2-1-1994

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A NEW SPECIES OF *HETERORHABDITIS* FROM THE HAWAIIAN ISLANDS

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**ABSTRACT:** A new species of nematode of the genus *Heterorhabditis* (Nemata: Heterorhabditidae) was found during a survey of the soil entomopathogenic nematode fauna of the Hawaiian Islands. *Heterorhabditis hawaiiensis* sp. n. can be separated from all other species of *Heterorhabditis* by the length of the infective juvenile and the morphological characters of the spicules, gubernaculum, and bursa. Random amplified polymorphic DNA (RAPD) fragment analysis showed that this species also has a distinct genetic pattern in RAPD bands relative to the other 6 species or isolates of *Heterorhabditis* that were compared.

Entomopathogenic nematodes of the genus *Heterorhabditis* Poinar, 1975, are being investigated as agents for biological control of soil-inhabiting insects (Gaugler and Kaya, 1990). Free-living infective third-stage juveniles (J₃) locate and enter a suitable insect host through natural body openings (mouth, anus, or spiracles) and penetrate into the hemocoel. Infective nematodes can also penetrate into the hemocoel through thin areas of the insect's cuticle (Bedding and Molyneux, 1982). Once in the insect, the nematodes release symbiotic bacteria, *Photorhabdus luminescens* (Poinar and Thomas, 1976), from their intestinal tracts. The bacteria multiply and kill the host insect, and the nematodes feed on bacteria, becoming hermaphroditic adults. Hermaphroditic adults produce progeny that develop into both males and females. These adults mate and females produce infective-stage juveniles that leave the cadaver of the insect (Poinar, 1990; Dix et al., 1992).

*Heterorhabditid* nematodes are cosmopolitan (Poinar, 1990; Poinar et al., 1992) with all described species showing allopatric distributions. Presently, 4 species of nematodes are assigned to the genus *Heterorhabditis*. *Heterorhabditis bacteriophora* Poinar, 1975, was described from specimens isolated from Brecon, Australia (Poinar, 1975). *Heterorhabditis heliothidis* (Khan, Brooks, and Hirschmann, 1976) from North Carolina subsequently was determined to be conspecific with *Heterorhabditis bacteriophora* (see Poinar, 1990). *Heterorhabditis zealandica* Poinar, 1990, originally described as a New Zealand population of *H. heliothidis* by Wouts (1979), was reassigned by Poinar (1990). *Heterorhabditis megidis* Poinar, Jackson, and Klein, 1987, was isolated from infected insects from Ohio. *Heterorhabditis indicus* Poinar, Karunakar, and David, 1992, was described from specimens collected in Tamil Nadu, India. In addition, a possible new species of *Heterorhabditis* recently has been isolated from Argentina.

Many other uncharacterized and taxonomically unstudied populations of nematodes of the genus *Heterorhabditis* exist in nature, and various "isolates," "strains," or "biotypes," with a percentage of them likely representing undescribed species, are being cultured in laboratories around the world.

During a survey of the diversity of entomopathogenic nematodes of Hawaii (Hara et al., 1991), several populations representing a new species of the genus *Heterorhabditis* were isolated from soil using trap-insects. Investigation of morphological and genetic variation in these nematodes revealed that characters were appreciably different from those described previously. Herein we describe these nematodes as a new species.

**MATERIALS AND METHODS**

Nematodes were isolated from soil using trap-insects, *Galleria mellonella* (Linnaeus), and maintained in culture in our laboratory (see Hara et al., 1991). Standard methods of in vivo culturing on *G. mellonella* were followed to obtain different stages that were measured (Woodring and Kaya, 1988). Nematodes recovered from *G. mellonella* were fixed in triethanolamine formalin (TAF) (Woodring and Kaya, 1988) and cleared in either lactophenol or glycerol and lactic acid. Quantitative measurements were made using a Leitz Ortholux II microscope equipped with an ocular micrometer and Jandel™ software and video digitizer. Drawings were made with the aid of a drawing tube. All measurements are given in micrometers with means following in parentheses unless otherwise indicated.

Received 26 March 1993; revised 13 September 1993; accepted 20 September 1993.

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Methods of random amplified polymorphic DNA (RAPD) extraction and analysis generally follow Caswell-Chen et al. (1992) with the following modifications: Several thousand infective juveniles collected from 14-day-old cultures of nematodes in G. mellonella were washed in buffered saline 5 times and then in 95% ethanol (EtOH) 5 times. They were then transferred to fresh 95% EtOH for storage. Several thousand alcohol-precipitated infective juveniles (0.07 g wet weight) were transferred to a glass homogenizing tube containing extraction buffer (100 mM ethylene diamine tetraacetic acid, pH 8.0; 0.5% sodium lauryl sulfate; 50 μg/ml proteinase K), homogenized on ice at 1–2 °C, and transferred to a 1.5-mL Eppendorf™ tube. Extraction buffer was added to make a final volume of 500 μl. This was incubated in a water bath at 50 °C for 2 hr. To remove proteins and other cellular debris, 500 μl of phenol/chloroform/isooamyl alcohol (25:24:1) was added to the tube and centrifuged at 16,000 g for 15 min at 21 °C. DNA was precipitated from the supernatant portion with 1/10 volume of 3 M NaAc, pH 5.5, and 3 volumes of 95% EtOH. The precipitate was resuspended in TE buffer (pH 8.0) and used directly for polymerase chain reactions (PCR) for the RAPD analysis. Operon™ random primers (A-05, A-13, B-1, B-4) of 10 bases long were used for all reaction experiments with an annealing temperature of 35 °C.

Spectrophotometric measurement of DNA samples extracted from each species was conducted to ensure that the solution concentration of template DNA remained at 10 ng/μl. Purified DNA from the nematode genome was subjected to the PCR reaction, and the amplified DNA was run on a 1.7% horizontal agarose gel containing extraction buffer (100 mM ethylene diamine tetraacetic acid, pH 8.0) and 21% acetic acid, pH 5.5, and 3 volumes of 95% EtOH. The precipitate was resuspended in TE buffer (pH 8.0) and used directly for polymerase chain reactions (PCR) for the RAPD analysis. Operon™ random primers (A-05, A-13, B-1, B-4) of 10 bases long were used for all reaction experiments with an annealing temperature of 35 °C.


**Amphimictic female** (n = 20): Length 1.3–2.3 mm (1.8 mm). Maximum width 104–171 (139). Stoma length 6–12 (9). Stoma width 5–10 (7). Distance from anterior end to nerve ring 78–116 (99). Distance from anterior end to excretory pore 116–175 (153). Esophagus 110–153 (137) long. Vulva situated from 49 to 56% (52%) of body length. Tail length 49–87 (63). Width at anus 20–35 (29).


**Taxonomic summary**

**Symbiotype:** Unknown in nature, from trap-insect in sandy loam under ironwood tree (Casuarina equisetifolia Linnaeus).

**Type locality:** Hanalei, 0.5 m, Island of Kauai, Hawaii (22°12'N, 159°30'W).
TABLE I. Similarity matrix based on Jaccards’ coefficient of shared random amplified polymorphic DNA (RAPD) bands among 7 species and isolates of *Heterorhabditis.*

<table>
<thead>
<tr>
<th>Species or strain of <em>Heterorhabditis</em></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. sp.</em> 1 Argentina</td>
<td>1.000000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. bacteriophora</em></td>
<td>0.4660194</td>
<td>1.000000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. megidis</em></td>
<td>0.2363636</td>
<td>0.1864407</td>
<td>1.000000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. sp.</em> 2 Davis</td>
<td>0.8987342</td>
<td>0.4509804</td>
<td>0.2363636</td>
<td>1.000000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. sp.</em> 3 Tasmania</td>
<td>0.2053571</td>
<td>0.1983471</td>
<td>0.8591549</td>
<td>0.1891892</td>
<td>1.000000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. hawaiiensis</em></td>
<td>0.1417323</td>
<td>0.1297710</td>
<td>0.1769912</td>
<td>0.1583333</td>
<td>0.1810345</td>
<td>1.000000</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. zealandica</em></td>
<td>0.1742424</td>
<td>0.1641791</td>
<td>0.2018349</td>
<td>0.1666667</td>
<td>0.2477064</td>
<td>0.2265625</td>
<td>1.000000</td>
<td></td>
</tr>
<tr>
<td><em>S. carpocapsae</em></td>
<td>0.1884058</td>
<td>0.2248062</td>
<td>0.2212389</td>
<td>0.1739130</td>
<td>0.2231405</td>
<td>0.1510791</td>
<td>0.1857143</td>
<td>1.000000</td>
</tr>
</tbody>
</table>

* Complete identity is 1 and complete difference is 0. Numbers in matrix represent percentage of shared RAPD bands ranging from a theoretical low of 0 to a maximum of 1.


**Specimens deposited:** Holotype male, University of California Davis Nematode Collection number (UCDNC) UCDNC 3234, field collection number KH3. Allotype amphimictic female, UCDNC 3235. Paratypes, 5 males UCDNC 3236, 5 amphimictic females UCDNC 3237, 5 hermaphroditic females UCDNC 3238, 5 infective juveniles UCDNC 3239.

**Etymology:** This species was named after the geographic locality from which it was found.

**Remarks**

*Heterorhabditis hawaiiensis* can be distinguished easily from all previously described species of *Heterorhabditis* except *H. indicus* by having much shorter infective juveniles. *Heterorhabditis hawaiiensis* can be separated from *H. indicus* by having spicules differing in overall shape, with *H. hawaiiensis* having a more well developed manubrium and the lamina with a ventral expansion, and in the more pronounced development of the bursal rays (cf. Figs. 11–15 with fig. 4 in Poinar et al. [1992]). In addition, the genetic profiles generated by RAPD fragment analysis show that this species is distinct, sharing a maximum of 18% of the RAPD bands (Table I) with the other 6 heterorhabditid isolates and/or species that we analyzed (Fig. 17).

**DISCUSSION**

Despite the importance of nematodes of the genus *Heterorhabditis* in biological control, few robust quantitative studies of morphological and genetic variation in *Heterorhabditis* have been published (see, however, Akhurst, 1987; Curran, 1990). Usually, some knowledge of the extent of morphological or genetic variation of the population and the species is required before a specimen can be assigned to its proper species.

The morphological characteristics of small rhhabditid bacterial-feeding nematodes are believed to be difficult to study (Dix et al., 1992). Relative to nematodes of the genus *Heterorhabditis*, Dix et al. (1992) stated "... the extent of overlap in morphometric characters is such that, with the possible exception of *H. megidis*, no one individual from a population can be reliably assigned to a particular species." The lack of comparative data makes it difficult to assign individuals of these nematodes to a correct species using only morphological characters. However, with the advent of PCR techniques, unambiguous and rapid identifications of even single juvenile nematodes now should be realized.

For diagnosis of species or strains of nematodes, RAPD fragments constitute a welcome addition to sometimes scarce morphological characters. Although extreme care must be taken in the collection, preparation, and analysis of DNA for study of RAPDs, patterns can be generated relatively easily and quickly (Williams et al., 1990; Ellsworth et al., 1992). RAPD markers have been shown to work well for genetic analysis with the absence of a band in 1 taxon being attributed to the lack of amplification of the DNA due to loss
of the DNA priming site by mutation, i.e., deletion or insertion (Williams et al., 1990). The RAPD patterns that we demonstrated in this study serve well for diagnosis and, in the future, may possibly be used for characters in phylogenetic analyses.

ACKNOWLEDGMENTS

We especially thank Frances F. Wu for technical assistance in DNA analyses. This work was supported in part by National Science Foundation grant BSR-9024816 to S.L.G., by grant number 9386 from the University of California Genetic Resources Conservation Program to S.L.G. and H.K.K., and by a grant from the International Foundation for Science, Stockholm, Sweden, to S.P.S.

LITERATURE CITED


15. Posterior end of male, right lateral view. 16. Whole amphimictic female, left lateral view. Scale bars same for Figures 12, 13.


