male:Female ratio	1 : 5			up to 1 : 1		
Spermatheca shape	round/oval when empty			round		
Lip number	3			3, offset		
Excretory pore position	at/posterior to nerve ring			at/posterior to nerve ring		
Phasmid-tail terminus	9 - 13 annules[3]			6* - 8 annules (n = 10)		
Pharynx	92 - 115	102.6 ± 7.7	(n = 10)	102 - 122	111 ± 7.2	(n = 10)
Pharyngeal overlap	25 - 35	30.2 ± 3.7	(n = 10)	30 - 40	34.1 ± 3.1	(n = 10)
b′	4.3 - 5.2	4.8 ± 0.4	(n = 10)	4.0 - 4.8	4.4 ± 0.2	(n = 10)
c′	1.7 - 2.2	2.0 ± 0.2	(n = 10)	2.0 - 2.4	2.2 ± 0.1	(n = 10)
Post-uterine sac	16 - 25	20.6 ± 3.4	(n = 10)	16 - 26	19.9 ± 2.6	(n = 10)
Vulva-anus distance	72 - 90	78.8 ± 6.1	(n = 10)	70 - 88	76.8 ± 6.5	(n = 10)
Vulval width	16 - 20	17.9 ± 1.3	(n = 10)	14 - 20	17.1 ± 1.6	(n = 10)
Vulva-spermatheca	28 - 42	32.6 ± 4.9	(n = 10)	23 - 50	34.2 ± 7.3	(n = 10)
Vulva-spermatheca/Vulva-anus × 100	31 - 58	42 ± 10	(n = 10)	33 - 59	44 ± 10	(n = 10)

tion (15.5 - 17 μm) overlapped the narrow range for both *P. fallax* (15 - 15.5 μm) and *P. convallariae* (16 - 17 μm). The *P. fallax* population also had male tails with a slight concavity on the posterior third of the bursa (Fig. 4I) as in the original drawing (Seinhorst, 1968). This *P. fallax* male bursal profile with the concavity after the papillar phasmid is different from the uniformly convex bursal profile descriptions (Seinhorst, 1959) and observations from *P. convallariae* (Fig. 4N). Measurement ranges (Table 2), supplementing those in the literature, include c′ and b′ values, post-uterine sac length, vulva-anus distance, vulva width, vulva spermatheca and vulva-spermatheca relative to vulva-anus distance. These measures are similar for *P. fallax* and *P. convallariae*, but pharynx length or overlap was slightly greater in *P. convallariae* than *P. fallax*. The pharyngeal overlaps occurred within the range of those already published (Loof, 1991). However, even the highest value of the 30 - 40 μm overlap (average = 34 μm) measured here for *P. convallariae* fell within the lower quarter of the 32 - 65 μm range for *P. penetrans* overlap (Loof, 1991). The number of tail annules from the terminus to the phasmid was another differentiating character between *P. fallax* (9 - 13 annules) and *P. convallariae* (less than nine annules) based on literature descriptions and observations in specimens from these two populations. Exceptional individuals had *P. fallax*-like tail annulation, with *P. convallariae*-like stylet knob-shape and length or bifurcated tail termini with more tail annules. One of these individuals had identical 28S rDNA D3 sequences to those of completely true-to-type *P. convallariae* and *P. fallax* from separate shipments. Conversely, we measured a population (n = 10) with all the other characteristics of *P. convallariae* except for smaller body length (0.46 - 0.58 mm) that bridges the originally non-overlapping published length ranges for *P. fallax* (0.42 - 0.56 mm) (Seinhorst, 1959) and *P. convallariae* (0.58 - 0.51 mm) (Seinhorst, 1968). A voucher specimen (UCDNC 3279) of this *P. crenatus* conformed to the species description.

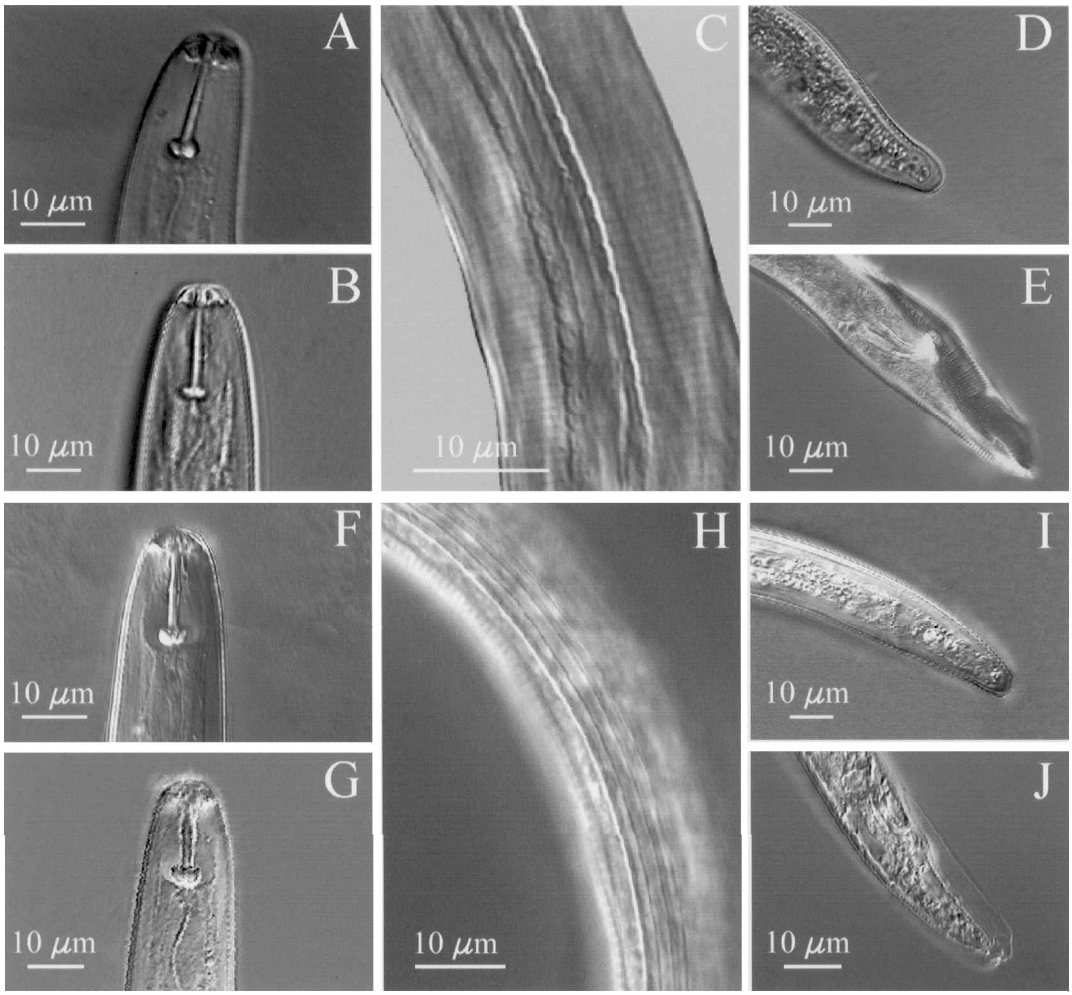

Fig. 4. *Comparison of* Pratylenchus fallax *and* P. convallariae *(lateral view), DIC A, B:* P. fallax *head; C:* P. fallax *lateral field, midbody; D:* P. fallax *female tail; E:* P. fallax *male tail; F, G:* P. convallariae *female heads; H:* P. convallariae *lateral field, mid-body; I, J:* P. convallariae *female tails.*

Molecular characterisation

Shown in Fig. 5 are aligned sequences of the D3 region of the LS 28S rDNA for *P. arlingtoni*, designated as 'arl', for *P. convallariae* as 'con', and for *P. fallax* as 'fal'. Also shown is the sequence for *P. penetrans* (Al-Banna *et al.*, 1997) from the GenBank database that most nearly resembled our new sequences.

The 28S rDNA sequences of typical specimens of *P. convallariae* and *P. fallax* were identical. *P. arlingtoni* n. sp. differed from that sequence by three base pairs (bp)/305 (1% difference), which included a G substitution from A at position 55 in the sequence or 56 in the alignment, and a T substitution from C at position 71 in the sequence or 73 in the alignment, both within the variable region of the sequence. The third change, a G insertion between G and T at position 251 in the sequence or 256 in the alignment, occurred in a highly conserved region of the molecule. The *P. arlingtoni* sequence differed by 9/305 bp (3% difference) from *P. penetrans* which included the previous changes, plus changes in common with *P. fallax* and *P. convallariae.* These shared differences among the three species relative to *P. penetrans* included a C substitution from G at position 39 in the alignment, C substitution from A at position 69, G from T at position 71, T from A at position 82, G from A at position 84, C from T at position 139, G from A at position 148, and A from G at position 231. The sequence

Fig. 5. *Sequence alignments of LS 28SrDNA with Clustal W for* Pratylenchus arlingtoni *n. sp.,* P. convallariae, P. fallax *and* P. penetrans. pen*: Closest sequence in Genbank to the others,* P. penetrans*, accession number U47546 (Al-Banna* et al.*, 1997);* arl*:* P. arlingtoni*;* con*:* P. convallariae*;* fal*:* P. fallax*; –: Gap. Nucleotides differing from the others are underlined and in bold.*

of *P. fallax/P. convallariae* differed from *P. penetrans* by 12/305 bp (4%). The sequence for *P. crenatus,* Accession number U47549 (not shown in Table 5) (Al-Banna *et al.*, 1997) was fairly distant from *P. arlingtoni* n. sp., differing by 49/305 bp (16%), where all but six major changes occurred in a variable region between alignment positions 60 and 175.

Discussion

The unusual character of six or eight lateral lines was reported (Roman & Hirschmann, 1969) in a small proportion of the populations of *P. brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941, *P. coffeae* (Zimmerman, 1898) Filipjev & Schuurmans Stekhoven, 1941, *P. penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941, *P. scribneri* Steiner, 1943, *P. vulnus* Allen & Jensen, 1951 and *P. zeae* Graham, 1951. However, *P. arlingtoni* n. sp. is the first nematode with a consistent six to eight, just as four to six have been found in a few other lesion nematode species.

Pharyngeal characters of *P. arlingtoni* n. sp. are important for its identification. The pharyngeal overlap was a diagnostically and phylogenetically reliable character in well-preserved specimens in recent morphological and molecular studies with multiple *Pratylenchus* species (Loof, 1991; Al-Banna *et al.*, 1997; Duncan *et al.,* 1999).

Despite the morphological similarity of *P. arlingtoni* n. sp. to *P. crenatus* populations described from Europe (Loof, 1960, 1991; Urek, 1999) and South America (Torres & Chaves, 1999), there is substantial 28S rDNA sequence difference between *P. arlingtoni* n. sp. and the *P. crenatus* population from Oregon (Al Banna *et al.*, 1997) and a population from Ohio (unpubl.). Just as Al-Banna *et al.* (1997) proposed the genus *Pratylenchus* to be polyphyletic, crenate-tailed nematodes with *P. crenatus*-like morphology might be polyphyletic as well.

Although *P. arlingtoni* n. sp. and *P. crenatus* are morphologically similar, they are molecularly distant. However, the identical DNA sequences here for *P. fallax* and *P. convallariae* could lend support to a proposal that *P. fallax* be considered a subspecies or synonym of *P. convallariae* due to morphological similarity. It would be premature to synonymise *P. fallax* as *P. cerealis,* as suggested by Frederick and Tarjan (1989), when both species might be better synonymised with *P. convallariae,* due to overlapping morphological and molecular characters and priority of description. Breeding studies might be helpful since the morphologically intermediate specimens in this report may have been sterile hybrids of two species, or simply natural variants within a single biological species.

P. fallax was originally described as being morphologically similar to the highly variable *P. penetrans* (Seinhorst, 1968), with fewer males, a generally longer pharyngeal overlap (Loof, 1991) and occasional populations with crenate tails (Tarté & Mai, 1976). *P. fallax* was also different from *P. penetrans* in lacking fertile hybrid progeny (Perry *et al*., 1980) and on isozyme gels (Ibrahim *et al.*, 1995) or with restriction-enzyme-digested fragments of the rDNA ITS region (Waeyenberge *et al.*, 2000). Here, it appears that *P. fallax* may share at least as close a biological relationship with *P. convallariae* as with *P. penetrans.* Both *P. convallariae* and *P. fallax* have relatively narrow, overlapping morphometric ranges compared to other lesion nematodes based on keys and information presented here, and both are found with similar soil types, hosts and overlapping geographic regions. However, careful comparative pathogenicity testing has been limited for *P. convallariae* and *P. fallax* (Webb, 1990).

Because of the morphological and molecular relationship of *P. arlingtoni* n. sp., *P. fallax* and *P. convallariae*, and their limited distribution or absence in the United States, there is a need for further survey of lesion nematodes, particularly in the United States, Canada and Eurasia. It is difficult to believe that *P. fallax* is restricted to the southern provinces of Canada but not the northern states of the United States. However, if true, there may be a parallel in *P. fallax* being common in Great Britain (Corbett, 1970), while both *P. convallariae* and *P. fallax* are found at lower latitudes in Europe (Seinhorst, 1959, 1977). The influence of temperature on morphological variability in these species as currently characterised might be considered. Further tests of European, Canadian and U.S. populations of these related nematodes are needed to evaluate current regulatory restrictions on *P. fallax.*

Acknowledgements

The authors thank Donna Ellington, Roberta Henegar and Sharon Ochs for technical assistance, and Joseph Cavey and Michael Firko, Animal and Plant Health Inspection Service, USDA, Riverdale, MD, USA, for regulatory information. The authors wish to thank Gerrit Karssen, Plant Protection Service, Wageningen, The Netherlands, for sharing information on *P. fallax* and *P. convallariae.* The authors thank Pierre Baujard, Gerrit Karssen and Michel Luc for manuscript review. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture.

References

AL-BANNA, L., WILLIAMSON, V. & GARDNER, S.L. (1997). Phylogenetic analysis of nematodes of the genus *Pratylenchus* using nuclear 26S rDNA. *Molecular Phylogenetics and Evolution* 7, 94-102.

BALDWIN, J.G., FRISSE, L.M., VIDA, J.T., EDDLEMAN, C.D. & THOMAS, W.K. (1997). An evolutionary framework for the study of developmental evolution in a set of nematodes related to *Caenorhabditis elegans. Molecular Phylogenetics and Evolution* 8, 249-259.

CAFÉ-FILHO, A.C. & HUANG, C.S. (1989). Description of *Pratylenchus pseudofallax* n. sp. with a key to species of the genus *Pratylenchus* Filipjev, 1936 (Nematoda: Pratylenchidae). *Revue de Nématologie* 12, 7-15.

CHOU, Q., RUSSELL, M., BIRCH, D.E., RAYMOND, J. & BLOCH, W. (1992). Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. *Nucleic Acids Research* 20, 1717-1723.

CORBETT, D.C.M. (1970). Cereal *Pratylenchus* spp. in England and Wales and their recognition. *Plant Pathology* 19, 6-10.

CORBETT, D.C.M. (1972). The effect of *Pratylenchus fallax* on wheat, barley and sugar beet roots. *Nematologica* 18, 303-308.

DUNCAN, L.W., INSERRA, R.N., THOMAS, W.K., DUNN, D., MUSTIKA, I., FRISSE, L.M., MENDES, M.L., MORRIS, K. & KAPLAN, D.T. (1999). Genetic and morphological relationships among isolates of *Pratylenchus coffeae* and closely related species. *Nematropica* 29, 61-80.

ELLIS, R.E., SULSTON, J.E. & COULSON, A.R. (1986). The rDNA of *C. elegans*: sequence and structure. *Nucleic Acids Research* 14, 2345-2364.

FILIPJEV, I.N. (1936). On the classification of the Tylenchinae. *Proceedings of the Helminthological Society of Washington* 3, 80-82.

FREDERICK, J.J. & TARJAN, A.C. (1989). A compendium of the genus *Pratylenchus* Filipjev, 1936 (Nemata: Pratylenchidae). *Revue de Nématologie* 12, 243-256.

GOLDEN, A.M. (1990). Preparation and mounting nematodes for microscopic observation. In: Zuckerman, B.M., Mai, W.F. & Krusberg, L.R. (Eds). *Plant nematology laboratory manual*. Revised Edition. Amherst, MA, USA, University of Massachusetts Agricultural Experimental Station, pp. 200-201.

GOLDEN, A.M., LOPEZ, CH.R. & VILCHEZ, R.H. (1992). Description of *Pratylenchus gutierrezi* n. sp. (Nematoda: Pratylenchidae) from coffee in Costa Rica. *Journal of Nematology* 24, 298-304.

HAQUE, M.M. (1965). Two new species of plant nematodes. *Doklady Timiryazevskoi Selskokhozyaistrennoi Akademii* 113, 209-219.

IBRAHIM, S.K., PERRY, R.N. & WEBB, R.M. (1995). Use of isoenzyme and protein phenotypes. *Annals of Applied Biology* 126, 317-327.

KHAN, E. & SINGH, D.B. (1974). Five new species of *Pratylenchus* (Nematoda: Pratylenchidae) from India. *Indian Journal of Nematol*ogy 4, 199-211.

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

LOOF, P.A.A. (1978). The genus *Pratylenchus* Filipjev, 1936 (Nematoda: Pratylenchidae): a review of its anatomy, morphology, distribution, systematics and identification. *Vaxtskyddsrapporter* 5, 50 pp.

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 Inc., pp. 363-421.

MINAGAWA, N. (1982). Descriptions of *Pratylenchus gibbicaudatus* n. sp. and *P. macrostylus* Wu, 1971 (Tylenchida: Pratylenchidae) from Kyushu. *Japanese Journal of Applied Entomology and Zoology* 17, 418-423.

NORTON, D.C. (1984). *Distribution of plant-parasitic nematode species in North America*. Hyattsville, MD, USA, Society of Nematologists, 199 pp.

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

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

SEINHORST, J.W. (1959). Two new species of *Pratylenchus*. *Nematologica* 4, 83-86.

SEINHORST, J.W. (1968). Three new *Pratylenchus* species with a discussion of the structure of the cephalic framework and of the spermatheca in this genus. *Nematologica* 14, 497-510.

SEINHORST, J.W. (1977). *Pratylenchus fallax*. CIH descriptions of plant parasitic nematodes, St Albans, UK, Commonwealth Agricultural Bureaux, Set 7, No. 98, pp. 1-2.

STIERNAGLE, T. (1999). Maintenance of *C. elegans*. In: Hope, I.A. (Ed). *C. elegans, a practical approach*. New York, NY, USA, Oxford University Press, pp. 51-67.

TARTÉ, R. & MAI, W.F. (1976). Morphological variation in *Pratylenchus penetrans*. *Journal of Nematology* 8, 185-195.

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* 22, 4673-4680.

TORRES, M.S. & CHAVES, E.J. (1999). Description of some Pratylenchidae (Nemata) from Argentina. *Nematologia Mediterranea* 27, 281-289.

UREK, G. (1999). The effect of host plants on the biopotential and the morphometric characteristics of *Pratylenchus crenatus* Loof, 1960. *Acta Phytopathologica et Entomologica Hungarica* 34, 253-262.

WAEYENBERGE, L., RYSS, A., MOENS, M., PINOCHET, J. & VRAIN, T.C. (2000). Molecular characterisation of 18 *Pratylenchus* species using rDNA restriction fragment length polymorphism. *Nematology* 2, 135-142.

WEBB, R.M. (1990). Effects of the nematode *Pratylenchus fallax* on roots of oilseed rape (*Brassica napus* var. *oleifera*). *Revue de Nématologie* 13, 115-117.

WILLIAMS, B.D., SCHRANK, B., HUYNH, C., SHOWNKEEN, R. & WATERSTON, R.H. (1992). A genetic mapping system in *Caenorhabiditis elegans* based on polymorphic sequence-tagged sites. *Genetics* 131, 609-624.

YU, Q., POTTER, J.W. & GILBY, G. (1998). Plant parasitic nematodes associated with turfgrass in golf courses in Southern Ontario. *Canadian Journal of Plant Pathology* 20, 304-307.

ZARINA, B. & MAQBOOL, M.A. (1998). Descriptions and observations on two new and two known species of the genus *Pratylenchus* Filipjev, 1936 (Nematoda: Pratylenchidae) from Pakistan. *Pakistan Journal of Nematology* 16, 13-24.

ZEIDAN, A.B. & GERAERT, E. (1991). *Pratylenchus* from Sudan, with the description of two new species (Nemata: Tylenchida). *Revue de Nématologie* 14, 261-275.