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Evaluating the predatory potential of carnivorous nematodes against *Rotylenchulus reniformis* and *Meloidogyne incognita*

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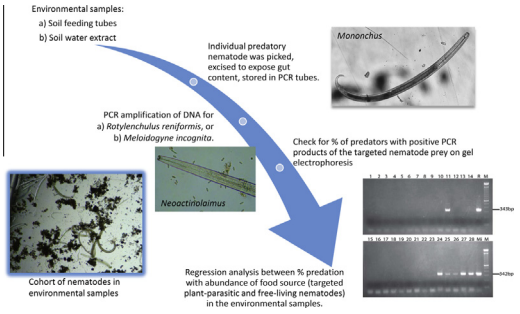
HIGHLIGHTS

- Predatory potential of carnivorous nematodes on target nematodes were assayed using PCR.
- Gut content of 28% of *Mononchus* were tested positive for *Rotylenchulus reniformis*.
- Gut content of 39.9% *Neoactinolaimus* were tested positive for *R. reniformis*.
- Fungivorous or other predatory nematodes distracted the predation of *Neoactinolaimus*.
- *Prismatolaimus*, *Mesodiplogaster* and *Eudorylaimus* also prey on *R. reniformis*.

GRAPHICAL ABSTRACT



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ABSTRACT

Predatory behavior of a nematode is usually determined through gut content observation or prey delimitation counts. In this experiment, *Mononchus* and *Neoactinolaimus* predation of *Rotylenchulus reniformis* or *Meloidogyne incognita* was determined using a PCR-based nematode gut content analysis. Soil samples naturally infested with *Mononchus* were placed in tubes and potential prey nematodes *R. reniformis*, *M. incognita*, or a mixture of both were introduced. The gut contents of *Mononchus* were assayed for the DNA from *R. reniformis* or *M. incognita* using PCR specific primers. A higher % of *Mononchus* tested positive for DNA of *R. reniformis* than for *M. incognita* when the prey were added alone. However, when provided with both prey species, *Mononchus* was tested positive for DNA of *M. incognita* more frequently than for *R. reniformis*. Percent *Mononchus* testing positive for DNA of *R. reniformis* correlated positively with the abundance of *R. reniformis*, but this relationship was not observed between *Mononchus* and *M. incognita*. *Neoactinolaimus* was added to aqueous solution containing a mixture of free-living nematodes and *R. reniformis*. More *Neoactinolaimus* tested positive for DNA of *R. reniformis* than other predatory or omnivorous nematodes in the same samples. Based on regression analysis, the presence of fungivorous and other predatory nematodes in the soil could distract *Neoactinolaimus* from predation on *R. reniformis*. Our results suggested that *Prismatolaimus*, *Mesodiplogasteroides* and *Eudorylaimus* could also prey on *R. reniformis*. Although less than 40% of the predatory or omnivorous nematodes tested preyed on *R. reniformis*, this level of predation could contribute to reducing the population densities of plant-parasitic nematodes in the soil.
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1. Introduction

Predatory nematodes are known to be potential nematode biological control agents. At the beginning of the 20th Century, Cobb (1917) reported the efficiency of *Mononchus* spp. as hunters and advocated their use in biocontrol against plant-parasitic nematodes. Since then, numerous research articles on predatory nematodes have been published and the potential of predatory nematodes as biocontrol agents against plant-parasitic nematodes has been both claimed and doubted (Khan and Kim, 2007). Bilgrami and Jairajpuri (1988) reported the advantages of predatory nematodes over other forms of nematode biocontrol agents, such as nematode-antagonistic fungi or bacteria parasites of nematodes. They claimed that predatory nematodes actively seek prey. However, predatory nematodes are opportunistic feeders consuming free-living nematodes and other micro- or mesofauna in addition to plant-parasitic nematodes. Bilgrami et al. (1986) reported that mononchida fed on several species of plant-parasitic nematodes although free-living nematodes were most abundant in the intestine of mounted specimens. In addition, they described mononchida as generalist predators that feed on rotifers and other invertebrates, and possess cannibalistic tendencies. Understanding the prey preference of specific predatory nematodes will aid in evaluating the biological control potential of predatory nematodes against plant-parasitic nematodes.

Historical data on prey preferences have been gathered by visually examining the gut contents of fixed predatory nematode specimens or from microscopic observations of in vitro cultures of predatory nematodes (Bilgrami et al., 2005). When observing mounted specimens, Bilgrami et al. (1986) reported that mononchida fed on free-living nematodes at a higher rate than dorylaimids although a conclusion could not be drawn on whether a preference existed because prey abundance was unknown. Predation studies conducted in vitro may be altered by variables in the artificial environment. Observations of *Neoactinolaimus agilis* by Khan et al. (1995) demonstrated differences in predation could be due to variations in prey number, temperature, and agar concentration. A thorough review on nematode predation by Mononchida, Dorylaimida, Diplogasteridae, and Aphelenchidae (*Seinura*) revealed that most studies were based on gut content observation under the microscope or by in vitro or greenhouse pot bioassays through prey delimitation counts (Khan and Kim, 2007). Some studies from the field were conducted to demonstrate the negative correlation between abundance of predatory nematodes and plant-parasitic nematodes (Azmi, 1983; Rama and Dasgupta, 1998) to suggest evidence of the impact of predatory nematodes.

A simple PCR assay was developed to analyze gut contents of predatory and omnivorous nematodes by probing for specific prey nematode DNA (Cabos et al., 2013). This PCR-based gut content analysis technique is a reliable method to detect target nematode prey consumed by predatory and omnivorous nematodes from different nematode guilds including *Mononchoides* (Diplogasteridae, P1 guild), *Mononchus* (Mononchidae, P4 guild), *Neoactinolaimus* (Actinolaimidae, P5 guild), *Mesodorylaimus* (Dorylaimidae, O4 guild) and *Aporcelaimellus* (Aporcelaimidae, O5 guild) (Cabos et al., 2013). Although this method allowed detection of prey DNA in predatory or omnivorous nematodes, potential predation against specific plant-parasitic nematodes remains unknown.

The overarching objective of this research was to ascertain the potential of *Mononchus* and *Neoactinolaimus* to prey upon *Rotylenchulus reniformis* or *Meloidogyne incognita* using the PCR-based gut content analysis technique on environmental samples. Specific objectives of the research were to use environmental samples to (i) compare the feeding preference of *Mononchus* towards *R. reniformis* and *M. incognita*, (ii) calculate predation efficiency of *Neoactinolaimus*, and (iii) determine if predation of plant-parasitic nematodes by *Mononchus* or *Neoactinolaimus* was affected by the abundance of free-living nematodes.

2. Materials and methods

2.1. Predation assay for Mononchus

The predation assays were conducted in tubes filled with soil to provide a condition similar to the natural environment. Soil samples naturally infested with *Mononchus* were collected from a taro (*Colocasia esculentum*) farm in Waianae, Oahu, HI. Soil was collected from six different sites in the farm. The soil collected was a Hanalei silty clay with 47.5% clay, 47.0% silt, 5.5% sand, and 4.5% organic matter. Each soil sample was gently mixed and a 100 cm^3 subsample was subjected to elutriation and centrifugal-flotation nematode extraction method (Jenkins, 1964) to estimate the abundance of all nematodes for each tube (Table 1). The remaining field soil was randomly placed into tubes. The tube (Cone-tainer, Hummert International, Earth City, MO) was a 3.75 × 15.2-cm plastic cone-shaped container, filled with 100 cm^3 of the field soil. Three types of tubes were established with the addition of: (i) 200 vermiform *R. reniformis*; (ii) 200 juveniles of *M. incognita*; or (iii) 100 vermiform *R. reniformis* and 100 juveniles of *M. incognita*. Four tubes were established at a time for each feeding type. The added plant-parasitic nematodes and the naturally occurring free-living nematodes served as potential food sources for the *Mononchus*.

Prey nematodes were collected from greenhouse cultures of *R. reniformis* maintained on cowpea (*Vigna unguiculata*) and *M. incognita* maintained on tomato (*Solanum lycopersicum*). Plant roots were removed from the pots, rinsed free of soil and then shaken in 0.5% NaOCl to extract eggs (Hussey and Barker, 1973). The nematode eggs were collected on a 25-μm pore sieve, rinsed, and then place in hatching chambers to collect juveniles (Wang et al., 2001). Freshly hatched nematodes were collected 48 h later, counted, and used as prey.

The tubes were maintained at 24 °C. One tube was randomly selected and emptied into a Baermann tray 1, 2, 3, and 4 days after prey addition to extract live nematodes from the soil. This was to determine if exposure time affected the feeding rate of *Mononchus*. Each Baermann tray incubation lasted for 24 h. Up to 15 *Mononchus* were then individually placed on a glass slide, mounted on an inverted microscope, and cut with a micro-surgical blade to obtain the gut contents. The gut contents for each nematode were stored individually in 10 μl dH_2O in a 200 μl PCR tube. The gut samples were then immediately processed or stored at −20 °C before

Table 1
Average (n = 36) abundance of free-living nematodes present in each soil tube used in the predation assay for *Mononchus*.

Nematodes	Abundance/ 100 cm^3 soil	Nematodes	Abundance/ 100 cm^3 soil
Bacterivores		Fungivores	
Acrobeles	57	*Aphelenchoides*	4
Acrobeloides	9	*Aphelenchus*	47
Cephalobus	23	*Filenchus*	13
Eucephalobus	28	*Tylenchus*	4
Panagrolaimus	2	Total fungivores	62
Prismatolaimus	87		
Rhabditidae	123		
Total bacterivores	332		
Omnivores		Predators	
Aporcelaimellus	10	*Cryptonchus*	2
Eudorylaimus	18	*Mononchus*	15
Paraxonchium	2	Total predators	17
Total omnivores	30		

PCR amplification to detect nematode prey DNA in the gut contents. Not all samples contained 15 *Mononchus*, thus % of *Mononchus* testing positive for prey nematode DNA was calculated for each sample. The experiment was repeated 3 times.

2.2. PCR amplification of target prey DNA in predation assays for *Mononchus*

Primer 3.0 software (http://biotools.umassmed.edu/bioapps/primer3_www.cgi) was used to design PCR primers for *R. reniformis* and *M. incognita*. The primers for *R. reniformis* (ncRenF and ncRenR) were designed from the sequence of the internal transcribed spacer 1 (ITS1) region of *R. reniformis* (Gen Bank# AY335192) submitted by Iwahori and Sano (2003). The sequence of ncRenF is 5′-CGGCT TAATTGCAATGGTTT-3′, whereas that of ncRenR is 5′-AGGGCGCTC ATTGAGTCTT-3′. The reverse and forward primers for *M. incognita*, Mi1 and Mi2, were designed from the ITS region of *M. incognita* as described by Saeki et al. (2003). The sequence of Mi1 is 5′-AAAC GGCTGTCGCTGGTGTC-3′, whereas that of Mi2 is 5′-CCGCTATAAG AGAAAATGACCC-3′. The PCR reactions generated amplification products of 343 and 342 base pairs (bp) of the ITS conserved region of *R. reniformis* and *M. incognita*, respectively.

PCR amplification was conducted in a 25 μl reaction mixture in 200 μl PCR tubes using an ABI thermo cycler (ABI Foster City, CA USA). The reaction mixture consisted of 2.5 μl of reaction buffer, 0.1 μM forward and reverse primers and 10.2 μl of the gut contents of the *Mononchus*. The PCR reaction began with 94 °C denaturation for 5 min followed by 30 cycles of 30 s of denaturation at 94 °C, 30 s of annealing at 55 °C, and 2 min of elongation at 72 °C using high fidelity taq polymerase (Invitrogen, Carlsbad, CA, USA.). After the cycling reactions, the final elongation was performed at 72 °C for 7 min. PCR products were size fractionated on 2% agarose gel stained with ethidium bromide.

2.3. Predation assay for Neoactinolaimus

Neoactinolaimus, originally collected from dracaena (*Dracaena deremensis*), were reared in vitro on ¼ strength corn meal agar (CMA) petri plates infested with non-sterile Rhabditidae nematodes and a sterile carrot disk as an additional carbon source for the bacteria. *Neoactinolaimus* was maintained and subcultured at 3-month intervals under laboratory conditions for 12 months prior to initiation of the predation assay.

Due to the ability of *Neoactinolaimus* to withstand a soil water aqueous solution over a period of time while maintaining viability and feeding integrity, the predation potential was determined by introducing in vitro cultured *Neoactinolaimus* into soil water extracts. These soil water extracts were collected from either potted dracaena 'Lisa' or turmeric (*Curcuma longa*) inoculated with *R. reniformis* for more than 3 months. Soil samples from dracaena or turmeric were placed on Baermann funnels (Walker and Wilson, 1960) for 48 h to collect nematodes. Fifteen soil water extracts of 20 ml each in 50 ml beakers were prepared. All nematodes collected were identified to the genus level and counted with the aid of an inverted microscope. To ensure the presence of *R. reniformis*, 48 additional vermiform stages of *R. reniformis* were added to each beaker. The initial nematode counts in each beaker prior to the introduction of *Neoactinolaimus* are averaged and shown in Table 2.

Fifteen *Neoactinolaimus* obtained from in vitro cultures were added to each beaker and allowed to feed for 1 week. Beakers were maintained covered at 24 °C and aerated once a day. Seven days after the introduction of *Neoactinolaimus*, *Neoactinolaimus* as well as other predatory and omnivorous nematodes (*Eudorylaimus*, *Mesodiplogasteroides*, and *Prismatolaimus*) present were hand-picked, placed on a glass slide, cut to release gut contents, as described for *Mononchus,* and stored at −20 °C in individual PCR tubes. All remaining nematodes in each beaker were then identified and counted to estimate prey delimitation.

Table 2
Average ($n = 15$) abundance of nematodes present in each beaker of water extract used in the study of predation by *Neoactinolaimus*.

Nematodes	Abundance	Nematodes	Abundance
Bacterivores		Fungivores	
Acrobeloides	14	*Aphelenchoides*	8
Alirhabditis	2	*Filenchus*	14
Eucephalobus	30	Total Fungivores	21
Pseudoacrobeles	2		
Prismatolaimus	1	Omnivores	
Rhabditidae	129	*Eudorylaimus*	15
Total bacterivores	177	*Mesodorylaimus*	2
		Total omnivores	17
Herbivores			
Rotylenchulus	94	Predators	
Helicotylenchus	23	*Mesodiplogasteroides*	94
Total herbivores	117	Total predators	94

2.4. PCR amplification of targeted prey in predation assays for *Neoactinolaimus*

The nematodes were subjected to PCR amplification using primers targeted for *R. reniformis*. All predatory or omnivorous nematodes collected from the *Neoactinolaimus* assay were cut on a glass slide as described in the *Mononchus* experiment and stored in PCR tubes individually. Each PCR reaction mixture contained 10.5 μl of the nematode and its gut contents, 12.5 μl GoTaq MasterMix, and 0.4 μM each of the forward and reverse primer. Primers ncRenF and ncRenR were used. The PCR reaction conditions were 95 °C for 2 min followed by 40 cycles of 95 °C for 45 s, 56 °C for 30 s, and 72 °C for 20 s, and a final extension of 5 min at 72 °C. PCR products were separated on a 1.2% agarose gel stained with GelRed in 1 × TAE and observed under UV light. A *R. reniformis* or *M. incognita* positive control and a no-template sterile water control were included in each electrophoresis run.

2.5. Statistical analysis

Percentage of *Mononchus* testing positive for either *R. reniformis*, *M. incognita* or both from each soil feeding tube was calculated. A 3 × 4 (prey types × days of exposure) factorial analysis of variance (ANOVA) was conducted using SAS (SAS Inc, Cary, NC). Subsequently, regression analysis between % of *Mononchus* testing positive for targeted prey DNA and abundance of bacterivorous, fungivorous, omnivorous and predatory nematodes was conducted using Proc GLM in SAS. For *Neoactinolaimus*, % of all predatory and omnivorous nematodes testing positive for DNA of *R. reniformis*, and the % reduction in population densities of the most abundant free-living nematodes were calculated by [(initial − final)/initial] × 100 and compared among genera using one-way analysis of variance. To eliminate potential predation from omnivorous *Eudorylaimus*, data were separated into groups with and without *Eudorylaimus*. Means of genera in each group were separated using a Waller–Duncan k-ratio ($k = 100$) t-test wherever appropriate. In addition, regression analysis between % *Neoactinolaimus* or % *Eudorylaimus* testing positive for DNA of *R. reniformis* and abundance of bacterivorous, fungivorous, herbivorous, omnivorous and predatory nematodes in the initial samples was also conducted using Proc GLM in SAS.

3. Results

This experiment confirmed that nematode prey could be identified from the gut contents of predatory as well as omnivorous nematodes using species-specific PCR primers. Band sizes of 343 and 342 bp were obtained for PCR products of nematodes that were offered *R. reniformis* and *M. incognita*, respectively on an electrophoresis gel as anticipated (Fig. 1).

3.1. Predation assay for Mononchus

Analysis of variance demonstrated that days of exposure did not affect % predation by *Mononchus* of *R. reniformis, M. incognita* or their combination ($P > 0.05$). Hence data from the 4 days of exposure were combined and one-way ANOVA was conducted. The highest predation rates (28%) were seen in *Mononchus* testing positive for DNA of *R. reniformis* when offered only this plant-parasitic nematode as compared to predation on *M. incognita* or the combination of *R. reniformis* and *M. incognita* (Table 3). Percent predation by *Mononchus* was reduced to 15.0% when offered only *M. incognita*. However, when offered *R. reniformis* and *M. incognita* together, a higher percentage of *Mononchus* tested positive for DNA of *M. incognita* (15.7%) than *R. reniformis* (5.6%) based on χ^2 0.05, df = 11 analysis ($P \leqslant 0.05$). Nonetheless, % predation by *Mononchus* on either *R. reniformis* or *M. incognita* was not different among the prey options ($P > 0.05$, Table 3).

Table 3
Percentage of Mononchus with gut contents testing positive for DNA of *Rotylenchulus reniformis*, Meloidogyne incognita, or both.

Prey	% *Mononchus* positive for		
	R. reniformis	*M. incognita*	*R. reniformis* + *M. incognita*
R. reniformis	28.00[z] a	0.00 b	28.00 a
M. incognita	0.00 b	14.96 a	14.96 a
R. reniformis + *M. incognita*	5.60 b	15.71 a	21.31 a

[z] Means are average of 12 replications. Means in a column followed by same letter(s) are not different according to Waller–Duncan *k*-ratio ($k = 100$) *t*-test.

3.2. Predation assay for Neoactinolaimus

The percent of *Neoactinolaimus*, *Prismatolaimus*, *Mesodiplogasteroides*, and *Eudorylaimus* testing positive for DNA of *R. reniformis* were 38.9%, 28.6%, 27.8%, and 20.2%, respectively. However, no difference in the presence of *R. reniformis* DNA was detected among these four nematodes ($P > 0.05$). *Neoactinolaimus* ranked the highest for the presence of DNA from *R. reniformis* whereas *Eudorylaimus* ranked the lowest.

R. reniformis, Helicotylenchus, Rhabditidae, *Eucephalobus, Acrobeloides, Aphelenchoides,* and *Filenchus* were the most common nematodes, and thus potential preys, for the predators. Assuming predation by nematodes was the main contribution to % reduction in prey nematode numbers after incubation, *Aphelenchoides* had the highest % reduction, whereas *Helicotylenchus* and *Eucephalobus* had the lowest % reduction (Table 4). *R. reniformis,* Rhabditidae, *Acrobeloides,* and *Filenchus* had similar reduction rates as *Aphelenchoides* (Table 4). Similar trends in the ranking of % reduction of the nematode prey species were observed between samples with and without *Eudorylaimus*, except that % reduction of *Helicotylenchus* was not different from the other prey ($P > 0.05$). Thus, *Neoactinolaimus* fed on *Helicotylenchus* at similar rates as it fed on *Aphelenchoides,* Rhabditidae, *Filenchus*, and *R. reniformis,* but it did not feed on *Eucephalobus* as indicated by the negative % reduction (Table 4).

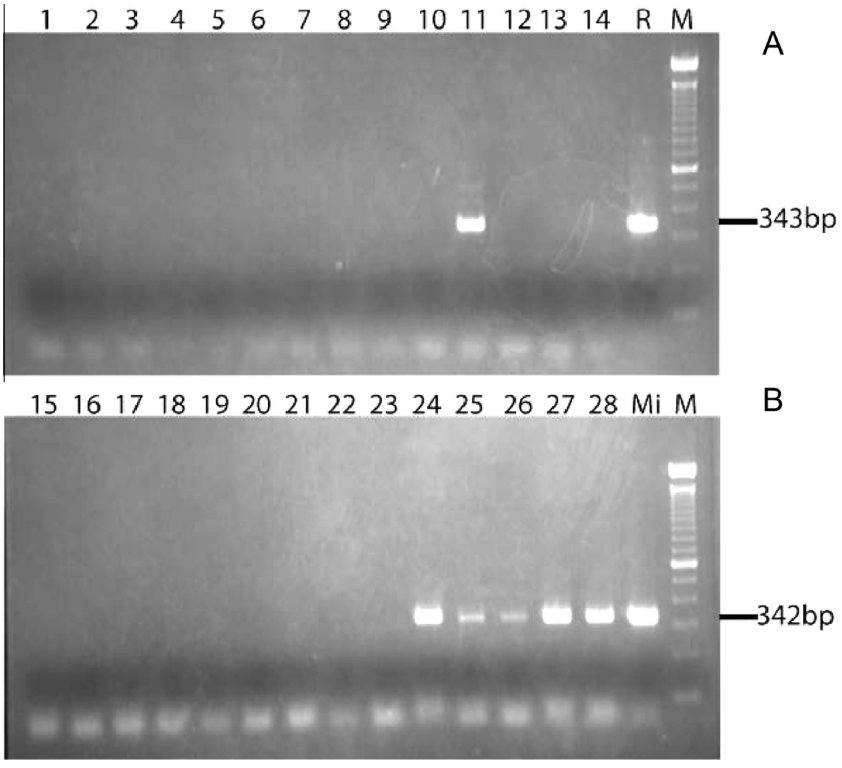

Fig. 1. Electrophoresis gels of PCR products of the gut contents of *Mononchus* amplified (A) by ncRenF/ncRenR primers specific for *Rotylenchulus reniformis* (lines 1–14); and (B) by ncRenF/ncRenR and Mi1/Mi2 (specific for *Meloidogyne incognita*) primers (lines 15–28). *R* = positive control of *R. reniformis*; Mi = positive control of *M. incognita*; and M = ladder marker.

Table 4
Percent reduction of prey nematodes after a 7-day incubation in the presence of *Neoactinolaimus* with and without *Eudorylaimus*.

Prey nematodes	% Reduction of prey nematodes[z]			
	With *Eudorylaimus*	*N*[y]	Without Eudorylaimus	*N*
Aphelenchoides	98.6 a[x]	7	100.0 a	3
Acrobeloides	83.3 ab	7	–	0
Rhabditidae	75.2 ab	10	89.8 a	6
Filenchus	74.4 ab	15	75.0 a	6
Rotylenchulus	48.6 ab	15	56.5 a	6
Helicotylenchus	22.1 b	15	47.0 ab	6
Eucephalobus	19.7 b	15	−30.6 b	6

[z] % reduction of prey nematodes are calculated from abundance of each nematode genus prior to the introduction of *Neoactinolaimus* minus that counted at 7 days after incubation of *Neoactinolaimus*.
[y] Numbers of soil samples containing the particular nematode prey.
[x] Means are average of n replications based on their presence in 15 and 6 soil samples for soil with and without *Eudorylaimus*, respectively. Means followed by same letter(s) in the same column are not different based on Waller–Duncan *k*-ratio ($k = 100$) *t*-test.

3.3. Regression analysis

Percentage of *Mononchus* testing positive for DNA of *R. reniformis* regressed positively with total abundance of *R. reniformis* ($r^2 = 0.20$, $P = 0.007$, df = 35, Fig. 2A), whereas % of *Mononchus* testing positive for DNA of *M. incognita* was negatively related to abundance of *R. reniformis* ($r^2 = 0.21$, $P = 0.005$, df = 35, Fig. 2B). However, if % of *Mononchus* testing positive for DNA of *R. reniformis* or *M. incognita* were combined, no significant relationship between % predation with total abundance and the abundance of *R. reniformis*, *M. incognita*, or that of any nematode trophic group was found ($P > 0.05$).

On the other hand, % of *Neoactinolaimus* testing positive for DNA of *R. reniformis* was negatively related to the abundance of fungivorous nematodes ($r^2 = 0.35$, $P = 0.0123$, df = 16, Fig. 2C). However, no significant correlation was observed between % of *Neoactinolaimus* testing positive for DNA of *R. reniformis* with total nematode abundance or abundance of other nematode trophic groups ($P > 0.05$). Unlike *Neoactinolaimus*, % of *Eudorylaimus* testing positive for DNA of *R. reniformis* was not related with total prey nematode abundance as well as abundance of any nematode trophic group. *Mesodiplogasteroides* was the only indigenous predatory nematode that existed in abundance (Table 2). These samples were also associated with the lowest % of *Neoactinolaimus* testing positive for DNA of *R. reniformis*. As a consequence, predation by *Neoactinolaimus* was negatively related with the abundance of other predatory nematodes in the same samples ($0.06 < P < 0.10$, data not shown).

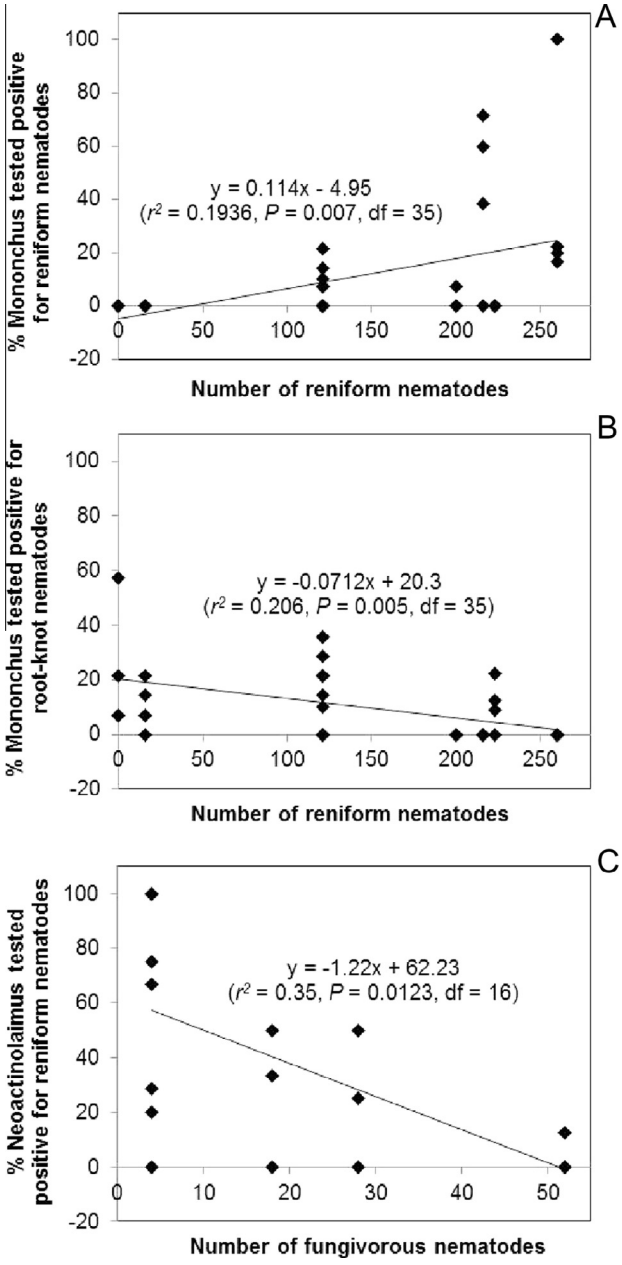

Fig. 2. Regression analysis between (A) % *Mononchus* testing positive for DNA of *Rotylenchulus reniformis* and (B) % *Mononchus* testing positive for DNA of *Meloidogyne incognita* and the abundance of *R. reniformis* in a sample, and between (C) % *Neoactinolaimus* testing positive for DNA of *R. reniformis* and abundance of fungivorous nematodes in a sample.

4. Discussion

Nematode prey can be identified from the gut contents of predatory as well as omnivorous nematodes using species-specific PCR primers. We have demonstrated that *Mononchus, Neoactinolaimus, Prismatolaimus, Mesodiplogasteroides,* and *Eudorylaimus* consume *R. reniformis*. We also demonstrated that *Mononchus* consumes juveniles of *M. incognita*. The use of prey-specific primers allowed for the detection of specific prey nematode in the gut contents of these predatory and omnivorous nematodes. The detection of the prey nematode was direct and did not depend on observation of the prey in the gut, observation of the predator consuming the prey, nor an indirect assessment of the remaining nematode prey population.

The current study also demonstrated that *Mononchus* exhibited prey preference. While predation on *R. reniformis* by *Mononchus* was density dependent, predation of *M. incognita* by *Mononchus* did not follow a similar density dependent curve. Instead, predation of *M. incognita* by *Mononchus* was negatively affected by the abundance of *R. reniformis*. This result suggests that *Mononchus* might have preferred to feed on *R. reniformis*. The lack of a significant relationship between total abundance of nematodes or abundance of nematodes in a trophic group with predation by *Mononchus* indicated that an abundance of free-living nematodes did not stimulate or suppress predation on *R. reniformis* or *M. incognita* by *Mononchus*. Similarly, no food source dilution effect was observed. Low predation rates of *R. reniformis* and *M. incognita* by *Mononchus* (<30%) supported the argument that predatory nematodes with relatively long life cycles are not likely to be effective biocontrol agents by themselves (Stirling, 2011).

Grootaert and Maertens (1976) suggested that predation by *Mononchus* varied depending on the age of the nematodes themselves. The early juvenile stages of *Mononchus* are smaller than late stage juveniles and adult nematodes. The smaller-size juveniles of *Mononchus* precludes consumption of those nematodes that are larger than them. Juvenile mononchida often feed on bacteria and only molt into adulthood in culture plates if adult *Mononchus* are present in the culture (Salinas and Kotcon, 2005). Nonetheless, top-down regulation of predatory nematodes on *M. incognita* has been reported in a relatively healthy soil condition where a portion of the population of *M. incognita* had been suppressed by a naturally occurring bacteria parasite of the nematode, *P. penetrans* (Wang et al., 2008). This phenomenon did not occur at the same field site when the infestation of *P. penetrans* was low (Wang et al., 2008). Some have suggested that the ratio of predators to prey is important for omnivorous and predatory nematodes to suppress plant-parasitic nematodes (Sánchez-Moreno and Ferris, 2007).

Yeates et al. (1993) categorized *Neoactinolaimus* as a predatory or omnivorous nematode. Khan et al. (1995) reported that *Neoactinolaimus* preferred to prey on the second stage juveniles of *M. incognita*, *Anguina tritici*, and *Tylenchulus semipenetrans*. On the other hand, Khan et al. (1995) reported that *Neoactinolaimus* consumed fewer *Helicotylenchus indicus* and *Rotylenchus robustus*, but preyed on *Paratrichodorus, Hirschmanniella oryzae, Xiphenema americanum*, and adult *Aphelenchoides* at a moderate level. The current research also indicated that *Neoactinolaimus* might have preferred to prey on fungivorous nematodes such as *Aphelenchoides* and *Filenchus* over *R. reniformis*. The reduction of *Aphelenchoides* was highest among the prey nematodes and it was entirely eliminated where *Neoactinolaimus* was the only predatory nematode. The % of *Neoactinolaimus* testing positive for DNA of *R. reniformis* was negatively related to the abundance of fungivorous nematodes, but positively related to the abundance of *R. reniformis*. Thus, fungivorous nematodes appear to distract *Neoactinolaimus* from preying on *R. reniformis*. Our prey delimitation results from the predation assay for *Neoactinolaimus* also suggested that *Neoactinolaimus* does not like to feed on *Eucephalobus*. In fact, during the incubation period, *Eucephalobus* increased in population density suggesting the hatch of eggs during the incubation.

In addition, the weak negative relationship between predation by *Neoactinolaimus* with the abundance of other predatory nematodes ($0.06 < P < 0.10$) suggested that the potential of *Neoactinolaimus* preying on plant-parasitic nematodes could be negated by the indigenous nematode community structure. It is interesting to find that without the present of *Eudorylaimus*, percent reduction of *Helicotylenchus*, a rather big nematode compared to the other preys present, became similar to that of *Aphelenchoides*. It is not clear what kind of interaction *Eudorylaimus* might have on the predation of *Neoactinolaimus*.

Although this study focused on evaluating predation potential of *Mononchus* and *Neoactinolaimus*, other omnivorous and predatory nematodes were also examined. We have confirmed the potential predation of *R. reniformis* by three additional predators, i.e., *Prismatolaimus*, *Mesodiplogasteroides*, and *Eudorylaimus*. Yeates et al. (1993) categorized *Prismatolaimus* as a bacterivore with a question mark due to the presence of a tooth in its stoma, suggesting potential as a predatory nematode. The PCR detection technique used here confirmed that *Prismatolaimus* is a predatory nematode, having the telltale DNA of *R. reniformis* present in its gut. Yeates et al. (1993) categorized *Mesodiplogasteroides* as a bacteria feeder and predator. *Mesodiplogasteroides* does prey on *R. reniformis* and may have the potential to be a predatory nematode biocontrol agent against plant-parasitic nematodes. *Eudorylaimus* has been categorized as an omnivorous nematode (Yeates et al., 1993). The fact that *Eudorylaimus* ranked lowest in terms of its predation rate on *R. reniformis*, and its predation on *R. reniformis* was not correlated with abundance of *R. reniformis* nor nematodes in other trophic groups, suggested that *Eudorylaimus* is a poor predator of *R. reniformis* at best. How to categorize a nematode as a predator or omnivore remains unclear but the predatory behavior of *Eudorylaimus* clearly differs from *Mononchus* and *Neoactinolaimus*.

In conclusion, the PCR-based gut content analysis technique is robust and has allowed for detection of specific prey ingested by predatory nematodes in environmental samples. While soil aqueous solutions directly extracted from environmental soil samples are suitable for the study of *Neoactinolaimus*, the soil tube technique is more suitable for *Mononchus*. Although it is discouraging that all the predatory or omnivorous nematodes assayed in this research revealed lower than 40% predation on the targeted nematode prey, this research suggested that many naturally occurring predatory as well as omnivorous nematodes could serve as natural enemies against plant-parasitic nematodes. While the presence of fungivorous and other predatory nematodes could distract *Neoactinolaimus* from predation upon *R. reniformis*, the presence of other free-living nematodes in the soil did not affect *Mononchus* predation of *R. reniformis* or *M. incognita*. Future research in this area should examine the relationship between predation rates and prey population densities, especially in the case of *Mononchus* vs *R. reniformis*. It is likely that predatory nematodes would be more effective as biocontrol agents if augmentation were to take place after planting when population densities of the plant-parasitic nematodes are high.

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