

# The rDNA Internal Transcribed Spacer Region as a Taxonomic Marker for Nematodes<sup>1</sup>

T. O. POWERS,<sup>2</sup> T. C. TODD,<sup>3</sup> A. M. BURNELL,<sup>4</sup> P. C. B. MURRAY,<sup>4</sup> C. C. FLEMING,<sup>5</sup>  
A. L. SZALANSKI,<sup>6</sup> B. A. ADAMS,<sup>6</sup> AND T. S. HARRIS<sup>6</sup>

**Abstract:** The ITS region from a wide taxonomic range of nematodes, including secernentean and adenophorean taxa, and free-living, entomopathogenic, and plant-parasitic species, was evaluated as a taxonomic marker. Size of the amplified product aided in the initial determination of group membership, and also suggested groups that may require taxonomic reevaluation. Congeneric species often displayed identically sized ITS regions, but genera such as *Pratylenchus* and *Tylenchorhynchus* had species with large differences in size. ITS heterogeneity in individuals and populations was identified in several nematode taxa. PCR-RFLP of ITS1 is advocated as a method of taxonomic analysis in genera such as *Helicotylenchus* that contain numerous species with few diagnostic morphological characteristics.

**Key words:** diagnosis, genetics, internal transcribed spacer region, ITS, molecular ecology, nematode, systematics, taxonomy.

The Internal Transcribed Spacer Region (ITS), located between the repeating array of nuclear 18S and 28S ribosomal DNA genes, is a versatile genetic marker. Among eukaryotes, including organisms as diverse as protozoa, plants, vertebrates, and fungi, ITS data have been used in constructing phylogenetic trees, estimating genetic population structures, evaluating population-level evolutionary processes, and determining taxonomic identity. The structure of the rDNA cistron contributes to its wide applicability. The rDNA cistron is divided into domains that evolve at different rates; thus, this region can be used to address diagnostic and evolutionary problems at different levels of divergence. The rDNA is a component of the middle repetitive family of the nuclear DNA genome, and the presence of multiple copies of these genes in the genome facilitate PCR amplification from single juvenile and adult nematodes. The ITS, intergenic

spacer (IGS), and rDNA genes appear to display concerted evolution so that copies of these genes from a single individual tend to be similar to one another, although generally being distinct from those of other species (Elder and Turner, 1995). The application of the ITS to identification has received the most attention by nematologists (Campbell et al., 1995; Cherry et al., 1997; Chilton et al., 1995; Epe et al., 1996; Fallas et al., 1996; Ferris et al., 1993, 1994, 1995; Gasser and Hoste, 1995; Hoste et al., 1995; Ibrahim et al., 1994, 1997; Joyce et al., 1994; Kaplan, 1994; Nasmith et al., 1996; Orui, 1996; Reid, 1994; Stevenson et al., 1995; Szalanski et al., 1997; Thiery and Mugniery, 1996; Vrain et al., 1992; Vrain and McNamara, 1994; Wendt et al., 1995; Zijlstra et al., 1995, 1997). The majority of these studies have focused upon agriculturally important plant-parasitic species, animal parasites, or beneficial insect parasites. Yet, to the best of our knowledge, there is not a single nematode species that has failed to provide an amplification product of the ITS region when amplified with "universal" PCR primer sets. Universal amplification coupled with the ability to amplify ITS from individual nematodes suggests that any species, population, or ecological community of nematodes can be analyzed using a molecular approach based on the rDNA ITS region (Vrain and McNamara, 1994). A standardized taxonomic marker would be particularly useful when populations contain a

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<sup>2</sup>Associate Professor, Department of Plant Pathology, 406 Plant Sciences Hall, University of Nebraska, Lincoln, NE 68583-0722.

<sup>3</sup>Nematologist, Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, KS 66506.

<sup>4</sup>Senior Lecturer and Graduate Student, Department of Biology, St. Patrick's College, Maynooth, Co. Kildare, Ireland.

<sup>5</sup>Zoologist, Department of Agriculture of Northern Ireland, Newforge Lane, Belfast, United Kingdom.

<sup>6</sup>Postdoctoral Research Associate, Graduate Student, and Research Technologist, Department of Plant Pathology, University of Nebraska—Lincoln.

E-mail: tpowers@unlinfo.unl.edu

large number of juvenile stages, when sexes are dimorphic, or when unfamiliar nematodes are encountered. In ecological studies, PCR-RFLP profiles of ITS from individual nematodes may be one method to assess nematode diversity in samples as well as provide critical taxonomic characters useful for species comparison and identification. In this study we have evaluated the diagnostic utility of the ITS region from a wide taxonomic range of nematodes, including representatives of both Secernentea and Adenophorea, among free-living, insect and plant-parasitic species. We demonstrate that the size of the amplified ITS product aids in the initial determination of group membership, and we investigate the occurrence of ITS heterogeneity in nematode populations and individuals and discuss its taxonomic implications.

#### MATERIALS AND METHODS

**Nematode samples:** Cephalobid and rhabditid nematodes were obtained from the *Caenorhabditis* Genetics Center at the University of Minnesota. *Steinernema* and *Heterorhabditis* spp. were maintained in the laboratory at the University of Nebraska.

**PCR-RFLP:** Amplification of the product including the entire ITS1, 5.8S rDNA gene, and ITS2 region was conducted using the primers described in Vrain et al. (1992).

This primer set consists of one primer, designated as rDNA2 (5'-TTGATTACGTC-CCTGCCCTTT-3'), located in the 3' portion of 18 S, the small ribosomal subunit gene, approximately 190 bp from its junction with ITS1, the first internally transcribed spacer. The second primer, designated as rDNA2.144 (5'-GTAGGTGAACCTGCAGATGGAT-3'), is located in the 5' portion of 28 S, the large ribosomal subunit gene, approximately 80 bp from the junction with ITS2, the second internal transcribed spacer. Between both spacers is the 5.8 S ribosomal gene that is generally around 155 bp in length. The ITS1 amplification procedure in this research used the 18 S primer of Vrain et al. (1992), rDNA2, together with a primer located in the first 20 bp of the 5.8 S gene flanking ITS1 (Cherry et al., 1997), designated as rDNA1.58s (5'-ACGAGCC-GAGTGATCCACCG-3').

PCR-RFLP was performed according to previously described methods (Cherry et al., 1997; Powers and Harris, 1993).

#### RESULTS AND DISCUSSION

**ITS size variation:** The phylum Nemata displays a wide range of ITS sizes. Figure 1 shows amplified product of the entire ITS1 and ITS2 region from representative rhabditid and cephalobid nematodes arranged according to size. The estimated size of the

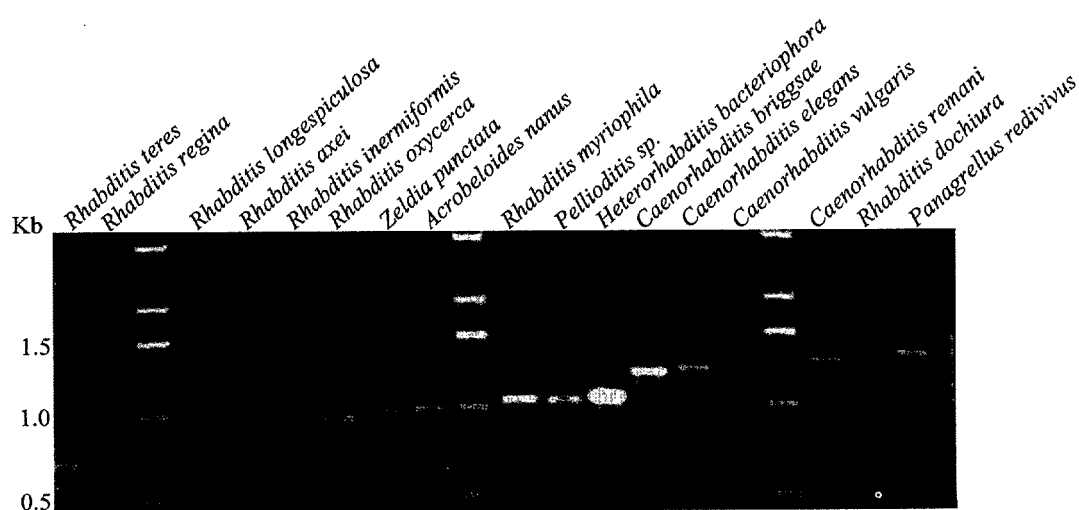


FIG. 1. Amplification of the ITS 1 and 2 regions of rhabditid and cephalobid nematodes.

product ranges from 0.7–1.3 kb. Assuming that the flanking 18S and 28S plus the internal 5.8S ribosomal gene sequences comprise approximately 425 bp of that amplified product, the combined size of ITS1 and ITS2 ranges from 275–875 bp. Slight size variation is observed among the four *Caenorhabditis* species; however, ITS size variation was not detected among seven species of *Heterorhabditis* (Joyce et al., 1994) or five species of *Steinernema* (T. Powers, unpublished). *Rhabditis* species include many ITS size classes (Fig. 1). The existence of size variation among rhabditid genera appears to be correlated with high levels of genetic divergence among members of this group. Nucleotide sequence variation of the 18S gene measured among some of the same rhabditid genera (Fig. 1) were estimated at eight times the level of divergence observed among tetrapod classes (Aves, Amphibia,

Reptilia, Mammalia) (Fitch et al., 1995). Large genetic distances between *Caenorhabditis* spp. also were estimated from a comparison of the mitochondrial cytochrome oxidase subunit II and calmodulin genes (Thomas and Wilson, 1991). The high levels of 18S divergence were presumed to be due to the ancient origin of nematodes, elevated rates of molecular evolution, or a combination of both factors (Fitch et al., 1995). Like 18S nucleotide divergence, ITS size polymorphism (Fig. 1) appears to reflect a high level of genetic divergence among rhabditid and cephalobid nematodes, although the magnitude of the size difference does not necessarily correspond in a linear fashion to the degree of divergence based on 18S analysis.

An equally large range of size variation is observed among the plant-parasitic nematodes. The ITS1 amplification products

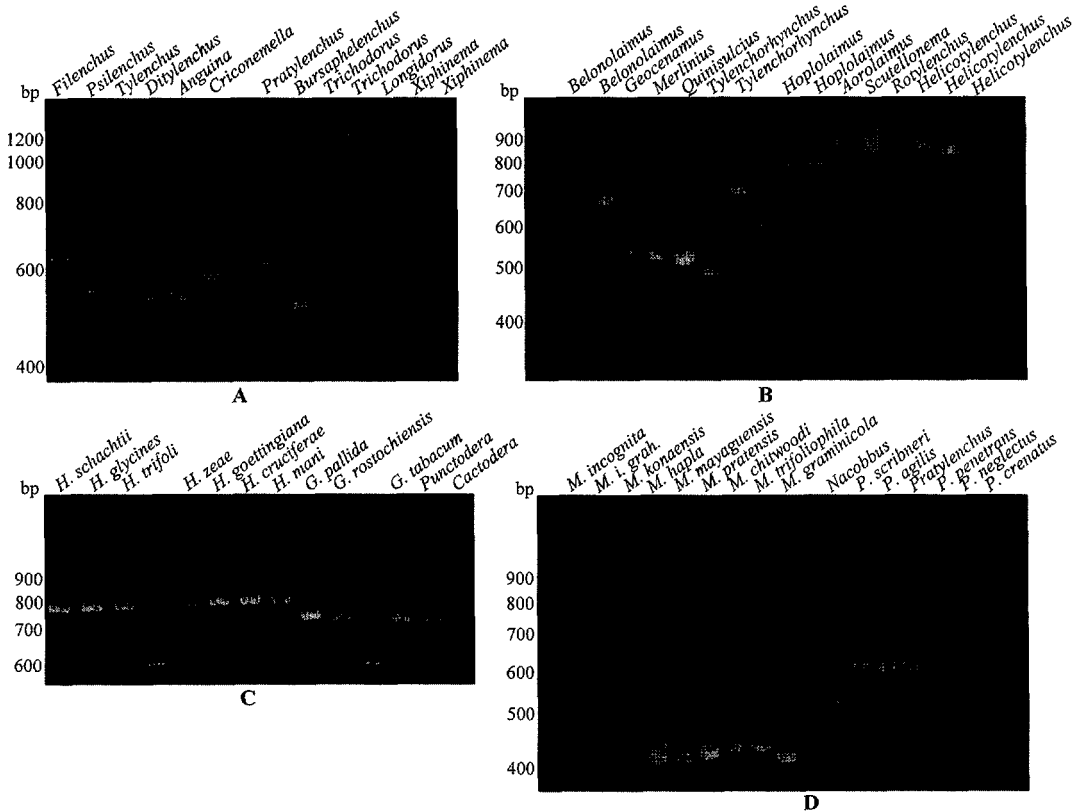


FIG. 2. A) ITS1 size variation among various tylenchid and dorylaimid nematodes. B) ITS1 size variation within Belonolaimidae and Hoplolaimidae. C) ITS1 size variation within Heteroderidae. D) ITS1 size variation among *Meloidogyne*, *Nacobbus*, and *Pratylenchus* species.

	1						60
<i>M. arenaria</i>	TTTGTATGGAA	ACCAATTTAA	TCGCAGTGGC	TTGAACCGGG	CAAAAGTCGT	AACAAGGTAG	
<i>M. incognita</i>	.....	.....	.....	.....	.....	.....	
<i>M. javanica</i>	.....	.....	.....	.....	.....	.....	
<i>M. chitwoodi</i>	.....	.....	.....	.....	.....	.....	
<i>M. hapla</i>	.....	.....	.....	.....	.....	.....	
	61						120
<i>M. arenaria</i>	CTGTAGGTGA	ACCTGCTGCT	GGATCATTAC	--TTTATGTG	ATGTTC--AA	ATTTGAATT-	
<i>M. incognita</i>	.....	.....	.....	--.....	.....--..	.....-	
<i>M. javanica</i>	.....	.....	.....	--.....	.....--..	.....-	
<i>M. chitwoodi</i>	.....	.....	.....C	--T.....	.....AA.T	T.....A	
<i>M. hapla</i>	.....	.....	.....C	--T.....	.....--..	...C...-A	
	121						180
<i>M. arenaria</i>	--CGCAA-TG	AAATGAT--C	GTTGTGAAAC	GGCTGTCGCT	GGTGTCTAAG	TGTTGCTGAT	
<i>M. incognita</i>	--.....-..	.....--..	.....	.....	.....	.....	
<i>M. javanica</i>	--.....-..	.....--..	.....	.....C...	.....	.....	
<i>M. chitwoodi</i>	TA....C---	..T.-----	-G....T...	.....C...	.....G.	.....	
<i>M. hapla</i>	GT.T....---	CGT.T--AT.	.....-..	.....	.....G.	.....	
	181						240
<i>M. arenaria</i>	ACGGTGTGTA	ACGTCCGTGG	CTGTATATGT	GGTGACATGT	TAGGACTCT-	-----AAT	
<i>M. incognita</i>	.....	.....	.....	.....	.....-	-----..	
<i>M. javanica</i>	.....	.....	.....	.....	.....-	-----..	
<i>M. chitwoodi</i>	T.A.....	G.....A	...A....A	.....	.....T..CT	TTATAAG...	
<i>M. hapla</i>	T.R.C...C.	.....	...A....A	.....	.....--CT	T-----..	
	241						300
<i>M. arenaria</i>	-GAG-TTAAG	ACCTAATGAG	CCTCTTAAGT	GAGGCCGCCA	GCAACCTTTT	TTT-TCTCTA	
<i>M. incognita</i>	---.....	.....	.....	.....	.....	.....	
<i>M. javanica</i>	---.....	.....	.....	.....	.....	.....	
<i>M. chitwoodi</i>	C.---.....	..T.....	.....	...A.....	...C..A...	...CA-AA..	
<i>M. hapla</i>	C.G.T.....	..T.....	.....	...A.....	...TA....	...CA-A...	
	301						360
<i>M. arenaria</i>	CA---TTTTA	AAAAAAAA--	---ACTAAAA	TTCTACCCTT	ATCGGTGGAT	CACTAGGCTC	
<i>M. incognita</i>	..---.....	.....--	---.....	.....	.....	.....	
<i>M. javanica</i>	..---.....	.....--	---.....	.....T...	.....	.....	
<i>M. chitwoodi</i>	A.TTT....T	T.TTC....--	---AAT...	..T..G....	.C.....	...C..T..	
<i>M. hapla</i>	---TT....T	..---.....	---..G....	..T..T....	.....	...C.....	
	361						420
<i>M. arenaria</i>	GTGGATCGAT	GAAGAACGCA	GCAAACCTGCG	ATAATTATTG	CGAACTGCAG	AAGTATTGAG	
<i>M. incognita</i>	.....	.....	.....	.....	.....	.....	
<i>M. javanica</i>	.....	.....	.....	.....	.....	.....	
<i>M. chitwoodi</i>	.....	.....	..T.....	.....TA..	T.....	..A.C....	
<i>M. hapla</i>	.....C..	.....	..T.....	.....TG..	.....	..AC.....	
	421						480
<i>M. arenaria</i>	CACAAAAGTT	TTGAACGCAA	ATGGCCGCAT	TGAGGTCAAA	CTCTTTGCAA	CGTCTGGTTC	
<i>M. incognita</i>	.....	.....	.....	.....	.....	.....	
<i>M. javanica</i>	.....	.....	.....	.....	.....	.....	
<i>M. chitwoodi</i>	..T.....	.....T	..T..G....	..G.....	.C....GC.	.....	
<i>M. hapla</i>	..T.....	.....T....	.CT..G...C	..G...AG..	.C....C.	.....	

FIG. 3. Alignment of 18S, ITS1, 5.8S, ITS2, and 28S sequences among five *Meloidogyne* species. Sequence identity is indicated by ".", deletions by "--", and coding regions are italicized.

from 56 nematode species were examined (Fig. 2A–D). The 1.2-kb product of *Trichodorus* is three times the size of the 0.4-kb *Meloidogyne* product (Figs. 2B,2D). The ITS1 size of *Meloidogyne*, exclusive of the flanking

18 S and 5.8 S ribosomal gene sequences, is approximately 215 bp (Fig. 3). This is among the shortest ITS1 sequences known for any eukaryotic organism. Congeneric species often display the same

	481					540
<i>M. arenaria</i>	AGGGTCATTT	TCTCTTATAG	CGGAAGCTTT	AATTTCTATA	A-TGATGTTG	T---T---GC
<i>M. incognita</i>	.....	.....	.....	.....	.....	.....
<i>M. javanica</i>	.....	.....	.....	.....	.....	.....
<i>M. chitwoodi</i>	.....	...TAC.A..	..A.....	T.A..T....	T...T-A..-	ATTG.ATAC.
<i>M. hapla</i>	.....	..TCTA..A..	TAT...-A..	TTA..T...T	T-..CCA..-	-----G..
	541					600
<i>M. arenaria</i>	TTTATA----	--TTTTAAAA	-GGATT----	--TTTGTTTA	----TT--CA	TGTATTAAAT
<i>M. incognita</i>	.....	.....	.....	.....	.....	.....
<i>M. javanica</i>	.....	.....	.....	.....	.....	.....
<i>M. chitwoodi</i>	.....A--	---...-TTC	-T.T..TGA-	TGCAAT....	-----T	..A..-....
<i>M. hapla</i>	AC....A--	AC.....TG	TT.G.ACGCA	GCGA.T.G..	-----A.	..A..-..C.
	601					660
<i>M. arenaria</i>	CTAACTGTGA	AAATCAAACA	A---TTTTGA	CCTGAACTCA	GTCGAGAGCA	CCCGCTGAAC
<i>M. incognita</i>	.....	.....	.....	.....	.....	.....
<i>M. javanica</i>	.....	.....	.....	.....	.....	.....
<i>M. chitwoodi</i>	A.TTG.A.C-	....ATGCTT	TATT.....	.....	.....T..	.....
<i>M. hapla</i>	..TT---CG	CTG...C.TT	TA-T.....	.....G....	.....T..	.....
	661				704	
<i>M. arenaria</i>	TTAAGCATAT	CAGTAAGCGG	AGGAAAAGAA	ACTAAATAGG	ATTG	
<i>M. incognita</i>	.....	.....	.....	.....	.....	
<i>M. javanica</i>	.....	.....	.....	.....	.....	
<i>M. chitwoodi</i>	.....	.....	.....	.....C...	.....	
<i>M. hapla</i>	.....	.....	.....	.....	.....	

GenBank Accession Numbers: *M. arenaria* U96301, *M. incognita* U96304, *M. javanica* U96305, *M. chitwoodi* U96302, *M. hapla* U96303.

FIG. 3. Continued

size amplification product. Three *Heterodera* spp. (Fig. 3) show a consistent ITS1 size that is slightly larger than that of *Globodera*, *Punc-todera*, and *Cactodera*. Little or no size variation was observed among congeneric species of the following genera: *Heterodera*, *Glo-bodera*, *Meloidogyne*, *Hoplolaimus*, *Ditylenchus*, *Belonolaimus*, *Trichodorus*, and *Xiphinema*. Both *Tylenchorhynchus* and *Pratylenchus* have species displaying extreme ITS1 size variation (Figs. 2B,2D). ITS size variants have been observed among *Aphelenchoides* (Ibrahim et al., 1994) and *Pratylenchus* (Orui, 1996) spp. When large size variation does occur in a genus, it may signal the need for taxonomic action. ITS size alone, however, is insufficient evidence upon which to base taxonomic reevaluation.

**ITS restriction site variation:** Restriction analyses of PCR-amplified rDNA ITS prod-ucts have been used in the diagnosis of

many species of nematodes that are difficult to identify morphologically. *Meloidogyne hapla* and *M. chitwoodi* can be distinguished by digestion of their ITS products with a number of restriction enzymes (Zijlstra et al., 1995). *Meloidogyne fallax*, a species closely related to *M. chitwoodi*, can be identified by a small length variation in digestion prod-ucts (Zijlstra et al., 1995). Differentiation among *M. arenaria*, *M. javanica*, and *M. incognita* by means of PCR-RFLP profiles has not been achieved (Xue et al., 1993; Zijlstra et al., 1995), and identical copies of the ITS region have been cloned and sequenced from these three *Meloidogyne* species (Figure 3). This sequence identity among the three major mitotic parthenogenetic species is in sharp contrast to sequence divergence observed when those species are compared with *M. hapla* or *M. chitwoodi*. Approximately 16% sequence divergence is found in these

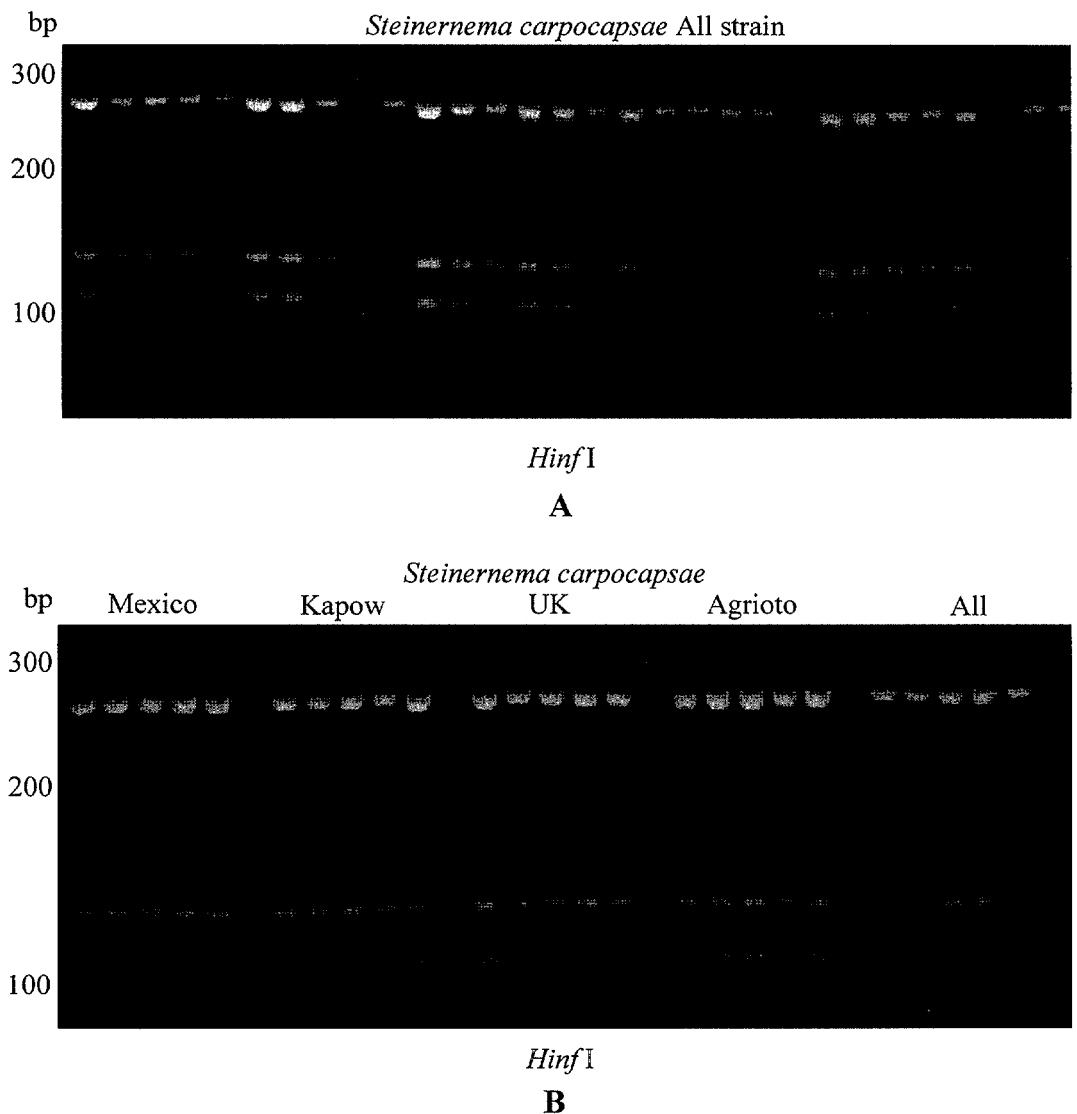


FIG. 4. A) *Hinf* I digest of ITS1 amplicon from *Steinernema carpocapsae* All strain. B) *Hinf* I digest of ITS1 amplicon from *Steinernema carpocapsae* Mexico, Kapow, UK, Agrioto, and All strains.

comparisons, underscoring the large number of discriminating restriction sites available for identification of those species by PCR-RFLP.

*Globodera* and *Heterodera* species have been examined by PCR-RFLP and nucleotide sequencing (Ferris et al., 1993, 1994, 1995; Thiery and Mugniery, 1996; Szalanski et al., 1997). The relatively low level of ITS sequence divergence between *H. glycines* and *H. schachtii*, reported as less than 1.0% dissimilarity (Ferris et al., 1993), is still of sufficient magnitude to allow discrimination by

the restriction enzyme *Fok* I (Szalanski et al., 1997). *Globodera pallida* and *G. rostochiensis* ITS can be separated by several restriction enzymes; however, identification is complicated if *G. "mexicana"* and *G. tabacum* isolates are included in comparisons (Thiery and Mugniery, 1996). French, U.S., and Mexican isolates of *G. tabacum* produced ITS profiles very similar to those of *G. rostochiensis*, whereas isolates identified as *G. "mexicana"* were similar to *G. pallida*. Each of the four taxa could be discriminated by at least one restriction pattern (Thiery and Mugni-

ery, 1996). Laboratory cross-mating between *G. pallida* and *G. rostochiensis* has demonstrated that composite ITS restriction patterns can be produced in hybrid offspring, further complicating interpretation of species status based on ITS restriction patterns alone (Thiery and Mugniery, 1996).

Interspecific ITS comparisons in *Radopholus* have been conducted in *R. similis*, *R. citrophilus*, and *R. bridgei* (Fallas et al., 1996; Kaplan, 1994). Limited variation was observed using six restriction enzymes in a survey of *R. similis* isolates from banana-growing regions around the world, but *R. similis* was readily separated from *R. bridgei* by PCR-RFLP (Fallas et al., 1996). Kaplan (1994) sequenced the ITS region of representative isolates of *R. similis* and *R. citrophilus* and considered the region too highly conserved for the development of diagnostic assays. However, among the seven nucleotide differences recorded between those species in the ITS1 region, one substitution is predicted to result in a restriction site polymorphism; further evaluation of the ITS1 and ITS2 regions of *Radopholus* could produce a taxonomic marker of the desired specificity.

Other plant-parasitic nematodes that have been successfully differentiated by PCR-RFLP of ITS include five species of *Ditylenchus* and eight described species of *Aphelenchoides* (Ibrahim et al., 1994; Wendt et al., 1995). Among insect-parasitic genera, both *Steinernema* (Nasmith et al., 1996; Reid, 1994) and *Heterorhabditis* (Joyce et al., 1994; Nasmith et al., 1996) species display species-specific digestion profiles. At subspecific levels, remarkable consistency is observed within isolates of one of these genera as demonstrated by a Hinf I digestion of ITS1 amplified from 53 infective juveniles representing five strains of *S. carpocapsae* (Fig. 4A,B). This same enzyme differentiates many of the described species of *Steinernema* (T. Powers, unpublished). The consistency in patterns among isolates of *Steinernema* and *Heterorhabditis* species may be due, in part, to homogeneity resulting from founder effects associated with the infection process, inbreeding by sexual stages in the infected cadaver, or, in the case of *Heterorhab-*

*ditis*, hermaphroditic reproduction by first-generation adult females.

Animal-parasitic nematodes for which ITS has shown to be a useful diagnostic tool include *Dictyocaulus* (Epe et al., 1996), *Hypodontus* (Chilton et al., 1995), *Trichostrongylus* (Gasser and Hoste, 1995; Hoste et al., 1995), *Strongylus* (Campbell et al., 1995), and *Haemonchus* (Stevenson et al., 1995).

ITS profiles may be particularly useful in large genera in which taxonomic characters are difficult to interpret. *Helicotylenchus* contained 184 described species in 1991 (Eb-sary, 1991), with 34 North American species (Nematode Geographical Distribution Committee, 1984). The morphological variability and small distinctions among *Helicotylenchus* spp. have practically eliminated dichotomous keys as an option for species identification and have led to the development of a probability-based computer identification system called NEMISYS (Fortuner, 1993). In our survey of *Helicotylenchus* isolates from the Great Plains region of North America, we have found ITS1 patterns correlated with plant hosts such as Hinf I patterns from eight individual nematodes representative of populations from agronomic and native hosts (Fig. 5A). Patterns A1 and A2 have been observed in more than 30 *Helicotylenchus* isolates from corn, soybeans, sorghum, and bluegrass lawns throughout eastern Kansas, Nebraska, and South Dakota. In northeast Nebraska and South Dakota counties bordering Nebraska, five corn fields were found with mixtures of patterns A1-A2 and B. Pattern C has been observed only from corn in western Kansas, whereas patterns D1 and D2 have been recorded from native and agronomic hosts from Florida to Hawaii. Both of these patterns, together with pattern E, are the predominant HinfI patterns found on the remnant, native, tall-grass prairies in the Great Plains of North America. D1 was the only pattern observed in samples taken from St. Augustine grass throughout Florida. D1 also has been recorded from saguaro cactus in Arizona, in culture on jade plant at Cornell University, and in pineapple fields in Hawaii. Pattern F has been observed from *Helicotylenchus* spp.

from bluegrass lawns in Brookings and Sioux Falls, South Dakota.

Species names can be placed only tentatively on the *Helicotylenchus* specimens from which these patterns were derived. For example, pattern F in Figure 5A has been exclusively associated with a *Helicotylenchus* species that conforms morphologically to *H. digonicus* Perry, 1959 and has been collected for molecular studies from localities previously identified by Thorne and Malek (1968). Similarly, many of the cornfield samples contained species that could be morphologically identified as *H. dihystra* (Cobb, 1893) Sher, 1961. Yet, from the PCR-RFLP patterns, it is clear that many populations identified as *H. dihystra* exist as mixtures of genotypes. Since many *Helicotylenchus* species are collections of parthenogenetic lineages, we believe application of species names is ill-advised before a detailed study that links morphological variation with molecular patterns is conducted.

**ITS heterogeneity:** In addition to size variation between species and genera and restriction site variation between genera, species, and populations, nematodes also appear to commonly possess ITS heterogeneity within individuals. Heterogeneous individuals, defined as nematodes containing more than one ITS pattern in their genome, have been reported in *Meloidogyne* (Zijlstra et al., 1995, 1997), *Heterodera* (Szalanski et al., 1997), *Belonolaimus* (Cherry et al., 1997), and have been demonstrated experimentally in hybridization studies between *G. pallida* and *G. rostochiensis* (Thiery et al., 1996). Amplification of ITS with two size classes within an individual could be inferred from two-banded amplification patterns of *Aphelenchoides besseyi* and *A. arachidis* (Ibrahim et al., 1994). Heterogeneity also may be inferred from digestion experiments in which digestion profiles exhibit "extra" fragments relative to a standard profile, without the expected alteration of another fragment in the

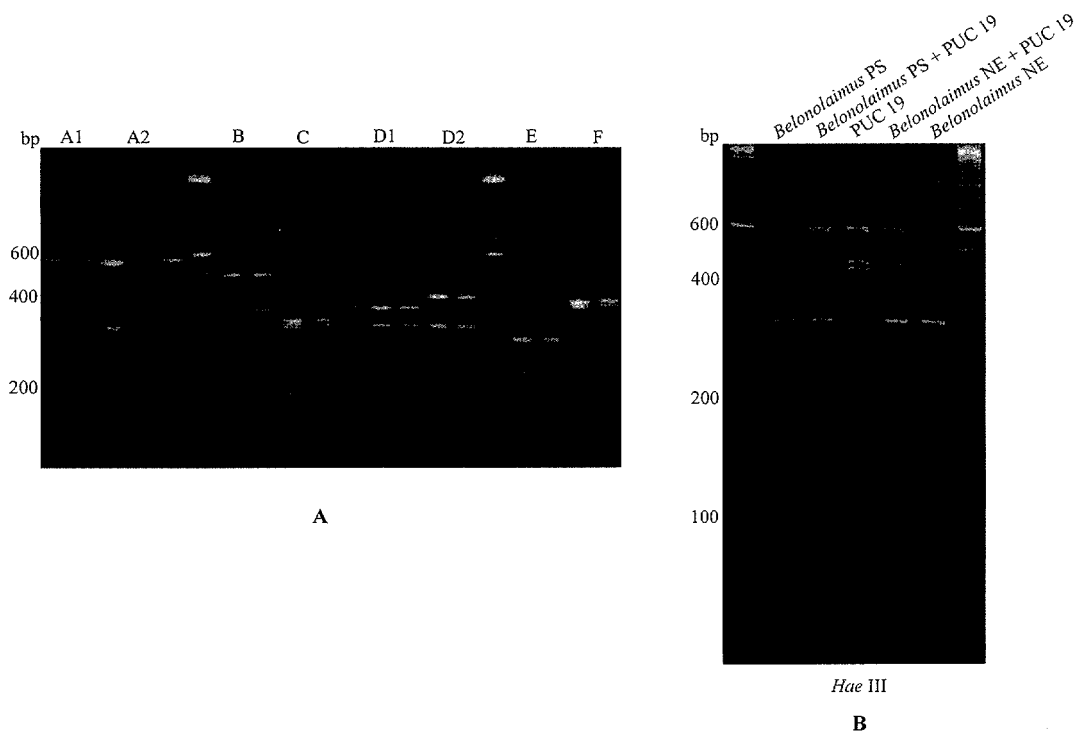


FIG. 5. A) Eight *Hinf* I patterns from individual *Helicotylenchus* nematodes representative of populations from agronomic and native hosts. B) *Hae* III digestion of *Belonolaimus* ITS1 amplified from individuals from Nebraska (NE) and Palm Springs, CA (PS) isolates. Nematode DNA was mixed with PUC 19 vector DNA prior to digestion to evaluate completeness of restriction digestion.

putative heterogeneous profile. These extra fragments also result in inconsistencies between the sum of the estimated size of digestion products and the size of the undigested amplification product, as in two ITS1 amplifications from *Belonolaimus* individuals (Fig. 5B): the Hae III profile of a nematode from Palm Springs, Florida (Lane 2), and the Hae III profile of a nematode from Nebraska (Lane 6). The Nebraska isolate is characterized by extra fragments of approximately 170, 70, 60, and 50 bp. Digestion of plasmid DNA mixed with the ITS1 amplification product (Fig. 5B, lanes 3,5) demonstrated that the additional products were not the result of incomplete restriction digestion. When plasmid DNA was mixed with either *Belonolaimus* isolate, a pattern that represents a mixture of each of the individual DNA digestions was produced. Digestion patterns with extra fragments also can be observed in comparisons of *Radopholus similis* isolates (Fallas et al., 1996). As a taxonomic marker in PCR-RFLP studies, heterogeneity provides added genetic resolution and may contribute information in studies of population structure and gene flow. However, ITS heterogeneity will complicate phylogenetic analyses unless questions of DNA sequence homology can be addressed (Baldwin et al., 1995).

Overall, the ITS region may become an important taxonomic feature in future nematode diagnoses. ITS versatility, specificity, ease of experimental manipulation, and growing ITS databases should accelerate its application in nematology. Its usefulness, however, will hinge on a careful evaluation of the relationship between ITS genetic variation and traditional taxonomic characters.

#### LITERATURE CITED

- Baldwin, B. G., M. J. Sanderson, J. M. Porter, M. F. Wojciechowski, C. S. Campbell, and M. J. Donoghue. 1995. The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82:247-277.
- Campbell, A. J. D., R. B. Gasser, and N. B. Chilton. 1995. Differences in a ribosomal DNA sequence of *Strongylus* species allow identification of single eggs. *International Journal of Parasitology* 25:359-365.
- Cherry, T., A. L. Szalanski, T. C. Todd, and T. O. Powers. 1997. The internal transcribed spacer region of *Belonolaimus* (Nemata: Belonolaimidae). *Journal of Nematology* 29:23-29.
- Chilton, N. B., R. B. Gasser, and I. Beveridge. 1995. Differences in a ribosomal DNA sequence of morphologically indistinguishable species within the *Hypodontus macropi* complex (Nematoda: Strongyloidea). *International Journal for Parasitology* 25:647-651.
- Ebsary, B. A. 1991. *Catalog of the Order Tylenchida* (Nematoda). Ottawa: Research Branch, Agriculture Canada.
- Elder, J. F., and B. J. Turner. 1995. Concerted evolution of repetitive DNA sequences in eukaryotes. *Quarterly Review of Biology* 70:297-320.
- Epe, C., G. von Samson Himmeltjerna, M. Stoye, and T. Schnieder. 1996. Comparative molecular characterization of *Dictyocaulus viviparus* and *Dictyocaulus eckerti*. *Berliner und Münchener Tierärztliche Wochenschrift* 109:227-231.
- Fallas, G. A., M. L. Hahn, M. Fargette, P. R. Burrows, and J. L. Sarah. 1996. Molecular and biochemical diversity among isolates of *Radopholus* spp. from different areas of the world. *Journal of Nematology* 28:422-430.
- Ferris, V. R., J. M. Ferris, and J. Faghihi. 1993. Variation in spacer ribosomal DNA in some cyst-forming species of plant-parasitic nematodes. *Fundamental and Applied Nematology* 16:177-184.
- Ferris, V. R., J. M. Ferris, J. Faghihi, and A. Ireholm. 1994. Comparisons of isolates of *Heterodera avenae* using 2-D PAGE protein patterns and ribosomal DNA. *Journal of Nematology* 26:144-151.
- Ferris, V. R., L. I. Miller, J. Faghihi, and J. M. Ferris. 1995. Ribosomal DNA comparisons of *Globodera* from two continents. *Journal of Nematology* 27:273-283.
- Fitch, D. H. A., B. Bugaj-Gaweda, and S. W. Emmons. 1995. 18S ribosomal RNA gene phylogeny for some Rhabditidae related to *Caenorhabditis*. *Molecular Biology and Evolution* 12:346-358.
- Fortuner, R. 1993. The NEMISYS solution to problems in nematode identification. Pp. 137-163 in R. Fortuner, ed. *Advances in computer methods for systematic biology: Artificial intelligence, databases, computer vision*. Baltimore: Johns Hopkins University Press.
- Gasser, R. B., and H. Hoste. 1995. Genetic markers for closely related parasitic nematodes. *Molecular and Cellular Probes* 9:315-319.
- Hoste, H., N. B. Chilton, and I. Beveridge. 1995. Differences in the second internal transcribed spacer (ribosomal DNA) between five species of *Trichostrongylus* (Nematoda: Trichostrongylidae). *International Journal for Parasitology* 25:75-80.
- Ibrahim, S. K., R. N. Perry, P. R. Burrows, and D. J. Hooper. 1994. Differentiation of species and populations of *Aphelenchoides* and of *Ditylenchus angustus* using a fragment of ribosomal DNA. *Journal of Nematology* 26:412-421.
- Ibrahim, S. K., J. G. Baldwin, P. A. Roberts, and B. C. Hyman. 1997. Genetic variation in *Nacobbus aberrans*: An approach toward taxonomic resolution. *Journal of Nematology* 29:241-249.
- Joyce, S. A., A. M. Burnell, and T. O. Powers. 1994. Characterization of *Heterorhabditis* isolates by PCR am-

plication of segments of mtDNA and rDNA genes. *Journal of Nematology* 26:260–270.

Kaplan, D. T. 1994. Molecular characterization of the burrowing nematode sibling species, *Radopholus citrophilus* and *R. similis*. Pp. 77–83 in F. Lamberti, C. De Giorgi, and D. M. Bird, eds. *Advances in molecular plant nematology*. New York: Plenum Press.

Nasmith, C. G., D. Speranzini, R. Jeng, and M. Hubbes. 1996. RFLP Analysis of PCR amplified ITS and 26S ribosomal RNA genes of selected entomopathogenic nematodes (Steinernematidae, Heterorhabditidae). *Journal of Nematology* 28:15–25.

Nematode Geographical Distribution Committee. 1984. *Distribution of plant-parasitic nematode species in North America*. Hyattsville, MD: Society of Nematologists.

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

Powers, T. O., and T. S. Harris. 1993. A polymerase chain reaction method for identification of five major *Meloidogyne* species. *Journal of Nematology* 25:1–6.

Reid, A. P. 1994. Molecular taxonomy of *Steinernema*. Pp. 49–57 in A. M. Burnell, R.-U. Ehlers, and J. P. Mason, eds. *Genetics of entomopathogenic nematode-bacterium complexes*. Publication EUR 15681 EN. Luxembourg: European Commission.

Stevenson, L. A., N. B. Chilton, and R. B. Gasser. 1995. Differentiation of *Haemonchus placei* from *H. contortus* (Nematoda: Trichostrongylidae) by the ribosomal DNA second internal transcribed spacer. *International Journal of Parasitology* 25:483–488.

Szalanski, A. L., D. D. Sui, T. S. Harris, and T. O. Powers. 1997. Identification of cyst nematodes of agronomic and regulatory concern by PCR-RFLP of ITS1. *Journal of Nematology* 29:255–267.

Thiery, M., and D. Mugniery. 1996. Interspecific rDNA restriction fragment length polymorphism in *Glo-*

*bodera* species, parasites of solanaceous plants. *Fundamental and Applied Nematology* 19:471–479.

Thiery, M., D. Mugniery, D. Fouville, and A. Schots. 1996. Hybridations naturelles entre *Globodera rostochiensis* et *G. pallida*. *Fundamental and Applied Nematology* 19:437–442.

Thomas, K. W., and A. C. Wilson. 1991. Mode and tempo of molecular evolution in the nematode *Caenorhabditis*: Cytochrome oxidase II and calmodulin sequences. *Genetics* 128:269–279.

Thorne, G., and R. B. Malek. 1968. *Nematodes of the northern Great Plains. Part 1. Tylenchida (Nemata: Secernentea)*. South Dakota Agricultural Experiment Station Technical Bulletin 31.

Vrain, T. C., and D. G. McNamara. 1994. Potential for identification of quarantine nematodes by PCR. *EPPO Bulletin* 24:453–458.

Vrain, T. C., D. A. Wakarchuk, A. C. Levesque, and R. I. Hamilton. 1992. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology* 15:563–573.

Wendt, K. R., A. Swart, T. C. Vrain, and J. M. Webster. 1995. *Ditylenchus africanus* sp. n. from South Africa—a morphological and molecular characterization. *Fundamental and Applied Nematology* 18:241–250.

Xue, B. G., D. L. Baillie, and J. M. Webster. 1993. Amplified fragment length polymorphisms of *Meloidogyne* spp. using oligonucleotide primers. *Fundamental and Applied Nematology* 16:481–487.

Zijlstra, C., A. E. M. Lever, B. C. Uenk, and C. H. Van Silfhout. 1995. Differences between ITS regions of isolates of root-knot nematodes *Meloidogyne hapla* and *M. chitwoodi*. *Phytopathology* 85:1231–1237.

Zijlstra, C., B. J. Uenk, and C. H. van Silfhout. 1997. A reliable, precise method to differentiate species of root-knot nematodes in mixtures on the basis of ITS-RFLPs. *Fundamental and Applied Nematology* 20:59–63.