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Phylogenetic Analysis of Nematodes of the Genus *Pratylenchus* Using Nuclear 26S rDNA

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Abstract

We used nucleotide sequences of the large subunit ribosomal genes (26S rDNA) to examine evolutionary relationships among species of the genus *Pratylenchus* (Order: Tylenchida, Family: Pratylenchidae), commonly known as root-lesion nematodes. Ten species of *Pratylenchus* were studied including, *P. penetrans*, P. crenatus, *P. minyus*, *P. vulnus*, *P. thornei*, *P. musicale*, *P. coffeae*, *P. bexincisus*, *P. scribneri*, and *P. brachyurus*. The species *Hirschmanniella belli*, *Meloidogyne javanica*, *Heterorhabditis bacteriophora*, *Nacobbus eberruns*, *Radopholus similis*, and *Xiphinema index* were used as outgroups. Based on parsimony analyses of approximately 307 aligned nucleotides of the D3 expansion region of the 26S rDNA, it is clear that species of *Pratylenchus* are a paraphyletic assemblage. The outgroup taxon *H. belli* shares a common ancestor with the clade that includes *P. vulnus* and *P. crenatus* while *N. aberrans* and *R. similis* share a common ancestor with 5 other species included in this study.

Introduction

Nematodes of the genus *Pratylenchus* Filipjev, 1936 are common endoparasites of plants world-wide. Second only to root-knot nematodes in causing economic losses in agricultural systems (Sasser and Freckman, 1987), these nematodes are migratory parasites that invade the cortex of roots, tubers, and bulbs of plants, resulting in necrotic lesions. This causes reduction of yields (Lownsberry, 1956; McKenry, 1989) or lowering of the market value of crops (Bernard and Laughlin, 1976), Filipjev (1936) first erected the genus *Pratylenchus* when he recognized the group of nematodes possessing a tylenchoid esophagus overlapping the anterior por-tion of the intestine and a uniovarial gonad in adult females. Ninety species of *Pratylenchus* have been described worldwide (Jatala and Bridge, 1990); however, Loof (1991) recognized only forty-six. These nematodes typically have wide host ranges and are highly variable in their pathogenicity (Pinochet *et al.*, 1993; Wheeler and Riedel, 1994).

Species in this genus are typically distinguished from each other by several characters such as number of annuli in the lip region, presence or absence of a spermatheca in the female, presence or absence of males, number of lines in the lateral field, and the shape of the tail (Handoo and Golden, 1989; Loof, 1991).

Morphologically, *Pratylenchus* species are differentiated from the other 8 genera in the family Pratylenchidae by the number of female gonads (one vs. two), whether or not the caudal alae reach to the tip of the tail, sexual dimorphism, presence or absence of deirids, and by the direction of overlap of the esophageal glands over the intestine (Luc, 1987). These diagnostic features applicable at the species or genus level are not based on presence of synapomorphies, as determined by careful studies of ancestor-descendant relationships (i.e., studies of phylogeny), but on phenetic or morphological similarities. In this group of nematodes, multistate characters commonly have been used to classify nematodes to one genus or another. However, species in one genus (as defined by past researchers) may actually have all states present. For example, the direction of overlap of the esophageal gland over the intestine (dorsal, ventral, or lateral) is used as a diagnostic character to define all genera in the family Pratylenchidae. However, several species, each with a different state of this character (esophageal gland overlap) have been included in one genus (*Pratylenchoides*). Therefore, we decided to perform a phylogenetic analysis on species of the genus *Pratylenchus* based on ribosomal DNA (rDNA) sequences. Evolution of rDNA is relatively independent of changes in morphology, and analysis of these genetic data have been shown to provide good phylogenetic resolution (Nadler, 1992; Heise *et al.*, 1995). In fact, several

recent studies of eukaryotes used rDNA sequences in phylogenetic analyses to make strong inferences of ancestor descendant relationships when analyses of morphological data only resulted in more unanswered questions (Carmean *et al.*, 1992; Sidow and Thomas, 1994). In addition, the analysis of rDNA nucleotide sequences has recently been used to assess phylogenetic relationships among taxa of both higher and lower organisms (Hillis and Dixon, 1991; Sidow and Thomas, 1994; Halanych *et al.*, 1995).

Choosing the appropriate segment of DNA within the genome of an organism is a critical step in any phylogenetic study (Hillis and Dixon, 1991; Derr *et al.*, 1992). The region of choice should have enough variability among the taxa in question to allow an estimation of their historical relationships. This variation must not be too great so as to obscure past ancestor-descendant relationships.

One of the rRNA genes that has been used in mo-lecular systematics is the large subunit rRNA gene (23S in prokaryotes and 26S or 28S in eukaryotes). This rRNA gene has been shown to be useful in estimating phylogeny because it contains regions that evolve slowly and other regions that evolve more quickly. Thus this gene can be used to infer both paleozoic and mesozoic divergences (Hillis and Dixon, 1991; Nadler, 1992). Nadler (1992) used rDNA to examine the evolutionary relationships among animal parasitic nematodes of the families Ascarididae and Anisakidae.

In the present study, phylogenetic relationships of several species of the genus *Pratylenchus* were investigated using nucleotide sequences of the D3 expansion region of 26S rDNA corresponding to the positions 3304-3648 in *Caenorhabditis elegans* (Maupas, 1900) (see Ellis *et al.*, 1986).

Materials and Methods

Collection of Nematode Species.

Nineteen populations representing 10 species of the genus *Pratylenchus* were obtained from various hosts and from different localities from North and Central America (Table 1). Six outgroup taxa were used, including *Hirschmanniella belli* Sher, 1968. *Radopholus similis* (Cobb, 1893), *Nacobbus aberrans* (Thorne, 1935), *Meloidogyne javanica* (Treub, 1885), *Heterorhabditis bacteriophora* (Poinar, 1975), and *Xiphinema index* Thorne and Allen, 1950.

Extraction and Culture.

Establishment of nematodes. Plant parasitic nematodes were extracted from their hosts or from soil using either centrifugation (Niblack and Hussey, 1985) or Baermann funnel techniques (Christie and Perry, 1951). Nematodes recovered from the extractions were either were killed and fixed in buffered formalin (Humason, 1972) or used to establish new cultures. Pure cultures of each species

TABLE 1

Nematode Species Studied with Collection Localities and Hosts

Nematode species	Locality	Host
Pratylenchus brachyurus (Godfrey, 1929)	FL, U.S.A.	*Aster* sp.
P. crenatus Loof, 1960	OR, U.S.A.	*Rubus ditifolius*
P. coffeae (Zimmermann, 1898)	Guatemala	*Coffeea arabica*
P. hexincisus Taylor and Jenkins, 1957	MD, U.S.A.	*Zea mays*
P. minyus Sher and Allen, 1953	Monterey Co., CA, U.S.A.	*Pyrethrum* sp.
P. minyus Sher and Allen, 1953	Siskiyou Co., CA, U.S.A.	*Solanum tubersom*
P. musicola (Cobb, 1919)	HI, U.S.A.	*Aglonema* sp.
P. musicola (Cobb, 1919)	FL, U.S.A.	*Citrus* sp.
P. penetrans (Cobb, 1917)	MD, U.S.A.	*Medicago sativa*
P. penetrans (Cobb, 1917)	Monroe Co., NY, U.S.A.	*Prunus avium*
P. penetrans (Cobb, 1917)	OR, U.S.A.	*Lilium eximium*
P. penetrans (Cobb, 1917)	OR, U.S.A.	*Menta* sp.
P. scribneri Steiner, 1943	Kern Co., CA, U.S.A.	*Vitis* sp.
P. scribneri Steiner, 1943	Wayne Co., NY, U.S.A.	*Solanum tubersome*
P. thornei Sher and Allen, 1953	Yolo Co., CA, U.S.A.	*Vicia faba*
P. thornei Sher and Allen, 1953	Yolo Co., CA, U.S.A.	*Carthamus tinctorius*
P. thornei Sher and Allen, 1953	Yolo Co., CA, U.S.A.	*Triticum aestivum*
P. thornei Sher and Allen, 1953	San Joaquin Co., CA, U.S.A.	*Lycopersicon esculentum*
P. vulnus Allen and Jensen, 1951	Yolo Co., CA, U.S.A.	*Juglans hindsii*
Hirschmanniella belli Sher, 1968	Yolo Co., CA, U.S.A.	*Typha* sp.
Nacobbus aberrans (Thorne, 1935)	NE, U.S.A.	*Beta vulgaris*
Radopholus similis (Cobb, 1893)	FL, U.S.A.	*Citrus* sp.
Meloidogyne javanica (Treub, 1885)	Yolo Co., CA, U.S.A.	*Lycopersicon esculentum*
Heterorhabditis bacteriophora Poinar, 1975	UT, U.S.A.	*Phyllophaga* sp.
Xiphinema index Thorne and Allen, *1950*	Napa Co., CA, U.S.A.	*Vitis* sp.

Note: Two individuals from each species from each host or locality sequenced.

of *Pratylenchus* were maintained on root ex-plants (Huettel and Robois, 1985). *Hirschmanniella* and *Meloidogyne* were maintained on potted plants in the greenhouse at UC, Davis. *Heterorhebditis becteriophora*, an entomopathogenic nematode was recovered following Gardner *et al.* (1994). *Nacobbus aberrans* and *R. similis* were obtained as specimens that were either fixed in ethanol (for analysis of rDNA) or TAF (formalin 7%, triethanolamine 2%, water 91%) for analysis of morphology (Courtney *et al.*, 1955). Permanent mounts were made of nematodes fixed in buffered formalin or TAF following Seinhorst (1959). Species of nematodes were identified using both diagnostic keys and original descriptions. Voucher specimens and samples of the amplified DNA and frozen individuals of each species were deposited at the University of California at Davis Nematode Collection (UCDNC) Nos. 3276-3291 and at the University of Nebraska State Museum, Lincoln, Nebraska, nos. 38797-38844. The sequences of these regions (D3 expansion region of 26S rDNA) for the nematodes that were examined were deposited in the GenBank database with the following accession numbers: *Hirschmanniella belli*, U47556; *Heterorhabditis bacteriophora*, U47560; *Meloi-dogyne javanica*, U47559; *Nacobbus eberrens*, U47557; *Pratylenchus brachyurus*, U47553; *Pratylenchus crenetus*, U47549; *Pratylenchus coffeae*, U47552; *Pratylenchus hexincisus*, U47554; *Pratylenchus minyus*, U47548; *Pratylenchus musicale*, U47555; *Pratylenchus penetrans*, U47546; *Pratylenchus scribneri*, U47551; *Pratylenchus thornei*, U47550; *Pratylenchus vulnus*, U47547; *Radopholus similis*, U47558; *Xiphinema index*, U47561. See Table 1 for a list of these species, localities from which they were collected, and associated host plants.

DNA amplification, purification, and sequencing.

The D-3 expansion region of the 26S rDNA fragment (345 bp) was directly amplified by the polymerase chain reaction (PCR) from individual females of each species. Two separate PCR runs were conducted for each species analyzed using one female each time. The primers for the amplification of the fragment were: D3A 5′-GAC CCG TCT TGAAAC ACG GA-3′, and D3B 5′-TCG GAA GGA ACC AGC TAC TA-3′. These primers were designed by W. Kelly Thomas of the School of Biological Sciences, University of Missouri, Kansas City, based on comparison of sequences of rhabditoid nematodes with *C. elegans*. Amplification was conducted using a 50-µl reaction mixture containing a single macerated nematode, 600 nM each of two primers, 250 µM of each dNTP, 2.5 units of Taq DNA Polymerase with the provided buffer (Fisher Scientific), and 2.5 mM $MgCl_2$. The PCR conditions were denaturation at 94°C for 5 minutes followed by 35 cycles of 94°C for one minute, 62°C for one minute, and 72°C for one minute, and an additional 7 minutes at 72°C. A positive control (10 ng of genomic DNA of *Meloidogyne javanica*) as well as negative controls (H_20) were included in each run. Five microliters of the amplification product were electrophoresed on a 1.7% agarose gel and visualized following staining with ethidium bromide. The remaining product was purified using the Wizard PCR Prep DNA Purification System (Promega). After purification 35 ng of the DNA fragment was sequenced in both directions with the automated cycle sequencing system at the DNA Sequencing Facility, Iowa State University. The same primers were used for both amplification and sequencing. An annealing temperature of 55°C was used for all sequencing reactions.

Sequence alignments and phylogenetic inference.

Nucleotide sequences were aligned (Figure 2) using the pileup option in the University of Wisconsin Genetic Computer Group (GCG) software package. Because there was some ambiguity in alignments of three species, alternative manual alignments were tried: *Xiphinema index* position numbers 35-36, 38-39, 45-47; *Pratylenchus penetrans* position numbers 75-77; *Pratylenchus musicola* position numbers 76-78.

Phylogenetic analyses were conducted using the computer program PAUP 3.1.1 (Swofford, 1993). The branch and bound algorithm option in PAUP was used to find the most parsimonious tree (MPT). Aligned sequences with base pair differences were treated as multistate characters. Gaps were treated as missing data and all characters were run unordered with no weighting. Ambiguous areas of alignment including positions 68-80 were analyzed by changing the positions of gaps and by excluding the whole region from one analysis with PAUP. Character state optimization "ACCTRAN" was used in tree descriptions.

Several outgroup taxa were used to polarize the data set. The test of monophyly of the ingroup was conducted by specifying only one taxon as an outgroup (*Xiphinema index*) and allowing the rest of the outgroups to float (Swofford, 1993).

Tree lengths and consistency indices (CI) were calculated automatically during phylogenetic analyses. Characters that were uninformative (either invariant nucleotides or autapomorphies) in a phylogenetic sense were deleted before calculating the consistency index.

The topology of the most parsimonious tree was evaluated by several methods: g1 statistics, bootstrapping, and the decay index. Tree length asymmetry was determined by evaluating one million trees using the random tree search option in PAUP to calculate the g1 statistic (Hillis and Huelsenbeck, 1992). Support of branches of the most parsimonious tree was evaluated by bootstrap (Felsenstein, 1985) and decay analysis (Hibbett and Vilgalys, 1993). Bootstrapping was conducted using the heuristic search (with branch swapping option) in PAUP on the full data matrix. Five hundred bootstrap replicates were run and a majority rule consensus was computed to provide an estimate of how the data support each clade on the most parsimonious tree (Felsenstein, 1985). The de-

cay index, a measure of the number of extra steps required for a particular clade to collapse as parsimony is relaxed (Hibbett and Vilgalys, 1993), was calculated by ob-taining the semistrict consensus of trees which are one or more steps longer than the shortest tree or by using the tree filtering option in PAUP.

Results

Amplification by PCR of the D3 expansion region of the large subunit 26S rRNA gene for each species of nematode yielded a single PCR product with an approximate length of 345 bp (Figure 1). This showed that our primers amplified only the target DNA. To assess both inter- and intraspecific variation that may exist in the D3 expansion region, we sequenced two individuals from every population examined. Identical sequences were obtained from individuals from each population of the same species.

Final alignments of the DNA sequences before phylogenetic analysis were relatively unambiguous (see Materials and Methods) because of the high degree of sequence conservation in the "stem" of the D3 expansion region (Figure 2). This region can be used for construction of primers because it is highly conserved, with extremely small amounts of variation evident among the metazoa (Ellis *et al.*, 1986).

Among the 16 species of nematodes studied, a total of 307 nucleotides were analyzed with 126 variable sites, 72 of which were phylogenetically informative. Of these 72 sites, 42 (60%) were hypothesized transitions and 30 (40%) were hypothesized transversions.

Parsimony analysis of aligned sequences using the branch and bound algorithm of PAUP 3.1.1 resulted in a single shortest tree with 295 steps. The consistency index (CI) was 0.67 or 0.56, excluding uninformative characters (Figure 3). Minimum branch lengths supporting the internal nodes are shown in Figure 3. Fifty-six unique characters (autapomorphies) were found in this region of which 18 were present within the *Xiphinema* index lineage while 13 were present within the *Heterorhabditis* lineage. The

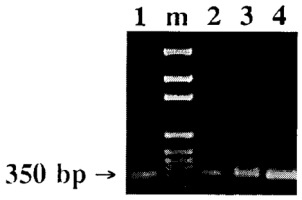


FIGURE 1. Visualization of PCR products using 5 milliliters from a total of 50 milliliters of amplified product representing the D3 expansion region of 26S rDNA of single nematodes from species of *Pratylenchus* and outgroups; 1. *Heterorhabditis bacterio-phora*; 2. *Pratylenchus vulnus*; 3, *Pratylenchus penetrans*; 4. *Xiphinema index*; m. PGEM marker (500 nanograms). The agarose gel (1.7%) is stained with ethi-dium bromide.

sister group *Meloidogyne javanica* was defined by five autapomorphies. Running PAUP with alternative alignments of the data set produced the same tree as in Figure 3. Excluding positions 68-80 produced 29 trees. A majority-rule consensus tree from these data show the same relationships as in Figure 3 except for a polytomy in the middle that included: ((*P. minyus*, *P. tbornei*, *H. belli*) (*P. vulnus*, *P. crenatus*)(*N. eberrens*, *R. similis*)). All other relationships were the same as in Figure 3.

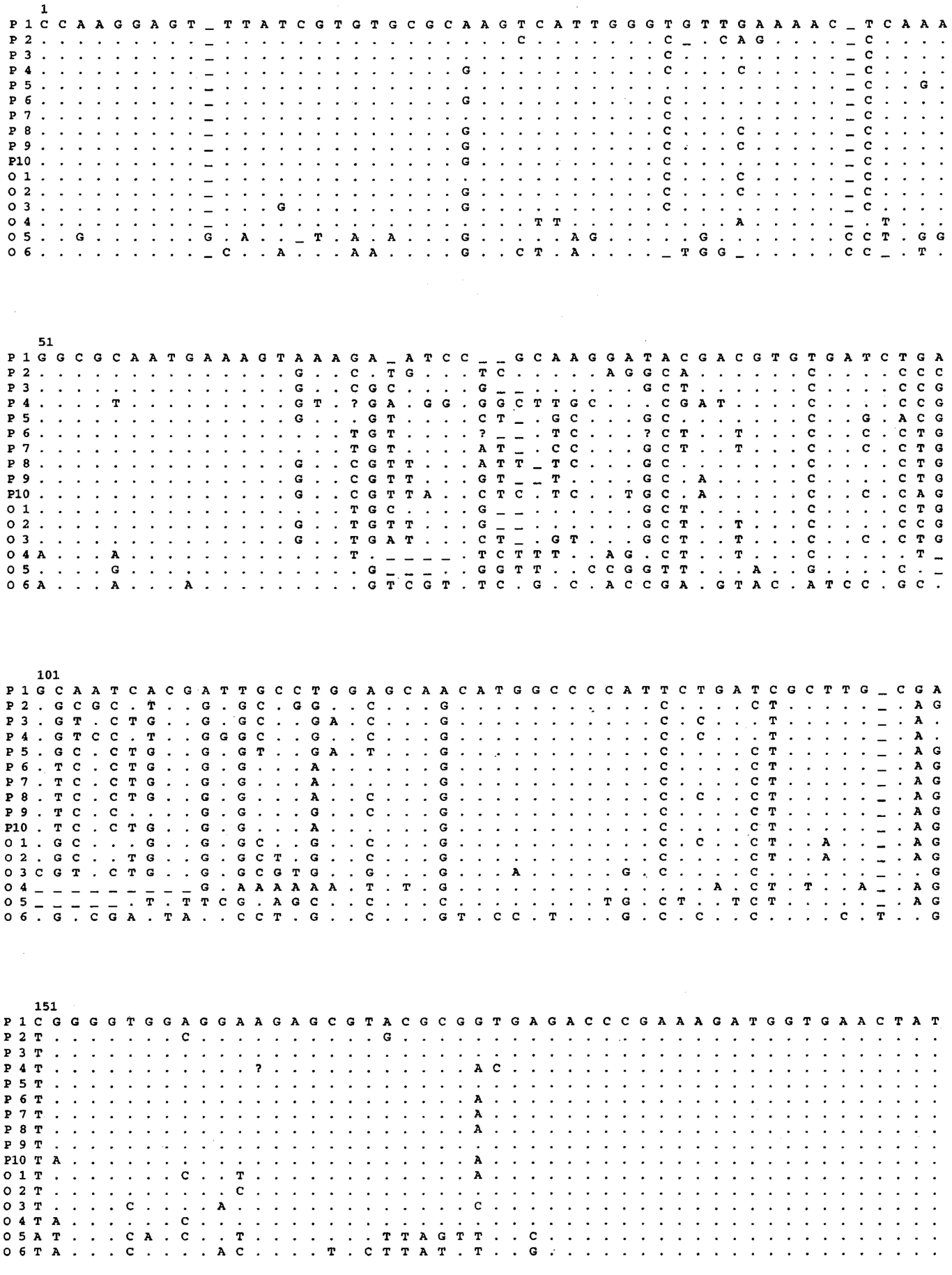
Calculation of the g1 statistic showed that the distribution of trees was appropriately skewed (as compared to a normal distribution) in the negative direction (g1 = -0.54; $P < 0.001$). Bootstrap analysis with 500 replicates resulted in a majority rule consensus tree (Figure 4) with exactly the same topological configuration as the shortest tree (Figure 3) found using branch and bound. Decay analysis showed that support for branches was similar to the result of bootstrap analysis (Figure 4), examination of less parsimonious topologies revealed 11 trees of 296 steps and 78 trees of 297 steps. Most relationships remained unchanged even in trees 3 steps longer than the shortest tree. The longer trees differ from the shorter tree (Figure 3) in placement of the species *Pratylenchus penetrens*, *P. thornei*, *H. belli*, and *R. similis* within the clade defining the Pratylenchidae. Because of limitations in computational resources it was not possible to pursue this analysis to the point at which the most parsimonious tree was totally collapsed.

Our results indicate that *H. belli*, *N. aberrans*, *R. similis*, and the 10 species of *Pratylenchus* studied form a monophyletic group supported by 5 synapomorphies. However, characters associated with species in this clade exhibited many reversals. Three species of nematodes (*P. vulnus*, *P. crenetus*, and *H. belli*; Table 1.)recovered from native plants in western North America formed a clade. Black walnut (*Jugulans hindsih* and cattail (*Typha* sp.) are the type hosts for *P. vulnus* and *H. belli*, respectively.

Pratylenchus thornei, *P. penetrans*, and *P. minyus* branch from the main *Pratylenchus* clade separately (Figure 3) with each sharing a common ancestor with the rest of the species in the tree (Figure 3). This pattern shows that *P. thornei*, *P. penetrens*, and *P. minyus* diverged from the main clade of the Pratylenchidae early in the evolution of these nematodes. The five remaining species of *Pratylenchus* (*P. musicale*, *P. scribneri*, *P. coffeae*, *P. brechyurus*, and *P. hexincisus*) share a common ancestor with both *R. similis* and *N. aberrans* (Figure 3).

Discussion

Our results indicate that the D3 expansion region for *Pratylenchus* is specific to the level of the species and



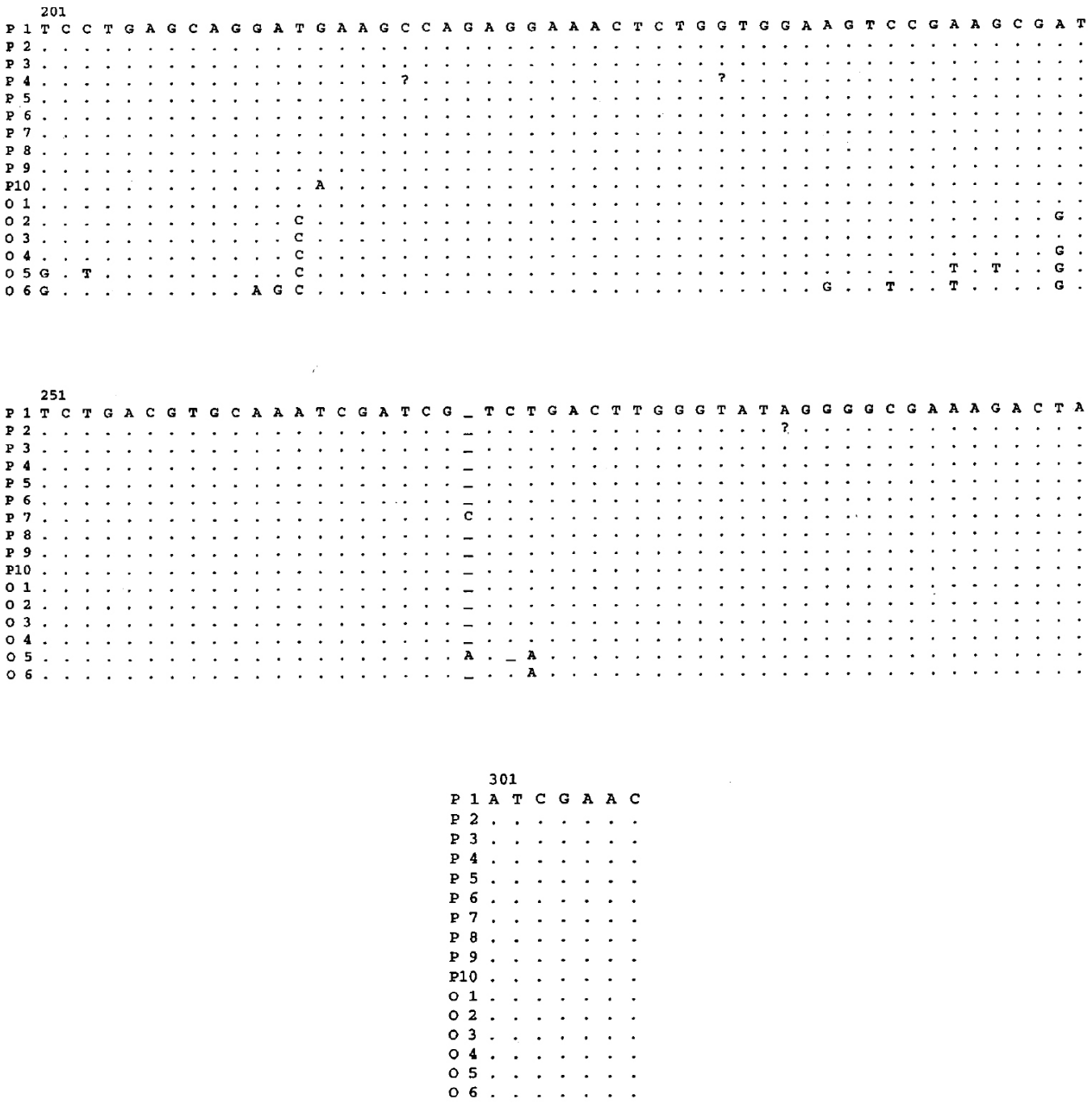


FIGURE 2. Aligned 03 expansion region sequences for each of the 10 species of *Pratylenchus* and outgroups; PI, *Pratylenchus penetrans*; P2, *P. minyus*; P3, *P. vulnus*; P4. *P. crenatus*; P5. *P. thornei*. Po, *P. hexincisus*; P7, *P. brachyurus*; P8, *P. scribneri*; P9, *P. musicola*; Pl0. *P. coffeae*, '01. *Hirschmanniella belli*; 02. *Nacobbus aberrans*; 03, *Radopholus similis*; 04, *Meloidogyne javanica*; 05, *Heterorhebditis bacteriophora*; 06. *Xiphinema index*. Periods (.....) indicate identity with *Pratylenchus penetrans*; Dashes ("-") indicate gaps in sequences; "7" indicates missing data or uncertainty of nucleotide sequence. Position number 1 corresponds to number 3324 of *C. elegans* 26S rRNA gene (Ellis *et al.*, 1986).

does not vary among populations of conspecifics. This specificity may be used to develop DNA probes that may allow researchers to more easily identify *Pratylenchus*. The transition/transversion bias of 60%/40% agrees with most previous analyses of rDNA sequences (Berbee and Taylor, 1992; Nadler, 1992). In our study,transitions and transversions were given equal weight because in the rDNA region of the genome, transitions have been shown to contain considerable phylogenetic information (Brown *et al.*, 1994; Reeder, 1995). Follwing Hillis and Huelsenbeck (1992) it appears that substantial phylogenetic infor-

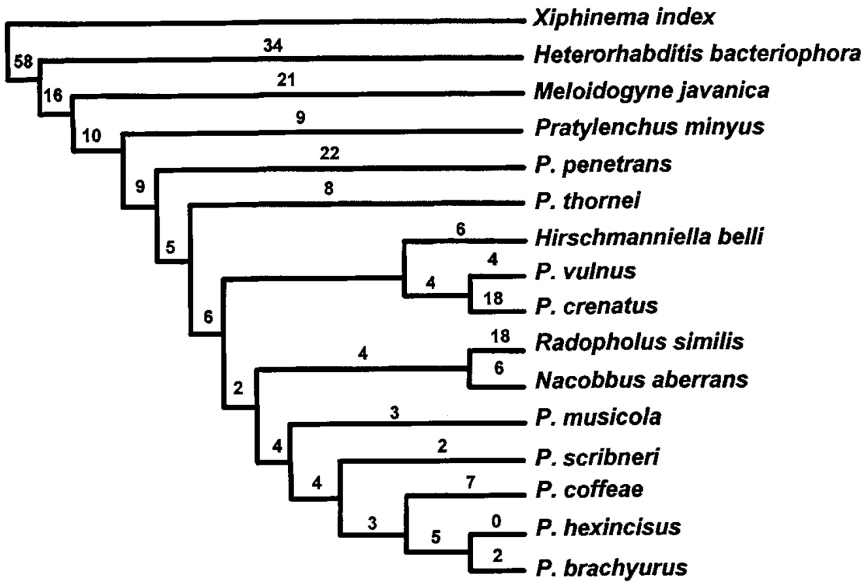


FIGURE 3. Single shortest tree generated from DNA sequences of the 03 expansion region of the 26S rRNA gene of *Pratylenchus* species and outgroups. Tree length, 295 steps: consistency index (excluding uninformative characters). 0.56. Minimum possible branch lengths are shown above each branch.

mation is present in our data-set, based on the value of the g1 statistic (g1 = -0.543, $P < 0.001$).

Although the majority rule consensus tree of the 500 resampled replicates had the same topology as the MPT, certain clades were supported more strongly than others (Figure 4). Low bootstrap values in certain clades could be due to homoplasy, low numbers of synapomorphies defining clades on the MPT, or because of the heuristic search method of estimating the most parsimonius trees of the different resampled replicates.

The cladogram resulting from analysis of the D3 expansion region showed that the ancestor-descendant relationships among the species included in our study were completely resolved (Figure 3). However, our analysis did not support the monophyly of the genus *Pratylenchus*. The species of *Pratylenchus* that were studied formed groups that share common ancestors with *Hirshmanniella belli*, *Nacobbus aberrans*, and *Radopholus similis*. *Hirshmanniella*, *Nacobbus*, and *Radophonlus* belong to the same family (Pratylenchidae) as does *Pratylenchus*, and they are differentiated

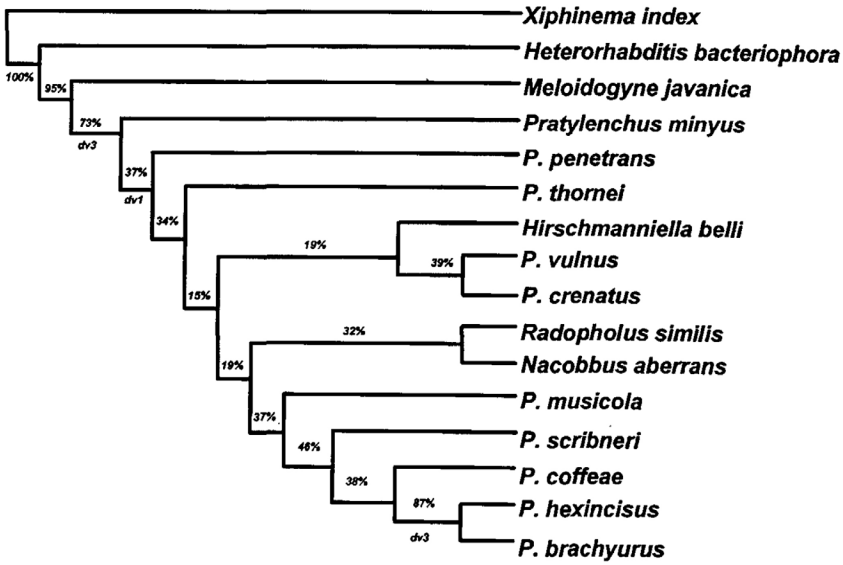


FIGURE 4. Bootstrap majority-rule consensus tree based on the same input data matrix used for Figure 3. Numbers at each node represent the percentage of bootstrap values supporting each internal branch. Decay values (dv) are shown below each internal branch.

Table 2

Characters Differentiating Genera in the Family Pratylenchidae

Character	*Hirschmanniella*	*Nacobbus*	*Pratylenchus*	*Radopholus*
Number of ovaries	2	1	1	1 and 2
Overlapping esophogeal glands	Ventral	Dorsal	Ventral	Dorsal
Male secondary sexual dimorphism	No	No	No	Yes
Caudal alae	Subterminal	Terminal	Terminal	Terminal and subterminal

by morphological characters that have also been used to define many diverse nematode taxa (Table 2). As an example, the character "number of ovaries" (one vs. two) has been used to differentiate genera in the family Pratylenchidae as well as in other families of the Nemata (i.e., Hoplolaimidae, Tylenchidae, Rhabditidae). Several reports indicated that the evolution from two ovaries to one is often convergent (Sternberg and Horvitz, 1982; Baldwin and Cap, 1991). An alternative explanation is that the presence of two ovaries is ancestral in all Nemata and one ovary may be reduced or lost since it may be easier to lose a complex biological function than to develop it several times. It has been shown that two gonads form in the fourth stage juveniles of all species of *Pratylenchus* after which the posterior gonad is reduced (with or without remnants of ovary cells) in the adult females (Roman and Hirschmann, 1969; Luc, 1987).

Our analysis shows that *P. minyus* is the sister taxon to the rest of the Pratylenchidae that were analyzed. This species has the smallest degree of overlap in the esophagus compared with all other species examined in this study. Therefore, the overlap of the esophageal glands over the intestine is probably a primitive character that may be used to infer phylogeny based on morphological characters.

Understanding the phylogenetic relationships among species of nematodes can clarify the bio-geographic history of these nematodes. *Nacobbus aberrans* occurs only in the Americas, mainly in South America (Luc, 1987) and has been reported from Europe only on potted tomatoes in green houses (Franklin, 1959). *Radopholus similis* is found in tropical and subtropical regions of the world (Florida, Australia, India) and is thought to have originated in the South Pacific region (Holdeman, 1986; Luc, 1987). In our analysis, *Nacobbus aberrans* and *Radopholus similis* form a clade that indicates divergence of these two species occurred relatively recently (Cretaceous). The common ancestor of these two species appears to have existed in southern Gondwana when Australia and South America were still in contact via Antarctica with latest contact tentatively estimated to be about 35 million years ago (Pitman *et al*., 1993).

Five species of *Pratylenchus* (*P. musicale*, *P. scribneri*, *P. coffeae*, *P. brachyurus*, and *P. hexincisus*) are included in one clade and are serious pests of many crops (banana, citrus, coffee, corn, pineapple, potato) in tropical and subtropical regions. This group shares a common ancestor with *N. aberrans* and *R. similis*, perhaps indicating an origin in Gondwanaland. Published evidence indicates that these seven species have a distribution primarily among the southern continents (Loof, 1991). The occurrence of these species outside their ranges (assuming that species were identified correctly) is probably due to anthropogenic events.

An expanded study of molecular phylogenetics of nematodes of the family Pratylenchidae including more species would clarify patterns of diversifica-tion of species in this genus and provide more information on which species actually belong in this family and in the genus *Pratylenchus*. Revision of the genera in the family Pratylenchidae is needed as it is clear from the present analysis that the genus, as presently conceived, is paraphyletic. Utilization of molecular data in the revision will be required since morphological characters used in the past may be less robust.

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