MOLECULAR, MORPHOLOGICAL AND THERMAL CHARACTERS OF 19 *PRATYLENCHUS* SPP. AND RELATIVES USING THE D3 SEGMENT OF THE NUCLEAR LSU rRNA GENE

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ABSTRACT

Carta, L. K., A. M. Skantar, and Z. A. Handoo. 2001. Molecular, morphological and thermal characters of 19 *Pratylenchus* spp. and relatives using the D3 segment of the nuclear LSU rRNA gene. Nematropica 31:195-209.

Gene sequences are provided for the D3 segment of the large subunit rRNA gene in *Pratylenchus agilis, P. hexincisus, P. teres,* and *P. zeae.* They were aligned with the closest comparable previously published molecular sequences and evaluated with parsimony, distance and maximum-likelihood methods. Different outgroups and more taxa in this study compared to a previous D3 tree resulted in improved phylogenetic resolution. Congruence of trees with thermal, vulval and lip characters was evaluated. A tropical clade of *Pratylenchus* with 2 lip annules was seen in all trees. Maximum-Parsimony and Quartet-Puzzling Maximum-Likelihood trees, with ambiguously-alignable positions excluded and *Radopholus similis* as an outgroup, had topologies congruent with species possessing 2, 3 or 4 lip annules. An updated sequence for *Pratylenchus hexincisus* indicated it was an outgroup of *P. penetrans, P. arlingtoni, P. fallax* and *P. convallariae. Pratylenchus zeae* was related to *P. neglectus* in a Neighbor-Joining tree, but was equivocal in others. The relatives of *P. teres* were *P. neglectus* and *Hirschmanniella belli* rather than morphometrically similar *P. crenatus.* The *P. agilis* sequence is more closely related to the nearly identical sequences of *P. pseudocoffeae* and *P. brachyurus,* than to that of *P. scribneri,* which is a species closely related morphologically.

Key words: Hirschmanniella, lesion nematode, molecular evolution, morphometrics, *Nacobbus,* nematode phylogeny, *Pratylenchus, Radopholus,* ribosomal DNA, systematics, taxonomy, thermal adaptation.

RESUMEN

Carta, L. K., A. M. Skantar, y Z. A. Handoo. 2001. Caracteres moleculares, morfológicos y térmicos de 19 especies de Pratylenchus y algunos parientes usando el segmento D3 del gen nuclear LSU rARN. Nematrópica 31:195-209.

Se dan las secuencias genéticas del segmento D3 del gene LSU rARN en *Pratylenchus agilis, P. hexincisus, P. teres,* y *P. zeae.* Se alinean con las secuencias moleculares comparables más cercanas que sean publicado previamente, y se evalúan con métodos de parsimonia, de distancia y de la probabilidad máxima. En este estudio, la comparación de diversos outgroups y otros taxas con los de un árbol D3 existente dieron lugar a una resolución filogenética mejorada. La congruencia de árboles con los caracteres termales, vulvales y labiales se evalúa. Un clade tropical de Pratylenchus con 2 anillos labiales se observó en todos los árboles. Los árboles de Parsimonia-Máxima y de Quartet-Puzzling de la probabilidad máxima, utilizando todas posiciones menos las ambiguas e incluyendo *Radopholus similis* como outgroup, tenían topologías congruentes a los especies que poseían 2, 3 ó 4 anillos labiales. Una secuencia actualizada para el *Pratylenchus hexincisus* indica que es un outgroup de *P. penetrans, P. arlingtoni, P. fallax,* y de *P. convallariae. Pratylenchus zeae* estuvo relacionado con *P. neglectus* en un árbol 'Neighbor-Joining,' pero resultó equivocado en los otros. Los relativos de *P. teres* fueron *P. neglectus* y *Hirschmanniella belli* en vez del morfométricamente similar *P. crenatus.* La secuencia de *P. agilis* es más cercana a las secuencias casi idénticas de *P. pseudocoffeae* y de *P. brachyurus,* que a *P. scribneri,* que es un especie morfométricamente semejante.

Palabras claves: adaptación térmica, ADN ribosomal, evolución molecular, filogenia nemátoda, *Hirschmanniella*, morfométricos, nematodo lesionador, *Nacobbus, Pratylenchus, Radopholus*, sistemática, taxonomía.

INTRODUCTION

Molecular identification of parasitic nematodes is valuable when there is insufficient material for microscopic diagnosis, such as in quarantine samples from plant shipments for national and international markets. Reliable molecular markers will be increasingly used by regulatory agencies as technology improves and costs for these molecular analyses are reduced. One such molecule which may be useful for species diagnosis or phylogenetic relationship determination in plant parasitic nematodes is the gene encoding the rRNA large subunit (LSU) (Duncan *et al.*, 1999; Kaplan *et al.*, 2000).

Morphological identification of lesion nematode species rarely or never identified in the U.S. that may be intercepted from international plant shipments, such as *P. convallariae* Seinhorst, 1959 and *P. fallax* Seinhorst, 1968 from Europe, or *Pratylenchus teres* Khan and Singh, 1975, from Africa or India, can be difficult. Molecular techniques facilitate the diagnosis of these species and their separation from related species *P. crenatus* Loof, 1960, *P. arlingtoni* Handoo, Carta and Skantar, 2001, and *P. penetrans* (Cobb, 1917) Filipjev and Schuurmans Stekhoven, 1941 found in the U.S.

Pratylenchus agilis Thorne and Malek, 1968 is also of diagnostic interest as one of five damaging lesion nematodes of soybean (Schmitt and Barker, 1981). A population identified as *P. agilis* from Maryland soybean (Golden and Rebois, 1978) that fits the minimal description of the original North Dakota population is believed by some taxonomists to require revision; diagnosis might be improved after inclusion in a phylogenetic study. Violent movement was the unique diagnostic character given in the original description by Thorne and Malek, but the Maryland population is merely very active. Our formal designation for this population is *Pratylenchus agilis sensu* Golden and Rebois since it fails to "throw itself into a coil and move violently when touched" (Thorne and Malek, 1968). However, we will continue to refer to this population as *P. agilis* until taxonomic revision is done. *Pratylenchus agilis* is often found in nature with either *P. hexincisus* Taylor and Jenkins, 1957 or *P. scribneri* Steiner, 1943. Both *P. agilis* and *P. zeae* Graham, 1951 were described long ago as relatives of *P. scribneri* (Thorne and Malek, 1968). Since many more related species have been described since then, identifying molecular relatives may help to update and improve diagnosis of all these species.

A better understanding of phylogenetic relationships may be useful for interpreting host or thermal preferences, biogeography, and assessing diagnostic value of morphological characters. A recent, partial LSU rRNA gene tree of lesion nematodes and relatives suggested that the genus *Pratylenchus* does not represent a monophyletic group (Al-Banna *et al.*, 1997). Although this proposal for polyphyly is intriguing, the bootstrap values for the most parsimonious tree were not definitive. Frequently, the addition of more taxa (Forey *et al.*, 1992) or different outgroups (Milinkovitch and Lyons-Weiler, 1998) improves the resolution of phylogenetic trees. In this study the D3 portion of LSU rDNA sequences are reported for *Pratylenchus agilis*, *P. hexincisus*, *P. teres*, and *P. zeae.* These and ten other previously sequenced lesion nematode species were aligned for phylogenetic analysis. Trees are interpreted in light of some morphological and thermal characteristics.

MATERIALS AND METHODS

Culture: Nematodes were raised in tissue culture on Gamborg's B5 Medium on excised Iowa Chief corn roots (Huettel and Rebois, 1985), grown in a Precision Dual-Program incubator. All cultures were kept at 28°C, although the optimum for *P. teres* is considerably higher (M. Botha-Greeff, pers. comm.). Active nematodes emerging from Baermann funnels (Southey, 1986) were processed to DNA extraction buffer in microcentrifuge tubes.

Population information, climate range and some morphological species characters from the literature were included in Table 1 (Acosta and Malek, 1979; Gotoh, 1974; Inserra *et al.*, 1978, Loof, 1991, 1978; Norton, 1984) for use in comparison with the trees. Morphometrics of sequenced populations were generated for *Pratylenchus agilis*, *P. hexincisus*, and *P. zeae* in Table 2 and for *P. teres* (unpublished results). Morphometrics are also given for *Pratylenchus* sp. U from voucher slide UCDNC 3280 (Table 2), originally identified as *P. coffeae*, Genbank U47552. *Pratylenchus* sp. U had lower 'b' and 'c' values than *P. coffeae* and *P. pseudocoffeae* (Inserra *et al.*, 1998), and did not correspond to any described species.

Based on preliminary sequencing results, the *P. agilis* culture was discovered to also contain *P. hexincisus* (Al-Banna, pers. comm.). By 1997, cultural purity was assured by taking single-paired males and females on separate plates and confirming the species morphologically. In this study, individual *P. hexincisus* were identified under a high-power light microscope from a mixed *Pratylenchus agilis*/*P. hexincisus* culture maintained from the original 1976-77 culture. After examining the voucher specimen for *P. hexincisus*, slide UCDNC 3285, with 3 adult females (Al-Banna *et al.*, 1997), it was determined that those specimens were actually *P. agilis*. Morphometrics of remaining voucher specimens were in accord with the identified species (Al-Banna *et al.*, 1997).

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 60 min. Proteinase K was denatured by heating to 95°C for 15 min.

Amplification and sequencing: The D3 expansion region of the LSU rDNA fragment (345 bp raw sequence; 298-303 bp/sequence; 308 alignment positions) 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 DisplayTAQ buffer (PGC Scientific, Gaithersburg, MD, USA), 250 µM dNTPs, 4 mM $MgCl_2$, and 600 µM of each ribosomal DNA primer, D3A (5'-GACCCGTCTTG AAACACGGA-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 (~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, 3min. (to allow hot start); (94°C, 1 min.; 52°C, 1 min.; 72°C, 1 min.) × 35 cycles; 72°C, 10 min.]. Reactions were analyzed by gel electrophoresis.

Table 1. Population, Morphological, and Climate Characteristics in the Pratylenchinae.

Nematode authority/synonymy	Pop./aln lbl[x]	Genbank Acc. #	Lip annule	Host and locality	Climate[y]	Species V%
Radopholus similis (Cobb, 1893) Thorne, 1949	O3 (rad)	U47558	4	*Citrus* sp., FL, USA	Tropical	55-61
Nacobbus aberrans (Thorne, 1935) Thorne and Allen, 1944	O2 (nac)	U47557	4	*Beta vulgaris*, NE, USA	Tmp-Trp[z]	91-94
Hirschmanniella belli Sher, 1968	O1 (hir)	U47556	4	*Typha* sp., Yolo Co., CA, USA	Tropical	50-60
Pratylenchus teres Khan and Singh, 1975	JK (tjk)	AF196353	3	*Gossypium hirsutum*, Jan Kempdorp, South Africa	Tropical	69-78
Pratylenchus neglectus (Reusch, 1924) Filipjev and Schuurmans Stekhoven, 1941 = *P. minyus* Sher and Allen, 1953	P2 (neg)	U47548	2	*Pyrethrum* sp. and *Solanum tuberosum*, Monterey and Siskiyou Counties, CA, USA	Tmp-Trp	75-84
Pratylenchus thornei Sher and Allen, 1953	(tho)	U47550	3	*Vicia faba*, *Carthamus tinctorius*, *Triticum aestivum*; Yolo Co., CA; *Lycopersicon esculentum*, San Joaquin Co., CA, USA	Tmp-Trp	74-79
Pratylenchus vulnus Allen and Jensen, 1951	P3 (vul)	U47547	3-4	*Juglans hindsii*, Yolo Co., CA, USA	Tmp-Trp	77-82
Pratylenchus crenatus Loof, 1960	P4 (cre)	U47549	3	*Rubus ditifolius*, Oregon, USA	Temperate	78-86
Pratylenchus arlingtoni Handoo, Carta and Skantar, 2001	(arl)	AF307328	3	*Poa pratensis*, *Festuca arundinaceae*, Arlington Co., VA, USA	Temperate	81-86
Pratylenchus convallariae Seinhorst, 1959	(con)	AF196351	3	*Convallaria majalis*, France	Temperate	78-81
Pratylenchus fallax Seinhorst, 1968	(fal)	AF264181	3	*Convallaria majalis*, France	Temperate	77-81
Pratylenchus penetrans (Cobb, 1917) Filipjev and Schuurmans Stekhoven, 1941	(pen)	U47546	3	*Medicago sativa*, MD; *Prunus avium*, Monroe Co., NY, *Lilium eximium*, *Menta.* sp., OR, USA	Tmp-Trp	75-84
Pratylenchus zeae Graham, 1951	(zea)	AF303950	3	*Zea mays*, Ohio, USA	Tropical	68-76
Pratylenchus hexincisus Taylor and Jenkins, 1957	NL6 (hxn)	AF303949	2-3	*Zea mays*, Wye, Eastern Shore, MD, USA	Trp-Tmp	75-82

Table 1. (Continued) Population, Morphological, and Climate Characteristics in the Pratylenchinae.

Nematode authority/synonymy	Pop./aln lbl[x]	Genbank Acc. #	Lip annule	Host and locality	Climate[y]	Species V%
Pratylenchus agilis sensu Golden and Rebois; *P. agilis* Thorne and Malek, 1968	NL7 (agi)	AF196352	2	*Zea mays*, Wye, Eastern Shore, MD, USA	Trp-Tmp	75-81 76
Pratylenchus pseudocoffeae Mizukubo, 1992	(pse)	AF170444	2	*Aster* sp., FL, USA	Trp-Tmp	74-84
Pratylenchus brachyurus (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941	(bra)	U47553	2	*Aster* sp., FL, USA	Tropical	82-89
Pratylenchus gutierrezi Golden, Lopez and Vilchez, 1992	K3 (gkt)	AF170442	2	*Coffea* sp. San Antonio, Costa Rica	Tropical	74-84
Pratylenchus coffeae (Zimmmerman, 1898) *sensu* Sher and Allen, 1953 = *P. musicola* (Cobb, 1919)	C4 (cfc) M (cfa)	AF170428 U47555	2	*Citrus* sp., FL, USA *Citrus* sp., FL, USA *Aglonema* sp., HI, USA	Trp-Tmp	76-84
Pratylenchus scribneri Steiner, 1943	(scr)	U47551	2	*Vitis* sp., Kern Co., CA; *Solanum tuberosum*, Wayne Co., NY, USA	Trp-Tmp	75-82
Pratylenchus loosi Loof, 1960	T (lot)	AF170439	2	*Camellia sinensis*, Sri Lanka	Tropical	79-85
Pratylenchus sp. U (= *P. coffeae* (Zimmerman, 1898) Filipjev and Schuurmans Stekhoven, 1941)	P10 (spu)	U47552	2	*Coffea arabica*, Guatemala	Tropical	75-82

[x]Alignment label.
[y]Thermal and climate information based on Acosta and Malek, 1979; Gotoh, 1974, Loof, 1990; and Norton, 1984. Morphological information in Handoo and Golden, 1989, Jatala, 1991 (*N. aberrans*), Loof, 1991 (*R. similis*, *H. belli*), Inserra *et al.*, 1998 (*P. pseudocoffeae*), Roman and Hirschmann, 1969 (*P. scribneri*), or original (*P. agilis sensu* Golden).
[z]Tmp = Temperate, Trp = Tropical.

Table 2. Morphometrics of Selected Populations of *Pratylenchus* spp.

Measure	*P. agilis* Thorne and Malek, 1968 *sensu* Golden and Rebois	*P. hexincisus* Taylor and Jenkins, 1957	*P. zeae* Graham, 1951	*Pratylenchus* sp. U
n =	10	10	16	6
Length mm	0.42-0.60	0.39-0.63	0.4-0.55	0.43-0.55
a ratio	18-30	17.7-24.6	18.6-25.4	25.5-28.7
b ratio	3.8-5.3	3.5-4.6	4.9-8.6	3.4-4.2
c ratio	12-22	15.1-22.6	11.4-14	15.2-18
V %	75-81	75-79	66.5-71	75-82
Stylet	15-15.5	14.6-16	15-16.5	15.5-17
Lips	2	2	3	2
TA #	20-25	20-22	25-29	22-24
c'	2.7-3.9	2.5-2.8	2.8-3.7	2.9

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 template. Prior to sequencing, the DNA was purified using the Qiaquick PCR purification kit (Qiagen, Valencia, CA, USA). Sequencing reactions included 100 ng template and 3.2 pmol D3A or D3B primer. To generate cloned templates 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 was performed on two or more individuals from the same nematode population. The DNA sequences from individuals of each nematode species were aligned to obtain a consensus sequence. Individual bases present in a minimum of three aligned sequences were chosen for the consensus. In general, sequences from cloned PCR products were examined. However, in some cases, sequences derived directly from PCR products were used to resolve base conflicts.

Phylogenetic analysis: Outgroup taxa for the Tylenchida, listed with alignment abbreviation and Genbank Accession number, were *Teratorhabditis palmarum* Gerber and Giblin-Davis, 1990 (tpl) TPU73455 (Baldwin *et al.*, 1997), *Acrobeloides bodenheimeri* Steiner, 1936, PS2160 (abh) AF147065 (De Ley *et al.*, 1999), and previously-used *Heterorhabditis bacteriophora* (Poinar, 1975) (hbc) U47560 (Al-Banna *et al.*, 1997).

Sequences for species of Tylenchida with alignment and accession numbers are listed in Table 1. These include *Pratylen-*

chus gutierrezi, P. loosi, P. pseudocoffeae, P. coffeae (Duncan *et al.*, 1999), *Radopholus similis, Nacobbus aberrans, Hirschmanniella belli, Pratylenchus brachyurus, P. crenatus, Pratylenchus* sp. U (= *P. coffeae*), *P. neglectus* (= *P. minyus*), *P. coffeae* (= *P. musicola*), *P. penetrans, P. scribneri, P. thornei, P. vulnus* (Al-Banna *et al.*, 1997), *P. arlingtoni, P. convallariae* and *P. fallax* (Handoo *et al.*, 2000).

New nematode sequences for the D3 portion of the LSU rRNA gene of *Pratylenchus agilis, P. hexincisus, P. teres,* and *P. zeae* were submitted to GenBank with accession numbers listed in Table 1. Voucher specimens are deposited in the USDANC, Beltsville, MD.

Sequences were aligned with the Clustal W (ver 1.4) program, (Clustal W at EBI, and Thompson *et al.*, 1994). Positions are numbered with 1 corresponding to number 3324 of the *C. elegans* LSU rRNA gene (Ellis *et al.*, 1986; Al-Banna *et al.*, 1997). Maximum-Parsimony, Neighbor-Joining and Quartet-Puzzling Maximum-Likelihood methods to construct phylogenetic relationships were used with the PAUP* program, ver. 4.0b4a. Each tree was constructed with a single outgroup (Swofford, 1998). Initially, a Neighbor-Joining (N-J) phylogram was constructed for all characters with bootstrap values based on 3 000 replicates with *H. bacteriophora* as outgroup. In subsequent trees, to further reduce homoplasy, *R. similis* was the outgroup, and ambiguously-aligned nucleotide positions 67-77 were excluded. A Maximum-Parsimony (M-P) tree was constructed using a heuristic search on parsimony-informative characters. Bootstrap values of >50% were provided based on 1 000 replicates for monophyletic groups. Options for branch-swapping and "ACCTRAN" character-state optimization were selected. A Quartet-Puzzling (Q-P) tree derived with settings based on the Hasegawa-Kishino-Yano (1985) model was made with Maximum-Likelihood support values for quartets from 1 000 puzzling steps on the whole data set, excluding ambiguously-aligned positions.

RESULTS

Fig. 1 shows a Clustal W alignment of the new sequences for *P. agilis, P. hexincisus, P. teres,* and *P. zeae* with the closest published sequences. Out of 308 nucleotide positions in this alignment of the D3 portion of the LSU rRNA, 184 are constant, 47 variable characters are parsimony uninformative, and 76 are parsimony-informative characters as defined by PAUP* ver. 4.0b4a (Swofford, 1998).

M-P and Q-P Maximum-Likelihood trees (Figs. 3 and 4), with ambiguous positions excluded and *R. similis* as outgroup, had topologies congruent with taxa possessing 4, 3 or 2 lip annules. *P. neglectus* was the only exception, having 2 annules within a group with 3 annules (Table 1).

A primarily tropical group of *Pratylenchus* spp. with 2 lip annules (Table 1), comprised of *P. agilis, P. brachyurus, P. coffeae* C4 and M, *P. gutierrezi, P. loosi, P. pseudocoffeae* and *P. scribneri,* occurred in all trees (Figs. 2-4, lower branch on page).

Within the outgroups *R. similis* and *N. aberrans* (4 lip annules), there were three monophyletic branches on the Maximum-Parsimony (M-P) tree (Fig. 3). In the first basal branch, *H. belli* (4 lip annules) was located outside *P. teres, P. neglectus, P. thornei* and *P. vulnus* (3 lip annules, but 2 in *P. neglectus*). The second, intermediate branch was composed of *P. zeae, P. crenatus, P. hexincisus, P. penetrans, P. arlingtoni,* and *P. fallax-P. convallariae* (3 lip annules, temperate climate except *P. zeae*). The third, derived, tropical branch of taxa with 2 lip annules was as described above.

The Q-P tree (Fig. 4) had a topology similar to the M-P tree (Fig. 3) except the basal taxa for the M-P tree no longer

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bra   CCAAGGAGTTTATCGTGT-GCGCGAGTCATTGGGCGTTGAAAACCC-AAAGGCGCAATGAAAGTAAATGTA-TCCATCCCGGAGCTGATGTGCGACCCTGGTCACTGCGGTG-GCCAGGA 116
pse   CCAAGGAGTTTATCGTGT-GCGCGAGTCATTGGGCGTTGAAAACCC-AAAGGCGCAATGAAAGTAAATGTA-TCCATCC-GGAGCTGATGTGCGACCCTGGTCACTGCGGTG-GCCAGGA 115
agi   CCAAGGAGTTTATCGTGT-GCGCGAGTCATTGGGCGTTGAAAACCC-AAAGGCGCAATGAAAGTAAATGTA-TCCGCTC-GGAACTGATGTGCGACCCTGGTCACTGCGGTG-GCCAGGA 115
cfa   CCAAGGAGTTTATCGTGT-GCGCGAGTCATTGGGTGTTCAAAACCC-AAAGGCGCAATGAAAGTGAACGTT--TCCGTTAGGAGCCAACGTGCGATCCTGGTCACCACGGTG-GCCGGGC 115
cfc   CCAAGGAGTTTNTTGTTTTGCGNGAGTCATTGGGCGTTCAAAACCC-AAAGGCGCAATGAAAGTGAACGTT--TCCGTTAGGAGCCAACGTGCGATCCTGGTCACCACGGTG-GCCGGGC 116
gkt   CCAAGGAGTTTATCGTGT-GCGCGAGTCATTGGGCGTTGAAAACCC-AAAGGCGCAATGAAAGTGAACGTT--TCCGTTAGGAGCCAACGTGCGATCCTGGTCACCACGGTGCATCAGGC 116
spu   CCAAGGAGTTTATCGTGT-GCGCGAGTCATTGGGCGTTGAAAACCC-AAAGGCGCAATGAAAGTGAACGTTACCCTCCTCGGTGCCAACGTGCGACCCAGGTCACTGCGGTG-GCCAGGA 117
lot   CCAAGGAGTTTATCGTGT-GCGCGAGTCATTGGGCGTTGAAAACCC-AAAGGCGCAATGAAAGTGAACGT--ATCCGCTAGGAGCCAACGTGCGATCCTGGTCATTGCGGTG-GCCAGGC 115
scr   CCAAGGAGTTTATCGTGT-GCGCGAGTCATTGGGCGTTCAAAACCC-AAAGGCGCAATGAAAGTGAACGTT-TCCATTTCGGAGCCGACGTGCGATCCTGGTCACTGCGGTG-GCCAGGC 116
tho   CCAAGGAGTTTATCGTGT-GCGCAAGTCATTGGGTGTTGAAAACCC-AGAGGCGCAATGAAAGTGAAGGTA-TCCCTCGCGGAGCCGACGTGCGAGCACGGGCACTGCGGTG-TCCGAGT 116
hir   CCAAGGAGTTTATCGTGT-GCGCAAGTCATTGGGTGTTCAAAACCC-AAAGGCGCAATGAAAGTAAATGCATCCGCA-AGG-AGCTGACGTGCGATCCTGGGCATCGCGGTG-CCCGGGC 115
nac   CCAAGGAGTTTATCGTGT-GCGCGAGTCATTGGGCGTTCAAAACCC-AAAGGCGCAATGAAAGTGAATGTTTCCGCA-AGGGAGCTGATGTGCGATCCCGGGCATTGCGGTG-CTCGGGC 116
tjk   CCAAGGAGTTTATCGTGT-GCGCGAGTCATTGGGCGTTGAAAACCC-AAAGGCGCAATGAAAGTGAATGC-TCCGCA-AGG-AGCTTACGTGCGATCCTGGGCACCGCGGTG-TCCGGGC 114
neg   CCAAGGAGTTTATCGTGT-GCGCAAGCCATTGGGC-TCAGAAACCC-AAAGGCGCAATGAAAGTGAACATGTCCTCGCAAGAGGCAGACGTGCGATCCCCGGCGCCTCGGTG-CCGGGGC 116
zea   CCAAGGAGTTTATCGTGT-GCGCGAGTCATTGGGCGTTGAAAACCC-AAAGGCGCAATGAAAGTGAA-GTG-CCACGCAAGTGGCTGATGTGCGATCGGTGTT-CCACGGAA-CACGTGC 114
vul   CCAAGGAGTTTATCGTGT-GCGCAAGTCATTGGGCGTTGAAAACCC-AAAGGCGCAATGAAAGTGAACGCATCCG--CAAGGAGCTGACGTGCGATCCCGGGTACTGCGGTG-CCCGAGC 115
cre   CCAAGGAGTTTATCGTGT-GCGCGAGTCATTGGGCGTTCAAAACCC-AAAGGCGTAATGAAAGTGTANGAAGGCGGCTTGCGATCGATCGTGCGATCCCGGGTCCCTCGGGG-CCCGGGC 117
arl   CCAAGGAGTTTATCGTGT-GCGCGAGTCATTGGGTGTTCAAAACTC-AAAGGCGCAGTGAAAGTAAA-GCAGCTGC--AAGGTTGCGACGTGTGATCTGAGCAATCACGATT-GCCTGGA 114
fal   CCAAGGAGTTTATCGTGT-GCGCAAGTCATTGGGTGTTCAAAACTC-AAAGGCGCAATGAAAGTAAA-GCAGCCGC--AAGGTTGCGACGTGTGATCTGAGCAATCACGATT-GCCTGGA 114
pen   CCAAGGAGTTTATCGTGT-GCGCAAGTCATTGGGTGTTGAAAACTC-AAAGGCGCAATGAAAGTAAA-GAATCCGC--AAGGATACGACGTGTGATCTGAGCAATCACGATT-GCCTGGA 114
hxn   CCAAGGAGTTTATCGTGT-GCGCAAGTCATTGGGTGTTCAAA-CCT-AAAGGCGCAATGAAAGTGAATGCCTCCGC--AAGGAGTTGATGTGTAACACTGTTGGCCACGGCT-GGCGGGT 114
rad   CCAAGGAGTTTAGCGTGT-GCGCGAGTCATTGGGCGTTGAAAACCC-AAAGGCGCAATGAAAGTGAATGAT-TCCCTCGTGGAGCTGATGTGCGACCCTGCGTACTGCGGTG-CGTGGGG 116
abh   CCAAGGAGTCTAGCGTAT-ACGCGAGTCATTGGGTG--GAAAACCC-ATAGGCGCAATGAAAGTGAAGGCTTCCTC--GCGGAGCTGATATGCGATCCGTTGCACTTCGGTGTACGCGGA 114
tpl   CCAAGGAGTCTACCGCAT-GCGCGAGTCATTGGGT--TGCAAACCT-AAAGGCGCAATGAAAGTAAAGGTCGACGTTCGCTCGGCTGATATGGGATGCGTGCGCTTGTCGCG----CGCC 112
hbc   CCGAGGAGTGTAATTTAT-ACGCGAGTCAAGGGGTGGTGAAAACCCTAGGGGCGGAATGAAAGTAAAGGC---CGGTTAACCGGTTGACATGGGATCC---GCTCTTCGGAG----CGGC 109
      ** ****** *      *  **  ** **  ***      ** *   *  **** * *******  *                       **  *

bra   GCAGCATGGCCCCATCCTGACTGCTTGCAGTGGGGTGGAGGAAGAGCGTACGCGATGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATGAAGCCAGAGGAAACTCTGGTGGAAGT 236
pse   GCAGCATGGCCCCATCCTGACTGCTTGCAGTGGGGTGGAGGAAGAGCGTACGCGATGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATGAAGCCAGAGGAAACTCTGGTGGAAGT 235
agi   GCAGCATGGCCCCATCCTGACTGCTTGCAGTGGGGTGGAGGAAGAGCGTACGCGATGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATAAAGCCAGAGGAAACTCTGGTGGAAGT 235
cfa   GCAGCATGGCCCCATCCTGACTGCTTGCAGTGGGGTGGAGGAAGAGCGTACGCGGTGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATGAAGCCAGAGGAAACTCTGGTGGAAGT 235
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gkt   GCAGCATGGCCCCATTCTGACTGCTTGCAGTGGGATGGAGGAAGAGCGTACGCGATGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATAAAGCCAGAGGAAACTCTGGTGGAAGT 236
spu   GCAGCATGGCCCCATCCTGACTGCTTGCAGTAGGGTGGAGGAAGAGCGTACGCGATGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATAAAGCCAGAGGAAACTCTGGTGGAAGT 237
lot   GCAGCATGGCCCCATCCTGACTGCTTGCAGTAGGGTGGAGGAAGAGCGTACGCGATGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATAAAGCCAGAGGAAACTCTGGTGGAAGT 235
scr   GCAGCATGGCCCCATCCCGACTGCTTGCAGTGGGGTGGAGGAAGAGCGTACGCGATGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATGAAGCCAGAGGAAACTCTGGTGGAAGT 236
tho   GCAGCATGGCCCCATCCTGACTGCTTGCAGTGGGGTGGAGGAAGAGCGTACGCGGTGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATGAAGCCAGAGGAAACTCTGGTGGAAGT 236
hir   GCAGCATGGCCCCATCCCGACTGCATGCAGTGGGGTGGCGGTAGAGCGTACGCGATGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATGAAGCCAGAGGAAACTCTGGTGGAAGT 235
nac   GCAGCATGGCCCCATCCTGACTGCATGCAGTGGGGTGGAGGCAGAGCGTACGCGGTGAGACCCGAAAGATGGTGAACTATTCGTGAGCAGGACGAAGCCAGAGGAAACTCTGGTGGAAGT 236
tjk   GCAGCATGGCCCCATCCTGACTGCTTGCAGTGGGGTG-CGGAAGAGCGTACGCGGTGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATGAAGCCAGAGGAAACTCTGGTGGAAGT 233
neg   GCAGCATGGCCCCATCCTGACTGCTTGCAGTGGGGTGGCGGAAGAGCGTGCGCGGTGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATGAAGCCAGAGGAAACTCTGGTGGAAGT 236
zea   GCAGCATGGCCCCATCCTGGCTGCTTGCAGCGGGGTGGAGGAAGAGCGTGCGCGATGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATGAAGCCAGAGGAAACTCTGGTGGAAGT 234
vul   GCAGCATGGCCCCATCCCGATTGCTTGCAATGGGGTGGAGGAAGAGCGTACGCGGTGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATGAAGCCAGAGGAAACTCTGGTGGAAGT 235
cre   GCAGCATGGCCCCATCCCGATTGCTTGCAATGGGGTGGAGGANGAGCGTACGCGACGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATGAAGTCAGAGGAAACTCTGNTGGAAGN 237
arl   GCAACATGGCCCCATTCTGGCCGCTTGCGGCGGGGTGGAGGAAGAGCGTACGCGGTGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATGAAGTCAGAGGAAACTCTGATGGAAGT 234
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hxn   GCAACATAGCCCCATTCGGATTGCTTGCAATCGGGTGGCGGAAGAGCGTACGCGACGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGATGAAGCCAGAGGAAACTCTGGTGGAAGT 234
rad   GCAGCATAGCCCCGTCTTGACCGCTTGCGGTGGGGCGGAGAAAGAGCGTACGCGCTGAGACCCGAAAGATGGTGAACTATTCCTGAGCAGGACGAAGCCAGAGGAAACTCTGGTGGAAGT 236
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      *** *** ****  *   *   ** ***    **     *   ******          ********************* * ********   * * * ********** * **** *

bra   CCGAAGCGATTCTGACGTGCAAATCGATCGCTCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 303
pse   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 301
agi   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 301
cfa   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 301
cfc   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGG-TATAGGG-CGAAAGACTAATCGAAC- 300
gkt   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 302
spu   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 303
lot   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 301
scr   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 302
tho   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 302
hir   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 301
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neg   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATNGGGGCGAAAGACTAATCGAAC- 302
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vul   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 301
cre   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 303
arl   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 300
fal   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 300
pen   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 300
hxn   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 300
rad   CCGAAGCGATTCTGACGTGCAAATCGATCG-TCTGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 302
abh   CCGAAGCGCTTCTGACGTGCAAATCGATCG-TCTGACTTGG---------------------------
tpl   CCGTAACGATTCTGACGTGCAAATCGATCG-TCTGACTGGCGTATAGGGGCGAAAA------------
hbc   CCGTATCGGTTCTGACGTGCAAATCGATCG-ATAGACTTGGGTATAGGGGCGAAAGACTAATCGAAC- 294
      *** * ** ********************      **** *
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Fig. 1. Clustal W (1.81) multiple sequence nucleotide alignment of the D3 expansion segment of the LSU 28S rRNA for new *Pratylenchus* and related nematode sequences over 308 alignment positions. Pearson sequence format, * = conserved sequence. Twenty-five taxa with alignment designations include *Heterorhabditis bacteriophora* (hbc), *Teratorhabditis palmarum* (tpl), *Acrobeloides bodenheimeri* (abh), *Radopholus similis* (rad), *Nacobbus aberrans* (nac), *Hirschmanniella belli* (hir), *Pratylenchus agilis sensu* Golden and Rebois (agi), *P. arlingtoni* (arl), *P. brachyurus* (bra), *P. coffeae* M (cfa), *P. coffeae* C4 (cfc), *P. crenatus* (cre), *P. fallax/P. convallariae* (fal), *P. gutierrezi* K3 (gkt), *P. hexincisus* (hxn), *P. loosi* (lot), *P. neglectus* (neg), *P. penetrans* (pen), *P. pseudocoffeae* (pse), *P. scribneri* (scr), *Pratylenchus* sp. U (spu), *P. teres* (tjk), *P. thornei* (tho), *P. vulnus* (vul), and *P. zeae* (zea).

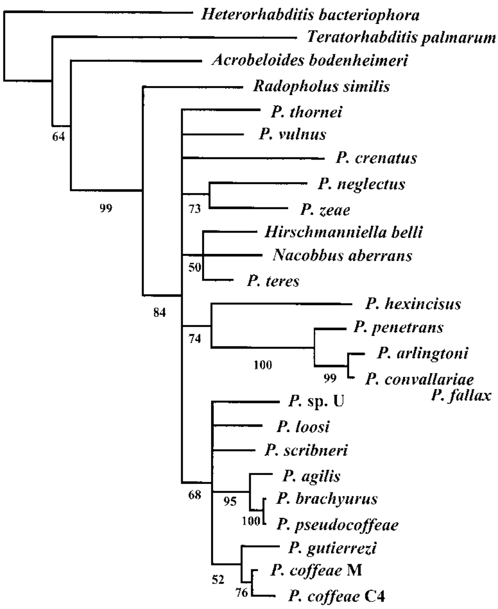

Fig. 2. Neighbor-Joining 50% majority-rule consensus phylogram of complete nucleotide sequence from the D3 expansion segment of the 28S rDNA gene from *Pratylenchus* spp. and six other genera, including outgroups *Heterorhabditis bacteriophora*, *Teratorhabditis palmarum*, and *Acrobeloides bodenheimeri*, with bootstrap values from 3 000 replicates as implemented in PAUP* 4.0b4 (Swofford, 1998).

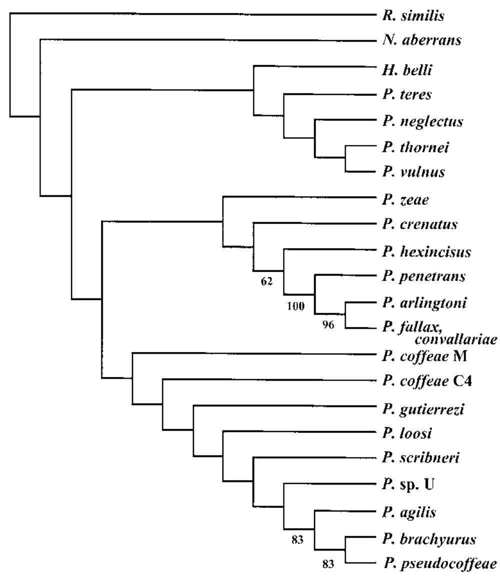

Fig. 3. Maximum-Parsimony Tree. One of seven most parsimonious trees from the D3 expansion segment of the LSU rDNA gene for *Radopholus similis* (outgroup), *Nacobbus aberrans Hirschmanniella belli*, and *Pratylenchus* spp. A heuristic search was made on 51 phylogenetically-informative nucleotide characters, excluding ambiguous alignment positions 67-77 in PAUP* 4.0b4 (Swofford, 1998). Tree length = 164, and CI = 0.53.

formed a monophyletic group. Instead, the first branch featured *N. aberrans* and *H. belli* as sister taxa (67% Maximum-Likelihood support), and the second branch had *P. teres* and *P. neglectus* as closely related (60% support). In the third branch *P. thornei* was most closely related (53% support) to *P. vulnus* and *P. crenatus* (54% support). The fourth monophyletic branch of the Q-P tree included the same taxa as the intermediate second branch of the M-P tree, but excluded *P. crenatus* (Fig. 4). In the M-P tree, *P. crenatus* was located between *P. zeae* and *P. hexincisus* (Fig. 3).

High statistical support values on tree branches occurred for the group of *P. convallariae*, *P. fallax*, *P. arlingtoni*, and *P. penetrans* (93-100% support in Figs. 2-4) where *P. hexincisus* was an outgroup (74, 62, 87% support in Figs. 2-4). Another strongly-supported group included *P. pseudocoffeae*, *P. brachyurus* and *Pratylenchus agilis* (95, 83, 85% support in Figs. 2-4), followed by *P. coffeae* M and *P. coffeae* C4 (76, 82% support in Figs. 2 and 4). *Pratylenchus loosi* was related to *Pratylenchus* sp. U in the N-J (Fig. 2) and Q-P trees (Fig. 4).

Pratylenchus teres was more closely related to *H. belli* (Figs. 2-4) and *P. neglectus* (Figs. 3 and 4) than to morphometrically similar *P. crenatus* or *P. convallariae* (Handoo and Golden, 1989).

Pratylenchus agilis differed by only 2 ambiguous (N) nucleotides from the reported sequence for the misidentified *P. hexincisus* in the original tree (Al-Banna *et al.*, 1997). Therefore the original "*P. hexincisus* = *Pratylenchus agilis*" sequence was

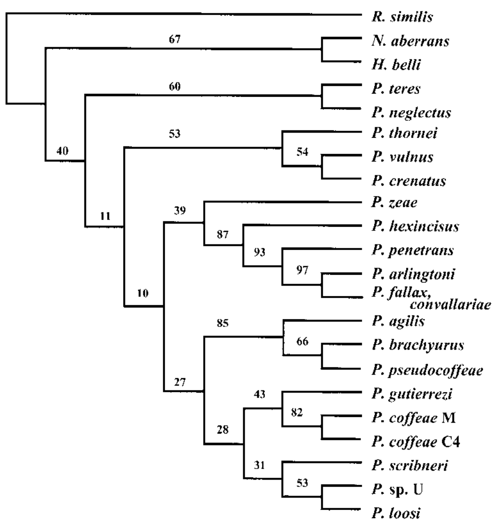

Fig. 4. Quartet-Puzzling tree from 1 000 puzzling steps using the substitution model on 298 nucleotide characters of the D3 expansion segment of the 28S rDNA gene, excluding variable alignment positions 67-77, for *Pratylenchus* spp. and three other genera with *Radopholus similis* as outgroup. Transition/transversion ratio = 2, and nucleotide ratios are A = 0.26630, C = 0.21398, G = 0.32904, T = 0.19068. Maximum-Likelihood support values for quartets provided on branches. Settings correspond to the Hasegawa-Kishino-Yano (1985) model as implemented in PAUP* 4.0b4 (Swofford, 1998).

not used in these trees, and the new *P. hexincisus* substituted. Although the distant sequence similarity of this *P. hexincisus* sequence was only slightly greater for *H. belli* (90%) than for *P. penetrans* (89%), there were 8 homoplasies in common with *H. belli* and none with *P. penetrans.* There was moderate bootstrap support (74% NJ, Fig. 2; 62% M-P, Fig. 3; 86% Q-P, Fig. 4) for *P. hexincisus* as an outgroup to *P. penetrans, P. convallariae, P. fallax* and *P. arlingtoni.* Both *P. hexincisus* and *P. arlingtoni* have at least 6 incisures in the lateral field (Handoo *et al.*, 2000).

The sequences and trees (Figs. 1-4) indicated *Pratylenchus agilis* was more closely related to *P. brachyurus* and *P. pseudocoffeae* than to *P. scribneri.* In *P. scribneri* there were twice the number of nucleotide differences compared to these other species, and an apomorphic C at conserved position 38 in the sequence. The sequence in *P. brachyurus* was identical except for 2 deletions compared to *P. pseudocoffeae* (Fig. 1).

The position of *P. zeae* was not clear. It was related to *P. neglectus* within a basal polytomy from a simple N-J analysis of all characters (support value 73% Fig. 2), but when ambiguous positions were excluded, *P. zeae* joined an intermediate, temperate branch to become an outgroup to *P. hexincisus* in M-P and Q-P analyses (<50% support values) (Figs. 3 and 4). In the M-P tree, *P. zeae* fell immediately outside *P. crenatus* (Fig. 3).

DISCUSSION

Selection of outgroup taxa may significantly affect the best ingroup tree topology (Milinkovitch and Lyons-Weiler, 1998; Baldwin *et al.*, 1997). By inspection, we found the outgroup taxa *Heterorhabditis bacteriophora, Teratorhabditis palmarum* and *Acrobeloides bodenheimeri* provide a tree topology more in line with classical morphological characters for *Pratylenchus* and related genera than the combination of *Meloidogyne javanica, Xiphinema index* and *Heterorhabditis bacteriophora.* These last outgroup taxa resulted in a polyphyletic *Pratylenchus* tree where *P. neglectus* floated as an outgroup to other *Pratylenchus* spp., *N. aberrans, R. similis* and *H. belli* (Al-Banna *et al.*, 1997). A very phylogenetically distant outgroup such as *Xiphinema index* may excessively increase homoplasy in the data set. Also a taxon such as *Meloidogyne javanica* that is not clearly an outgroup to the other parasites may increase uncertainty (Forey *et al.*, 1992). In these trees, good bootstrap support was provided for *R. similis* as a close outgroup to *N. aberrans, H. belli,* and *Pratylenchus* spp. when *H. bacteriophora* was used as a single distant outgroup to construct an initial tree (Fig. 2, and trees not shown). To further reduce homoplasy, *R. similis* was then designated as the outgroup in both M-P (Fig. 3) and Q-P (Fig. 4) trees when ambiguously-aligned characters were removed. *Radopholus similis* was also the outgroup used to root the D2-D3 sequences of *P. coffeae* populations and relatives (Duncan *et al.*, 1999). *Nacobbus aberrans* and *H. belli* remained within the *Pratylenchus sensu lato* group in these trees only when ambiguously aligned characters were not excluded (Fig. 2 and trees not shown). Outgroups *Radopholus* spp., *Nacobbus* spp. and *Hirschmanniella* spp. have 4 lip annules compared to *Pratylenchus* spp. with 2, 3 or occasionally 4 (Table 1). Both *Radopholus* spp. and *Nacobbus* spp. have a dorsal esophageal overlap compared to *Hirschmanniella* spp., which shares a ventral esophageal overlap with *Pratylenchus* spp. (Loof, 1990; Al-Banna *et al.*, 1997). Other molecules and taxa in the future may confirm the close relationship of *H. belli* to *Pratylenchus sensu lato.* For example, *Pratylenchus morettoi* is morphologically similar to *Hirschmanniella* except for its one-armed gonad (Luc *et al.*, 1986). The genetic change involved in switching between a two to one-armed gonad is not as developmentally complex as once believed (Horvitz and Sternberg, 1982). The closest relative to *H. belli* in this data set, *P. teres*, has a distinctly more anterior vulva position (69-78%) than most other *Pratylenchus* spp., approaching the 50-60% position of *H. belli* (Table 1).

Character information from Table 1 and molecular information from the trees in Figs. 2-4 indicate a gradual progression from four lip annules to two. The best-supported group is essentially tropical, as noted before (Al-Banna *et al.*, 1997) and all its members have 2 lip annules. Since the original tree was constructed (Al-Banna *et al.*, 1997), *P. penetrans* moved into a well-supported intermediate temperate group that included the new sequences for *P. fallax*, *P. convallariae*, *P. arlingtoni* (Handoo *et al.*, 2001) and *P. hexincisus.* This intermediate group (Figs. 3 and 4), including *P. zeae* at its base, is the rough equivalent of the "*pratensis* Group" (Frederick and Tarjan, 1989), except for the inclusion of *P. hexincisus* with 2 lip annules and *P. penetrans.* The basal groups still had more temperate representatives, although tropical species such as *P. teres* and *P. zeae* existed as well. Besides the 3 lip annules and generally shorter esophageal overlap, the basal and intermediate taxon groups included nematodes with 6 or more lateral lines (*P. arlingtoni*, *P. hexincisus*, *P. crenatus*, *P. teres*) or crenate tail tips (previous 3 species, plus *P. fallax*, and *P. convallariae*) except for *P. penetrans.* These characters were generally not found in the tropical, 2-annules group of nematodes (Handoo and Golden, 1989), with the exceptions of the crenate tail of *P. gutierrezi* and the 4-6 lateral lines of *P. coffeae* and *P. loosi. Pratylenchus neglectus* and *P. hexincisus* were the only members outside the tropical clade with 2 lip annules, although *P. hexincisus* may have three annules (Loof, 1978). Conversely, although *P. zeae* and *P. teres* have high thermal optima, they did not fall in the tropical tree group. However, warm-climate *P. zeae* with 3 lip annules was positioned outside *P. hexincisus* with 2-3 lip annules (Figs. 3 and 4); both taxa might be interpreted as sister groups outside the tropical tree taxa with 2-annules (Figs. 2 and 3).

There is some correspondence of the molecular tree groups to Groups 1-3 based on SEM head patterns (Corbett and Clark, 1983). Group 1 of Corbett and Clark (undivided face sectors) corresponds to the tropical tree group for *P. brachyurus*, *P. loosi* and *P. coffeae* which have 2 lip annules, but not *P. crenatus* and *P. zeae* (3 lip annules) which occur in the basal tree group. From Group 2 of Corbett and Clark (fused submedial and large lateral sectors), *P. neglectus* and *P. thornei* occur in the basal tree group. From Group 3 of Corbett and Clark (prominent fused submedial and small lateral sectors), *P. penetrans* and *P. fallax* occur in the medial tree group, with *P. vulnus* in the basal tree group.

One application of these preliminary results of overlaying the phylogeny of *Pratylenchus* and related taxa with thermal characterization is to identify closely related thermal opposites for studies using a "recovery from supercooling" assay (Wergin *et al.*, 2000). It may be possible to determine the degree of correspondence of this assay with more time-consuming thermal-optima studies. Identifying related thermal pairs might be helpful in this exercise. Based on these phylogenetic trees, related nematodes with somewhat different thermal preferences include *P. neglectus* (temperate) compared to *P. teres* (tropical), *P. hexincisus* (temperate) compared to *P. zeae* (tropical), *Pratylenchus agilis* (temp-tropical) compared to *P. brachyurus* (tropical), and *P. crenatus* (temperate) compared to *P. vulnus* (temperate-tropical) (Table 1, Figs. 2-4).

One interest we had in the population of *P. teres*, a warm-climate South African cotton pathogen, was in determining its position among other members of the classical Pratylenchinae. *Pratylenchus crenatus,* a morphologically similar species present in temperate zones, has also been associated with cotton in the literature (Kir'yanova and Krall, 1980), but morphometrics are often misleading for the determination of phylogenetic relationship. This is especially true when few populations have been characterized morphologically as shown by Duncan *et al.*, 1999. It is possible that *P. crenatus* might be a temperate sister to the tropical *P. teres*. Most of the *P. crenatus* in the U.S. have been detected in the northern states that are geographically discontinuous with detections in the Central Valley of California (Norton, 1984). The semi-tropical populations might be transitional between cold-adapted populations of *P. crenatus* and warm-adapted populations of *P. teres*-like nematodes not yet recognized in the U.S. However, the morphological similarities between *P. teres* and *P. crenatus* may be symplesiomorphic or convergent. *Pratylenchus convallariae* from Europe and the northwestern U.S. also share a somewhat similar morphology with these nematodes. We wanted to determine whether *P. crenatus*, *P. convallariae* or some other taxon was more closely related to *P. teres*. The V ratio (length from anterior end to vulva/total body length expressed as a percentage) has generally been a more reliable morphometric measure than the other ratios of de Man (Siddiqi, 1997). The V ratio of *P. convallariae* has an intermediate range between that of the anterior values of *P. teres* and the posterior values of *P. crenatus* (Table 1), although the b ratio was quite different between *P. teres* and *P. convallariae* (Handoo and Golden, 1989).

Pratylenchus teres (V = 69-78%) has one of the most anterior vulval positions of any lesion nematode species (Handoo and Golden, 1989) comparable only to the more precariously positioned *P. zeae* (V = 68-76%) within these trees (Table 1). *Hirschmanniella belli* has an even more anterior vulva (V = 50-60%), which may be related to the presence of the two-armed gonad (Baldwin *et al.*, 1997). A vulva position of less than 74% (the lower limit for other taxa in Table 1) might be considered a symplesiomorphic character for *H. belli* with other *Pratylenchus* species.

The sequence information here did not support the reliability of the similar morphology of *P. teres* and *P. crenatus* or *P. convallariae* for inferring a close phylogenetic relationship. In fact, *P. teres* shared a face pattern (unpublished results) and tree position (Figs. 1-4) with greater similarity to *P. neglectus* (divided Group 2) than to *P. crenatus* (Group 1) (Corbett and Clark, 1983). *Pratylenchus neglectus* and *P. teres* are also sister groups just inside the *H. belli* branch at the base of M-P and Q-P trees (Figs. 3 and 4). More taxa, such as California populations of *P. crenatus* and the likely transitional species *P. morettoi* Luc, Baldwin and Bell, 1986, would be valuable in filling in evolutionary gaps.

In the original diagnosis of *P. agilis*, *P. scribneri* was described as a close relative with a shorter stylet, finer body striae, and lower, but more massive head structure (Thorne and Malek, 1968). Furthermore *P. agilis* was recently synonymized under *P. scribneri* based on similar SEM face view, isozyme pattern and rDNA ITS gel band position (Hernández *et al.*, 2001). The sequences and trees from this study indicate *P. agilis sensu* Golden and Rebois is less closely related to *P. scribneri* than to *P. brachyurus* and *P. pseudocoffeae*. *P. brachyurus* is a parthenogen with an undivided head (Corbett and Clark, 1983) unlike *P. pseudocoffeae* (Inserra *et al.*, 1998). Although *P. gutierrezi* and *P. pseudocoffeae* differ mainly in the length of the pharyngeal overlap, this was not reflected in their tree position.

Taxa with identical sequences included *P. convallariae* and *P. fallax* although their species status is uncertain (Handoo *et al.*, 2001). All trees in this study also supported the close morphological relationships of *P. convallariae* and *P. fallax* to *P. penetrans* within the "pratensis group." Morphologically similar *P. crenatus* is placed a few branches outside *P. arlingtoni* within this "*pratensis group*" in the M-P tree (Fig. 3), although the molecular sisters of *P. arlingtoni* are *P. fallax* and *P. convallariae* (Figs. 2-4, Handoo *et al.*, 2001).

The *P. coffeae* M isolate (originally = *P. musicola*) (Al-Banna *et al.*, 1997) came from the same host (citrus) and locality (Florida) as the molecularly related *P. coffeae* C4 isolate (Duncan *et al.*, 1999). *Pratylenchus musicola* was synonymized under *P. coffeae* based on morphology (Sher and Allen, 1953). *Pratylenchus* sp. U (= putative *P. coffeae*) from Guatemalan coffee (Al-Banna *et al.*, 1997) had the closest molecular relationship to *P. loosi* from tea in Sri Lanka, although it had lower 'a,' 'b,' and 'c' values (Table 2) than *P. loosi* (Duncan *et al.*, 1999). It has been very difficult to identify *P. coffeae*-like populations based on morphometrics without the aid of SEM face patterns or molecular markers (Duncan *et al.*, 1999).

Although the D3 region may have sufficient characters to aid in diagnosis of many taxa, improved phylogenetic resolution will likely occur through supplementing the current information with the D2 and ITS regions of the rDNA molecule.

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