RESEARCH NOTE/NOTA INVESTIGATIVA

DETECTION OF PLANT-PARASITIC NEMATODE DNA IN THE GUT OF PREDATORY AND OMNIVOROUS NEMATODES

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ABSTRACT

Cabos, R. Y. M., K.-H. Wang, B. S. Sipes, W. P. Heller, and T. K. Matsumoto. 2013. Detection of plant-parasitic nematode DNA in the gut of predatory and omnivorous nematodes. Nematropica 43:44-48.

A protocol for molecular gut analysis of nematodes was developed to determine if predatory and omnivorous nematodes from five different guilds prey on *Rotylenchulus reniformis, Meloidogyne incognita*, and *Radopholus similis. Mononchoides, Mononchus, Neoactinolaimus, Mesodorylaimus,* and *Aporcelaimellus* were collected from Hawaii agroecosystems. Gut contents were released by slicing nematodes and pipetted into a PCR tube. Appropriate species-specific primers for the PCR reaction were determined by the predominant plant-parasitic nematodes associated with the source of the predatory and omnivorous nematodes. Predator and omnivore samples containing ingested *R. reniformis, M. incognita* and *R. similis* amplified a band of the desired size of 224, 342, and 269 bp, respectively. All predator and omnivore genera tested were positive for plant-parasitic nematode DNA in at least 22% of the specimens assayed. The highest percentage of positive detections (55%) was in *Neoactinolaimus*. This study confirmed that species-specific PCR primers could detect targeted plant-parasitic nematode prey in the gut of excised nematodes. This protocol could be adopted to further our understanding of the role of nematodes in soil food web interactions.

Key Words: Aporcelaimellus, burrowing nematode, molecular gut content analysis, *Mesodorylaimus, Mononchoides, Mononchus, Neoactinolaimus*, plant-parasitic nematodes, predator-prey, predatory nematodes, reniform nematode, root-knot nematode.

RESUMEN

Cabos, R. Y. M., K.-H. Wang, B. S. Sipes, W. P. Heller, and T. K. Matsumoto. 2013. Detección de DNA de nematodo fitoparásitos en el intestino de nematodos predadores y omnivoros. Nematropica 43:44-48.

Un protocolo para el análisis molecular de los intestinos de nematodos omnivoros y predadores de cinco comunidades diferentes, fue desarrollado para determinar si éstos se alimentan de *Rotylenchulus reniformis, Meloidogyne incognita* y *Radopholus similis. Mononchoides, Mononchus, Neoactinolaimus, Mesodorylaimus* y *Aporcelaimellus* fueron colectados en agroecosistemas de Hawaii. Los nematodos omnívoros y predadores fueron cortados y sus contenidos intestinales fueron transferidos a tubos de PCR. Los cebadores específicos para la reacción de PCR fueron determinados por las especies de nematodos fitoparásitos asociados con la fuente de nematodos omnívoros y predadores. Muestras intestinales de éstos nematodos contenian DNA ingerido de *R. reniformis, M. incognita* y *R. similis*, amplificando bandas del tamaño esperado de 224, 342, y 269 bp, respectívamente. Todos los géneros de nematodos omnívoros y predadores evaluados fueron positivos para DNA de nematodos fitoparásitos en mínimo 22% de los especímenes evaluados. El más alto porcentaje de detecciones positivas (55%) fue en *Neoactinolaimus*. Éste estudio confirma que los cebadores específicos de PCR pueden detectar nematodos fitoparásitos ingeridos por los nematodos examinados. Éste protocolo puede ser adoptado para futuras investigaciones acerca de el rol de los nematodos en las interacciones de la cadena alimenticia del suelo.

Palabras clave: Aporcelaimellus, nematodo barrenador, análisis molecular de contenidos intestinales, *Mesodorylaimus, Mononchoides, Mononchus, Neoactinolaimus*, nematodos fitoparasitos, predador-presa, nematodo reniforme, nematodo agallador.

Categorization of nematode feeding habits has been based on stylet types or the morphology of the buccal cavity (Yeates *et al.*, 1993). Predators as described by Yeates *et al.* (1993) are nematodes that feed on invertebrates, such as rotifers, enchytraeids, protozoa, and other nematodes, whereas omnivores consume a range of food including plants, bacteria, fungi, unicellular eukaryotes, and invertebrates. It is generally assumed that predatory or omnivorous nematodes are not intentionally preying on specific nematode prey, and thus their potential as biological control agents against plant-parasitic nematodes has not been widely appreciated. Several publications have reported on the efficiency of predatory nematodes and advocated their use in biological control (Cobb, 1917; Small, 1979; Bilgrami *et al.*, 2008). Historical data on prey of predatory and omnivorous nematodes has been gathered by 1) examining the gut contents of fixed predator specimens (Bilgrami *et al*., 1986), 2) observations from *in vitro* cultures or feeding chambers (Yeates, 1969; Small and Grootaert, 1983), and 3) pot experiments with known inoculum of predators and prey (Small, 1979; Khan and Kim, 2005). These approaches have been challenged by the inability to recognize partially digested prey in the gut contents of predators, obstacles in establishing in vitro cultures of predators and prey concurrently (especially for plant-parasitic nematodes), difficulties in creating a favorable artificial feeding environment for predatory nematodes, and the uncertainty of total recovery of nematodes from pot experiments. We have used a simple PCR assay to analyze gut contents of predatory and omnivorous nematodes and document their predation of plant-parasitic nematodes.

To confirm that predatory and omnivorous nematodes prey on economically important plant-parasitic nematodes, a protocol for molecular gut analysis using species-specific primers for targeted plant-parasitic nematodes was developed. We targeted reniform (*Rotylenchulus reniformis*), root-knot (*Meloidogyne incognita*), and burrowing nematodes (*Radopholus similis*) that are commonly found in agricultural soil in Hawaii. To ensure predatory nematodes had a favorable niche for feeding, specimens were collected directly from their natural environment, thus avoiding the challenges of maintaining predatory nematode cultures. To demonstrate the versatility of this PCR-based gut analysis technique, predatory and omnivorous nematodes were assayed from five different guilds that are commonly found in the Hawaii agroecosystem. The predatory nematodes were *Mononchoides* (Diplogasteridae, P1 guild), *Mononchus* (Mononchidae, P4 guild), and *Neoactinolaimus* (Actinolaimidae, P5 guild) (Bongers and Bongers, 1998). Two omnivorous nematodes, *Mesodorylaimus* (Dorylaimidae, O4 guild) and *Aporcelaimellus* (Aporcelaimidae, O5 guild) (Bongers and Bongers, 1998), were also assayed.

Predatory or omnivorous nematodes were collected from field soil where the targeted nematodes were known to occur. Samples from sweet potato (*Ipomoea batatas* 'Okinawan'), papaya (*Carica papaya* 'Kapoho'), and dracaena (*Dracaena deremensis* 'Lisa') soils infested with *R. reniformis* were collected, as well as, samples from a beet (*Beta vulgaris* 'Detroit dark red') field infested with both *M. incognita* and *R. reniformis*. Volcanic cinder beds used to grow anthuriums (*Anthurium andraeanum* 'Tropic Ice') and infested with *Radopholus similis* were also sampled. All of the soils collected contained an abundance of microbivorous nematodes (bacterivores and fungivores), rotifers, enchytraeids, protozoa, fungal hyphae, and other microorganisms that could also serve as food for the predatory or omnivorous nematodes. Three samples from each crop were taken bimonthly over a 6-month period.

A total of 45 *Mononchus* were collected from sweet potato, papaya, dracaena, beet, and anthurium soil samples. *Mononchoides* were extracted from the rhizosphere of dracaena, beets, and anthuriums resulting in 32 nematodes. *Mesodorylaimus* were recovered from sweet potato, papaya, dracaena, beet, and anthurium soils for a total of 43 nematodes. Twenty-three *Aporcelaimellus* were recovered from beets. All nematodes were assayed by PCR.

Neoactinolaimus, collected from the rhizosphere of Hawaiian native sedge, `Ahu`awa (*Cyperus javanicus*), were initially established as a co-culture with unsterile Rhabditidae on quarter strength corn meal agar. *Neoactinolaimus* were picked and added to a beaker with 20 mL water and a soil community from cantaloupe (*Cucumis melo*) containing root-knot, reniform, and microbivorous nematodes. *Neoactinolaimus* were allowed to feed for 1 week prior to the assay and 20 specimens were harvested for evaluation by PCR.

Nematodes were extracted from the soil samples using Baermann funnels (Walker and Wilson, 1960) and collected on a 20-µm mesh sieve. Individual predators or omnivores were picked from the nematode mixture and placed in a 10.5-µL drop of water on a clean glass slide. Nematodes were sliced in half with a scalpel to release the gut contents. The solution including nematode parts was pipetted into a 200 µL PCR tube for immediate processing or stored at -20°C.

The appropriate primers for the PCR reaction were determined by visually identifying the predominant plant-parasitic nematodes contained in the sample from which the predators and omnivores were extracted. Primers for *R. reniformis*, RrF (5'-AAAGTGGCTGTTCGCCACACTAAC-3') and RrR (5'-AACCAGGGCGCTCATTGAGTCTTA-3') were designed from the sequence of the internal transcribed spacer 1 (ITS1) region (Genbank #AY335192) submitted by Iwahori and Sano. Primers were created using IDT's PrimerQuest Primer3 software (Rozen and Skaletsky, 1998). *Radopholus similis* primers, RadF (5'-TATGATTCCGTCCTTTGGTGGGCA-3') and RadR (5'-CCACCGATAAGGCTAGAATTCCTGT-3')

were designed from the ITS1 region of isolate RSHZ (Genbank #EU728661) submitted by D. Peng *et al.* The *M. incognita* primer set, Mi1 (5'-AAACGGCTGTCGCTGGTGTC-3') and Mi2 (5'-CCGCTATAAGAGAAAATGACCC-3'), created by Saeki *et al.* (2003) was used to amplify the ITS region of *M. incognita* prey.

PCR amplification was conducted in 25 µL reactions containing 12.5 µL GoTaq MasterMix, 0.4 µM of each primer, and the 10.5 µL macerated predator nematode solution. Appropriate controls were included with each sample set processed including a positive control of a macerated plant-parasitic (prey) nematode and negative controls consisting of either a predatory nematode isolated from soil observed to be free of the target prey or a macerated Rhabditidae or Tylenchidae and a no-template sterile water control. The conditions of the PCR reaction were 95°C for 2 min followed by 40 cycles of 95°C for 45 sec, 56°C for 30 sec, and 72°C for 20 sec, and a final extension of 72°C for 5 min. PCR products and a phiX174 DNA/HaeIII marker were separated on a 1.2% agarose gel stained with GelRed in 1 × TAE and visualized under a UV light.

To verify the food source detected, selected PCR products were sent to Eurofins MWG Operon (Huntsville, AL) for sequencing. To confirm the identity of predatory and omnivorous nematodes, individual specimens were prepared as described above and used in PCR reactions with the universal Nem_18SF (5'-CGCGAATAGCTCATTACAACAGC-3') and Nem_18SR (5'-GGGCGGTATCTGATCGCC-3') primer set (Floyd *et al*., 2005). The resulting PCR products were sequenced by Eurofins MWF Operon and the results compared to predatory and omnivorous nematode genomic sequences entered in the GenBank DNA sequence database.

PCR assay results indicated that plant-parasitic nematode prey could be identified from the guts of predators and omnivores using species-specific PCR primers. This is the first documentation of feeding habits of predatory nematodes using a PCR-based approach. In separate assays, the positive samples containing ingested *R. reniformis, M. incognita* and *R. similis* produced a band of the desired size of 224 (Fig. 1), 342, and 269 bp (Fig. 2), respectively. No band was detected in the predators not exposed to the targeted plant-parasitic nematode, Rhabditidae or Tylenchidae nematode samples, or in the no-template controls further confirming the reliability of this PCR assay. Sequencing results confirmed the PCR products were plant-parasitic nematode DNA and verified the identity of the predatory and omnivorous nematodes assayed.

It is generally believed that predatory and omnivorous nematodes are opportunistic feeders

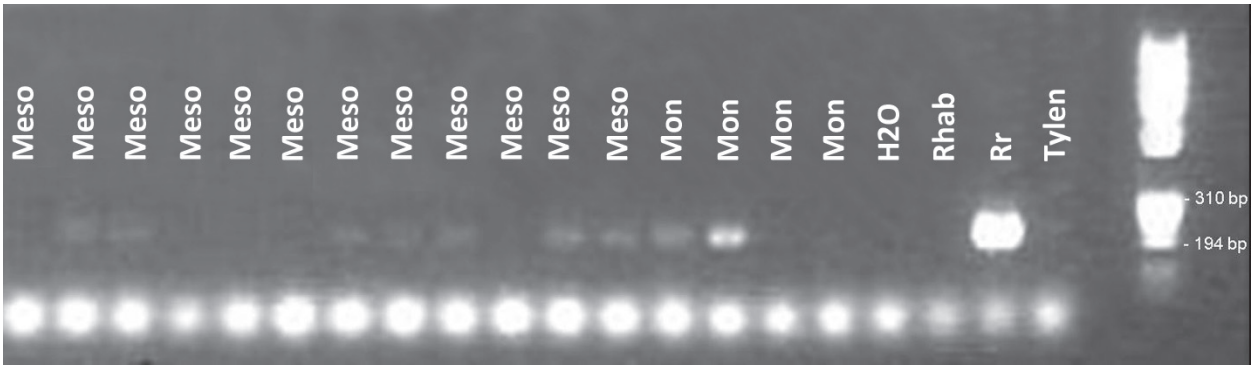

Fig. 1. Agarose gel of PCR products obtained from predatory nematodes using *Rotylenchulus reniformis* nematode primers RrF and RrR. Meso: *Mesodorylaimus*, Mon: *Mononchus*, Rhab: Rhabditidae, Tylen: Tylenchidae, Rr: *R. reniformis*.

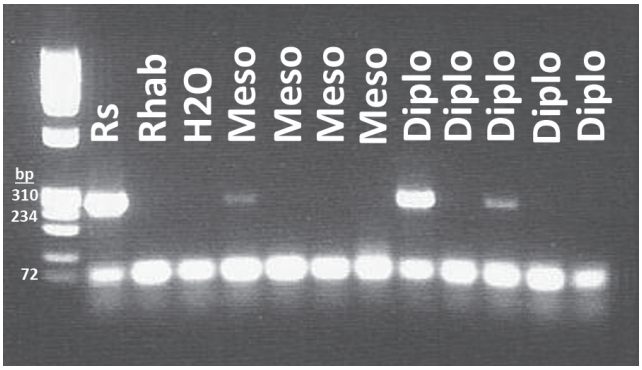

Fig. 2. Agarose gel of PCR products obtained from predatory nematodes using *Radopholus similis* nematode primers RadF and RadR. Meso: *Mesodorylaimus*, Diplo: *Mononchoides*, Rhab: Rhabditidae, Rs: *R. similis*.

Table 1. Percentage of predatory and omnivorous nematodes that tested positive for *Rotylenchulus reniformis, Radopholus similis*, or *Meloidogyne incognita* DNA using species-specific PCR primers.

		Rotylenchulus reniformis			*Radopholus similis*			*Meloidogyne incognita*		
Guild[z]	Predator/Omnivore	PCR +	PCR -	Percent +	PCR +	PCR -	Percent +	PCR +	PCR -	Percent +
P1	*Mononchoides*	7	18	28.0	2	5	28.6	-	-	-
P4	*Mononchus*	18	20	47.4	0	6	0.0	1	9	10.0
P5	*Neoactinolaimus*	11	9	55.0	-	-	-	-	-	-
O4	*Mesodorylaimus*	15	17	46.9	1	10	9.1	-	-	-
O5	*Aporcelaimellus*	2	9	18.2	-	-	-	3	9	25.0

[z]Guilds of predatory (P) and omnivorous (O) nematodes were assigned by Bongers and Bongers (1998).

consuming many types of nematodes and other micro- or mesofauna. The current experiment indicated that when predatory nematodes were exposed to the rhizosphere of plants, they feed on plant-parasitic nematodes. All predatory and omnivorous nematodes tested in this experiment were positive for plant-parasitic nematode DNA in at least 22% of the specimens assayed (Table 1). The highest percentage of positive PCR reactions (55%) was from the *Neoactinolaimus* samples. With the exception of *Mononchoides*, predators tested positive more often for plant-parasitic nematode DNA than omnivores. However the data are insufficient to draw conclusions on feeding preferences or to select the most promising predators for use in biocontrol of plant-parasitic nematodes. Additional research such as assaying a higher volume of samples, evaluating more genera of predatory and omnivorous nematodes, and determining feeding preferences based on known food sources is needed to further our understanding in this area.

In comparison to examining fixed specimens and observing in vitro cultures, more predatory nematode samples can be evaluated with less work hours by using this PCR assay. However, this technique is not quantitative and can only test if plant-parasitic nematode DNA is present in the gut of a predatory or omnivorous nematode at the time of the assay; thus a negative result does not indicate a predator has never fed on the target nematode prey. Treonis *et al.* (2010) identified bacterial rRNA within the pharynx of bacterial-feeding nematodes using taxon-specific fluorescent *in situ* hybridization but could not detect fungi in fungal-feeding nematodes likely due to the rapid digestion of the fungal hyphae ribosomes. In our work, negative results could occur if the PCR reaction was not sufficiently sensitive, the predatory or omnivorous nematode was degraded, the prey had denatured due to digestion, or the nematode had not fed recently. Strike rates and predation by *Mesodorylaimus bastiani* on plant-parasitic and saprophagous nematodes in petri dish assays has been reported (Bilgrami, 1995); however, the impact of this predation on the natural soil food web is unknown. More research needs to be conducted to examine the percentage of plant-parasitic nematodes being preyed upon by predatory and omnivorous nematodes under field conditions.

This work further supports Yeates *et al.* (1993) conclusions that categorizing feeding habits based on mouth structure is an accurate foundation for analyzing nematode communities. We confirmed that the predatory and omnivorous nematodes assayed were correctly grouped as animal predators in his outline for soil ecologists.

This study confirmed that species-specific PCR primers could detect targeted plant-parasitic nematode prey in the gut of excised predatory and omnivorous nematodes. This protocol could be adopted to further our understanding of the role of nematodes in soil food web interactions.

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LITERATURE CITED

Bilgrami, A. L. 1995. Numerical analysis of the predatory relations between *Mesodorylaimus bastiani* (Nematoda: Dorylaimida) and different prey trophic categories. Nematologia Mediterranea 23:81-88.

Bilgrami, A. L., I. Ahmad, and M. S. Jairajpuri. 1986. A study of the intestinal contents of some mononchs. Revue de Nématologie 9:191-194.

Bilgrami, A. L., C. Brey, and R. Gaugler. 2008. First field release of a predatory nematode, *Mononchoides*

gaugleri (Nematoda: Diplogastrida), to control plant-parasitic nematodes. Nematology 10:143-146.

Bongers, T., and M. Bongers. 1998. Functional diversity of nematodes. Applied Soil Ecology 10: 239-251.

Cobb, N. A. 1917. The *Mononchus*: a genus of free living predatory nematodes. Soil Science 3:431-486.

Floyd, R. M., A. D. Rogers, J. D. Lambshead, and C. R. Smith. 2005. Nematode-specific PCR primers for the 18S small subunit rRNA gene. Molecular Ecology Notes 5:611-612.

Khan, Z., and Y. H. Kim. 2005. The predatory nematode, *Mononchoides fortidens* (Nematode: Diplogasterida), suppresses the root-knot nematode, *Meloidogyne arenaria*, in potted field soil. Biological Control 35:78-82.

Rozen, S., and H. J. Skaletsky. 1998. Primer3. Code available online at http:///sourceforge.net/projects/primer3/.

Saeki, Y., E. Kawano, C. Yamashita, S. Akao, and Y. Nagatomo. 2003. Detection of plant parasitic nematodes, *Meloidogyne incognita* and *Pratylenchus coffeae* by multiplex PCR using specific primers. Soil Science and Plant Nutrition 49:291-295.

Small, R. W. 1979. The effects of predatory nematodes on populations of plant parasitic nematodes in pots. Nematologica 25:94-103.

Small, R. W., and P. Grootaert. 1983. Observations on the predation abilities of some soil dwelling predatory nematodes. Nematologica 29:109-118.

Treonis, A. M., E. H. Michelle, C. A. O'Leary, E. E. Austin, and C. B. Marks. 2010. Identification and localization of food-source microbial nucleic acids inside soil nematodes. Soil Biology and Biochemistry 42:2005-2011.

Walker, J. T. and J. D. Wilson. 1960. The separation of nematodes from soil by a modified Baermann funnel technique. Plant Disease Reporter 44:94-97.

Yeates, G. W. 1969. Predation by *Mononchoides potohikus* (Nematoda: Diplogasteridae) in laboratory culture. Nematologica 15:1-9.

Yeates, G. W., T. Bongers, R. G. M. De Goede, D. W. Freckman, and S. S. Georgieva. 1993. Feeding habits in soil nematode families and genera- an outline for soil ecologists. Journal of Nematology 25:101-313.

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