

**DAUER JUVENILES OF *POIKILOLAIMUS FLORIDENSIS* (RHABDITIDA: RHABDITIDAE)  
ISOLATED FROM DRYWOOD TERMITES (KALOTERMITIDAE)**

Natsumi Kanzaki <sup>1,\*</sup>, Robin M. Giblin-Davis <sup>1</sup>, Rudolf H. Scheffrahn<sup>1</sup>, and Barbara J. Center <sup>1</sup>

<sup>1</sup> Fort Lauderdale Research and Education Center, University of Florida/IFAS, Davie, Florida 33314 USA; <sup>2</sup> Forest Pathology Laboratory, Forestry and Forest Products Research Institute, Ibaraki Japan.  
\*Corresponding author: nkanzaki@affrc.go.jp

---

ABSTRACT

Kanzaki, N., R. M. Giblin-Davis, R. H. Scheffrahn, and B. J. Center. 2009. Dauer juveniles of *Poikilolaimus floridensis* (Rhabditida: Rhabditidae) isolated from drywood termites (Kalotermitidae). *Nematropica* 39:305-310.

The dauer juvenile of *Poikilolaimus floridensis* is described and illustrated. The dauer juveniles were isolated from the foregut of *Incisitermes schwarzi* (nymphs), *I. milleri* (alates and workers) and *Cryptotermes cavifrons* (alates) collected from the upper Florida Keys and identified as *P. floridensis* using an SSU molecular barcode sequence. The dauers isolated from the termites have a closed stoma and degenerative digestive system, suggesting that the dauer juvenile stage is phoretic and not parasitic. The number of nematodes per infected termite and the infection ratio of termites were low, *i.e.*, three or fewer and 0-33%, respectively. These low numbers and association ratios appear relatively weak for maintenance of the termite-nematode association without local extinctions. Further studies on the life history are needed to understand the dynamics of the association between *P. floridensis* and kalotermitid termites.

*Key words:* Dauer juveniles, new host, phoretic nematode, termite.

---

RESUMEN

Kanzaki, N., R. M. Giblin-Davis, R. H. Scheffrahn, and B. J. Center. 2009. Juveniles dauer de *Poikilolaimus floridensis* (Rhabditida: Rhabditidae) aislados de termitas (Kalotermitidae). *Nematropica* 39:305-310.

Se describe e ilustra el juvenil dauer de *Poikilolaimus floridensis*. Estos juveniles dauer se aislaron del sistema digestivo de *Incisitermes schwarzi* (ninfas), *I. milleri* (alados y obreros) y *Cryptotermes cavifrons* (alados) colectados en Florida Keys y se identificaron como *P. floridensis* utilizando la secuencia de la unidad ribosomal pequeña (SSU). Los juveniles aislados de las termitas poseen un estoma cerrado y aparato digestivo atrofiado, lo cual sugiere que este estadio juvenil es forético y no parasítico. La cantidad de nematodos por termita infectada y la tasa de infección de termitas fue baja, con tres o menos y 0 a 33%, respectivamente. Estas bajas cantidades y tasas de asociación son relativamente débiles para el mantenimiento de la asociación sin extinciones locales. Se requieren más estudios del ciclo de vida para entender la dinámica de la asociación entre *P. floridensis* y las termitas kalotermitidas.

*Palabras clave:* juveniles dauer, nematodo forético, nuevo hospedante, termita.

---

In a previous study, the authors conducted a field survey of the termite-associated nematodes in Secret Woods Nature Center, Fort Lauderdale, Florida, and described a phoretic rhabditid nematode,

*Poikilolaimus floridensis* Kanzaki and Giblin-Davis, 2009 from *Incisitermes snyderi* (Light), *Neotermes castaneus* (Burmeister), and *N. jouteli* (Banks) with a note that the nematode was associated with *I. schwarzi*

(Banks), *I. milleri* (Emerson), and *Cryptotermes cavifrons* Banks as our “unpublished data”, because the numbers of nematodes associated with an individual termite, organ of residence within those termites and the morphology and biology of the dauer juveniles were not clear at the time (Kanzaki *et al.*, 2009).

In the present study, we conducted a follow-up field survey at a different locality in south Florida and obtained the phoretic dauer juveniles of *P. floridensis* from the foregut of five species of termites. Herein, detailed morphology of the dauer juvenile is described and illustrated.

#### *Termite Samples*

Field colonies of termites were collected along trails at the Dagny Johnson Key Largo Hammock State Park (DJ) (25.17608N, 80.36945W) and John Pennekamp State Park (PSP) (25.12515N, 80.40722W) on 10 April 2008. Up to 15-30 individual termites were randomly chosen from each colony for dissection.

#### *Isolation of Nematodes from Termites and Molecular Identification*

Termites were placed in the water droplet for several minutes and observed for external/casual associations. Dissections were conducted in a drop of deionized water under a dissecting microscope, and insects were carefully examined for nematodes. The potential nematode association with each termite haemocoel, head capsule and digestive system was examined separately.

The nematodes isolated from each termite were microscopically examined. During the observation, several individuals were photographed and/or drawn with a camera lucida drawing tube. The observed nematodes were heat-killed (65°C) and fixed in formalin-glycerol, processed

through a glycerin-ethanol series using Seinhorst’s method (Hooper, 1986) and mounted in glycerin according to the method of Maeseneer and d’Herde (Hooper, 1986), or collected individually into nematode lysis buffer (Ye *et al.*, 2007) for subsequent molecular identification.

The termite species name, colony number and nematode infestation levels are reported in Table 1. Only *P. floridensis* was recognized in the present study, and no other nematode species was isolated. All three termite species (*I. milleri*: an alate and a worker, *I. schwarzi*: nymphs and *C. cavifrons*: alates) collected at Dagny Johnson Key Largo Hammock State were associated with *P. floridensis*. All nematodes were isolated from the foregut of the termite, regardless of caste. No nematode was isolated from the termites collected at John Pennekamp State Park.

The molecular barcode sequence, ca. 600 bps of SSU rDNA (Giblin-Davis *et al.*, 2007; Kanzaki *et al.*, 2008), of three individual dauers from *I. milleri*, *I. schwarzi*, and *C. cavifrons*, were identical to that of *P. floridensis* deposited at the GenBank database with accession number, AB370214.

#### *Description of Dauer Juveniles*

The morphology of the living specimens of the dauer juveniles are shown in Figs. 1 and 2. Measurements of permanent-mounted specimens are shown in Table 2.

Body cylindrical, slender. Cuticle smooth, thin, with shallow transverse annulations. Lateral field not observed clearly. Six equal-sized lips fused to form a dome-shaped anterior end. Anterior end of stoma closed. Amphidial apertures not observed clearly. Stoma long, thin, tube-like, simple, lacking clear glottoid apparatus, teeth or denticles. Cheilostom short, ring-like, closed completely. Gymnostom tube-like, occupying ca. one-fourth of total stoma. Stegostom long,

Table 1. Collected termites and their nematode association.

| Colony ID <sup>*</sup> | Termite species               | Number and stage of dissected termites <sup>†</sup> | Number of termites with nematodes <sup>†</sup> | Number of nematodes per termite <sup>‡</sup> |
|------------------------|-------------------------------|---|--|--|
| DJ-1                   | <i>Incisitermes milleri</i>   | 3 a   | 1 a  | 3  |
| DJ-2                   | <i>I. milleri</i>             | 13 w  | 1 w  | 1  |
| DJ-3                   | <i>I. schwarzi</i>            | 12 w  | 0  | —  |
| DJ-4                   | <i>I. schwarzi</i>            | 4 a, 11 n   | 3 n  | 1, 1, 2                                      |
| DJ-5                   | <i>Cryptotermes cavifrons</i> | 11 a  | 2  | 1, 1   |
| DJ-6                   | <i>C. cavifrons</i>           | 10 a  | 0  | —  |
| DJ-7                   | <i>C. cavifrons</i>           | 13 w  | 0  | —  |
| PSP-1                  | <i>I. milleri</i>             | 18 w  | 0  | —  |
| PSP-2                  | <i>I. schwarzi</i>            | 10 w  | 0  | —  |
| PSP-3                  | <i>C. cavifrons</i>           | 15 w  | 0  | —  |
| PSP-4                  | <i>Neotermes castaneus</i>    | 6 w   | 0  | —  |
| PSP-5                  | <i>N. jouteli</i>             | 9 w   | 0  | —  |

<sup>\*</sup>DJ: Dagny Johnson Key Largo Hammock State Park; PSP: John Pennekamp State Park.

<sup>†</sup>a: alate; w: worker; n: nymph.

<sup>‡</sup>Only *Poikilolaimus floridensis* was found.

tube-like, *ca.* twice as long as gymnostom. Pharyngeal sleeve visible. Pharynx somewhat degenerate, but observed clearly. Procorpus cylindrical, muscular. Metacorpus appears as weakly developed median bulb. Isthmus long, slender, muscular. Terminal bulb muscular, elongated oval to polygonal in form, with clear valvular (grinder) apparatus at middle. Cardia distinguishable, closed. Nerve ring surrounding isthmus at middle or a little posterior to midpoint. Excretory pore visible, heavily sclerotized, adjacent to or a little anterior to terminal bulb. Deirid and post-deirid not observed. Entire intestine occupied by lipid-like storage material. Genital anlagen occurs around the middle part of the ventral body. Rectum and anus visible. Tail slightly ventrally arcuate, conical with bluntly pointed tip.

#### Voucher Material

Three individuals isolated from *I. milleri* and three individuals from *I. schwarzi* were

available for the morphometrics. The six individuals were deposited at Fort Lauderdale Research and Education Center, University of Florida, Fort Lauderdale, Florida, USA.

#### Remarks

Currently, several authors have reported a variety of nematode-termite associations (summarized by Carta *et al.*, 2005; Fürst von Lieven, 2003; Fürst von Lieven and Sudhaus 2008; Kanzaki *et al.*, 2009). However, except for a report by Pemberton (1928), a "*Rhabditis* sp." from *N. connexus* Snyder, *P. floridensis* is the only nematode species isolated from termites in the family Kalotermitidae (dampwood or drywood termites) (Kanzaki *et al.*, 2009). Because of its wide-ranging association with kalotermitid termites (two species of *Neotermes*, three species of *Incisitermes*, and *C. cavifrons*), the vector specificity of *P. floridensis* does not seem high within the

Table 2. Morphometrics of the dauer juveniles of *Poikilolaimus floridensis* from two different termite colonies. All measurements are in  $\mu\text{m}$ .

|  | DJ-1 ( <i>Incisitermes milleri</i> ) | DJ-4 ( <i>I. schwarzi</i> ) |
|--|--------------------------------------|-----------------------------|
| n  | 3 or 2                               | 3                           |
| L  | 410, 400, 500                        | 450, 440, 490               |
| a  | 29.3, 28.6, 30.3                     | 32.1, 33.8, 32.7            |
| b  | — <sup>x</sup> , 4.0, 4.4            | 4.2, 4.3, 4.6               |
| c  | — <sup>x</sup> , 13.3, 12.8          | 13.2, 12.9, 13.6            |
| c'                                       | — <sup>x</sup> , 3.3, 3.9            | 3.8, 4.0, 4.0               |
| Maximum body diam.                       | 14, 14, 16.5                         | 14, 13, 15                  |
| Stoma length                             | — <sup>x</sup> , 12.5, 14            | 13, 13, 14                  |
| Anterior pharynx (pro + meta corpus)     | — <sup>x</sup> , 44, 50              | 47, 44, 46                  |
| Posterior pharynx (isthmus + basal bulb) | — <sup>x</sup> , 43, 49              | 47, 45, 47                  |
| Nerve ring from anterior end             | — <sup>x</sup> , 74, 79              | 77, 70, 77                  |
| Excretory pore from anterior end         | — <sup>x</sup> , 83, 93              | 91, 87, 860                 |
| Anal body diam.                          | — <sup>x</sup> , 9, 10               | 9, 8.5, 9                   |
| Tail length                              | — <sup>x</sup> , 30, 39              | 34, 34, 36                  |

<sup>x</sup>Not available because of sample condition.

kalotermitids occurring sympatrically. Nevertheless, the nematode has not been isolated from termites in the other families at the same locality (Kanzaki *et al.*, 2009). The habitat preference (= relatively dry condition) of the nematode seems to be a limiting factor of its vector range.

Morphologically, the stoma of the dauer juveniles of *P. floridensis* is closed and the digestive system is degenerative (Figs. 1 and 2). A plug-like object in the stoma (Fig. 1B) is considered to be an artefact, because this structure was not observed in the other specimens. These morphological traits suggest that the dauer juveniles are not a “parasitic juvenile”, but are endophoretic dauers.

The number of nematodes per infected termite was one to three, and the nematodes were isolated from the foregut of *I. milleri* workers and *I. milleri*, *I. schwarzi* and *C. caviifrons* alates (Table 1). In comparison with the previous study, the nematode

infection manner does not differ between worker, soldier, and alates, *i.e.*, less than five individual nematodes were isolated from foregut (Kanzaki *et al.*, 2009). The colony of kalotermitid termites is established by a mated pair of adults (Nutting, 1969). Thus, the infection ratio and number of nematodes, *i.e.*, only two individual alates out of 11 examined had one nematode each in *C. caviifrons* colony DJ-5, appears too low for vertical transmission and establishment of a new nematode population in a termite nest. We do not have any clear explanation for this phenomenon. More field surveys and laboratory studies are necessary to understand the life history of this highly specialized nematode species.

#### ACKNOWLEDGEMENTS

This study was supported in part by National Science Foundation (NSF) Biotic

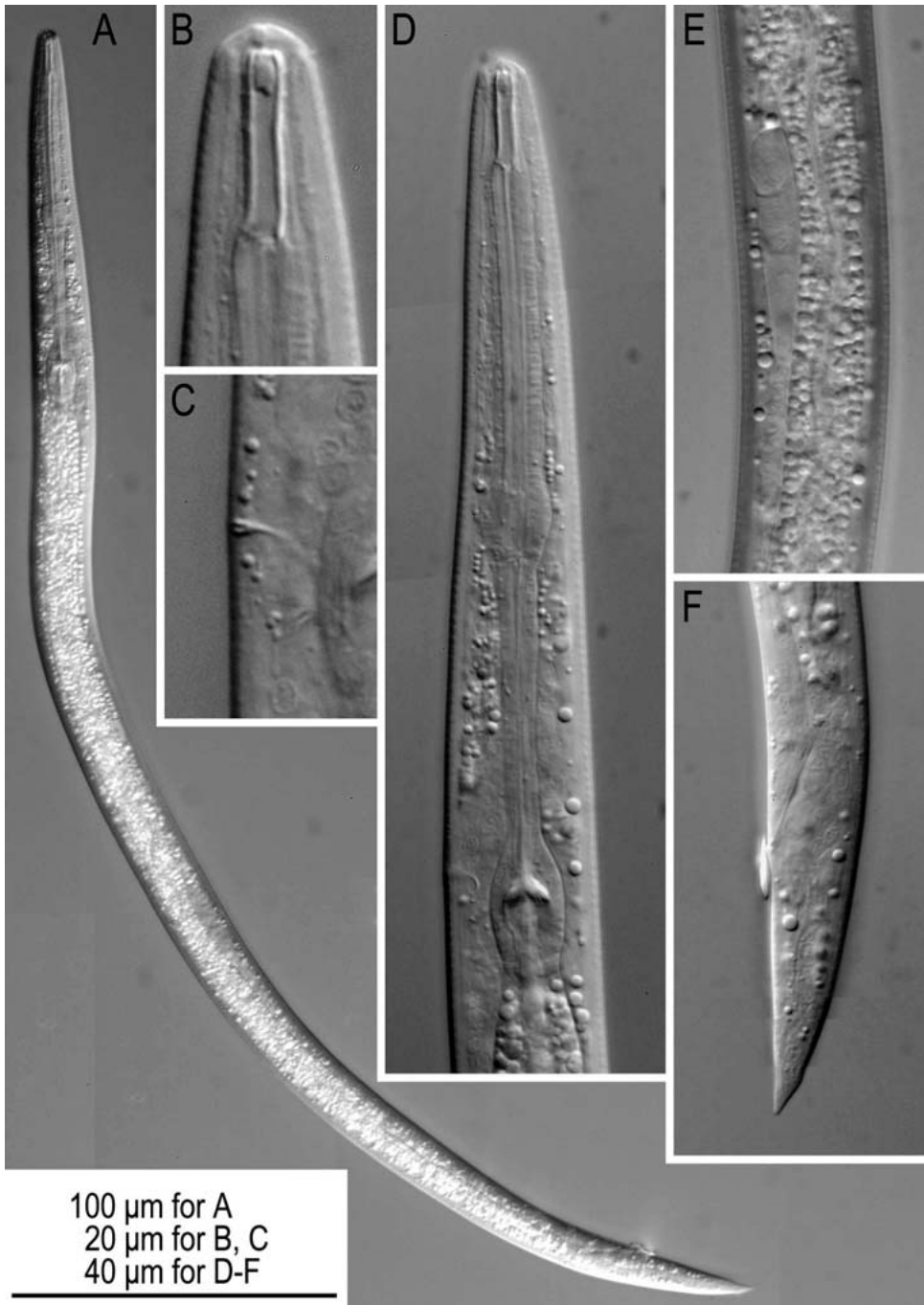


Fig. 1. The dauer juvenile of *Poikilolaimus floridensis* A: Whole body; B: Stoma; C: Excretory pore; D: Anterior part; E: Genital anlagen; F: Tail region.

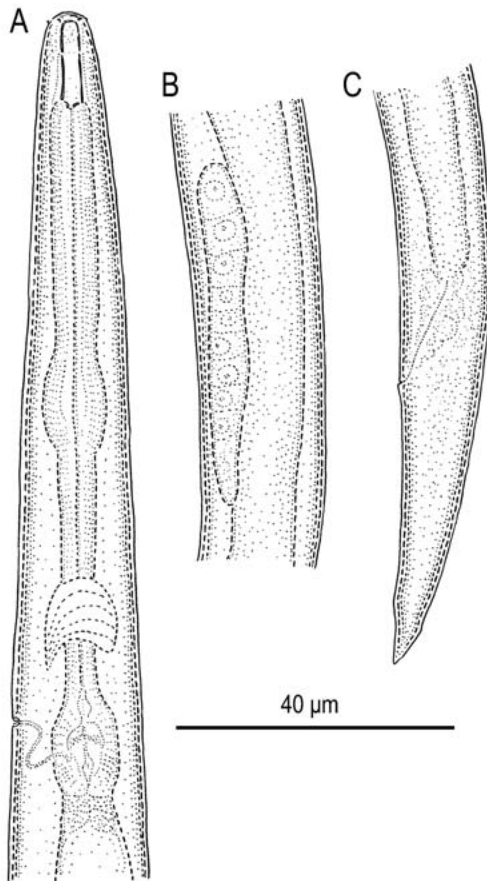


Fig. 2. The dauer juvenile of *Poikilolaimus floridensis* A: Anterior part; B: Genital Anlagen; C: Tail region.

Surveys and Inventories projects (DEB-0450537; DEB-0640807).

#### LITERATURE CITED

- Carta, L. K., and W. Osbrink. 2005. *Rhabditis rainai* n. sp. (Nematoda: Rhabditida) associated with the Formosan subterranean termite, *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *Nematology* 7:863-879.
- Fürst von Lieven, A. 2003. The genus *Oigolaimella* Paramonov, 1952 (Nematoda: Diplogastridae) and description of *O. kruegeri* n. sp. and *O. ninae* n. sp. *Nematology* 5:583-600.
- Fürst von Lieven, A., and W. Sudhaus. 2008. Description of *Oigolaimella attenuata* n. sp. (Diplogastridae) associated with termites (*Reticulitermes*) and remarks on life cycle, giant spermatozoa, gut-inhabiting flagellates and other associates. *Nematology* 10:501-523.
- Giblin-Davis, R. M., N. Kanzaki, W. Ye, R. H. Scheffrahn, and T. O. Powers. 2007. Focal sampling of termites to test the latitudinal gradient hypothesis for entomophilic nematode diversity. *Journal of Nematology* 39:96.
- Hooper, D. J. 1986. Handling, fixing, staining and mounting nematodes. Pp. 59-80 in J. F. Southey, ed. *Laboratory methods for work with plant and soil nematodes*. London: Her Majesty's Stationery Office.
- Kanzaki, N., N. Machara, T. Aikawa, R. M. Giblin-Davis, and B. J. Center. 2008. The first report of a putative "parasitic adult form" in *Bursaphelenchus*. *Journal of Parasitology* 95:113-119.
- Kanzaki, N., R. M. Giblin-Davis, R. H. Scheffrahn, and B. J. Center. 2009. *Poikilolaimus floridensis* n. sp. (Rhabditida: Rhabditidae) associated with termites (Kalotermitidae). *Nematology* 11:203-216.
- Nutting, W. L. 1969. Flight and colony foundation. Pp. 233-282 in K. Krishna and F. M. Weesner, Eds. *Biology of termites Vol. 1*. New York: Academic Press.
- Pemberton, C. E. 1928. Nematode associated with termites in Hawaii, Borneo and Celebes. *Proceedings of the Hawaiian Entomological Society* 7:148-150.
- Ye, W., R. M. Giblin-Davis, H. Braasch, K. Morris, and W. K. Thomas. 2007. Phylogenetic relationships among *Bursaphelenchus* species (Nematoda: Parasitaphelenchidae) inferred from nuclear ribosomal and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution* 43:1185-1197.

Received:

5/XII/2008

Accepted for publication:

9/VIII/2009

Recibido:

Aceptado para publicación